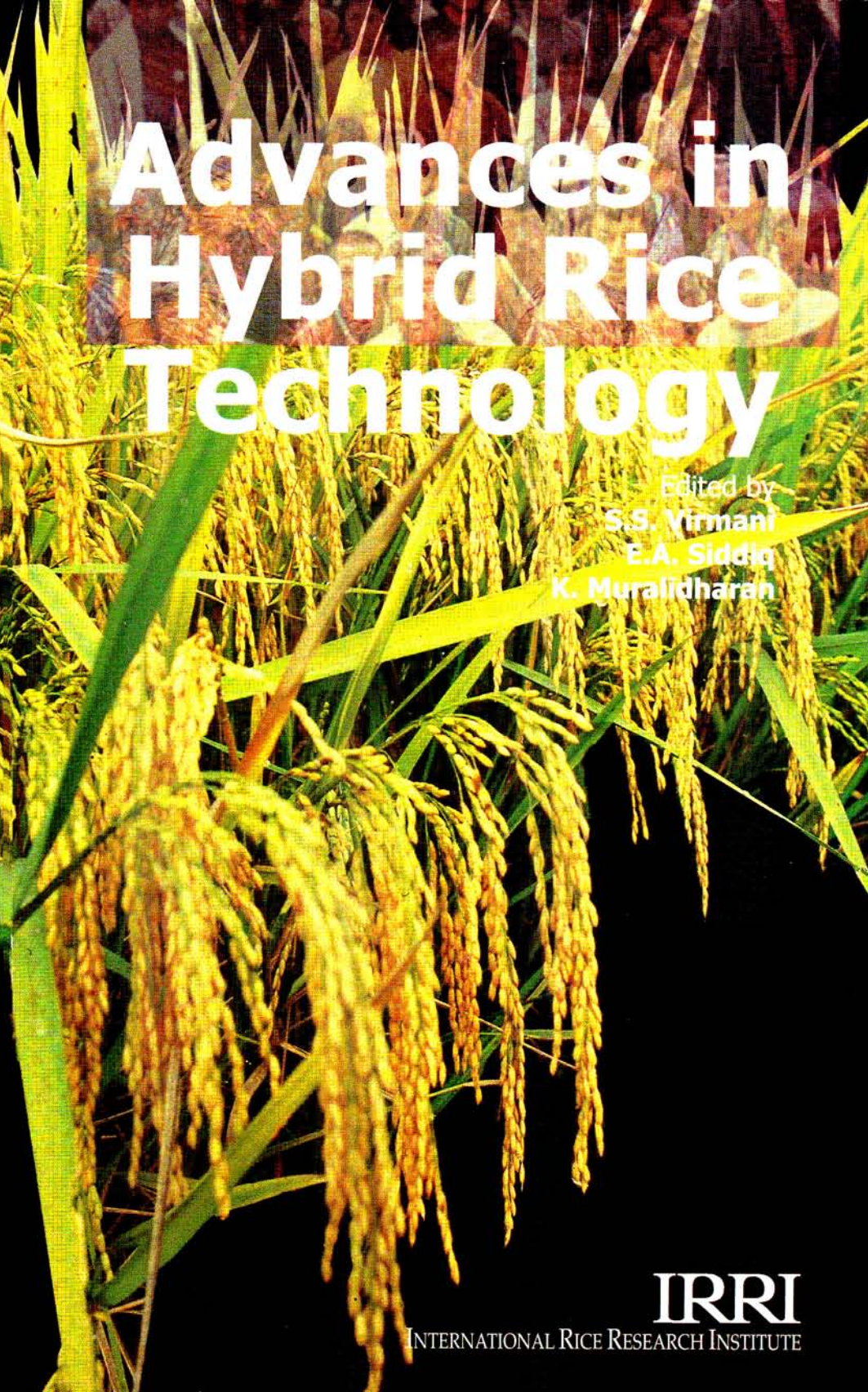
Advances in Hybrid Rice Technology
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Foreword

The world will need at least 40% more rice than what is produced today to feed the extra billions who will rely on it within the next three decades. Tomorrow's technology and management must not only enhance the production of rice per unit area, with less water and less pressure on the natural resource base, but also maintain rice as an attractive crop for future generations of farmers. Research must respond to the need for many rice growers to change from subsistence farming to farming for profit to produce the surplus needed to feed rapidly growing urban populations; to cope with the globalization of agriculture, which may cause farmers to abandon rice in favor of more lucrative crops or to release land for nonagricultural uses; to overcome growing shortages of rural workers and increase their wages; and to adapt to the changing tastes of consumers whose incomes are growing.

China has for a long time produced hybrid rice, particularly for the more temperate regions. Now, based on that experience, but adapted to the tropics, other countries such as India, Vietnam, and the Philippines are developing viable hybrid rice programs in collaboration with IRRI. Hybrid rice can contribute significantly to increasing rice production, especially in irrigated ecosystems, and to generating rural employment opportunities through associated labor-intensive seed production by companies in the public, private, NGO, and/or cooperative sectors.

The 3rd International Symposium on Hybrid Rice was held at Hyderabad, Andhra Pradesh, India, 14-16 November 1996 to review and discuss opportunities for enhancing and sustaining hybrid vigor in rice. It was cosponsored by the Indian Council of Agricultural Research, United Nations Development Programme, IRRI, the Food and Agriculture Organization of the United Nations, and the MAHYCO Research Foundation of India. The 1st and 2nd International Symposia on Hybrid Rice were held in China (1986) and at IRRI (1992); the latter was organized under the umbrella of the International Rice Research Conference.

About 200 hybrid rice scientists from 20 countries participated in the 3rd symposium and discussed different aspects of improving hybrid rice technology and making it available outside of China. Contributions covered the current scenario on hybrid rice, approaches and strategies for increasing breeding efficiency and enhancing yield heterosis, and sustainability of hybrid rice technology. Several country reports on the current status of developing and

adopting hybrid rice technology were also presented and a meeting of the International Task Force on Hybrid Rice was held during the symposium. Papers presented at the symposium are compiled in this book. In addition, summaries of the posters presented at the symposium were published separately in *International Rice Research Notes,* Vol. 23(1) and 23(2). This book provides valuable information on the new technology and serves as a useful reference for researchers and students interested in investigating the subject.

We are grateful to the many individuals who worked on the international and national organizing committees to prepare the symposium and to the authors for presenting the invited papers published in this book.

KENNETH S. FISCHER
Deputy Director General for Research

INTRODUCTION

IRRI's role and vision for hybrid rice

G.H.L. Rothschild

Achieving self-sufficiency in rice production and maintaining price stability are important objectives in low-income countries, where rice as the staple food provides the basis for national food security and generates employment and income for poor people. Asia produces and consumes 90% of the world's rice. Most rice-growing countries in this region have done remarkably well in meeting their rice needs over the past three decades using Green Revolution technologies. But the future poses a major challenge. By 2030, the world must produce 70% more rice than it produced in 1995 to meet demand created by increasing populations and rising incomes. This production increase must be achieved on less land, with less labor, less water, and less pesticide, and it must be sustainable. Increasing the yield potential of rice varieties is considered an important strategy for meeting this challenge.

In the 1970s, Chinese scientists amply demonstrated that the use of hybrid rice could increase rice yields in China by 15–20%. Hybrid rice is now used extensively in China. But hybrid rice from China was neither adapted to tropical conditions nor was it available freely to countries outside China. Therefore, IRRI began research in 1979 to explore the potential of adapting this technology for the tropics. Soon, it was concluded that hybrid rice offered an important option to increase varietal yields in the tropics. This encouraged several tropical rice-growing countries to develop this technology either independently or in collaboration with IRRI and/or China. Currently, 17 national programs are involved in developing hybrid rice: Bangladesh, Brazil, Colombia, Egypt, India, Indonesia, DPR of Korea, the Republic of Korea, Japan, Malaysia, Myanmar, Pakistan, the Philippines, Sri Lanka, Thailand, the United States, and Vietnam.

Hybrid rice research programs in national agricultural research systems (NARS) are in different stages of development. Although China has the strongest national network in hybrid rice, programs in Brazil, India, Japan, and the United States are well established. Other countries may take 3–5 yr to develop such programs. Several private companies in Brazil, India, Japan, and the United States have developed a strong research base in hybrid rice while some are still developing this and others are involved solely in seed production activities.

IRRI's role in the past

Over the years, IRRI has developed more than 100 cytoplasmic male sterile (CMS) lines and identified several hundred restorers of diverse genetic background for use by NARS. The two most widely used CMS lines in NARS for developing commercial hybrids are IR58025A and IR62829A, which were developed at IRRI. The hybrids released for commercial cultivation in India so far are derived from IRRI-bred CMS lines. More than 100 elite heterotic rice hybrids have also been identified at IRRI and shared with NARS along with their parents for evaluation and use.

Since 1980, IRRI has helped about 160 rice researchers from 17 countries to receive training in breeding, seed production, pathology, and entomology of hybrid rice. A number of training materials such as a seed production manual, a video on seed production technology, and a slide tape module on seed production have been developed for the purpose and shared with trainees. In collaboration with the Mahyco Research Foundation of India, the hybrid seed production manual has been copublished in six Indian languages. The monograph on *Heterosis and Hybrid Rice Breeding,* by Dr. S.S. Virmani, was published jointly by Springer-Verlag and IRRI. This monograph has also been translated into Chinese by Prof. Yang Rencui for use by hybrid rice scientists in China.

IRRI scientists have been conducting strategic research in hybrid rice breeding. This includes diversification of CMS, thermosensitive genic male sterility (TGMS), development of composite populations of TGMS, maintainers, and restorers, and biotechnology applications (e.g., anther culture, tagging of restorers, and TGMS and wide compatibility—WC—genes with molecular markers). Research is also under way on increasing seed production efficiency and agronomic management of hybrids to support the development and use of the technology by NARS.

In collaboration with Chinese scientists, our economists have comprehensively analyzed the economics of hybrid rice cultivation and seed production in China. They have also conducted ex ante economic analyses of the technology in India and Vietnam to extrapolate the results for use in other NARS interested in using this technology.

IRRI scientists have also provided consultancy services to India, Vietnam, Bangladesh, Myanmar, and Sri Lanka bilaterally or through FAO to develop and/or review hybrid rice research programs in these countries.

IRRI has been involved in cosponsoring and hosting all three international symposia on hybrid rice: the first in Changsha, China, in 1986; the second at IRRI in 1992; and the third in Hyderabad, India, in 1996. These symposia have provided an excellent forum for discussion of pertinent issues related to the development and use of this technology.

IRRI has bilateral collaboration agreements with China, India, the Philippines, Thailand, Vietnam, Indonesia, Sri Lanka, and the Republic of Korea. Several other countries (e.g., Colombia, Egypt, Myanmar, and Pakistan) are also seeking such collaboration.

Vision for the future

Hybrid rice technology has two major components—research and seed production. Both components must be strong to ensure an appropriate impact of this technology at the farm

level. Although IRRI's strength lies in research, it must link its research activities with the seed industry in national programs. IRRI has established collaboration with FAO in order to help national programs strengthen their seed industries. The IRRI-FAO-NARS collaboration model is being developed to help expedite the development and use of hybrid rice technology, which will contribute significantly to increased rice production in the 21st century.

While strengthening IRRI-FAO-NARS collaboration, we also hope to establish NARS-NARS collaboration to develop and use hybrid technology. The International Task Force on Hybrid Rice established for this purpose under the umbrella of the proposed Irrigated Rice Consortium will link this project with other components pertaining to the Integrated Pest Management and Integrated Nutrient Management Networks. Issues related to increased productivity and sustainability can thus be dealt with jointly and not in isolation.

The transfer of hybrid rice technology requires active participation by the seed industry in the public, private, and NGO sectors. IRRI therefore believes that public-sector research institutions working on hybrid rice should consider private-sector seed companies as partners rather than as adversaries, and establish linkages with them accordingly.

To transfer the available technology expeditiously, mass-scale training in seed production is needed in national programs. IRRI can help train the trainers in NARS for this purpose. In some NARS, applied research programs on hybrid rice are well established. IRRI will focus on training their scientists in strategic research areas, such as the use of TGMS and WC genes and diversification of CMS.

Over a period of time, as NARS capacity for conducting applied research on hybrid rice is improved, especially for the irrigated ecosystem, IRRI will devolve its work on identifying heterotic rice hybrids to NARS. IRRI will concentrate on developing parental lines possessing cytoplasmic and genetic diversity, good combining ability, and higher outcrossing potential. Research on two-line hybrid breeding will also be strengthened.

With the development of new plant types and availability of elite tropical japonica rice cultivars, it may be possible to develop indica/tropical japonica hybrids using the CMS or TGMS systems. These hybrids will yield significantly higher than indica/indica hybrids and tropical japonica hybrids. For japonica rice-growing countries (such as Japan, the two Koreas, and Egypt), IRRI has also proposed to explore prospects for exploiting heterosis in tropical japonica/temperate japonica crosses.

When hybrid rice technology is established and widely accepted under the irrigated ecosystem, its potential in direct-seeded and unfavorable ecosystems (such as boro, inland salinity-prone, and certain rainfed lowland ones) will be explored. IRRI will carry out this research using the shuttle breeding approach with selected NARS interested in developing rice hybrids for these ecosystems.

The ultimate goal of hybrid rice research at IRRI is to develop apomictic hybrids, or hybrids that produce seeds asexually. Seeds from such hybrids will breed true to type, and farmers can use the F_1 harvest as seed for the next crop. This will enable even resource-poor farmers to benefit from hybrid rice technology. Special efforts are under way at IRRI in collaboration with advanced laboratories to search for and develop apomixis in rice.

China's success in exploring the use of hybrid rice to meet its increasing demand for rice has been phenomenal. India's demonstrated success in using the same technology adapted to its conditions is equally inspiring and encouraging. We hope that other tropical

countries will also succeed in developing hybrid rice adapted to their environments with such strong partnerships. This will also help them in encouraging and supporting the development of private seed industries, which are essential for the success of hybrid rice technology.

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CHAPTER 1

Hybrid rice technology in India: problems and prospects

R.S. Paroda

Rough rice production in India has exceeded 100 million t annually since 1988. Total production in 1995 was almost 122 million t, with yield averaging 2.9 t ha^{-1}. Irrigated rice yields in northern India can reach 5.5 t ha^{-1}. But the future poses challenges. Despite the rice yield increases of the 1970s and 1980s and the stable production achieved in the 1990s, the yield ceiling of irrigated rice must be raised again. This production increase must be achieved from less land, with less labor, less water, and fewer pesticides. It must also be sustainable. Productivity in less-favorable rainfed environments and in rice-based systems must also be increased, while protecting the environment and the natural resource base. Simultaneously, rice production must be made profitable for farmers so that they do not join the rapidly expanding, highly explosive communities of urban poor. To meet this challenge, India-IRRI collaboration, with sponsorship from the United Nations Development Programme (UNDP) and technical support from the Food and Agriculture Organization of the United Nations (FAO), has led to the commercialization of hybrid rice technology in India. About 60,000 ha were planted to rice hybrids in 1996, boosting the average yield ha^{-1} by 15% over that of today's modern varieties. This chapter discusses the problems and prospects of hybrid rice cultivation in India and the strategies adopted to increase production.

Background

Agricultural scientists need to continuously seek and develop new technologies to increase food production. Simultaneously, planners and policymakers have to establish policies and strategies to provide an atmosphere conducive to increasing food production to satisfy demand from the growing population. This task has become more difficult because the resource base is diminishing, particularly land, water, and fertilizer. Although India achieved food self-sufficiency during the past decade, the

country must increase food production by at least 5 million t and rice by 2 million t every year to sustain this self-sufficiency. But the options available to accomplish this task are limited. In eastern India, under rainfed upland and lowland ecosystems, production can be increased marginally by adopting appropriate high-yielding varieties and management technologies.

Rice production has remained stable in the irrigated ecosystem. To increase production and productivity in this ecosystem, new efforts are needed. Some genetic options involve (1) a new plant type based on physiological and genetic manipulation, (2) new biotechnological tools to increase potential yields, and (3) yield heterosis in hybrid rice. Of these options, hybrid rice technology is the most practical to raise production in the irrigated ecosystem.

During the past two decades, in the People's Republic of China, hybrid rice technology has been demonstrated successfully on a large scale. Although planted on only 17 million ha (55% of the total area), hybrids account for more than 66% of rice production in China. But these hybrids were not suitable for the tropical irrigated ecosystem in India. Therefore, the Indian Council of Agricultural Research (ICAR) started a time-bound and goal-oriented project on the "Development and Use of Hybrid Rice Technology." This project began in 1989 in collaboration with IRRI, and was strengthened in 1991 with sponsorship from UNDP and technical collaboration from FAO. The project has a national research network with 12 centers across the country, each having a specific responsibility. After six years, seven public-bred hybrids had been released. The hybrid seed production technology was developed to obtain seed yields of 1.5–2.0t ha^{-1}. Close and effective collaboration with private seed companies has enabled them to develop and market six additional rice hybrids. The hybrid seed is currently sold at Rs. 70–100 (US$2–3) kg^{-1}. During the 1995-96 dry season, 1,300 t of F_1 seed were produced, enough to plant more than 60,000 ha.

Potential

The extensive cultivation of rice hybrids on research farms and in farmers' fields has established the yield superiority of hybrids under good management. Hybrids produced 15–20% more grain than the highest-yielding variety. Several accomplishments listed below show the potential for increased rice production through hybrid rice cultivation in India.

- The F_1 seed harvest of 1.5–2.0t ha^{-1} in seed production plots has clearly demonstrated the feasibility of hybrid rice seed production.
- Most of the released hybrids have acceptable, if not very desirable, grain quality.
- To facilitate heterosis breeding and hybrid seed technology, a national research network with well-qualified and trained personnel was established.
- The private-sector seed industry has the necessary human resources and infrastructure to produce adequate quantities of hybrid seed.

- There is now a relatively good demand for hybrid seeds from farmers in the target areas. This demand will certainly increase if the cost of the F_1 seed is reduced to around Rs. 50 (US$1.50) kg^{-1} of seed.
- Promising hybrids with a higher magnitude of heterosis, better grain and cooking qualities, and resistance to major pests and diseases are in the final stages of evaluation.

Problems

To increase the area under hybrid rice, the following problems need to be solved:

- An inadequate sustainable supply of pure breeder seed of commercial cytoplasmic male sterile (CMS) and restorer lines.
- Nonsynchronization of parental lines, particularly in large-scale hybrid rice seed production plots at new locations.
- Lack of proper facilities for pilot seed production of promising hybrids identified for conducting national hybrid trials, on-farm trials, and front-line demonstrations in farmers' fields.
- Low head-rice recovery, which causes low consumer acceptability and marketability of the produce from hybrids.
- Lack of free exchange of promising germplasm between public research institutions and the private seed sector.

Strategies

To solve these problems, the following strategies are being adopted:

- The Directorate of Rice Research (DRR), Hyderabad, in collaboration with the Mahyco Research Foundation has begun a project to produce nucleus and breeder seed of all promising parental lines for sharing with interested public and private-sector seed agencies.
- For the free exchange of parental lines between public research institutions and private seed agencies and others, a system of registration of parental lines is being introduced.
- Testing of cooking and eating quality of hybrids in national trials has been made an integral part of evaluation to ensure marketability, eating quality, and consumer acceptance of hybrids.
- The hybrid seed production technology will be pilot-tested prior to large-scale seed production at new locations. The required guidance for pilot-testing will be extended by the DRR and other research centers of the hybrid rice network.

Future outlook

We hope to have 2 million ha under hybrid rice in the favorable irrigated ecosystem by the year 2000 (Table 1). During the 1996 wet season, hybrid rice was planted on more than 60,000 ha, far exceeding the target.

Table 1. Targeted area coverage under hybrid rice and seed requirement in India, 1995-2000.

Year	Area (ha)[a]	Hybrid seed requirernent (t)
1995	5,000 (10,000)	100
1996	10,000 (60,000)	200
1997	40,000 (120,000)	800
1998	150,000	3,000
1999	500,000	10,000
2000	2,000,000	40,000

[a] Numbers in parentheses denote the actual area covered during the specific year.

There is now some curiosity among the farming community; many progressive farmers are eager and enthused to adopt this technology. We therefore urgently need to effectively transfer the technology already developed by conducting a large number of on-farm and front-line demonstrations. There is also a need to motivate, mobilize, activate, and coordinate the seed production programs. Simultaneously, we need to conduct more training programs for seed production personnel.

The large-scale adoption and future spread of the hybrid technology will, however, primarily depend on its economic attractiveness. We need to develop rice hybrids with a still higher magnitude of heterosis, better cooking and eating quality, and resistance to major pests and diseases. The hybrid rice seed production technology should be refined further to obtain $1.5–2.0\,t\,ha^{-1}$ of seed yield in large seed plots on a sustainable basis. This would stabilize the cost of the hybrid seed. Hybrid rice scientists need to increase the magnitude of heterosis by using conventional breeding and biotechnological tools.

The screening of parental lines for resistance to major pests and diseases should be emphasized. Parental lines possessing multiple disease and pest resistance will be increasingly needed to develop resistant hybrids. Similarly, appropriate parental lines should be selected to obtain hybrids with desirable cooking and eating qualities, because these parameters determine acceptability and the price of produce in the market.

Seed production research should give priority to increasing the outcrossing potential of CMS lines and seed yield per unit area. Seed production technology should continue to be improved to attain higher and higher seed yields. Because gibberellic acid (GA_3) is the costliest input in seed production in India, research should focus on economizing the use of GA_3 and finding a suitable alternative to it.

A comprehensive package of practices for the cultivation of hybrids in target areas in different seasons should be developed. This would help farmers exploit the full yield potential of hybrids and make hybrid rice cultivation more profitable. The possibilities of adopting hybrid technology on a large scale in the favorable rainfed

shallow lowland ecosystem and in the irrigated boro (winter) season in eastern India should also be explored. Appropriate parental lines and hybrids need to be developed for this purpose.

Astrong network of hybrid rice research, seed production, and technology transfer has been established. This has been achieved by developing the necessary infrastructure for research and developing human resources by training a large number of scientists, seed production personnel, progressive farmers, and farm women; through the active participation of several public and private seed agencies; and via timely support and encouragement from policymakers and the government. This will help the country forge ahead in developing and using hybrid rice technology.

A private seed company, Hybrid Rice International Ltd., was established recently, exclusively for research, development, and large-scale seed production of rice hybrids. Aprivate research foundation, Mahyco Research Foundation, has provided substantial grants to support research and development activities on hybrid rice in India. With the well-directed research now under way, rice hybrids with a still higher magnitude of heterosis, resistance to major pests and diseases, and better cooking and eating quality will be made available and seed production technology improved further to obtain higher seed yields at a lower cost. Prospects for the large-scale adoption of hybrid rice technology therefore appear bright in India.

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CHAPTER 2

Prospects for hybrid rice in tropical Asia

P.L. Pingali, M. Morris, and P. Moya

Demand for rice in Asia continues to rise, although the supply has begun to level off as productivity gains from Green Revolution technologies show signs of becoming exhausted, especially in irrigated zones. Without an immediate shift in the yield frontier for rice and increased rice production, many countries risk that rice supplies will not keep up with demand. Recent breakthroughs in tropical hybrid rice technology provide some hope for sustaining future production growth.

This chapter assesses the economic potential for hybrid rice in tropical Asia. The objective is to examine the determinants of and constraints to hybrid rice adoption, taking into account technical, economic, and institutional factors. Lessons are drawn from the only successful case of hybrid rice adoption—in China—and implications are spelled out for the likely future dissemination of hybrid rice technology elsewhere in Asia. In addition, the development of the global maize seed industry is scrutinized for patterns that may be relevant to the emerging hybrid rice seed industry. The chapter also discusses a set of key policy issues that will have to be addressed if hybrid rice is to realize its potential to significantly increase rice production in tropical Asia.

China's pioneering success in developing hybrid rice

First-generation offspring of a cross between genetically different parents of the same plant species are called *hybrids.* Many hybrids demonstrate a phenomenon called *heterosis* (also commonly known as hybrid vigor), which can be described as the tendency for offspring of genetically diverse parents to perform better than their parents in one or more physical or agronomic traits. Because these traits often have commercial value (as in the case of high yield, improved grain quality, and short growth cycle), heterosis represents one of the most important practical applications of genet-

ics in agriculture. In rice, the immediate attraction of heterosis is increased genetic potential. Hybrid rice yields more than regular self-pollinating rice varieties because it produces a larger total biomass and more grains per unit area (Ponnuthurai et al 1984).

The successful development of hybrid maize during the 1930s in the United States provided an important impetus for breeders of other commercial crops. But the rapid progress achieved in maize proved difficult to match in cereals such as rice and wheat because of the greater technical difficulty of controlling reproduction in these self-pollinating species. This slowed the rate of genetic gains that could be achieved by researchers and greatly increased the cost of seed production.

China's hybrid rice research program, under the innovative leadership of Professor Yuan Long Ping, made an important breakthrough in 1970 following the discovery in a wild rice population of a single male sterile plant. After the genetic mechanism controlling male sterility in rice was identified and controlled, the technical difficulty (and expense) of producing hybrid seed was greatly reduced. In 1976, hybrid rice was made available to Chinese farmers (Zhu 1988). The early Chinese hybrids outyielded the varieties that farmers were growing by an average of 15%. Since the mid-l970s, China has managed to sustain output growth in rice by encouraging farmers to switch from conventional modern varieties to hybrids. In irrigated zones, hybrid rice was adopted rapidly, resulting in a marked increase in average yields. By 1991, more than 50% of China's rice area was planted to F_1 hybrids (Lin and Pingali 1994).

China's initial success with hybrid rice technology generated considerable interest in other Asian countries, especially in India. Unfortunately, the Chinese hybrids were developed for subtropical conditions and therefore could not be easily transferred to the rest of Asia. When grown under tropical conditions, the Chinese hybrids were very susceptible to insect and disease damage. With the recent appearance of hybrids suitable for tropical conditions, however, it may now be possible to duplicate China's success elsewhere.

Hybrid rice adoption and diffusion: lessons from the Chinese experience

In considering the prospects for hybrid rice in tropical Asia, valuable lessons can be drawn from the Chinese experience. On the demand side, it is important to understand the conditions under which hybrid rice would be profitable relative to conventional modern varieties. On the supply side, it is important to recognize the constraints associated with technology generation, seed production and distribution, and farmer adoption.

Yield advantage and relative profitability of hybrid rice

Detailed household-level studies carried out in a number of China's most important rice-producing zones have found that the yield advantage of hybrid rice over the conventional semidwarf variety is about 15%, without major differences in material costs and labor requirements. He et al (1984, 1987) compared farmers growing hybrid rice

and farmers growing conventional modern varieties in Jiangsu Province for the 1984 crop season. Hybrid rice yields were found to be at least 1 t ha^{-1} higher than those of conventional varieties, which averaged around 6.5 t ha^{-1}. Returns to labor were higher for hybrid rice, although returns to nonlabor inputs, as well as total costs, were estimated to be similar to those associated with conventional varieties.

These results from Jiangsu Province were corroborated by a subsequent survey of 500 farm households conducted in Hunan Province during the 1988 crop season (Lin 1990). Mean yields of hybrids were found to be significantly higher than those of conventional varieties for middle- and late-season rice, although the difference was not statistically significant for early season rice. Hybrid rice did not require more labor input than conventional rice, but the yield advantage of hybrids was partly offset by added requirements for chemical inputs and the higher expenditure on seed. These economic studies revealed that even though the cost per ton of producing hybrid rice was higher than that of conventional rice, the higher yielding ability of hybrids allowed increased production to be achieved from the same piece of land. For China, this has proved important because it has enabled farmers not only to meet their grain quota but also to generate a surplus that can be sold in the open market at favorable prices.

Irrigated versus rainfed environments

The Chinese experience suggests that the demand for yield increases associated with hybrid rice technology will generally come from irrigated environments rather than from rainfed lowland and upland environments. In China, hybrid rice is grown exclusively in irrigated areas, and the switch from conventional varieties to hybrids, from a farmer's point of view, involves only a change in seed. In contrast, in upland and rainfed lowland areas, switching to hybrids involves changes in seed, in the type and quantities of inputs used, and in cultivation practices—which implies a fundamental alteration of the entire farming system. Without changes in management practices, hybrid rice is unlikely to be profitable in upland and rainfed lowland areas.

Significant heterosis, commonly observed for vegetative vigor and root characteristics, suggests that the use of hybrid rice should also be explored for certain stress environments (rainfed, lowland, drought-prone) where transplanting is practiced or some seeding equipment is available for direct seeding with a reduced seed rate (Virmani 1996).

Prospects for yield improvements

In irrigated areas, the higher genetic potential of hybrid rice is likely to be appreciated only when the genetic potential of conventional high-yielding varieties is completely exhausted. In irrigated rice fields of South and Southeast Asia, particularly in the so-called rice bowl provinces, prospects are limited for further yield gains through Green Revolution-type modern varieties, because the economically exploitable yield gap between the technological frontier and farmers' yields has for the most part been bridged. In these intensively cultivated areas, further yield improvements will have to come from the adoption of hybrids and/or the "new plant type" varieties (Pingali et al

1997). But in some places where the prospects are good for further yield improvements through the use of conventional semidwarf varieties, adoption of hybrids is likely to be limited.

Seed costs

Because the variable cultivation costs involved in growing hybrid rice and conventional rice are similar, the relative profitability of the two crops depends to a large extent on the relative price of seed. Hybrid seed production is labor-intensive; therefore, hybrid seed prices are likely to be relatively lower in low-wage countries. As agricultural wages are determined by the labor-land ratio (among other factors), the profitability of hybrid rice production relative to the production of conventional rice is likely to be influenced by the prevailing labor-land ratio. To the extent that hybrid seed production is characterized by economies of' scale, prices of hybrid seed are also likely to be relatively lower when the absolute size of a country's irrigated rice area is large.

Characterizing countries in terms of hybrid rice demand

Potential demand for hybrid rice technology can be anticipated by classifying countries in terms of their land-labor ratio and the proportion of their crop land that is irrigated. In countries that have a high labor-land ratio and a high proportion of irrigated crop land (e.g., India, Indonesia, Philippines, Sri Lanka, Vietnam), hybrid rice production is likely to be profitable, and demand for hybrid rice technology is therefore likely to be strong. In countries with a high proportion of irrigated crop land but a low labor-land ratio (e.g., Malaysia, Pakistan), agricultural wages will tend to be high, thus undermining the profitability of hybrid rice production. Similarly, hybrid rice production is unlikely to be profitable in countries with a low proportion of irrigated crop land (e.g., Bangladesh, Nepal, Myanmar, Thailand), irrespective of wage rates. In these last two groups of countries, demand for hybrid rice technology can be expected to be weaker.

Characterizing countries in terms of hybrid rice supply

In estimating the potential supply of hybrid rice technology, we need to consider the same two factors that determine demand: (1) the size of the irrigated rice area, and (2) the availability of labor. Of the total irrigated area in tropical Asia, 31% is in India and 13% in Indonesia. If we assume that the supply of hybrid rice technology will respond to demand, then India and Indonesia are potentially the most important suppliers of tropical hybrid rice technology. Given the size of the potential market for hybrid seed, commercial seed producers in these two countries also stand to benefit from any economies of scale associated with hybrid rice research and seed production.

The Chinese experience has shown that hybrid rice offers an attractive alternative to conventional varieties only if the hybrids are well adapted to local agroclimatic conditions. This suggests that location-specific research will be needed to develop (or adapt) hybrids that perform well under local conditions. In countries such as India

and Indonesia where irrigated rice area is extensive, often it will be economically feasible to set up research facilities and seed production organizations at the regional level. But regional organizations will not always be cost-effective in countries in which irrigated rice area is limited. For example, in the Philippines it would not be economical to establish a hybrid rice research institute and seed production capacity for every ecoregion (with the possible exception of Central Luzon).

Rather than establish their own hybrid rice research capacity and seed production infrastructure, countries in which rice area is modest should seek to take advantage of research spillover from their larger neighbors. Sri Lanka, for instance, could benefit from technological spillover from hybrid rice research conducted in southern India. Similarly, Malaysia could benefit from the research being carried out in West Java, Indonesia. The costs of developing regionally adapted hybrid rice technology will be substantially lower if regional cooperation based on ecological similarities can be fostered.

Profitability of hybrid rice and distribution of benefits

Significant technical and institutional hurdles remain to be overcome for hybrid rice to demonstrate its commercial viability in tropical Asia. So it is still too early to conduct a definitive economic evaluation of the technology, much less estimate the potential impact on production and productivity. But it may be useful to speculate on the conditions under which the switch to hybrid rice from conventional varieties would be profitable, both from the farmer's perspective and from society's.

As soon as reliable farm-level input-output data become available. one of the first questions that needs to be answered is whether the shift in the productivity frontier associated with the adoption of hybrid rice represents a parallel shift or a pivotal shift skewed toward higher input levels. In other words, will the productivity gains associated with hybrid rice be the same in absolute terms across the entire range of input levels (an intercept shift), or will farmers who are using high levels of inputs realize proportionally higher yield gains than farmers who are using low levels of inputs (a slope shift)? If the shift in the production frontier is parallel, then adoption of hybrid rice will bring equal benefits to all farmers—meaning that the benefits will be relatively greater (in percentage terms) for those who are currently using low levels of inputs. But if the shift in the production frontier is pivotal, then adoption of hybrid rice would bring proportionally greater benefits to farmers who are currently using high levels of inputs. If the shift in the production frontier is pivotal, then the greatest gains of all would be achieved by farmers who are able to combine hybrid adoption with changes in management practices.

Figure 1 provides a diagrammatic representation of these different potential shifts in the productivity frontier. It is quite likely that in the early stages of hybrid rice adoption, input use and management practices would be the same as with conventional varieties. but over time farmers would learn to adapt their management practices to the particular requirements of hybrid materials.

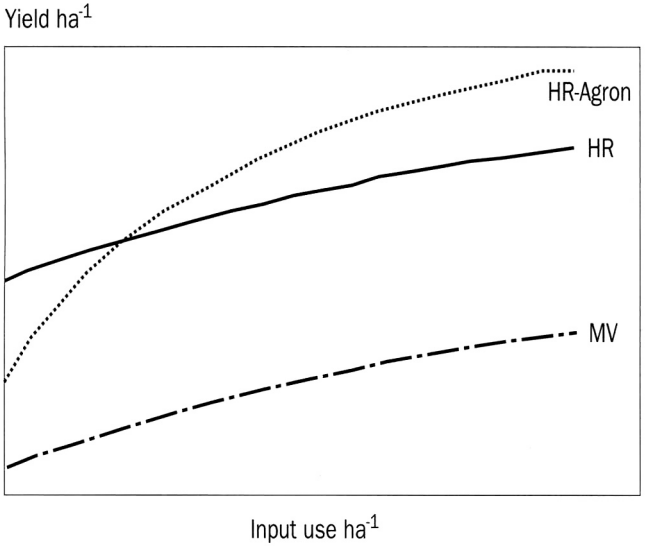

Fig. 1. Input responsiveness of modern varieties (MV), hybrid rice (HR), and hybrid rice with improved crop management (HR-Agron).

Effects of a parallel shift in the production frontier

If adoption of hybrid rice results only in a parallel shift in the production frontier, the profitability of adopting hybrid rice will depend largely on the cost of seed. Empirical evidence from China suggests that the shift in the production frontier has been a parallel shift, because similar absolute yield gains have been achieved across a wide range of yield levels with little or no change in input use and no change in crop management practices. Early experiences from Vietnam and India seem to validate the results from China. In Vietnam's Red River Delta, the use of hybrids brought from China (Thao 1995) resulted in yield increases averaging around 13%, whereas total production costs rose by 16% (Tables 1, 2). Seed accounted for about 54% of the increase in costs. In India, results of several on-farm trials show that the switch to hybrids resulted in a yield increase of about 24%, whereas production costs rose by 18%. The only significant increases in input costs were related to seed and pesticides.

If the shift in the production frontier is parallel (implying that all farmers achieve the same absolute yield gain simply by switching to hybrid seed, without having to change their management practices), the main factor determining profitability will be the price of seed. Future advances in seed production technology, to the extent that they lead to increases in seed yields or reduce variable production costs, could contribute substantially to reductions in seed prices. Some progress has been achieved in lowering seed production costs. Researchers are currently working on seed production methods that would reduce labor requirements and eliminate the need for expensive inputs such as gibberellic acid.

Table 1. Yield and farm-level resource use of hybrid rice and conventional rice, 1994-95, Red River Delta, Vietnam.

Factor	Wet season			Dry season		
	Hybrid rice	Conventional rice	Difference	Hybrid rice	Conventional rice	Difference
Yield (t ha^{-1})	4.95	4.49	0.46***	5.93	5.15	0.78
Fertilizer (kg ha^{-1})						
Nitrogen	211	193	18***	208	178	31***
Phosphorus	365	331	34***	261	226	35***
Potassium	80	59	21	68	47	21
Organic manure (t ha^{-1})	7	6	1**	9	7	2**
Pesticide (VND OOO)[a]	260	249	11 ns	221	229	(9) ns
Seed	28	70	(42)	31	83	(52)
Labor (days ha^{-1})	227	228	1 ns	228	227	1 ns

[a] US$1 = 16,315 Vietnamese dong. **, *** significant at 5% and 1%, respectively, ns = not significant.
Source: Thao 1995.

Table 2. Comparative farm-level profitability of hybrid rice vs. conventional rice, 1994-95, Red River Delta, Vietnam (dong ha^{-1})[a].

Factor	Wet season			Dry season		
	Hybrid rice	Conventional rice	Difference	Hybrid rice	Conventional rice	Difference
Value of production	10,103	10,052	51 ns	12,203	11,353	850***
Costs of production						
Fertilizer	1,811	1,609	202***	1,947	1,530	417**
Pesticide	260	249	11***	221	229	(9) ns
Seed	589	237	353**	653	288	365***
Rent, fuel, etc.	1,756	1,756	0	2,397	2,397	0
Total costs	4,416	3,850	566	5,217	4,445	773
Family income	5,688	6,202	(515)	6,986	6,908	77

[a] US$1 = 16,315 Vletnamese dong. **, *** significant at 5% and 1%, respectively, ns = not significant.
Source: Thao 1995.

Of course, if rice output prices were to increase substantially, the profitability of adopting hybrids would be enhanced even at current high seed prices. Although the absolute level of rice output prices is difficult to predict, hybrid rice in general is subject to significant price discounts because of its lower grain quality. If hybrid rice continues to have lower grain quality than conventional varieties (traditional or modern), and if the quality differential continues to be reflected in lower prices for grain, then hybrids will have to deliver relatively greater yield increases in order to replace conventional varieties.

Effects of a pivotal shift in the production frontier

If the adoption of hybrid rice leads to a pivotal shift in the production frontier (implying that larger absolute yield gains will be realized at higher input use levels), then

profitability will depend not only on seed costs but also on the costs of other inputs. In addition to purchased inputs (e.g., fertilizer, pesticide), management time will have to be taken into account, especially if successful cultivation of hybrid rice requires knowledge-intensive crop management practices. In irrigated environments, the steeper the slope of the new productivity frontier, the lower the unit costs of production for hybrids relative to current modern varieties. Productivity growth can be expected as irrigated farmers move toward and then along the new productivity frontier, until diminishing returns set in again. The size of the productivity gains, and the length of time during which they can be sustained, will depend partly on complementary investments in the construction of new irrigation infrastructure or maintenance of current infrastructure.

Hybrid rice and the competition for resources

What will be the likely effects of widespread adoption of hybrid rice on the competition for resources, specifically land, labor, and water? If a quantum leap in productivity is realized in favorable irrigated environments, this would reduce pressure to intensify production in more fragile environments, especially upland ones. With increased production from irrigated areas, high-cost (in terms of human labor) production of subsistence rice in upland areas is likely to become displaced, to the point that upland areas would eventually be diverted to nonrice activities. But in fragile rainfed environments, there are fewer alternatives to rice, especially during the wet season. These areas could therefore be negatively affected to the extent that productivity growth in irrigated areas depresses output prices generally. On the other hand, in the favorable rainfed shallow lowland environments, where the use of modern rice varieties is already extensive, adoption of hybrids could be highly profitable.

Widespread adoption of hybrid rice would almost certainly increase the demand for labor. Labor requirements would be significantly increased for crop establishment, harvest, and postharvest operations. Labor needed for crop establishment would increase because of limitations on the use of direct seeding in hybrid rice production systems, whereas labor needed for harvest and postharvest operations would increase because of greater output per unit land area. Mechanization of certain operations could potentially alleviate labor constraints during periods of peak labor demand and reduce dependence on costly hired laborers. In the case of family labor, especially that used for management and supervisory activities, the consequences are harder to predict. If hybrids can be adopted without significant changes in crop management techniques, then the labor needed for management activities would not increase significantly. But if productivity gains can be achieved only with the concurrent adoption of knowledge- or management-intensive technologies (e.g., timing nitrogen applications to match the available supply of nitrogen to the plants' changing needs), then the labor needed for management activities could rise substantially.

Adoption of hybrid rice technology can be expected to have little impact on the competition for water. Whether farmers continue to grow conventional varieties or

switch to hybrids, improved water management and water use efficiency will be required to achieve long-term sustainability in intensive rice production systems (Pingali et al 1997). Improved water management will require additional management time at both the farm and system levels. Farmer participation in decision making for system-level water allocation will become increasingly crucial to ensuring an adequate and timely water supply. Given the increasing cost of farmer time (Pingali et al 1997), a lack of water management expertise could become a constraint to sustaining the productivity gains from hybrids.

Development of the hybrid rice seed industry

Because farmers who adopt hybrids must replace their seed every year, the development and spread of hybrid rice technology will have important implications for the rice seed industry. Historically, seed industries in developed countries have expanded by developing and selling hybrids. Nearly 40% of total global revenues from commercial seed sales (currently estimated to total about US$15 billion) are generated through sales of hybrid seed (Sehgal 1992).

What will an efficient hybrid rice seed industry look like? Obviously it is likely to be different from the existing rice seed industry. Whereas farmers themselves now carry out most rice seed multiplication, on-farm seed production will no longer be a viable option in the case of hybrids. Instead of producing their own seed, farmers will have to purchase fresh seed annually, thus making them dependent on external sources and greatly increasing the importance of the formal seed industry.

In most tropical Asian countries, the seed industry is either still in an embryonic stage of development (e.g., Indonesia, Philippines, Vietnam) or is just beginning to enter the growth phase (e.g., India, Thailand). But the situation is dynamic, and new seed companies are springing up daily. Changes in the structure of many national seed industries have been precipitated with the relaxation of restrictions pertaining to the activities of private firms (Sehgal 1992). For example, policy reforms embodied in the "New Seed Policy" enacted in India in 1988 encouraged many national and transnational companies to enter the seed business (Singh et al 1995). Similar deregulation in Thailand and the Philippines led to an expansion in the seed industry during recent years in those two countries (Sehgal 1992). Sri Lanka also plans to strengthen its seed industry during the next 5 years by encouraging private investment. Several other countries have taken steps to improve seed production capacity, which would benefit the introduction of hybrid technologies.

Among the few tropical rice-growing countries that have released hybrids for commercial cultivation (India, Vietnam, Philippines), only India has the infrastructure needed for successful production, processing, certification, and distribution of hybrid rice seed. Although Vietnam does not have an established seed industry, experience suggests that if hybrid rice is determined to be a priority, the government will be able to mobilize the public resources needed to handle seed production, processing, and distribution. In the Philippines, the strategy for producing hybrid rice seed is

likely to involve the mobilization of private seed companies and seed growers' cooperatives. Indonesia already has a reasonably good seed industry infrastructure, which could be mobilized once suitable rice hybrids are identified.

Lessons from the hybrid maize seed industry

The organization and performance of the future hybrid rice seed industry are difficult to predict with certainty. In attempting to anticipate its growth, however, it is useful to examine the hybrid maize seed industry. Hybrid maize was first exploited commercially in the United States more than 50 years ago; since then, it has spread throughout the industrialized world and made important inroads in many developing countries. Because it has been in existence much longer, the hybrid maize seed industry is worth examining for clues to the likely future growth path of the hybrid rice seed industry.

Most national maize seed industries appear to have followed the same basic growth path, which can be characterized as an industry life cycle with four stages: (1) preindustrial stability, (2) emergence, (3) growth. and (4) maturity. Each stage tends to be associated with a characteristic mix of farming practices, farmer knowledge, seed technology, seed production practices, seed market structure, supporting legal systems, etc. (Table 3).

The roles played by public, private, and participatory organizations tend to change over time as the seed industry moves through these four stages. During the early part of the seed industry life cycle, when farmers are still largely unfamiliar with hybrid technologies and when effective demand for hybrid seed is still weak, public organizations must usually take the lead in conducting research, producing improved seed, distributing seed to farmers, and educating farmers about its use. This has been the experience even in countries whose maize seed industry later came to be dominated by the private sector. In the United States, for example, early research on hybrid maize was carried out primarily in the public research system, seed production took place on government-funded agricultural experiment stations, and the federal extension service conducted literally thousands of demonstration trials designed to educate farmers about the new hybrid technologies (Duvick 1997). In most developing countries, the initial investment in hybrid maize research, seed production, and extension has likewise been made by the public sector. But the evolutionary process has not been uniform across countries; because of historical and political factors, the primary beneficiaries of early hybrid maize development efforts have tended to vary. In some countries (such as Kenya and Zimbabwe), early hybrid maize development efforts were targeted at large-scale commercial farmers, whereas in other countries (such as China) they were targeted at smallholders.

Private-sector participation has usually been lacking during the emergence stage of the seed industry life cycle because of the absence of profit opportunities. Until farmers appreciate the benefits of hybrid technology and are willing to invest in hybrid seed, the cost of developing improved germplasm, producing improved seed, and distributing that seed to farmers far exceeds any revenues that can be earned through

Table 3. Characteristics associated with the stages of maize seed industry development.

Characteristics	Stage 1: preindustrial	Stage 2: emergence	Stage 3: growth	Stage 4: maturity
Orientation of agriculture	Subsistence	Semisubsistence	Mostly commercial	Completely commercial
Predominant seed technology	OPVs[a]	OPVs, some hybrids	Some OPVs, hybrids	Hybrids
Seed procurement practices	On-farm production, farmer-to-farmer exchange	On-farm production, farmer-to-farmer exchange, some purchases	Frequent purchases	Annual purchase
Seed production	On-farm	On-farm, public organizations	On-farm, public organizations, private companies (national)	Private companies (global)
Seed market coverage	Local	Local, regional	Local, regional, national	Local, regional, national, global
Sources of seed information	Direct experience, other farmers	Public agencies	Private seed companies	Private seed companies
Locus of seed R&D	On-farm	Public organizations	Public and private organizations	Public and private organizations (specialized)
Supporting legal systems	Customary law	Civil	Commercial (domestic)	Commercial (global)
Intellectual property rights	None	None	Trade secrets	Plant variety protection, patents

[a]OPVs = open-pollinated varieties.
Source: Morris (1997, in press).

seed sales. Public organizations have been willing to carry out these activities during the emergence stage of the seed industry only because they are motivated by goals other than making profits, such as enhancing national food security or reducing inequalities in income distribution.

As more farmers learn about the advantages of hybrids and begin to purchase commercial seed regularly, the industry moves into the growth phase of its life cycle. Public organizations continue to dominate research, but private companies begin to produce and distribute commercial seed in direct competition with public agencies. Interestingly. although a thriving private seed industry has been a recurring pattern, the structure taken by the industry has varied considerably. In countries such as India and Thailand, where a thriving indigenous commercial sector already existed, the emergence of the maize seed industry was spearheaded by large numbers of small local companies. But in countries such as Malawi and Zimbabwe, where the indigenous commercial sector was and continues to be relatively weak, the maize seed industry has been dominated by a handful of large transnational companies that moved quickly to fill the void.

As we would expect, one major difference between public seed agencies and private seed companies has been the attention they have paid to the bottom line. Motivated by the quest for profits, private seed companies have usually been adept at carefully targeting selected commercial markets. Their attentiveness to production efficiency and their responsiveness to changes in market conditions have usually allowed them to dominate seed production and distribution activities in those markets. Unable to compete effectively in commercial markets, public seed agencies have been relegated to less profitable markets characterized by weak demand and higher real marketing costs. They have sometimes succeeded in serving those markets, but in most cases they have not been able to do so without incurring significant financial losses.

Where the cost of delivering seed to noncommercial growers has turned out to be unacceptably high, budgetary pressures have sometimes required a scaling back in public support. In such cases, participatory organizations (such as local producers' associations and NGOs) have sometimes provided an effective mechanism for producing improved seed. In parts of northeastern Brazil, for example, community-level seed multiplication schemes have been introduced to promote local production of improved maize seed (Garcia 1997). But the success of these schemes often depends on the complexity of the seed production technology. Community-level seed multiplication schemes have been particularly effective in producing seed of open-pollinating varieties, but they have been less effective in producing seed of hybrids, which requires a much greater degree of technical expertise.

Because they lacked their own breeding programs, most private maize seed companies at first handled only public hybrids, the parental seed of which could be obtained from government breeding programs at minimal cost. But as competition in the industry intensified, the need to differentiate their products eventually forced them to obtain their own materials, either by breeding them in-house or by importing them. Many seed companies consequently launched their own research programs or established links to foreign seed companies. The speed with which the private sector moved into the development of proprietary hybrids was often remarkable. In India, for example, dozens of seed companies formed maize research divisions during a 5-year period; many of these companies simultaneously developed links to foreign firms with hopes of gaining access to additional sources of improved germplasm. Following a lag of 3–5 yr, the more successful private breeding programs began to generate commercial materials, which were immediately promoted in preference to the public hybrids. With the help of aggressive marketing and distribution tactics, sales of these proprietary hybrids grew rapidly, while the market share of public materials steadily eroded.

The Indian experience involving the gradual displacement of public-bred varieties and hybrids by proprietary hybrids developed in the private sector has been a consistently recurring pattern in the hybrid maize seed industry. But the rate at which this displacement has occurred, and the degree to which it has been completed, has been influenced in each country by the ability of seed companies to protect their intellectual property. Seed companies have demonstrated an understandable reluc-

tance to invest in research unless they believe that they will be able to appropriate the returns to their investment. In the case of hybrid maize, a primary concern has been to protect the inbred lines used in producing commercial hybrids. In the past, seed companies have sought to protect their inbred lines through a "trade secrets" strategy (based on restricting physical access to the seed). More recently, as the trade secrets strategy has become increasingly impractical, seed companies have turned to the courts to seek legal protection for their materials through plant variety protection laws. Where plant variety protection laws have been absent (or where they have been unenforceable), the effect on hybrid seed technology has been evident. For example, many transnational seed companies have declined to release their best germplasm in countries where they believe that it would be difficult to keep the germplasm from falling into the hands of competitors; instead, they have chosen to market less valuable products. As a result, farmers have been denied access to the best germplasm. This has been a problem particularly in India, where most private companies have been reluctant to market single-cross hybrids because of the difficulty of ensuring the security of the parental lines.

By the maturity stage of the seed industry life cycle, when a large proportion of farmers know how to manage hybrid technology and are regularly replacing their seed, private companies come to dominate the market, and public seed agencies all but vanish. With mature seed industries such as those found in the United States and in Western Europe, public organizations no longer produce and sell commercial maize seed, and their involvement in applied plant breeding research continues to decrease. In mature seed industries in which products and services are being produced, exchanged, and consumed in response to market-transmitted price signals, private firms can conduct economic activity more efficiently than public and participatory organizations, and sooner or later they displace the other types of economic organization.

In fully mature hybrid maize seed industries, private companies tend to concentrate on research activities that are likely to have immediate commercial applications, such as screening new materials, inbreeding, test crossing, and evaluating experimental hybrids. Although over time public organizations tend to become displaced from the applied end of the research spectrum, they continue to play an important role in producing goods and services that offer limited profit opportunities for private companies, such as germplasm collection and conservation, prebreeding, basic population improvement activities, and the development of special-trait materials that can be used as inputs into commercial breeding programs.

Implications for the hybrid rice seed industry

Although there is no reason to believe that the hybrid rice seed industry will evolve in exactly the same way as the hybrid maize seed industry, developments to date suggest similarities between the two. With the notable exception of China, most national hybrid rice seed industries are beginning to enter into the emergence stage of the seed industry life cycle. As was true in the case of hybrid maize, research and seed production activities during this emergence stage are still heavily dominated by the public

sector. A few private seed companies have begun to produce commercial seed on an experimental basis, but virtually all of the commercial hybrids are based on germplasm developed in public breeding programs.

If the use of hybrid rice turns out to be profitable at the farm level, interest in the technology is likely to increase. This would increase the demand for commercial seed and create a fertile environment for the emergence of a flourishing private seed industry. Because private seed companies will almost certainly be able to operate more efficiently than public seed agencies, over time the seed production function for hybrid rice is likely to be taken over by the private sector. But market coverage may be inconsistent, because private companies will have strong incentives to concentrate on areas where demand for commercial seed is strong (such as irrigated production zones that have a good transportation infrastructure). Special efforts may be needed to ensure that hybrid rice seed becomes available to farmers located in areas that do not represent attractive commercial markets.

As the demand for hybrid rice seed continues to grow and competition in the seed industry intensifies, private companies will come under increasing pressure to differentiate their products from those of their competitors. Eventually, a few companies are likely to launch their own in-house research programs aimed at developing proprietary hybrids that can be sold on an exclusive basis. The number of companies that will be able to develop a successful in-house research capacity will depend in part on the economics of research. Research start-up costs are known to be high and hybrid rice research is likely to be characterized by economies of scale. In the absence of government interventions, the seed industry could therefore over time become extremely concentrated unless companies lacking their own in-house research capacity continue to have access to public germplasm.

Emerging policy issues

As national hybrid rice seed industries grow and mature throughout Asia, the following key policy issues are likely to require attention:

1. Research issues

- Private companies will undoubtedly be willing to take on applied research functions (such as the test crossing of inbred lines and local testing of experimental hybrids), but they will probably continue to look to public breeding programs for inherently unprofitable activities such as germplasm collection and conservation, prebreeding, and inbred line development. Mechanisms will therefore be needed to ensure continuing public investment in hybrid rice research at socially optimal levels.
- Socially optimal levels of private-sector investment in hybrid rice research will be forthcoming only if effective systems are in place to allow the protection of intellectual property rights; many developing countries still lack such systems.

- To the extent that hybrid rice research turns out to be characterized by economies of scale, there is a danger that a small number of firms could dominate the seed industry (this appears to be happening in the maize seed industry, although thus far there is little evidence that the high degree of industry concentration has led to oligopolistic pricing practices). It will therefore be important to monitor the industry for signs of socially undesirable pricing behavior.

2. Seed production and distribution issues

- The commercial production of hybrid rice seed still depends heavily on controlling pollination through cytoplasmic male sterility (CMS). Because only a few sources of CMS have been identified, commercial rice hybrids share a common genetic heritage that makes them vulnerable to a catastrophic disease or pest epidemic (as occurred with the southern maize blight epidemic that devastated the North American maize crop during the early 1970s). Until alternative methods of seed production that do not involve the use of CMS are developed, policymakers may want to consider measures to reduce the risk of epidemics.
- As long as the seed industry remains competitive, private companies are likely to be effective in serving commercial farmers in favorable production environments that have a good transportation infrastructure. As the seed industry matures, these private companies will have greater and greater incentives to maintain the quality of their seed products, and the need for governmental quality control activities (such as seed certification) will decline.
- Profit-oriented private companies are unlikely to serve resource-poor farmers in marginal production environments that lack a good transportation infrastructure. For these farmers, alternative seed delivery mechanisms may be required (such as public seed agencies or participatory organizations).
- At any point in time within a given country, different regions or different groups of farmers are likely to find themselves at different stages of seed industry growth. This means that a mix of different types of organizations (public, private, participatory) will be needed to develop and deliver hybrid rice technologies.

3. Farmer education issues

- To the extent that management of hybrid rice technology turns out to be information-intensive, increased public investment may be needed to better educate farmers so that they can properly manage the technology and exploit the crop's full genetic potential.

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CHAPTER 3

Hybrid rice breeding in China

Long Ping Yuan

From 1976 to 1995, hybrid rice helped China to increase rice production from 129 million t to 200 million t annually. Hybrid rice varieties yield on average 6.6 t ha^{-1} compared with 5 t ha^{-1} for conventional rice varieties. Hybrid rice seed production technology has been well developed to achieve a nationwide average seed yield of 2.4 t ha^{-1}. Most existing commercial rice hybrids belong to the category of intervarietal hybrids based on the CMS system. Future emphasis is on developing two-line rice hybrids using PGMS and TGMS systems. To increase the yield potential of hybrid rice in China, emphasis is given to intersubspecific hybrids (indica/japonica, indica/javanica, and japonica/javanica). The discovery of QTLs for yield in wild rice species has opened up a new avenue for raising the heterosis level by using distant genes.

Current status of hybrid rice in China

Since 1976, when hybrid rice was first released commercially, its area has increased consistently (Fig. 1). Farmers growing hybrid rice obtain more than a 30% yield advantage over conventional pure-line varieties (Table 1). From 1976 to 1995, hybrid rice helped China to increase rice production from 129 million t to 200 million t. In recent years, hybrid rice has yielded about 6.6 t ha^{-1} versus 5 t ha^{-1} for conventional rice. In 1994, hybrid rice covered 15.7 million ha, 50% of the total rice area, and hybrid rice production was 57% of the total rice output in China. The largest hybrid rice-growing province is Sichuan, where 3 million ha (95% of the rice area) is under hybrids and the average yield has remained at 7.5 t ha^{-1} for years. Hunan is the second-largest hybrid rice-growing province, where the average yield of the second crop grown on 2 million ha is 6.8 t ha^{-1} . The highest yield recorded for hybrid rice from a single crop on a large scale (1,000 ha) was 11.2 t ha^{-1} and from a small plot (0.1 ha) 16.8 t ha^{-1}. So far, the highest yield recorded for double-cropped hybrid rice was

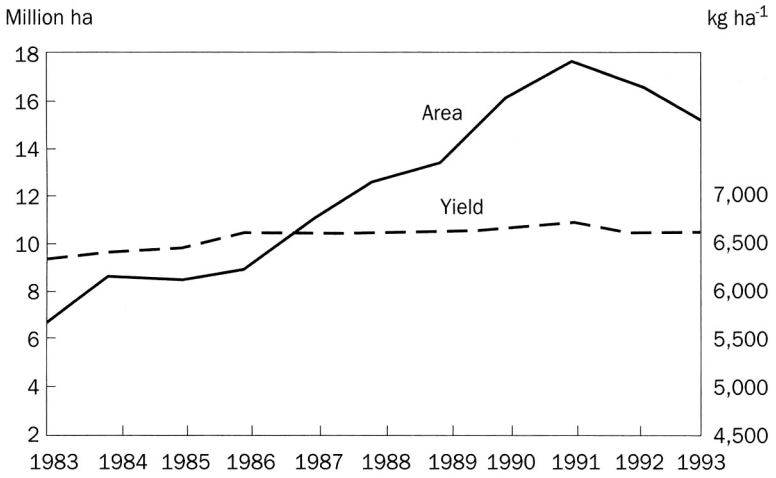

Fig. 1. Area and yield of hybrid rice in China.

Table 1. Yield of hybrid rice compared with that of conventional rice from 1986 to 1993 in China.

Year	Conventional variety (kg ha^{-1})	Hybrid rice (kg ha^{-1})	Hybrid over conventional (%)
1986	4,857	6,600	36
1987	4,779	6,615	38
1988	4,539	6,600	45
1989	4,787	6,615	38
1990	5,315	6,675	26
1991	4,551	6,565	44
1992	4,986	6,636	33
1993	4,950	6,675	35

23.3 t ha^{-1}. In Hunan Province, more than 0.2 million ha of double-cropped hybrid rice produce 15 t ha^{-1} of grain every year.

The technology of hybrid rice seed production has been well developed. The nationwide average seed yield is 2.4 t ha^{-1} (Table 2). Many new cytoplasmic male sterile (CMS) lines with a high outcrossing rate and good grain quality have been developed recently. Many new rice hybrids with good grain quality and multiple resistance have been released to farmers. The field area ratio of A line multiplication, hybrid seed production, and F_1 commercial cultivation was 130: 1,000 in the late 1970s. This has been increased to about 1:50:6,000 recently. The highest hybrid seed yield recorded (7.4 t ha^{-1}) was obtained in 1993 by the Zixing Seed Company of Hunan Province on a small plot (0.2 ha).

Table 2. Area and yield of hybrid rice seed production from 1986 to 1993 in China.

Year	Area (ha)	Yield (kg ha^{-1})
1986	100,500	1,995
1987	154,100	2,010
1988	135,800	1,628
1989	171,900	1,956
1990	192,000	2,250
1991	124,700	2,252
1992	146,600	2,438
1993	105,900	2,214

Constraints and challenges

Research on the commercial use of heterosis in rice has made tremendous achievements during the past 20 years. From a strategic point of view, however, it is still in the juvenile stage because the high yield potential of hybrid rice has not yet been fully tapped. Hybrid rice breeding still has a bright future. Based on our studies, to derive full benefit from hybrid rice breeding, future developments may involve intensifying research on breeding methods and increasing the degree of heterosis. Three approaches are involved:

1. The three-line method using the CMS system.
2. The two-line method using the photoperiod-sensitive genic male sterility (PGMS) or thermosensitive genic male sterility (TGMS) system.
3. The one-line method using the apomixis system.

The rice hybrids used in commercial production belong to the category of intervarietal hybrids based on the CMS system. Many years of practice and experience have proved that the CMS system or three-line method is an effective way to develop rice hybrids and will continue to play an important role in this century. But this system has some constraints and problems.

- In all the rice hybrids developed so far, the level of yield harvested has stagnated for years (Fig. 1). This means that we have already reached the yield plateau for rice hybrids. It would be difficult to further increase the yield potential in new rice hybrids if no new methods and materials are invented and adopted.
- The sources of male sterility-inducing cytoplasm that can be used to develop better CMS lines are poor. Currently, about 85% of the A lines used in commercial production still belong to the wild abortive (WA) type. The dominant cytosterility situation of the WA type could produce a crisis in the long run, which could make hybrid rice susceptible to destructive pests.
- The heterosis level in japonica hybrids is not as good as in indica hybrids. In addition, the currently used CMS lines (BT type) in japonica are not stable enough to produce pure F_1 seeds. Therefore, the planting area of japonica

hybrids has been limited to about 0.1 million ha for many years and, what is worse, the area of japonica hybrids is declining.

- We do not have very early maturing combinations with strong heterosis suitable for the first crop in rice double-cropping regions, which is one of the major reasons why the area under hybrid rice cannot increase.

To increase the yield potential of hybrid rice, the magnitude of heterosis must be increased by adopting the following strategies:

1. Intersubspecific hybrids.
2. Distant hybrids (interspecific or intergeneric hybrids).

In each of these phases, if the objectives are achieved, this will mark a new breakthrough in rice breeding and will result in a large increase in yield.

Strategies for the 21st century

Development of two-line hybrids

Taking the long-range strategy of rice heterosis breeding into account, many Chinese rice scientists have been exploring new technological approaches to replace the CMS system. So far, the most successful outcome is the development of two-line hybrids.

This method is based on two new kinds of rice genetic tools: photoperiod-sensitive genic male sterile (PGMS) lines and thermosensitive genic male sterile (TGMS) lines that have been successfully developed in China recently. Their male sterility is mainly controlled by one or two pairs of recessive nuclear genes, and it has no relation to cytoplasm. Exploitation of these P(T)GMS lines to develop rice hybrids has the following advantages over the classical three-line or CMS system:

- The maintainer line is avoided. The PGMS lines under longer daylength or the TGMS lines under higher temperature show complete pollen sterility; therefore, they can be used for hybrid seed production in these conditions. Under shorter daylength or moderate temperature conditions, they show almost normal fertility and can thus multiply themselves by selfing.
- The choice of parents in developing heterotic hybrids is greatly broadened. Studies showed that more than 95% of varieties tested (within the same subspecies) can restore such GMS lines. In addition, PGMS and TGMS genes can be easily transferred into almost any rice lines with desirable characteristics.
- No negative effects are caused by sterile cytoplasm and the dominant cytoplasm situation of WA will be avoided.

Several achievements in this research area have been made.

To develop P(T)GMS lines that can be used commercially, one important criterion is that the male-sterility-inducing temperature (critical t^o) must be relatively low (mean temperature 23 °C in the temperate zone and 24 °C in the subtropics). If the critical temperature is relatively high (such as 26 °C) for these male sterile lines, regardless of PGMS or TGMS, the temperature is not safe in hybrid seed production because a temperature below this point, which can induce sterile pollen into fertile pollen, sometimes occurs in the hot season. After nine years' research, considerable

Table 3. Area and yield of two-line hybrid rice from 1993 to 1997 in China.

Year		Area (ha)	Yield (kg ha^{-1})
1993		17,190	7,170
1994		60,000	7,005
1995		75,330	7,215
1996	(estimated)	120,000	–
1997	(estimated)	600,000	–

progress has been made. Now more than 20 P(T)GMS lines have been registered in China. Among these, two japonica lines belong to PGMS and the others are indica TGMS lines. Seven combinations have been certified and released for commercial production. The area under the two-line system for hybrid rice has been increasing steadily (Table 3). Experimental tests and commercial practices have proved that the best two-line hybrids outyield three-line hybrids by 5–10%.

Another advantage of the two-line system over the three-line system is that the yield area ratio of P(T)GMS line multiplication, seed production, and commercial use of F_1 is 1 : 100: 12,000–15,000. The expansion of this ratio can reduce seed cost.

The area of two-line rice hybrids will be extended to 3.4 million ha by the end of the 20th century. We believe that two-line hybrids will replace 70% of the three-line hybrids in the first decade of the 21 st century.

Development of intersubspecific hybrids

Our studies have indicated that the degree of heterosis in different kinds of rice hybrids has the following general trend: indica/japonica > indica/javanica >japonica/javanica > indica/indica > japonica/japonica (Yuan 1994).

The first three kinds are intersubspecific hybrids and the latter two are intervarietal hybrids. Indica/japonica hybrids possess the highest yield potential, considering their sink and source. Their theoretical yield may be 30% higher than that of the existing intervarietal hybrids (Yuan, 1994). Exploiting the strong heterosis in indica/japonica hybrids has been the major goal of our two-line system hybrid breeding program. To achieve this, however, five barriers commonly found in such F_1 hybrids must be overcome: low seed set rate, too tall plant height, very long growth duration, many poorly filled grains, and poor grain quality. So far, progress has been encouraging in our attempts to overcome these barriers.

By using wide compatibility (WC) genes, the low seed-setting rate caused by semisterility from incompatibility between indica and japonica lines can be raised to nearly normal levels. A large number of japonica lines and several indica TGMS lines possessing WC genes have been developed recently.

Transferring an allelic dwarf gene (Sd_1) into male and female parents can lower the plant height of indica/japonica hybrids to a semidwarf level, and still allow the hybrids to express strong heterosis.

By crossing parental lines of different growth duration, except photosensitive late varieties, indica/japonica hybrids with medium and even shorter growth duration can be obtained.

Efforts are now focused on solving the last two problems, that is, the poor filling of a number of fertilized grains and poor grain quality. Within a year or two, revised breeding strategies are expected to help overcome these barriers. The new strategies emphasize developing indica/javanica hybrids rather than typical indica/japonica hybrids in the indica rice-growing region and japonica/javanica hybrids in the japonica rice-growing region. The superiority of this strategic change involves:

- Fewer fertility problems.
- Ecological adaptability, which will solve the problem of poor grain filling.
- The indica/javanica hybrids have similar or improved grain quality compared with that of indica rice; the japonica/javanica hybrids also have similar or better grain quality.

Several combinations that performed well in the experimental field in 1995 underwent regional trials in 1996. The intersubspecific hybrids with super high yield potential (100 kg ha^{-1} d^{-1}) are expected to be released to farmers by the end of this century. These hybrids will play a major role in increasing rice yield in the 2 I st century.

Development of the one-line system of hybrid rice

Theoretically, there could be several approaches to fixing heterosis; among these, the use of apomixis to develop true-breeding F_1 hybrids appears to hold promise. Although this research program began in the late 1980s, it is still tentative. After extensive screening, some apomictic rice lines with a low frequency of apomixis (adventitious embryo and apospory) were found. But their frequency is too low (only 1–5%) for practical use. Transferring the obligate apomixis gene from wild grasses (such as *Pennisetum*) via genetic engineering combined with conventional breeding should be an effective way to develop the one-line system of rice hybrids. This could be achieved in the 21st century.

Using distant genes to raise the heterosis level

To use stronger heterosis observed in distant crosses, a marker-assisted advanced breeding strategy can be used to rapidly discover and transfer valuable novel genes for high yield potential and grain quality from wild species into elite combinations of hybrid rice. Based on careful evaluation in the experimental field and with the help of molecular markers, we discovered two important quantitative trait loci (QTL) from a wild rice in 1995 (Xiao et al 1996). The two genes are located on chromosomes 1 and 2, respectively, each bringing about an increase in grain yield of 20% compared with the control hybrid. We have just started the new strategy to exploit heterosis of distant hybrids. We expect that the skillful combining of conventional breeding methods with molecular techniques could lead to another breakthrough in hybrid rice breeding in the first decade of the 21st century.

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Notes

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CHAPTER 4

Hybrid rice research and development in the tropics

S.S. Virmani

Hybrid rice technology has enabled China to increase its rice production significantly during the past 20 years. This technology also offers an economically viable option for increasing rice yields beyond the level of semidwarf inbred rice varieties in the tropics. During the past 16 years, IRRI, in collaboration with several NARS, has developed this technology for the tropics and helped India, Vietnam, and the Philippines to start commercializing it. Several other countries in the tropics are also developing this technology. By the year 2000, about 3 million ha are expected to be covered with rice hybrids, which should produce about 3 million t of extra rice (worth $450 million) annually. This chapter highlights the current status of research and development, the major constraints experienced, and strategies to expedite the development of hybrid rice technology in the tropics.

Achieving self-sufficiency in rice production and maintaining price stability are important political objectives in countries where rice provides food security and generates employment and income for people (Hossain 1995). In the past three decades, most rice-growing countries, particularly in Asia, have done remarkably well in meeting their rice needs. But by 2030, the world must produce 60% more rice than it produced in 1995 to meet the demand created by increasing population and rising income. This production increase must be achieved using less land, less labor, less water, and fewer pesticides, and it must be sustainable. To meet this challenge, increasing the yield potential of rice beyond the level of semidwarf varieties is considered an important strategy.

Chinese rice scientists have amply demonstrated the role of hybrid rice technology in increasing rice production in the country (Yuan 1977, Lin and Yuan 1980, Yuan et al 1989, 1994). But hybrid rice from China was neither adaptable to tropical conditions nor available freely to countries outside China. Therefore, IRRI investigated the

potential use of and problems arising from this technology in the tropics (IRRI 1980, Virmani et al 1981).

Hybrid rice was identified as an important option to increase rice yields in the tropics (Virmani et al 1982, Virmani 1987, Yuan and Virmani 1988a). As a result, several other tropical rice-growing countries collaborated actively with IRRI to develop this technology. Progress made in developing hybrid rice was reviewed frequently (Virmani and Edwards 1983, Virmani 1987, 1994a, 1994b, Yuan and Virmani 1988a, Virmani et al 1993). This chapter highlights the current status of research and development, the major constraints experienced, and strategies to expedite the development and use of hybrid rice in the tropics.

Current status of research

Besides IRRI, 17 national agricultural research systems (NARS) are involved in the development of hybrid rice technology; nine of these are in the tropics (Table I). In Brazil, India, Indonesia, Japan, the Philippines, and the United States, private seed companies are also actively involved. NARS research programs are in various stages of development. For example, programs in India and Brazil are well established, whereas programs in other countries may require 3–5 yr to develop. Several private companies have also developed a strong research base in India and Brazil. In some countries, they are involved in seed production only. IRRI has been helping these research efforts by freely supplying breeding materials such as cytoplasmic male sterile (CMS), maintainer, restorer, and thermosensitive genic male sterile (TGMS) lines and hybrids for evaluation and use by public and private institutions in all national programs.

Table 1. Hybrid rice research in countries outside China.

Country	Institutions Involved (no.)			
	Private	Year begun	Government	Year begun
Bangladesh	–	–	1	1993
Brazil	1	1994	1	1984
Colombia	–	–	1	1985
Egypt	–	–	1	1987
India	7	1988	15	1981
Indonesia	1	1986	1	1992
DPR Korea	–	–	1	1976
Rep. of Korea	–	–	3	1982
Japan	1	1989	2	1983
Malaysia	–	–	1	1985
Myanmar	–	–	1	1993
Pakistan	–	–	3	1993
Philippines	2	1980	1	1988
Sri Lanka	–	–	1	1991
Thailand	–	–	1	1993
United States	1	1980	1	1980
Vietnam	–	–	2	1985
Total	13	–	37	–

Breeding parental lines

During the past 5 yr, we have developed 84 new CMS lines. All these lines are maintained, multiplied, and evaluated at IRRI before they are shared with NARS. Two IRRI CMS lines—IR58025A and IR62829A—were made available in 1991 to NARS for developing commercial rice hybrids. These lines have been successfully used to develop and release commercial rice hybrids in India, the Philippines, and southern Vietnam. Up to 1991, all the CMS lines were derived from elite lines bred in conventional breeding programs of IRRI and NARS. The number of IRRI-bred CMS lines has increased significantly during 1991 -95 (Fig. 1) as a result of specific efforts made to breed maintainer lines from B x B crosses. Currently, 74% of the IRRI-bred CMS lines are derived from such lines. Eleven new IRRI-bred CMS lines are stable for complete pollen sterility and are adapted to tropical conditions. These new CMS lines have a relatively good outcrossing rate and combining ability (Table 2) and hence are commercially usable.

To increase cytoplasmic diversity among IRRI-bred CMS lines, we have included additional CMS sources. In 1991, CMS lines were derived from only three such sources (Fig. 2). The CMS lines in the backcross nursery grown in 1996 consisted of lines derived from seven CMS sources. In recent years, CMS lines are bred at IRRI and in India to develop heterotic hybrids for the rainfed lowland ecosystem, basmati grain quality, and new plant type.

As reported earlier, many restorers are available for WA, ARC, and mutagenized IR62829B cytoplasm sources (IRRI 1996). But no restorers have been found for *O. perennis* or *O. glumaepatula* cytoplasm. Restorer frequency was reasonably high among elite breeding lines developed for the irrigated and rainfed lowland ecosys-

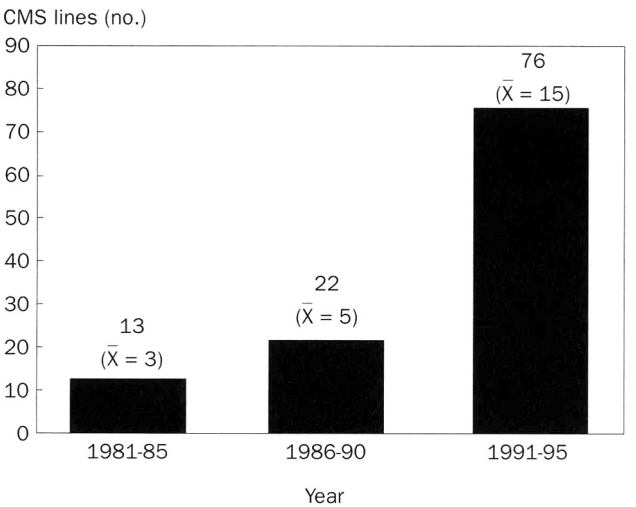

Fig. 1. Number of CMS lines developed at IRRI during 1981-95.

Table 2. New CMS lines developed at IRRI having good combining ability and desirable floral and agronomic traits.

CMS line	Pollen sterility (%)		Outcrossing rate (%)		Phenotype acceptability	
	100	99–99.9	>30	25–30	Above av	Average
IR68886A		X	X			X
IR68888A	X			X	X	
IR68890A	X		X			X
IR68897A	X		X			X
IR68899A		X		X		X
IR68902A	X			X	X	
IR69620A		X	X			X
IR69622A	X		X		X	
IR69624A	X		X		X	
IR69627A	X		X		X	
IR69628A	X		X		X	

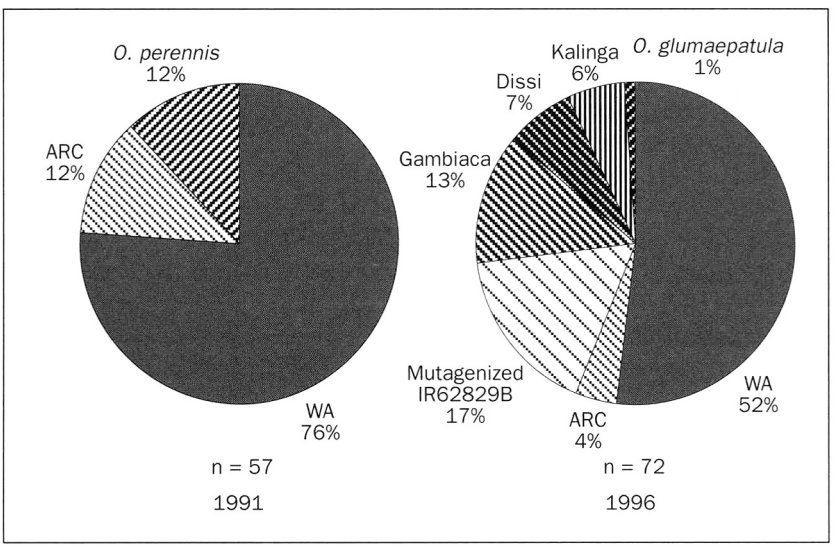

Fig. 2. Composition of backcross nursery according to source of cytoplasm. WA = wild abortive.

terns and the *boro* (spring) season. Restorer frequency was low, however, in lines derived from indica/japonica and negligible in both basmati and new plant type lines. We have therefore started breeding restorer lines by making pedigree selection in R x R crosses, deploying anther culture in A x R crosses, and using male-sterility-facilitated recurrent selection to develop restorer composite populations.

To increase the efficiency of hybrid rice breeding in the tropics, the TGMS system is being developed at IRRI and in India. Some TGMS lines have been bred and shared with NARS for evaluation (see Lu Xinggui et al, this volume, Chapter 9).

Seed production for experimental rice hybrids

After identifying suitable parental lines, breeders have to produce sufficient seeds of numerous experimental rice hybrids for evaluation in observation and preliminary yield trials. For this challenging task, the procedures used in China (Yuan and Virmani 1988b) were tested at IRRI. The chimney isolation approach was not successful because it increased the temperature inside the chimney and reduced the outcrossing rate on male sterile parents. The incidence of sheath blight and bacterial blight diseases under high humidity in the tropics further reduced seed set in the enclosed plants. In addition, chimneys could not be retained intact because of strong winds and typhoons in the wet season. For the same reasons, even isolation barriers constructed on larger seed production plots to avoid contamination with foreign pollen were difficult to maintain.

Virmani and Casal (1993) therefore developed an isolation-free system for producing seeds of experimental rice hybrids. By using this system (illustrated in Fig. 3), we now produce an adequate quantity of seeds in experimental hybrids every season

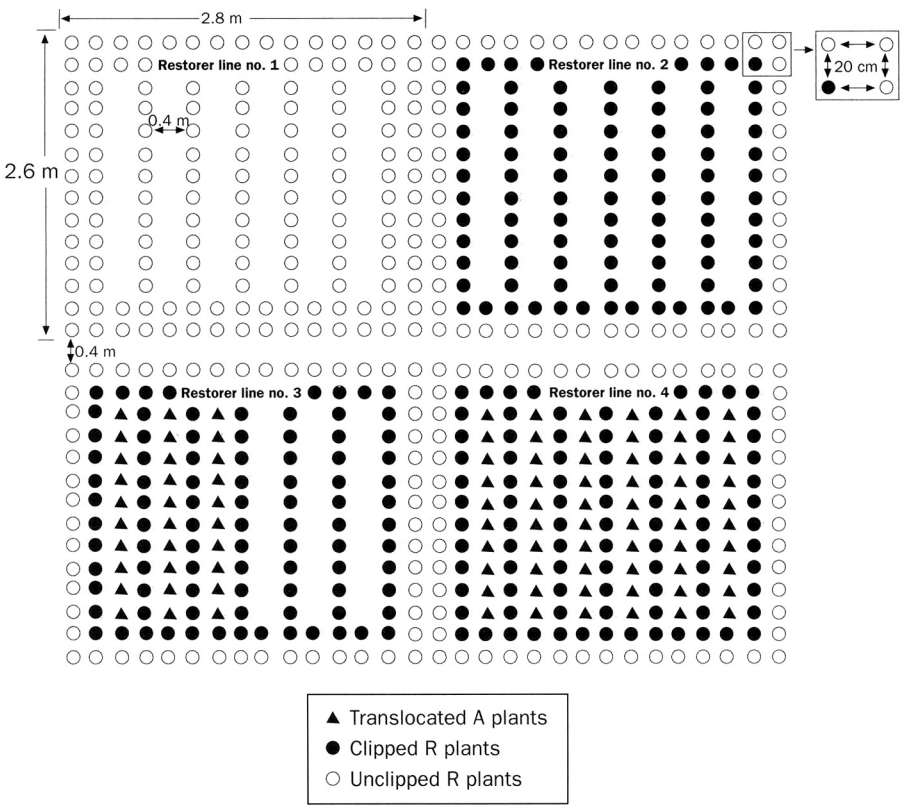

Fig. 3. Layout for isolation-free system of producing F_1 seeds of experimental rice hybrids for preliminary yield trials.

with 20–200 g of seed per hybrid. Such seed increases for experimental hybrids are routinely made for evaluation in observation yield trials (500–600 hybrids season $^{-1}$), preliminary yield trials (100–150 hybrids season $^{-1}$), and combining ability trials (50–100 hybrids season $^{-1}$). The extent of contamination observed in the plots raised from hybrid seed produced through this system was 0–8% (mean 1.8%), which is tolerable in the initial stage of evaluation of hybrids. This system is now used in NARS also.

Combining ability of parental lines

We have been studying the combining ability of elite CMS lines since 1987. IR58025A is still the best general combiner for yield and some new CMS lines—IR68886A, IR68888A, IR68897A, and IR68902A—are equally good.

Performance of experimental rice hybrids

Since 1991, about 4,000 experimental rice hybrids have been evaluated at IRRI in observation yield trials. The deployment of more genetically diverse CMS lines and a few TGMS lines (Fig. 4) has resulted in a diverse genetic profile for these hybrids. On average, about 25% of these hybrids are selected for further testing in replicated preliminary yield trials. Hybrid entries in preliminary yield trials also show a corresponding change in their profile (Fig. 5). Newer hybrids have been found to perform better than the check varieties (Fig. 6). Improvement in the mean yield of checks over the years could be attributed to the modification introduced in the agronomic management strategies of the trials, such as the increase in N rate and number of splits, use of a chlorophyll meter to decide on time of N application, use of integrated pest management for insects, etc.

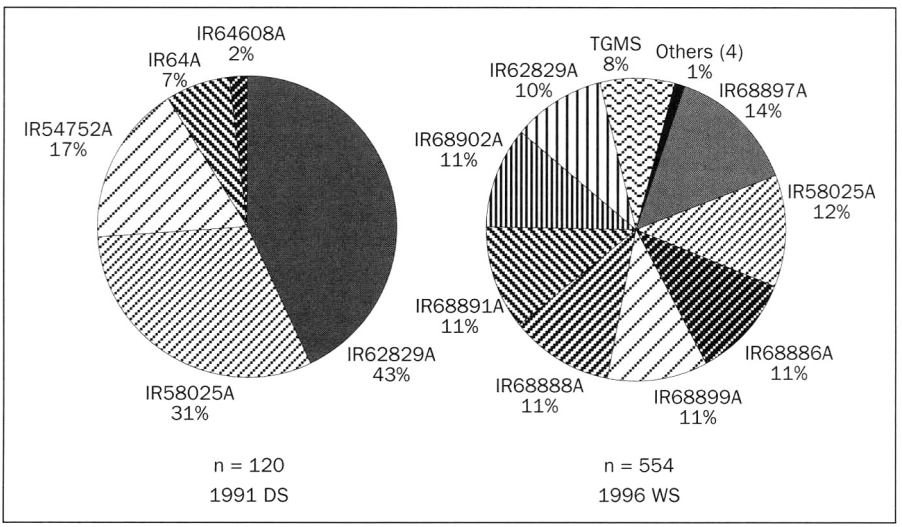

Fig. 4. Comparative profile of hybrid combinations derived from CMS lines in observational yield trials conducted during the 1991 dry season (DS) and 1996 wet season (WS) at IRRI.

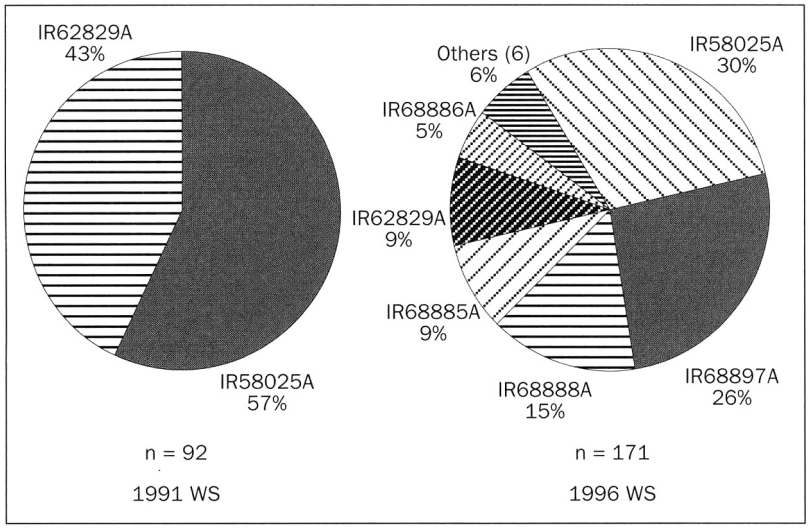

Fig. 5. Comparative profile of hybrid combinations derived from CMS lines in preliminary yield trials conducted during the 1991 and 1996 wet season (WS) at IRRI.

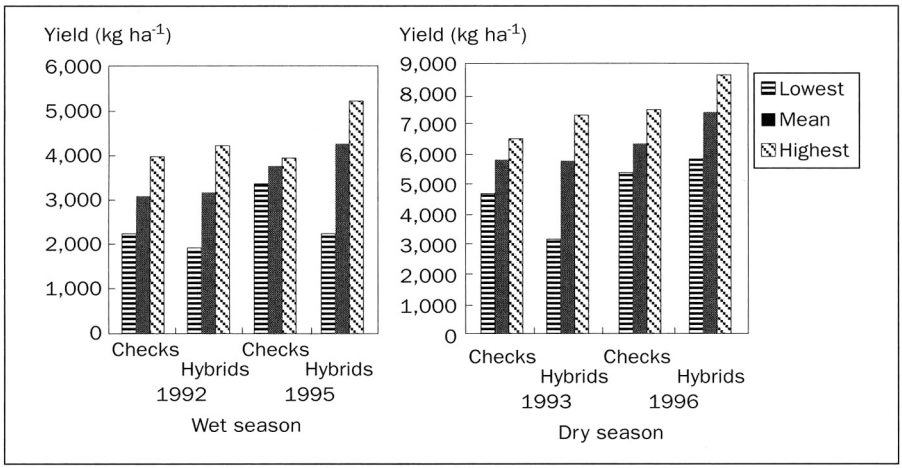

Fig. 6. Comparison of lowest, mean, and highest yields of hybrids and check varieties evaluated in advanced yield trials, IRRI, 1992-96.

We have shared more than 200 experimental rice hybrids with NARS for evaluation in yield trials. Several of these hybrids have yielded >1 t ha^{-1} higher than the local check varieties used in different countries. Some IRRI-bred rice hybrids have been released for commercial cultivation in India, the Philippines, and the Mekong Delta of Vietnam (Table 3). A few of these hybrids also performed well in the boro season in eastern India, Bangladesh, and Vietnam's Red River Delta (Table 4). Some IRRI-bred

Table 3. Heterotic rice hybrids released for commercial cultivation in Vietnam, India, and the Philippines up to 1997.

Country	Hybrid	Released as	Year
Vietnam	IR58025A/IR29723	UTL-1[a]	1993
	IR62829A/IR29723	UTL-2[a]	1993
India	IR58025A/Vajram	APHR-1[b]	1994
	IR62829A/MTU 9992	APHR-2[b]	1994
	IR62829A/IR10198	MGR-1[c]	1994
	IR58025A/IR9761-19-1R	KRH-1[d]	1994
	IR62829A/Ajaya	CNHR-3	1995
	IR58025A/IR40750-82	DRR-H1	1996
	IR58025A/KMR3	KRH-2	1996
Philippines	IR62829A/IR29723-143-3-2-1R	Rc26H[e] (or Magat)	1994
	IR58025A/IR34686-179	Rc72H (or Mestizo)	1997

[a]For regional adaptability trials in Mekong Delta. [b]For Telangana and Rayalseema districts of Andhra Pradesh, India. [c]For May/June and September/October planting in Tamil Nadu, India. [d] For irrigated areas of Karnataka, India. [e] For Cagayan valley region.

Table 4. Rice hybrids identified as promising in the boro season in West Bengal, India, Bangladesh, and the Red River Delta in Vietnam from 1993 to 1996.

Hybrid	Yield advantage of at least 1 t ha^{-1} over check		
	West Bengal, India	Bangladesh	Red River Delta, Vietnam
IR62829A/IR54742R	X[a]	–	–
IR58025A/IR29723R	X[a]	–	–
IR62829A/IR29723R	X[b]	X[b,c]	–
IR62829A/IR46R	X[b]	X[b,c]	–
IR62829A/IR47310R	X[b]	X[b]	–
IR62829A/IR48491R	X[b]	X[c]	–
IR58025A/IR21567-18-1R	–	X[b]	X[b]
IR58025A/IR34686-179R	–	X[b]	–
IR62829A/BR 736-20-3-1	–	X[c]	X[b]
IR58025A/IR54742-22-19-3	–	–	X[b]
IR58025A/RP 633-76	–	–	X[b]
IR62829A/IR54791R	–	–	X[b]

[a] 1993. [b] 1995. [c] 1996.

rice hybrids have also shown yield superiority over inbred rice under rainfed lowland conditions in the Philippines and eastern India (Table 5).

Since 1994, IRRI rice hybrids have also been tested in INGER trials. Hybrids IR67693H, IR69672H, IR69679H, IR68689H, and IR68691H in the first International Rice Hybrid Observation Nursery (IRHON) 1994, and IR70404H and IR70397H in the second IRHON 1995, showed at least a 1 t ha^{-1} yield advantage over the local checks (Table 6) in at least three locations (Chaudhary and Virmani 1996). But none of the hybrids showed significant yield superiority over the checks at Sakha, Egypt.

Table 5. Yield of rice hybrids under rainfed lowland conditions in Tarlac, Philippines, and Cuttack, India.

Hybrid	Year tested	Yield (t ha^{-1})	Yield advantage (t ha^{-1}) over check
Tarlac, Philippines			
IR58025A/IR29723R	1993	5.6	0.6 (IR46)
IR58025A/IR29723R	1994	5.5	0.7 (Mahsuri)
Cuttack, India			
IR58025A/IR29723R	1993	5.1	1.8 (Jaya)
IR58025A/IR54742R	1994	5.1	0.7 (Swarna)
PMS 8A/IR46R	1994	5.7	1.3 (Swarna)
IR58025A/RP 1057R	1994	5.1	0.7 (Swarna)

Table 6. Rice hybrids showing yield advantage of at least 1 t ha^{-1} in International Rice Hybrid Observation Nurseries evaluated in 1994 and 1995.

Hybrid	Yield advantage of at least 1 t ha^{-1} over check at different locations[a]																
	1994								1995								
	1	3	8	9	10	11	12	13		3	5	6	7	11	13	14
IR64616H		+						+	IR65488H					+		+
IR67161H	+	++							IR65489H			+		+		
IR67693H		+				+		++	IR67265H		+		++			
IR68284H		+	+						IR67693H	+						++
IR68285H			+					+	IR70404H			+			+	++
IR68286H		+						+	IR69671H					+		
IR69614H		+						++	IR69679H	+						++
IR69672H			+			+		+	IR70397H		+					+
IR69679H		+				+		++	IR70398H						+	++
IR69684H		+	++						IR70400H	+						
IR68689H			+		+	+		++	IR70402H						+	++
IR68690H						++	++									
IR68691H					+		+									
IR68696H		+		++												
IR68698H		+			++											
IR68699H		+			+											

[a] + = yield advantage over check is 1–2t ha^{-1}; ++ =yield advantage over check is >2 t ha^{-1}; locations reporting are 1 =Yangon (Myanmar), 3 = Los Baños (Philippines), 5 = San Mateo (Philippines), 6 = Omon (Vietnam), 7 = An Khand (Vietnam), 8 = Kapurthala (India), 9 = Masodha (India), 10 = Karnal (India), 11 = Mandya (India), 12 = Coimbatore (India), 13 = Pantnagar (India), 14 = Thatta (Pakistan).

Genotype × environment interaction analysis of hybrids and inbreds showed that both groups of cultivars were affected similarly and the hybrids were not more widely adapted than inbred rice (Bartolome et al 1996).

Increased yield obtained in hybrid rice is due to increased dry matter production arising from higher leaf area index and crop growth as well as increased harvest index arising from increased spikelet number or grain weight. The physiological basis of higher yield potential in rice hybrids and its implication for their agronomic management practices have been discussed by Peng et al (this volume, Chapter 14). Chinese

scientists have reported considerable differences in agronomic management of hybrid rice compared with inbred rice, especially for management of seedlings in the seedbed and N application (Yan 1988).

Resistance to biotic stresses in hybrids

At IRRI (Virmani 1996) hybrid rice was found to be resistant if one of the parents was resistant to biotic stresses. The hybrids were resistant or susceptible depending on whether the gene imparting resistance was dominant or recessive. If both parents were susceptible, then the hybrids derived from them were also susceptible. No evidence is available so far to indicate whether the vigor of hybrids makes them more tolerant of or susceptible to biotic stresses than their parental lines. Some IRRI-bred CMS lines (Table 7) and many restorer lines have been found to possess multiple resistance to biotic stresses (Table 8). Therefore, elite rice hybrids derived from them

Table 7. IRRI-bred CMS lines found to possess multiple resistance to biotic stresses.

CMS line	Stress resistance scores[a]							
	RTV	GLH	BPH1	BPH2	BPH3	BB1	BB2	BI	YSB
IR68886A	R/S[b]	5	3	5	6	7	7	4	3
IR68888A	R/S			7	7	3	7	1	3
IR68897A	S	5	3	9	5	5	7	4	3
IR69618A	R/S	3	5	5	5	1	7	4	3
IR69627A	R	3	7	9	9	1	7	6	3
IR69628A	R/S	5	9	9	9	1	7	4	3
IR70959A	MS	3	5	7	9	1	7	4	5
IR72078A	MR	3	3	3	3	1	7	5	7
IR72079A	S	5	3	3	3	1	7	5	3
IR72080A	R/S	5	5	7	7	7	1/7	5	3

[a]RTV = rice tungro virus, GLH =green leafhopper, BPH1 = brown planthopper biotype 1, BPH2 = brown planthopper biotype 2, BPH3 = brown planthopper biotype 3, BB1 = bacterial blight pathotype 1, BB2 = bacterial blight pathotype 2, BI = blast, YSB = yellow stem borer. On a scale of 1–9,where 1 = resistant and 9 = highly susceptible. [b] R = resistant, S = susceptible, MR = moderately resistant, MS = moderately susceptible, and R/S = segregating.

Table 8. IRRI-bred restorer lines found to possess multiple resistance to biotic stresses.

Restorer line	Stress resistance scores[a]							
	GLH	BPH1	BPH2	BPH3	BB1	BB2	BI	YSB
IR42221-14-1-3-1-2R	3	3	7	5	1	7	1	5
IR21567-16-3R	3	3	7	9	1	7	1	5
IR58103-62-3R	5	1	3	3	1	7	1	5
IR65514-5-3-1-2R	5	1	5	7	1	7	4	5
IR65515-56-1-3-1R	3	1	3	3	1	7	4	5
IR65509-22-1-2-1R	5	1	1	3	1	7	7	5
IR65516-67-3-3-1R	3	1	3	3	7	7	4	5
IR65514-5-3-3-3R	5	3	5	7	1	7	4	3
IR65514-5-1-2-1R	5	1	3	3	1	7	4	5
IR33509-26-2-2R	5	1	3	3	1/7	7	4	5

[a]GLH = green leafhopper, BPH1 = brown planthopper biotype 1, BPH2 = brown planthopper biotype 2, BPH3 = brown planthopper biotype 3, BB1 = bacterial blight pathotype 1, BB2 = bacterial blight pathotype 2, BI = blast, YSB = yellow stem borer. On a scale of 1–9, where 1 = resistant and 9 = highly susceptible.

Table 9. Elite rice hybrids found to possess multiple resistance to biotic stresses.

Hybrid	Stress resistance scores[a]								
	RTV	GLH	BPH1	BPH2	BPH3	BB1	BB2	B1	YSB
IR58025A/IR42221-14-1-3-1-2R	MR[b]	5	3	5	3	1	7	1	5
IR58025A/IR21567-16-3R	MR	3	3	7	5	1	7	1	5
IR58025A/IR58103-62-3R	R	5	3	9	5	1	7	3	6
IR58025A/IR65514-5-3-1-2R	MS	5	3	5	5	1	7	4	3
IR58025A/IR65515-56-1-3-IR	R	5	3	3	5	1	7	4	3
IR58025A/IR65509-22-1-2-IR	R	5	3	5	5	1	7	9	3
IR58025A/IR65516-67-3-3-19R	R	3	5	9	9	7	7	4	3
IR58025A/IR65514-5-3-3-3R	MR	5	5	7	9	1	7	4	3
IR58025A/IR65514-5-1-2-1R	R	3	5	7	5	1	7	4	5
IR62829A/IR33509-26-2-2R	R	3	1	3	1	1	7	7	3

[a] RTV = rice tungro virus, GLH =green leafhopper, BPH1 = brown planthopper biotype 1, BPH2 = brown planthopper biotype 2, BPH3 = brown planthopper biotype 3, BB1 = bacterial blight pathotype 1, BB2 = bacterial blight pathotype 2, BI = blast, YSB = yellow stem borer. On a scale of 1–9, where 1 = resistant and 9 = highly susceptible. [b] R = reststant, MR = moderately resistant, MS = moderately susceptible.

also possess multiple resistance to biotic stresses (Table 9). Rice hybrids with the required level of resistance to biotic stresses can be developed by the appropriate choice of parental lines. No evidence indicates any association of disease or insect susceptibility in hybrid rice with any of the CMS system deployed to develop commercial rice hybrids.

Hybrid rice seed production

Hybrid rice seed yields of 0.2–2.5 t ha^{-1} have been obtained in the tropics using the available seed production technology (Virmani and Sharma 1993, Virmani 1996). A further update on improvements made in hybrid rice seed production technology in the tropics is presented by Mao et al (this volume, Chapter 11).

Current status of development

Hybrid rice in the tropics was commercialized in 1993. The first set of tropical rice hybrids developed at IRRI was released in Vietnam as UTL 1 and UTL 2 for the Mekong Delta (Luat et al 1993, 1995). At about the same time, some rice hybrids from Guangxi Province, China (Shan You Gui 99, Bo You 64, Kim You 99, Te You 63, and Shan You 12), were also introduced for commercial cultivation by rice farmers of Vietnam's Red River Delta. In 1994, four rice hybrids—APHR-1, APHR-2, MGR-1, and KRH-1—were released in India and one hybrid—Rc 26H or Magat—was released in the Philippines. In 1995, India released another rice hybrid—CNHR-3 IR62829A/Ajaya—for the boro season in West Bengal. In 1996, Andhra Pradesh, India, released one more rice hybrid, DRR-H-1. Some private seed companies in India, such as Pioneer, Hybrid Rice International Ltd., and Mahyco, have also released additional rice hybrids for commercial cultivation. These hybrids have covered a sizable area of 60,000 ha in India and 100,000 ha in Vietnam.

Because the seed production infrastructure has yet to develop fully in the Philippines, coverage under released hybrids is poor. India has mobilized its own public and private seed companies to produce the required quantities of hybrid seed. Vietnam has been meeting most of its hybrid seed requirements by importing seed from China, although it is currently strengthening its own seed production capabilities. India and Vietnam have a target of 2 million and 0.5 million ha, respectively, under hybrid rice by 2000. Several other countries in the tropics such as the Philippines, Bangladesh, Sri Lanka, and Indonesia in Asia, and Brazil and Colombia in Latin America, may also release rice hybrids for commercial cultivation. Tropical rice-growing countries may produce hybrid rice from approximately 3 million ha by 2000. Considering the 1 t ha^{-1} yield advantage of rice hybrids, this should contribute 3 million t (worth $450 million) annually to world rice production.

Major constraints

The following factors were responsible for the slow development of hybrid rice technology in the tropics.

- Slow and inadequate investment to develop the technology.
- Slow breeding progress in commercially usable CMS and TGMS lines during the initial stages.
- Limited yield heterosis in commercial rice hybrids.
- Low and inconsistent seed yields in hybrid seed production plots.
- Inadequate human resources in terms of number and quality of personnel available in NARS for hybrid rice research and seed production.
- Lack of collaboration among national programs involved in hybrid rice research and development.
- Inadequate and ineffective seed production infrastructure in NARS.
- Lack of effective coordination between hybrid rice research and seed production systems.
- Inadequate incentives for establishment or expansion of the private seed industry.

Strategies to expedite research and development

Progress of hybrid rice research and development has been determined by the extent to which steps have been taken to overcome the constraints mentioned. From 1980 to 1988, India did not commit enough resources to develop this technology and hence progress was limited. Once the government made a whole-hearted commitment to develop and use this technology in 1989, progress has been phenomenal. India has set a good example for other countries to follow.

The restricted availability and nonadaptability of the Chinese CMS and TGMS lines in the tropics caused IRRI and NARS to develop suitable lines for the tropics. A lower frequency of maintainer lines found in elite indica rice cultivars did not allow

the development of the required number of commercially usable CMS lines. At IRRI, the maintainer breeding program began in 1992. This problem was soon solved and 10–20 new CMS lines are now developed every year for sharing with hybrid rice breeding programs around the world. In addition, hybrid rice breeders in India have also developed CMS lines and made them available to other countries. More recently, TGMS lines have also started coming out of IRRI's hybrid rice breeding program. To strengthen three- and two-line hybrid breeding programs further, IRRI has begun a male-sterility-facilitated recurrent selection program to develop composite populations for maintainers, restorers, and TGMS lines. NARS can use these populations to extract locally adapted B, R, and TGMS lines on a regular basis.

Yield heterosis in most of the indica rice hybrids released so far for commercialization ranges between 15% and 20% (equivalent to about 1 t ha^{-1}). With this yield advantage, hybrid rice technology has been found economical only in irrigated rice, where farmers use the transplanting method of stand establishment with a 15–20 kg ha^{-1} seed rate. To use hybrid rice under direct-seeded irrigated and rainfed lowland conditions, yield heterosis must be enhanced. Indica/tropical japonica hybrids could provide a solution. The discovery of apomixis in rice will make this technology useful even for resource-poor farmers.

The quantity of hybrid seed yield obtained by seed growers basically determines the economic viability of hybrid rice technology. With seed yields of 1–2 t ha^{-1}, the technology is viable. But to popularize it, hybrid seed yields must be raised consistently beyond 2 t ha^{-1}. This can be done by developing male sterile lines with higher outcrossing potential and improving seed production practices. The Chinese experience has proved that this is achievable.

Both China and IRRI have been helping countries around the world to develop their human resources for hybrid rice research and development. More recently, India has also developed the capacity to provide training to interested NARS. The Food and Agriculture Organization (FAO) of the United Nations has been providing financial resources for training and to support hybrid rice development in NARS (Vietnam, Myanmar, and Bangladesh) through special Technical Cooperation Projects.

IRRI has been helping NARS through bilateral collaboration to strengthen their research capabilities in hybrid rice. But NARS can benefit further if intercountry collaboration is also established. The International Task Force on Hybrid Rice (INTAFOHR) established by IRRI and FAO should help to obtain such collaboration.

There is a need to improve the seed production infrastructure in several tropical rice-growing countries around the world. FAO has been extending help to strengthen this infrastructure in some countries. Such support should not only be continued but also intensified for all needy countries interested in hybrid rice technology. Once an effective seed production infrastructure is established in the public, private, or NGO sectors in a country, its seed production units should be closely linked with its hybrid rice research units to expedite the transfer of this technology.

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CHAPTER 5

Ushering in an era of hybrid rice in India

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Using a goal-oriented and time-bound research network at 12 centers across the country launched in 1989, four public-bred rice hybrids were released in India in 1994. Later, three more public-bred hybrids were released. The private sector also markets more than six other hybrids. These hybrids have a convincing yield advantage of about 1 t ha^{-1} over the highest-yielding inbred cultivars with similar maturity duration. The additional yield of hybrid rice results in an additional net profit of US$100 (Rs. 3,500) ha^{-1}. During the 1996 wet season, hybrid rice was planted on 50,000 ha. Because hybrid rice seed production technology has been optimized over the past 5 years, seed yields of 1.5–2.0 t ha^{-1} are now being obtained. Five public-sector seed agencies and about 10 private seed companies are engaged in large-scale hybrid rice seed production. In the 1995-96 dry season, 1,300 t of hybrid seed were produced. Having pure seed of parental lines available for public-sector seed agencies and obtaining the proper synchronization of parental lines are some of the problems encountered in large-scale seed production. To sustain hybrid rice technology, attempts are being made to enhance heterosis by using diverse parental lines, developing cytoplasmic male sterile lines with better outcrossing traits, incorporating resistance to major pests and diseases, and improving cooking and eating quality characteristics. Efforts are also under way to develop hybrids suitable for northwestern India—basmati rice hybrids and hybrids suitable for the favorable shallow lowland ecosystem. The large-scale adoption and further spread of hybrid rice depends primarily on the economic attractiveness of the technology. This chapter considers various issues, options, and strategies for future research and development and discusses prospects for the large-scale adoption of hybrid rice technology in India during the 21st century.

From 1970 to 1980, research covered several aspects of hybrid rice in some agricultural universities and national institutes. But these efforts were mostly of an academic nature. The parental lines introduced and hybrids from China were found to be unadapted to Indian conditions because they were highly susceptible to pests and diseases and their grain quality was very poor. Stable cytoplasmic male sterile (CMS) lines for the tropics were not available then. Progress was therefore not made on applied aspects of heterosis breeding in rice.

The Indian Council of Agricultural Research (ICAR), New Delhi, identified hybrid rice as a priority area and launched a goal-oriented and time-bound project for the development and use of hybrid rice technology in December 1989. In September 1991, this project was strengthened by support from the United Nations Development Programme and the Food and Agriculture Organization of the United Nations. This project now operates as a national network with 12 research centers across the country coordinated by the Directorate of Rice Research (DRR), Hyderabad.

Hybrids released

Since 1990, almost 700 experimental rice hybrids, developed indigenously, as well as those from the International Rice Research Institute (IRRI) have been systematically evaluated in National Hybrid Rice Trials at 12 centers. About 30 of these hybrids were identified as promising, with a yield advantage of 1 t ha^{-1} or more over inbred check varieties of similar maturity duration. Performance of the hybrids was found to be location-specific. Ten hybrids were selected based on yield potential, grain quality characteristics, and ease in production of hybrid F_1 seed. Large quantities of seeds of these hybrids were produced and extensive on-farm trials were conducted in the area of their adaptation. The yield advantage of the hybrids observed in the national hybrid trials at the research station farms was further confirmed in on-farm trials. Farmers' opinions of the hybrids were also collected. Based on their convincing yield advantage, seven rice hybrids were released by the respective state variety release committees for commercial cultivation (Table 1).

The private seed sector was also actively involved as an equal partner right from the start of the network project on hybrid rice. All the parental lines and the required technical guidance were provided to it. This enabled private companies to develop and produce large quantities of seed and market rice hybrids at the rate of Rs. 70–105 (US$2–3) kg^{-1} of hybrid seed. All these hybrids made available to farmers for commercial use heralded an era of hybrid rice in Indian agriculture. During the I996 wet season, hybrid rice was grown on nearly 50,000 ha. Because of the switchover to the cultivation of hybrids, the average additional profit obtained was Rs. 3,500 (US$100) ha^{-1}.

Table 1. Salient features of hybrids released.

Hybrids/year	Parentage	Duration (d)	Yield (t ha^{-1}) [a]		Yield advantage over check	
			Hybrid	Check	t ha^{-1}	%
APHR-1 (1994)	IR58025A/ Vajram	130–135	7.14	5.27 (Chaitanya)	1.87	35.4
APHR-2 (1994)	IR62829A/ MTU 9992	120–125	7.52	5.21 (Chaitanya)	2.31	44.2
MGR-1 (1994)	IR62829A/ IR10198	110–115	6.08	5.23 (IR50)	0.85	16.2
KRH-1 (1994)	IR58025A/ IR9761	120–125	6.02	4.58 (Mangala)	1.44	31.4
CNRH-3 (1995)	IR62829A/ Ajaya	125–130	7.49	5.45 (Khitish)	2.04	37.4
DRRH-1 (1996)	IR58025A/ IR40750	125–130	7.30	5.50 (Tellahamsa)	1.80	32.7
KRH-2 (1996)	IR58025A/ KMR 3	130–135	7.40	6.10 (Jaw)	1.30	21.3

[a] In on-farm trials.

Seed production technology developed

To develop an efficient and economical seed production package, several experiments were conducted at the network research centers. As a result, a standard methodology was developed for hybrid rice seed production and CMS multiplication to ensure a hybrid seed yield of 1.5–2.0 t ha^{-1}.

In hybrid seed production technology (Siddiq et al, this volume, Chapter 24), it is necessary to raise healthy and vigorous seedlings with 3–4 tillers in 25 d. This can be achieved by adopting sparse sowing (20 g m^{-2}) in the nursery and maintaining a proper layout of the seed production plot. Applying an optimum dose (60 g ha^{-1}) of gibberellic acid (GA_3) at the right stage, supplementary pollination at peak anthesis, thorough roguing at various stages of the crop, and careful handling during harvest are essential for obtaining higher seed yields. By adopting these methods during the 1995-96 dry season (DS), private and public-sector seed agencies produced 1,300 t of hybrid seed (Table 2).

Several private seed companies, such as Nath Seeds, Omega Seed Limited, Ganga Agriseeds, Nagarjuna Seed Company, Amareshwari Agrotech Limited, Cosmo Plant Gene, Mahendra Seed Company, EID Parry Company, and Nuzveedu Seeds Limited, also produced another 5 t of hybrid rice seed during the 1995-96 DS.

The private sector is taking the lead in the large-scale seed production of hybrid rice in India. Various public-sector seed agencies are also being encouraged to produce seed on a large scale to meet the huge demand anticipated in the coming decades. Five public-sector seed agencies and 8–10 seed companies in the private sector are now engaged in large-scale hybrid rice seed production. In addition, 15 new seed companies have recently begun producing seed on a smaller scale.

Table 2. Seed produced by various agencies during the 1995-96 dry season.

Seed agency	Rice hybrids	Seed produced (t)
Private sector		
Hybrid Rice International Company	6201	500
Mahyco Seed Company	MPH 516, MPH 517, MPH 518	480
Spic-PHI Biogene Limited	PHB-71	100
Hindustan Lever Limited	PRS- 101	98
Vikki's Agrotech Limited	VRH-4	5
Public sector		
DRR/KVK Gaddipally	DRRH-1	100
Department of Agriculture, Tamil Nadu, and EID Parry Company	MGR-1	5
Karnataka State Seed Development Corporation	KRH-1	5
Andhra Pradesh State Seed Development Corporation	APRH-2	5
Department of Agriculture, West Bengal, and Pallashree Seed Company	CNRH-3	2
Total		1,300

The two major problems in large-scale seed production are:

- Nonavailability of pure seed of parental lines for public-sector seed agencies.
- Failure to obtain proper synchronization in parental lines.

Steps are being taken to solve these problems.

Training programs conducted

Training a large number of seed production personnel at various levels from the private and public seed sector, along with progressive farmers, seed growers, and farm women, in hybrid rice seed production technology is a prerequisite for undertaking large-scale seed production. Realizing this critical need, the research network centers and nongovernmental organizations have conducted extensive training programs on seed production technology for a diverse clientele (Table 3).

The government of India plans to intensify the frequency and coverage of these training programs during the next 3–5 yr, to make available a large number of trained personnel for undertaking large-scale F_1 seed production to meet the anticipated demand of 40,000 t of hybrid seed by the year 2000.

Sustaining hybrid rice technology

Hybrid rice was introduced in Indian agriculture recently and is now cultivated commercially on a small scale. For the widespread and large-scale adoption of this technology, certain constraints must be overcome and challenges must be met successfully.

At present, the heterosis observed for yield is in the range of 15–20% only. The cost of hybrid seed is also high. Therefore, the economic advantage of cultivating rice

Table 3. Training programs conducted at different centers in India.

Type of training	Programs (no.)	Duration (d)	Number	Participants and category
DRR Hyderabad				
Hybrid rice technology	10	6	255	Personnel from private- and public-sector seed production agencies, development officers from departments of agriculture, various states
KVK Gaddipally				
Hybrid rice seed production	2	1 yr	30	Science graduates
	2	6 mo	120	Rural unemployed youth
	8	10	2,192	Progressive farmers, agricultural officers, rural youth, and farm women
	3	3–5	1,134	Farm women and progressive farmers
Hybrid rice cultivation	3	1	907	Progressive farmers
Mandya				
Hybrid rice seed production	9	6	251	Seed growers, personnel from the State Seed Corporation, University of Agriculture, Bangalore and Dharwad, personnel from private seed companies
Hybrid rice cultivation	15	3	410	Farmers and staff from Department of Agriculture
Coimbatore				
Hybrid rice seed production and cultivation	20	2–5	315	Personnel from private- and public-sector seed agencies, Department of Agriculture, and progressive farmers
Karjat				
Hybrid rice seed production	2	3	61	Personnel from agricultural universities and private seed companies
Maruteru				
Hybrid rice seed production (state level)	9	3–5	410	Staff from Department of Agriculture and progressive farmers
Pantnagar				
Hybrid rice seed production	4	6	150	Personnel from private- and public-sector seed agencies, NGOs, and progressive farmers
Chinsurah				
Hybrid rice seed production	6	2–6	210	Staff from private- and public-sector seed agencies, NGOs, and progressive farmers

hybrids is marginal. Increasing the profit in cultivating rice hybrids, enhancing yield heterosis, reducing the cost of hybrid seed, improving grain quality, and incorporating resistance to major pests and diseases in hybrids would help sustain hybrid rice technology in the future.

Enhancement of heterosis

If the magnitude of heterosis can be increased from 15–20% to 25–40%, this innovative technology is likely to be adopted widely in a short time. Two approaches are now being followed to enhance yield heterosis:

- Developing two-line hybrids.
- Developing intersubspecific indica/tropical japonica hybrids.

A preliminary evaluation of heterosis in two-line hybrids and intersubspecific hybrids was carried out recently and the results appear promising.

Reducing the cost of hybrid seed

Yields in hybrid seed production plots have to be increased to 2.0–2.5 t ha^{-1} to reduce the seed cost. New CMS lines with a higher outcrossing potential, better management of seed plots, and a search for alternatives to GA_3, which is now the costliest input in seed production, are some aspects that are receiving immediate attention for reducing the cost of seed.

Improving grain quality in hybrids

Consumer acceptance of hybrid rice depends largely on grain quality characteristics. In some of the currently cultivated hybrids, the milling percentage is reported to be rather low. In some other hybrids, the keeping quality of the grain after cooking is poor. The ultimate quality of the cooked rice is the most important character. Therefore, greater emphasis is being given to improving eating quality in all the hybrids. Panel tests by consumers are conducted rigorously before advancing hybrids to on-farm trials.

Incorporating resistance to pests and diseases

The hybrids released up to now do not possess specific resistance to major pests and diseases. Therefore, all parental lines involved in developing hybrids are screened for resistance to major pests and diseases. Increased emphasis is being given to using parental lines that possess resistance to at least one major pest and one major disease in the target area.

Hybrid rice seed multiplication

At present, the public-sector seed agencies cannot produce a large volume of hybrid seed. Therefore, even though the public-bred hybrids are on a par with or even better than the private hybrids, they do not occupy large areas because of the nonavailability of seed. We therefore need to develop a mechanism for activating public-sector seed agencies for the large-scale seed production of rice hybrids. We also need to identify an agency for the maintenance and supply of nucleus seed of parental lines of public-bred hybrids on a sustainable basis.

Developing hybrids for specific situations

Special emphasis is given to developing hybrids for northwestern India. This high-productivity area includes Punjab, Haryana, and western parts of Uttar Pradesh. The cultivation and release of hybrids in this region will make an impact on overall productivity and production of rice in the country. So far, three hybrids—Punjab Rice Hybrid- 1 (PRH- 1), Uttar Pradesh Rice Hybrid-27 (UPRH-27), and Haryana Karnal Rice Hybrid-I (HKRH-1) were tested in on-farm trials. The yield potential of the inbred varieties currently grown in this region is very high (8–10 t ha^{-1}). The average yield advantage from cultivating hybrids was observed to be limited (0.5 t ha^{-1}). Another requirement for introducing hybrids in the region is resistance to bacterial leaf blight, as this disease is endemic in the area. Efforts are now being made to develop and release a hybrid suitable for the region in the near future.

Hybrids suitable for the favorable rainfed shallow lowland ecology and basmati-type hybrids possessing high grain quality are also being developed. The requisite parental lines have already been developed. These experimental hybrids are now being evaluated to ascertain their yield advantage over the inbred check varieties. These hybrids for special situations are likely to become available to farmers for commercial cultivation during the next 5 yr.

Future outlook

A sound base has been created for the development of hybrid rice technology in the years ahead. The network research project has helped many scientists from the various hybrid rice research centers under the different universities to undergo training in heterosis rice breeding and seed production technology at the National Hybrid Rice Research and Development Center, Changsha, China; IRRI, Los Baños, Philippines; and other leading laboratories around the world. International experts provided consultancy for more than 20 mo in research. Many centers have developed all the infrastructure required for an active and dynamic heterosis breeding program. Many seed production personnel from public- and private-sector seed agencies, seed growers, progressive farmers, and farm women have been trained in all aspects of seed production technology.

For the effective and rapid transfer of hybrid rice technology to large areas, the availability of an adequate quantity of hybrid seed is a prerequisite. Seed production agencies, particularly in the public sector, therefore need to be motivated to produce large quantities of hybrid seed. For this, personnel are trained in various aspects of seed production technology.

Research teams in the future will focus on enhancing the magnitude of heterosis and increasing average seed yields to >2.5 t ha^{-1}. The research programs organized to achieve these objectives have already been discussed briefly. Initially, the objective was to develop hybrids for the high-productivity areas in the irrigated ecosystem only. But hybrids suitable for favorable shallow lowlands in the rainfed ecosystem and

hybrids with basmati-type quality are also being developed. The target is to cover 2 million ha in the next 4 yr, that is, by 2000, and to cover 10 million ha by 2010.

Considering the recent developments in heterosis breeding and seed production technology, with the establishment of an extensive mechanism in the country to develop and use hybrids, and the active interest taken by the seed sector, the targets for coverage under rice hybrids are likely to be achieved. Prospects for the large-scale adoption of hybrid rice in Indian agriculture during the 21 st century therefore appear to be bright.

Notes

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CHAPTER 6

Using tropical japonica germplasm to enhance heterosis inrice

G.S. Khush, R.C. Aquino, S.S. Virmani, and T.S. Bharaj

The current level of heterosis (15–20% or 0.75–1.0 t ha^{-1}) in indica rice hybrids developed for the tropics is economically viable, but a higher level would be more attractive. Studies at IRRI have showed a higher heterosis for yield in tropical japonica/indica crosses than in indica/indica crosses. Increased heterosis for yield in tropical japonica/indica crosses is possible only if they exhibit normal spikelet fertility. Several tropical japonica rice cultivars that possess the wide compatibility (WC) gene have been identified. Suitable parental lines in improved tropical japonica germplasm possessing the WC gene are being bred at IRRI. These parental lines would help to develop heterotic tropical japonica/indica hybrids. We discuss the progress made in improving tropical japonica germplasm and strategies used at IRRI to develop tropical rice hybrids with enhanced yield heterosis.

Genetic diversity between parents, within limits, is related to magnitude of heterosis in crosses derived from them. Rice hybrids grown widely in China and those released in recent years for commercial cultivation in India, Vietnam, and the Philippines are based on indica germplasm. These hybrids show 15–20% standard heterosis (Yuan et al 1994, Virmani 1994, 1996). In japonica rice hybrids cultivated commercially in China, standard heterosis is even lower (less than 10%) and, as a result, the area under such hybrids is limited. To raise the level of yield heterosis, Chinese and Japanese rice scientists proposed using indica/japonica crosses (Anonymous 1988, Maruyama 1988, Ikehashi 199 1).

Preliminary studies conducted at IRRI showed enhanced yield of crosses involving semi-improved bulu or javanica (now designated as tropical japonica) and indica rice compared with indica/indica crosses (IRRI 1989). Khush and Aquino (1994) discussed the prospects for and problems of developing tropical japonica for hybrid rice. This chapter highlights the progress made in improving tropical japonica

germplasm and strategies used at IRRI to develop tropical rice hybrids with enhanced yield heterosis.

Improving tropical japonica germplasm

To increase the yield potential of rice cultivars, IRRI scientists conceived a new plant type with the following attributes (Khush 1995):

- Lower tillering capacity (3-4 tillers when direct seeded, 8–10 tillers when transplanted)
- No unproductive tillers
- 200–250 grains panicle^{-1}
- 90–100 cm tall
- Very sturdy culms
- Dark green thick and erect leaves
- Vigorous root system
- 100–130 d growth duration
- Multiple disease and insect resistance
- Acceptable grain quality

Most of these attributes were found in tropical japonica germplasm (IRRI 1992). A Chinese japonica rice breeding line, Shen Nung 89-366, and a tropical japonica semidwarf selection, MD 2 from Madagascar, were used as sources of dwarfism. Numerous advanced-generation breeding lines with the new plant type (improved tropical japonica) are now being evaluated for yield potential. But their yield expression is constrained by their susceptibility to insects (such as brown planthopper, green leafhopper, and stem borer) and diseases (such as tungro virus, sheath blight, and bacterial blight), and their partial grain filling. Intensive efforts are being made at IRRI to incorporate multiple disease and insect resistance into such lines and to study the causes of partial grain filling to overcome this problem.

Most of the tropical japonica germplasm possesses short bold grains. Therefore, improved tropical japonica lines bred so far at IRRI also have short bold grains. But consumers in the tropics and subtropics prefer long slender grains. Some tropical japonica rice cultivars with long slender grains have been identified, and are now used to breed new-plant-type lines with long slender grains.

Using tropical japonica germplasm to enhance yield heterosis

Tropical japonica rice germplasm is genetically diverse from indica rice germplasm (Glaszmann 1987). Hybrids derived from intergroup crosses are expected to show stronger heterosis than intragroup crosses. But the commercial exploitation of the enhanced heterosis in such crosses is hampered by intervarietal hybrid sterility. Studies conducted at IRRI on these topics are summarized below.

Heterosis studies

We made crosses among indica (I) and improved tropical japonica (TJ) rice breeding lines developed at IRRI during 1993 and 1994. Because selected TJ rice was inherently lower tillering than indica rice, we also studied genotype × spacing interaction. Test entries in each group (TJ/I, I/I, TJ/TJ, I, and TJ) yielded significantly higher at 20 × 10 cm (inter- and intrarow) spacing than at 20 X 20 cm spacing (Table 1). The difference was greater for TJ/I and TJ/TJ crosses and TJ lines than for I/I crosses and I lines. At closer (20 × 10 cm and 15 × 10 cm) spacing, TJ/I crosses showed higher yield and standard heterosis than I/I crosses (Table 2). The higher yield of TJ/I hybrids was attributed to higher total biomass, harvest index, and 1,000-grain weight. Various groups of hybrids and inbreds yielded in order TJ/I > I/I > TJ/TJ = > I > TJ. Yield of the TJ inbred was lower than that of the I inbred because of susceptibility to pests and diseases and partial grain filling as discussed earlier.

Searching for the wide compatibility gene among tropical japonica germplasm

Increased heterosis for yield in indica/tropical japonica rice hybrids is possible only if they exhibit normal spikelet fertility. Generally speaking, indica/japonica or indica/tropical japonica F_1s exhibit partial spikelet sterility because of allelic interaction at the *S-5* locus, which causes female gametes carrying the japonica allele to be eliminated (Ikehashi and Araki 1984). Varieties with wide compatibility (WC) genes carry a neutral allele at the *S-5* locus (Ikehashi and Araki 1984, 1986). Several tropical japonica rice cultivars have been identified as possessing the WC gene (Ikehashi and Araki 1984, Luo et al 1990, Vijaya Kumar and Virmani 1992, IRRI 1996). The $S\text{-}5^n$ locus was found to be closely linked with marker gene *C* (chromogen for pigmentation) and *wx* (waxy endosperm), which are located on chromosome 6 (Ikehashi and Araki 1986, 1987). Recent studies at IRRI have also showed a close linkage (4.1 cm)

Table 1. Yield of hybrids and inbreds evaluated at two plant spacings, IRRI, 1993 wet season.

Group	Number	Yield ($g\ m^{-2}$)[a]		
		S_1 (20 × 20 cm)	S_2 (20 × 10 cm)	Difference $S_2 - S_1$
Tropical japonica/indica	5	497 a	573 a	+76**
Indica/indica	5	518 a	542 b	+24**
Tropical japonica/tropical japonica	5	394 c	482 c	+88**
Indica	9	447 b	476 c	+30**
Tropical japonica	8	307 d	389 d	+82**
Mean		423	452	+29**

[a]Numbers followed by different letters were significantly different from each other using Duncan's multiple range test. **Significant at the 1% level using the least significant difference test.

Table 2. Total biomass, grain yield, harvest index, and 1,000-grain weight of inter- and intravarietal group hybrids and their inbreds evaluated at IRRI, 1993 wet season and 1994 dry season (Bharaj, Virmani, and Khush, unpublished).

Group	Number	Total biomass[a] ($g\ m^{-2}$)	Grain yield ($g\ m^{-2}$)	Harvest index	1,000-grain wt. (g)
1993 wet season (spacing 20 × 10 cm)					
Tropical japonica/indica	3	1,816 a	890 a	0.49 a	31.0 b
Indica/indica	5	1,540 b	710 b	0.46 b	28.0 c
Tropical japonica/ tropical japonica	5	1,489 b	643 c	0.43 c	32.6 a
Indica	9	1,418 b	603 c	0.42 c	26.3 d
Tropical japonica	9	1,116 c	412 d	0.37 d	28.8 c
1994 dry season (spacing 15 × 10 cm)					
Tropical japonica/indica	8	2,087 a	1,030 a	0.50 a	28.1 a
Indica/indica	8	1,834 b	894 b	0.48 b	27.0 b
Tropical japonica/ tropical japonica	8	1,724 bc	822 c	0.48 b	27.6 b
Indica	8	1,651 c	726 d	0.44 c	24.4 c
Tropical japonica	8	1,453 d	566 e	0.39 d	25.0 c

[a] Numbers followed by different letters were significantly different from each other using Duncan's multiple range test.

of the WC gene with *Amp 3* and *Est 2* (Malik and Khush 1996). All the WC varieties (WCVs) have allele 2 and all the non-WCVs have allele 1 of *Amp 3*. Such tight linkage with an isozyme marker is of great practical significance for selecting WC individuals in a segregating population. We use this information in a marker-based selection program to develop tropical japonica breeding lines possessing the WC gene.

Bharaj et al (1994) identified two (among 10) improved tropical japonica rice cultivars possessing the WC gene. As most traditional tropical japonica rice cultivars possess the WC gene, it is logical to expect a large number of WCVs among the improved tropical japonicas derived from them. A continuous search for WCVs among improved tropical japonica rice cultivars is in progress at IRRI for the selection of suitable parents for developing I/TJ rice hybrids.

Hybrids from TJ/I crosses involving TJ parents with the WC gene showed a significantly higher yield than TJ/I crosses involving TJ parents without the WC gene (Table 3). Therefore, the presence of the WC gene in one of the parents of I/TJ crosses is essential for exploiting indica-tropical japonica heterosis.

Table 3. Yield of tropical japonica (TJ)/indica (I) hybrids involving TJ parents with the wide compatibility (WC) and without the WC (NWC) gene, at two spacings, IRRI, 1993 wet season (Bharaj, Virmani, and Khush, unpublished).

Hybrid	Number	Yield (g m^{-2})[a]		
		S_1 (20 × 20 cm)	S_2 (20 × 10 cm)	Difference S_2 - S_1
TJ (WC)/I	3	544 a	622 a	+78**
TJ (NCW)/I	2	428 b	500 b	+73**

[a] Numbers followed by different letters were significantly different from each other using Duncan's multiple range test. **Significant at the 1% level using the least significant difference test.

Developing tropical japonica parental lines for hybrid rice breeding

The enhanced yield heterosis in I/TJ hybrids over that of I/I hybrids at IRRI has encouraged us to begin a breeding program to develop such hybrids. Numerous A, B, and R lines, and some thermosensitive genic male sterile (TGMS) lines, are already available in indica rice. Such lines, however, need to be bred in improved tropical japonica germplasm.

To identify maintainer and restorer lines among improved tropical japonica breeding lines bred at IRRI, 293 test crosses have been made. Almost all the TJ lines were found to be maintainers or at least partial maintainers; none of the I28 lines tested so far could restore the fertility of WA, ARC, and mutagenized IR62829B CMS lines (Casal and Virmani, unpublished). Some improved TJ lines are being converted into CMS lines at IRRI (Table 4). Like their parents, these improved TJ lines also do not possess the desired level of disease or insect resistance and acceptable grain quality. In addition, they have a low outcrossing rate and are therefore not yet suitable for developing commercial TJ/I hybrids by crossing them with indica restorers. To search for fertility restorers among traditional tropical japonica varieties, about 225 test crosses were made at IRRI with indica CMS lines carrying CMS-WA and CMS-*O. perennis* cytoplasm. None of the lines was found to be an effective restorer (Tai 1995).

The apparent lack of restoration ability in japonica germplasm indicated that japonica lines cannot be used as a pollen parent of an I/TJ rice hybrid. If the outcrossing rate of tropical japonica CMS lines also turns out to be low, such CMS lines will also be of limited use for developing commercial I/TJ hybrids. Therefore, concurrently, prospects of the TGMS system are also being explored at IRRI. Some indica TGMS lines have been bred at IRRI (IRRI 1995) and crosses have been made with improved tropical japonica lines possessing the WC gene to incorporate the TGMS gene into them. Anther culture is being used to expedite the breeding process. Both indica and tropical japonica TGMS lines possessing the WC gene would be useful in developing I/TJ rice hybrids.

Table 4. List of improved tropical japonica breeding lines in a backcross nursery being converted into cytoplasmic male sterile lines with different CMS sources at IRRI, 1996 wet season.

TJ line	BC generation	CMS source	
IR65564-44-2-3	4	Mutagenized	IR62829B
IR655697-17-4-3-3	4	Mutagenized	IR62829B
IR65600-7-2-3-2	4	Mutagenized	IR62829B
IR65600-164-3-2	4	Mutagenized	IR62829B
IR65600-1-3-2	4	ARC	
IR65600-21-2-2-2	2	ARC	
IR65600-54-6-3-2	4	ARC	
IR65600-96-1-2-2	4	ARC	
IR65600-129-1-1-2	4	WA	
IR67963-42-3	1	WA	
IR67963-42-3	1	WA	
IR67964-58-1	1	WA	

Future outlook

Hybrid rice is already a great success in China. During the next few years, India, Vietnam, and the Philippines also expect to benefit from this technology. The technology is useful not only because it gives the rice crop a higher yield potential, but also because of its capability to increase rural employment opportunities through a hybrid seed industry.

The current level of heterosis (15–20% or 0.75–1.0 t ha^{-1}) in tropical rice hybrids is economically viable, but a higher level, observed in indica/tropical japonica crosses, would be more attractive. The prospects for developing such hybrids appear bright because improved tropical japonica lines possessing the new plant type and a higher yield potential are on the horizon and should be available within 4–5 years. Such lines can be converted into suitable parental lines and used as one of the parents of I/TJ or reciprocal-cross hybrids. The problem of intervarietal hybrid sterility in these crosses can be overcome by developing a tropical japonica parental line possessing the WC gene. This should not be difficult because the WC gene is widely distributed in tropical japonica germplasm. The lack of restorers for the available CMS systems among tropical japonica germplasm makes the TGMS system more practical than the CMS system for developing I/TJ hybrids. The outcrossing potential of tropical japonica lines when used as female or male parents has not yet been studied thoroughly. We hope that this would not pose problems in hybrid seed production.

The yield potential of modern high-yielding varieties grown under the best tropical conditions is 9–10t ha^{-1} . Tropical rice hybrids under similar conditions have shown about 1 t ha^{-1} higher yield. The new-plant-type rice cultivars expected to be available by the year 2000 should have a yield potential of about 12 t ha^{-1} (Khush 1995). These new-plant-type cultivars will be used to produce hybrid rice, which is expected to have a yield potential of 13 t ha^{-1} .

In temperate rice-growing countries such as Japan, Korea, and Egypt, hybrid rice technology has not been successful so far. The major constraint is the limited extent (up to 10%) of heterosis in japonica/japonica crosses. Hybrids derived from improved tropical japonica lines and locally bred temperate japonica lines may have a better performance than temperate japonica rice hybrids (Virmani 1996). Studies on this should be carried out in temperate rice-growing countries.

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CHAPTER 7

The wide compatibility system: current knowledge of its genetics and use for enhanced yield heterosis

H. Ikehashi and J. Wan

The genetic basis for hybrid sterility is presented with methods to analyze it. Several independent loci have been identified that cause partial abortion of female gametes carrying an allele in heterozygotes. Similar genetic mechanisms were also identified for male gametes that affect pollen fertility. The hybrid sterility in indica/japonica crosses was mostly attributed to an *S-5* locus. Therefore, a single wide compatibility allele can be used to obtain fertile hybrids between the two groups. But hybrid sterility in crosses between rice cultivars from the Indian subcontinent and other areas was controlled by several loci, as detected in Basmati 370 crosses. In hybrid rice breeding, hybrid sterility genes in cytoplasmic male sterile lines affect the screening of potential restorers but not maintainers. It is important to analyze hybrid sterility genes in maintainers to achieve enhanced heterosis in hybrid rice breeding.

Progress in understanding the genetics of hybrid sterility has been slow because of its uniqueness. In typical indica/japonica crosses, hybrid sterility is caused by an allelic interaction at locus *S-5.* Indica and japonica varieties carry $S\text{-}5^i$ and $S\text{-}5^j$, respectively. For hybrid sterility, some other varieties have a neutral allele, $S\text{-}5^n$. The $S\text{-}5^i/S\text{-}5^i$ genotype produces semisterile panicles because of the partial abortion of female gametes carrying the allele $S\text{-}5^j$. Such abortion does not occur in $S\text{-}5^n/S\text{-}5^i$ and $S\text{-}5^n/S\text{-}5^j$ genotypes. The donor of $S\text{-}5^n$ is referred to as a wide compatibility variety (WCV) (Ikehashi and Araki 1986). As soon as the simple monogenic nature of hybrid sterility was understood, it was applied to hybrid rice breeding to enhance the level of heterosis. The WC allele has been incorporated into indica and japonica varieties to overcome hybrid sterility and achieve a pronounced heterosis in intersubspecific hybrids (Ikehashi 1991, Yuan 1992, Zou et al 1992). In hybrid rice breeding, distant crosses are handled by examining the fertility status of pollen and spikelets. Therefore, understanding the genetics of hybrid sterility would help to develop better rice hybrids.

Genetics of hybrid sterility

So far, the allele of S-5^n has been effective in a large number of indica/japonica crosses. In tests using more than 1,000 varieties from China, a few showed hybrid sterility in their crosses with WCVs (Table 1). But such WCVs exhibited hybrid sterility when crossed with varieties from the Indian subcontinent or with native rice varieties in China. Therefore, further genetic analyses were conducted on hybrid sterility gene loci.

A large number of three-way crosses (A/B//C) were made after confirming that a hybrid A/C produced semisterile panicles, whereas the hybrid B/C was fertile. The progeny of A/B//C segregated for semisterile plants as expected from A/C, and for fertile plants as expected from B/C, in a ratio of 1:1. When a backcross, A/C//C, was made, the progeny segregated into semisterile plants as expected from NC, and into fertile plants as expected from C/C, in a certain ratio. Then, genetic markers cosegregating with the semisterility were surveyed to identify a locus for the semisterility. In the backcrosses, F_1 plants were used as females to find distortion of marker genotypes, caused by abortion of the female gamete carrying one allele.

Table 1. Applicability of wide compatibility gene (S-5^n) to Chinese varieties.

Type	Source	Varieties (no.) classified by F_1 fertility in crosses with tester varieties					
		K. Nangka		Dular		Nekken 2	
		Fer.[a]	Ster. (%)	Fer.	Ster. (%)	Fer.	Ster. (%)
Indica	Yunnan	158	5 (3.1)	163	0	157	6 (3.7)
	Tai-hu	127	1 (0.8)	128	0	127	1 (0.8)
	Cultivated	185	0 (0)	184	1(0.5)	185	0
Japonica	Yunnan	113	7 (5.8)	120	0	112	8 (6.7)
	Tai-hu	275	9 (3.2)	284	0	274	10 (3.5)
	Cultivated	126	0 (0)	126	0	126	0
Total		984	22 (2.2)	1,005	1(0.01)	981	25 (2.5)

Type	Source	Varieties (no.) classified by F_1 fertility in crosses with tester varieties			
		Nekken 1		02428	
		Fer.	Ster. (%)	Fer.	Ster. (%)
Indica	Yunnan	156	7 (4.3)	141	22 (13.5)
	Tai-hu	126	2 (1.8)	128	2 (1.5)
	Cultivated	185	0	160	25 (13.5)
Japonica	Yunnan	112	8 (6.7)	113	7 (5.8)
	Tai-hu	274	10 (3.5)	278	6 (2.1)
	Cultivated	125	1 (0.8)	125	1 (0.8)
Total		978	28 (2.8)	945	63 (6.3)

[a] Fer. = panicles of a hybrid had fertility higher than 70%, ster. = panicles of a hybrid had fertility lower than 70%.

Allelic differences at a new locus were estimated following the model of allelic interaction at *S-5*. For three given varieties—A, B, and N—if a hybrid A/B showed gamete abortion at hybrid sterility gene locus *S-X,* but hybrids N/A and N/B showed no distorted segregation of markers for *S-X,* variety N was determined to possess a neutral allele, $S\text{-}X^{n}$, at the new locus.

Identified hybrid sterility gene loci and markers

A number of new hybrid sterility gene loci were identified (Table 2). A locus *S-7* was detected in hybrids between *aus* varieties (summer rice on the Indian subcontinent) and some javanicas (Yanagihara et al 1992). Locus *S-8* was detected in a hybrid between a Korean indica variety and some javanicas (Wan et al 1993). In hybrids between *aus* varieties and javanicas, *S-9* was detected (Wan et al 1996a). Locus *S-15* was found in hybrids between *aus* variety Dular (WCV) and IRRI line IR2061 (Wan et al 1996a). *S-16* was identified near *Est-1* on chromosome 1 in hybrids between Ketan Nangka and local varieties in the Tai-hu Lake region or Yunnan Province in China (Wan and Ikehashi 1995a). One more locus, *S-17(t),* was identified in crosses between Penuh Baru II and japonicas (Wan and Ikehashi 1995c). Isozyme analyses to find markers cosegregating with hybrid sterility gene loci were conducted according to Ishikawa et al (1989) and Glaszmann et al (1988). Table 3 lists the estimated allelic constitution for a set of tester varieties. Because the alleles at hybrid sterility gene loci have been characterized, these tester varieties can be used to identify corresponding alleles in other varieties.

Three alleles were initially identified at the *S-5* locus. Further analyses, however, showed that allelic differentiation at hybrid sterility gene loci consisted of a number of alleles at a single locus. Especially in Indian varieties, more than five alleles were identified at *S-7* using the set of testers. The diversity of alleles at hybrid sterility gene loci and in isozymes in Chinese and *aus* Indian rice varieties was studied. The highest diversity in terms of alleles at hybrid sterility gene loci (Wan and Ikehashi 1997) was found in the Indian rice. For example, Basmati 370 showed several loci—*S-8, S-7,* and *S-5*—for hybrid sterility (Table 4) (Wan and Ikehashi 1995b). The markers *Cat-1, Est-9,* and *Amp-3* are linked to *S-8, S-7,* and *S-5,* respectively (Table 2). In contrast, hybrid sterility in typical indica/japonica crosses was predominantly controlled by alleles at *S-5* (Table 1).

Table 2. Alleles at hybrid sterility gene loci and markers in tester varieties [a].

Tester varieties	Marker gene and chromosome										
	Chrom. 6			Chrom. 7		Chrom. 4		Chrom. 12			Chrom. 1
	Amp-3 (S-5)	Est-2 (S-5)	Cat-1 (S-8)	Pox-5 (S- 7)	Est-9 (S- 7)	Est-1 (S-9)	Mal-1 (S-9)	Acp-1 (S-15, S-17(t))	Pox-2 (S-15, S-17(t))	Sdh-1 (S-15, S-17(t))	Est-5 (S-1 6)[b]
IR36	1	2 (i)[c,d]	1	1 (n)[e]	2 (n)[e]	1	2 (n)[e]	1	1 (n)	1 (n)	1 (n)
Akihikari	1	0 (j)	2	2 (n)	1 (n)	0	1 (n)	2	0 (n)	2 (j)	1 (n)
K. Nangka	2	1 (n)	2	2 (kn)	1 (kn)	0	1 (kn)	2	1 (n)	2 (n)	1 (kn)
Dular	2	1 (n)	2	2 (n)	1 (n)	0	1 (n)	2	0 (du)	2 (n)	1 (n)
02428	2	1 (n)	2	2 (n)	1 (kn)	0	1 (kn)	2	0 (n)	2 (n)	1 (kn)
CY85-26	2	2 (n)	1	1 (n)	2 (kn)	1	2 (n)	1	1 (n)	1 (n)	1 (n)
Yeong Pung	1	2 (i)	1	1 (yp)	2 (ai)	1	2 (n)	1	1 (n)	1 (-)	1 (-)
N22	2	1 (n)	2	2 (n)	1 (ai)	0	1 (ai)	2	0 (n)	2 (n)	1 (-)
IR2061-628	1	2 (i)	1	1 (n)	2 (n)	1	2 (n)	1	1 (i)	1 (-)	1 (-)
Fengjingdao	1	0 (j)	2	2 (n)	1 (ai)	0	1 (n)	1	0 (n)	2 (n)	2 (j)
P. Baru II	1	1 (j)	2	2 (pb)	1 (kn)	1	1 (n)	2	0 (n)	2 (pb)	1 (-)
Panbira	2	1 (n)	1	1 (n)	2 ai)	1	2 (n)	1	1 (n)	1 (n)	1 (-)
DJ123	1	0 (j)	2	2 (n)	1 (ai)	0	1(i)	2	0 (n)	2 (n)	1 (n)

[a]Isozyme allele systems quoted from Morishima and Glaszmann (1991). [b]Seven hybrid sterility loci are shown under the marker loci. [c]Parentheses indicate allele at the hybrid sterility locus; (-) = no data. [d]i = indica, j =japonica, n = neutral, kn = Ketan Nangka. pb = Penuh Baru II, du = Dular, ai = Ingra, yp = Yeong Pung. [e]Not neutral to *aus* varieties.

Table 3. Estimated allelic constitution at hybrid sterility gene loci in tester varieties.

Varieties	*S-5*	*S-8*	*S-7*	*S-9*	*S-15*	*S-16*	*S-17(t)*
IR36	i[a]	n	n	n	n	n	n
Guichao 2	i	n	n	n	n	n	–
Akihikari	j	n	n	n	n	n	n
Miyukimochi	j	n	j	n	n	n	n
Ketan Nangka	n	kn	kn	kn	n	kn	n
Nekken 2	n	kn	kn	kn	n	kn	n
Dular	n	n	n	n	du	n	n
Pei Ai 64	n	n	n	n	n	n	n
CY 85-26	n	n	n	kn	n	n	n
Basmati 370	ba	ba	n	n	n	n	–
Ingra	i	n	i	n	n	n	n
Yeong Pung	i	yp	ai	n	n	n	–
N22	n	n	n	i	n	n	–
Jaya	i	n	i	i	n	–	–
IR2061-628	i	n	n	n	i	n	n
Fengjingdao	j	n	ai	n	n	j	n
Penuh Baru II	j	yp	n	n	n	n	j

ai = Indica, J = japonca, n = neutral, kn = Ketan Nangka, ba = Basmati.
yp = Yeong Pung, ai = Ingra, du = Dular.

Table 4. Distribution of spikelet fertility (SF) classified by marker genotype in basmati crosses.

Genotype	No. of plants with spikelet fertility										Overall mean (%)	T test
	10	20	30	40	50	60	70	80	90	100		
Basmati 370/IR36//IR36												
Cat-1^2*/Cat-1*^{1}	2	3	3	5	4	6	3	3	2	0	31**	52.3**
Cat-1^1*/Cat-1*^{1}	2[a]	1	2	1	1	2	1	17	18	16	61	75.8
SF was not differentiated at *Est-2, Est-9, Est-1,* and *Sdh-1*												
Basmati/IR2061-628//Basmati												
Cat-1^2*/Cat-1*^{2}	1	1	1	1	0	2	8	10	8	6	38**	76.3**
Cat-1^1*/Cat-1*^{2}	3	2	2	4	7	9	8	8	8	4	55	51.7
SF was not differentiated at *Est-2, Est-9, Est-1,* and *Sdh-1*												
Basmati/IR2061-418//Basmati												
Est-9^2*/Est-9*^{1}	2	2	3	3	6	3	14	8	5	4	50**	56.3**
Est-9^1*/Est-9*^{1}	1	1	1	2	1	1	4	10	10	6	37	74.3
SF was not differentiated at *Est-2, Cat-1, Est-1,* and *Shd-1*												
Basmati/Ketan Nangka//Akihikari												
Amp-3^2*/Amp-3*^{1}	0	1	0	0	3	2	14	11	7	5	43	76.5**
Amp-3^1*/Amp-3*^{1}	2	1	2	3	4	2	4	7	5	3	33	52.3

[a]Numbers underlined are assumed to be recombinants.
Source: Wan and Ikehashi 1995c.

Differentiation of hybrid sterility genes

A genetic analysis was made of mutant Miyukimochi from variety Toyonishiki (Toda 1982). The semisterility in hybrids between Toyonishiki and IR36 was caused only by an allelic interaction of $S\text{-}5^{i}/S\text{-}5^{j}$. But the semisterility in F_1 hybrids between Miyukimochi and IR36 was attributed to allelic interactions by both $S\text{-}5^{i}/S\text{-}5^{j}$ and $S\text{-}7^{i}/S\text{-}7^{j}$. Thus, the neutral allele $S\text{-}7^{n}$ in Toyonishiki was found to be mutated into $S\text{-}7^{j}$ following irradiation with ^{60}Co (Wan and Ikehashi 1996b).

Another case of mutational change of a hybrid sterility allele was found for an experimental line, 02428 from China, that possessed the $S\text{-}5^{n}$ allele (Zou et al 1992). The parents for 02428—Pangxiegu and Jibangdao—were shown to possess $S\text{-}5^{j}$. The allele $S\text{-}5^{n}$ in 02428 must have been induced from $S\text{-}5^{j}$ as a result of ^{60}Co irradiation of the parents (Wan and Ikehashi 1996b).

These two instances of mutations explain how new alleles are conserved. In the first case, mutant allele $S\text{-}7^{j}$ was induced in the background of neutral allele $S\text{-}7^{n}$ in Toyonishiki, and the heterozygote produced $S\text{-}7^{n}/S\text{-}7^{n}$ and $S\text{-}7^{j}/S\text{-}7^{j}$ without showing any sterility. Similarly, a number of new alleles might have been conserved in rice (Wan and Ikehashi 1996b). Interestingly, different series of new alleles may be fixed under different genetic backgrounds of neutral alleles. Because a varietal group contains a series of alleles, a high level of hybrid sterility understandably found in intergroup hybrids is not encountered in crosses among them.

Hybrid sterility in pollen and hybrid rice

Hybrid sterility is also known to be expressed in pollen. Because a large number of pollen genotypes are found in a single spikelet, genetic analysis for hybrid sterility in pollen was difficult. Recently, however, a locus for pollen sterility on chromosome 7 and another on chromosome 12 were identified (Wan and Ikehashi 1996c). At the *ga-11* locus on chromosome 7 (Table 5, Fig. 1), pollen carrying an indica-type allele was found to be aborted on the heterozygote. Thus, the same mechanism operates for both

Table 5. Distribution of pollen fertility classified by marker genotypes in KL10005/ /IR36/Dular.

Genotype	No. of plants with pollen fertillty[a] (%)							Total	Mean (%)
	<40	50	60	70	80	90	100		
rfs^{+}/rfs^{+}	6	7	9	3	2	8	14	49	70.3
rfs/rfs^{+}	5	7	12	1	1	9	13	48	71.6
$Est\text{-}9^{2}/Est\text{-}9^{1}$	8	11	18	2	0	4	7	50	56.8**
$Est\text{-}9^{1}/Est\text{-}9^{1}$	3	3	3	2	3	13	20	47	83.3
$Acp\text{-}4^{1}/Acp\text{-}4^{1}$	10	14	18	3	0	1	2	48	50.2**
$Acp\text{-}4^{1}/Acp\text{-}4^{2}$	1	0	3	1	3	16	25	49	89.3

[a] Numbers underlined are assumed to be recombinants or individuals showing recombination between the marker and sterility gene.
Source: Wan and Ikehashi 1996c.

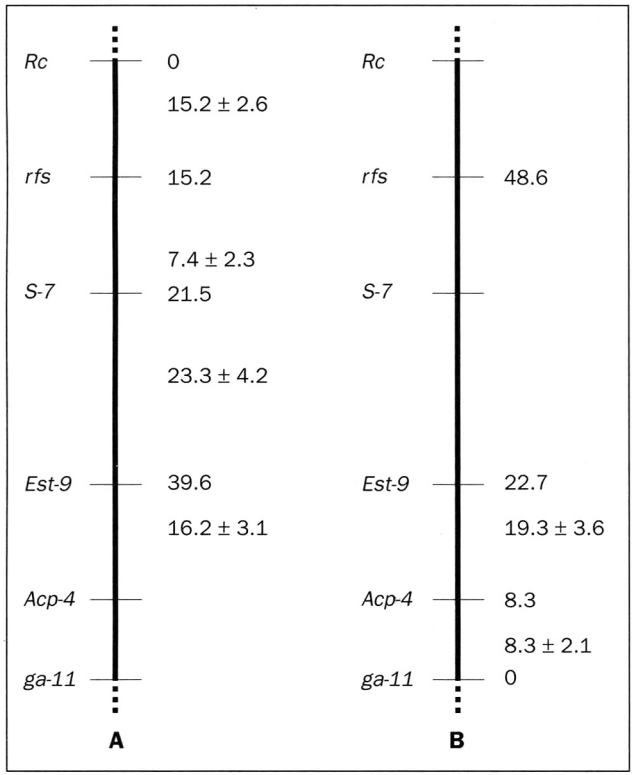

Fig. 1. Linkage map for the markers on chromosome 7. (Adapted from Wan and Ikehashi 1996c.)

male and female gametes. Evidence clearly indicates that hybrid sterility for pollen and hybrid sterility for spikelets are independently controlled, perhaps because of the nonconservation of a new gene responsible for both female and male abortion.

By using the WC gene, indica/japonica hybrids successfully attained a high level of heterosis. But pollen fertility was very low because of hybrid sterility expressed in their pollen. These hybrids also produced unstable yields in adverse conditions because of decreased pollen viability. Thus, the importance of the WC gene for pollen was recognized. One solution was to develop indica/javanica hybrids, as many javanica varieties showed a normal pollen fertility in their crosses with indicas and japonicas (Ikehashi and Araki 1987).

Strategy for overcoming hybrid sterility

Screening potential maintainers and restorers is a basic operation in hybrid rice breeding. Pollen and spikelet fertility is regularly examined in F_1s in this screening process after crossing CMS lines as female testers.

Selection of maintainers

In the search for maintainers, varieties showing a very low level of pollen fertility (<1–2%) are selected. Hybrid sterility may not be the cause of such a low level of pollen sterility. Spikelet sterility in initial test crosses can be eliminated by repeated backcrosses. Interference from hybrid sterility in the selection of maintainers is therefore negligible.

Selection of restorers

Test varieties or lines are pollinated to CMS lines. The F_1s are examined for pollen and spikelet fertility. F_1s showing a high level of pollen and spikelet fertility (80–90%) are selected as potential restorers. If the F_1 between a tester CMS line and the tested variety exhibits a clear hybrid spikelet sterility, such a variety is rejected from the potential pool of restorers. Thus, the status of hybrid sterility genes of CMS lines determines the range of restorers. Only indica lines possessed the *Rf* gene, whereas japonica CMS lines produced hybrid sterility with restorers. This problem was solved by incorporating the WC gene into the CMS line. With this knowledge, breeders in Japan started hybrid rice work.

Need for analysis of hybrid sterility gene loci of maintainers

The range of restorers is determined not only by *Rf* genes but also by hybrid sterility gene loci. Therefore, it is important to analyze these gene loci in key maintainers to choose restorers.

Although Basmati 370 is identified as a maintainer, it has interacting alleles at *S-8, S-7,* and *S-5.* CMS lines derived by using Basmati 370 as a maintainer would show hybrid sterility when pollinated by IR36 and a few other IRRI lines (Table 4).

Incorporation of WC genes into CMS lines

To generate a large pool of restorers for use, it is important to incorporate the WC gene into CMS lines. Several attempts have been made in hybrid rice breeding. An improved japonica line, TML 1, possessing $S\text{-}5^n$ from Suweon 258/Tainung67//Nekken 2 was developed in Japan with the cytoplasm of Chinsurah Boro II and restorer Habataki, a high-yielding indica line. The hybrid yielded more than the control (15% in 1993 and 48% in 1994). The yield was also 14% higher than the yield obtained with the high-yielding parent (Takita 1995).

Hybrid Liangyou Peite (Pei Ai64S/Teqing) was registered in 1994 by the Hunan Varieties Evaluation Committee for national release as the first two-line hybrid rice combination. It showed a high yield potential, good grain quality, and resistance to multiple diseases and insects. Liangyou Peite had about a 10% yield advantage over similar three-line hybrids. The female parent, Pei Ai 64s (Bai Delang and Luo Xiaohe 1996), is the first Chinese TGMS line with the WC gene.

Use of the WC gene in India

In East Asia, a single WC gene is more or less effective. If CMS lines are developed in the genetic background of javanicas and restorers are obtained by screening indica

lines, there will be a good level of heterosis. The data showed hybrid sterility in the cross of Basmati 370 and IR36. The hybrid sterility gene loci in Basmati 370 (Table 4) have already been analyzed. The narrow range of potential restorers under the genetic background of Basmati may be due to hybrid sterility gene loci. In view of the complexity of hybrid sterility genes on the Indian subcontinent, the search for WC genes for a number of hybrid sterility gene loci may not be easy.

On the other hand, if CMS lines are isolated from genetically diverse restorers, as indicated by hybrid sterility gene loci, heterosis can increase. Evidence of polymorphism at isozyme loci indicated that Basmati 370 was closer to japonicas. Therefore, if the genetic background of Basmati 370 is maintained in CMS lines, it will provide a base for enhanced heterosis.

Toward a systematic enhancement of heterosis

Because hybrid sterility genes are diverse in Indian rice, such as in Basmati 370, the range of potential restorers may be narrowed by hybrid sterility in their crosses with CMS lines. In a long-term project, it may be advisable to incorporate maintainers having WC genes or different hybrid sterility gene loci to generate a wide range of restorers (Fig. 2). It may be possible to enhance heterosis by testing the combining ability of potential maintainers and restorers. The role of hybrid sterility gene loci in such a system remains to be investigated.

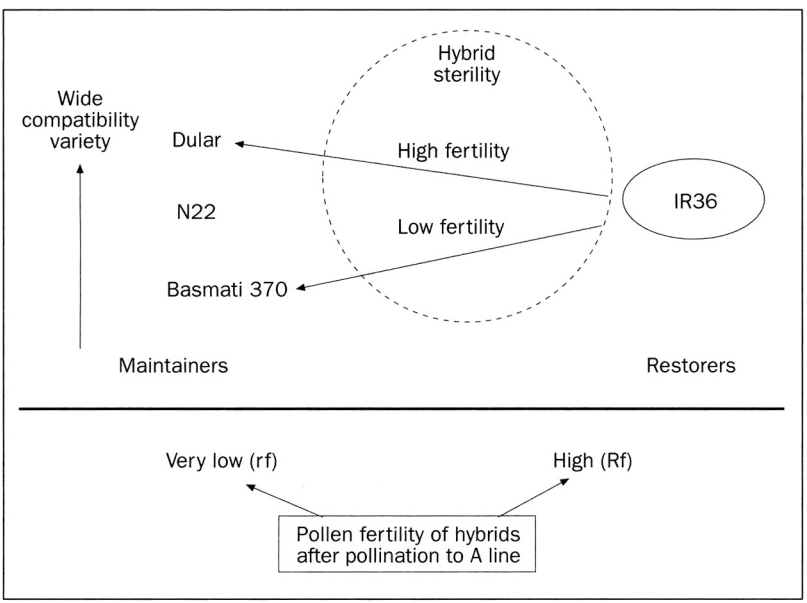

Fig. 2. Scheme to generate maintainers and restorers possessing wide compatibility for hybrid rice breeding.

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CHAPTER 8

Breeding and characterizing indica PGMS and TGMS lines in China

Mou Tongmin, Li Chunhai, Young Guocai, and Lu Xinggui

Hubei photoperiod-sensitive genic male sterile (PGMS) rice Nongken 58S, which was used as the donor of genic male sterile genes, was crossed, backcrossed, and multicrossed with various target indica varieties. Sterile plants were selected under different environments. In the past 15 years, eight thermosensitive genic male sterile (TGMS) lines and four PGMS lines were developed. The characteristic response of these lines to photoperiod and temperature was studied under various environments in the field and in phytotrons. The results showed that most of them had a completely stable sterile period of about 45 days at Wuhan. The temperature-sensitive stage ranged from the differentiating stage of the pollen mother cell to the early ripe stage of pollen. The critical temperature points (CTP) of fertility alteration varied in different TGMS lines: 26.5 °C (daily mean) for W6154S, W6184S, W6111S, W6417S, and W8103S; 25.5 °C for W9046S and W9056S; and 24 °C for W91607S. The fertility expression of PGMS lines was controlled simultaneously by photoperiod and temperature. PGMS line W7415S had a CTP of 26 °C and a critical photoperiod point (CPP) of 13.5 h. The CTP and CPP for W9451S and W9461S were 24 °C and 14.0 h, and for W9593S they were 24 °C and 13.0 h, respectively. A practical and effective procedure for breeding PGMS and TGMS lines has been established.

China cultivates about 33 million ha of rice. In about 90% of this area, indica rice is grown. Shi (1981) first reported discovering photoperiod-sensitive genic male sterile (PGMS) rice from a japonica variety—Nongken 58. We began introducing PGMS genes into indica rice varieties in 1982 to breed PGMS lines in order to develop two-line hybrids in indica rice. When several breeding lines developed in 1988 were subjected to different photoperiods and temperatures, we found that the fertility expression of indica PGMS lines was controlled not only by photoperiod but also by temperature (Fang and Lu 1990). In some cases, the traits of fertility alteration in indica

lines were controlled completely by temperature. These lines were called thermosensitive genic male sterile (TGMS) lines.

Most rice-growing areas around the world are distributed in the tropics and warm-temperate regions, where differences in photoperiod are marginal but differences in temperature over a year are striking. TGMS lines are more widely used geographically than PGMS lines. PGMS lines, particularly japonica PGMS lines, are very useful at higher latitudes (above 30°N) because of their more stable sterility and easier multiplication than TGMS lines. The critical temperature point (CTP) of fertility alteration is the most important index in both TGMS and PGMS lines (Yuan 1992). This chapter describes work done in China during the past 15 yr to breed and characterize PGMS and TGMS lines.

Breeding approaches for TGMS and PGMS lines

Cross breeding and anther culture are the main approaches for breeding TGMS and PGMS lines. Improvements took place mainly in techniques and test environments for selecting and identifying sterile plants. Before 1989, sterile plants from crossed offspring were generally selected in fields at lower altitude locations in the summer season. In environments where daylength was longer and temperature was higher, we could not differentiate the selected sterile plants into TGMS and PGMS lines because of the lack of special equipment to identify the critical temperature point and critical photoperiod point (CPP) of fertility alteration. Breeding lines usually had a higher CTP.

Sterile plants were selected starting in 1990 in various environments, which included lower temperature/longer daylength at higher altitude locations in the higher latitude region in summer; higher temperature/shorter daylength at lower altitude locations in the lower latitude region in summer or autumn; and lower temperature/shorter daylength at lower altitude locations in the lower latitude region in winter. Artificial climate rooms and phytotrons were also used for supplementary selection and identification. An effective procedure (Table 1) for breeding TGMS and PGMS lines has been established and practiced in recent years. A breeding cycle could be completed in 5 yr by applying this procedure. Different breeding lines according to breeding objectives could also be developed.

To breed various types of lines, many conventional indica varieties were used for breeding target parents. Cultivars Zhenshan 97, V20, and Xie-Qing-Zao have good combining ability and are the maintainers of CMS lines Zhenshan 97A, 20A, and Xie-Qing-Zao A, respectively.

Table 1. Selection procedure for TGMS and PGMS lines.

Step	Time (yr)	Environment	Method	Tissue culture approach
1	1st	Any	PGMS & TGMS donors x target parents	
2		Any	F_1 planted	
3	2nd	Long day and lower temperature	F_2 planted and selection of sterile plants (first selection in F_2)	
4		Short day and higher temperature	Ratooning selected sterile plants (F_2), distinguishing sterile and fertile plants (second selection in F_2)	Anther culture
5	3rd	Long day and lower temperature	F_3 planted in artificial climate room, selecting sterile plants (first selection in F_3)	H0 planting in field
6		Short day and higher temperature	Ratooning selected sterile plants (F_3), further distinguishing sterile and fertile plants	H1 planting
7	4th	Short day and lower temperature	F_4 planting and harvesting seed	H2 planting
8		Long day and higher temperature	F_5 planting in rows and identifying genetic stability for sterility expression	H3 planting and selection of sterile plants
9	5th	Short day and lower temperature	Multiplication (F_6)	H4 multiplication
10		Combination of different photoperiod and tempera-ture in phytotron	Formal identification (F_7)	H5 formal identification

Breeding TGMS and PGMS lines

Table 2 lists the TGMS and PGMS lines bred and their pedigrees. These cultivars have been used widely in China for three-line hybrid rice. TGMS line W6154S possessed the best combining ability among the inbred lines (Mou and Lu 1991). Lines with fewer than 80 d from sowing to 50% flowering are classified as early lines in the Yangtze valley and others (between 80 and 100 d) as medium lines. W6154S, W6184S, and W6111S were applied to two-line hybrid rice seed production in some areas from 1988 to 1992. They are not used in production now because of their higher CTP. W91607S, W9451S, W9461S, and W9593S, with a lower CTP, are being used to breed two-line hybrid combinations.

Characterizing TGMS and PGMS lines in fields

TGMS and PGMS lines were sown sequentially from late March to mid-July at Wuhan. Changes in pollen and spikelet fertility were investigated at heading. Figures 1-4

Table 2. Pedigree, flowering duration, and plant height in PGMS and TGMS lines.

Name	Pedigree[a]	HDG[b]	HT (cm)	Year developed
W6154S	NK58S/CS253-2-3-2//Zhenshan 97	69	66.8	1988
W6184S	NK58S/CS253-2-3-2//Zhenshan 97	64	68.6	1988
W6111S	NK58S/CS253-2-3-2//Zhenshan 97	66	64.7	1988
W7415S	V20/NK58S//Zhenshan 97/// Jing Nan-Tel 43	76	74.1	1988
W6417S	NK58S/IR2588	83	79.4	1988
W8013S	W6154S/Xie Qing Zao//Xie Qing Zao	67	65.4	1990
W9046S	NK58S/CS253-2-3-2//Zhenshan 97/// Xie Qing Zao////IR46830	77	73.7	1992
W9056S	NK58S/CS253-2-3-2//Zhenshan 97/// 3268	79	72.9	1992
W91607S	W6154S/CP-SL017	87	93.7	1994
W9451S	8902S/Yu Hong 231-8	76	88.2	1995
W9461S	8902S/Jw-15	82	78.4	1995
W9593S	8902S/W9056S//W7415S	88	83.9	1996

[a]NK58S = Nongken 58S, the orignal japonica PGMS rice, 8902S = a derivative from NK 58S bred at Wuhan University, CS253-2-3-2 = a conventional Indica breeding line from India. [b]HDG = number of days from seeding (mid-May to 50% flowering at Wuhan), HT = plant height.

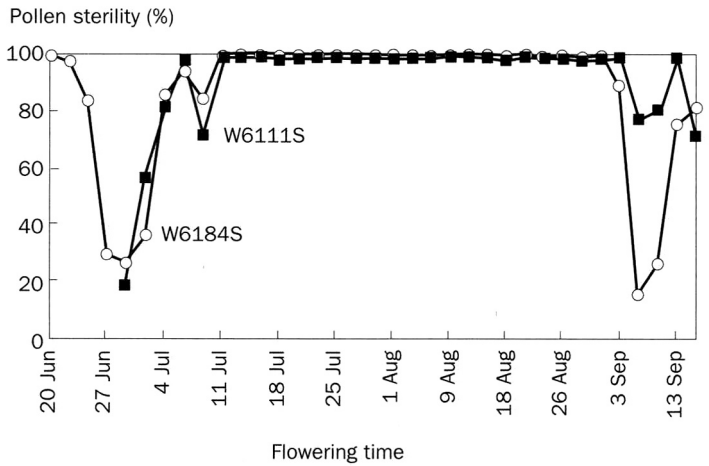

Fig. 1. Expression of W6184S and W6111S in field in 1988 at Wuhan.

show the variable expressions of fertility in seven lines grown in fields in different years at Wuhan. The curves in the figures indicate that, during the period from mid-July to early September, these lines usually remain sterile at Wuhan. During this period, sterility was stable in most lines. Sharp fluctuations in temperature caused a fluctuation in sterility in a few lines.

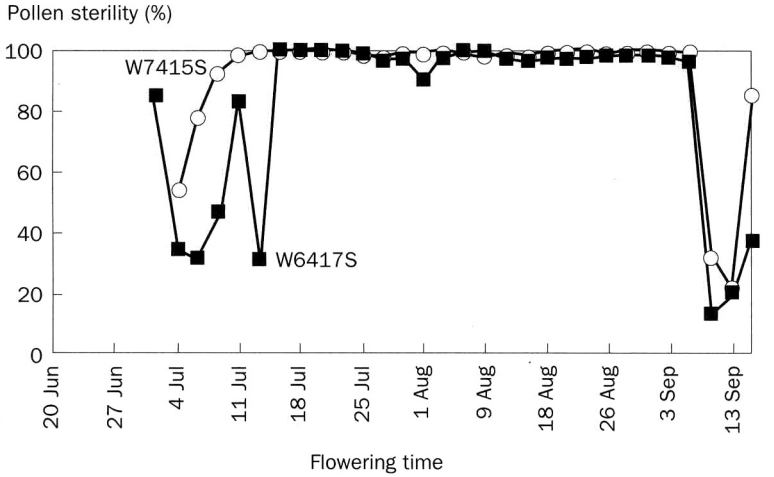

Fig. 2. Expression of W7415S and W6417S in field in 1988 at Wuhan.

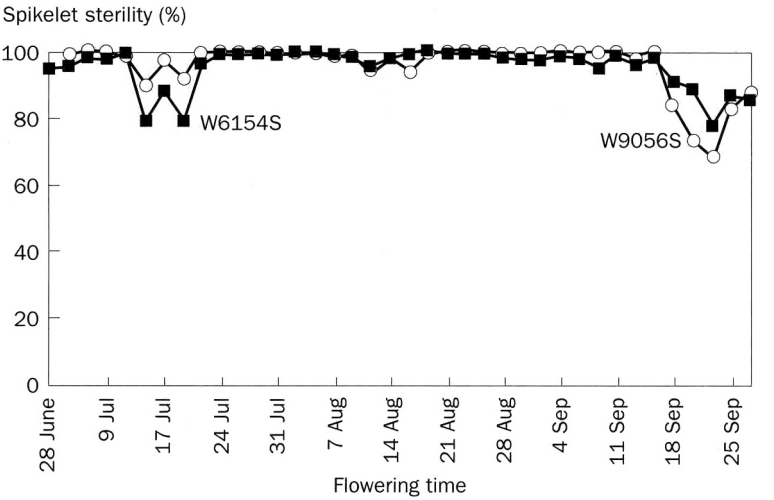

Fig. 3. Expression of W9056S and W6154S in field in 1991 at Wuhan.

Experiments with various photoperiod durations imposed artificially (Table 3) showed that lines have a different CPP requirement for sterility expression. The lines were seeded on May 9, transplanted on June 5 in the field, and from June 25 the rice was covered with a black cloth in the morning and evening until the heading of all lines on August 3. The CPP for W9451S, W9461S, 899S, and W91608S was about 14 h, and for W9593S it was 13 h. TGMS line W91607S-5 was almost insensitive to photoperiod, with a range of 12–14 h.

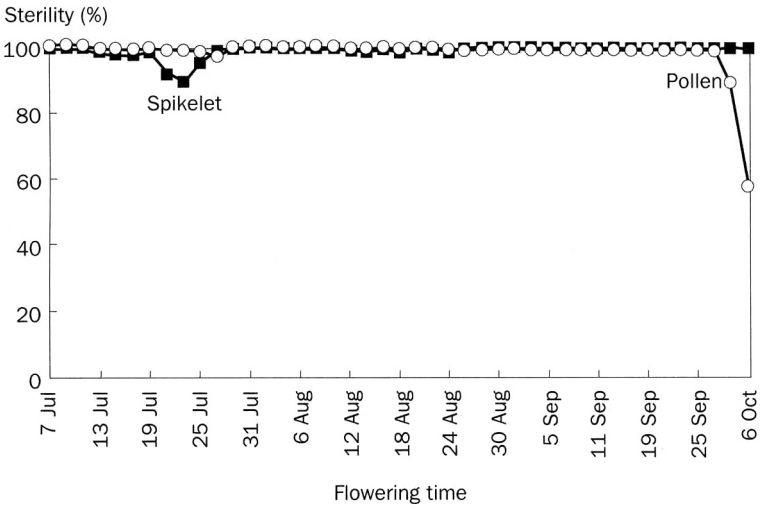

Fig. 4. Expression of pollen and spikelet sterility of W91607S in field in 1995 at Wuhan.

Table 3. Pollen (spikelet fertility) of PGMS and TGMS lines under different photoperiod treatments in the field (1996, Wuhan).

Line	Pollen (spikelet fertility) (%)			
	10 h	12 h	13 h	14 h
W9451S	65.4	24.3	12.7	1.4
	(16.1)	(22.2)	(2.3)	(0.5)
W9461S	59.9	33.8	23.0	2.2
	(36.0)	(57.2)	(21.9)	(0.7)
W9593S	57.4	48.2	2.7	0.9
	(27.2)	(7.1)	(0.0)	(0.0)
W91607S-5	13.0	1.0	1.1	4.1
	(10.3)	(3.1)	(2.2)	(1.1)
899S	88.7	90.7	89.7	1.0
	(52.2)	(61.5)	(41.8)	(0.0)
W96108S	55.7	35.9	47.0	0.5
	(23.1)	(23.9)	(17.8)	(0.5)

A correlation analysis was made in a time series between fertility and temperature from 15 d before heading to the heading date (Table 4). Sterility expression in TGMS lines W9046S and W9056S was significantly correlated to the daily mean, minimum, and maximum temperatures during the period from 14 d to 4 d before heading. This corresponded physiologically to the period of pollen mother cell differentiation and ripe pollen stages. The value of daily mean temperature is usually used

Table 4. Correlation between sterility and temperature over a time series (1991, Wuhan).

Days before heading	W9046S			W9056S		
	Tmean[a]	Tmin	Tmax	Tmean	Tmin	Tmax
15	0.2200	0.0543	0.2846	0.0855	0.0264	0.0293
14	0.3597*	0.2870	0.3964**	0.2026	0.2332	0.1810
13	0.3946*	0.1794	0.4563*	0.2209	0.1655	0.2204
12	0.4651**	0.3964*	0.4954**	0.3284*	0.2673	0.3795*
11	0.6243**	0.4649**	0.6630**	0.4660**	0.4098*	0.4973**
10	0.6131**	0.5550**	0.5732**	0.5206**	0.4140*	0.5396**
9	0.5436	0.5191	0.5321	0.6267	0.5533	0.6299
8	0.5347	0.5700	0.5105	0.5612	0.5031	0.5411
7	0.4788	0.4618	0.4665	0.5248	0.5413	0.4405
6	0.3316	0.4489	0.3283	0.5052	0.5587	0.4502
5	0.3189	0.2926	0.3096	0.4397	0.5440	0.3512
4	0.2650	0.2981	0.1981	0.4671	0.5866	0.3730
3	0.1981	0.2861	0.1009	0.3871	0.3931	0.2109
2	0.1113	0.1608	0.0642	0.2196	0.4413	0.1138
1	0.0079	0.0261	−0.0787	0.1047	0.3198	0.0203
0	0.0048	0.0149	−0.1101	0.1658	0.3956	0.0072

[a] Tmean, Tmin, and Tmax represent the daily mean, minimum, and maximum temperatures. respectively. *, ** Statistically significant at 5% and 1%, respectively.

as a CTP index of fertility alteration because it is a function of the minimum and maximum temperature.

Characterizing TGMS and PGMS lines in phytotrons

TGMS and PGMS lines were grown to the stage that is sensitive to photoperiod and temperature under field conditions. Then they were treated in a phytotron under different photoperiod and temperature combinations (Tables 5–7). W6154S and W7415S were completely sterile when the daily mean temperature surpassed 28 °C (Table 5). W7415S was sensitive to photoperiod in the range of 23–26 °C (Tables 5 and 7) and W6154S was weakly sensitive. W91607S, W9046S, and W8013S were almost insensitive to photoperiod in the range of 23.1–30.1 °C (Tables 6 and 7). W9451S was more sensitive to photoperiod than to temperature (Table 6).

The results of experiments in the field (Figs. 1–4, Table 3) and in the phytotron (Tables 5–7) indicated that when the male sterile genes from NK58S were introduced into the genetic background of different indica varieties, expressions of fertility alteration in the breeding lines differed. All male sterile lines were sensitive to higher temperature. In lower temperature conditions, some male sterile lines were insensitive or weakly sensitive, whereas others were sensitive to photoperiod. Based on their sensitive or insensitive (including weakly sensitive) response to photoperiod under lower temperature conditions, these male sterile lines were classified into thermo- and photoperiod-sensitive types (Table 8). TGMS line W91607S, with a CTP of 24 °C, can be used in a large geographic area. PGMS lines W9451S, W9461S, and

Table 5. Spikelet fertility (%) of W6154S and W7415S under different daylength and temperature regimes.

Line	Temp. (°C)	Daylength (h)						
		13.00	13.33	13.67	14.00	14.33	14.67	15.00
W6154S	22	19.4	16.9	17.9	21.5	21.8	23.0	19.5
	24	56.8	49.5	29.6	27.8	30.6	28.5	28.7
	26	43.2	24.8	15.4	20.1	17.9	12.8	16.4
	28	1.3	0.0	0.3	0.0	0.0	0.0	0.2
	30	0.0	0.0	0.0	0.0	0.0	0.1	0.0
	32	0.0	0.0	0.2	0.0	0.0	0.0	0.0
	34	0.0	0.0	0.0	0.0	0.0	0.1	0.0
W7415S	22	16.4	15.8	21.2	18.5	18.5	21.6	17.6
	24	43.3	38.6	25.6	23.7	25.8	23.8	24.7
	26	34.6	5.8	5.3	4.9	4.2	2.6	3.8
	28	0.8	0.0	0.0	0.0	0.0	0.0	0.0
	30	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	32	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	34	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Source: Zhang et al (1992).

Table 6. Spikelet fertility (%) of W91607S and W9451S under different temperature and daylength regimes in the phytotron.

Line	Temp. (°C)	Daylength (h)				
		15.0	14.0	12.5	12.0	10.0
W91607S	30.1	0.2	0.2	0.8		
	24.0	0.2	1.1	7.6		
	23.1	32.2	58.7	58.4		
W9451S	30.1	0.0	0.0	0.0	–	–
	28.0	0.0	–	–	12.5	13.7
	24.0	0.0	1.7	26.4	–	27.7
	23.1	2.6	5.0	16.0	–	–

Table 7. Spikelet fertility (%) of four lines bred at Wuhan under different daylength and temperature regimes in the phytotron.

Line	Temp. (°C)	Daylength (h) 15.0	14.8	14.0	13.3	12.5
W6154S	30.1	0.1	–	0.0	–	0.0
	29.6	–	0.3	0.3	1.3	–
	25.7	–	51.0	29.6	28.7	–
	24.1	58.5	–	53.7	–	47.6
	23.5	–	42.6	23.1	32.2	–
	23.1	67.8	–	19.9	–	53.1
W8013S	30.1	0.0		–	0.0	– 0.0
	29.6	–	0.0	0.0	0.0	–
	25.7	–	46.1	14.3	11.9	–
	24.1	31.4	–	47.8	–	33.4
	23.5	–	46.3	19.4	57.8	–
	23.1	38.7	–	32.3	–	11.8
W9046S	30.1	0.0	–	0.0	–	0.0
	29.6	–	0.0	0.0	0.0	–
	25.7	–	0.4	0.3	1.6	–
	24.1	23.0	–	29.0	–	29.7
	23.5	–	49.0	38.9	59.5	–
	23.1	34.5	–	27.2	–	30.2
W7415S	29.6	–	0.0	0.0	0.0	–
	25.7	–	1.0	11.8	29.7	–
	23.1	–	7.8	0.5	30.3	–

Table 8. Critical points of photoperiod and temperature of PGMS and TGMS lines bred at Wuhan.

Line	Type[a]	CTP[b] (°C)	CPP (h)
W6154S	T	26.5	
W6184S	T	26.5	
W6111S	T	26.5	
W6417S	T	26.5	
W8013S	T	26.5	
W9046S	T	25.5	
W9056S	T	25.5	
W91607S	T	24.0	
W7415S	P	26.0	13.5
W9451S	P	24.0	14.0
W9461S	P	24.0	14.0
W9593S	P	24.0	13.0

[a]T = thermosensitive, P = photoperiod-sensitive. [b]CTP = critical temperature point, CPP = critical photoperiod point of fertility alteration.

W9593S can be used in higher latitude areas and easily multiplied. W7415S may not be useful anywhere. Other TGMS lines can be used with care in special higher temperature regions.

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CHAPTER 9

Advances in two-line hybrid rice breeding

Lu Xinggui, S.S. Virmani, and Yang Rencui

The discovery of environment-sensitive genic male sterility in rice led to the development of a simpler and more efficient two-line hybrid breeding system compared with the cytoplasmic male sterility or three-line system. Several elite photoperiod-sensitive (PGMS) and thermosensitive genic male sterile (TGMS) lines have been developed in China. The commercial two-line hybrids developed using these lines occupied about 330,000 ha in 1996. Multiplying PGMS and TGMS lines in a pure form requires some special handling. These methods have been developed in China. Similar methods of seed production have been adopted for both two-line and three-line hybrids. The key point is to determine the time when a PGMS or TGMS line will show complete sterility for about 1 mo at a given location. Seed yield of two-line hybrids in China is 2.25–3.0 t ha^{-1}. Under tropical conditions, in which daylength differences are marginal, the TGMS system is considered to be more useful than the PGMS system. Genetic analysis at IRRI confirmed the monogenic recessive control of the TGMS trait. The TGMS gene of the IRRI mutant, IR32364 TGMS, was found to be nonallelic to the TGMS genes identified in China *(tms1)* and Japan *(tms2)*. IRRI has developed some TGMS lines in indica rice that possess the *tms2* gene; these lines are being evaluated in national agricultural systems. As expected, the TGMS system gave a higher frequency of heterotic hybrids than the CMS system. This chapter also discusses constraints and the future outlook for two-line hybrid rice breeding.

Two-line hybrid rice breeding in China

Breeding of PGMS and TGMS lines

The rice belt of China extends from south (17°30'N) to north (53°27'N) under five temperature zones: tropics, subtropics, warm-temperate, intermediate-temperate, and cool-temperate. The scope for using only photoperiod-sensitive genic male sterility

(PGMS) in China is limited. The original Hubei PGMS rice (HPGMR), Nongken 58S, for example, has no stable sterile period at Sanya City (17°30'N). Thermosensitive genic male sterility (TGMS) is as practical as PGMS in two-line hybrid rice breeding in China. PGMS lines derived from HPGMR are used in the Yangtze valley and in areas farther north, where daylength differences are more striking. TGMS lines derived from Annong S. and 5460S are mainly used in South China, where daylength differences are marginal. Chinese scientists have worked out a strict fertility index for commercial TGMS lines as follows:

- More than 1,000 identical plants should be tested, and the proportions of both sterile and fertile plants must be 100% during both the sterile and fertile periods.
- Pollen and spikelet sterility must be ³ 99.5% during the sterile period and seed set must be ³ 30% during the fertile period.
- The critical sterility-inducing temperature is 23.5–28.0 °C for TGMS lines.
- The critical daylength for fertility induction is shorter than 13 h for PGMS lines; however, long days interact with lower temperature and short days with higher temperature to have a complementary effect on fertility alteration.

In China, the methods used to breed PGMS and TGMS lines include hybridization followed by anther culture and somaclonal variation. To increase selection efficiency for breeding commercially usable sterile lines, early generations (F_2, F_3 are selected under long day/lower temperature and short day/higher temperature conditions. Breeding materials are planted in the summer at higher altitudes where the temperature (mean 24 °C) is low at the sensitive stage for fertility alteration. Then, sterile plants with desirable agronomic traits are selected at flowering. The selected sterile plants are transplanted under short day/higher temperature conditions at low altitude. These plants are ratooned and secondary selections are made. The sterile plants selected under long day/lower temperature conditions, when ratooned under short day/higher temperature conditions, usually show two types at heading: one type reverts back to fertility and the other remains sterile. The former is probably PGMS and the latter TGMS (Yuan 1992, Lu 1992).

Characterization of PGMS and TGMS lines for their critical fertility/sterility points for daylength or temperature regimes is done in a phytotron. Because of differences in latitude and daylength in China, the field characterization of such lines is done at Hangzhou and at Guangzhou simultaneously. Photoperiods are 11.5, 12.5, 13.5, and 14.5 h and mean day temperatures are 29, 28, 24, and 23 ºC. When testing materials reach the initial stage of stamen and pistil primordial differentiation, they are transferred into a phytotron and retained there until 3 d after the late stage of pollen mother cell meiotic division. Pollen and spikelet fertility are determined after heading.

Sterile lines for which fertility alterations are confirmed in the phytotron are grown in fields at different locations and sterile lines with ecological adaptability are identified. The fertility variation in tested sterile lines is investigated through their sequential sowing at Wuhan (30º30'N), Guiyang (26º35'N), and Sanya (17º30'N). After ecological adaptability is tested for 2 yr, the range of geographic adaptation for each sterile line can be defined. By 1995, more than 40 sterile lines had been devel-

Table 1. Some elite photoperiod- and thermosensitive lines developed in China.

Name	Sub-species	Type of reaction[a]	Origin of gene[b]	Place where developed[c]
N5088S	Japonica	PGMS	HPGMR	Hubei AAS
7001S	Japonica	PGMS	HPGMR	Anhui AAS
Peiai 64S	Indica	P(T)GMS	HPGMR	Hunan HRRC
GD2S	Indica	TGMS	HPGMR	Guangdong AAS
KS1S	Indica	TGMS	HPGMR	Guangxi AAS
6442S	Indica	TGMS	HPGMR	Jiangxi AAS
Shuguang 612S	Indica	TGMS	HPGMR	Sichuan Univ.
W91607S	Indica	TGMS	HPGMR	Hubei AAS
W9451S	Indica	PGMS	HPGMR	Hubei AAS
3418S	Indica	PGMS	HPGMR	Anhui AAS
1103S	Indica	PGMS	HPGMR	Wuhan Univ.
Anxiang S.	Indica	TGMS	Annong S.	Hunan HRRC
Xiang 125S	Indica	TGMS	Annong S.	Hunan HRRC
9201	Indica	TGMS	560S	Fujian Univ.
HS-1	Indica	PGMS	HPGMR	Fuijan Univ.

[a]PGMS = photoperiod-sensitive genic male sterility, TGMS = thermosensitive genic male sterility. [b]HPGMR = Hubei photoperiod genic male sterile rice. [c]AAS = Academy of Agricultural Sciences, HRRC = Hybrid Rice Research Center.

oped in China, and some of these lines (Table 1) were found to give several good combinations.

Breeding of two-line hybrids

Breeding wide compatibility (WC) lines is the key to successfully developing male parents of new two-line rice hybrids. The major WC donors are Ketan Nangka, CP-SLO 17,02428, Peidy, and Varylava with the gene *S-5^n*. In the past few years, several WC sterile lines, such as Peiai 64S, 3502S, 02428S, and XingguangS, have been developed. A number of WC restorers, such as Lunhui 422, Linlun, JW3044, BP98, MCP231, 9022, 418, 501, and 1001, have also been developed and used for breeding intersubspecific combinations. The evolution and differentiation of cultivated rice are reflected in the differentiation of the WC loci. It is now known that the WC genes of Aus 373 and Dular are nonallelic to *S-5^n*. Chinese breeders have developed several WC lines with non- *S-5^n*. Because *S-5^n* cannot completely overcome the problem of low seed set in indica-japonica hybrids, new WC genes that are nonallelic to *S-5^n* assume significance in breeding intersubspecific hybrids. Recombination between nonallelic WC genes is being used in commercial breeding programs.

To develop two-line rice hybrids, there are many types of PGMS and TGMS lines, which pose no restrictions for the restorer-maintainer relationship as in the case of CMS lines. It is therefore relatively easier to develop desirable new two-line rice hybrids than three-line rice hybrids. In early work in breeding two-line rice hybrids, Chinese breeders usually used the newest restorers, such as Minhui 63, Wanhui 9, and 6078, from the three-line breeding program, and the newest cultivars, such as Teqing,

Table 2. Some two-line hybrid rice combinations in commercial production and regional trials in China.

Combination	Cross	Subspecies	Place where developed[a]	Status
70 You 9	7001S/Wanhui 9	Japonica	Anhui AAS	Commercialized
E-Jing-Za No.1	N5088S/R187	Japonica	Hubei AAS	Commercialized
Hua-Jing-Za No.1	N5088S/1514	Japonica	Huazhong Agric. Univ.	Commercialized
Liang-You-Pei-Te	Peiai 64S/Teqing	Indica	Hunan HRRC	Commercialized
Pei-Za-Shan-Qing	Peiai 64S/ Shanqing 11	Indica	Hua Mao Company, Guangdong Province	Commercialized
Pei-Liang-You 288	Peiai 64S/288	Indica	Hunan Univ.	Commercialized
Pei-Za 77	Peiai 64S/77 Zhan	Indica	Guangdong AAS	Regional trial
Liang-You 681	Shuguang 612S/881	Indica	Sichuan Univ.	Regional trial

[a]AAS =Academy of Agricultural Sciences, HRRC = Hybrid Rice Research Center.

Shanqing 11, Te-san-ai, and 77 Zhan, from the conventional breeding program as two-line system restorers. A number of new two-line hybrid rice combinations with a yield increase of 5–10% over the three-line combinations (Table 2) have been developed and commercialized (Wang and Li 1992, Luo et al 1994, Wang et al 1995, Chen et al 1996).

Seed production for two-line hybrids

Seed production of two-line rice hybrids is not more difficult than that of three-line rice hybrids. The key is to determine the stable sterile period for a given sterile line at a certain location through sequential sowing. All plants of the sterile line must head only during the stable sterile period and complete flowering 15 d prior to the end of this period. At present, seed production in these two-line hybrids is about 3 t ha^{-1}, comparable to seed yields of three-line hybrids.

Multiplication of PGMS lines is also not difficult. The key is to determine the stable fertile period through sequential sowing at certain locations and make plants in PGMS lines head during that period. Fertility alteration of PGMS lines is mainly controlled by photoperiod and partially regulated by temperature. If photoperiod and temperature satisfy the requirements of PGMS, high yield can be obtained in seed multiplication. Seed multiplication yield of N5088S and 7001S, for example, has reached 4.5 t ha^{-1} with adequate management. The fertility of TGMS lines is completely controlled by temperature. For multiplication of these lines, the optimal temperature must be between the critical temperature of fertility alteration and the critical temperature of chilling injury. Indica TGMS lines generally require a temperature regime of 22–23.5 °C. Because it is difficult to meet this requirement under natural conditions, multiplication yields of TGMS lines fluctuate.

Chinese scientists (Lu 1992) found that fertility alteration of TGMS lines was most sensitive to the temperature on the field surface (0 cm). According to this finding, while TGMS lines develop into the secondary rachis-branch primordial differentiation stage, cool water from the deep layer of a reservoir irrigates the field continu-

ously. Water temperature at the entrance of the field is about 19–20 °C, and the exit water temperature is about 24 °C. The depth of the water is adequate to immerse the growing point of rice and the irrigation lasts about 12–15 d. This method successfully solved the problem of unsteady multiplication yield in TGMS lines. The first multiplication base for TGMS lines with a cool-water continual irrigation device has been established in Maomin City, Guangdong Province. The multiplication yield of TGMS line Peiai 64S reached 3.75–4.0 t ha^{-1} (Zhou and Liu 1993).

Seed production of PGMS and TGMS lines

Multiplication of PGMS and TGMS lines in a pure form also requires some special procedures. When PGMS and TGMS lines were reproduced for several generations without any selection, plants in a population would segregate for critical temperature point and the proportion of the plants that would require a higher critical temperature would increase. As a result, the average critical temperature value in a population would become higher and higher. Ultimately, this makes the line useless. This phenomenon occurred conspicuously in TGMS lines. For this reason, Yuan (1994) proposed a seed multiplication program for PGMS and TGMS lines based on nucleus seed multiplication. The general operational procedure is as follows:

- Take out about 100 plants with typical characteristics of the original line and plant them in pots.
- When the rice plants develop into the secondary rachis-branch primordial differentiation stage, transfer the pots into a glasshouse with a controlled temperature or phytotron until heading. Daylength is 12.5 h in South China and 13.5 h in the Yangtze valley. Daily temperature ranges from 19 to 27 °C and the average is 23 °C. These changes are simulated to depict natural conditions and are controlled by computers in the climate room or phytotron.
- When plants begin heading, pollen and spikelet fertility are investigated and plants with 100% sterility are selected.
- Selected plants are cut and ratooned in suitable short light/low temperature. The self-pollinated seeds are harvested. These seeds are called nucleus seeds.
- The nucleus seeds from each selected plant are planted in rows. Their agronomic traits and fertility are compared with those of the original lines, and the rows that are identical to the original lines are harvested. The harvested seeds are also called nucleus seeds.
- Nucleus seeds are used for multiplication and the multiplied seeds are called breeders' seeds. These breeders' seeds are used to produce foundation seeds of PGMS and TGMS lines.
- Foundation seeds are used directly for seed production for F_1 hybrids.

The establishment of a seed-reproducing program can prevent mixtures and ensure seed purity for PGMS and TGMS lines and hybrid seeds.

Two-line hybrid rice breeding in the tropics

For breeding two-line rice hybrids under tropical conditions where daylength differences are marginal, the TGMS system is considered more useful than the PGMS system (Virmani et al 1991). IRRI scientists introduced the TGMS mutant, Norin PL 12, developed by Maruyama et al (1991) from Japan, for evaluation in the tropics and its transfer into indica rice cultivars (IRRI 1992). Concurrently, gamma irradiation was also deployed successfully to induce a TGMS mutant in an indica rice cultivar, IR32364-20-1-3-2B (Virmani and Voc 1991, IRRI 1992, Voc 1993). Both TGMS mutants (Norin PL 12 and IR32364 TGMS) showed complete pollen sterility at maximudminimum temperature (30–32 °C/23–25 °C) and partial fertility at 24–27 °C/18–19 °C in IRRI trials. Subsequently, the critical stage for fertility alteration was found to be during 6–15 d after panicle initiation (PI) in Norin PL 12 and 6–10 d after PI in IR32364 TGMS (Borkakati 1994, Borkakati and Virmani, unpublished). On reversion, IR32364 TGMS showed much higher pollen fertility than spikelet fertility. Perhaps spikelet fertility in this mutant was also affected by some other factors besides pollen fertility.

Genetic studies at IRRI (Borkakati and Virmani 1993, 1996) indicated that the TGMS trait in Norin PL 12 and IR32364 TGMS was controlled by a single recessive gene. Allelic relationship studies indicated that the TGMS genes in the two mutants were different (Borkakati and Virmani 1996). Because the TGMS gene in line 5460s from China was designated as *tms1,* and the one in Norin PL 12 from Japan as *tms2* (Kinoshita 1992), the TGMS gene in the IR32364 TGMS mutant was tentatively designated as *fms3(t).* Its allelic relationship with the *tms1* gene present in TGMS mutant 5460S could not be studied due to nonavailability of the mutant, 5460S. Recently, the *tms3(t)* TGMS gene has been located on the short arm of chromosome 6 using molecular markers (Subudhi et al 1997). Because the *tms1* gene of 5460S TGMS is now known to be located on chromosome 8 (Wang et al 1995). we can conclude that *tms3(t)* of IR32364 TGMS is not allelic to *tms1.* Thus, *tms3(t)* can now be designated as *tms3.*

Transfer of the TGMS gene *tms2* from Norin PL 12 into indica rice at IRRI has also resulted in several indica TGMS lines (Table 3) that are being shared with collaborating national programs interested in developing two-line rice hybrids. Three of these TGMS lines, along with the IR32364 TGMS mutant developed at IRRI, were evaluated by monthly planting at Hyderabad, India. All four lines were sterile when they flowered in June and July (Table 4). In planting where flowering occurred from August until February, these lines turned fertile. Fertility reversion in IR32364 TGMS was much lower than in other TGMS lines. The temperature at Hyderabad during March to July is consistently higher (max. above 30 °C); that of August to October fluctuates depending on clouds and rainfall; and the November to February temperature remains consistently lower (max. lower than 28 °C). These results further confirm the TGMS nature of IRRI-bred indica TGMS lines. Their utility in breeding two-line hybrids will depend on their stability of sterility expression, extent of fertility reversion, and outcrossing rate under field conditions.

Table 3. Parentage and agronomic traits of some TGMS lines bred at IRRI.

TGMS line	Parentage	Agronomic traits		Spikelet fertillty (%) when seeded in		
		Days to 50% flowering	Plant height (cm)	Nov 1994[a]	Jan 1995[b]	Nov 1995[a]
IR68945-4-33-4-14	Norin PL 12/IR36	78	112	65.4	0	72.8
IR68949-11-5-31	Norin PL 12/BG90-2	96	108	67.2	0	81.7
IR71018-13-73-2	Norin PL 12/IR46830B	88	98	70.2	0	80.6
IR68294-1-18	Norin PL 12/IR62829B	83	125	80.7	0	67.8
IR682971-13-15	Norin PL 12/IR9761-61-IR	88	123	84.8	0	85.8
IR68948-4-14-1-4	Norin PL 12/IR47686-17-2	82	102	–[c]	0	77.3
Norin PL 12	Induced mutant of Reimei	68	98	38.5	0	91.3

[a]Maximum temperature from panicle initiation to heading = 28.0–28.8°C. [b]Maximum temperature from PI to heading=30.6–31.0°C. [c] No data.

Table 4. Sterility/fertility behavior of IRRI TGMS lines evaluated at Hyderabad, India, during 1994–95.

TGMS line	Sterility/fertility[a] behavior when flowered during												
	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	MSS[b]
				1994							1995		(%)
IR68945	S	S	S/F	F	F	F	S/F	F	F	S/F	S	S	85
IR68949	S	S	F	F	F	F	F	F	F	F	S	S	85
IR68294	S	S	S	F/S	F	F	F	F	F/S	S	S	S	65
IR32364	S	S	F/S	F	F	F/S	F	S	F	S	S	S	20

[a]S = sterile, F = fertile. [b]MSS = mean seed setting.
Source: DRR 1996.

When TGMS lines were evaluated at the IRRI farm over the years, it was observed that during the dry season, TGMS lines flowering in February-March (temperature during the thermosensitive stage 27.6–30.8/21.1–22.2 °C) in the field showed significant seed setting, whereas the same lines flowering in April-May (temperature during the thermosensitive stage 30.7–33.5/22.0–24.6 °C) showed complete pollen sterility. During the wet season, consistently high temperatures (>30 °C) in the field were recorded in September-October. Occasionally, in July-August, however, the maximum temperature dropped below 30 °C because of excessive rains and/or cloudy weather. Thus, by adjusting the time of planting at IRRI, it is possible to multiply seeds of TGMS lines and use these to produce two-line hybrids in both the dry and wet seasons. Breeding of TGMS lines and two-line rice hybrids would become even less cumbersome if such behavior could be confirmed in other tropical countries.

In test crosses made at IRRI, the frequency of heterotic rice hybrids derived from the TGMS system was higher than from the CMS system (Table 5). Some two-line rice hybrids yielding 1 t ha^{-1} higher than inbred check varieties were identified in

Table 5. Relative frequency of heterotic hybrids derived from cytoplasmic male sterility (CMS) and thermosensitive genic male sterility (TGMS) systems in a test-cross nursery, IRRI, 1993-94.

Male sterile system	Number of crosses		Number and frequency (%) of heterotic hybrids	
	1993	1994	1993	1994
CMS	103	106	17 (16)	64 (31)
TGMS	131	115	47 (36)	77 (67)

Source: Lopez and Virmani, unpublished.

preliminary yield trials at IRRI. Bulk quantities of seeds of these hybrids are now produced to enable multilocational evaluation in tropical rice-growing countries.

Two-line hybrid rice breeding in the tropics faces several constraints:

- Limited TGMS germplasm available for use.
- Insufficient training and experience of researchers in breeding and using TGMS lines.
- Lack of knowledge of target seasons or locations suitable for expression of complete sterility (for hybrid seed production) and fertility (for TGMS line multiplication).
- Lack of knowledge of stability of TGMS lines under field conditions.

Most of these constraints are temporary and can be overcome by intensifying research efforts at IRRI and at collaborating NARS. But if TGMS lines under field conditions do not express complete sterility, then the usefulness of this breeding approach would have to be reassessed.

Future outlook

The two-line hybrid rice breeding technique in China has evolved successfully through studies over the past 10 years. The main achievements have been in:

- Developing a number of practical PGMS and TGMS lines.
- Developing a number of two-line hybrid combinations with a 5–10% yield increase over three-line hybrid combinations.
- Establishing preliminary seed multiplication and production programs and the technique of preventing mixtures and preserving purity for two-line hybrid rice. The efficiency of seed production for two-line hybrid rice has slightly exceeded that of three-line hybrid rice.

Now, however, two-line hybrid combinations developed and commercialized in China are limited in number and mainly intervarietal. They cannot meet the needs of diverse ecological conditions and various cropping systems in large geographic areas because of their lack of genetic diversity. We are focusing our attention on developing intersubspecific two-line hybrids that are needed to increase yield by 15% over that of

three-line rice hybrids with the same growth duration. Research on the following subjects will be conducted in the next five years.

- Developing early intervarietal two-line hybrids suitable for cultivation as the first crop in the double-cropped Yangtze valley. The breeding target is 110–120 d maturity duration and a 10% yield increase over that of the leading conventional varieties.
- Developing intersubspecific two-line rice hybrids for cultivation as a medium- or late-season crop in the Yangtze valley.
- Developing intersubspecific two-line rice hybrids for early and late-season crops in southern China.
- Developing intersubspecific two-line rice hybrids for the single-cropped area in northern China.

The government agenda at all levels in China now involves developing and spreading two-line rice hybrids. By the end of the 20th century, about 2 million ha in China are expected to be planted to two-line hybrids.

Deployment of the two-line method of hybrid breeding involving the TGMS system has shown interesting prospects for increasing the efficiency of hybrid rice breeding in the tropics. This method would have a decided advantage over the three-line method for breeding indica/tropical japonica and basmati rice hybrids because of the lack or inadequacy of restorers (of CMS systems) among tropical japonica and basmati inbred lines. Work has begun at IRRI to transfer TGMS genes into indica, tropical japonica, and basmati rice cultivars for this purpose. Anther culture is deployed in such crosses to expedite the breeding process. Tagging of TGMS genes with molecular markers (Wang et al 1995, Subudhi et al 1997) should be helpful in using marker-aided selection to increase the efficiency of breeding TGMS lines.

A major concern in the use of the two-line method of hybrid rice breeding is to see whether the TGMS lines show stable expression of complete sterility under field conditions. It is therefore important to develop TGMS lines possessing a diverse genetic background and test these in different environments for their stability. Different countries must identify locations and seasons under which sterility expression of TGMS lines is complete and fertility transformation is maximum. TGMS lines that require a somewhat lower maximum temperature (about 28 °C) for expression of complete sterility would be more widely adapted. The fertility-altering temperature regimes for these lines should be assessed frequently in tropical rice-growing countries. The outcrossing potential of TGMS lines should be comparable to that of the CMS lines.

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Notes

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CHAPTER 10

Improving parental lines to increase efficiency of hybrid rice breeding: some new approaches

Xiao C. Liu, S.S. Virmani, and B.C. Viraktamath

Because of the potential of hybrid rice to increase rice production and productivity, many countries are currently working to exploit the benefits of this technology. The major problems in hybrid rice breeding are the limited number of parental lines with specific desirable traits, a lower frequency of maintainers and restorers among elite breeding lines, the narrow genetic base, a lack of resistance to biotic stresses, and poor grain quality of some parental lines. Therefore, improving parental lines must be an integral part of hybrid rice breeding to develop heterotic hybrids and improve breeding efficiency. The frequency of maintainers and restorers has been significantly increased after initiating specific maintainer and restorer breeding programs. Diverse parental lines with better grain quality and multiple resistance have been developed in China and at IRRI. Random mating of composite populations of maintainers and restorers developed at IRRI has helped to widen the genetic base of parental lines. Transferring restorer genes into tropical japonicas and incorporating wide compatibility genes into promising elite lines are considered essential to developing indica/japonica hybrids. Thermosensitive genic male sterile lines are being generated in indica, tropical japonica, and basmati genetic backgrounds. Anther culture and marker-aided selection can be deployed to expedite the parental line improvement program. This chapter discusses the limitations in current parental lines, progress made in improving them, and strategies envisaged to increase the efficiency of hybrid rice breeding.

Food shortages may pose a threat worldwide as a result of rapid population growth. Therefore, increasing rice production becomes more important. Using hybrid rice breeding, China achieved a marked increase in rice production and productivity. This spectacular success encouraged the International Rice Research Institute (IRRI) and several national agricultural research systems (NARS) to invest in research and devel-

opment of this technology (Virmani and Edwards 1983, Virmani 1996). A high level of standard heterosis is one of the prerequisites for the economic viability of rice hybrids. Yield advantage, resistance to biotic and abiotic stresses, adaptability, grain quality, and other traits in rice hybrids are largely determined by the type of parental lines used. Strategies and approaches for breeding hybrids vary depending on yield levels, pest prevalence, and consumer needs of the target environments. For example, commercial rice hybrids introduced from China during the late 1970s were found to be unadapted to the tropics. These hybrids were unacceptable to farmers on account of their poor grain quality and susceptibility to major diseases and insects. Such susceptibility was inherited from their female parents—V20A, Zhenshan 97A, and V41 A (Virmani et al 1982, Virmani and Edwards 1983). Improving parental lines by incorporating resistance and adaptability to the tropics is therefore crucial to hybrid breeding. This chapter presents some new approaches for improving parental lines to increase breeding efficiency of hybrid rice.

Limitations in parental lines available in China

Narrow genetic base

Restorer lines used in China are derived from a very limited number of elite donors, such as Minhui 63, Ce64-7, and several IR lines (Table 1). Although they possess a similar genetic base (Wang et al 1995), these lines have a good general combining ability. It is difficult, however, to obtain a higher specific combining ability, which is a major contributor to heterosis. The male sterile lines used in production have similar serious limitations. The genetic similarity among male sterile lines is relatively higher than among restorer lines (Fig. 1). Though a large number of hybrids are made annually, effective yield heterosis is not achieved in them.

Table 1. Restorer lines and their donors developed in China during 1991–96.

Restorer line	Donor
402	Zhi 3-6/Ce 64-7 (IR9761-19-1-64)
438	75P12/Ce 64-7
Gang No. 9	Huaai 17/1R24
102	Zheyeqing 8//IR36/IR24
Wan 3	Minhui 63 / Erliuzhezao
Minhui 72	C Bao/N//Minhul 63
Minhui 77	Minhui 63/Ce 64-7
Minhui 78	Minhui 63/1R26
501	Minhui 63//Taiyin No. 1/IR26
92	IR209/Ce 64-7
96	Ce 64-7/518
432	Ce 64-7/1R56
Duoxi No. 1	Minhui 63/TTP//Mlnhui 63/// Minhul 63
5111	IR209/IR36

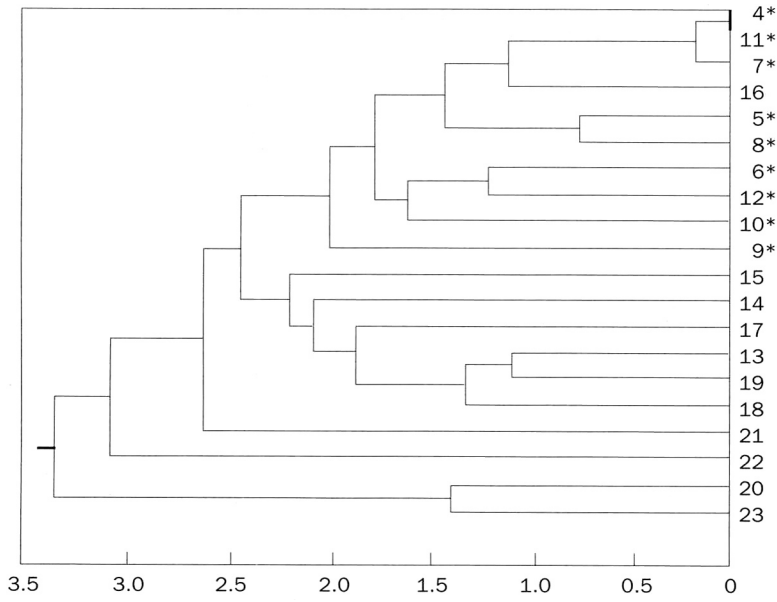

Fig. 1. Phenogram showing the genetic diversity and relationship among parental lines in hybrid rice. The * indicates male sterile lines, and the rest are restorer lines.

Indica/indica hybrids have excellent adaptability and yield stability and acceptable quality (Wang and Xu 1996). But the genetic distance between male and female parents is relatively small. As a result, the heterosis level of their hybrids is not as good as that of indica/japonica hybrids. Although indica/japonica hybrids show remarkable biological heterosis, problems such as semisterility, poor adaptability, long growth duration, and poor grain filling limit their expression.

Male sterile cytoplasm resources

With the introduction of hybrid rice in 1974, WA (wild abortive) male sterile cytoplasm has been used every year in more than 93% of the total area under hybrid rice in China. Several reports (Xing 1990, Shi et al 1996, Liu et al, unpublished) indicate a negative effect on yield heterosis following the deployment of this male sterile cytoplasm. Hybrid rice production faces a potential threat from pests and diseases. The homogeneous genetic base for hybrids makes them genetically vulnerable. Both PGMS- and TGMS-derived hybrids may be able to avoid this risk, but their fertility-sterility behavior is unstable, which may limit their use in hybrid rice production.

Grain quality of hybrid rice

In developing hybrid rice, grain quality is a major concern. The ultimate economic product of hybrid rice is a bulk of segregated endosperm of the F_2 generation and this makes parental breeding complicated. An improvement in rice grain quality has been

Table 2. Level of disease and insect resistance[a] of elite hybrids used in China.

Hybrid	Blast	Bacterial blight	Sheath blight	Brown plant-hopper	White-backed plant-hopper
Weiyou 64	MR	MR		MR	MR
Shanyou 63	MR-R	MR	MR		MR
Dyou 63	MR	MR	MR		
Ganyou 63	R	MR	MR		
Xieyou 56	R	R	R		
Weiyou 35	MR			MS	MR
Dyou 10	R	MR	MR		

[a]R = resistant, MR = moderately resistant, MS = moderately susceptible.

achieved in some recently released hybrids. The male sterile lines currently used in China have a higher amylose content than the restorer lines.

Pest and disease resistance

Blast, bacterial blight, and planthopper are the major pests that affect both hybrid and conventional varieties. Table 2 describes the level of resistance to major diseases and insects in some elite rice hybrids cultivated in China. For indica/japonica hybrids, the emphasis has been on yield improvement and growth duration, but not on incorporating resistance. As a result, these hybrids may be more prone to losses from pests and diseases than indica/indica hybrids.

Limitations in available parental lines in other countries

To develop rice hybrids in countries outside China, the major constraint is the limited number of currently available parental lines and their narrow genetic base. The number of CMS lines in indica rice is limited, mainly because of a low frequency of maintainer lines among elite rice cultivars developed for tropical conditions. For the widely used WA-CMS system, maintainer frequency was 0–5% in elite rice cultivars originating from IRRI and NARS located in the tropics (Virmani 1994). But the lines developed in India showed a relatively higher maintainer frequency (0–38%) (DRR 1995, 1996). They included lines developed for both the tropics and subtropical and temperate regions. Similarly, restorer frequency is also quite low among elite lines of basmati grain type and American long-grain types, which are known to be derived from bulu (tropical japonica) rice varieties. Although the frequency of occurrence of restorers among elite indica rice originating from the tropics is much higher (20–30%), hybrid rice breeders waste considerable time and energy in identifying good restorers.

To breed improved heterotic rice hybrids, we need to adopt new strategies to enhance the frequency of occurrence of maintainers and restorers, and ensure a con-

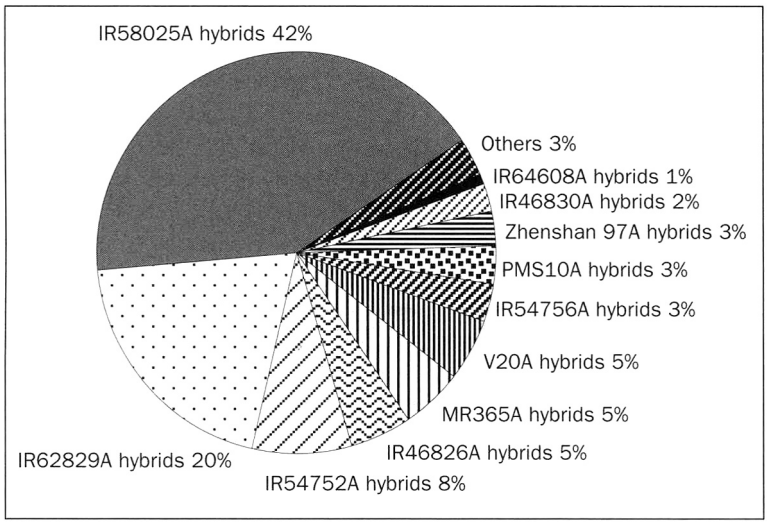

Fig. 2. Relative frequency of different cytoplasmic male sterile lines used as female parents in the IRRI-bred hybrids evaluated in replicated advanced yield trials conducted at IRRI during 1986-95.

stant supply of genetically diverse parental lines. Despite the availability of diverse CMS sources, most of the commercial rice hybrids released in India, Vietnam, and the Philippines (Virmani 1996) are based on WA-CMS cytoplasm as in China (Zhou 1994). Only two WA-CMS lines, IR58025A and IR62829A, developed at IRRI, are the most commonly used parents in IRRI-bred rice hybrids, evaluated in replicated advanced yield trials conducted during 1986-95 (Fig. 2). To achieve the desired productivity and sustainability in rice hybrids, such an overdependence on two CMS lines is undesirable. Hybrid rice programs must therefore broaden the cytoplasmic and nuclear diversity of parental lines.

To develop heterotic rice hybrids possessing the required resistance to diseases and insect pests with acceptable grain quality and economic seed yields, the selected parental lines must possess specific traits—resistance to specific diseases and insects. desired grain quality, and a high outcrossing rate. In indica/japonica rice hybrids, it is necessary to incorporate the wide compatibility gene into one of the parental lines. Similarly, restorer genes must be incorporated into japonica lines to use them as pollen parents in developing three-line indica/japonica hybrids.

Progress made in improving parental lines

In the past five years, considerable improvement in grain quality, pest resistance, and growth duration has been achieved in rice hybrids in China although their yield level has remained static. In 48 three-line indica hybrids released after the 2nd International Hybrid Rice Symposium, the average increase in yield was approximately 2.5%. The hybrids V you wan 3 and V you 77 increased yields markedly, by 7.3% and 6.6%,

respectively, compared with other elite hybrids. Although these hybrids have not attained the most desirable grain quality, they at least have acceptable grain quality.

Current restorer lines in China were mainly improved by R × R crosses and a pedigree selection strategy; maintainer lines were improved by B × B crosses. Some breeders have used (A × R) × elite cultivar crosses to improve isoplasmic restorer lines. This approach proved more efficient in improving restorer lines because it is easy to identify restorers and maintainers in the progeny. A number of donors for resistance to major diseases and insects have been found and used to improve parental lines and hybrids. Improved resistance in the parental lines was obtained through recurrent selection methods. At the China National Rice Research Institute, the anther culture technique was used successfully to transfer WC genes into the female lines, such as male sterile line 064A. A novel approach uses molecular marker-aided selection to incorporate heterotic genes into parental lines and to make heterotic gene combinations in the available parental lines.

At IRRI and in India, attention was given to increasing the number of available maintainer lines suitable to the tropics. The frequency of the occurrence of maintainer lines was found to be much higher among the elite lines derived from B × B crosses than in selected lines from the IRRI inbred rice breeding program (Fig. 3). Later, a similar strategy was deployed to breed restorer lines from R × R and A × R crosses. To expedite the breeding process, anther culture was also used in selected A × R crosses. Some elite isoplasmic restorer lines were also bred (Table 3). At the Directorate of Rice Research, restorer improvement using A × R crosses has shown promise (Table 4). To recombine desirable traits and pyramid restorer genes, crosses were made be-

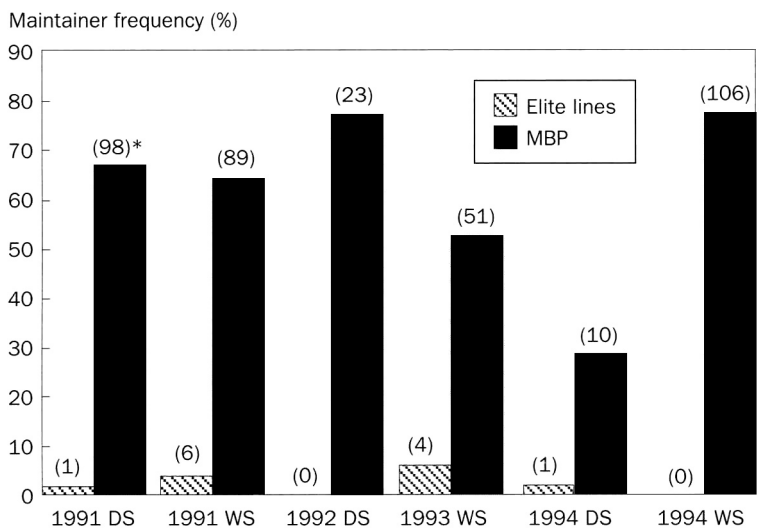

Fig. 3. Maintainer frequency among IRRI elite lines and lines developed from the maintainer breeding program (MBP). Numbers in parentheses denote maintainers identified (no.). DS = dry season, WS = wet season.

Table 3. List of restorers developed using anther culture of F_1 hybrids and promising hybrids involving some restorers as male parents, IRRI.

Restorer	Promising hybrids in PYT[a] and OYT
IR64615H-AC-1-4	IR68897A/IR67265H-AC-18-5*
IR64615H-AC-7-1	IR68885A/IR65489H-AC-2-2*
IR65489H-AC-4-1	IR68897A/IR65489H-AC-2-1
IR65489H-AC-2-1	IR68897A/IR65489H-AC-2-3
IR65489H-AC-2-2	IR68897A/IR65489H-AC-2-4
IR65489H-AC-2-3	IR68897A/IR65489H-AC-2-5
IR65489H-AC-2-4	IR68897A/IR65489H-AC-2-9
IR65489H-AC-2-5	IR68897A/IR64615H-AC-7-1
IR67265H-AC-10-1	IR68886A/IR65489H-AC-2-2
IR67265H-AC-10-2	IR62829A/IR65489H-AC-2-5
IR67265H-AC-10-5	IR58025A/IR67265H-AC-10-1
IR67265H-AC-10-6	IR68897A/IR67265H-AC-10-2
IR67265H-AC-10-7	IR58025A/IR67265H-AC-10-6
IR67265H-AC-10-8	IR68902A/IR67265H-AC-10-10
IR67265H-AC-10-9	IR58025A/IR67265H-AC-18-5
IR67265H-AC-10-10	IR68099A/IR65489H-AC-2-2
IR67265H-AC-10-13	IR68897A/IR65489H-AC-2-4
IR67265H-AC-12-1	IR68899A/IR65489H-AC-2-5
IR67265H-AC-17-3	IR68897A/IR67265H-AC-10-10
IR67265H-AC-18-5	IR58025A/IR67254-AC-10-13

[a]PYT = preliminary yield trial, OYT = observational yield trial.

Table 4. Details of isocytoplasmic restorer lines developed in India.

Cross	Fertile lines selected (no.)	Current stage	Spikelet fertility (%)
IR58025A/IR54742	15	F_5	76.8–89.2
IR62829A/IR40750	50	F_6	80.6–91.4
IR58025A/IR21567	25	F_6	82.4–90.6
IR58025A/IR29723	41	F_5	81.9–91.8
IR58025A/9303	40	F_5	79.8–91.7
IR62829A/IR34686	31	F_4	80.9–91.7
IR62829A/WGL 3962	25	F_4	79.9–89.6
IR62829A/IR29723	26	F_5	80.4–90.1
Total	253		

Source: DRR 1996.

tween different restorers. More than 200 F_5 lines have been identified from seven R × R crosses (Table 5).

Pedigree selection is used at IRRl and in India to transfer wide compatibility genes to promising maintainer and restorer lines. It uses the purple apiculus marker reported to be linked with the WC (*S-5*^{n}) allele (Ikehashi and Araki 1986). Three CMS lines (Table 6) possessing the WC gene have been developed at IRRI. Twenty-seven

Table 5. List of R × R crosses made in India to develop restorer lines possessing desired characteristics.

Cross	Selected lines in F_6 (no.)	Desired target characteristics to be combined in the R lines
IR9761/ARC 11353	25	Grain quality and good combining ability
IR29723/1R9761	29	Yield and blast resistance
IR29723/WGL 3962	15	Yield and resistance to gall midge
IR29723/1R10198	44	Yield and improved restoration ability
IR29723/ARC 11353	46	Yield and good combining ability
IR29723/IR BB7	31	Yield and resistance to bacterial blight
IR10198/ARC 11353	26	Good restoration and long duration
Total	216	

Source: DRR 1996.

Table 6. CMS lines developed at IRRI possessing the WC gene.

Designation	Parentage
IR67701A	IR46830A///6*BPI 76/Moroberekan//Taichung 65
IR68277A	IR46830A///7*BPI 76/Moroberekan//Taichung 65
IR68884A	IR58025A///8*BPI 75/Palawan//Taichung 65

Table 7. Number of restorer lines selected in India from crosses made between restorer and wide compatibility (WC) donor lines, DRR, Hyderabad, 1995.

Recipient restorer	WC donor	Selected lines (no.)
Swarna	9310	8
IR40750-87-2-2-3R	9310	8
Swarna	9312	3
IR31802-48-2-2-2	9313	1
IR34686-179-1-2-1	9312	4
IR46	9314	3

F_6 lines derived from R × WC donor crosses have been selected in India to transfer WC genes into restorers (Table 7).

Two random-mating composite populations of restorers-IR69701 CP 138 and IR69702 CP 139—have been developed at IRRI using the male sterility-facilitated recurrent selection procedure (Fig. 4). lR69701 CP 138 was derived from nine early maturing (<120 d) elite restorer lines and genic male sterile IR36 (ms). IR69702 CP 139 was derived from 14 medium-maturing (120–140 d) elite restorer lines and genic male sterile IR29723-143-3-2-1 (ms). The male sterile lines used were in the genetic background of restorers. These populations are now used to extract genetically diverse improved restorer lines for WA-CMS cytoplasm. Seeds of these composite populations have also been shared with India. This will help Indian breeders to extract

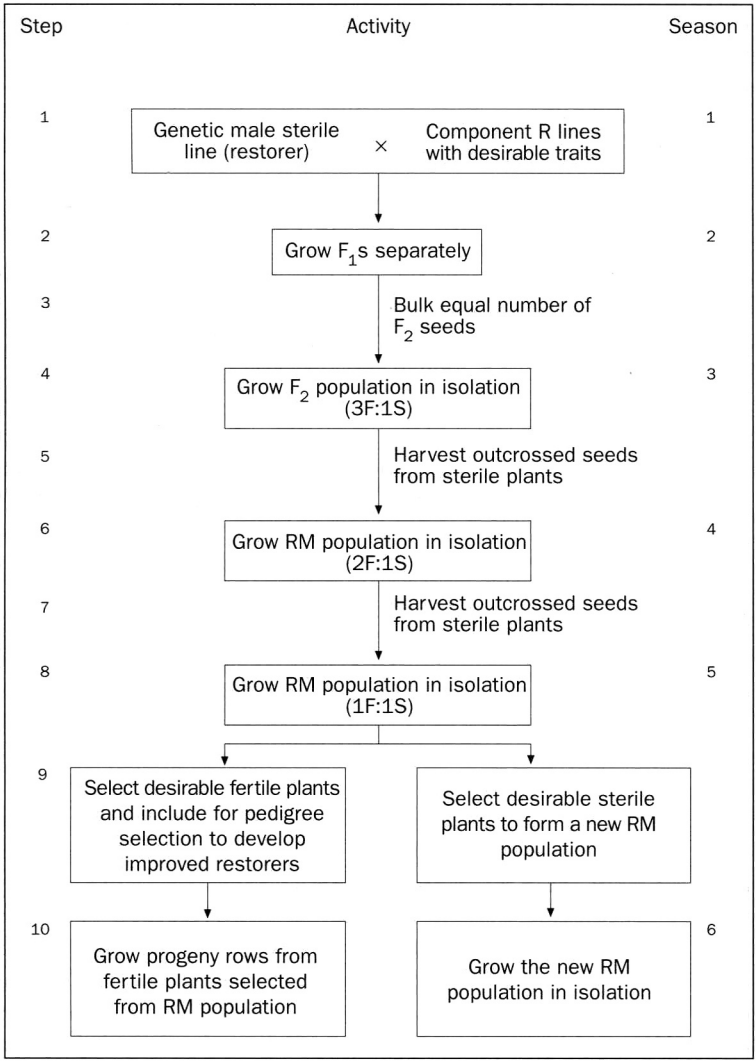

Fig. 4. Procedure used at IRRI to develop random-mating (RM) composite populations for improvement of restorer lines. F = fertile, S = sterile.

genetically diverse, locally adapted R lines for their hybrid rice program. The elite R lines selected from these populations at IRRI and NARS can be processed by repeating this procedure to develop new random-mating composite populations for further improvement of restorer lines.

A similar procedure was also used at IRRI to develop two random-mating composite populations of maintainers (Fig. 4). Instead of using a nuclear male sterile stock in a restorer background, however, we used a nuclear male sterile stock in a

maintainer background. This stock, designated as IR70413 (ms), was bred at IRRI in a maintainer background developed from the cross IR36 (ms) × IR58025B by following the procedure published by Bharaj and Virmani (1997).

Future strategies for parental improvement

Conventional rice breeding programs can provide parental lines for hybrid breeding. But if we rely solely on this important source, it may not be adequate to ensure a continuous supply. To be efficient and effective, parental line improvement should be made an integral part of a hybrid rice program itself. This is ensured in the strategies adopted in China and India and at IRRI to develop good parental lines.

Diversifying CMS sources and widening the genetic base of both CMS and restorer lines hold the key to achieving sustainability in hybrid rice technology. New heterotic genetic resources and heterotic groups have to be identified in different regions. Parental lines must be continuously improved for disease and insect resistance to develop hybrids with a stable performance. These parental lines must also possess acceptable grain quality to develop derived hybrids with the desired grain quality. Therefore, evaluation of parental lines for their grain quality should be done routinely.

Yield performance, genetic diversity, and combining ability of parental lines are the criteria used to breed heterotic rice hybrids. The genetic diversity of parental lines can now be better determined by using molecular marker technology, which should aid in selecting parents suitable for making hybrids. Molecular markers can also help in tagging heterotic gene blocks, accumulating favorable genes, and making heterotic combinations (Zhang et al 1994).

Parental line improvement is a prerequisite for developing indica/japonica hybrids when suitable lines are not readily available. For example, japonica lines possessing restorer genes must be bred by transferring R genes from indica rice. To facilitate this transfer, marker-aided selection for the tagged R genes should be useful. Indica/japonica hybrids also require the presence of the WC gene in one of the parents. Transfer of the WC gene to indica or japonica parental lines can also be facilitated by marker-aided selection. The WC gene has already been tagged with random fragment length polymorphism markers (Zheng et al 1992, IRRI 1993) for this purpose.

In the tropics, the TGMS system is considered more efficient for developing indica, basmati, and indica/tropical japonica rice hybrids (Virmani 1994, 1996). Therefore, we need to develop a large number of TGMS parental lines in indica, tropical japonica (possessing WC genes), and basmati rice. The TGMS genes *tms1* (Wang et al 1995) and *tms3* (Subudhi et al 1997) have been tagged with molecular markers. It should now be possible to use marker-aided selection to incorporate these genes to develop genetically diverse TGMS lines.

Anther culture is known to expedite the development of japonica rice restorers in Korea (H.P. Moon, personal communication). It has also been used successfully to develop isoplasmic indica restorer lines at IRRI (Virmani et al, unpublished). This

can be deployed routinely in selected crosses and random-mating composite populations of maintainers and restorers to expedite the development of improved parental lines.

We also need to develop methods to predict heterosis to reduce wasted labor and increase hybrid breeding efficiency. Xiao et al (1996) identified genes from wild rice *O. rufipogon* that improved yield in cultivated rice. The lines so developed, when used as parents, may help to improve heterosis. This strategy may also help to improve the combining ability of the best available parental lines, which, in turn. would improve heterosis.

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Notes

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CHAPTER 11

Technological innovations to lower the costs of hybrid rice seed production

C.X. Mao, S.S. Virmani, and Ish Kumar

The economic viability and adoption rate of hybrid rice technology depend on the level of hybrid rice seed yields in a country. Over the years (1976-94), mean seed yields in China have increased from 0.27 to 2.25 t ha^{-1}, with a high of 7.39 t ha^{-1}. At IRRI, hybrid rice seed yields increased from 0.15 t ha^{-1} in 1989 to 1.09 t ha^{-1} in 1994; the highest was 2.05 t ha^{-1}. India has also recorded a similar improvement in hybrid seed yield. In 1991, the average seed yield was 0.50 t ha^{-1}. In 1995, the maximum hybrid seed yield was 3.30 t ha^{-1}. The same trend in increased seed yield in hybrid rice was also seen in the Philippines and Vietnam. This increase in seed yield can be attributed to improved seed production technology and increased familiarity with it, experience with parental lines, and the selection of better locations and seasons. This chapter presents some technological innovations made worldwide to increase hybrid seed yields and decrease the cost of hybrid seed production.

Seed yield obtained in a hybrid rice seed production plot is a function of (1) the yielding ability of the fertile counterpart of the male sterile line used, (2) the proportion of male sterile lines in relation to the pollen parent, and (3) the outcrossing rate of the male sterile line. Improving any of these functions can help to increase hybrid rice seed yields. This would also improve seed production economics if input costs remained unchanged (Virmani 1996).

During the past three years, India, Vietnam, and the Philippines have started commercializing hybrid rice technology with the help of public, private, and nongovernmental seed production agencies. Their experience is being watched closely by several other national agricultural research systems whose major concern is the economic viability of hybrid rice seed production. Analyses of hybrid rice technology in China (He et al 1984, 1987a, Lin 1990) established that an increased yield of at least 1 t ha^{-1} of rice hybrids over inbred check varieties was profitable. This profit accrued

after compensating for extra investment in seed and minor chemical inputs. Similar economic analyses have been conducted for hybrid rice seed production in China (He et al 1987b, 1988), lndia (Govindaraj 1993, DRR 1995), and the Philippines (Lara et al 1994). They showed that hybrid seed production was profitable if seed yields ranged from 1.5 to 2 t ha^{-1}. The economic viability and adoption rate of hybrid rice technology will improve further if hybrid rice seed yields increase beyond 2 t ha^{-1} and the price drops below the current level of \$2–3 kg^{-1}.

In China, nationwide average seed yields were barely 0.27 t ha^{-1} when the technology was introduced to farmers in 1976. Since then, tremendous improvements increased average yields to 2.25 t ha^{-1}, with a high of 7.39 t ha^{-1} (Xu 1994). At IRRI, from 1989 to 1994, hybrid rice seed yields averaged 0.15–1.09 t ha^{-1}, with a high of 2.05 t ha^{-1} (Virmani 1996). Similarly, in the Philippines, India, and Vietnam, hybrid rice seed yields ranged between 0.2 and 3.0 t ha^{-1}. The increased seed yields can be attributed to improved seed production technology, increased familiarity and experience with A and R lines, and selection of better locations and seasons. This chapter presents technological innovations that were made worldwide to increase hybrid seed yields and decrease the cost of seed production.

Landmarks in developing hybrid rice seed production technology in China

In China, three parental lines—cytoplasmic male sterile (CMS), maintainer, and restorer lines—essential to producing F_1 hybrids were successfully developed in 1973. Suitable rice hybrids were commercialized on a large scale in 1976 (Lin and Yuan 1980). Chinese scientists also studied hybrid seed production technology, including parental line multiplication and F_1 seed production. The techniques for hybrid seed production were developed primarily in 1975 (Yuan 1985), after which this technology developed in three major stages in China.

The first stage (1973 to 1980) was a trial stage, when primary studies were made on techniques for parental line multiplication, purification, and F_1 hybrid seed production. Nationwide average seed yields ranged from 0.1 to 0.7 t ha^{-1}, which were not profitable for seed growers if the government did not provide subsidies (Lou and Mao 1994).

The second stage (1981 to 1985) further perfected the seed production technology system established in the first stage. The nationwide average yield for hybrid rice seed reached 1.5–1.7 t ha^{-1} by 1985. The increased yield and decreased cost had reached the economic threshold for seed growers without any subsidies in this period.

The most important stage was the third stage, which began in 1986. The goal of this stage was to obtain super high yields in high-yielding areas, where the average seed yield had reached 1.5 t ha^{-1}, through further technological improvements. The targeted yield was 3 t ha^{-1} or more on a large scale for the super-high-yielding program (CAAS and HAAS 1991). Through intensive efforts from scientists and seed growers, nationwide seed yield averaged 2.0–2.25 t ha^{-1} by the mid-1990s (Table 1). Many seed growers have obtained super high yields surpassing 6 t ha^{-1} (Table 2). The

Table 1. Hybrid rice seed production area and yield in China, 1973-95.

Year	Area (ha)	Yield (t ha^{-1})	Increase/ decrease over previous year (%)
1973	0.67	0.09	–
1974	11.20	0.20	122.2
1975	4,160	0.25	25.0
1976	85,120	0.27	8.0
1977	200,610	0.36	33.3
1978	270,120	0.48	33.3
1979	218,430	0.54	12.5
1980	172,350	0.69	27.8
1981	110,400	0.67	–2.9
1982	154,600	0.91	35.8
1983	138,800	1.29	41.8
1984	104,730	1.41	9.3
1985	87,670	1.65	17.0
1986	100,530	2.00	21.2
1987	154,070	2.01	0.5
1988	135,830	1.63	–18.9
1989	171,870	1.96	20.2
1990	191,990	2.25	14.8
1991	124,730	2.26	0.4
1992	158,300	2.19	–3.1
1993	146,450	2.20	0.5
1994	150,220	1.89	–14.1
1995	163,300	2.24	18.5

Table 2. Super-high-yield records of hybrid rice seed production in China.

Location	Year	Yield (t ha^{-1})	Area (ha)
Xuning, Hunan	1989	6.23	0.22
Taojiang, Hunan	1990	6.50	0.11
Youx, Fujian	1990	6.35	0.10
Wugang, Hunan	1990	6.07	0.20
Longhui, Hunan	1990	6.26	0.07
Zhixing, Hunan	1990	6.33	0.09
Youxi, Fujian	1991	6.77	0.10
Zhixing, Hunan	1992	6.13	0.11
Zhixing, Hunan	1993	7.39	0.11
Zhixing, Hunan	1994	6.78	0.11

highest yield (7.39 t ha^{-1}) was obtained by the Zhixing Seed Company, Hunan Province, in 1993 (Xu 1994).

Besides technological innovations, changes in seed production systems and government policies also played a vital role in enhancing hybrid rice seed production in China. In the early stage (in 1976), county seed companies were in charge of purification and multiplication of parental lines, and communes were responsible for F_1 hybrid seed production. In 1979, this changed. Prefecture seed companies became responsible for parental line purification, and county seed companies took charge of parental line multiplication. Both the county seed company and the commune produced F_1 hybrid seeds. Despite such changes, seed yield and purity could not be ensured. Therefore, an improved system was established in 1982. The provincial seed company became responsible for foundation seed production of parental lines and for purifying those parental lines continuously. The prefecture seed company was put in charge of large-scale multiplication of CMS line seed (foundation seed phase 2). A county seed company produced F_1 hybrid seeds only. A few suitable locations were usually selected to serve as seed production bases and the farmers living around the bases were organized in groups to produce hybrid seed according to a contract with the company (Mao 1988). This improved seed production system is still in place.

In the mid-1980s, the Chinese government cancelled subsidies for hybrid rice seed production, which forced seed companies to begin efforts to increase seed yield, improve seed quality, and reduce the seed production cost.

The technological innovations made in the past 20 years increased seed yield considerably. The field area ratio of CMS line multiplication to F_1 seed production and commercial cultivation of hybrid rice changed from 1:30:1,000 in the 1970s to 1:50:2,500 in the mid-1980s and 1:50:5,000 in the 1990s.

The seed production cost also decreased gradually and steadily (Table 3). Compared with its cost in 1976 and 1985, the seed cost was 87% and 25% lower, respectively. The cost kg^{-1} of F_1 hybrid seed in 1995 was estimated at US$0.80 (Table 4).

Hybrid rice seed production is a complex technology. Its establishment and improvement have been a gradual process. From 1973 to 1975, after successfully devel-

Table 3. Hybrid rice seed production cost estimated in 1995 in China.

Type of cost	Items	$ kg^{-1} F_1 seed
Direct (production cost)	Production materials such as parental seed, fertilizer, pesticides, GA_3, etc.	0.23
	Labor	0.25
	Processing and transportation	0.05
	Seed tests and packaging	0.04
Indirect (management cost)	Wages, training, Interest on loans, storage, etc.	0.22
Total		0.79

Table 4. Hybrid rice seed yield and cost changes in China, 1976-95.

Year	Yield		F_1 seed cost	
	t ha^{-1}	+versus 1976 (%)	$ kg^{-1}	- versus 1976 (%)
1976	0.30	0	5.96	0
1981	0.67	123	2.55	57
1985	1.65	459	1.05	82
1995	2.25	650	0.79	87

oping CMS, maintainer, and restorer lines, Chinese scientists made intensive efforts to develop basic guidelines and procedures for hybrid seed production technology. Experiments were conducted to answer the following questions:

- Which season and which places are the best ones for hybrid rice seed production?
- What kind of weather is favorable during the flowering period in hybrid rice seed production?
- How can we obtain perfect synchronization between CMS and R lines?
- What should be the safe distance isolation and time isolation for parental line multiplication and F_1 seed production?
- What are the suitable row ratio and direction for CMS line multiplication and hybrid seed production?
- How can we predict and adjust flowering of parental lines?
- How can we remove or reduce the barrier, caused by flag leaves, to outcrossing and cross pollination?
- How can we get panicles in CMS lines to fully emerge from the leaf sheath?
- How can we do supplementary pollination'?
- How can we do field roguing and purify parental lines'?
- What are the specific agronomic management and disease and pest control measures for hybrid rice seed production?

Based on the results of these experiments, detailed guidelines for seed production were established (Yuan 1985, Xu and Li 1988).

The national average yield of seed production surpassed 1.5 t ha^{-1} in the mid-1980s. The new challenge was to increase seed yield further and reduce the cost when there were no more problems in choosing ideal climatic and site conditions, safe isolation, good synchronization, and perfect field management.

A super-high-yield program for hybrid rice seed production was launched in 1986. The hybrid seed yield target in large areas was set at more than 3 t ha^{-1} (Xu et al 1991). Approaches used to reach this goal included increasing the per-unit population size of parents, increasing the outcrossing rate, and ensuring proper field management.

Increasing the per-unit area population size of parents

The pollen load of the male parent and spikelet number of the female parent per unit area should be increased. The R line should have 1.2 million productive panicles and the A line should have 3.0–3.5 million or more ha^{-1}. The spikelet ratio between R and A lines should be 1:2 to 1:2.5, with a total spikelet number of 300 million in the A line (Yuan and Fu 1995). This can be achieved by:

- Using one sowing of an R line instead of two or three sowings as in the past.
- Increasing the row ratio of R:A to 2:16-20.
- Using wider spacing of double rows of R lines instead of one row.
- Raising more tillered seedlings.
- Transplanting R lines with 1–3 seedlings $hill^{-1}$ and A lines with 2–3 seedlings $hill^{-1}$.
- Applying fertilizers during the early growth stage after transplanting and using more N in the vegetative stage, with a balanced use of NPK and micro-elements to produce more effective tillers and increased panicle weight.
- Using effective water and pest management.

Increasing the outcrossing rate

The average outcrossing rate increased from about 10% in the late 1970s to about 30% in the early 1980s and 50% in the 1990s. In many super-high-yielding plots, the outcrossing rate has reached 70–85%, which is similar to that of conventional varieties (Xu 1994). This was achieved by:

- Developing high-outcrossing CMS lines with a very high stigma exsertion rate (for example: II-32 has 80% and U-1A 85%), early flowering and a centralized blooming period, and smaller and erect flag leaves.
- Properly applying GA_3 to make panicles in CMS lines less enclosed or fully emerged from the flag leaf sheath and to increase the rate of stigma exsertion. The correct time and proper dosage for the GA_3 application vary depending on the A line used and the season, mainly because of the sensitivity of the A line, stage of the plants, and environmental conditions. The use of a ULV (ultra low-volume) sprayer could reduce the dosage and increase the effectiveness of GA_3. The dosage of GA_3 in the late 1970s in China was only 7.5–15 g ha^{-1} due to its high cost, but it increased to 270–300 g ha^{-1} in the 1980s. The proper use of GA_3, even at 90–130 g ha^{-1}, could also give good results (Xu et al 1991).
- Mixing some micro-fertilizers and plant hormones (such as trade names Tiao Hua Ling, Tiao Hua Ji, Dong Ke No. 1, and Hua Shi Tiao Jie Ji) with GA_3. The application of this mixture has played an important role not only in enhancing panicle exsertion and stigma exsertion but also in adjusting and extending flowering time (Mao and Zhou 1990).
- Undertaking supplementary pollination at the peak flowering of both parental lines. The rod-driving method is much better than the rope-pulling method. In the morning, shaking the female panicles before flowering could make the

spikelets open uniformly earlier, which helps to attain a synchronous peak flowering of both parental lines.

Proper field management and pest control

Proper field management and pest control are needed from seeding to harvesting to ensure high yields. These include:

- Raising vigorous and healthy seedlings with more tillers in the seedbed by sowing parental seeds sparsely and evenly. The seeding rate should be less than 150 kg ha^{-1}. Spraying multi-effects triazole (MET) is very helpful in promoting tillering and reducing seedling height in China. R line seedlings should be raised in a two-step method to obtain more tillers.
- Fertilizing and irrigating less in the mid to late stage to inhibit the growth of flag leaves and reduce the possibility of pest incidence.
- Paying more attention to disease and insect pest control, especially for kernel smut *(Tilletia barchyanta)* in China. Spraying 20% Traidimefeon EC 1000X, 75% Topsin-M WP 500X, and 50% carbendazim WP 500X during the heading and flowering stages effectively controls kernel smut disease.

Hybrid rice seed production in the tropics

Virmani (1994) reviewed the outcrossing mechanism and hybrid seed production practices and described guidelines for hybrid rice seed production in the tropics. Simultaneously, IRRI published a manual on hybrid rice seed production (Vinani and Sharma 1993). Huang et al (1994) presented the advances in hybrid rice seed production technology in China as well as at IRRI. These publications adequately summarized seed production technology, which could help achieve yields of up to 2 t ha^{-1} of seed in the tropics (Virmani 1996).

To further economize the cost of hybrid rice seed production in the tropics, the following technological innovations are important.

- Improving the outcrossing potential of male sterile lines.
- Increasing the pollen load released from the male parent.
- Synchronizing flowering of the female and male parent.
- Increasing the proportion of area in the female parent versus that of the male parent.
- Making a more economical use of gibberellic acid or finding a cheaper substitute to improve panicle exsertion and prolong the duration of floret opening in male sterile parents.
- Selecting ideal seasons and areas for seed production.

IRRI scientists subjectively monitoring the outcrossing rate of CMS lines in the BC_4 generation onward and only those progenies of CMS lines that have a moderate (roughly 15%) to high (above 30%) outcrossing are maintained. This is helpful in improving the outcrossing potential of CMS lines during their breeding process. In 1995, an experimental layout was developed at IRRI (Fig. 1), which helped to assess the outcrossing potential of promising CMS lines. This involved growing promising CMS

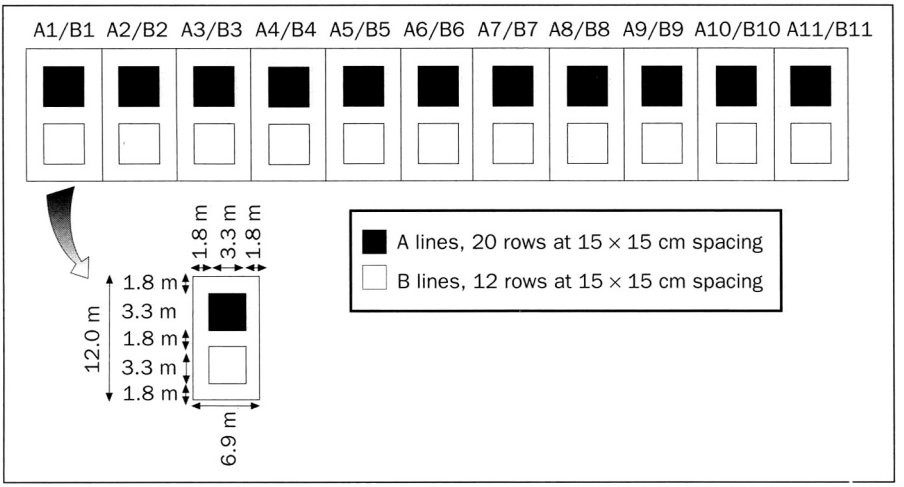

Fig. 1. Field layout of the experiment to study outcrossing and seed yield potential of different CMS lines, IRRI, 1995 dry season.

lines in 9-m^2 blocks bordered with 12 rows of their respective maintainer lines all around to ensure adequate pollen availability for outcrossing. CMS lines were sprayed with GA_3 to maximize their outcrossing rate. A paired plot unsprayed with GA_3 was included to select CMS lines that would show high outcrossing even without a GA_3 spray. The seed yield potential of CMS lines was based on their perfect stand. The highest yielder was IR58025A (3.3 t ha^{-1}), followed by IR68886A (2.9 t ha^{-1}) and IR62829A (2.7 t ha^{-1}); the lowest yielders were IR68891A (0.4 t ha^{-1}) and PMS 4A (0.5 t ha^{-1}). Achievable seed yield from a CMS line was estimated after deducting 15% from the area on account of the space occupied by the male parent.

The outcrossing rate for a male sterile line is also influenced by the number of pollen grains deposited on its stigma, which in turn is affected by pollen load or the number of pollen grains $liter^{-1}$ of air in a flowering seed production plot. Normally, most pollen grains are shed out of the anthers at the time of floret opening. But some pollen grains remain inside the anther. The residual pollen grains per anther and number of blooming spikelets per unit area (Namai and Kato 1987) affect the pollen load in the air. Kato and Namai (1987), using pollen samplers, showed that anther length did not correlate with percentage or number of residual pollen grains per anther exserted from the spikelets. Virmani (1996) also observed elite restorer lines with similar anther length and pollen number per anther showing a different percentage of residual pollen (Table 5). Further studies have been made on pollen load variation in trials conducted at IRRI. The following observations are important.

The number of air-borne pollen $liter^{-1}$ was negatively correlated ($r = -0.76^*$) with distance from the pollen source (Virmani 1996). Pollen load was depressed in the middle rows of the female plots and maximum at the downward side of the male plots (Fig. 2). Pollen load was higher in a seed production plot when seedlings of three

Table 5. Floral traits of some promising restorer lines of rice (IRRI, 1991 dry season).

Restorer line	Anther length (mm)	Pollen anther^{-1}	Residual pollen (%)
IR46R	2.84 ± 0.07	1,628 ± 104	34.1
IR9761R	2.99 ± 0.07	1,729 ± 45	70.0
IR10198R	2.63 ± 0.04	1,071 ± 88	12.5
IR28238R	2.61 ± 0.03	1,604 ± 160	73.2
IR29723R	2.46 ± 0.04	1,407 ± 80	20.5
IR54742R	3.00 ± 0.07	1,667 ± 64	48.2

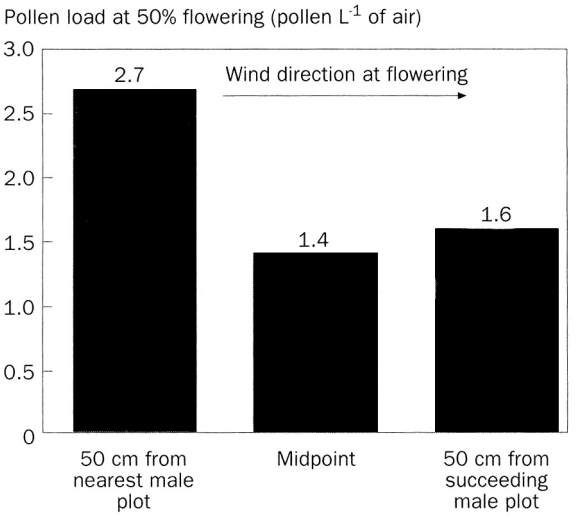

Fig. 2. Pollen load in a seed production plot at varying distance from pollen source.

different ages were planted as in pattern 3 (Fig. 3) than in pattern 2 and was comparable with pattern 1, in which seedlings of one age corresponding to age 2 were planted. Pattern 1, however, carries a risk of nonsynchronization and is therefore not recommended.

After selecting female and male parents suitable for hybrid rice seed production, the most important factor for obtaining high seed yields is the synchronization of flowering of the two parents. The seed yield obtained in hybrid seed production plots varied from 70 to 750 kg ha^{-1} depending on the difference in days to flowering between CMS line IR62829A and the pollen parents (Virmani 1994).

We recommend the following measures to achieve good synchronization of flowering of female and male lines:

1. Spray urea at the panicle initiation stage to delay flowering by 1–2 d, or spray boric acid and KNO_3 to advance flowering by 2–3 d (Huang et al 1994).

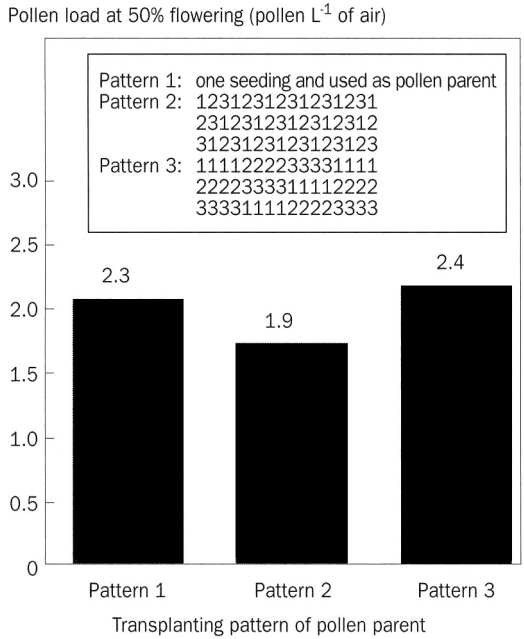

Fig. 3. Pollen load in seed production plots with different transplanting patterns of pollen parent.

2. Keep the water level in the seed production plot at a minimum until the desired tiller number hill^{-1} is attained for both parents. This prevents the undesirable alteration of growth duration of the parents.
3. Minimize the application of fertilizer at stages past mid-tillering as this differentially affects the growth duration of parents.

Studies conducted at IRRI (Virmani 1996) have shown that seed yields of rice hybrids were directly proportional to an increase in the area of the seed parent from 16% to 78%. If the female parent area increased beyond 78%, seed yield declined. To accommodate the maximum number of panicles of the female parent in proportion to the male parent, a planting density of 45 hills m^{-2} using 15 × 15-cm spacing was found useful for IRRI-bred CMS lines (IRRI 1992). To economize on GA_3 dosage in hybrid rice seed production, a ULV sprayer was found to be extremely useful. Spraying GA_3 at 15 g ha^{-1} with a ULV gave results similar to spraying 45 g ha^{-1} with a knapsack sprayer (IRRI 1992, Huang et al 1994).

Environmental factors that influence natural outcrossing in rice include temperature, relative humidity, light intensity, and wind velocity. The seed set percentage and seed yield of CMS lines were negatively correlated to relative humidity (Virmani 1994). The highest seed yield in the trial was obtained when the seed and pollen parents flowered from the end of February to early March, when the relative humidity was 50–60%, the max/min temperature was 28–30/2 1–22 °C, and wind velocity was above 2.5 m s^{-1}. Seed yields at IRRI are generally higher in the dry season than in the

Table 6. Mean seed yield and outcrossing rate for IRRI-bred CMS line IR58025A at various locations in India, 1994-95.

Year (season)	Location	Yield (t ha^{-1})	Outcrossing rate (%)
1994-95 dry season	Hyderabad	1,586	33
	Coimbatore	959	30
	Bangalore	2,375	26
1995 wet season	Hyderabad	515	17
	Coimbatore	1,068	33
	Bangalore	2,229	26
	Maruteru	707	19
	Pantnagar	2,521	-

Source: DRR 1995.

wet season. Studies conducted in India showed a variation in seed yield with changes of location (Table 6). Therefore, to achieve high seed yields in rice hybrids, seed production should be undertaken in ideal seasons and locations, which provide favorable weather conditions for high outcrossing in male sterile lines.

Commercial-scale hybrid rice seed production: a case study of Hybrid Rice International Ltd.

Hybrid Rice International Ltd., a joint venture of Proagro Seed Company Ltd. and a consortium of Japanese companies, is dedicated to developing hybrid rice technology. The company has nearly three years of experience in hybrid rice seed production and has produced 500 t of seed in a single season. This section presents a case study for its success in hybrid seed production.

Identifying suitable female lines

For economical hybrid rice seed production, Chinese female lines were not found suitable under tropical conditions because of their low seed production potential, susceptibility to biotic and abiotic stresses, and poor growth under a tropical climate. The use of such lines in a commercial production program was found to be uneconomical on account of very low seed set. Indian CMS lines having a poor outpollination potential were also found to be uneconomical because they do not yield more than 0.5 t ha^{-1} in farmers' fields. Identifying female lines with a high outpollination potential is the major factor for commercial success. Currently, the female lines developed at IRRI—IR58025A and IR62829A—showed a high outpollination potential in hybrid rice seed production fields and were thus very economical for commercial use.

Female-to-male ratio

In China, a row ratio of up to 16 female:2 male rows is followed. Under Indian conditions, the pollen in central rows with a higher row ratio (16:2 or 8 females:2 males) did not effectively fertilize and produce enough seed for female lines. We have observed that a row ratio of 6 female:2 male rows sown three times is the most profit-

able. We recommend a total population of 350,000 plants ha^{-1} (260,000 female plants:90,000 male plants), with a spacing of 15 cm within female or male rows but 30 cm between female and male rows. In successful plots, we observed nearly 1.99 million panicles of female rows and 0.97 million panicles of male rows. This provided sufficient pollen load to yield up to 3.3 t ha^{-1} of hybrid seed in addition to about 1.9 t ha^{-1} of male seed in commercial plots, if another package of practices was followed. The row ratio of 6:2 may be increased after farmers gain sufficient experience to grow hybrid seed production plots or when only one sowing of a male row is used instead of the current practice of three sowings. The pollen load with one sowing would increase, thus offering a possibility to increase the female-to-male row ratio.

Synchrony in flowering of A and R lines

Synchrony in the flowering of A and R lines proved to be the most important factor influencing yield. When the male line flowered earlier than the female line, there was always a setback for seed set. But if the female line flowered 2–3 d earlier than the male line, good seed set always resulted. Many heterotic combinations show differences between A and R lines in days to flowering. Therefore, we always study the staggering period of these lines at every production location for commercial success. The significance of staggering parental lines can be better realized by considering the example of seed production of commercial rice hybrids of Hybrid Rice International (HRI). The behavior of its parental lines varied to an extent of 13 d with season, change in age of seedlings, and location.

Effect of date of sowing on flowering synchrony

The date of sowing of male and female lines of a hybrid has a significant effect on their flowering behavior, and thus seed production. Both the male and female parents of our commercial hybrid were sown in a nursery at 5-d intervals, from 16 May to 30 June 1996. The seedlings of these parents were transplanted when they reached an age of 30 d. The male parent showed more sensitivity to date of sowing than the female parent. Between the first and last nursery sowing, days to flowering in the male line was 13 versus 9 in the female (Table 7). Because the male line has to be sown earlier than the female line, the exact information on flowering behavior at different sowing dates must be well understood. In the absence of such information, predicting flowering in both the parental lines would be difficult.

Effect of age of seedling on flowering synchrony

Because of the difference in days to flowering of A and R lines, the staggering of parental lines has to be followed. Seedlings of the longer duration parent are to be sown and planted before the shorter duration parent so that they flower at the same time. The difference in sowing and planting of the early maturing parent depends on the difference in days to flowering of the male and female parent. Farmers normally prefer to transplant the male and female nursery in hybrid seed production plots with a minimum gap because it is easier for them to arrange labor and keep puddles soft. From an experiment in which male seedlings of 30-d age were transplanted every 5 d,

Table 7. Effect of date of sowing on flowering duration of male and female lines of rice hybrid 6201 (1996 wet season, Hyderabad, India).

Date of sowing[a]	Days to flower		Difference in flowering of male and female lines (d)
	Male line	Female line	
May 16	118	102	16
May 21	118	102	16
May 26	116	100	16
May 31	113	99	14
June 5	112	97	15
June 10	111	98	13
June 15	109	95	14
June 20	109	96	12
June 25	109	95	14
June 30	105	93	12
Difference in flowering between first and last sowing (d)	13	9	

[a]30-d-old seedlings were used for transplanting.

Table 8. Effect of sowing dates on flowering synchrony. Male and female seedlings were 30-d old at planting.

Date of sowing		Date of flowering		Diff. in male & female sowing
Male	Female	Male	Female	
May 16	June 5	Sep 11	Sep 9	20
May 21	June 10	Sep 16	Sep 13	20
May 26	June 15	Sep 19	Sep 19	20
June 5	June 20	Sep 24	Sep 23	15
June 10	June 25	Sep 28	Sep 25	15
June 15	June 30	Sep 30	Sep 28	15

along with female seedlings aged 20, 25, 30, 35, 40, and 45 d, the following observations were recorded on their flowering synchrony.

1. Male and female seedlings of 30-d age at transplanting:
   - When the male nursery was sown in the second half of May (May 16-30), and transplanted after attaining the age of 30 d, it flowered between September 11 and 19. The flowering of this male nursery synchronized well with the female nursery sown between June 5 and 15 (i.e., 20 d after the male sowing) and transplanted after attaining the age of 30 d (Table 8).
   - For the male nursery sown between June 5 and 15, it was suggested that the gap between male and female staggering be reduced to 15 d instead of 20 d. The male nursery sown between June 5 and 15 flowered between Sep-

tember 24 and 30, whereas the female nursery sown 15 d after the male sowing flowered between September 23 and 28, thus resulting in good synchrony (Table 8).

- It is therefore suggested that for the male nursery sown in May, the staggering period between the male and female line should be 20 d. This period should be reduced to 15 d if the male nursery is sown in the first half of June. This would help to achieve better synchrony of male and female line flowering.

2. Male seedlings of 30-d age and female seedlings of varying ages at transplanting:
   - For the male nursery sown in the 3rd week of May and transplanted in the 3rd week of June (after attaining 30-d age), the female nursery sown 20 d after male sowing and transplanted after attaining the age of 30-40 d synchronized well in flowering with the male nursery (Table 9).
   - The male nursery sown in the 4th week of May and transplanted in the 4th week of June (after attaining the age of 30 d) and the female nursery sown 20 d after the male nursery and transplanted after attaining the age of 25–30 d synchronized well in flowering. Because the nursery of 25-d age flowered at the same time as the 30-d-old seedlings, the gap between male and female transplanting can be reduced to 15 d instead of 20 d like in the 3rd week of June planting.
   - The male nursery sown at the end of May or in the 1 st week of June and transplanted after attaining the age of 30 d and the female nursery sown 20 d after the male nursery and transplanted after attaining the age of 20 d synchronized well in flowering. Because the female seedlings were only 20 d old, the gap between male and female transplanting in the main field was shortened by 10 d.

Table 9. Effect of age of seedling on flowering synchrony.

Male				Female			
Sowing date	Transplanting date	Flowering date	Age at planting (d)	Sowing date	Transplanting date	Flowering date	Age at planting (d)
May 16	June 15	Sep 11	30	June 5	July 5	Sep 10	30
				June 5	July 10	Sep 12	35
May 21	June 20	Sep 16	30	June 10	July 10	Sep 16	30
				June 10	July 15	Sep 16	35
				June 10	July 20	Sep 16	40
May 26	June 25	Sep 19	30	June 15	July 10	Sep 19	25
				June 15	July 15	Sep 18	30
May 31	June 30	Sep 21	30	June 20	July 10	Sep 21	20
June 5	July 5	Sep 25	30	June 25	July 15	Sep 24	20
June 10	July 10	Sep 29	30	June 30	July 20	Sep 28	20
				June 30	July 25	Sep 29	25
June 15	July 15	Oct 2	30	June 25	July 25	Sep 28	30

Table 10. Optimum package for HRI's commercial hybrid rice seed production.

Activity	HRI technology
Best season	Dry season (rabi)
Seed rate	A line, 10 kg ha^{-1}; R line, 5 kg ha^{-1}
Nursery	Sparse sowing: 2.5 kg seed 100 m^{-2} to obtain seedlings with tillers in 25–30 d in wet season and 35 d in dry season
Sowings	Male, 3 times with 3–d interval Female, 1 time
Staggering	Dry season: A line sown 10 d later than R line Wet season: A line sown 10–20 d later than R line depending on date of sowing
Row ratio	6A:2R
Seedlings $hill^{-1}$	1 seedling $hill^{-1}$ for both A and R lines
Planting pattern	Alternate planting of male seedlings of 3 ages after the middle sowing of R line attains age of 30 d
Spacing	Between R lines, 15 cm Between R & A lines, 30 cm Between A lines, 15 cm Plant to plant, 15 cm
GA_3 application	50 g ha^{-1} at 5–10% heading in two split doses of 30 and 20 g ha^{-1} on consecutive days
Supplementary pollination	Two or three times a day during anthesis for 8–10 d, with the help of a stick or rope
Roguing	Once during vegetative phase, three times during flowering, and twice after flowering
Harvesting	R lines harvested 3–5 d before A line. A line harvested and threshed in a clean threshing yard before threshing of male line to avoid any admixtures.
Seed yield	1.2–3.3 t ha^{-1} of hybrid seed 1.2–1.9 t ha^{-1} of male seed

- For the male nursery sown in the 2nd week of June and transplanted after attaining the age of 30 d, the female seedlings sown 20 d after the male nursery and transplanted after attaining the age of 20 or 25 d synchronized well with the males in flowering. The older seedlings (30–40 d) of the female line did not synchronize in flowering with the male line as well as did the 20–25-d-old seedlings.
- It is obvious from the above results that for the early sown and early transplanted male lines, the age of seedlings of the female line has to be 30–40 d, but for late-sown males, the age of the female nursery should be less, i.e., up to 20–25 d. This suggests that flowering synchrony can be manipulated both through time of planting and age of seedling.

Optimum package for seed production

The available package of seed production technology has been standardized for HRI's commercial hybrids under tropical conditions. Table 10 summarizes the practices.

Future outlook

Although rice is a self-pollinated crop, significant cross-pollination occurs on male sterile plants, depending on their flowering behavior, floral characteristics, amount of pollen available from the pollen parent, and prevailing weather conditions. Hybrid seed yields of up to 7.39 t ha^{-1} have been reported from China and up to 2.5 t ha^{-1} in other countries in the tropics. China's experience has shown that both genetic improvement for flowering behavior and floral traits of seed and pollen parents and modifications in seed production practices are useful for pushing seed yields beyond 2 t ha^{-1}. These strategies should be adopted to increase hybrid seed yields in other countries. Moreover, the flowering behavior of parental lines of hybrids must be studied critically across locations. For economical seed production, a specific staggering schedule for a particular production environment must also be worked out before embarking on production. The prohibitive cost of GA_3 in many countries requires us to identify a cheaper substitute or improve male sterile lines for their panicle exsertion (using the elongated uppermost internode gene), duration of floret opening, and stigma exsertion rate.

Seed discoloration of hybrid rice seed caused by several fungi occurring in the tropics is an important problem that needs to be tackled to produce good-quality seeds. A higher incidence of seed-borne diseases (such as paddy bunt caused by *Neovassia horrida* and false smut caused by *Ustilaginoidea virens*) on male sterile lines compared with the pollen parent can cause a serious outbreak of these diseases on commercial crops of hybrid rice. This also hampers the exchange of seeds of male sterile lines and rice hybrids for research purposes among collaborating countries. Therefore, this problem must be given more attention.

In countries such as the United States, Japan, Korea, and Malaysia, which have a low labor-to-land ratio and high wages, hybrid rice seed production needs to be mechanized to make it cost-effective and economically viable. In this context, strategies such as using a facultative female sterile line as a pollinator (Maruyama and Oono 1983), incorporating a herbicide-sensitive recessive gene or phenol reaction gene in the pollen parent, and practicing mixed planting of seed and the pollen parent followed by mechanized harvesting (Maruyama et al 1991) should be investigated critically.

In countries lacking a suitable seed industry infrastructure, the self-sustaining hybrid rice seed production system developed at IRRI (Virmani et al 1993) can be deployed initially to introduce hybrid rice technology.

Apomixis is the ultimate genetic tool to develop true-breeding hybrids that would have a seed cost comparable to that of inbred rice and thus affordable even to resource-poor farmers. Research efforts are under way in China and at IRRI, but these need to be intensified to discover, induce, and/or genetically engineer apomixis in rice.

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Notes

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CHAPTER 12

Diversifying the CMS system to improve the sustainability of hybrid rice technology

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Cytoplasmic male sterility (CMS) resulting from nuclear-cytoplasm interaction has been commercially exploited for the production of F_1 hybrid seed in a number of crops such as maize, sorghum, sunflower, and sugarbeet. More recently, major progress has been made in developing high-yielding hybrid rice varieties based on the CMS system, occupying more than 18 million ha in China. The first CMS line used to develop commercial F_1 hybrids was developed in China in 1973 from a single male sterile plant (*Oryza sativa* f. *spontanea*) designated as wild abortive (WA). Since then, more than 20 CMS sources have been developed from various accessions of cultivated rice and wild species. A large number of CMS lines have been produced in China, at IRRI, and in several other countries from these cytosterility systems. But 95% of the total area planted to hybrid rice has a single CMS source, WA cytoplasm. This cytoplasmic uniformity of hybrid varieties could result in disease epidemics, such as the outbreak of Southern corn blight due to *Helminthosporium maydis* on U.S. maize hybrids carrying T-type cytoplasm.

To overcome the danger of genetic vulnerability of hybrid rice to diseases and insects, we urgently need to diversify the cytoplasm of the male sterility sources. Conventional backcrossing and somatic cell hybridization are possible avenues to achieve diversification using cultivated and wild species as CMS donors. Two major problems are (1) the lack of efficient techniques for characterizing CMS sources and (2) nonavailability of effective restorers, particularly when the cytoplasmic donors (wild species) and recipient parents are distantly related. Under such situations, the donor species itself should be explored as a possible restorer. CMS sources are distinguished from each other on the basis of the fertility of crosses of CMS lines with restorers. These procedures are laborious and fertility is affected by environment.

Advances in molecular biology offer potential to develop precise, reliable, and quick molecular techniques to characterize different CMS sources. Fertile and male sterile lines including different sources of CMS can be distinguished from each other using molecular diagnostic probes based on the restriction endonuclease frag-

ment pattern of mtDNA, including the nature of mitochondrial protein products and ultrastructure of the mitochondrial genome. But the role of these elements in producing cytosterility is not well understood. Protoplast fusion offers promise to produce cybrids and new alloplasmic male sterile lines. Moreover, this technology can further enhance efficiency through the quick transfer of CMS from one source to other elite breeding lines. Otherwise, this requires 5–6 generations of conventional backcrossing. Both two-line (photoperiod-sensitive and thermosensitive genic male sterility) and one-line (apomixis) systems offer a good alternative to the current three-line (CMS) system for producing hybrid seed. But until these systems become available for commercial production of hybrid seed, CMS continues to be the best strategy and thus needs diversification for the sustainability of hybrid rice technology.

Cytoplasmic male sterility (CMS) is a maternally inherited trait in which plants fail to produce normal fertile pollen. Although CMS has been observed in more than 150 plant species, the mechanism by which it interrupts normal pollen development is not well understood. A cytoplasmic-genetic male sterility system, controlled by the interaction of cytoplasmic and nuclear genes, was discovered by Jones and Emsweller (1937) in onion. The role of cytoplasm in causing male sterility in rice was first reported by Sampath and Mohanty (1954). Shinjyo and Omura (1966), from indica × japonica crosses, developed a japonica CMS line with indica cytoplasm (Chinsurah Boro II). Since then, several CMS lines have been developed from various sources (see Virmani and Shinjyo 1988, Virmani 1994, 1996). Some of the described sources may be genetically similar. The first CMS line used to develop commercial F_1 rice hybrids was developed in China in 1973 from a male sterile plant occurring naturally in a population of wild rice (*Oryza sativa* f. *spontanea*) growing on Hainan Island in 1970 (Yuan 1977). This plant was designated wild rice with aborted pollen (WA). Since then, numerous CMS lines have been developed in China, at IRRI, and elsewhere from various wild and cultivated accessions (Lin and Yuan 1980, Virmani and Edwards 1983, Li and Zhu 1988, Virmani and Wan 1988, Virmani 1994, 1996).

It is now well accepted that CMS is associated with the mitochondrial (mt) genome. CMS is encoded by the mt genome and probably some defect in mitochondrial function arrests normal pollen development observed in male sterile genotypes. The mitochondrial genomes of higher plants are much larger, ranging from 218 kb in *Brassica campestris* to 2,400 kb in muskmelon, and more complex than those of other eukaryotic organisms (Lonsdale et al 1984). The genome size of mtDNA of rice is as large as 280 kb (Kadowaki et al 1988). The mt genome consists of relatively large and heterogeneous circular molecules, which are often present together as plasmid-like DNAs. The complex structure of the mt genome is thought to be the result of intra- and intermolecular recombinations that not only alter gene organization but also affect morphological traits such as CMS (Dewey et al 1986). The existence of sequences that apparently originated from chloroplast DNA also increases the com-

plexity of mt genomes. Iwahashi et al (1992) constructed a genetic map of rice mtDNA. The genome is organized as five basic circular DNAs, each of which shared homologous sequences with others, and the total length of a unique sequence is 374 kb. Nakazono et al (1996) found 16 chloroplast fragments in rice mtDNA that ranged from 32 bp to about 6.8 kb in length. About 6% (22 kb) of rice mtDNA excluding repeat sequences is made up of chloroplast sequences.

New sources of CMS are continually being evaluated. Until recently, CMS cytoplasms were classified primarily on the basis of their interactions with nuclear restorer genes. CMS types can now be categorized based on the presence or absence of specific mtDNA sequences or restriction fragments.

CMS systems in rice

A number of CMS systems have been reported in the literature (Table 1). Virmani and Edwards (1983) described various sources, their genetic properties, and the relation

Table 1. Male sterile cytoplasms in rice and their sources and designation. (Adapted from Virmani and Shinjyo 1988.)

Cytoplasm donor		Nuclear donor	Reference	Proposed designation
Species	Strain			
O. sativa	Chinsurah Boro II	Talchung 65	Shinjyo and Omura 1966	*cms-bo*
"	Lead rice	Fujisaka 5	Watanabe et al 1968	*cms-ld*
"	Tadukan	Norin 8	Kitamura 1962	*cms-TA*
O. sativa f. *spontanea*	A Chinese strain	Fujisaka 5	Katsuo and Mizushima 1958	*cms-CW*
"	Wild abortive	(Several)	Lin and Yuan 1980	*cms-WA*
"	Red-awned wild	Lien-Tong-Tsao, etc.	Lin and Yuan 1980	*cms-HL*
O. sativa	Akebono	*O. glaberrima*	Yabuno 1977	*cms-ak*
O. rufipogon	W1080 (India)	Taichung 65	Shinjyo et al 1981	*cms-W18*
"	W1092 (India)	Taichung 65	Shinjyo and Motomura 1981	*cms-W19*
"	KR7	Taichung 65	Cheng and Huang 1979	*cms-KR*
O. sativa f. *spontanea*	Ya Cheng	Guang Xuan 3	cf. Virmani and Wan 1988	*cms-YC*
"	Tian Dong	Zhen Shan 97	cf. Virmani and Wan 1988	*cms-TD*
"	Lie Zhou	Zhen Shan 97	cf. Virmani and Wan 1988	*cms-LZ*
"	Indian	Jin Nan Te 43	cf. Virmani and Wan 1988	*cms-IN*
"	Dong Pu	Jin Nan Te 43	cf. Virmani and Wan 1988	*cms-DP*
"	Jun Niya	Chao Yang 1	cf. Virmani and Wan 1988	*cms-JNY*
"	He Pu	Li Ming	cf. Virmani and Wan 1988	*cms-HP*
"	Teng Qiao	Er-Jin-Qing	cf. Virmani and Wan 1988	*cms-TQ*
"	San Ya	Jing Yin 1	cf. Virmani and Wan 1988	*cms-SY*
"	Rao Ping	6964	cf. Virmani and Wan 1988	*cms-RP*
"	Guangzhou	6964	cf. Virmani and Wan 1988	*cms-GZ*
"	Dwarf aborted	Xue Qin Zhao	cf. Virmani and Wan 1988	*cms-DA*

Table continued

Table 1 continued

Cytoplasm donor		Nuclear donor	Reference	Proposed designation
Species	Strain			
O. sativa	Taichung N1	Pankhari 203	Athwal and Virmani 1972	*cms-TN*
	Gambiaca	Chao Yang 1, etc.	Lin and Yuan 1980	*cms-GAM*
"	Birco (P1279120)	Calrose	Erickson 1969	*cms-BI*
"	ARC 13829-16	IR10179-2-3-1	IRRI 1986	*cms-ARC*
"	E Shan Ta Bei Cu	Hong Mao Ying	cf. Virmani and Wan 1988	*cms-STB*
"	Tian Ji Du	Fujisaka 5	cf. Virmani and Wan 1988	*cms-TJD*
"	IR24	Xiu Ling	cf. Virmani and Wan 1988	*cms-IR24*
"	Jing Chuan Nao	Nan Tai Geng	cf. Virmani and Wan 1988	*cms-JCN*
"	Sheng Qi	Nong Ken 8	cf. Virmani and Wan 1988	*cms-SQ*
"	Li Up	Jing Yin 83	cf. Virmani and Wan 1988	*cms-LU*
"	Zhao Jin Feng	Lan Bery	cf. Virmani and Wan 1988	*cms-ZJF*
"	Zhao Tong Bei	Ke Ching 3	cf. Virmani and Wan 1988	*cms-ZTB*
"	Dissi Hatif	Zhen Shan 97	Wan et al 1988	*cms-DIS*
"	V20B	Zhunghua-1	Pradhan et al 1990a	*cms-V20B*
"	Kalinga-I	Krishna	Pradhan et al 1990b	*cms-KAL*
"	Lalruma	Krishna	Jachuck PJ (pers. commun.)	*cms-LAL*
"	Khiaboro	Akihikari	Nagamine et al 1995	cms-KHB
"	Gamma irradiation	IR62829B	IRRI 1995	*cms-mutant*
O. perennis	Acc. 104823	IR64	Dalmacio et al 1995	*cms-PER*
O. glumaepatula	Acc. 100969	IR64	Dalmacio et al 1996	*cms-GLU*
O. rufipogon	–	IR66	Ahmed MI (pers. commun.)	*cms-RUF*
	–	RMS2B	Ahmed MI (pers. commun.)	*cms-RUF*
O. nivara	–	IR66	Ahmed MI (pers. commun.)	*cms-NIV*
O. sativa	Indonesia 6	32A	Zhu Y-G (pers. commun.)	*cms-IND*
	Dwarf WA	Zhen Shan 97	Zhu Y-G (pers. commun.)	*cms-WA dwarf*
	Ma-Wei-Zhan	Xie-Qing Zhao	Zhu Y-G (pers. commun.)	*cms-WA dwarf*

between restorer and maintainer lines. Male sterile lines have been classified into three basic groups:

- Group I. The WA-CMS lines are typical of this group. The male sterility gene is sporophytic and pollen grains abort at the uninucleate stage.
- Group II. This group consists of BT-type male sterile lines. It is gametophytic; pollen grains abort between the binucleate and trinucleate stages.
- Group III. This group consists of some *O. sativa* f. *spontanea.* The CMS-Hong-Lein is typical of this group. Pollen grains abort at the binucleate stage.

During the past two decades, some 20 CMS sources have been identified. The WA source, however, has been used predominantly in the production of commercial hybrids. Some recently identified sources include CMS-ARC, *O. perennis* (Dalmacio et al 1995) *O. glumaepatula* (Dalmacio et al 1996), and a gamma-ray-induced mutant from IR62829B (IRRI 1995). Pradhan et al (I990a) identified two new CMS sources—V20B and Sattari—through indica × japonica hybridization. V20B is a

maintainer of a CMS-WA source but was also found to be a source of CMS with japonica cultivar Zhunghua 1 (Pradhan et al 1990b). More recently, two CMS sources from *O. rujipogon* and one from *O. nivara* have been identified (Ahmed et al, unpublished). The three new CMS sources are designated as RPMS-1 (*O. rufipogon*), RPMS-2 (*O. nivara*), and RPMS-4 (*O. rufipogon*). Six CMS lines have been developed from these sources. In these highly stable CMS lines, panicle exsertion is almost complete, unlike CMS lines based on a WA source. No restorer could be identified from the cultivated germplasm for these CMS lines and the search for restorer genes from the respective donor wild species is being made.

Two CMS lines — Pushpa A and Mangla A — are available with MS577A cytoplasm derived from *O. rufipogon*. These lines do not possess good agronomic and floral traits. New CMS lines are therefore being developed using elite breeding lines as recurrent parents.

Results show that male sterility-inducing cytoplasmic factors are widely distributed in wild and cultivated rice. It is therefore feasible to develop CMS lines possessing diverse cytoplasmic and nuclear backgrounds.

CMS diversification

Although many CMS sources have been identified in rice (Table 1), more than 90% of commercial hybrid production involves only a WA cytoplasmic source for male sterility (Yuan 1993). Because CMS-WA cytoplasm gives stable CMS lines for which a high frequency of restorers is available, rice breeders tend to deploy this CMS system in hybrid rice breeding more frequently. Most commercial rice hybrids cultivated in China during 1988-92 were based on CMS-WA cytoplasm covering 87.9% of the area, followed by CMS-D and *Gam* (7.8%), CMS-dwarf WA (2.6%), CMS-IP (0.49%), and others (1.6%) (Table 2).

Hybrids released recently in India, Vietnam, and the Philippines also carry WA cytoplasm. Such a cytoplasmic uniformity of hybrid rice varieties could cause genetic

Table 2. CMS systems used in commercial production of indica hybrid rice in China.

Cytoplasmic donor	Cytoplasmic source	CMS line
MS wild rice	WA	Zhen Shan 97A, V20A
Dwarf MS wild rice	Dwarf WA	Xie-Qing-ZhaoA
Dissi D_{52}	D	Zhen Shan 97A
Gambiaca	Gam	Shao-Yang 1A
Indonesia 6	Indonesia	I-32A
MS Ma-Wei-Zhan	Ma-Xie	Ma-XieA
K52	K	K17A Huan-Ai 15A
Red-awned wild rice	Hong-Lian (HL)	Zong-Quang 41A Rei-TaiA

Source: Zhu Y.-G. (unpublished).

vulnerability to diseases and insects. The epidemics of Southern corn blight due to *Helminthosporium maydis* in 1969 and 1970 devastated U.S. maize hybrids carrying T-type cytoplasm (Hooker et al 1970, Ullstrup 1972). To overcome the danger of genetic vulnerability of hybrid rice to diseases and insects, we urgently need cytoplasmic diversification of male sterility sources. Cytoplasmic diversity will reduce the probability of an epidemic arising from cytoplasmically inherited susceptibility to pests.

Approaches for diversifying CMS systems

Various approaches involving intra- and interspecific hybridization and induced mutations are available for diversifying CMS systems. Embryo rescue and protoplast fusion techniques offer new opportunities to produce hybrids among distantly related species and to develop alloplasmic lines with diverse CMS sources (see Brar et al 1994).

Intraspecific hybridization

In this approach, cultivars of *O. sativa* are used as cytoplasmic donors. Crosses could be made between indica x indica, japonica x japonica, and indica x japonica to identify nuclear-cytoplasmic combinations giving rise to male sterility. Several sources have been identified through intraspecific hybridization. The procedure consists of making a cross between two parents (reciprocal) and then backcrossing the F_1 to the recurrent parent until normal-looking progenies like the recurrent parent become available and consistently show male sterility. Pradhan et al (1990a) obtained three new CMS sources—Kalinga I, Lalruma, and V20B—through intraspecific crosses. Pradhan et al (1990b) developed a CMS line carrying cytoplasm of indica cultivar Kalinga and the nuclear genome from another indica cultivar, Krishna. Genetic tests showed that the Kalinga cytoplasm source was different from four other cytoplasmic sources (WA, Gambiaca, TN-1, and *O. sativa* f. *spontanea).*

Indica × japonica crosses are quite promising for isolating additional sources of CMS. A number of CMS lines have been developed at CRRI, Cuttack, with different CMS sources (Table 3). Nagamine et al (1995) crossed four boro varieties from Bangladesh (Tupaboro, Khiaboro, Poshusail, and Habiganjboro VII) with japonica variety Akihikari. One of the lines with Khiaboro cytoplasm and the nuclear genome of Akihikari was found to be male sterile. Test crosses based on fertility restoration showed that the cytoplasm of this line was different from the CMS-bo source. Southern hybridization of mtDNA with eight probes showed that the mtDNA of Khiaboro and that of Chinsurah Boro II differ from each other at the region around the *atp6* only.

Induced mutation

This approach involves mutagenesis of maintainer or elite breeding lines. Kinoshita (1990) induced CMS by gamma irradiation from the N cytoplasm strains of sugarbeet. CMS has been induced in rice through gamma irradiation of IR62829B, a maintainer

Table 3. CMS lines with different CMS sources developed at the Central Rice Research Institute (CRRI), Cuttack, India.

CMS source	CMS lines developed
Wild abortive	CRMS5A, 6A, 7A, 8A, 10A, 11A, 13A, 15A, 16A, 17A, 18A, 19A, 23A, 24A, 25A, 26A, 27A, 28A, 29A, 30A, 31A, and 34A
O. perennis	CRMS22A, 35A, 36A
V20B	CRMS20A
Kalinga I	CRMS21A, 32A, 33A
Lalruma	CRMS37A

of WA cytoplasm (IRRI 1995). More research is needed to isolate cytoplasmic mutants using induced mutation.

Interspecific hybridization

The genus *Oryza* has 22 wild species. These wild species are an important source of CMS. The first CMS line commercially used in hybrid rice with WA cytoplasm was obtained from *O. sativa* f. *spontanea* (Lin and Yuan 1980). More recently, *O. perennis, O. rufipogon,* and *O. nivara* have been used successfully to isolate new CMS sources. We crossed 46 accessions of A-genome wild species with IR64, a restorer of WA. Backcrossing was used to isolate normal-looking male sterile lines.

Two CMS lines have been identified (Dalmacio et al 1995, 1996). One line—IR66707A—has the cytoplasm of *O. perennis* (Acc. 104823) and the nuclear genome of IR64. This line is stable for complete male sterility and resembles IR66707B in morphological characters, except that it is 5–7 d later in flowering. The wild species, *O. perennis* (Acc. 104823), has long awns, a black hull, and a purple pericarp, and is a shattering type. But the derived CMS line (IR66707A) is free of these weedy traits. Similarly, another CMS line (IR69700A) having cytoplasm of *O. glumaepatula* (Acc. 100969) with an IR64 nuclear background has been developed (Dalmacio et al 1996). Genetic tests show that the male sterility source of these two lines (IR66707A, IR69700A) is different from WA cytoplasm.

Southern hybridization was carried out on IR66707A using eight mtDNA specific probes and five restriction enzymes. In all 40 combinations, identical restriction fragment length polymorphism patterns were observed in IR66707A and *O. perennis* Acc. 104823 (Dalmacio et al 1995; Fig. 1). The results indicate that IR66707A has the same cytoplasm as the donor, *O. perennis,* and suggest that CMS was not caused by any major rearrangement or modification of mtDNA but was probably caused by modified transcription or translation of messenger RNA. This is a valuable addition to diversification of the CMS system. A search for restorers of these lines is under way.

The Directorate of Rice Research, Hyderabad, India, has an active program on diversification of the CMS system in rice. In this program, 132 crosses involving A-genome wild species and cultivated rice were evaluated. Initially, six male sterility-

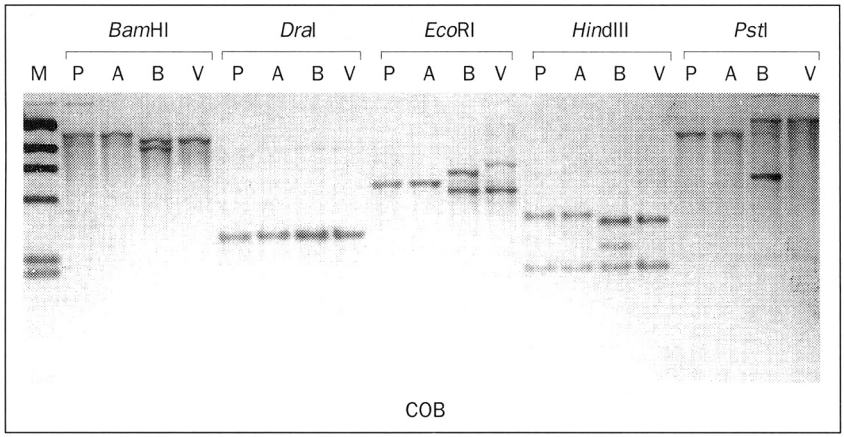

Fig. 1. Southern hybridization patterns with mitochondrial DNA-specific probe (COB) using five restriction endonucleases, *Bam*HI, *Dra*I, *Eco*RI, *Hind*III, and *Pst*I. M: lambda DNA digested with *Hind*III as molecular marker; P: *O. perennis* Acc. 104823; A: IR66707A; B: IR66707B; V: V20A (WA cytoplasm). Reproduced from Dalmacio et al (1995) with permission from Kluwer Academic Publishers, The Netherlands.

Table 4. New CMS sources identified from crosses of cultivated rice and wild *Oryza* species.

Line	Source	Restorer	Maintainer	Reference
RPMS 1	*O. rufipogon*	None	IR66, IR70, PMS 2B. V20B	Directorate of Rice Research (DRR), Hyderabad (unpublished)
RPMS 2	*O. nivara*	None	IR66, IR70	DRR (unpublished)
RPMS 4	*O. nivara*	None	IR66, PMS 2B	DRR (unpublished)
IR66707A	*O. perennis*	None	IR64	Dalmacio et al 1995
IR69700A	*O. glumaepatula*	None	IR64	Dalmacio et al 1996

inducing sources from *O. nivara* and *O. rufipogon* were identified. Of these, three sources different from WA have been identified (Table 4). By using these three new sources of sterility-inducing cytoplasms, six CMS lines—RPMS1-1, RPMS 1-2, RPMS 1-3, RPMS 1-4, RPMS-2, and RPMS-4—have been developed. These lines are completely stable for male sterility. Efforts to identify restorers for CMS lines derived from A-genome wild species have been largely unsuccessful. One approach could be to transfer restorer (nuclear) genes from the respective wild species (the cytoplasmic donor itself).

Two CMS lines, Pushpa A and Mangla A with MS577A (*O. rufipogon*) cytoplasm, have been developed. Restorers for the MS577A source have been identified from the A-genome wild species, *O. sativa* f. *spontanea,* which could restore 83%

Table 5. Restorers identified for MS 577A having *O. rufipogon* cytoplasm.

CMS line	Restorer	1993 DS[a]		1994 WS	
		Pollen fertility	Spikelet fertility	Pollen fertility	Spikelet fertility
		(%)			
Pushpa A	VN2 *(O. rufipogon)*	90.7	70.2	85.6	77.9
Pushpa A	DRW-22016 *(O. rufipogon)*	82.4	52.5	78.4	70.4
Pushpa A	DRW-22017-5 *(O. rufipogon)*	54.3	30.4	84.2	65.5
Mangla A	RPW-2001 *(O. sativa* f. *spontanea)*	95.2	80.4	85.7	83.0

[a] DS = dry season, WS = wet season.
Source: M. Ilyas Ahmed (unpublished).

spikelet fertility. Similarly, 77.9% fertility could be achieved using *O. rufipogon* as a restorer (Table 5).

Protoplast fusion

Protoplast fusion is another important approach for achieving cytoplasmic diversification and for developing alloplasmic lines from distant species of *Oryza* and related genera. Protoplast culture has made it possible to transfer CMS into elite breeding lines in one generation instead of the 5–7 repeated backcrosses required in conventional procedures. Some notable examples of successful CMS transfer by protoplast fusion include tobacco, *Brassica,* citrus species, and, more recently, rice. Somatic hybrids have been produced through protoplast fusion between cultivated rice and four wild species (Hayashi et al 1988).

The production of cybrids following protoplast fusion is one of the most exciting developments. In cybridization, the nuclear genome of one parent is combined with the organelles of a second parent. In effect, organelles are transferred from one parent to the other in a single step. Protoplast fusion provides a unique opportunity to produce cybrids and recombine cytoplasmically inherited traits. Cybrids have been produced through protoplast fusion in several species. Cybridity is confirmed on the basis of mtDNA restriction patterns, morphological traits, and isozyme and cytological tests.

Protoplasts of the donor CMS line are exposed to high doses of irradiation and fused with the iodoacetamide-treated protoplasts of the recipient line. Irradiation inactivates the nucleus and chemical treatment with iodoacetamide prevents cell division. As a result, metabolically complementary cells are capable of developing into plantlets after fusion treatment. The donor-recipient method has been used successfully to transfer CMS sources into fertile lines of rice (Yang et al 1989, Kyozuka et al

1989). This method does not require the use of a selectable cytoplasmic marker of the donor.

Yang et al (1988, 1989) produced cybrid plants in rice by electrofusing gamma-irradiated protoplasts of A-58 CMS and iodoacetamide-treated protoplasts of the fertile cultivar Fujiminori. Cybrids had the peroxidase isozyme of the fertile parent (Fujiminori) but had four plasmid-like DNAs (B1, B2, B3, and B4) from the sterile A-58 CMS parent in their mitochondrial genomes. Cybrids produced through protoplast fusion using the donor-recipient method had the mitochondrial genome of the CMS line and the nuclear genome of the fertile variety (Akagi et al 1989). Kyozuka et al (1989) also used the donor-recipient method and transferred CMS of Chinsurah Boro II, an indica rice, into japonica variety Nipponbare.

Akagi and Fujimura (1992) have developed an efficient and highly reproducible system for transferring CMS into an array of breeding lines. The method inactivates protoplasts of the recipient parent with 3 mM of iodoacetamide for 15 min, and those of the donor (CMS) parent with a high dose of X-rays (125 kr). Using this method, indica CMS has been introduced into 35 japonica cultivars. Professor E.C. Cocking (personal communication) is employing protoplast fusion techniques to produce alloplasmic lines in rice using various wild species as cytoplasmic donors.

Characterizing CMS sources

CMS sources can be distinguished from each other using the following approaches:

1. A classical approach involving fertility restoration by nuclear genes.
2. Molecular characterization of mtDNA and variation in the restriction fragment pattern of mtDNA/diagnostic DNA probes.
3. Detection of variation in mitochondrial translation products.
4. Ultrastructure analysis of the mt genome.

Classical approach

In this approach, CMS sources are classified solely on the basis of their interaction with nuclear restorer genes. This involves crossing male sterile lines (unknown CMS source) with a series of inbred lines and with restorers of known CMS sources. Most lines are found to restore fertility with some sources of cytoplasm, but not others. On the basis of fertility restoration, the CMS sources are classified into different groups. Plant breeders commonly use this approach to identify CMS sources. Based on fertility restoration, CMS sources in rice have been classified into four cytoplasmic types: S1 (originally Chinsurah Boro II), S2 (derived from WA), S3 (Gambiaca), and S4. In maize, three cytoplasmic sources (C, S, T) have been identified based on restoration of fertility by nuclear genes (Duvick 1965). Although the identification procedure based on restoration of fertility by nuclear genes is laborious, the process could be accelerated if new CMS cytoplasms could be identified using molecular approaches.

Molecular approaches

CMS is thought to be encoded by the mt genome. Some defects in mitochondrial function result in the arrest of normal pollen development observed in male sterile genotypes. Although mtDNA regions correlated with CMS have been identified in maize, *Petunia,* and other species, their role as causative in producing sterility is not understood. It is also possible that the "plasmid-like mtDNA" molecules either encode or in some way induce the synthesis of additional polypeptides, which in turn may cause CMS.

Molecular analysis of mtDNA from maize, sorghum, and rice has shown differences in the occurrence of small linear or circular DNA molecules in CMS and male fertile lines. Correlations between CMS types and the presence or absence of specific mtDNA sequences or restriction fragments have been reported for a number of species. Umbeck and Gengenbach (1983) observed a 6.6-kb *Xho*I mtDNA restriction fragment diagnostic for CMS-T cytoplasm in maize, a *Bgl*II restriction fragment diagnostic for CMS in *Petunia,* and an *Nco*I fragment of *Ogura* radish mtDNA for CMS in *Brassica* (Bonhomme et al 1991).

Kemble et al (1980) classified 31 maize lines into four groups—C, T, S, and N—based on an mtDNA restriction fragment pattern. The four cytoplasmic types found to give mtDNA banding pattern characteristics of T cytoplasm were also verified as members of the T group by using the *H. maydis* race T pathotoxin test. Mikami et al (1986) could distinguish N and S cytoplasms based on the restriction pattern of mtDNA in sugarbeet. The male sterile cytoplasms could be classified into three groups according to the constitution of the low molecular weight mtDNA fragment. The male fertile lines contain a 1.3-kb DNA fragment that is absent from all nine CMS lines, regardless of the source of cytoplasm. An organelle DNA assay provides a convenient way to recognize and characterize cytoplasmic variation.

Mann et al (1989), through restriction endonuclease analysis, could identify BMC-CMS, a cytoplasm derived from the wild beta, *Beta maritiama,* which is distinguishable from both N- and S-CMS. Kiang et al (1993) identified and cloned a 4.5-kb *Bam*HI-*Hind*III restriction fragment from the mtDNA of the CMS line in *Lolium perenne.* The cloned fragment failed to hybridize with the sequences in the mtDNA of fertile plants. Such diagnostic probes need to be identified in rice to distinguish fertile and sterile plants in the seedling stages.

In rice, four kinds of circular plasmid-like DNA—B1ß2, B3, and B4—have been detected in rice mitochondria (Yamaguchi and Kakiuchi 1983, Shikanai et al 1987, 1989, Shikanai and Yamada 1988). Yamaguchi and Kakiuchi (1983) identified two small mtDNAs, B-1 and B-2, in the BT cytoplasm of male sterile rice, which were absent in normal fertile cytoplasm. Sequence analysis has revealed that these are related to 1.9-kb and 1.4-kb circular plasmid-like DNA found in maize (Shikanai et al 1989). These molecules do not show extensive homology to either the main mitochondrial genome or the chloroplast genome, but they do exhibit extensive homology to the nuclear genome.

Differences in the occurrence of linear or circular DNA molecules have been reported in rice. Kadowaki et al (1986) found that Chinsurah Boro II cytoplasm con-

tained two plasmid-like low molecular weight DNAs and the mtDNA restriction patterns were different from those of N cytoplasm. Kadowaki and Harada (1989) studied 10 strains of rice with different CMS sources: CMS-Bo, CMS-R, CMS-UR89, CMS-UR93, CMS-UR102, CMS-UR104, CMS-UR106, WA, CMS-Gam, and MS577. Plasmid-like DNAs were observed in all the male sterile cytoplasms along with high molecular weight mtDNAs. In contrast, four types of low molecular weight plasmid-like DNAs have been identified in mitochondria associated with S2 CMS WA (Mignouna et al 1987).

Wang et al (1987) analyzed mtDNA of four CMS sources—*cms-WA, cms-bo* (BT), *cms-GAM,* and *cms-HL.* A 1.9-kb mtDNA band was found in sterile line *cms-bo* (BT) but was absent in the maintainer line. This band was also absent in the mtDNA of *cms-WA, cms-GAM,* and *cms-HL.*

So far, molecular characterization has been based on a restriction fragment using purified mtDNA from fertile and sterile lines. But the efficiency of such characterization could be enhanced if mtDNA-specific probes could be developed to detect differences in total DNA, as has been reported for sugarbeet (Weihe et al 1991).

Mitochondrial translation products

Detection of mitochondrial translation products is another approach to characterizing CMS sources. The ability of the mitochondrion to continue protein synthesis after isolation from the cell allows translation products to be labeled with radioactive amino acids with high specific activity. In maize, T, S, and C male sterile cytoplasms have been found to synthesize variant polypeptides that distinguish them from each other and from normal (N) mitochondria (Forde et al 1978). Forde et al (1980) used this method in combination with sodium dodecyl sulfate-polyacrylamide gel electrophoresis to compare the translation products of mitochondria from 28 cytoplasmic sources.

The translation products of mitochondria from five cytoplasms were indistinguishable from male fertile N cytoplasm of CO 192. Based on this, 18 of the cytoplasms were identical to S cytoplasm, 3 to T cytoplasm, and 2 to C cytoplasm. No variation was observed in the mitochondrial translation products within each of the four cytoplasmic groups, which is in contrast to the differences observed in fertility restoration patterns of members of the same group. This method has some advantages over the nuclear fertility restoration procedure, including its speed (4-d-old etiolated seedlings are used). With this method, identification was possible in different nuclear backgrounds. The apparent lack of variation in translation products within each cytoplasmic group suggests that there may be no advantage in using one source of male sterility rather than another in the same group.

Dixon and Leaver (1982), based on mitochondrial translation products in sorghum, distinguished fertile (Kafir) cytoplasm from male sterile (Milo) cytoplasm and from three alternative sources of CMS. Milo cytoplasm synthesized a 65,000-Da polypeptide that was not synthesized by Kafir cytoplasm. In addition, alternative sources of CMS cytoplasm could be distinguished from each other as well as from Milo and Kafir cytoplasm. It is also possible that plasmid-like mtDNA molecules

either encode or in some way induce the synthesis of additional polypeptides, which in turn may cause cytoplasmic male sterility.

Ultrastructure analysis of the mitochondrial genome

A few studies have been made to compare the variation in ultrastructure of the mitochondrial genome of fertile and sterile cytoplasm. Mikami et al (1986) isolated mtDNA of normal and male sterile cytoplasms of sugarbeet and examined it by electron microscopy. Mitochondrial DNA was composed of a heterogeneous population of circular molecules. MtDNA of the S cytoplasm plants lacks the minicircles of 0.28 to 0.4 mm size, which are found in the N mtDNA. Electron-microscopy analysis showed that the tapetal cells of CMS lines in *Petunia* synthesize a lower level of rough endoplasmic reticulum than the fertile counterparts.

Pring et al (1977) found that S cytoplasm had two small DNAs and electron microscopy indicated that these unique DNAs were of different lengths.

Constraints to cytoplasmic diversification

1. Developing new CMS sources and diversifying cytoplasm through intra- and interspecific crosses require extensive crossing and backcrossing. The whole process is quite laborious and time-consuming. One of the major constraints to developing CMS through wide crosses is the lack of restorers and transfer of restorers into a desirable agronomic background. Furthermore, chances of heterotic combination become restricted because of the narrow base of CMS and restorers. Extensive efforts needed to identify new CMS sources and the low probability of obtaining heterotic combinations make wild species of limited use in developing CMS lines for heterosis breeding.
2. The lack of efficient techniques for characterizing different CMS sources is another constraint to cytoplasmic diversification. Identifying individual CMS sources through the fertility restoration method is laborious and time-consuming. Fertility is also affected by environmental factors.
3. Transferring CMS into different breeding lines through protoplast fusion (donor-recipient method) has had limited success because of poor plant regeneration in several indica rice lines.
4. Several CMS sources show instability for male sterility and thus cannot be used in commercial heterosis breeding.

Future research on CMS diversification

1. We need to precisely characterize individual CMS sources based on restriction patterns of mtDNA. A catalogue should be prepared based on these patterns from all available CMS sources in rice, including those classified through fertility restoration as well as unclassified ones. We also should emphasize developing diagnostic molecular markers to identify CMS sources.

2. Although several CMS sources have been identified, only a few have been used to develop commercial rice hybrids. Because japonica rice has few restorers, it will be important for breeders of japonica rice to identify new CMS sources for which sufficient restorers are available among japonica rice cultivars.
3. Protoplast fusion technology should be extended to produce cybrids for the quick transfer of CMS sources into an array of elite breeding lines of rice.
4. Many CMS sources derived from wild species of *Oryza* lack good restorers. It is thus important to induce mutations for cytoplasmic male sterility in elite breeding lines and maintainer stocks of rice.
5. The molecular mechanism of CMS and causes for instability of some CMS sources need to be investigated. The role of low molecular weight DNAs (plasmid-like DNAs) in causing CMS and their use as a diagnostic molecular marker to characterize different CMS sources should also be investigated.

Until two-line (TGMS or PGMS) or one-line (apomixis) systems become available for commercial hybrid rice breeding, the CMS system continues to be the most appropriate strategy for heterosis breeding in rice. Therefore, CMS diversification is necessary for sustainable hybrid rice technology.

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CHAPTER 13

Managing vulnerability of hybrid rice to biotic stresses in China and India

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Hybrid rice is grown mainly along the middle and lower reaches of the Yangtze River. It is also grown in southwest and southern China. At least three diseases and five insect pests that cause economic losses are known to occur widely. Compared with conventional varieties, rice hybrids that are highly responsive to fertilizer are more prone to pest damage. Rice yield losses also occur in India because of several diseases and insect pests in conventional varieties. Most of the currently released rice hybrids in India are susceptible to the major diseases and insect pests. These hybrids are now grown in relatively less pest-prone areas. Rice hybrids from China and India have been screened to identify resistance. Several parental lines and hybrids showed resistance to some diseases and pests. This chapter discusses pest problems that could affect hybrid rice production in the future.

Rice is the main food crop in China and India, the two largest rice producers and consumers in the world. Both countries have variations in environmental, socioeconomic, and rice production conditions.

The genetic tools (cytoplasmic male sterile, maintainer, and restorer lines) essential to producing F_1 rice hybrids were successfully developed in 1973. Hybrid rice was then rapidly popularized in China. The annual cropped area under hybrid rice surpassed 15 million ha. Hybrid rice produced about 20% more than conventional varieties. This helped China to increase total rice production (Yuan et al 1994).

Hybrid rice is grown mainly along the middle and low reaches of the Yangtze River as well as in southwest and southern China, which are in the subtropical zone with mild climate and abundant rainfall. Rice production in China is increasingly exposed to serious biotic stresses. About 70 diseases and 350 insect pests are known to attack rice in fields. At least five insect pests and three diseases are widespread in China and cause economic losses every year (Table 1).

Compared with conventional inbred varieties, rice hybrids are apparently more responsive to fertilizers and exhibit vigorous growth with higher tillering capacity. These attributes are closely associated with vulnerability to disease epidemics and insect outbreaks (Mew et al 1988). Some conventional varieties such as IR26, IR30, and their derivatives are widely used as restorers (Shen 1993). Most rice hybrids therefore show resistance to rice brown planthopper but are susceptible to whitebacked planthopper. This has resulted in a serious problem for rice production in China (Table 2). Sampling in fields planted to hybrid rice showed the severe incidence of whitebacked planthopper (Table 3).

Table 1. Rice area (million ha) affected by major diseases and insect pests in China, 1990-94.

Year	Blast	Sheath blight	Bacterial blight	Striped stem borer	Yellow stem borer	Brown planthopper	Whitebacked planthopper	Leaf-folder
1990	3.92	16.05	1.20	10.46	3.54	5.09	11.36	12.86
1991	3.13	15.75	0.85	11.08	4.43	8.50	14.74	16.55
1992	4.95	15.82	0.64	9.79	3.42	4.59	9.77	11.21
1993	6.19	15.07	0.64	10.88	4.06	3.56	10.83	12.85
1994	4.47	15.40	0.60	10.80	4.85	4.01	9.94	9.97

Table 2. Yield losses of rice caused by brown planthopper and whitebacked planthopper in China during 1990-94.

Year	Yield loss (000 t)	
	Brown planthopper	Whitebacked planthopper
1990	152	390
1991	594	1,076
1992	106	306
1993	151	431
1994	133	333

Table 3. Two planthoppers sampled by a suction apparatus in hybrid rice at the National Rice Research Institute farm, 1996.

Date	Hybrid[a]	Planthoppers 0.25 m^{-2} sample (no.)	
		Brown planthopper	Whitebacked planthopper
July 23	Shan You 63	1.8 ± 0.9	199.5 ± 78.4
	Xie You 9308	1.3 ± 0.6	339.2 ± 129.5
July 30	Shan You 63	8.1 ± 11.8	171.0 ± 116.1
	Xie You 9308	7.3 ± 10.3	192.7 ± 123.3

[a] Shan You 63 = Zhen Shan 97A/MR63 (MR63 = Gui 630/1R30); Xie You 9308 = Xie You Zao A/9308R (9308R = C 57/300/IR26).

Table 4. Potential pest problems in hybrid rice-growing states in India.

Disease or pest	States
Blast	Andhra Pradesh, Karnataka, Tamil Nadu, and West Bengal in late kharif and rabi seasons
Bacterial blight	Punjab, Haryana, western Uttar Pradesh, Andhra Pradesh, Karnataka, and West Bengal
Sheath blight	Andhra Pradesh, western Uttar Pradesh, Punjab, Haryana, West Bengal in kharif season
False smut	Coastal Andhra Pradesh, western Uttar Pradesh, Haryana, and West Bengal
Kernel bunt	Punjab, western Uttar Pradesh, and Haryana
Stem borer	Punjab, Haryana, Andhra Pradesh, and Tamil Nadu
Gall midge	Andhra Pradesh, Karnataka, and West Bengal
Brown planthopper	Andhra Pradesh, Kerala, West Bengal, and Karnataka
Whitebacked planthopper	Haryana, Punjab, and Andhra Pradesh
Leaffolder	Andhra Pradesh, Karnataka, Tamil Nadu, and Haryana
Hispa	Andhra Pradesh and West Bengal

Table 5. Reaction pattern of hybrid rice to major insect pests and diseases at DRR, India.

Hybrid	Designation	Blast	Bacterial leaf blight	Sheath blight	Brown planthopper	Gall midge
APRH1	R58025A/Vajram	S[a]	S	S	S	S
APRH2	IR62829A/MTU992	S	S	S	S	S
MGR1	IR62829A/IR10198	S	S	S	R	S
DRRH1	IR58025A/IR40750	MS	S	S	S	S
6201	Proagro 6201	R	S	S	S	S
PHB 71	OR161	MS	S	R	R	S
Jaya		S	S	S	S	S
Rasi		R	S	S	S	S

[a]S = susceptible, MS = moderately susceptible, R = resistant.

In its search for a new yield frontier, India also explored the possibility of developing hybrid rice. During the initial phase of development in India, the extent of hybrid vigor for yield was the major concern. Developing pest resistance in hybrids was relegated to the second phase of the program.

India now has several insect pests and diseases that cause yield losses (20–30%) in rice. Punjab, western Uttar Pradesh, Haryana, West Bengal, Karnataka, Andhra Pradesh, and Tamil Nadu have potential for hybrid rice cultivation. So far, only a limited area is planted to hybrid rice. Based on experiences with pest management for conventional inbred varieties, estimates were made on potential problems (Table 4). Currently, a few public- and private-sector hybrids are grown in small areas in Andhra Pradesh, West Bengal, and Tamil Nadu. Like check varieties Jaya and Rasi, most are susceptible to major insect and disease problems (Table 5). The vulnerability of hybrid rice to pests and diseases will be known only when it is grown in large areas. For conventional varieties, the major approach for disease management was based on the use of host-plant resistance and need-based pesticide application.

Insect pest management

An insect pest management strategy is devised to keep major pests below threshold levels. In this strategy, the use of host resistance and adoption of nonchemical methods including biological control of pest population buildups play a crucial role. Chemical control is resorted to only when it is essential.

For a wider adoption of hybrid rice technology, it is essential that the hybrids evolved have resistance to major diseases and insect pests. Inbred conventional varieties and hybrids have been screened in China and India. Rice hybrids and conventional varieties have been evaluated in China to identify resistance to brown planthopper, whitebacked planthopper, green leafhopper, gall midge, stem borer, and leaffolder. Only 1% of the material screened was resistant to gall midge and leaffolder; 6–14% was resistant to other pests (Table 6). The hybrids found resistant to brown planthopper were Wei-You 35, Wei-You 64, IR58025A/IR21567, IRS8025A/IR29723, IR62829A/IR10198, OI 161, and OR 146. The hybrids resistant to whitebacked planthopper were Shan-You 63, Wei-You 35, and Wei-You 64. Some rice hybrids were found to possess multiple disease and insect resistance at 10 locations in China (Table 7). Only

Table 6. Evaluation of hybrids and inbred varieties for resistance to major insect pests of rice in China.

Target pest	Number screened	Resistant	Moderately resistant	Total	Resistant and moderately resistant (%)
Brown planthopper	60,740	4,036	1,898	5,934	10
Whitebacked planthopper	31,755	755	2,162	2,917	9
Green leafhopper	1,144	4	60	64	6
Gall midge	21,437	157	83	240	1
Stem borer	945	43	90	133	14
Leaffolder	11,698	16	126	142	1

Table 7. Rice hybrids with multiple disease and insect resistance selected in China.

Hybrid	Location	Resistance [a]				
		Blast	Bacterial blight	Sheath blight	Brown planthopper	Whitebacked planthopper
Wei-You 64	Hunan	MR	MR	–	MR	MR
Shan-You 63	Jaingsu, Sichuan	MR-R	MR	MR	–	MR
D-You 63	Hunan, Sichuan	MR	MR	MR	–	–
Gan-You 63	Sichuan	R	MR	MR	–	–
Xi-You 56	Guangdong	R	R	R	–	–
Wei-You 35	Hunan	MR	–	–	T	MR
D-You 10	Sichuan	R	MR	MR	–	–

[a] MR = moderately resistant, R = resistant, T =tolerant.

rice hybrid Xi-You 56 showed resistance to blast, bacterial leaf blight, and sheath blight disease.

Because most rice hybrids released in India are susceptible to major pests and diseases, there is a need to incorporate multiple resistance to biotic stresses in them using parental lines possessing such resistance. The reaction of a few parental lines to blast, bacterial leaf blight, and kernel bunt (Table 8) indicated the possible reason for the lack of resistance in hybrids.

In the hybrid rice network project in India, a large number of restorers and hybrids have been evaluated for resistance to major insect pests (Table 9); some of these

Table 8. Reaction[a] of parental lines to blast, bacterial blight, and kernel bunt in India.

CMS parental line	Blast	Bacterial blight	Kernel bunt (%)
PMS 1A	9	9	9
PMS 1B	8	9	1
PMS 2A	8	9	10
PMS 2B	7	9	1
PMS 3A	8	9	15
PMS 3B	8	9	1
IR58025A	9	9	11
IR58025B	9	9	1
IR62829A	9	9	1
IR62829B	9	9	1

[a] Blast and bacterial blight have a disease score on a scale of 1 to 9, where 1 = highly resistant and 9 =highly susceptible. Percentage of kernel bunt was recorded by Sharma 1992 (personal communication); blast incidence recorded at DRR in 1995.

Table 9. Screening restorers and hybrids for resistance to major insect pests of rice in India.

Year	Trial[a]	Screened (no.)	Resistant entries (no.)		
			Gall midge	Brown plant-hopper	White-backed planthopper
1991	Restorer nursery	100	24	15	—
1992	NHRT	77	2	8	—
1993	AHRT kharif 93	16	2	0	0
	IHRT-1 & 2	44	6	1	1
	AHRT rabi 93	24	1	0	0
	IHRT rabi 93	29	3	0	0
1994	NHRT-1	17	0	0	0
	NHRT-2	21	0	0	2
	NHRT-3	21	0	0	2
	NHRT-4	22	0	0	0

[a] NHRT = national hybrid rice trial: AHRT = advanced hybrid rice trial; IHRT = Initial hybrid rice trial.

showed resistance to brown planthopper and gall midge (Tables 8,9, and 10). Among the restorers, IR35366, Suweon 332, and HKR 120 possessed resistance to brown planthopper. For gall midge, the hybrids involving restorer line WGL 3962 were resistant.

Besides the use of host-plant resistance, other pest management methods could be adopted to prevent damage to hybrid rice. Practices such as adjusting sowing and harvesting time to avoid the vulnerable stages that allow pest incidence, planting with alley ways, field sanitation, and weed-free crops reduce pest incidence. The adoption of optimum spacing, moderate doses of N application, split application of N, and mid-season drying up to field capacity for moisture level in water-stagnant areas help to contain pests. China has adopted several methods for integrated pest management (Table 11).

Biological control involves the use of insecticide strictly on the basis of the need to conserve the natural enemies that are abundant in the rice ecosystem. For example,

Table 10. Promising restorers and hybrids possessing resistance to major insect pests in glasshouse screening tests at DRR, India.

Brown planthopper	
Restorers	Narendra 80, Pratap, T 1154, IET 9188, IET 10380, IR27315R, IR20226-24R, IR21916-12, IR35366-62, Suweon 332R, Milyang 46, Nanhigora, HKR 120, White Ponni, BG 367-4
Hybrids	IR58025A/IR21567, IR58025A/IR29723, IR58025A/IR54, IR58025A/Nanhigora, PMS 8A/IR31432, PMS 8A/IR27315, ORI 161, ORI 146
Gall midge	
Restorers	IET 9976, IET 9815, IET 9576, IET 9691, IET 10508, IET 8679, IET 9206, IET 9803, IET 10370, IR50, IR21015-12, IR29692-65, IR31858-90, IR35368-40-3-3, WGL 48684, WGL 3935, T 1154, Dhanyalaxmi, Mahaveer, Narendra 80m, Pal 579, Kalipidicham, Pratap, Sarathi
Hybrids	IR62829A/WGL 3962, IR62829A/IR50, IR58025A/WGL 3962, IR62829A/IR13603-30-IE-P-8R, IR62829A/IR40750-82, IR62829A/IR53915, IR62829A/IR54883-43, IR62829A/IR54883-100, IR58025A/IR10198-662 R, IR58025A/IR46, IR62829A/IR28238-109, IR62829A/IET 11680R, IR62829A/IET 11688R

Table 11. Management of biotic stresses on rice in China, 1994.

Practice	Area (ha)
Cultivated area	28,939,970
Chemical control	6,859,503
Spray	6,066,933
Seed treatment	6,747,940
Soil treatment	1,174,760
Biological control	1,448,369
Protection of natural enemies	1,647,420
Release of natural enemies	64,560
Spray of biofungicides	1,222,862
Spray of bioinsecticides	543,090
IPM demonstration	729,608

dipping of seedling roots in the insecticide before planting helps to conserve natural enemies. Releases of the egg parasites *Trichogramma japonicum* and *T. chilonis* were found to suppress populations of yellow stem borer and leaffolder. Insect sex pheromones show potential for pest monitoring and as a direct control tool through annihilation by mass trapping and mating disruption. A single application of a slow-release pheromone formulation at 40 g ai ha^{-1} within 2 wk after planting gave a reasonable control of yellow stem borer.

Disease management

Several diseases affect rice either alone or in combination with other diseases or insect pests. Many old disease problems are likely to persist. Several new ones could arise when hybrid rice is grown in large areas. This section discusses some potential problems that could affect hybrid rice and research on disease management.

Blast

Blast caused by *Pyricularia grisea* is the most destructive disease and is widely prevalent in prospective hybrid rice-growing areas. Rice blast affects all stages of crop growth. Losses are substantial when blast occurs at flowering.

The CMS parental lines currently used are highly prone to blast disease (Table 8). A few recently developed CMS lines that are highly resistant to leaf blast are IR68890A, IR68891A, IR68896A, and CMRS 4A. Nearly 250 potential restorers were screened and several were identified as highly resistant (with disease scores of 0–3). These were IR46R, IR29723-143-3-2, IR51078-33-2- 1- 1-3, IR21567-18-1 3, IR5472-22-19-3, IR32809-26-3-3, IR34686-179-1-2-1, IR55838-132-2-3-2-3, IR58841-48-b-3-2, IR57312-119-2-1, IR50400-64-1-2-2-2, IR49461-129-3-3-3, IR40750, IR13419, IR59685, IET 10462, IET 9302, R 312, ARC 11353, Suweon, IR64, and HR 12. Each year since 1991, 75–100 experimental hybrids were evaluated for blast resistance. Several hybrids-IR58025A/IR36R, IR58025A/IR32809-26-3-3R, IR58025A/IR57742-22-19-3R, IR58025A/IR32809-314-2-3-IR, IR58025A/IR21567-18-3R, IR58025A/IR29723-143-3-2-IR, IR58025A/IR39323-182-2-3-3R, IR58025A/IR54R, IR64608A/IR53915-5 1 -2-2-2R, IR64608A/IR53970-96-3-3-3 1 R, IR62829A/IR46R, IR62829/IR32809-26-3-3R, IR62829A/IRS4742-22-19-3-R, IR62829A/IR29723-143-3-2-1R, IR62829A/IR40750-82-2-3R3, IR62829A/IR44675-101-3-3-2-2R3, IR62829A/IR47310-94-4-3- I R, IR62829A/IR49461- 128-3-3-3R. IR62829A/IR53915-43-3-3R-3, IR62829A/IR54883-43-1-3R, OR 146, PA 103, PA 113, KMRH 3, HKRH 1001, PKRH 4, PKRH 7, MTU RH 2013 l, and AHl80l—were found to be resistant.

Generally, whenever blast-resistant R lines were used, the derived hybrids were also found to be resistant. A few highly resistant hybrids have also been identified in China (Table 7). Blast could be effectively managed in susceptible commercial hybrids by monitoring and need-based application of fungicides.

Sheath blight

Sheath blight disease caused by *Rhizoctonia solani* develops on infected tillers at first, and later moves rapidly to neighboring plants. The pathogen *R. solani* is a polyphagous competitive saprophyte and has a wide host range. Continuous rice cropping favors disease development. A crop with a high plant density normally associated with high leaf N content favors disease buildup from panicle initiation to flowering. Sheath blight has now become the most serious disease problem for rice production in China, where every year more than 15 million ha under rice are damaged by sheath blight (Table 1). Because resistance to sheath blight is not yet available, management of this disease assumes greater importance.

Rice hybrids are usually more susceptible to sheath blight. This increased susceptibility has been attributed to a shorter latent period, faster lesion development, and a larger number of sclerotia developed on rice hybrids. At present, the most common methods for management involve the use of clean seed, pathogen monitoring, cleaning of field bunds, field sanitation, and fungicide application based on disease development.

Bacterial blight

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is also a production constraint in China and India. Seven pathotypes have been identified in China. Types I and II are widely prevalent in north China and types II and IV are common along the Yangtze River. The disease occurs as a vascular wilt and leaf blight in northern India and as leaf blight in southern and eastern India. Bacterial blight can become a problem in the wet season, or after a cyclone or typhoon. Therefore, the success of hybrid rice also depends on the development of bacterial blight resistance in the hybrids. At present, most commercial hybrids are prone to bacterial blight, although several widely planted ones showed moderate resistance to it in China.

Minor insects and diseases

Many minor insect pests and diseases previously considered unimportant severely affect hybrids and CMS lines. Both sheath rot and bunt cause more damage to A lines than to B lines. Poor panicle emergence is considered to be one reason for a higher incidence of sheath rot. Similarly, bunt occurrence is also reported to be high on CMS lines in northern India because florets remain open for longer periods during anthesis. Armyworm and stink bug are also potential threats to hybrid rice cultivation.

Future outlook

Hybrid rice is now grown in most favored areas. We have limited knowledge on cytoplasmic-related susceptibility to insects and diseases. When large areas are planted to hybrids that have a narrow genetic base, this will certainly alter the balance in various ecosystems and favor pest outbreaks. Because of the susceptibility of hybrids to pests and pathogens, programs are needed for improving both parental lines and hybrids.

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CHAPTER 14

Physiology-based crop management for yield maximization of hybrid rice

S. Peng, J. Yang, F.V. Garcia, R.C. Laza, R.M. Visperas, A.L. Sanico, A.Q. Chavez, and S.S. Virmani

High grain yield of hybrid rice is attributed to high vegetative biomass production, high leaf area, large panicles, and, in some cases, high tillering capacity. Crop management strategies based on physiological characteristics of temperate hybrid rice have been developed to maximize yield in China. Early growth of temperate hybrids is promoted by proper seedbed management, plant spacing, and nutrient supply. Unproductive tillers are controlled by proper timing and rate of N application and by mid-season drainage or deep irrigation. Nitrogen topdressing based on leaf N status is practiced to regulate canopy size and architecture. Nutrient and water management even during late grain filling and delayed harvest is important for improving the grain filling of temperate hybrid rice.

Hybrid rice has recently been released for commercial cultivation in the tropics. Crop management practices used for conventional rice are also commonly used in tropical hybrid rice. Knowledge is limited on management strategies to maximize yield of tropical hybrid rice. The methods developed to maximize yield of temperate hybrid rice cannot be adopted for tropical hybrid rice. In 1992, IRRI began research on crop management of hybrid rice, especially N management for tropical hybrid rice based on physiological characteristics. Applying N at the basal and mid-tillering stages improved biomass production and sink size for both the tropical hybrid and the inbred cultivar. As sink size increased, however, the grain filling percentage of the inbred cultivar declined more than that of the tropical hybrid. Therefore, the tropical hybrid responded more to N application at the basal and mid-tillering stages than the inbred cultivar. Application of N at flowering increased leaf N concentration, Rubisco content, and photosynthetic rate in flag leaves. The grain filling percentage and yield of tropical hybrid rice were improved significantly by late-season N application. The inbred cultivar did not respond to N application at flowering in terms of grain yield. Rectangular planting and increased hill density improved sink size and grain yield only in hybrid rice. These results suggest that crop management strategies should be developed based on the physiological characteristics of tropical hybrids to fully express their yield potential.

China's success in hybrid rice has catalyzed research and development for hybrid rice in the tropics. In the past five years, some hybrid combinations developed at the International Rice Research Institute (IRRI) have shown a higher yield potential than the best indica inbred checks under tropical conditions. A few of these hybrids have been released for commercial production in tropical countries (Virmani 1994b). The magnitude of yield heterosis is similar between tropical and temperate hybrids. The major physiological traits responsible for yield heterosis are common between tropical and temperate hybrids. Hybrid vigor can be divided into favorable and unfavorable (Yang et al 1991). Favorable vigor contributes to the yield advantage of hybrids over conventional varieties. To fully express the yield potential of hybrid rice, management strategies should be directed to maximizing its favorable vigor and minimizing unfavorable vigor. Physiological differences between hybrids and conventional varieties should be used as the basis to develop management strategies specifically for hybrid rice. Most studies on optimizing hybrid rice management were conducted with temperate hybrid rice in China. These strategies cannot be adopted directly for tropical hybrid rice. With rapid progress in developing tropical hybrid rice, we must develop physiology-based management strategies for maximizing its grain yield.

Physiological traits responsible for yield heterosis

The yield advantage of hybrids is attributed to higher biomass production compared with the best conventional varieties. Patnaik et al (1994) reported that the vegetative growth rate measured at maturity (GRM) was highly correlated with the grain yield of hybrid rice. GRM was measured by dividing vegetative dry weight by days to 50% flowering. High biomass was the result of a greater accumulation of dry matter in the vegetative and reproductive stages. Dry matter accumulation was comparable between hybrids and conventional varieties after heading (Yan 1988). The higher growth rate of hybrid rice in the vegetative and reproductive stages was due to its higher optimum leaf area index (LAI) and greater leaf area duration (Xu et al 1984, Yan 1988). Ponnuthurai et al (1984), Yamauchi and Yoshida (1985), and Blanco et al (1986) also reported strong heterosis in leaf area development in tropical hybrid rice and a high correlation between LAI and shoot dry weight at the early vegetative stage. Hybrid rice often shows high parent heterosis for plant height (Virmani 1994a). Tall plant height is associated with high biomass production (Kuroda et al 1989). Significant positive heterosis in tillering capacity was reported by Govindaraj and Siddiq (1986), and Virmani et al (1981) found that tropical hybrids had a lower tiller number than the mid-parent, better parent, and check variety.

Heterosis in single leaf photosynthetic rate (P_{max}) was inconsistent (Akita 1988). Although there was usually no difference in P_{max} between hybrids and conventional varieties (Kabaki et al 1976, Yamauchi and Yoshida 1985), positive heterosis in the early vegetative stage and negative heterosis in the ripening stage were reported (Lin and Yuan 1980, Zhou 1994). These inconsistent results may be due to differences in N content of leaf tissue (Akita 1988) and growth stage. In respiration and photorespiration, negative heterosis was reported (Murayama et al 1984, Zhou 1994).

Total spikelet number is the component that contributes to higher yield of hybrid rice. The greater sink size of hybrids is the result of a larger number of spikelets panicle^{-1} rather than panicle number (Virmani et al 1981, Ponnuthurai et al 1984, Song et al 1990a, Patnaik et al 1991). Vigorous growth in the early and middle growing season led to the development of larger panicles (Cao et al 1980). Hybrid rice required lower plant dry matter to develop a spikelet than conventional varieties (Cao et al 1980). Kabaki (1993) also reported that japonica/indica hybrid rice showed an efficient sink formation in terms of unit dry matter compared with its parents.

Hybrid rice reaches the ripening phase with a higher biomass and larger sink size than conventional varieties. But the harvest index of hybrid rice was reported to be the same or slightly higher than that of conventional varieties (Ponnuthurai et al 1984. Blanco et al 1990). The grain filling percentage of hybrid rice was comparatively high in spite of its large number of spikelets (Yan 1988, Song et al 1990a). This was attained by the high ratio of reserves translocated from the culm and sheath to the spikelets and by the high LAI during the ripening period (Yan 1981, Song et al 1990a). Song et al (1990b) also reported that nonstructural carbohydrate content in the culm and sheath was higher for hybrid rice than for conventional varieties. Such differences did not exist in the leaf blade.

Management strategies for temperate hybrid rice

Promoting early growth

The final grain yield of hybrid rice depends more on panicle size than panicle number. The proportion of tillers that produced panicles is higher for hybrid rice than for conventional varieties because hybrid rice is planted at a lower density to reduce the cost of seeds. Only 10–20% of the panicles were produced by the main stems in hybrid rice (Yan 1978). Promoting crop growth in the very early stage is important for early and rapid tiller production. Matsushima (1980) reported a negative relationship between the number of ripened grains panicle^{-1} and the emergence date of tillers. In other words, the earlier a tiller emerges after transplanting, the larger and stronger it is likely to be. Seedling health and seedling age at planting, plant spacing, and fertilizer management are crucial to promoting early crop growth. Strong seedlings have the ability to tolerate stresses and reduce transplanting shock (Wen 1990).

Seedbed management. A low seeding rate is usually used to promote tillering and seedling vigor (150–300 kg ha^{-1} seedbed). Fertilizers of N, P, and K are needed, especially P and K (Wen 1990). A shallow water depth promotes root growth and is important for raising short and thick seedlings (Matsushima 1980). Tillering starts simultaneously with the emergence of the fourth leaf. Wen (1990) reported that tillers that emerged in the seedbed produced more spikelets panicle^{-1} (+20–25) than tillers that emerged after transplanting.

Transplanting. Transplanting is usually done when the sixth to seventh leaves are fully expanded, which corresponds to a seedling age of 35–40 d. At this time, most plants have 3–4 tillers. Hybrid rice is usually transplanted at 1 plant hill^{-1}. A rectangular planting geometry of 10 × 30 cm is used for better canopy ventilation, light distri-

bution, and development of heavy panicles. Transplanting shock is minimized if tillering occurs 4–5 d after transplanting. Deep transplanting prevents a quick recovery from transplanting shock and delays tillering (Matsushima 1980).

Fertilizer management. Organic fertilizers are applied at 10-15 t ha^{-1} (fresh weight) before plowing or puddling in addition to inorganic P and K (Jiang et al 1993d). From 50% to 70% of the total chemical N is applied basally or 5–7 d after transplanting (DAT). Before the mid-tillering stage (20 DAT), if the crop produces fewer than 15 tillers m^{-2} d^{-1}, additional N should be applied to stimulate tillering (Yan 1988).

Reducing unproductive tillers

Unproductive tillers compete for light and nutrients with productive tillers. At the maximum tillering stage, leaf area, dry matter, and N of unproductive tillers were about 24% of total aboveground plants (S. Peng 1994, IRRI, unpublished data). A hybrid that yielded 13.5 t ha^{-1} produced about 57% unproductive tillers (Xu et al 1984). Although C and N of unproductive tillers were translocated during their death to productive tillers (Mar 1964), the efficiency of translocation was not thoroughly determined. Reducing unproductive tillers at the middle growth stage promoted the development of heavy panicles and improved canopy structure and photosynthetic efficiency at the late growth stage (Jiang et al 1993d). To increase the number of productive tillers, tiller control is practiced when the stem number reaches 80% of the targeted final panicle number.

N fertilizer. The timing and rate of N fertilizer application have the greatest effect on tiller production and unproductive tiller percentage. The dilemma of N fertilizer management in the early vegetative stage is to promote early tillering and reduce unproductive tillering percentage. The content of NH_4^+-N in the soil solution is positively correlated with tillering ability (Jiang et al 1993d). Tiller production stops when NH_4^+-N in the soil solution drops below 30 ppm. The amount of N applied basally and after transplanting largely determines when the soil reaches this threshold. No nitrogen is applied after 20 DAT until the crop reaches panicle initiation to prevent excessive tiller production (Yan 1988).

Deep irrigation. When leaf number "n" is emerging, the tiller of the n-3 node emerges, the tiller bud of the n-2 node starts elongation, and the tiller bud of the n-1 node is very sensitive to adverse environmental conditions (Jiang et al 1993b). Deep irrigation applied when leaf number "n" is emerging stops differentiation of the tiller bud of the n-1 node. When the stem number reaches 80% of the targeted final panicle number, deep-water treatment at 10–20 cm for 15 d improved productive tiller percentage to 90–95% (Jiang et al 1993c).

Drainage. Drainage reduces NH_4^+-N content in the soil solution and therefore reduces tillering (Jiang et al 1993c). A drained field reached 30 ppm of NH_4^+-N 7 d earlier than a continuously flooded field. Tillering in the drained field stopped 9 d earlier than in the flooded field. Irrigation resumes when soil water content drops to 40-45%. Intermittent irrigation and drainage are repeated until the emergence of the second leaf of the main stem.

Regulating and controlling the rice canopy

Dry matter accumulation between panicle initiation and heading was highly correlated with sink formation (Kropff et al 1994). Leaf area index, leaf orientation, leaf N concentration, and N profile within the canopy affect canopy photosynthesis, respiration, and dry matter production. The rice crop reaches maximum LAI around booting or heading. Canopy photosynthesis is positively correlated with LAI until the crop reaches optimal LAI. An erect leaf and optimal hill spacing improve light distribution inside the canopy and increase optimal LAI. A maximum LAI of 10 is enough to produce 13.5 t ha^{-1} of yield (Xu et al 1984, Jifeng Yin 1995, IRRI, unpublished data). The duration that LAI is maintained above 6 or 7 could be more important than maximum LAI. Yan (1978) and Xu et al (1984) reported that maintaining LAI above 6 or 7 for 50–60 d was needed to achieve maximum grain yield. Leaf area of the second leaf of the main stem and flag leaf was highly correlated with spikelet number $panicle^{-1}$ (Yang et al 1992). The optimal length of the flag leaf was reported to be 40 cm (Yan 1978). Leaf area of the top three leaves at heading should be 75% of the total leaf area (Yang et al 1992).

Leaf N concentration should be about 4% at mid-tillering and should remain at 3% between panicle initiation and heading (Yan 1978). Simulation modeling suggested that a steeper slope in the vertical N concentration gradient in the leaf canopy with more N present in the uppermost stratum enhanced canopy photosynthesis (Dingkuhn et al 1991). Excessive leaf N reduces leaf thickness and causes more droopy leaves. It stimulates excessive leaf expansion and tiller production, and therefore causes mutual shading. High leaf N causes more dry matter to be partitioned to leaf blades than to the leaf sheath (Yan 1978). Less dry matter accumulation in the leaf sheath results in weak stems. High leaf N concentration is associated with high respiratory losses and high susceptibility to pest damage, especially when LAI is large. Jiang et al (1993a) developed a canopy index that was the product of LAI and leaf N concentration (%) at heading. The threshold limit of the canopy index was 39.4, below which filled spikelets and grain yield increased as the canopy index increased. This threshold should be adjusted according to total radiation level and leaf orientation. Nitrogen management and drainage between panicle initiation and booting are the most effective ways to regulate and control canopy size and architecture. Applying N around panicle initiation to increase spikelet number is normally not practiced in China because the large amount of organic fertilizer applied before transplanting supplies stable and sufficient nutrients for sink formation. About 10–20% of total inorganic N is applied when the second leaf emerges to reduce spikelet abortion.

improving grain filling

Grain filling is affected by photosynthesis after flowering and remobilization of stored carbohydrates. Cao et al (1988) classified rice varieties into sink-limiting, source-limiting, and intermittent types according to source-sink relationship. Most hybrid rice belongs to the source-limiting type, with a high ratio of spikelet number to leaf area at heading.

In source-limiting varieties, the active filling phases of superior and inferior spikelets were separated by 16–29d compared with 8–9d for the sink-limiting type (Zhu et al 1988). Gu et al (1981) found that the filling pattern and complete filled grain percentage of superior spikelets were similar between a hybrid and a conventional variety. The difference in grain filling between them was due to inferior spikelets. Filling of the inferior spikelet started immediately after pollination for the conventional variety but started 8 d after flowering for the hybrid. "Two-step grain filling" is observed in hybrid rice, which means that pollinated spikelets stop development for several days but maintain the ability to fill later (Wen 1990). Liu (1980) reported that some inferior spikelets of hybrid rice start active filling 20–30 d after heading. If C and N are sufficient in the late grain-filling stage, these spikelets still have a chance to develop into filled grains. Zhu et al (1981), using soft X-ray continuous photography, observed that the kernel size of inferior spikelets continued to increase until 36-44 d after heading. Therefore, grain-filling duration of a panicle is generally longer for hybrids than for conventional varieties.

Water and nutrient management, even during late grain filling and delayed harvest, was important for increasing the number of filled spikelets in hybrid rice (Liu 1980, Zhu et al 1981). Zhu et al (1981) reported that filling duration of a single spikelet was negatively related to its final grain weight. Application of N at heading reduced the filling duration of inferior spikelets and increased their final weight. Increasing N uptake after flowering to 30% of total N uptake delayed leaf senescence and improved grain filling (Jiang et al 1993a). Common management strategies for improving grain filling of hybrid rice include (1) applying 10–20% of total chemical N at heading, (2) foliar spraying of P and K during active grain filling, and (3) delaying final drainage and harvest to extend grain-filling duration.

Management strategies for tropical hybrid rice

Commercialization of tropical hybrid rice has just begun. Knowledge on management strategies to maximize yield of tropical hybrid rice is limited. A few studies were conducted to determine the effect of different N rates on grain yield of tropical hybrid rice (Patnaik et al 1994, Ali and Khan 1995, Om et al 1996). Various transplanting spacings, seedling numbers $hill^{-1}$, and seedling ages were evaluated in tropical hybrid rice (Suprihatno and Sutaryo 1993, Durga Rani and Murthy 1994, Ali and Khan 1995, Om et al 1996). Crop management practices of conventional rice are commonly used in tropical hybrid rice (S.S. Virmani 1996, IRRI, personal communication). Simulation modeling suggested that high-yielding cultivars expressed their yield potential only under improved crop management practices (Agarwal et al 1997). Since 1992, IRRI has studied crop management, especially N management, for tropical hybrid rice based on its physiological characteristics.

Both temperate and tropical rice hybrids exhibit vigorous vegetative growth as reflected by positive heterosis in crop growth rate before flowering. After flowering, early and fast leaf senescence result in a limited C source for grain filling. A large panicle is the component responsible for yield heterosis of temperate and tropical

hybrid rice. Physiological differences between temperate and tropical rice hybrids are mainly caused by different environmental conditions. Tropical hybrid rice does not show as strong a heterosis in tillering as temperate hybrid rice because of early season high temperature and rapid N uptake. Leaf senescence during ripening is more severe and grain filling duration is shorter for tropical than temperate hybrid rice because of high temperature in the tropics. Crop management strategies for tropical hybrid rice should be developed based on the differences between hybrid and inbred rice as well as between tropical and temperate conditions.

Nitrogen management

Application of basal N. In the Philippines, farmers usually do not apply basal N (Fujisaka 1994). In the field, where yields of 4–5 t ha^{-1} can be achieved without fertilizer N, basal N application is not necessary for cultivars with a high tillering capacity such as IR72. Delaying N application until mid-tillering often prevented excessive tiller production for the tropical indica inbred cultivar (Peng et al 1996). Most tropical hybrid rice produced fewer tillers than tropical indica inbred rice (Fig. 1). We hypothesize that basal N application is important for tropical hybrid rice. In addition, stimulating early tillering by using basal N can reduce the cost of hybrid seeds.

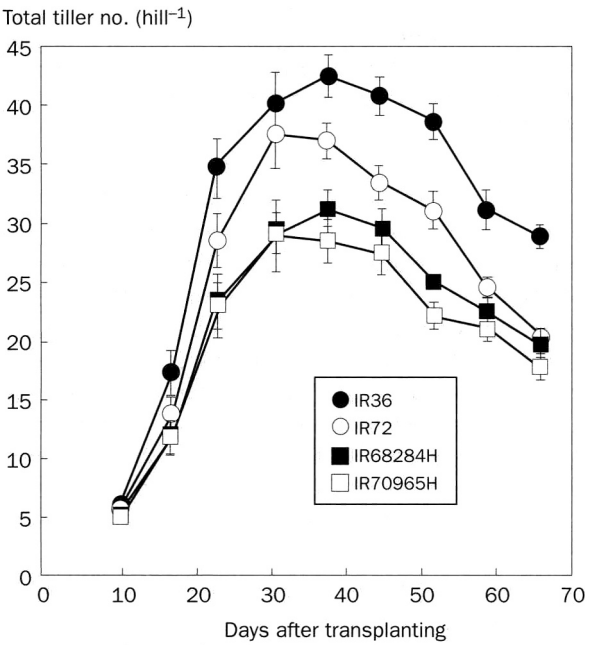

Fig. 1. Tiller number (including mother tillers) hill^{-1} of two indica inbreds (IR36 and IR72) and two tropical indica/indica hybrids (IR68284H and IR70965H) grown at IRRI in the 1996 dry season. Plants were transplanted at 20 × 20 cm with 4 seedlings hill^{-1}. Total N input was 200 kg ha^{-1} with four split applications: basally, at mid-tillering, at panicle initiation, and at flowering. Bar intervals represent ± one standard error and are smaller than the data points in some cases.

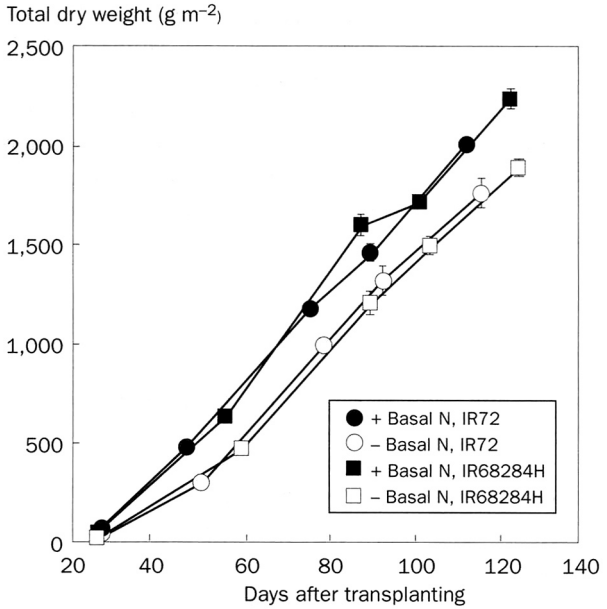

Fig. 2. Total dry weight of IR72 and IR68284H grown with and without basal N application of 60 kg ha^{-1} at the Philippine Rice Research Institute (PhilRice), 1996 dry season. All plots received 60 kg N ha^{-1} at mid-tillering and panicle initiation plus 45 kg N ha^{-1} at flowering.

In the 1996 dry season, we compared the importance of basal N application for an indica inbred cultivar (IR72) and a tropical hybrid (IR68284H). Fertilizer N treatments were the main plots and the two rice genotypes were subplots, with four replications. Fertilizer N was applied in four splits: basally (60 kg ha^{-1}), at mid-tillering (60 kg ha^{-1}), at panicle initiation (60 kg ha^{-1}), and at flowering (45 kg ha^{-1}). In each treatment, one split application—basally, at mid-tillering, or at flowering—was omitted to determine performance. In the control plots, fertilizer N was applied in all four stages. Applying N at panicle initiation is crucial for yield formation of irrigated rice; therefore, its importance was not determined for IR72 and IR68284H in this study. Fourteen-day-old seedlings were transplanted at 20 × 20 cm with 4 seedlings hill^{-1}. Both genotypes responded significantly to basal N application in terms of increased biomass production (Fig. 2).

Basal N application increased total sink size (Table 1) because of increases in both panicle number (Fig. 3) and spikelet number panicle^{-1} (data not shown). The increase in sink size was slightly more for IR68284H than for IR72. Increased sink size often resulted in a decrease in grain-filling percentage. The decrease in grain-filling percentage due to basal N application was significantly higher in IR72 than in IR68284H (Table 1). Therefore, filled spikelets of IR68284H increased more because of basal N application than did those of IR72. Basal N application was more important in increasing yield for the hybrid than for the inbred cultivar (Table 2).

Table 1. Effect of N application basally (60 kg ha^{-1}), at mid-tillering (60 kg ha^{-1}), or at flowering (45 kg ha^{-1}) on yield and yield components of IR72 and IR68284H grown at the Philippine Rice Research Institute in the 1996 dry season. The control plot received 60 kg N ha^{-1} basally and at mid-tillering plus 45 kg N ha^{-1} at flowering. All treatments, including the control, received 60 kg N ha^{-1} at panicle initiation. Yield components were determined from a 0.5m^2 harvest area.

Genotype	Without basal N	Without mid-tillering N	Without flowering N	Control	Effect of basal N[a] (%)	Effect of mid-tillering N (%)	Effect of flowering N (%)
Spikelets m^{-2}							
IR72	47,745 ± 2,383[b]	50,501 ± 1,268	56,820 ± 1,082	57,160 ± 603	19.7	13.2	0.6
IR68248H	46,252 ± 1,661	48,305 ± 289	55,934 ± 1,326	56,446 ± 1,319	22.0	16.9	0.9
Grain-filllng percentage (%)							
IR72	87.3 ± 0.9	86.7 ± 0.5	79.3 ± 1.3	79.1 ± 0.8	-10.4	-9.6	-0.3
IR68248H	79.8 ± 0.3	78.6 ± 0.5	72.9 ± 0.7	77.4 ± 0.7	-3.1	-1.6	6.2
Filled spikelets m^{-2}							
IR72	41,647 ± 1,721	43,779 ± 1,016	45,070 ± 1,044	45,214 ± 773	8.6	3.3	0.3
IR68248H	36,935 ± 1,444	37,969 ± 187	40.752 ± 867	43,683 ± 856	18.3	15.0	7.2
Filled spikelet dry weight (g m^{-2})							
IR72	954 ± 37	1,001 ± 20	1,025 ± 26	1,031 ± 14	8.1	3.0	0.6
IR68248H	949 ± 38	967 ± 11	1,053 ± 23	1,137 ± 24	19.8	17.6	8.0

[a] Calculated based on the control. [b] Mean ± standard error.

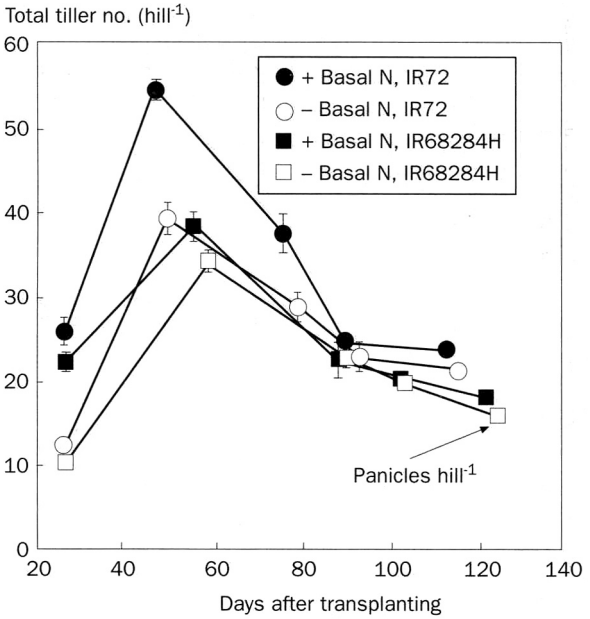

Fig. 3. Tiller number (including mother tillers) hill^{-1} of IR72 and IR68284H grown with and without basal N application of 60 kg ha^{-1} at PhilRice, 1996 dry season. All plots received 60 kg N ha^{-1} at mid-tillering and panicle initiation plus 45 kg N ha^{-1} at flowering.

Topdressing N at mid-tillering. The recommended N management in the Philippines is a standard split application of 2/3 incorporated before transplanting and 1/3 broadcast into shallow floodwater at 5–7 d before panicle initiation (De Datta et al 1974). Nitrogen is often not applied at mid-tillering if it is applied basally. In the same experiment conducted at PhilRice in the 1996 dry season, we compared the effect of N application (60 kg ha^{-1}) at mid-tillering on growth and yield of IR72 and IR68284H.

Both genotypes responded to mid-tillering N application by producing more biomass (Fig. 4). Without an N application at mid-tillering, leaf N concentration was very low at panicle initiation, especially for IR68284H. N application at panicle initiation and flowering did not change this pattern during the rest of the growth period (Fig. 5). Low leaf N reduced biomass production and sink formation. Mid-tillering N application increased total sink size (Table 1). The increase in sink size was slightly more for IR68284H than for IR72. The decrease in grain filling because of mid-tillering N application was significantly higher in IR72 than in IR68284H (Table 1). Therefore, filled spikelets of IR68284H increased more by mid-tillering N application compared with IR72. The increase in grain yield by mid-tillering N application was greater for the hybrid than for the inbred cultivar (Table 2).

Table 2. Grain yield of IR72 and IR68284H grown under different N management at PhilRice, 1996 dry season. Grain yield was measured from a 5-m^2 harvest area and expressed at 14% moisture content. In chlorophyll meter (SPAD)-based N management, N was applied only when the SPAD value dropped below the threshold of 35.

N treatment				Total N input	Grain yield		
BS[a]	MT	PI	FL		IR72	IR68284H	Heterosis
		(kg ha^{-1})			(t ha^{-1})		(%)
0	60	60	45	165	9.22 ± 0.30	9.45 ± 0.41	2.5
60	0	60	45	165	9.50 ± 0.14	9.62 ± 0.25	1.3
60	60	60	0	180	10.43 ± 0.19	10.80 ± 0.21	3.5
60	60	60	45	225	10.57 ± 0.05	11.19 ± 0.16	5.9
SPAD-based[b]				173–178	10.31 ± 0.13	10.10 ± 0.11	-2.0
N applied weekly at 15 kg ha^{-1}[c]				165	10.46 ± 0.12	11.14 ± 0.19	6.5
Effect of basal N (%)[d]					14.6	18.4	
Effect of mid-tillering N (%)					11.3	16.3	
Effect of flowering N (%)					1.3	3.6	

[a] BS = basal, MT = mid-tillering, PI = panicle initiatlon, FL = flowering. [b]N was applled at 14, 28, 42, 50, and 71 DAT with a total of 173 kg ha^{-1} for IR72 and at 14, 21, 36, 50, and 63 DAT with a total of 178 kg ha^{-1} for IR68284H. [c]N was applied weekly from 0 to 77 DAT at 15 kg ha^{-1} with a total input of 165 kg ha^{-1}. [d]Calculated using N treatment of 60-60-60-45 as the control.

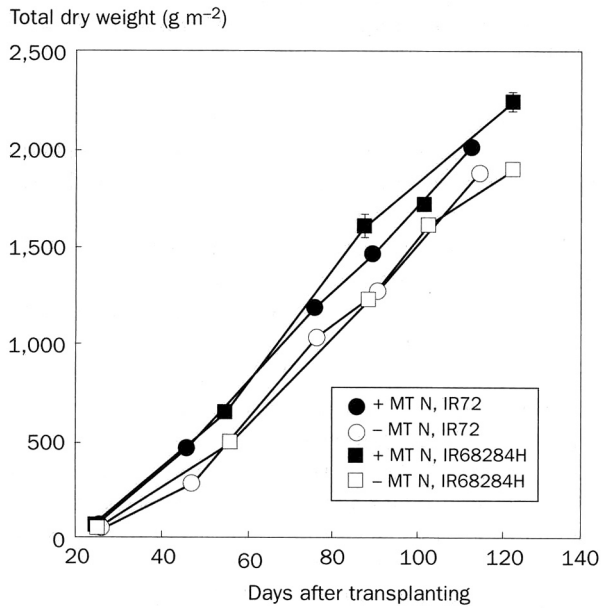

Fig. 4. Total dry weight of IR72 and IR68284H grown with and without mid-tillering N application of 60 kg ha^{-1} at PhilRice, 1996 dry season. All plots received 60 kg N ha^{-1} basally and at panicle initiation plus 45 kg N ha^{-1} at flowering. MT = mid-tillering.

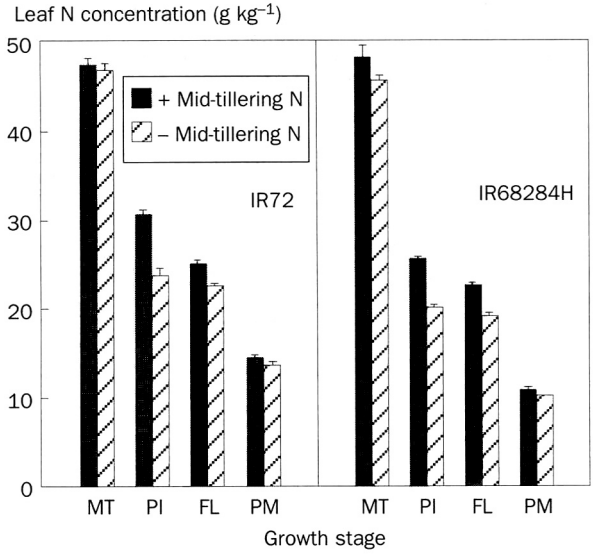

Fig. 5. Leaf N concentration at different growth stages in IR72 and IR68284H grown with and without mid-tillering N application of 60 kg ha^{-1} at PhilRice, 1996 dry season. All plots received 60 kg N ha^{-1} basally and at panicle initiation plus 45 kg N ha^{-1} at flowering. MT = mid-tillering, PI = panicle initiation, FL = flowering, PM = physiological maturity.

Topdressing N at flowering. Applying N at flowering is usually not recommended for semidwarf indica cultivars because yield response is often insignificant. This might be due to the unlimited source or limited sink size for most semidwarf indica cultivars such as IR72. Leaves of hybrid rice, especially the flag leaves, generally contain less N than the inbred cultivar (Fig. 6). Hybrid rice usually has large panicles and therefore greater sink size. It might be necessary to apply N at flowering for hybrid rice. We compared the effect of N (45 kg ha^{-1}) applied at flowering on yield and yield components of IR72 and IR68284H.

Nitrogen applied at flowering increased flag leaf N content significantly for both the inbred and hybrid rice (Fig. 7). This increase was also observed for the rest of the leaves besides the flag leaf (Fig. 8). Rubisco (ribulose- 1,5-bisphosphate carboxylase/oxygenase) content of the flag leaves was higher for the plants that received N at flowering (Fig. 9). A close correlation existed between photosynthetic rate and leaf N concentration of the flag leaves (Fig. 10). N applied at flowering enhanced the photosynthetic rate of flag leaves in both genotypes.

Nitrogen applied at flowering did not affect sink size but improved grain-filling percentage of IR68284H. Therefore, filled spikelet number and weight m^{-2} were increased by applying N at flowering for IR68284H (Table 1). Yield components of IR72 were not affected by application of N at flowering. Grain yield of IR68284H increased slightly by application of N at flowering. In the 1992 dry season, N applied at flowering increased the yield of IR64616H by 9% and of IR72 by 4% (IRRI 1993).

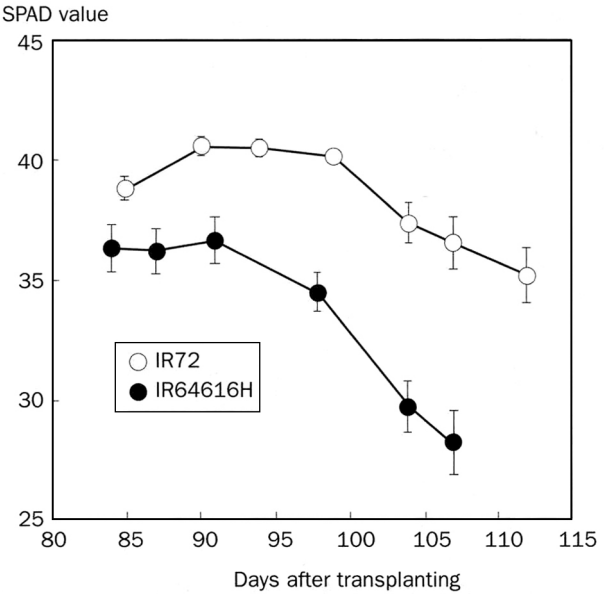

Fig. 6. Chlorophyll meter readings (SPAD values) of flag leaves of IR72 and IR64616H grown at IRRI, 1993 dry season. All plots received 60 kg N ha^{-1} basally, at mid-tillering, and at panicle initiation plus 45 kg N ha^{-1} at flowering.

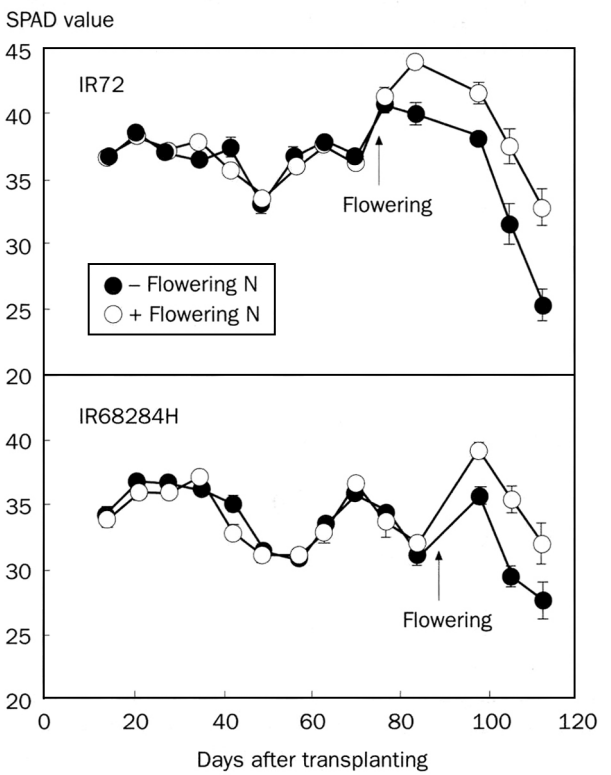

Fig. 7. Chlorophyll meter readings (SPAD values) of topmost fully expanded leaves of IR72 and IR68284H grown with and without N application at flowering of 45 kg ha^{-1} at PhilRice, 1996 dry season. All plots received 60 kg N ha^{-1} basally, at mid-tillering, and at panicle initiation.

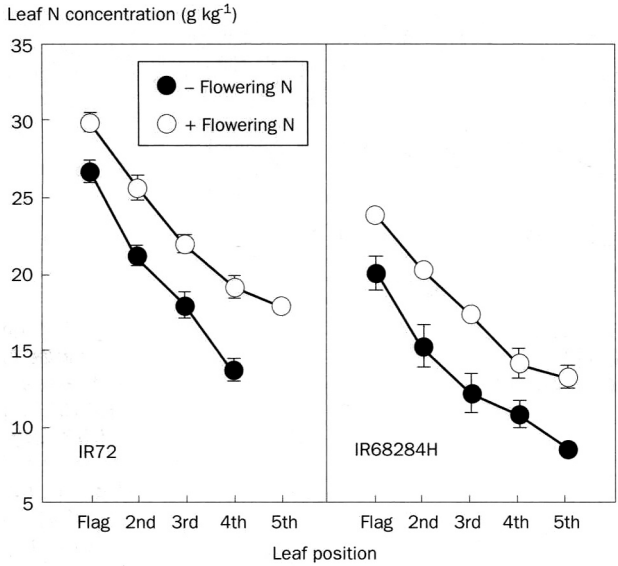

Fig. 8. Leaf N concentration of different leaves of IR72 and IR68284H grown with and without N application at flowering of 45 kg ha^{-1} at PhilRice, 1996 dry season. Samples were taken at the middle of grain filling. All plots received 60 kg N ha^{-1} basally, at mid-tillering, and at panicle initiation.

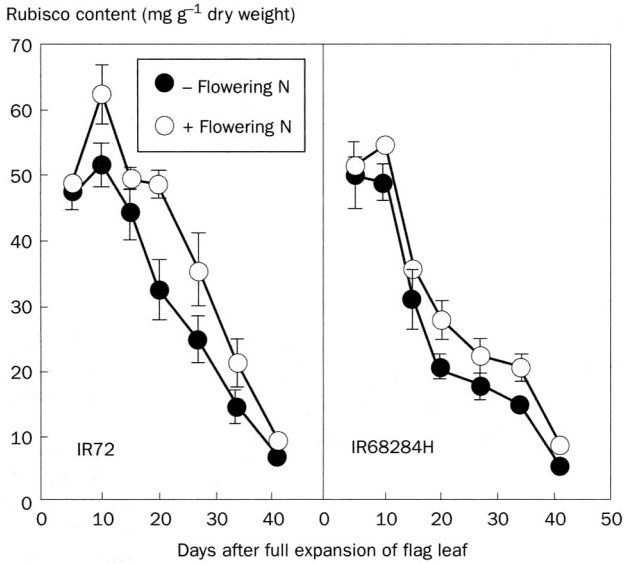

Fig. 9. Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) content of flag leaves of IR72 and IR68284H grown with and without N application at flowering of 45 kg ha^{-1} at IRRI, 1996 dry season. All plots received 60 kg N ha^{-1} basally, at mid-tillering, and at panicle initiation.

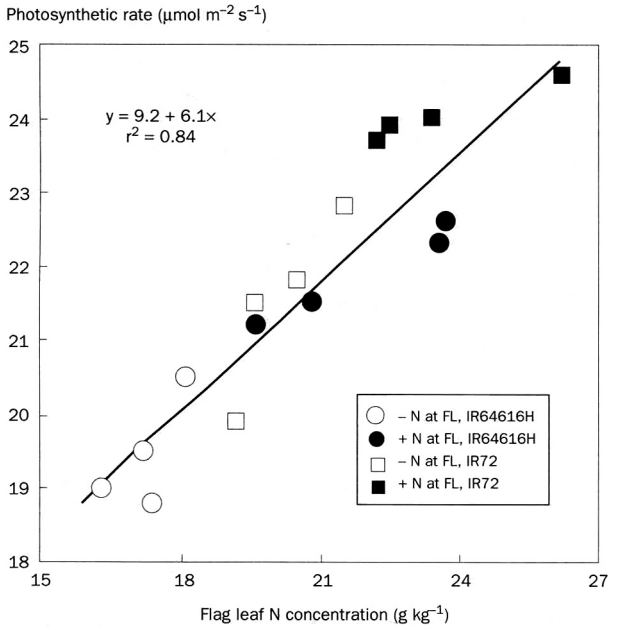

Fig. 10. Relationship between photosynthetic rate and leaf N concentration of flag leaves of IR72 and IR64616H grown with and without N application at flowering of 45 kg ha^{-1} at IRRI, 1992 dry season. FL = flowering.

Chlorophyll meter-based N management. A chlorophyll meter (SPAD-502) has been used to determine the timing of N topdressing for increasing N use efficiency and yield of indica inbred cultivars (Peng et al 1996). In this meter-based N management, N is applied only when the SPAD value drops below the threshold of 35 for IR72. Nitrogen is usually not applied basally if the soil N supply is enough at the early growth stage. The flag leaves of IR72 contain more N and their SPAD values are above 40 in the first-half period of grain filling. Therefore, monitoring N status using SPAD stops before the emergence of flag leaves and no N is applied after heading for IR72. We applied the same strategy for IR68284H.

Nitrogen was applied five times at 14, 28, 42, 50, and 71 DAT for IR72 and at 14, 21, 36, 50, and 63 DAT for IR68284H when the SPAD value was below 35. Total N was 173 kg ha^{-1} for IR72 and 178 kg ha^{-1} for IR68284H. Both genotypes had a low SPAD value in the early vegetative stage because of the lack of basal N application (Fig. 11). The SPAD value of flag leaves was much lower for IR68284H than for IR72 although neither received any N at flowering. Compared with the treatment that received the highest amount of N (225 kg ha^{-1}), IR72 produced 2.5% less grain with a N input of 173 kg ha^{-1}, whereas IR68284H produced 10% less grain with an N input of 178 kg ha^{-1} under SPAD-based N management (Table 2). The SPAD-based N treatment failed to improve grain filling and harvest index of IR68284H (data not shown).

The SPAD-based N management strategy needs modifications to be suitable for hybrid rice. First, basal N should be applied to promote early tillering of hybrid rice.

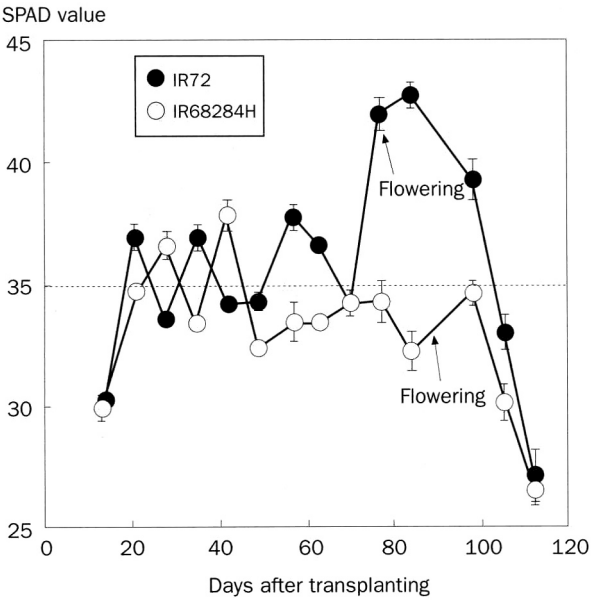

Fig. 11. Chlorophyll meter readings (SPAD values) of topmost fully expanded leaves of IR72 and IR68284H grown under chlorophyll meter-based N management at PhilRice, 1996 dry season. With this management, N was applied only when the SPAD value dropped below the threshold of 35. Total N input was 173 kg ha^{-1} for IR72 and 178 kg ha^{-1} for IR68284H.

Second, monitoring of the SPAD value should continue until mid-grain filling and N should be applied during flowering if the SPAD value drops below the threshold. Third, the threshold of the SPAD value should be determined specifically for different rice hybrids. The steady-state N supply mode achieved by applying N weekly from 0 to 77 DAT at 15 kg ha^{-1} with a total input of 165 kg ha^{-1} resulted in high grain yields for both IR72 and IR68284H (Table 2).

Plant geometry and hill density

For most semidwarf indica varieties with high tillering capacity, such as IR36, IR64, and IR72, the optimal transplanting spacing is 20 × 20 cm with 4–5 seedlings hill^{-1} in the Philippines (Mabbayad et al 1983). It may be necessary to increase hill density for genotypes with moderate tillering capacity such as most of the rice hybrids. We believe that rectangular planting facilitates the formation of large panicles.

We determined the effects of rectangular planting and increased hill density on yield and yield components of IR68284H versus IR72 at IRRI. Plant geometry and hill density were the main plots and the two rice genotypes were subplots, with four replications. Fourteen-day-old seedlings were transplanted at 10 × 16 cm with 2 seedlings hill^{-1}. The control plots were transplanted at 20 × 20 cm with 5 seedlings hill^{-1}. Both treatments had the same plant density—125 seedlings m^{-2}. All plots received 60 kg N ha^{-1} at mid-tillering and panicle initiation plus 40 kg N ha^{-1} at early flowering.

Table 3. Yield and yield components of IR72 and IR68284H transplanted at 10 × 16 cm with 2 seedlings hill^{-1} and at 20 × 20 cm with 5 seedlings hill^{-1} at IRRI in the 1994 dry season. Each plot received 60 kg N ha^{-1} basally, at mid-tillering, and at panicle initiation plus 40 kg N ha^{-1} at flowering. Yield components were determined from a 0.5m^2 harvest area. Grain yield was measured from 5 m^2 of harvest area and expressed at 14% moisture content.

Genotype	10 x 16 cm. 2 seedlings hill^{-1}	20 x 20 cm, 5 seedlings hill^{-1}	Effect of rectangular and dense planting (%)
Panicles m^{-2}			
IR72	463 ± 7	442 ± 10	4.8
IR68248H	416 ± 19	339 ± 4	22.7
Spikelets panicle^{-1}			
IR72	90.0 ± 2.8	90.9 ± 1.3	-1.0
IR68248H	113.0 ± 5.0	104.8 ± 1.5	7.3
Spikelets m^{-2}			
IR72	41,700 ± 1,600	40,200 ± 1,000	3.7
IR68248H	47,000 ± 3,800	35,500 ± 250	32.4
Grain-filling percentage (%)			
IR72	89.0 ± 1.0	89.9 ± 1.0	-1.0
IR68248H	79.9 ± 2.2	83.2 ± 1.3	-4.0
Filled spikelets dry weight (g m^{-2})			
IR72	856 ± 23	818 ± 30	4.6
IR68248H	985 ± 48	822 ± 16	19.8
Grain yield (t ha^{-1})			
IR72	9.08 ± 0.21	8.87 ± 0.17	2.4
IR68248H	10.06 ± 0.15	9.29 ± 0.27	8.3

Rectangular planting and high hill density increased the total sink size of IR68284H because of the increases in both panicle number m^{-2} and spikelet number panicle^{-1} (Table 3). The increase in sink size was not significant for IR72. Treatments of plant geometry and hill density did not affect grain filling percentage significantly. Rectangular planting and high hill density increased filled spikelet weight m^{-2} determined from 0.5 m^2 of harvest area and grain yield measured from 5 m^2 of harvest area more for IR68284H than for IR72 (Table 3). Therefore, rectangular planting and increased hill density improved grain yield of hybrid rice.

Conclusions

Our studies suggest that crop management strategies should be developed based on the physiological characteristics of tropical hybrids to fully express their yield potential. Crop management strategies differ across locations and genotypes. It is imperative that national agricultural research systems develop crop management systems for individual hybrid combinations and for their specific environmental conditions.

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CHAPTER 15

Improving grain quality of hybrid rice: challenges, strategies, and achievements

S.S. Virmani and F.U. Zaman

A major challenge in indica rice hybrid breeding is to ensure that the heterotic rice hybrids possess grain quality that is at least comparable, if not superior, to that of inbred check varieties grown by farmers. For this, a close linkage with an inbred rice breeding program is important to provide continuous access to newly developed elite inbred lines possessing desired grain quality characteristics. Most IRRI-bred cytoplasmic male sterile and restorer lines have grain quality similar to that of the check varieties. Therefore, grain quality in derived hybrids should be acceptable to farmers. To breed basmati rice hybrids, both parents need to possess basmati grain quality. Preliminary results from India and IRRI indicate the possibility of developing heterotic basmati rice hybrids. Deploying the thermosensitive genic male sterility system and anther culture could expedite the breeding of basmati rice hybrids.

Major determinants of grain quality in rice are (1) milling and head rice recovery, (2) grain size, shape, and appearance, and (3) cooking and eating characteristics. Hybrid rice developed in China, when introduced in Japan, Korea, and the United States, where consumers are highly conscious of quality, was rejected outright on account of its larger grain size, excessive chalkiness, and low milling yield. Even in countries like Vietnam, where consumers are less quality conscious, hybrid rice did not increase farmers' profitability. The reason was poor grain quality, although hybrids yielded 1–2 t ha^{-1} higher than inbred rice (Luat et al 1995). In several other tropical countries, Chinese rice hybrids were found to be unacceptable because of their susceptibility to major diseases and insects. Thus, higher yield of rice hybrids by itself would not make hybrid rice technology acceptable outside China. Rice hybrids must also have acceptable grain quality and resistance to major diseases and insects before rice farmers would accept them.

Rice is primarily consumed as a whole grain. The harvested grains from commercial F_1 rice hybrids represent the F_2 seed generation, which shows segregation for some grain characteristics. Therefore, a concern was expressed about consumer preferences because of the possible effect of this segregation on grain quality. Khush et al (1988) studied grain quality of several rice hybrids compared with that of the respective parental lines possessing diverse grain quality characteristics. They concluded that the genetic heterozygosity of hybrids did not impair grain quality in terms of physical and chemical characteristics as long as one of the parents was not a glutinous rice or poor in grain quality. To develop glutinous rice hybrids, both parents must have glutinous grains.

Hybrid rice breeding programs in the tropics develop parental lines of acceptable grain quality so that hybrids derived from them will also have this quality. Similarly, intensive efforts have been made in Japan and the United States to develop suitable parental lines that can result in rice hybrids with the required grain quality. In India and Pakistan, where special-quality basmati rice is grown for specific local and export markets, preliminary research has begun to develop basmati rice hybrids. IRRI scientists have also been involved in this initiative for the past two decades. A basmati rice breeding program has also been in progress at the Indian Agricultural Research Institute in New Delhi for three decades. It has resulted in the release of Pusa Basmati-1, a rice variety that combines basmati grain quality traits with a 50-l00% yield advantage over traditional basmati varieties such as Basmati 370 and Taraori Basmati. But few high-yielding basmati varieties are available for rice breeders even after three decades of intensive breeding efforts, indicating that breeding rice cultivars for basmati grain quality is quite complex and time-consuming. This chapter describes challenges, strategies, and achievements in improving the grain quality of hybrid rice.

Challenges in developing indica rice hybrids possessing improved grain quality

A major challenge in indica rice hybrid breeding is to ensure that heterotic rice hybrids possess grain quality that is at least comparable, if not superior, to that of inbred check varieties grown by farmers. Juliano and Villareal (1993), when studying grain quality characteristics of popular world rice varieties, observed that a wide variation in quality preferences existed between countries and between regions within large countries. All types of grain size and shape (except round shape) were represented among these rice varieties. Medium-sized grains were more common than long grains; short and medium-shaped grains were more common than slender ones and then bold ones. Extra-long grains were important mainly in Surinam. The long slender grains were the preferred ones in the Americas and exporting countries such as Myanmar, Thailand, Pakistan, and India (basmati growing areas). More people preferred rice grain with intermediate amylose content. Breeders in the tropics thus have to develop indica rice hybrids with grain quality specific to the target area. Because the hybrid rice breeding program at IRRI has to cater to the needs of various collaborating countries, IRRI-bred parental lines must have a range of quality characteristics.

Strategies used to breed indica rice hybrids with good grain quality

To breed indica hybrids with good grain quality in the tropics, the hybrid rice breeding program at IRRI has established a close linkage with inbred breeding programs for the irrigated rice ecosystem. This permits continuous access to newly developed elite inbred lines possessing different grain sizes, shapes, and chemical characteristics. The hybrid rice program also has access to elite lines from other countries through the International Network for Genetic Evaluation of Rice nursery materials. Elite lines from IRRI and outside IRRI are test-crossed to different sources of cytoplasmic male sterility (CMS) to identify maintainer and restorer lines. Promising maintainer and restorer lines are evaluated for grain quality characteristics.

The most promising of these lines are also crossed with available thermosensitive genic male sterility (TGMS) sources to develop TGMS lines with good grain quality. Heterotic hybrids derived from CMS and TGMS systems are evaluated for grain quality traits such as milling recovery (%), head rice (%), size, shape, chalkiness, amylose content, gelatinization temperature, and gel consistency, and are then compared with check varieties having acceptable grain quality.

Achievements in breeding hybrids with good grain quality for the tropics

To date, most IRRI-bred experimental rice hybrids introduced in national programs, including those released for commercial cultivation, are derived from two CMS lines, IR58025A and IR62829A. The grain quality of some IRRI-bred CMS lines and their maintainers was compared with that of inbred check varieties. Results (Table 1) indicate that in many cases the grain quality of IRRI CMS lines was similar to that of the check varieties and superior to that of the maintainer line, V20B, whose CMS line has been widely used in developing heterotic rice hybrids in China. Therefore, hybrids derived from IRRI-bred CMS lines can satisfy consumer preferences in the tropics, provided their male parents also possess acceptable grain quality. The grain quality of some IRRI-bred rice hybrids has been evaluated in India and it was found to be comparable with that of inbred check varieties (Siddiq et al, this volume, Chapter 24).

Perez et al (1996) studied the effect of quantity and timing of applied nitrogen on rough rice yield and some grain quality characteristics of a hybrid and two inbreds. The hybrid IR64616H not only yielded significantly higher but also had significantly higher head rice yield, protein content, and grain translucence than inbreds IR72 and IR58109-113-3-3-2 during the dry season (Table 2).

Table 1. Grain quality characteristics of commercial lines and some newly bred CMS lines compared with V20B and inbred check varieties at IRRI.

Line	Grain quality characteristics				
	Length[a]	Shape[b]	Chalkiness[c]	Gelatinization temperature[d]	Amylose content
IR58025A	3	1	5	L	17.2
IR58025B	3	1	1	L	16.7
IR62829A	5	5	1	I	22.6
IR62829B	5	5	1	I	20.6
IR66707A	3	1	9	I	17.4
IR66707B	3	1	5	I	20.0
IR67684A	3	1	0	L	23.2
IR67684B	3	1	1	L	23.6
IR68275A	5	5	5	L	22.2
IR68275B	5	5	1	L	22.7
IR68281A	3	1	1	I	19.8
IR68281B	3	1	5	I	20.6
IR68887A	3	1	5	L	21.2
IR68887B	3	5	1	L	22.5
IR68888A	5	1	9	L	21.7
IR68888B	5	1	5	L	22.6
IR68889A	5	5	0	I/L	21.7
IR68889B	5	5	0	I/L	22.4
IR68890A	5	5	0	L	21.7
IR68890B	5	5	5	L	23.0
IR68886B	3	1	9	I	22.2
IR68892B	5	5	1	L	23.0
IR68897B	3	1	5	L	22.7
IR68899B	3	1	5	L	23.0
IR68902B	3	1	5	L	22.0
IR69620B	5	5	1	L	16.5
IR69622B	5	5	9	L	22.4
IR69627B	3	1	5	I/L	16.0
IR69624B	3	5	5	L	23.2
IR96928B	3	1	0	L	16.4
PSBRc 4	3	1	1	HI/I	22.0
IR72	3	1	5	I	26.5
IR68	1	1	1	L	27.4
IR64	3	1	5	I	22.9
V20B	3	5	9	I/L	23.2

[a] Brown rice length: 1 = extra long (>7.5 mm), 3 = long (6.6–7.5 mm). 5 = medium (5.51–6.6 mm), and 7 = short (<5.5 mm). [b] Brown rice shape (length to width ratio): 1 = slender (>3.0), 3 =medium (2.1–3.0), 5 = bold (1.1–2.0), and 9 = round (<1.1). [c] Chalkiness of endosperm: 0 = none, 1 = small (<10%), 5 = medium (11–20%), and 9 = large (>20%). [d] Gelatinization temperature: H = high (not affected but chalky or swollen), HI = high or intermediate (swollen with collar incomplete and narrow), I = intermediate (swollen with collar complete and wide or split or segmented), and L = low (dispersed, merging with collar or completely dispersed and cleared).

Table 2. Effect of quantity and timing of applied nitrogen on rough rice yield, brown rice weight, milled rice yield, and protein content, whiteness, and grain translucence of milled rice of IR72, IR58109-113-3-3-2, and IR64616H, 1992 dry season, IRRI (Perez et al 1996).

N fertilizer treatment[a] (kg N ha^{-1})					Rough rice yield[b] (t ha^{-1})	Brown rice 100-grain wt (g)	Total milled rice yield (%)	Head rice yield		Milled rice		
										Protein content	White-ness	Trans-lucence
B	MT	PI	FL	Total				(%)	(t ha^{-1})	(%)	(%)	(%)
0	0	0	0	0	5.26 c	2.04 b	68.4 c	37.5 c	1.97 c	5.62 c	51.1 a	58.2 c
120	0	60	0	180	9.33 b	2.13 a	70.3 b	47.1 b	4.39 b	7.58 b	44.3 b	76.4 b
60	60	60	45	225	9.89 a	2.13 a	70.8 a	57.7 a	5.69 a	9.56 a	40.4 c	85.5 a
Variety												
IR72					7.98 b	2.04 b	70.3 a	38.8 b	3.10 c	7.63 b	45.2 a	69.5 b
IR58109-113-3-3-2					7.92 b	2.23 a	69.3 b	50.4 a	4.00 b	7.07 c	45.8 a	65.5 b
IR64616H					8.58 a	2.03 b	69.8 ab	53.0 a	4.55 a	8.05 a	44.8 a	85.2 a
Significance[c]												
N fertilizer					***	**	***	**	***	***	***	***
Variety					***	***	*	**	**	***	ns	***
N fertilizer × variety					**	ns	ns	ns	ns	ns	**	ns
CV (%)					3.3	1.8	1.1	19.4	22.5	4.4	3.0	6.7

[a] B = basal, MT = maximum tillering, PI = panicle initlation, FL = flowering. [b] Means in the same column followed by the same letter are not statistically different (P<0.05) by Duncan's multiple range test. [c] Level of significance: * = 0.01<P<0.05. ** = 0.001<P<0.01, and *** = P<0.001. ns = nonsignificant.

Achievements in breeding basmati rice hybrids

A number of elite basmati rice cultivars bred at IRRI were test-crossed to identify maintainer and restorer lines for CMS-WA cytoplasm. Most of these lines were maintainers and a few were restorers (Fig. 1). A number of these maintainer lines and Pusa Basmati-1, a commercial basmati rice variety introduced from India, have been converted into basmati CMS lines at IRRI (Table 3). These CMS lines do not have a high outcrossing rate comparable with that of IR58025A. At the Indian Agricultural Research Institute (IARI) in New Delhi, a number of basmati restorers have been developed (Table 4) by using the breeding strategy illustrated in Figure 2. Most of these lines are isocytoplasmic. Scientists at IARI have also converted Pusa Basmati-1 into a basmati CMS line and named it Pusa-3A. One experimental rice hybrid derived from a basmati CMS line and a basmati restorer at IRRI showed a higher yielding ability than Basmati 370 and comparable grain quality (Table 5). Seeds of this hybrid are being produced for evaluation in multilocational trials. Experimental basmati rice hybrids bred at IARI from Pusa 3A and basmati restorers have grain and cooking quality characteristics similar to those of popular basmati check varieties (Table 6).

Considering the low restorer frequency among basmati rice cultivars, crosses have been made at IRRI with available TGMS sources to develop basmati TGMS lines that would not need restorers to breed heterotic basmati rice hybrids.

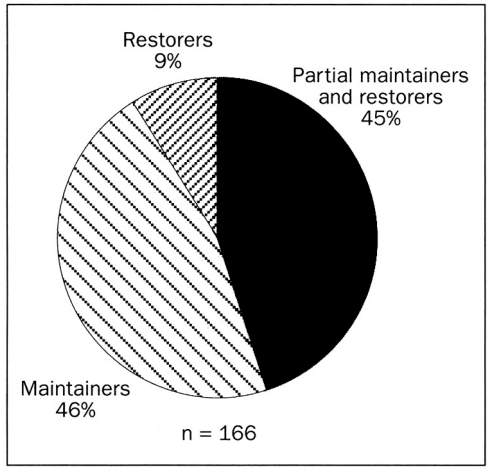

Fig. 1. Relative frequency of maintainers and restorers among basmati-type elite breeding lines at IRRI.

Table 3. CMS lines with basmati-type grain developed at IRRI. All have a slender grain shape.

CMS line	Days to flowering	Outcrossing[a]	GE ratio[b]	Aroma	Length	Chalkiness	Gelatinization temperature	Amylose content (%)
IR67684A	87	5	1.71	Strong	Long	None	Low	23.2
IR68280A[c]	88	4	1.92	Strong	Long	None	Intermed./low	22.3
IR68281A	88	5	1.64	Moderate	Long	None	Intermed.	19.8
IR69617A[c]	78	5	1.66	Strong	Long	Small	Intermed.	19.8
IR70372A[c,d]	96	6	1.90	Strong	Extra long	Small	Low	22.0

[a] On a 1–9 scale, where 1 = excellent and 9 = poor. [b]GE ratio = grain elongation ratio after cooklng. [c]Grain quality of the maintainer line is Indicated. [d] Derived from Pusa Basmati-1.

Table 4. Cooking quality characteristics of some basmati restorer lines developed at the Indian Agricultural Research Institute, New Delhi, India. All lines have an aroma.

Restorer line	Kernel length (mm)		Kernel breadth (mm)		Length/breadth ratio	
	Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking
SPS 95-694-2	7.07	12.00	1.67	2.33	4.23	5.15
SPS 95-694-3	6.73	12.20	1.74	2.26	3.87	5.40
SPS 95-81-1	7.60	12.60	1.87	2.50	4.06	5.04
SPS 95-81-3	7.80	13.80	1.44	2.33	5.42	5.92
SPS 95-81-4	7.33	12.14	1.44	2.44	5.09	4.97
SPS 95-81-5	7.33	13.33	1.47	2.40	4.99	5.55
SPS 95-81-6	7.20	12.00	1.40	2.47	5.14	4.86
SPS 95-167-1	7.60	11.74	1.80	2.87	4.22	4.09
SPS 95-768	7.07	12.40	1.40	2.00	5.05	6.20
Pusa Basmati-1	6.07	12.62	1.60	1.94	3.79	6.50
Karnal local	6.20	12.26	1.80	2.28	3.44	5.38

Table 5. Yield and grain quality characteristics of an experimental basmati rice hybrid evaluated in preliminary yield trials, IRRI, 1996 dry season.

Hybrid/check	Yield (t ha^{-1})	Length	Shape	Chalkiness	GT[a]	Amylose content (%)	Aroma
IR68280A/IR60975-46-1-2R	3.4	Long	Slender	None	I/L	21.0	Strong
Basmati 370 (check)	2.2	Long	Slender	Medium	I	20.7	Strong
IR68	3.9	Extra long	Slender	Small	L	27.4	None

[a]GT = gelatinization temperature: I = Intermediate (swollen with collar complete and wide or split or segmented) and L = low (dispersed, merging with collar or completely dispersed and cleared).

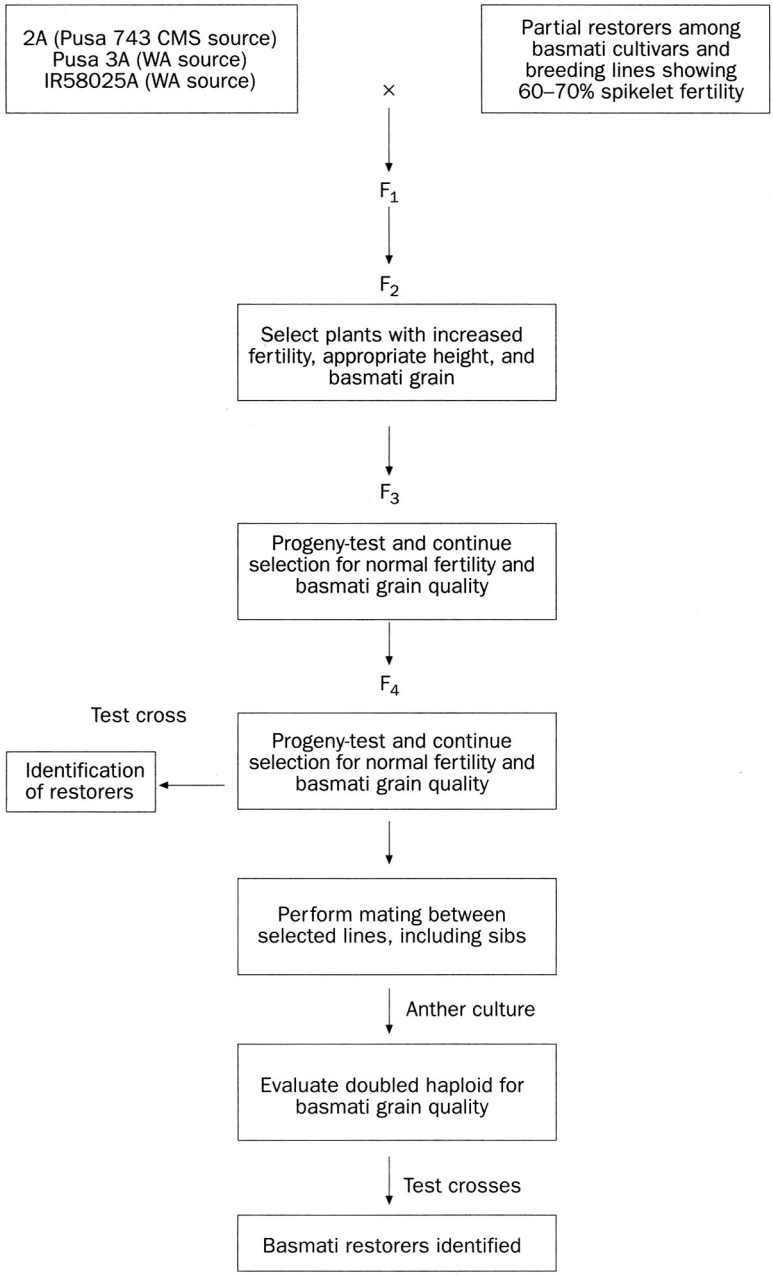

Fig. 2. Breeding strategy followed for developing basmati-quality restorers at the Indian Agricultural Research Institute, New Delhi.

Table 6. Grain and cooking quality characteristics of some basmati hybrids developed at the Indian Agricultural Research Institute, New Delhi. All hybrids have an aroma.

Hybrid	Kernel length (mm)		Kernel breadth (mm)	
	Before cooking	After cooking	Before cooking	After cooking
TC 85-23H	7.53	12.40	1.74	2.47
TC 95-30H	6.73	12.60	1.87	2.53
TC 95-35H	6.60	13.53	1.74	2.33
Pusa Basmati-1	6.07	12.62	1.60	1.94
Taraori Basmati	6.20	12.26	1.70	2.28

Future outlook

The grain quality of rice hybrids depends on the grain quality of the parents. It is therefore important that only parents that show consumer acceptability are chosen to make hybrids. Parents having a widely different endosperm appearance should not be chosen. It has been proven that segregation for different starch characteristics in bulk F_2 samples does not pose any problem for cooking and eating quality (Khush et al 1988). In order to develop rice hybrids possessing premier grain quality like that of basmati ones, both parents must possess basmati quality. The low frequency of restorer lines among basmati rice cultivars can be a serious handicap to developing basmati hybrids. But this problem can be overcome by using a TGMS system that does not require restorer lines. The pace of breeding basmati TGMS lines can also be improved by deploying anther culture in the desired crosses. Because most of the basmati restorer lines bred in India are isocytoplasmic, and have a narrow genetic base, it is important to develop a basmati restorer breeding population using a diverse cytoplasmic and genetic base. Composite populations using genetic male sterility are being developed for that purpose in India as well as at IRRI.

In temperate rice-growing countries such as Japan and Korea, indica rice is neither adaptable nor acceptable. Only japonica rice hybrids possessing the grain quality of premier japonica varieties (such as Koshihikari and Dongjinbyeo) are acceptable. Developing such hybrids also requires strategies similar to those used for basmati rice hybrids in which both parents need to have premier grain quality. Anther culture can also play an important role in expediting the breeding of parental lines for japonica rice hybrids.

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CHAPTER 16

Molecular and genetic approaches to understanding and engineering apomixis: *Arabidopsis* as a powerful tool

U. Grossniklaus, J.M. Moore, and W.B. Gagliano

Introducing apomixis into sexual crops will have far-reaching implications for seed production and crop improvement. Apomixis will allow the immediate fixation of any desired genotype and its clonal propagation through seeds. The manipulation of apomixis through biotechnology will only be possible through an interdisciplinary and multifaceted approach to investigating the regulatory mechanisms underlying plant reproduction. Apomictic and sexual reproduction are intimately related to each other, and the engineering of apomixis will require a detailed understanding of the genetic basis and the molecular mechanisms that control megasporogenesis, megagametogenesis, fertilization, and seed development. *Arabidopsis thaliana,* a member of the Brassicaceae, is particularly suitable for molecular and genetic analyses. Here we report on novel approaches that allow the identification and rapid molecular isolation of genes that control female reproduction in this model system. We describe genetic screens aimed at the isolation of mutants that display certain characteristics of apomixis and discuss their potential for engineering apomixis in agriculturally important crops.

A better understanding of the molecular and genetic basis underlying plant reproductive development will transform current breeding strategies and seed production. Despite the relatively limited knowledge of the molecular mechanisms that control plant gametogenesis, some of the most spectacular advances in plant breeding and agriculture have come from a manipulation of the reproductive system. These include the use of cytoplasmic male sterility and restorer genes for the production of hybrid seeds (Wych 1988, Hanson 1991, Levings 1993, Brar et al, this volume, Chapter 12), haploidization (de Buyser and Henry 1986, Snape et al 1986, Cowen et al 1992), and the manipulation of self-incompatibility (Olesen et al 1992). Over the past few years, the harnessing of apomixis, an asexual form of reproduction through seeds (Gustafson

1947, Nogler 1984a, Asker and Jerling 1992), has become an important goal of plant research (Hanna and Bashaw 1987, Koltunow et al 1995, Vielle Calzada et al 1996a).

The transfer of apomixis to sexual crops will have far-reaching implications for plant breeding, and the resulting commercial and agricultural benefits would be enormous (Hanna and Bashaw 1987, Dickinson 1992, Jefferson 1993, Savidan 1992, Vielle Calzada et al 1996a, Hanna et al, this volume, Chapter 22). Gametophytic apomixis can be viewed as a developmental variation of the sexual pathway where certain developmental steps are short-circuited (Koltunow 1993, Vielle Calzada et al 1996a). Its manipulation will require a better understanding of fundamental biological principles governing female gametogenesis. This chapter reviews developmental events during sexual and apomictic reproduction. It also reports on recent advances toward the understanding of these processes at the genetic and molecular level through studies in *Arabidopsis thaliana,* a member of the Brassicaceae, which has been widely adopted as a model system for plant developmental biology, and genetic and molecular investigations (Meyerowitz 1989).

Sexual reproduction and apomixis

Plants have evolved a characteristic life strategy with alternating generations, continuous postembryonic development, and the absence of a distinct germ line where somatic cells that have undergone many division cycles can ultimately give rise to reproductive cells. These specialized features have important implications for the development of plant gametes and embryogenesis (Walbot 1996). The plant life cycle alternates between a diploid and a haploid generation, the sporophyte and the gametophyte. Unlike in animals, where the meiotic products differentiate directly into gametes, the spores of plants undergo several division cycles to form a multicellular haploid organism. The differentiation of gametes occurs later in the development of the gametophytes. In angiosperm apomixis, the plant life cycle is short-circuited and sporophytic cells give rise to an unreduced megagametophyte (gametophytic apomixis) or directly to an embryo (sporophytic apomixis).

Sexual reproduction and development of the female gametophyte

The gametophytes of angiosperms consist of a small number of cells that develop in the sexual organs of the sporophyte. Male gametophytes are produced in the anthers and usually consist of three cells. The female gametophyte (megagametophyte or embryo sac) most often consists of seven cells and develops within the ovule, a specialized organ derived from the placental tissues of the ovary wall. Whereas male gametogenesis has been studied extensively at the genetic and molecular level (Albertsen and Phillips 1981, Kaul 1988, Mascarenhas 1992, Bedinger 1992, Goldberg et al 1993, McCormick 1993, Chaudhury 1993), the mechanisms controlling the development of the female gametophyte are largely unknown. Female gametogenesis has been difficult to study because of the inaccessibility of the embryo sac and the small number of cells involved. Many studies have focused on morphological descriptions (Cass and Jensen 1970, Russell 1985, Mogensen 1988, Huang and Russell

1992), but little emphasis has been given to the genetic regulation and molecular mechanisms controlling ovule development and megagametogenesis (Robinson-Beers et al 1992, Vollbrecht and Hake 1995, Nadeau et al 1996).

Megasporogenesis and megagametogenesis. The formation of ovules that harbor the female gametes is a key step in sexual reproduction. Much attention has been given to the ovule and its development in higher plants for more than a century (Hofmeister 1949, Maheshwari 1950, Bouman 1984, Reiser and Fischer 1993, Herr 1995, Schneitz et al 1995). Within an ovule primordium, a single hypodermal cell enlarges, differentiates into a megaspore mother cell, and undergoes meiosis to produce four megaspores (Fig. 1; Hill and Lord 1994). In *Arabidopsis,* the two meiotic nuclear divisions occur before cytokinesis, leading to the formation of tetrads with a linear or multiplanar arrangement (Webb and Gunning 1990, Schneitz et al 1995). Only the chalazal-most megaspore is not isolated by the deposition of callose, remains in contact with the sporophytic tissue of the ovule, and forms the megagametophyte (Webb and Gunning 1990). It has been suggested that the deposition of callose suppresses the nonfunctional megaspores by isolation, ensuring that only the chalazal megaspore enters megagametogenesis (Haig and Westoby 1986, Summer and van Caeseele 1988). Whereas the other three megaspores degenerate, the functional megaspore gives rise to an eight-nucleated syncytium in three mitotic cycles. Nuclear migration and the relative position of the division planes appear to play an important role in cell determination (Cass et al 1985, Russell 1993). Cellularization partitions the eight nuclei into seven cells (Fig. 1): an egg cell and two synergids at the micropylar pole, three antipodals at the chalazal pole, and a binucleate central cell whose nuclei fuse prior to fertilization in the center (Misra 1962, Reiser and Fischer 1993). After fertilization of both the central cell and egg, the ovule develops into a seed. Megagametogenesis is highly coordinated with the development of the surrounding sporophytic tissue of the ovule, the endothelium, and the inner and outer integuments.

Organization of the megagametophyte. The seven sister cells of the embryo sac are highly specialized and many studies have focused on ultrastructural and immunocytochemical analyses (Jensen 1965, Cass and Jensen 1970, Russell 1985, Mogensen 1988, Huang et al 1990, Huang and Russell 1992, Webb and Gunning 1990, 1991, Mansfield et al 1991) and in vitro fusion of isolated gametes (Kranz et al 1991, Faure et al 1993). In *Arabidopsis,* the two synergids and the egg cell are arranged in a triangular configuration to form the egg apparatus (Mansfield et al 1991, Webb and Gunning 1988). Synergids are highly specialized cells that constrain pollen tube attraction and the transport of male gametes into the egg and central cell (Mogensen 1988, Huang and Russell 1992). One of the synergids typically degenerates prior to fertilization, but the moment at which the degenerative process begins varies (Russell 1992). The egg cell is located at the micropylar end at a slightly chalazal position with respect to the synergids (Mansfield et al 1991). The egg cell and the synergids lack cell walls in the chalazal region, and their plasma membrane is in direct contact with the central cell. The central cell is the largest cell of the megagametophyte and is binucleated (Webb and Gunning 1991). Its nuclei originate from two different poles (chalazal and micropylar) and typically fuse before fertilization. Three antipodal cells

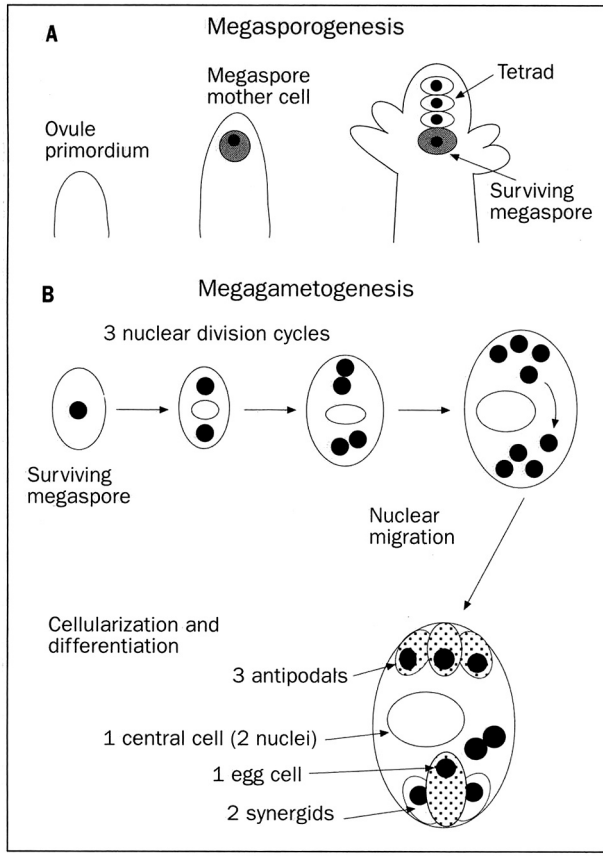

Fig. 1. Megasporogenesis and megagametogenesis in *Arabidopsis.* (A) A single megaspore mother cell differentiates in the nucellus and undergoes meiosis to produce four megaspores. Only the most chalazal one survives and gives rise to a megagametophyte of the *Polygonum* type. (B) Development of the megagametophyte. The surrounding sporophytic tissue of the ovule is not shown. Three free synchronous nuclear division cycles produce an 8-nucleated embryo sac. Cellularization produces the seven cells of the mature female gametophyte.

differentiate at the chalazal pole, but degenerate prior to the fusion of the two polar nuclei (Webb and Gunning 1988, 1990). The ephemeral nature of antipodals is intriguing and a clear role for these cells remains to be determined.

Mutants affecting megagametophyte development. Although genetic screens for female steriles have identified a number of mutants that disrupt ovule formation and yielded important insights into the molecular and genetic control of ovule development (Angenent and Colombo 1996), no systematic screens for mutants that affect female gametogenesis have been reported. Deficiency analysis of megagametogenesis

in maize (Vollbrecht and Hake 1995) and several transmission studies of chromosomal deletions (Patterson 1978, Coe et al 1988, Buckner and Reeves 1994, Vizir et al 1994) suggest that a large number of loci essential for female gametogenesis are dispersed throughout the genome. Nevertheless, only a few fortuitously identified mutants affecting this process have been described. In maize, megagametophytes carrying *lethal ovule* (*lo1* and *lo2*) mutations do not give rise to viable seeds (Singleton and Mangelsdorf 1940, Nelson and Clary 1952). *lo2* gametophytes arrest at the two or four nuclear stage of megagametogenesis (Vollbrecht 1994). Embryo sacs mutant for *indeterminate gametophyte (ig)* and the *r-X1* deficiency undergo abnormal nuclear division patterns and are transmitted through the female gametophyte at a reduced frequency (Kermicle 1971, Lin 1978, 1981, Weber 1983. Huang and Sheridan 1996). Two *Arabidopsis* mutations, *Gametophyte factor 1 (Gf1)* and *prolifera (prl),* are known to affect megagametogenesis (Redei 1965, Springer et al 1995). *Gf1* is not transmitted through the megagametophyte and shows reduced transmission through the male. Its developmental role is poorly understood. The *prl,* as a member of the *MCM2-3-5* family and a putative component of the DNA replication licensing factor, is required in all dividing cells.

Apomixis, an asexual method of reproduction through seeds

Apomixis occurs in more than 400 species belonging to about 40 families of the plant kingdom (Bashaw and Hanna 1990, Asker and Jerling 1992, Carman 1995). It is thought to have arisen in several different taxa independently. Apomictic embryos are derived from an unreduced cell lineage and are formed independent of fertilization. Therefore, apomictic seeds are genotypically identical to the mother plant and represent a genetically stable clone, a feature of utmost importance for agriculture. The developmental processes leading to apomictic reproduction are diverse and have been described in detail elsewhere (Nogler 1984a, Asker and Jerling 1992, Koltunow 1993, Naumova 1993). Here, we briefly describe the main classes of apomixis and illustrate their relationship to the sexual pathway.

Modes of apomictic reproduction. Apomictic processes can be divided into two fundamentally different classes (Gustafson 1947, Nogler 1984a, Koltunow 1993). In sporophytic apomixis, an embryo forms directly from a nucellar or integumentary cell in the ovule (adventive embryony). In gametophytic apomixis, the embryo results from an unreduced embryo sac whose egg cell develops parthenogenetically. In the latter case, the unreduced gametophyte originates either directly from nucellar cells (apospory) or from a megaspore mother cell that underwent aberrant meiosis resulting in the formation of two unreduced megaspores (diplospory). The unreduced egg cell in an apomictic embryo sac autonomously initiates embryogenesis in the absence of fertilization. Whereas some apomictic species are truly autonomous, most require the fertilization of the central cell to produce the nutritive endosperm (pseudogamous apomicts).

Genetic control of apomixis. In the few cases in which the inheritance of apomixis has been studied in hybrids of sexual and apomictic plants, it was found to behave as a single dominant Mendelian trait (Savidan 1980, Nogler 1984b, Gadella

1987, Sherwood et al 1994). Apomixis is closely associated with polyploidy. In many apomictic species with isolates of several ploidy levels, diploids are sexual whereas polyploid plants are apomictic (Asker and Jerling 1992). The dominant trait controlling apomixis is often not transmitted by haploid female gametophytes, suggesting that the locus causes megagametophyte lethality (Nogler 1984a). It has been proposed that apomixis is controlled by dominant mutant alleles of genes that play an essential role during sexual megasporogenesis and megagametogenesis (Mogie 1988). Alternatively, a locus controlling apomixis may be linked to gametophyte lethal mutations rather than directly causing megagametophyte arrest, as suggested by the recent isolation of diploid apomictic derivatives in *Hieracium* (Bicknell 1994). This and earlier observations suggest that polyploidy is not an absolute requirement for apomixis (Savidan 1980, Nogler 1982, Hashemi et al 1989).

Interrelationship of sexual and apomictic reproduction

Apomictic and sexual development are not mutually exclusive and, in facultative apomicts, both forms of reproduction coexist in the same plant or even the same ovule (Asker 1980, Nogler 1984a, Vielle Calzada et al 1995). The developmental regulation of sexual reproduction appears to be largely conserved during apomixis. Although an apomictic gametophyte or embryo has a distinct developmental origin, megagametophyte development, embryogenesis, and the development of the endosperm and seed coat are identical in sexual and apomictic reproduction. The sexual pathway is altered at two key points—meiosis and fertilization, the transitions between the two phases

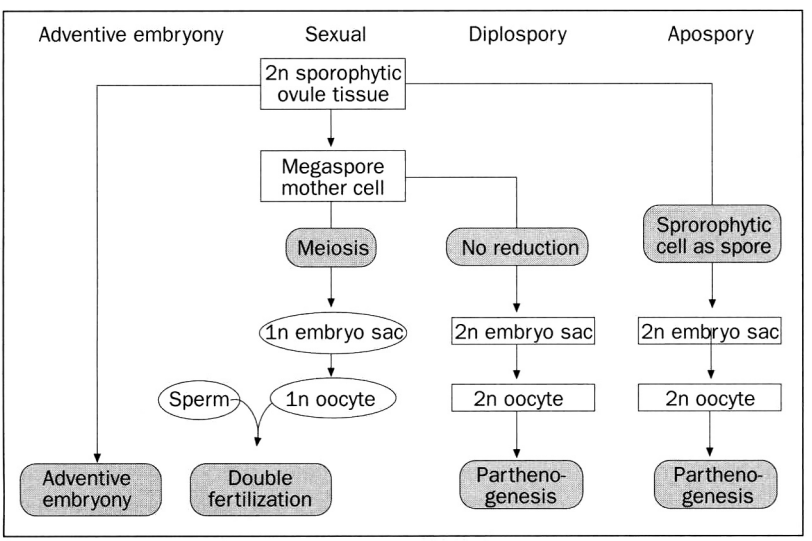

Fig. 2. Comparison of sexual and apomictic development. During apomictic reproduction, the sexual pathway is short-circuited (shaded boxes). In a first step, gametophytic apomicts form an unreduced embryo sac. In a second step, the egg cell is activated in the absence of fertilization or a sporophytic cell forms an embryo directly (adventive embryony).

of the plant life cycle (Fig. 2): (1) megasporogenesis is aberrant or aborted and (2) the unreduced egg cell or a sporophytic cell of the ovule (a nucellar or integumentary initial) develops autonomously into an apomictic embryo. Thus, apomixis can be viewed as a short-circuited sexual pathway (Vielle Calzada et al 1996a) in which part of the sexual developmental program begins at the wrong time or in the wrong cell. It is possible that apomictic reproduction results from the heterochronic or heterotopic expression of regulatory factors that control megasporogenesis and egg cell activation in sexual species (Peacock 1992, Koltunow 1993).

Although no fully apomictic mutants have been recovered in sexual species, several mutants and spontaneously occuring variations of sexual reproduction display individual components of apomixis, such as the production of unreduced spores (Rhoades and Dempsey 1966, Franke 1975, Jongedijk 1985), the formation of parthenogenetic haploids (Kimber and Riley 1963, Turcotte and Feaster 1963, Sarkar and Coe 1966, Chase 1969, Hagberg and Hagberg 1980), and the autonomous activation of endosperm development (Ohad et al 1996, Chaudhury et al 1997, R. Pruitt, S. Lolle, and U. Grossniklaus, unpublished results). The versatility found in sexually reproducing plants suggests that sexual and apomictic modes of reproduction share many regulatory components. At the level of gene expression, few differences could be detected between sexual and apomictic genotypes of *Pennisetum ciliare* (Vielle Calzada et al 1996b). The engineering of apomixis will require a better understanding of the genetic basis and molecular events that control megasporogenesis, megagametogenesis, and fertilization in sexual plants. What determines the commitment of a cell to a particular developmental pathway such as meiosis or megagametophyte development? What events lead to egg cell activation and the initiation of embryogenesis? How are these processes connected to the control of the cell cycle? The answers to these and related questions will provide important insights into the control of sexual reproduction and provide valuable tools for the engineering of apomixis.

The importance of apomixis for agriculture

The introduction of apomixis into sexual crops would completely revolutionize seed production and plant breeding, and is perceived as one of the most important scientific challenges facing modern agriculture (Dickinson 1992, Koltunow et al 1995, Vielle Calzada et al 1996a, Hanna et al, this volume, Chapter 22). Apomixis would allow the development of one-line hybrids—true breeding lines with permanently fixed heterosis (Bashaw 1980, Hanna and Bashaw 1987). This would greatly facilitate hybrid seed production and bears great promise for developing countries, where the high cost of hybrid seed is prohibitive to most farmers (David 1991). Most importantly, apomixis would allow the immediate fixation of any desired genotype (Koltunow et al 1995, Vielle Calzada et al 1996a). True breeding lines carrying complex multifactorially inherited traits could be established by selecting individual plants with the desired phenotype and fixing their genotype through apomixis. The development of true breeding cultivars carrying polygenic traits is difficult although much progress has been made through marker-assisted breeding. Apomixis would greatly

accelerate the breeding of crops adapted to specific agricultural, climatic, or economic needs and would allow the use of adapted local cultivars for breeding (Jefferson 1993, Koltunow et al 1995). Moreover, apomixis would allow true seed propagation of vegetatively propagated crops, as well as trees, flowers, and crops that are clonally propagated by tissue culture procedures (Savidan 1992).

The engineering of apomixis in sexual species

Although apomixis occurs in many plant families, it is found only in a few species of agricultural importance, such as forage grass crops, *Citrus,* apple and mango, as well as orchids (Wakana and Uemoto 1987, Bashaw and Hanna 1990, Naumova 1993). Apomixis has been described in wild relatives of several important grain crops, and breeding programs have focused on introgressing apomixis into economically important crops such as wheat and maize from distant relatives. The introduction of apomixis into a wide range of sexual plants, however, will have to rely on genetic engineering (Koltunow et al 1995, Vielle Calzada et al 1996a) and this requires a detailed knowledge of the genes and molecules involved in the regulation of sexual and apomictic reproduction. The interrelation of apomictic and sexual development suggests that mutants displaying certain aspects of apomictic reproduction that have been identified in sexual plants will provide important tools for the engineering of apomixis.

Introgression

Apomictic relatives have been identified for a number of important grain crops. Apospory is found in *Pennisetum squamulatum,* a relative of pearl millet, and diplospory has been reported in *Elymus* and *Tripsacum,* wild relatives of wheat and maize, respectively (Bashaw and Hanna 1990). To date, no apomictic relatives have been identified for rice (Khush et al, this volume, Chapter 23). Apomixis has been successfully introduced from wild relatives into pearl millet (Dujardin and Hanna 1989, Ozias-Akins et al 1993, Hanna et al, this volume, Chapter 22) and maize (Grimanelli et al 1995). But only apomictic plants with a high degree of seed abortion and additional chromosomes have been recovered. Although slow, introgression of apomixis into crop plants is attractive, because it is immediately applicable and does not depend on the identification and molecular isolation of the gene(s) controlling the trait. Introgression does, however, depend on the occurrence of apomixis in wild relatives and it can be severely impeded by breeding barriers (Koltunow et al 1995).

Biotechnology

The transfer of apomixis to a wide range of sexual species will be possible if we gain more insight into the genetic basis and the molecular mechanisms that control sexual and apomictic reproduction. Once key regulatory genes have been isolated, apomixis can be engineered by introducing such genes directly into crop plants by transformation. Ideally, the dominant locus controlling this trait would be isolated from an apomictic species. This approach is difficult, however, because little is known about the control and regulation of apomixis, and because of the poor characterization of apomictic

species at the genetic and molecular levels. Moreover, the isolation of the locus controlling apomixis could be difficult if it turned out to be complex and physically large. Major advances toward developing an apomictic model system have come from studies on *Hieracium,* a facultative aposporous apomict with autonomous endosperm development (Richards 1986). Recently, transformation procedures, tissue culture techniques, and heterologous transposable element systems have been developed for *Hieracium* (Bicknell 1994, Bicknell and Borst 1994, Koltunow et al 1995) and a genetic map is being established (R. Bicknell, personal communication). Alternatively, regulatory genes can be identified and isolated molecularly from sexual model systems with a firm basis for genetic and molecular research. A biotechnological approach toward the engineering of apomixis will have to target multiple steps during reproductive development, such as determination of the megaspore mother cell and its commitment to the meiotic pathway, egg cell activation, and the initiation of embryogenesis, as well as endosperm development.

Candidate regulatory genes

Important insights into the early phase of apomictic development can be gained from mutants that display some characteristics of apomixis. For instance, maize plants homozygous for *mac1 (multiple archegonial cells1)* contain multiple megaspore mother cells in an ovule (Sheridan et al 1996). A detailed study of this mutant may shed light on the determination of the megaspore mother cell, a process affected in some aposporous apomicts.

At the genetic level, the subsequent steps of meiosis have been studied extensively in *Zea mays* (Golubovskaya 1979, Golubovskaya et al 1992), and in the yeast *Saccharomyces cerevisiae,* for which a large body of knowledge on the molecular mechanisms governing meiosis has been accumulated (Mitchell 1995, Roeder 1995). Many yeast mutants have been isolated that regulate entry into meiosis and differentiate between meiotic and mitotic division (Mitchell 1995, Jefferson and Nugroho, this volume, Chapter 17). These mutants share some characteristics with apospory or diplospory of the *Antennaria* type (Koltunow 1993), and their plant homologs could be instrumental in the engineering of apomixis. In maize, similar mutants have been identified but their molecular nature is not known.

In plants homozygous for the *ameiotic1 (am1)* gene, meiosis does not occur and is replaced by a mitotic division (Palmer 1971). In *absence of first division (afd)* mutants, the first meiotic division is replaced by a mitosis (Golubovskaya 1979). Such a reversion to mitosis of a cell already committed to meiosis shows similarity to apomixis, characterized by a restitution nucleus at meiosis I *(Taraxacum* and *Ixeris* type, Koltunow 1993). A few mutants in maize and yeast produce unreduced spores reminiscent of diplospory. The yeast mutant *spo12* (Klapholz and Esposito 1980) and the maize mutant *elongate (el)* (Rhoades and Dempsey 1966) affect the second meiotic division and thus produce genetically diverse progeny. In *spo13,* however, meiosis I is omitted and a dyad of unreduced spores is formed by an equatorial division (Klapholz and Esposito 1980) in a process closely resembling the *Taraxacum* type of diplospory (Koltunow 1993).

To date, no homologs of *spo13* have been reported in other species, and we could not detect cross-hybridizing DNA in *Arabidopsis* and the closely related budding yeast *Kluveromyces lactis* by low stringent hybridization (H. Sims, U. Grossniklaus, unpublished results). It is important to stress that although meiosis in *spo13* mutants closely resembles an apomictic process, recombination still occurs and the progeny are genetically diverse. Thus, only a combination of *spol3* with a recombination-less mutant would produce clonal progeny. Such mutants have been extensively studied in yeast (Malone et al 1991) and homologs of one of these have been isolated recently from *Arabidopsis* (Sato et al 1995, Klimyuk and Jones 1997).

In addition to these regulators of megasporogenesis, a study of genes that control the cell cycle may provide useful tools for the engineering of apomixis. Recent studies in *Arabidopsis* have shown that misexpression of *cyclin1At* in root cells can trigger extra rounds of cell division (Doerner et al 1996) and it will be interesting to see whether similar experiments could induce proliferation of the egg cell.

Dissection of megagametogenesis by enhancer detection in *Arabidopsis thaliana*

The weed *Arabidopsis thaliana,* a member of the Brassicaceae, has been widely adopted as a model system for the developmental biology and genetics of flowering plants (Meyerowitz 1989). The small size of the plant, its rapid life cycle, and the large number of seeds it produces make it ideal for the isolation and study of mutants that affect biochemical and developmental pathways. The small genome size (~100 Mb), the high percentage of single-copy DNA (Pruitt and Meyerowitz 1986), and the rapid progress toward the completion of a physical map (Schmidt et al 1995) have made *Arabidopsis* a powerful system for molecular studies.

Highly efficient transformation methods (Bechtold et al 1993) and heterologous transposon systems for targeted gene tagging and genome-wide insertional mutagenesis are available (reviewed in Feldmann et al 1994). The *Arabidopsis* genome is likely to be the first plant genome to be completely sequenced. In light of the recent report on conserved gene order in large genomic regions (synteny) between dicotyledonous and monocotyledonous taxa (Paterson et al 1996), the information obtained in this project will also be of great importance for molecular studies in cereals. The amenability of *Arabidopsis* for genetic and molecular analysis makes it an ideal system for the identification and molecular isolation of genes that control sexual reproduction and for the isolation of mutations that interfere with this developmental process.

Enhancer detection

To identify genes expressed during megagametogenesis, we use enhancer detection, a novel technology that allows us to identify developmentally regulated genes based on their pattern of expression. Enhancer detection is one of the most powerful tools to identify tissue-specific genes and their regulatory sequences. The technique was developed in *Drosophila* and relies on a mobile genetic element carrying a reporter gene

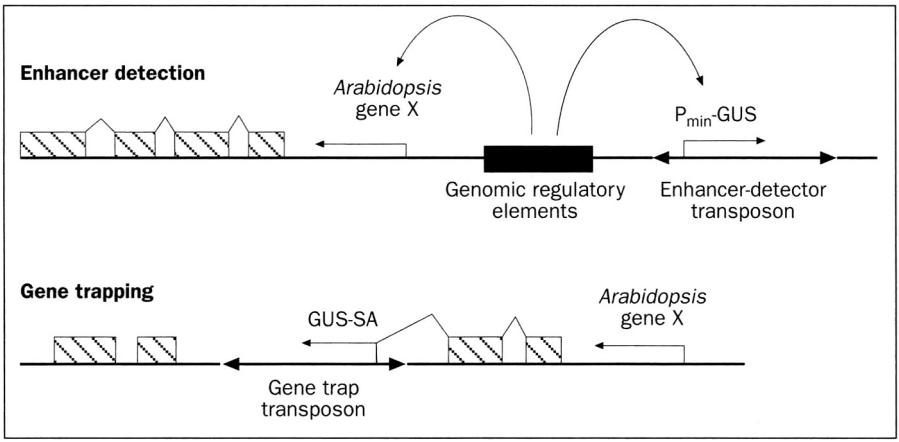

Fig. 3. Enhancer-detector and gene trap elements in *Arabidopsis.* In enhancer traps, the reporter gene (*GUS*) is controlled by a weak minimal promoter (P_{min}), which can respond to genomic regulatory sequences. In gene traps, GUS is preceded by an intron and splice accepter sites (SA) in all three reading frames.

under the control of a weak constitutive promoter (O'Kane and Gehring 1987). If this promoter comes under the control of genomic *cis* -regulatory elements (e.g., enhancers), the reporter gene is expressed in a specific temporal and spatial pattern (Fig. 3). This pattern reflects the expression of a nearby gene controlled by the same regulatory elements and thus allows us to identify genes based on their pattern of expression rather than on a mutant phenotype (Bellen et al 1989, Bier et al 1989, Grossniklaus et al 1989, Wilson et al 1989).

Gene traps are a modification of this approach, involving the generation of transcriptional fusions to the reporter gene (Gossler et al 1989, Skarnes 1990, Friedrich and Soriano 1991). Applying this approach in angiosperms should lead to the identification of many genes that control gametogenesis and cellular differentiation in the female gametophyte. In *Arabidopsis,* similar techniques based on T-DNA insertional mutagenesis (Topping et al 1991, Fobert et al 1991, Kertbundit et al 1991) or the *Ac/Ds* transposable element system from maize have been developed (Sundaresan et al 1995, Springer et al 1995, Smith and Fedoroff 1995).

Advantages of enhancer detection

Enhancer detection and gene trap systems also allow us to identify genes that are not easily amenable to classical genetic analyses (Bellen et al 1989, Bier et al 1989, Grossniklaus et al 1989, Wilson et al 1989). They have been especially useful in studying developmental processes that occur late in development, that is, after the effective lethal phase of a corresponding mutation, and in processes characterized by redundancy and high complexity. To identify the genes required in the gametophytic phase of the life cycle, enhancer detection has some important advantages over classical genetic screens:

1. Many essential genes that encode components of the basic cellular machinery will display a gametophyte lethal phenotype if disrupted. Essential genes with housekeeping functions are expected to show widespread, although not necessarily ubiquitous, expression during ovule and megagametophyte development, whereas expression in particular cell types of the megagametophyte suggests a function in cell specification and differentiation.
2. Because a large percentage of enhancer-detector insertions do not disrupt gene function, genes required for both micro- and megagametogenesis, for which only rare, partially penetrant mutations could be recovered, can be isolated.
3. By focusing on the cells and tissues where a gene is expressed, subtle phenotypes can be identified that may not be recognized easily in phenotypic screens. Moreover, reporter gene activity allows us to identify genes expressed in single cells, a powerful advantage for the study of sexual reproduction where single cells are differentiated from the neighbors.
4. Most importantly, enhancer-detector and gene trap transposons greatly facilitate the molecular cloning of genomic sequences flanking the insertion site and allow a detailed genetic analysis of the detected gene through remobilization and the recovery of additional alleles and regional chromosomal rearrangements (Grossniklaus et al 1992, Springer et al 1995, Tsugeki et al 1996).

Generating transposants

Transposants carrying enhancer-detector *(DsE)* or gene trap *(DsG)* elements were generated as described by Sundaresan et al (1995). Six independent starter lines carrying either *DsE* or *DsG* were used in combination with three independent *Ac*-lines. For positive-negative selection, approximately 700 seeds were plated on square 100 × 100 × 15-mm plates (Fisher) containing 1x or 0.5x MS salts (Carolina Biological Supply Company) adjusted to pH 5.7 with KOH, 1% sucrose (Sigma), 0.7% agar, 50 mg mL^{-1} kanamycin (Sigma), and 3.5 mM naphthalene acetamide (Sigma). The plates were kept at 4 °C for 4 d and then placed in a plant tissue culture incubator (Percival Scientific, modified model CU-32L with six instead of four shelves) with fluorescent light tubes (Philips TL70 or Trimline T8) at 22 °C under constant light. The distance between the shelves and the fluorescent tubes was 10 cm. The plates were scored for double resistant seedlings after 4–5 d in the incubator. They were transplanted to smaller plates and rescreened after another 4 d before transplanting them to soil. In brief, an enhancer or gene trap transposon can be mobilized by crossing a homozygous *Ds*-containing line to an *Arabidopsis* plant bearing a T-DNA insertion, producing *Ac*-transposase. Self-pollination of the F_1 plants will result in some F_2 progeny containing a transposed *Ds* element (transposants).

By selecting positively for the presence of *Ds* but selecting negatively against the donor *Ds* locus and *Ac,* unlinked stable transposition events can be recovered (Sundaresan et al 1995). Negative selection against the donor locus ensures that only unlinked or loosely linked transposition events are recovered, a prerequisite for genome-wide random insertional mutagenesis. Using six independent starter lines, we

generated approximately 45,000 F_1 seeds, of which more than 35,000 were grown up to harvest their F_2 seeds. About 20,000 of these F_2 families have been put through the positive/negative selection system to recover transposants. Between 20% and 25% of the F_2 families yielded an unlinked transposition event. As of February 1997, we have isolated 2,882 enhancer trap and 1,304 gene trap transposants (U. Grossniklaus, J. Moore, W. Gagliano, J.-P. Vielle Calzada, unpublished results).

Identifying genes expressed in cells of the megagametophyte

Very few genes with expression in the megagametophyte have been described (Nadeau et al 1996, Belostotsky and Meagher 1996). Genes expressed in individual cells of the embryo sac are yet to be identified and characterized. Enhancer-detector and gene trap transposons carry the *uidA* reporter gene encoding ß - glucuronidase *(GUS).* The expression of *GUS* can be visualized by histochemical staining (Jefferson et al 1987, Kavanagh et al 1988). To identify genes expressed during ovule development and female gametogenesis, we analyzed reporter gene expression in mature ovules of the first 1,000 transposants (U. Grossniklaus, J. Moore, W. Gagliano, unpublished results). Approximately 10% of the enhancer trap lines (n=511) and 3% of the gene trap lines (n=478) show spatially restricted *GUS* expression in mature ovules. Approximately 5% of the enhancer traps show expression restricted to either the sporophytic or gametophytic tissues of the ovule, respectively, whereas only two transposants show regional expression in cells derived from both generations of the life cycle.

Expression patterns in the megagametophyte. About half of the enhancer transposants with *GUS* activity in the ovule are expressed in the megagametophyte. Some are expressed in all cells of the embryo sac (Fig. 4A), whereas in others *GUS* expression is shared by only a subset of them, for example, the three cells of the egg apparatus (Fig. 4B). Most importantly, we also identified transposants with expression in individual cells of the megagametophyte such as the synergids, the egg cell, and the antipodals. Considerably more transposants stain in the synergids than in any other cell type of the embryo sac. This finding is consistent with the synergid being the metabolically most active cell of the megagametophyte (Jensen 1974, Russell 1993) while serving important functions for pollen tube attraction, sperm transport, and fertilization (Jensen et al 1985, Dumas and Mogensen 1993, Russell 1993).

Cell-type specific promoters. Some of the genes that are expressed in individual cell types or in the female gametophyte may serve important regulatory functions during sexual reproduction and could be involved in cell specification and differentiation processes. Alternatively, they may fulfill specific functions in these cells. If the corresponding genes control important developmental decisions during megagametogenesis as suggested by their expression pattern, mutant phenotype, and/or sequence, they may be useful for the engineering of certain aspects of apomictic reproduction. Importantly, the regulatory regions of these genes will be invaluable tools for the misexpression of candidate genes in particular cell types, We are currently identifying the regulatory regions that direct egg cell–specific expression (R. Baskar and U. Grossniklaus, unpublished results) and intend to use them to probe the

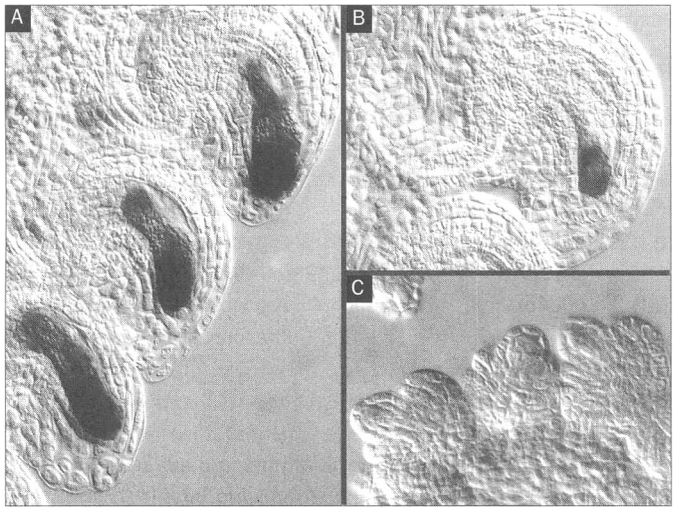

Fig. 4. Enhancer-detector lines with reporter gene expression during female gametogenesis. (A) Mature ovule before fertilization. *GUS* expression in all cells of the megagametophyte. (B) Mature ovule before fertilization. Reporter gene expression is restricted to the cells of the egg apparatus (egg cell and synergids). (C) Earlier stage of ovule development. The integuments have initiated and megasporogenesis is completed. The reporter gene is expressed in the dying megaspores but not the functional megaspore, which will give rise to the embryo sac.

potential of the egg cell for autonomous activation through misexpression of candidate genes such as cell cycle regulators. To identify genes involved in megasporogenesis (Fig. 4C), we are screening our lines for reporter gene expression at this stage of reproductive development (J.-P. Vielle Calzada and U. Grossniklaus, unpublished results).

Thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR)

Genomic fragments flanking the *Ds* insertions were isolated by TAIL-PCR (Liu et al 1995). Genomic DNA was isolated as described by Liu et al (1995); the precipitated DNA was resuspended in 50 mL TE (10 mM Tris, pH = 7.4; 1 mM EDTA, pH = 8.0). Alternatively, genomic DNA was prepared as described by Edwards et al (1991) and the precipitated DNA was resuspended in 25 mL TE. Three nested primers complementary to the 5' and 3' end of Ds were designed and used in consecutive rounds of PCR in combination with either the AD1 or AD2 primer described by Liu et al (1995). PCR conditions were essentially as described by Liu et al (1995), with minor modifications: the primary PCR reaction was incubated at 95 °C for 2 min before initiating the first 5 high-stringency cycles. In the secondary reaction, 15 instead of 12 supercycles, and in the tertiary reaction 25 instead of 20 low-stringency cycles, were performed.

A shift in size between a secondary and tertiary PCR product as predicted from the nesting of the Ds primers indicates the successful amplification of a border frag-

ment. The other half was used for subcloning the fragments into the pTAdvantage vector (Clontech) according to the instructions of the manufacturer or for direct sequencing of the successful reaction products. Sequencing was performed after purification of the reaction products with the QIAquick PCR purification kit (Qiagen) using the dRhodamine terminator cycle sequencing kit (ABIPrism) and Ds5-3 or Ds3-3 as sequencing primers. The nested Ds primers were Ds5-1 (5'-CCGTTTACCGTTTTGTATATCCCG-3'), Ds5-2 (5'-CGTTCCGTTTTCG-TTTTTTACC-3'), Ds5-3 (5'-GGTCGGTACGGAATTCTCCC-3'), Ds3-1 (5'-CGATTACCGTATTTATCCCGTTCG-3'), Ds3-2 (5'-CCGGTATATCC-CGTTTTCG-3'), and Ds3-3 (5'-GTTACCGACCGTTTTCATCC-3'). The degenerate primers were the same as described in Liu et al (1995) and AD1 (5'-NTCGA(G/C)T(A/T)T(G/C)G(A/T)GTT-3') and AD2 (5'-NGTCGA(G/C)(A/T)GANA(A/T)GAA-3').

We have isolated flanking genomic regions for 87 transposants (93% success rate for the recovery of at least one border fragment) using two different arbitrary primers (U. Grossniklaus, W. Gagliano, M. Hoeppner, unpublished results). Thus, TAIL PCR is a highly efficient method for rapidly isolating genomic regions flanking a *Ds* insertion (see also Tsugeki et al 1996). The average length of the products using a 128-fold degenerate arbitrary primer was 580 bp, ranging from 140 bp to 1.4 kb. The use of a less degenerate primer (64-fold) yielded larger products with an average of 775 bp (range: 120 bp to 1.5 kb), but the success rate for recovering at least one border fragment per locus was slightly lower (74% vs 82%). TAIL PCR products can be directly labeled for use in gel blots and library screens, and can be sequenced without subcloning.

Approximately 170 TAIL PCR products representing 87 loci that show reporter gene expression in developing ovules and embryo sacs or disrupt megagametogenesis were directly sequenced (M. Lodhi, R. McCombie, U. Grossniklaus, unpublished results). We identified 27 insertions into or nearby known genes or expressed sequence tags (EST) from *Arabidopsis,* 8 sequences with significant homology, and about 13 with weak homology to other sequences in the databases. Many of the sequences showed homology to ESTs of unknown function, while others were similar to genes encoding basic cellular factors involved in metabolism, as well as basic factors involved in transcription and translation. Importantly, we also identified genes encoding putative regulatory proteins involved in signal transduction processes and transcriptional regulation, which may serve key regulatory functions in sexual reproduction. As expected, gene traps expressing *GUS* are inserted in the transcribed regions because reporter gene expression depends on a chromosomal promoter. In contrast, enhancer-detector transposons are inserted within, as well as outside of, transcribed regions. Enhancer traps work in both orientations because they respond to genomic regulatory sequences.

Genetic screens for mutants displaying apomictic traits

An alternative to the isolation of regulatory genes controlling sexual development is the isolation of mutants displaying apomictic traits in a sexual species (Peacock 1992). Such an approach has been taken in several laboratories using *Arabidopsis thaliana* as a model system. Although no apomictic species have been described in this genus, the close relative *Arabis holboelli* is apomictic (Asker and Jerling 1992).

Arabidopsis mutants with autonomous seed development

Screens for mutants allowing seed development in the absence of fertilization took advantage of male sterile mutants and aimed at identifying second-site mutations that pseudo-suppress sterility. In *Arabidopsis,* unpollinated pistils do not elongate, such that pistil elongation is an easily scorable phenotype correlated with seed development. Different male sterile mutants have been used, such as *pistillata,* a homeotic flower mutant lacking a stamen and petals (Chaudhury and Peacock 1993, Koltunow et al 1995), the wax biosynthetic mutant *cer6* (Dellaert 1979, Preuss et al 1993), a conditional male sterile mutant that is fertile under high relative humidity (Ohad et al 1996), and the temperature-sensitive mutant *TH154,* isolated in R. Pruitt's laboratory (R. Pruitt, S. Lolle, and U. Grossniklaus, unpublished results), that is male sterile at high temperature but fully fertile at 18 °C.

Silique elongation under the restrictive condition indicates an asexual mode of reproduction with full or partial seed development, or the development of a fruit without concomitant seed production (parthenocarpy). Surprisingly, mutants displaying seed development without the participation of a pollen parent are recovered at a very high frequency. In a saturation screen performed in collaboration with R. Pruitt (Harvard University), we identified close to 500 putative mutants among a population of 15,000 to 20,000 mutagenized homozygous *TH154* plants. These mutants are currently being rescreened and characterized in more detail. Based on a pilot screen performed in 1995 in which we identified 26 mutants in 1,582 MI plants, we expect that at least half of the putative mutants will breed true. Three classes of mutations will be recovered:

1. Mutations suppressing the male sterility defect, which can be identified easily because they produce functional pollen.
2. Mutants displaying apomictic traits with autonomous development of the embryo, endosperm, or both.
3. Mutants displaying parthenocarpy.

In the majority of this type of mutations (e.g., 21 of 26 in our pilot screen), the mutant allele is not transmitted through the female gametophyte upon pollination (Ohad et al 1996, Chaudhury et al 1997) and can only be recovered through the pollen. Several mutants have been studied in detail and were shown to allow autonomous endosperm development, but they do not initiate embryogenesis in the absence of fertilization (Ohad et al 1996, Chaudhury et al 1997). Although these mutants do not produce fertile seeds, they show important characteristics of autonomous apomictic

reproduction, and their characterization constitutes an important step toward the engineering of apomixis.

A screen for mutants with pseudogamous apomixis

In cereals, the endosperm is permanent and of great economic value. Therefore, engineered apomixis in grain crops should allow for normal development of the endosperm, which is likely to require fertilization of the central cell (pseudogamy). In maize, proper development of the endosperm is strictly dependent on the presence of maternal and paternal genomes in a ratio of 2m: 1p, because of differential imprinting of the two parental genomes (Lin 1984, Kermicle and Alleman 1990). This is likely to be true for most agriculturally important grain crops (Haig and Westoby 1991). Imprinting phenomena may explain the high degree of sterility observed in apomictic hybrids, and have to be considered in introgression programs. Apomictic species may have a relaxed requirement for imprinting or may have evolved specific adaptations of the fertilization process that do not exist in sexual species.

Fertilization of an apomictic unreduced central cell with normal pollen will only sustain normal endosperm development if the correct ratio of maternal to paternal genomes is maintained. This can only be achieved if both sperm cells delivered by the pollen tube fuse with the central cell (Nogler 1984a, Reddy and d'Cruz 1969) or only one of the two polar nuclei and a single sperm nucleus participate in karyogamy (Savidan 1980). Alternatively, unreduced pollen could serve as the male parent (Chao 1980).

Although screens for *Arabidopsis* mutants displaying pseudogamous apomixis have been proposed (Chaudhury and Peacock 1993), they would be labor-intensive because they are based on outcrossing and scoring the progeny for exclusively maternal inheritance. In maize, such screens are greatly facilitated by the natural outcrossing mode of reproduction, the availability of embryo-specific markers that can be scored on whole kernels, and the multitude of genetic tools available to the geneticist. Over the past two years, we have developed maize stocks to perform a genetic screen that may lead to the isolation of mutants with characteristics of pseudogamous apomictic reproduction. Our ongoing screens aim to isolate parthenogenetic mutants, mutants causing nonreduction of the megaspore, or a combination thereof (U. Grossniklaus, unpublished results).

Conclusions and prospects

In summary, a multifaceted approach will be required for the engineering of apomixis in sexual plants through biotechnology. Various aspects of reproductive development will have to be addressed at the molecular and genetic level. A combination of (1) studies on the genetic basis and control of reproductive development in apomictic species such as *Hieracium,* (2) a detailed characterization of developmental events during sexual reproduction and the molecular mechanisms that control them, and (3) an analysis of mutants displaying certain characteristics of apomixis will provide

important insights into the regulatory control of plant reproduction and provide invaluable tools for the engineering of apomixis.

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Notes

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CHAPTER 17

Molecular strategies for hybrid rice: male sterility and apomixis

R.A. Jefferson and S. Nugroho

There is an urgent need for germplasm enhancement in rice to improve yield, quality, and adaptability to diverse environments. One promising approach is to exploit heterosis and develop high-yielding rice hybrids. Current hybrid technology is based on three-line systems involving a limited number of CMS sources. Efforts are being made to exploit the two-line system using TGMS and PGMS sources. We review some existing or proposed molecular technologies, and outline the potential for developing a robust two-line hybrid system. We also indicate the strategies needed to generate autonomous apomixis for fixing different hybrid combinations and thus take the next steps toward a new type of highly diversified and decentralized plant breeding. In particular, this chapter focuses on two areas of research under way at CAMBIA.

To meet projected demands for food security in the next 10–20 years, there is an urgent need for germplasm enhancement to improve yield and yield stability. The most promising approach to achieve this has been to introduce hybrid rice cultivation. Hybrids have been of great importance in the development of modern agriculture. In particular, large gains have been made by exploiting hybrid vigor in maize. In rice and wheat, however, the impact of hybrids has been much less, not because of a lack of potential, but rather because of a lack of opportunity to circumvent the self-pollinating growth habit of these crops. In China, where labor costs are modest, and where rice breeding is aggressively pursued, hybrids are proving to be of great importance. But even in such successful circumstances, there are major needs for increases in performance and, more importantly, for breakthroughs in methodology.

This chapter reviews some existing and proposed molecular technologies, and outlines the potential for developing a robust two-line hybrid system. We also indicate the strategies needed to generate autonomous apomixis for fixing different hybrid combinations and thus take the next steps toward a new type of highly diversified

and decentralized plant breeding. In particular, we focus on two areas of research under way at CAMBIA.

- The first research area covers strategies for developing a two-line hybrid system using transposon-mediated recessive male sterility coupled with conditional restoration, and a conditional transgenic protoxin activation method for male sterility of rice.
- The second research area is based on developing molecular apomixis for rice, and covers some of the steps necessary for generating a comprehensive "toolbox" to allow spatial, temporal, and exogenous control of transgenes associated with the development of the meiotic megaspore mother cell. This suite of tools will also have diverse uses ranging from plant breeding to plant protection.

Transgenic methodology: new approaches

The advent of genetic transformation in plants has dramatically increased the number of options and methods available for producing male sterility. Some of the criteria that can be set to establish a hybrid rice protocol based on male sterility that is economically viable and environmentally sound follow:

- Nearly 100% penetrance in male sterility, leading to pure hybrid seed production.
- 100% restoration of fertility of F_1 to ensure optimum grain yield.
- Male fertility rescued at least at moderate levels (>25%) for propagation.
- Environment-independent sterility and rescue.
- Trait should behave genetically as a single locus.
- Trait should be conditional, but have a tightly linked dominant genetic marker.
- Trait should be easily monitored and seed production verified.

This set of criteria is optimal, but many variants have now been proposed and promoted that do not meet these criteria. Several recent patents and publications have indicated new directions for overcoming many of the limitations.

Molecular approaches to creating male sterility have been patented or filed for patent by almost every major multinational involved in agriculture. A few of these approaches for transgenic male sterility include:

- Dominant destruction of male fertility through toxin expression, e.g., PGS Barnase system (three lines dominant, two transgenic lines)
- Antisense, cosuppression, or ribozyme inactivation of essential male fertility gene (three lines, two transgenic lines)
- Conditional male-specific expression of toxin (two lines, one transgenic line)
- Conditional antisense suppression of essential male genes (two lines, one transgenic line)
- Conditional rescue of dominant transgenic male sterility (two lines, one transgenic line)
- Conditional rescue of recessive male sterile mutation (two lines, one transgenic in a mutant background)

- Protoxin activation by male-specific transgene function (two lines, one transgenic locus)

Method 1

Among the first and most widely publicized methods was that of Plant Genetic Systems (PGS, now owned by AgrEvo) (Mariani et al 1992). This system involves the production of a toxic enzyme—an RNA-degrading enzyme from *Bacillus amyloliquefaciens* (BaRNAse) in the tapetum of transgenic plants, which destroys the pollen-forming ability of the organism. This trait is dominant, however, and both a restorer and a maintainer line are required. One of these lines must be transformed with a DNA construct that produces a stoichiometrically binding inhibitor of the RNAse.

This approach, although superficially attractive, is fraught with problems, as it requires two transgenic lines, and is thus a three-line system. It also involves production in the female of a highly toxic protein—relying only on specificity of a transgene promoter to maintain the normal health of the female line. Transgene function can be altered by environmental stresses; therefore, this system is, besides unwieldy, potentially risky (Brandle et al 1995). Three-line systems are also genetically more troublesome because they greatly limit the choice of parental material.

Method 2

Many other approaches, almost all involving a need for maintainer and restorer lines, have been published (mostly in the patent literature). They hinge on using one of two "mechanisms" of homology-dependent gene inactivation, often called antisense and sense suppression. In addition, some methods have been proposed that use the fascinating ability of certain RNA structures (often called ribozymes) to mediate sequence-specific RNA cleavage (Haseloff and Gerlach 1988). These methods have not been widely adopted in plants, however, because of problems of penetrance and a lack of robustness (Matsuda et al 1996). All of these methods assume sufficient inactivation of a gene or gene product associated with the male structures or function to render the transgenic line male sterile. Restoration of fertility by a second transgenic line is proposed, but is highly problematic. In the PGS system, at least a stoichiometric binding of a known inhibitor of the toxin is available. In the antisense approaches (with all three mechanisms of transgene inactivation lumped into this category), however, there is no such restoration function. Thus, restoration is typically cumbersome and of limited penetrance.

Methods 3, 4, and 5

These methods can be considered together because all three have a common weakness—the requirement for "conditional" transgene expression to affect sterility (Crossland 1994, Fabijanski and Arinson 1995, Robert et al 1994). This is a common thread throughout plant molecular biology and its applications. In some systems, topical or general application of a chemical compound can result in inducible (or suppressible) activity of a transgene. But few of these transgenes have been shown to operate

with adequate penetrance under field conditions. In addition, our understanding of the structure of plant promoters makes the combination of an inducible function with a cell- or stage-specific promoter difficult at this time, although some progress is on the horizon. The existing inducible systems, including the tetracycline repressor/operator (Gatz et al 1992), the copper-inducible system of the yeast metallotheionin gene (Mett et al 1993, 1996), and the glucocorticoid receptor (Schena et al 1991, Aoyama and Chua 1997) have some serious drawbacks, such as a poor dynamic range, a high background of noninduced expression, expensive inducers, poor access of the inducing molecule to target tissue, and/or limited penetrance.

Method 3 is particularly problematic because it requires strict cell- and stage-specific expression as well as inducibility, and control of transgene function is far too poorly understood for this method to be practical at this point.

Method 4 is better in that it proposes to inactivate a gene that has previously been shown to function only in the male flower parts or pollen. Thus, inappropriate ectopic (i.e., leaky) expression of the "antisense" or inactivation mechanism will not be lethal. A common feature of both of these methods, however, is that, in both systems, the default (resting state) condition is fertile. Thus, while facilitating 100% seed set (grain filling) in the hybrid, any limitation in the penetrance of sterility will result in less than 100% pure hybrid seed, a highly undesirable trait.

Method 5 avoids the latter problem because the default condition is male sterile. But it is seriously limited by another difficulty. Because the male sterility is dominant, the presence of the transgene in at least 50% of the F_1 hybrids will require extremely high penetrance of the restoration function, which is conditional. Therefore, broad area application of an inducing compound may be required. But some strategies can be envisioned that would use transgenic recombination systems such as cre/lox (Russell et al 1992, Osborn et al 1995) or FLP/FRT (Lloyd and Davis 1994, Sonti et al 1995) to facilitate irreversible restoration of fertility. Again, however, the reliability and penetrance of the transgene function under field conditions may make even this a suboptimal situation.

Method 6

This is a substantial improvement to the earlier methods, with few caveats. Pioneer Hi-Bred International has recently described what appears to be an efficient and effective method for transgenic two-line hybrids in maize, which may well point to a very effective method for rice as well (Albertsen et al 1995a, 1995b). From a commercial point of view, the production of hybrid seed must be optimized to ensure nearly 100% pure seed, and that seed must give 100% fertile progeny. The improvement by Pioneer seems to achieve this by first obtaining a mutation that exhibits fully penetrant but recessive male sterility. The recessive nature of this mutation (in the case of the Pioneer method, induced by the maize *Ac* transposable element [McClintock 1950]) assures that the F_1 hybrid will recover full fertility for effective grain yield. It also ensures 100% penetrance by empirically screening for just such a character and choosing a mutant allele accordingly. Thus, with the right mutation, many of the important criteria, except for restoration of male fertility in the homozygous male sterile

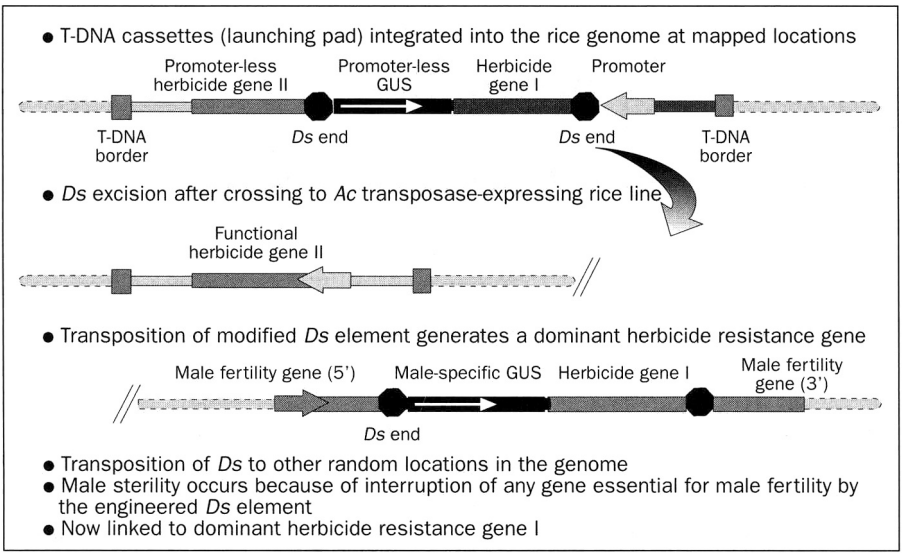

Fig. 1. Using the *Ds* enhancer-trap mutagen to create a male sterile line.

line, are met. By using the *Ac*-induced mutation as a "molecular handle," Pioneer then cloned the corresponding wild-type cDNA, and used it in a fusion with an inducible promoter—in their case the glucocorticoid system (Schena et al 1991, Aoyama and Chua 1997)—and reintroduced the chimeric inducible construct into the mutant line. The resulting line, especially if first screened for a line in which the transgene has integrated very close to the original mutation, is thus a conditional male fertile that meets most of the criteria for effective fertility restoration in the hybrid.

The transposon-mediated approach for recessive male sterility (modified from Albertsen et al 1995a, b) is given in Figures 1 and 2. This approach has several advantages: (1) transposons can be engineered to carry a dominant herbicide marker and introduced into rice, (2) transposon insertions can be obtained that cause a recessive male sterile mutation, (3) the transposon can be used to clone the wild-type gene, and (4) male fertility can be restored by fusion of the wild-type allele with an inducible promoter and introduction into a transgenic line.

As indicated above, certain key features are available to rice breeders that are not available to maize breeders. In particular, Pioneer's method used a "natural" *Ac*-induced mutation, and the corresponding presence of *Ac* to find the wild-type gene. But because *Ac* is found in many maize lines (reviewed in Gierl and Saedler 1992), and because *Ac*-induced mutations are not always stable and can revert, it was necessary to find nonreverting stable mutant lines that resulted from an imprecise excision of the *Ac* element. In addition, having either an active *Ac* element or even a nonautonomous *Ds* element in a parental line is not desirable for stable breeding of inbred parental lines.

Stable mutant lines that left a frame-shift footprint in the genome of the maize plant were obtained. Thus, the *Ac* was now missing from the male fertility gene, but

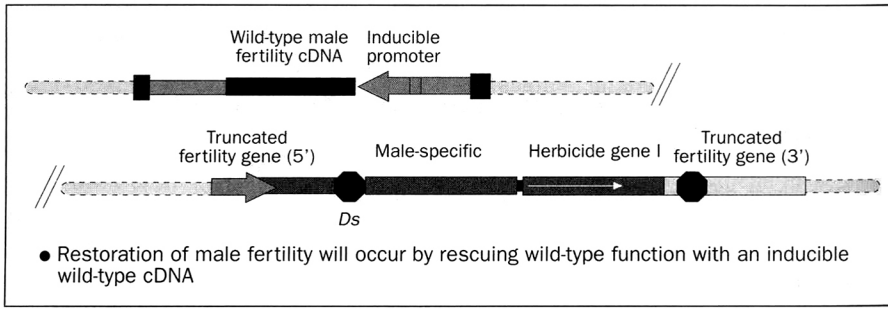

Fig. 2. Restoration of male fertility in the male sterile line by subsequent transformation.

the gene was irreversibly inactivated and "marked" by the insertion of an out-of-frame DNA fragment left behind. The only marker associated with the male sterility lesion was therefore the DNA sequence of this footprint. For advanced high-value breeding in maize, this is acceptable, because PCR-mediated diagnostics can detect this new DNA easily. But for the low-margin requirements of hybrid rice breeding, such a high-technology marking is not suitable.

In rice, *Ac* is not naturally present; therefore, leaving a nonautonomous *Ds* element is feasible and, in fact, desirable (Shimamoto et al 1993). As Figure 1 indicates, the strategy outlined uses a dominant herbicide marker within the *Ds* element to mark the male sterile mutation. This combines in a single step two desirable features—namely, a recessive male sterility mutation that can be followed readily in crosses because of the causally linked dominant herbicide resistance.

Potential spinoffs of a *Ds* population

Although obtaining male sterile mutations by such a *Ds* insertional mutagen is certainly going to be valuable, we can imagine an enormous increase in the value of such a mutational screen by adjusting conditions to develop a nearly saturated population of "enhancer-trap" insertions of *Ds,* heavily concentrated in particularly well-mapped locations on the rice genome. As Grossniklaus et al have described (Chapter 16, this volume), such an enhancer-trap *Ds* population in *Arabidopsis* has been and will continue to be a marvelous resource for plant breeders. A population using premapped launching pads, and with a number of key improvements, but maintained in rice in the field, could be the single greatest resource for rice breeders worldwide. Indeed, since the work of Kilian and others (Chen et al 1997, Kilian et al 1995), it is clear that the synteny between rice and other major cereals and grasses, including maize, wheat, barley, sorghum, millet, and sugarcane, is so great that the saturated rice population could also be of extraordinary utility for all breeders of graminaceous crops. This is particularly the case if the population is developed explicitly to facilitate such use, for instance, by including recombinational systems such as FLP/FRT.

Automated identification of mutant lines associated with virtually any sequenced fragment of DNA, such as the expressed sequence tags being developed through the

Rice Genome Project of Japan, could become routine. In addition, the ability to screen for traits under field conditions could be a great advantage.

Such a saturation strategy is now being undertaken in collaboration between CAMBIA and China's National Hybrid Rice R&D Center and Huazhong Agricultural University. Other participants are anticipated as well. The spinoff of this particular transgenic male sterility scheme makes it a most attractive method for some purposes.

Method 7

The concept of protoxin activation is an important synthesis of both genic male sterility and chemical hybridizing agents, which can reduce or eliminate the drawbacks of each method. Rather than the difficult and expensive quest for a toxic compound (CHA) with intrinsic specificity for male parts (e.g., pollen toxicity), without compromising female fertility or normal growth and yield, this approach uses a toxin of broader specificity (e.g., a herbicide) and renders it chemically inactive with a particular chemical modification to it, thus generating an inert protoxin. When this toxin is combined with a transgene whose function is to act on the protoxin and thereby render it toxic in the male parts, we have a facultative (conditional) two-line hybrid system. Unlike the more traditional spray-and-pray approaches to finding CHAs, this approach relies instead on the specificity of the transgene to provide the dynamic range to discriminate between dead pollen (or anthers) and live, female fertile plants. The facultative nature of the system is completely penetrant—until application of the protoxin, the plants are potentially completely normal and unaffected in their fertility. Only when the protoxin is applied to the plants, and acted on by the enzyme encoded by the transgene, is male sterility induced.

The advantages of this male sterility system are many. First, it promises to be a true two-line system. But instead of environmental variability in the restoration of fertility (which is typical of conventional systems, for instance with rice) and the huge burden of genetic drag and multilocus complexities, we have a fully facultative, monogenic trait for which the default condition is fertility, and only upon field application of a prepared (and potentially proprietary) biochemical will sterility arise. The quality of the system, however, will be determined by both the properties of the transgene product and the protoxin—in terms of chemistry, biology, and economics of production.

Dupont (O'Keefe et al 1994) has described a method that proposes a modification of a sulfonylurea herbicide and the expression of a transgenic enzyme to reactivate the herbicide. But the effect of the protoxin-activating enzyme on other endogenous compounds and the dynamic range afforded by the combination leave much to be desired. Also, the particular enzyme, a cytochrome P450, is not easy to monitor under field conditions, and would certainly be inappropriate to measure in most rice production systems.

Another method recently published (Kriete et al 1996) uses the popular herbicide phosphinothricin (marketed as Basta or Liberty) and renders it inactive by N-acetylation. This inactive herbicide is then reactivated in a male-specific manner by the tape-

tum-specific expression of a gene from *Escherichia coli,* the argE encoded L-ornithine N-deacetylase. The merits of this system have yet to be established in plants other than tobacco, but it shows promise because the herbicide is of limited environmental toxicity, is cheap and readily available, and in the published report the transgenic expression on argE is stated to have an effect on fertility in the absence of the protoxin. But few data are available yet. In addition, the method is proprietary, as is the protoxin.

Another method is to use the *gusA* gene, encoding b -glucuronidase, as a protoxin activator (Jefferson 1989, 1993, 1997, Hsu et al 1992).

GUS as the protoxin activator

Glucuronides (GUS substrates) are simple glycosides—Conjugates of virtually any compound with glucuronic acid, typically but not always through a 1-O glycosidic bond—and are among the most stable glycosides known. They are often as much as 30 times more stable than corresponding glucosides. In general, they are also water soluble due to the carboxylic acid group on the glycoside. They are often highly phloem-translocatable. In theoretical and experimental studies by Hsu et al (1995) at Dupont, glucuronidation dramatically increased the phloem translocation of most compounds.

Glucuronides are ubiquitous in animal biology—they are the natural detoxification forms of most xenobiotics, including many herbicides and invertebrates—and as such have been extensively characterized (e.g., Dutton 1966). More interestingly, they are natural biochemicals—often found in urine or bile—and as such it will be much easier to achieve approval for them for environmental use. There is a very good empirical method for preparing such compounds that involves feeding precursors to animals and collecting urine. A key advantage of the approach lies in the ability to prepare and test many different compounds for efficacy. We are not restricted to a single compound, and for differing production systems different compounds may be more suitable.

Another advantage of using GUS as the protoxin activator is that it probably has the highest field-test track record of any enzyme, and has been found to be innocuous in any plant tested, and any organ or stage. Because GUS activity is largely absent from all plant species, and because the glucuronides are very stable, even the issue of timing of application may be avoided with the proper choice of protoxin. There have been reports of endogenous GUS activity in a few field crops, but these have either not been repeated, or the activity reported is at very low levels and occurs under conditions of low pH (Alwen et al 1992, Hodal et al 1992). Even if such activity were present, the secretion of GUS or the import of substrates using permease into the cytoplasm would eliminate the caveat.

The expression of GUS under field conditions has now been documented for virtually all crops, with no loss of fitness or varietal characters associated with its expression. Thus, introducing the trait—or more specifically the "pro-trait"—into virtually any crop or breeding line can result in that line's potential use as a female parent in a breeding scheme. More specifically, because the testing of the trait (as GUS staining) is also robust and straightforward, monitoring the introgression of the

trait by conventional breeding becomes easy, and policing and verifying the quality of seed production and avoiding pilferage also become relatively trivial.

Although the concept of transgenic protoxin activation is straightforward, certain key features must be addressed to turn any of the schemes into a practical economic success.

The first is ensuring tight and properly regulated expression of the transgene in the appropriate cells and stages of the target crop. So far, the majority of the promoters that show anther/tapetum or even pollen-specific effects tend to be tightly reglated, but can function in a wide variety of crops (Koltunow et al 1990, Tsuchiya et al 1994). Also, in the GUS system, testing batches of transgenic plants for tapetum specificity using a trivial histochemical stain allows routine verification of trait stability. Verification of the stability of transgene expression becomes simple, thus greatly decreasing the risk.

Another key issue is ensuring that the protoxin and the activating enzyme come into suitable contact. If the enzyme is located intracellularly, the precursor protoxin must be transported or must diffuse across the membrane. Alternatively, if the enzyme is secreted, and localized in the periplasmic space, the protoxin need only have access to the apoplastic fluids. CAMBIA has a new technology that will likely provide an efficient secretion of a new GUS protein and thus provide access to the protoxin throughout the plant (A. Kilian, unpublished results). CAMBIA has developed another new technology that uses an integral membrane transporter to actively transport a wide variety of glucuronides, including protoxins. across living cell membranes (Jefferson 1995). We are optimistic that. when suitably engineered, this will provide an even higher dynamic range than the secretion approach.

The issue of protoxin choice can be dealt with through fairly empirical methods. In the case of the glucuronide protoxin. this can involve preparation and testing of candidates to find those that show adequate GUS-dependent toxicity or pollen inhibition, phloem translocatability, suitable persistence in the plant, and little background effect (i.e., highly stable). The goal is to find a protoxin that is affordable and easily formulated for modest-area applications.

It must be clear, however, that no matter what method of male sterility and restoration is chosen, it will at best only allow hybrid rice to benefit from conventional heterosis and hybrid breeding. The constraints of large-area evaluation and limited parental materials in various breeding schemes will apply, and the ability to breed specifically for local-area adaptation will still be elusive.

Apomixis

Hybrids will be vastly increased in value, scope, and utility if any heterozygous combination can be “fixed” and thus breed true, thereby allowing single-plant-level evaluation and propagation. This will be achieved by developing apomixis in rice. Apomixis can be considered formally as a combination of a failure to meiotically reduce the female gamete, the egg cell, and a precocious embryo and endosperm formation with the absence of fertilization by the male gamete (Koltunow et al 1995, Vielle-

Calzada et al 1996). It is not clear that these are necessarily separate or separable traits, however, and they could easily be envisioned as arising from an altered developmental pathway. Although apomixis in nature often seems to occur as a dominant trait that segregates as a single locus, if it is caused by two or more potentially deleterious events in combination, the only way these combined lesions would be fixed in a population under natural selection is if they were recombinationally joined as a single segregational locus.

A conditional nonpseudogamous apomixis trait (i.e., one requiring no pollination whatsoever) that allows the introgression of nontransgenic parental material, with a reversion to an apomictic habit, would open almost limitless possibilities. Even a pseudogarnous apomixis, however, which still requires pollen for endosperm formation, could provide substantial benefits. Some of the advantages that a properly designed apomixis could provide include: (1) production of large numbers of new hybrid cultivars from highly diverse parental materials, (2) single-plant evaluation, making plant breeding extremely rapid and responsive and encouraging "boutique breeding" for microenvironments, (3) propagation of hybrid seed directly by the farmer, (4) greatly expanded diversity of genetic resources used, and (5) reducing or eliminating fertilization and male-meiosis-related crop losses or anthesis-related crop losses.

Apomixis has long been the subject of study of a few visionary and dedicated breeders and crop geneticists who, with the tools available to them, have been trying to introgress apomixis into crop plants (Hanna et al 1993). To do so has required the availability of a related sexually compatible species that has components of apomixis. Although in plants with reasonable degrees of natural outcrossing, such as maize or pearl millet, we can find relatives (e.g., *Tripsacum,* LeBlanc et al 1995a, b, 1996; *Pennisetum,* Ozias-Akins et al 1993) with apomictic traits, we are confined to the properties of those particular traits. In this scenario, the best we can expect is that the target crop develops exactly the sexuality of the apomictic relative. But in no case is the natural apomixis in the existing crop relatives suitable. Penetrance is often low and the trait usually shows environmental dependence, is often pseudogamous (requiring pollen for fertilization of the central cell and production of endosperm), and is not controllable by plant breeders. Achieving goals with such starting material through introgression is highly unlikely.

Even more unlikely would be to find apomixis in wild *Oryza* populations related to a highly inbred species such as rice. The advantages of apomixis under natural selection would be negligible in such a circumstance; thus, expecting that evolution would produce apomictic rice is perhaps naive. Rather, using the power and potential of modern biological research to understand and then develop the apomictic trait through transgenesis is the most promising approach. It is this approach that we describe briefly here.

We can summarize an integrated strategy for developing apomixis through genetic engineering as follows:

- Genetic screens for and analysis of apomictic characters in *Arabidopsis thaliana.*
- Molecular, genetic, and cellular analysis of a model apomict: *Hieracium* spp.

- Tools to delimit and control expression in developing rice embryo sacs and megagametophytes: cell-type and stage-specific promoters.
- Complementation and homology analysis of meiotic mutants and genes in yeast.
- Mechanisms for control of transgene expression in the field: inducible/repressible promoter systems.

An integrated strategy for molecular apomixis

Programs in molecular, cellular, and genetic approaches to apomixis are increasing in number and quality, but most attention is being given to the upstream biology and genetics of the trait, and little attention is being given to developing the necessary tools to capture the trait for field use. This strategic goal is the focus of the research components being investigated by CAMBIA.

Arabidopsis as a genetic tool for apomixis

One approach in the search for apomixis or its components involves a detailed molecular and genetic analysis of *Arabidopsis thaliana,* the model system for plant genetics. Remarkable progress has been made in this direction in the past few years. Notably, Dr. Abed Chaudhury and his colleagues at the CSIRO Plant Industry have obtained, mapped, and characterized several independent mutations, called *fis* (for fertilization-independent seed), that show varying degrees of fully pollen-independent seed development (Chaudhury, personal communication). Although these mutant lines do not produce viable seed, they represent a very important start for understanding the nature of the parthenogenetic process. These lines were generated in a *pistillata* background, however, and are not easy to work with, although the cloning of the genes is well in hand.

In collaborative work, Dr. Robert Pruitt at Harvard University and Dr. Ueli Grossniklaus at Cold Spring Harbor Laboratory (Grossniklaus et al, Chapter 16, this volume) have accumulated a large number of lines that show fertilization-independent seed, in a genetic background with a conditional temperature-sensitive male sterility lesion. This background permits straightforward genetic manipulation, and hence will be a great resource because it can be readily analyzed and introgressed into material to investigate the effects when combined with meiotic lesions. In independent work at the University of California, Dr. Robert Fischer's lab has also isolated a mutant (*fie*) showing fertilization-independent endosperm formation (Ohad et al 1996).

Study of a model apomict

The second point in the fivefold path of the integrated strategy involves the rigorous analysis of an existing apomictic species chosen for both the quality of its apomixis approximating the ultimately desired trait and the ease of manipulation of the system. Much progress has been made through the efforts of Dr. Ross Bicknell, of the Crop and Food Research Institute in New Zealand (Bicknell and Borst 1996, Koltunow et al 1995). Bicknell has chosen to work with the small composite, *Hieracium,* which

had originally been described by Gregor Mendel, and was further studied by G. Nogler (1984) in Switzerland. Among the *Hieracium* species, some show a dominant, aposporous apomixis, which has been shown genetically to behave as a single locus. Bicknell has developed many of the key experimental tools in *Hieracium* to study and isolate this locus or the region, including *Agrobacterium*-mediated transformation, transposon mutagenesis, regeneration, and anther culture (Bicknell 1994, Bicknell and Borst 1994).

The approach CAMBIA is taking (described here) will generate a comprehensive tool kit of molecular technologies that will facilitate robust two-line hybrid systems, and will also allow us to apply and test apomictic gene candidates and combinations under field conditions. This should encourage the rapid development of hybrid rice for diverse Asian conditions and varieties, whether two-line or one-line in origin. In addition, new information essential to the understanding and manipulation of plant breeding systems will be provided.

Using yeast to understand and manipulate rice meiosis for apomixis

Apomixis and meiosis. Several studies show that apomixis seems to occur through the combination of the failure to reduce the female gamete via meiosis and the ability to initiate embryogenesis without pollen-mediated fertilization (Nogler 1984, Koltunow 1993). But endosperm formation differs from one apomictic species to another. In some apomictic species, such as *Hieracium,* the endosperm can be formed without fertilization (autonomous apomixis), but, in many others, fertilization is needed to initiate endosperm formation (pseudogamous apomixis) (Nogler 1984, Koltunow 1993, Asker 1979, 1980, Bashaw and Hanna 1990). In sexual plants, in order to develop a viable seed, a diploid cell (megaspore mother cell) has to undergo meiosis to reduce its chromosome number, to produce four haploid megaspores. Only one megaspore will survive, which will undergo mitosis, after which fertilization has to occur to enable the formation of a viable seed. In apomictic plants, however, only the mitotic division of the megaspore mother cell occurs. Meiosis is avoided and seeds are produced from the unreduced egg cell.

This fact shows that at a certain time during plant development, a signal or loss of signal determines whether the progenitor of the megaspore mother cell is to undergo meiosis, then mitosis, or continue directly with mitosis. This signal or loss of signal, which allows seeds to develop normally even though meiosis and gamete fertilization have been eliminated, is presumably a crucial factor in deciding whether to reproduce sexually or apomictically. Thus, to develop apomictic plants, a solid understanding of the early meiotic gene functions in plants is required. Because meiosis in yeast is well described, early meiotic genes in yeast might be used for this purpose to study their *complement in plants.*

Meiotic processes in yeast. Yeast (*Saccharomyces cerevisiae*) is a well-studied single-cell organism that undergoes meiosis in its life cycle. Three specialized cell types play distinctive and important roles in the life cycle of yeast (Herskowitz 1988). Two of these types are the haploid a and a cells, which are the mating-type cells. These cells mate efficiently when positioned next to each other, to produce a diploid

cell with a single nucleus. The third specialized cell type is the a/a diploid cell, which is unable to mate but is capable of undergoing meiosis. The decision to enter meiosis is governed by the presence of genetic and environmental signals. The genetic signal comes from the mating-type locus *(MAT),* which determines how the cells respond to the environmental signal (Herskowitz 1988). Haplid a and α cells have *MAT*a and *MAT* a alleles, respectively. For spore production, *MAT*a and *MAT* a must be present. Thus, only a/a diploid cells have the ability to sporulate (Kassir and Simchen 1976, Roman et al 1955). The principal environmental factor is the availability of a fermentable carbon source. In the absence of a carbon source, a and a cells will become arrested, but a/a cells will enter meiosis (Herskowitz 1988, Esposito and Klapholz 1981).

Many genes that play roles in yeast meiosis have been characterized, generally by identifying mutants with specific meiotic defects and studying the level of transcripts of the corresponding genes in sporulating cells, or by identifying the expression of genes in starved a/a cells using differential hybridization or lacZ fusion protein expression screens (Mitchell and Bowdish 1992, Mitchell 1994).

Based on their time of expression, meiotic genes have been divided into three classes: early meiotic genes, such as *HOP1, IME1, IME2, SPO11,* and *SPO13,* which are expressed at the beginning of meiotic prophase; middle meiotic genes, such as *SIT2, SPO12,* and *SPS1,* which are expressed later at prophase; and late meiotic genes, such as *SGA1, SPR1,* and *SPS100,* which are expressed around the time of meiotic division and spore packaging (Mitchell 1994, Wang et al 1987, Magee 1987, Clancy et al 1983). Among the products of early meiotic genes, the one that has been identified is the meiotic activator *IME1* (Kassir et al 1988, Smith and Mitchell 1989, Mitchell et al 1990, Kawaguchi et al 1992). To be functional, however, *IME1* has to be activated by phosphorylation by another protein, called RIM11 (Bowdish et al 1994).

Although genetically less well understood, meiotic processes in plants could be expected to share most key features with yeast. In yeast, upon the activation of early meiotic genes by the phosphorylation of *IME1* by RIM11, a starved a/a diploid cell undergoes meiosis to produce four haploid cells; each will be able to either mate to produce a diploid cell or undergo mitosis (Fig. 3). In plants, particularly in the female sexual organ, a diploid megaspore mother cell undergoes meiosis to produce four haploid cells. Only one of those cells survives (the megaspore), and it will undergo mitosis to produce an embryo sac. Finally, a double fertilization is needed to produce a viable seed. But it is not known whether the homolog of RIMl1 is present in plants and acts in a similar way to induce meiosis.

There is a small rice EST (expressed sequence tag) with a striking sequence similarity to the yeast RIM11, but does it share a similar function? Assuming that an RIM11 homolog is present in rice and induces meiotic processes, what would happen if its function were deleted from rice or altered in some way? Would it cause the megaspore mother cell to go through a mitotic pathway and yet still be capable of producing viable seed with or without fertilization (apomixis)?

In sexual plants (Fig. 4), at point b, a specialized cell, the megaspore mother cell, will undergo meiosis to form four reduced cells. Three undergo cell death and the

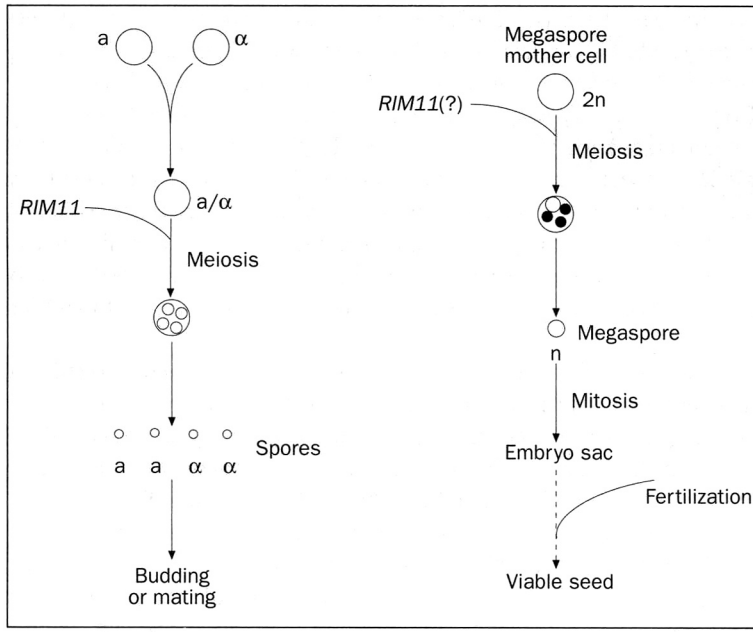

Fig. 3. Comparison of the meiotic division in yeast and in plant, with the putative entry of the product of the homolog of *RIM11* gene in rice.

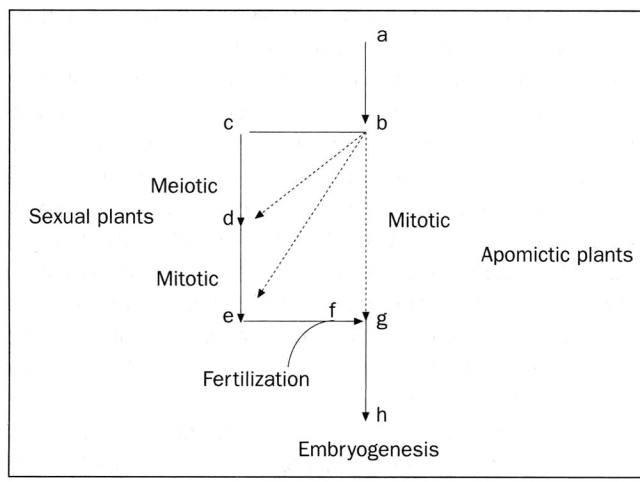

Fig. 4. Model of different pathways with a putative entry in which the decision to enter meiosis or mitosis is decided: (a) all cells are mitotic, (b) decision to enter meiosis or mitosis depends on the presence or absence of signals, (c) and (d) a megaspore is produced after undergoing meiosis, (e) an embryo sac containing 8 nuclei is produced after mitotic divisions, (f) a double fertilization occurs, (g) a viable seed is produced, and (h) an embryo develops

remaining one is the megaspore (point d), which will then undergo mitotic divisions to produce an embryo sac with eight nuclei consisting of one egg cell (embryo precursor), two polar nuclei (endosperm precursor), two synergids, and three antipodals (point e), which will be double-fertilized (point f) to obtain a viable seed.

When the entry of the megaspore mother cell to meiosis is blocked because of deletion of the RIM11 homolog function at point b (Fig. 4), one of three scenarios might take place. In the first scenario, the megaspore mother cell becomes nonfunctional and seed production is aborted, resulting in a complete disruption of the female sexual organ, which causes the inability to produce viable seeds. In the second scenario, the megaspore mother cell bypasses meiosis to undergo mitosis, in which case two different pathways (a, b, d, e, f, g, h or a, b, e, f, g, h) might be followed. The megaspore mother cell might follow pathway a, b, d, e, f, g, h, where it will undergo mitotic divisions by directly entering point d to produce the embryo sac, or the megaspore mother cell might follow pathway a, b, e, f, g, h, which will also produce the embryo sac by mitotic divisions. By going through either pathway, viable seed might be produced following fertilization, which might induce the formation of the endosperm. Although in some apomictic species single fertilization (point f) is required for endosperm development, in other apomictic species fertilization is not necessary to be able to produce viable seeds (Nogler 1984, Koltunow 1993, Asker 1979, 1980, Bashaw and Hanna 1990). In the third scenario, the megaspore mother cell bypasses meiosis to undergo mitosis, but fertilization is completely omitted in the production of a viable seed (pathway a, b, g, h). In this scenario, both the embryo and endosperm develop without fertilization (autonomous endosperm development).

The second and third scenarios lead to the development of apomictic plants, in which viable seeds are produced without any reduction in chromosome number through meiosis. The third scenario is probably more desirable, because fertilization is omitted completely, which will make the involvement of male sperm unnecessary for the development of viable seeds. But endosperm function must be maintained, and endosperm/embryo ploidy ratios must be investigated for their role in the process. Although there is a possibility to obtain viable seeds via the second and third scenarios, the formation of the embryo sac could still stop at any point for any number of reasons, including the absence of a signal(s) because of the change in the seed development pathway. If the blocking of the pathway occurs concurrently, however, it might be possible to trick the plant (female sexual organ) into bypassing meiosis, but still following the rest of the seed development processes as though it is undergoing normal seed production.

Analysis of the rice gene homolog of yeast Saccharomyces cerevisiae, *regulator of the inducer of meiosis RIM11.* To isolate rice genomic DNA that is homologous to yeast RIM11, two primers that correspond to the highly conserved region of the gene were constructed. Polymerase chain reaction (PCR) amplification of rice genomic DNA using the primers resulted in the amplification of an 815-bp fragment, which consists of four exons and three introns. The putative deduced protein sequence has 90% similarity to yeast RIM11. The copy number of the gene in rice genomic DNA was estimated by Southern hybridization analysis using the 815-bp fragment to probe

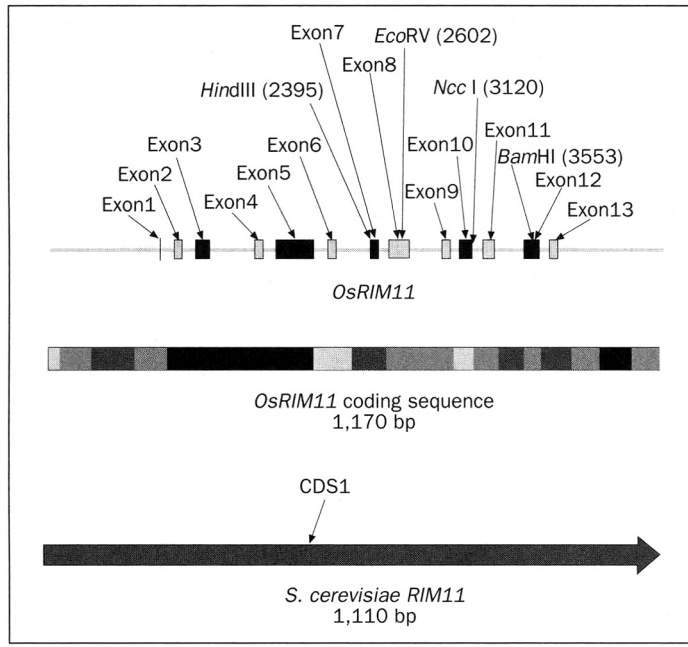

Fig. 5. Alignment of the *Oryza sativa OsRIM11* and *S. cerevisiae RIM11.*

genomic DNA digested by restriction enzymes *Eco*RV and *Hind* III that cut inside the 815-bp fragment, and *Bam* HI, *Eco* RI, and *Bgl* II that do not cut inside the fragment. The results show that the gene is a single-copy gene.

To isolate the whole rice gene homolog, the inverse-PCR technique was employed (Earp et al 1990). Four primers were constructed to amplify the 5' and 3' regions flanking the 815-bp known sequence following digestion with *Bam* HI, *Eco* RI, or *Eco* RV and self-ligation of rice genomic DNA. A rice gene of about 4.5 kb has been sequenced (Fig. 5), which most likely covers the whole gene homolog of the yeast RIM11. The putative sequence contains 13 exons and 13 introns. The deduced amino acid sequence is 68% similar and 50% identical to yeast RIM11, with a conserved protein kinase subdomain.

To study the specificity of the expression pattern of the promoter region, a 900-bp 5' end putative regulatory region of the gene has been cloned to a plasmid containing the *gusA* gene and selectable marker and has been transformed into rice by employing an *Agrobacterium* transformation method. Research to study the gene is still under way.

Cell- and stage-specific promoters

In CAMBIA'S collaboration with U. Grossniklaus (Grossniklaus et al, Chapter 16, this volume), Mr. Yang Wei is isolating rice homologs to key *Arabidopsis* genes that show megaspore mother cell-, megaspore-, or embryo-sac-specific gene expression.

We anticipate studying their function, through antisense and cosuppression approaches, and developing gene expression cassettes with these genes to provide a crucial component of the tool kit needed for control of apomixis.

The need for inducible and repressible systems

One of the principal bottlenecks to the successful implementation of apomixis and to many of the male sterility methods being proposed is to have a highly efficient and specific means of inducing or repressing transgene function under field conditions. There are several avenues toward this goal, but no solution is available yet. To develop inducible systems that can effectively control plant sexuality will require meeting stringent criteria. Some of these criteria for the inducing compound are summarized below:

- Low cost
- Environmentally and physiologically benign
- Stable compound in plant, or with varying stability according to need
- Good phloem mobility
- Effective formulation possibilities for field application

Likewise, for an effective exogenous induction system, the following criteria are important:

- High dynamic range of induction or repression
- Effective means of transducing signal across membranes
- High specificity for target transgene
- Low or zero basal levels of expression
- Effective in whole plants under field conditions

Unfortunately, existing systems, such as the tetracycline repressor (Gatz et al 1992), copper induction (Mett et al 1993,1996), and glucocorticoid receptors (Schena et al 1991, Aoyama and Chua 1997) fail to meet many of these criteria, although their performance under laboratory conditions is often promising.

CAMBIA is pursuing research strategies that begin by considering the properties of the inducing compound(s), and then use molecular biology to fine-tune the genetic performance. In particular, we are using the newly discovered gusR system, which encodes a glucuronide repressor (Wilson et al 1992), and another completely new system that also recognizes glucuronides (Jefferson, unpublished).

As described earlier, glucuronides meet many of the criteria for inducing compounds in that they are cheap, benign, stable, phloem-mobile, and soluble, and are not usually components of plant cells. In addition, they can be membrane-permeant through the use of the glucuronide permease (Jefferson 1995), or they can transfer information across the membrane in another system.

The glucuronide repressor is a 21,000-Da protein encoded by the gusR locus of *E. coli.* It shows the typical repressor structure composed of an amino-terminal DNA-binding domain and a larger C-terminal effector (glucuronide) binding region. In the absence of glucuronides, the glucuronide repressor binds a 16-bp palindromic sequence upstream of the gus operon, and in so doing inhibits transcription of the operon. When any of a wide variety of glucuronides is present, however, the repressor

disengages from the DNA, allowing transcription to proceed. We are now engineering this system into transgenic rice plants by placing the operator sequences within plant promoters, or upstream of a truncated promoter, and using the gusR protein either as a repressor or in a hybrid form as a transcriptional activator.

The advantage of starting with a system that has suitable inducing-compound characteristics is that these characteristics are usually fixed, whereas the improvements of the molecular biology, namely, the DNA:protein interactions and the fusions with transcriptional activators, are an option that can be approached readily with technologically sophisticated tools. We are optimistic that this system will provide a solution for an exogenous induction/repression system for hybrid rice.

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Notes

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CHAPTER 18

Developing and using novel sources of male sterility

K.K. Narayanan

In most grain crops, the exploitation of heterosis hinges on the availability of a good male sterility system. In the tropics, cytoplasmic uniformity, caused mainly by the exclusive and extensive use of the wild abortive (WA) type of CMS source, makes hybrid rice potentially vulnerable to the ravages of pests and diseases. To diversify CMS sources and solve problems such as incomplete panicle exsertion, plant cell and molecular manipulations are attempted. The earliest study involved the transfer of tissue-specific expression of the "toxin" gene that disrupted normal pollen development. The antisense RNA technique has been widely used to inhibit the expression of a locus, like that of male sterility. Genetic rearrangements in plant mitochondria have been associated with CMS. These techniques have been used extensively in rice and tobacco. The development of new CMS sources through either wide hybridization or protoplast fusion would also require the identification of good fertility restorers. This chapter outlines attempts to design and construct transposon traps to isolate fertility restorer genes.

In most grain crops, the exploitation of heterosis hinges on the availability of a good male sterility system. Over the past several decades, many cytoplasmic male sterility (CMS) sources have been successfully developed and used for hybrid seed production in various crop species, including rice. The wild abortive (WA) type of CMS source is used extensively for hybrid rice seed production, in China as well as in tropical rice-growing areas.

The increase in the area cultivated to hybrid rice will be the key to increasing the global productivity of rice in the coming decade. The use of hybrid varieties can, however, lead to cytoplasmic uniformity, mainly because of the limited availability of good CMS sources. This can make the crop potentially vulnerable to the ravages of pests and diseases. Furthermore, some currently employed CMS sources, including

WA, have incomplete panicle exsertion. Therefore, we need to identify and develop new sources of male sterility for hybrid rice production on a large scale.

Recent developments in plant cell and molecular manipulations have opened up several novel ways for engineering male sterile plants. Before long, these techniques will find application in heterosis exploitation of rice. Therefore, I will discuss some of the strategies used in cell and gene manipulations to generate male sterile plants and restore fertility in such sterile plants, to enable hybrid seed production.

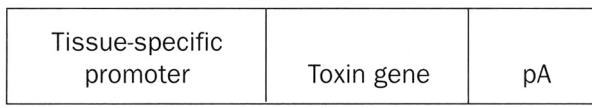
Engineered male sterility

Over the past decade, considerable progress has been made in understanding the organization and expression of plant genes. Efficient methods for transforming many plant species have also been developed. It is now possible to introduce virtually any genetic sequence into a plant genome and modulate its expression with a certain degree of precision. Via these tools, several methods have been developed for disrupting normal pollen development (for male sterility) and for restoring normal pollen development (fertility restoration) in the hybrid.

Disruption of pollen development by transgene expression

One of the earliest and successful attempts to induce male sterility by genetic engineering involved the transfer and tissue-specific expression of a "toxin" gene that disrupted normal pollen development. The toxin gene in this case was an RNase from a fungal source, Barnase, which was made to express itself specifically in the tapetal tissue of developing tobacco anthers (Mariani et al 1990). This was achieved by driving the expression of the gene with a tapetum-specific promoter, TA 29. Fertility restoration in these male sterile transgenic plants could he achieved by crossing with another transgenic tobacco line that expressed the specific RNase inhibitor, Barstar (Mariani et al 1992). This system has also been employed successfully to engineer male sterility in rapeseed, *Brassica napus* (Denis et al 1993).

The general design of a gene construct that can be used to engineer male sterility by disrupting pollen development using a toxin gene is illustrated below.



The construct consists of the toxin gene driven by a tissue-specific promoter and with appropriate transcription termination signals (pA). The toxin could be any protein that interferes with the normal growth and development of a tissue. We are trying to use the maize sterility-associated protein, T-URF 13, which is a trans-membrane protein, known to disrupt tissue function by inducing membrane leakage. Furthermore, the expression of this protein renders the tissue extremely susceptible to damage by

either the HmT fungal toxin, produced by the Southern maize leaf blight pathogen, *Cochliobolus heterostrophus,* or the insecticide methomyl.

The toxin has to be expressed in the anther tissues, more specifically in the sporogenous tissue, or in the nourishing tissue, the tapetum, by using an appropriate promoter. Several such promoters have been isolated from different species. LAT 52 is an anther-specific promoter from tomato (Twell et al 1989) that drives gene expression in developing pollen. ZM 13 is a promoter sequence from maize that drives gene expression in developing pollen at a late stage, possibly after meiosis (Hanson et al 1989). A tapetum-specific promoter, Osg6B, was characterized from rice and was used recently to drive the expression of the reporter gene, *GUS,* in rice tapetal tissues (Yokoi et al 1997). The effect of the toxin gene on pollen development can be nullified if an appropriate "antitoxin," also under the same temporal and spatial regulation, is expressed in the tissues. The antitoxin cold be a protein that binds to the toxin and inhibits its activity, like the Barstar, which inhibits the RNase, Barnase. In practice, the antitoxin construct is engineered into another line, which is crossed with the male sterile line for hybrid seed production. This method therefore requires the use of two transgenic lines in hybrid seed production.

The need for a second transgenic line can be obviated if the toxin gene can be activated by the presence of an external stimulus such as a chemical spray. Several promoters that turn on genes as a response to external stimuli such as heavy metals, salicylic acid, and heat shock have been identified and used in heterologous host systems to drive reporter gene expression. An easy technique for identifying genetic sequences whose expression is stimulated by an external agent is differential display reverse transcription-polymerase chain reaction (DDRT-PCR). This technique employs the power of PCR to amplify and differentiate between genetic regions that are either induced or repressed by a specific stimulus such as exposure to salicylic acid (Fig. 1).

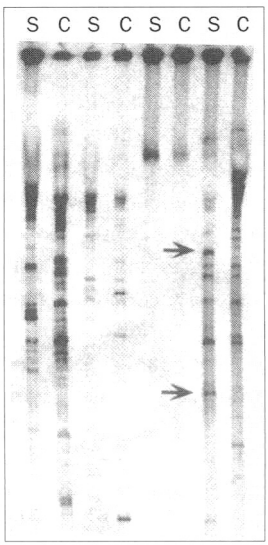
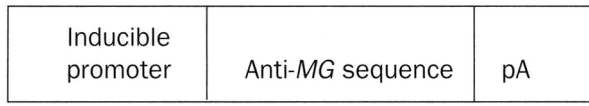


Fig. 1. Differential display reverse transcription-polymerase chain reaction. RNA isolated from salicylic acid-treated (S) and control (C) rice seedlings was reverse-transcribed using a poly (T) oligonucleotide and PCR-amplified using another random primer. The arrows show genetic regions amplified from the treated seedlings, which could represent regions specifically expressed on exposure to salicylic acid. (Data provided by S. Bharadwaj.)

Inhibition of male gametogenesis *(MG)* genes

Male gametogenesis is a complex development process that involves the expression of many penes in a coordinated manner. Some of the genes may be involved in this process only, whereas others may be components of other pathways as well. Genes whose function seems to be specifically associated with male gametogenesis (*MG* genes) have been reported in a few crops. Aarts et al (1993) isolated a gene from *Arabidopsis,* using transposon mutagenesis, the disruption of which leads to male sterility; this locus was designated *MS2.* With the availability of efficient molecular gene-hunting tools such as gene traps (Sundaresan et al 1995), which are improvements over the earlier transposon tags, it is now relatively easy to identify loci involved specifically in male gametogenesis.

Several molecular methods inhibit the expression of a locus. One of the more powerful and widely tested methods is the antisense RNA technique, which involves the expression of the sequence of the gene to be inhibited in an antisense (complementary to the mRNA sequence) orientation through transgenesis. A plant can thus be made male sterile by inhibiting any of the *MG* genes through the antisense RNA technique. The general design of a gene construct that can be used for this purpose follows.



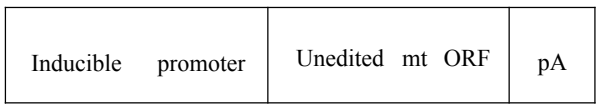
The antisense sequence has to be driven by an inducible promoter, which is turned on by a suitable external stimulus. The plants will be normal in the absence of the external stimulus. For hybrid seed production, when the plants have to be male sterile, the external stimulus can be provided.

Indirect modification of mitochondrial function

Genetic rearrangements in plant mitochondria have been associated with CMS. Several reports indicate that interference with the normal functioning of the mitochondrial genome of plants can cause male sterility. The mitochondrial genome codes for many proteins that form subunits of protein complexes, which are components of the electron transport chain. But not all the subunits that constitute the functional complexes are mitochondrially encoded; many are nuclear-encoded and imported into the mitochondrion. Therefore, suppressing expression of nuclear-encoded proteins that function as part of the mitochondrial electron transport chain can lead to mitochondrial dysfunction. This could in turn lead to male sterility. The antisense approach described earlier could be one of the ways of suppressing nuclear-encoded mitochondrial protein subunits.

Most of the mitochondrial-encoded genes undergo extensive RNA editing before translation. RNA editing brings about changes in the sequence of the mRNA, which may be necessary for translation of the functional protein. Unedited mRNA may lead

to the translation of an erroneous polypeptide, which may not just be nonfunctional but, more importantly, would compete with the correct protein, thus diminishing its functional efficiency. Researchers found the accumulation of partially edited and unedited transcripts of atp6 in the mitochondria of the sterile rice line and suggested that RNAediting plays a role in the expression of CMS. With this in view, one way of interfering with mitochondrial function and thus inducing male sterility is to increase the competition between the edited and unedited mRNA in the mitochondria. This can be achieved by genetically transforming an unedited version of the gene into the nucleus and directing the accumulation of this product in the mitochondria by providing an appropriate mitochondrial targeting sequence. The general design of the gene construct that can be used for transformation follows.



Mt targeting sequence

The unedited *mt* gene, fused with a mitochondrial targeting sequence, has to be driven by an inducible promoter so that the plants are normally fertile. Male sterility can be induced by applying the appropriate inducer.

A number of plant mt presequences have been characterized and used to target different nuclear-encoded proteins into the mitochondria. Hernould et al (1993) developed male sterility in transgenic tobacco by transforming it with an unedited version of the *atp9* gene from wheat, having the mt presequence of *coxIV,* a nuclear-encoded subunit of the cytochrome oxidase complex.

Diversification of CMS cytoplasm

It is now common knowledge that CMS can be induced by cytoplasmic substitution. Repeated backcrossing of a cultivar as the male recurrent parent with a wild relative as the female cytoplasmic donor can produce CMS lines. Several new CMS sources have been synthesized in different crops via this approach. In rice, two new sources, IR66707A with the *O. perennis* cytoplasm and IR69700A with the *O. glumaepatula* cytoplasm, have been developed at IRRI recently. The use of such lines will depend on identifying good fertility restorers.

Protoplast fusion for CMS

Sexual barriers to cross fertilization could be overcome through protoplast fusion and regeneration of the somatic hybrid. Protoplast fusion has been used to generate male sterile regenerants in some plant species. Male sterile rice plants were obtained by fusing iodoacetamide-treated Nipponbare protoplasts with X-ray irradiated Chinsurah Boro II protoplasts (Kyozuka et al 1989). Iodoacetamide treatment affects the cytoplasmic genomes whereas X-ray exposure inactivates the nuclear genome. The resultant fusant is therefore presumed to have obtained the functional nucleus from

Nipponbare and the cytoplasmic genomes, particularly the mitochondrial genome, from Chinsurah Boro.

A major limitation to the protoplast fusion method for male sterile rice is the recalcitrance of many genotypes to protoplast culture and regeneration. Developing protoplast regeneration protocols that are genotype-independent will go a long way toward the widespread application of this method.

Identification of good restorers

Developing new CMS sources, through wide hybridization or protoplast fusion, would entail the identification of good fertility restorer lines for use in hybrid seed production. If restorer genes are not available in the crop's varietal germplasm, they may have to be transferred from cytoplasmic donor species, either by backcrossing or through protoplast fusion. This may often prove to be more difficult than developing CMS lines.

Even though the genetics of fertility restoration has been well studied for many CMS systems in various species, and restorer genes are widely used in hybrid seed production, little is known about the molecular mechanism. Genetic studies indicate that the mechanism of fertility restoration will be different for different CMS systems. Only recently, one of the loci responsible for fertility restoration of T-CMS in maize, Rf_2, was cloned using transposon tagging (Cui et al 1996). The sequence of Rf_2 encoded a protein that showed homology to mammalian mitochondrial aldehyde de-

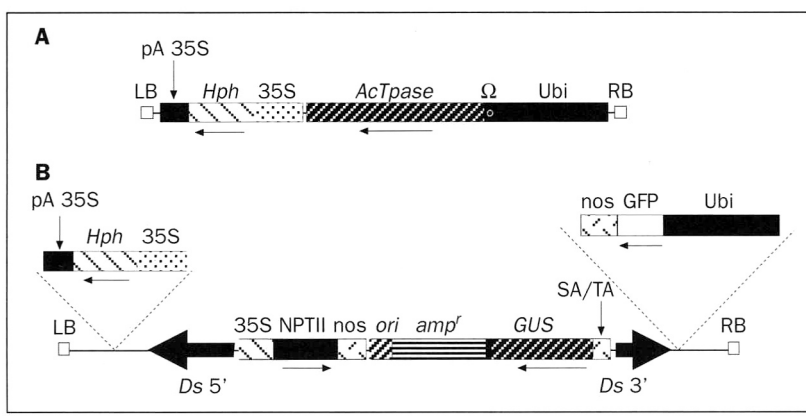


Fig. 2. Transposon trap constructs for transforming rice. (A) Maize *Ac* transposase (*AcTpase*) with its own termination sequence driven by the Ubiquitin promoter (Ubi). Selectable marker is hygromycin (*Hph*). (B) Gene trap construct having a reporter gene *GUS* with a minimal promoter (TA) or the splice acceptor (SA). A bacterial origin of replication (*ori*) and ampicillin resistance gene (*amp*) are provided for rescue of the tagged genomic region in an *E. coli* host. Within the *Ds* 5' and 3' termini, a kanamycin resistance marker, NPTII, is also provided. Outside the *Ds* termini, in addition to the *Hph* on one side, another reporter, the green fluorescent protein (GFP) driven by the Ubi promoter, is also provided on the other side. These constructions have been made in the vector pCAMBIA 1300 suitable for *Agrobacterium* - mediated transformation of rice.

hydrogenases, which catalyze the oxidation of the broad range of aldehydes to acids. The significance of this function in fertility restoration is not yet fully understood.

Transposon traps have become powerful tools for gene isolation and could be particularly useful for isolation of genes such as the fertility restorer, which does not have an obvious phenotype. We have designed and constructed the components of a transposon trapping system for rice using the maize *Ac/Ds* transposable elements (Fig. 2). We are now transforming these constructs in rice line AS 89044, which is a good restorer for the WA cytoplasm to develop the trap lines. Figure 3 outlines a strategy for using these trap lines to identify and isolate mutants for the fertility restorer locus (loci). The genetic regions involved in fertility restoration can then be isolated, cloned, and characterized using standard molecular biology techniques. Such studies may provide vital information for engineering ideal fertility restorer lines.

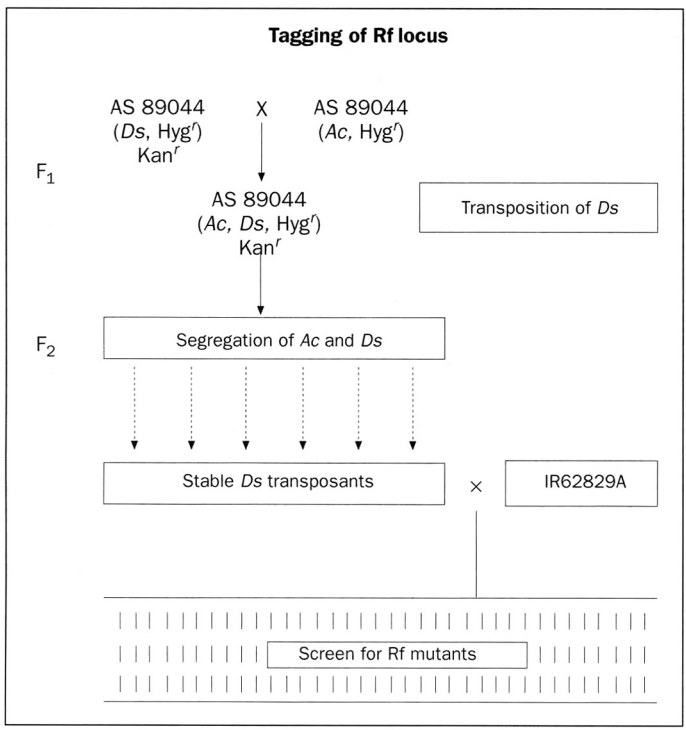


Fig. 3. Scheme for tagging the restorer locus using transposon trap lines of rice. Crossing of the trap lines initiates transposition in the F_1 generation. Stable transposants can be isolated in the segregating generations. R1 mutants can be screened by crossing with a CMS line and analyzing the progeny for pollen and seed fertility.

Conclusions

Studies on the use of cellular and molecular genetic tools to engineer male sterility in plants and to use such plants in hybrid seed production have already started bearing fruit. Hybrids of oilseed rape produced by crossing transgenic lines, one expressing the Barnase gene (male sterile) and the other expressing the Barstar gene (fertility restorer), are being field-tested. Several institutes are working toward engineering male sterility in rice by adopting ingenious methods. If these efforts succeed, they will make a useful contribution to hybrid rice breeding in the near future.

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CHAPTER 19

Mapping and molecular marke-based genetic analysis for efficient hybrid rice breeding

Qifa Zhang and Ning Huang

Work in mapping and molecular marker-based genetic analysis of fertility-related genes currently employed in hybrid rice breeding is reviewed. This includes genes for fertility restoration of wild abortive (WA) cytoplasmic male sterility (CMS), photoperiod-sensitive genic male sterility (PGMS), and thermosensitive genic male sterility (TGMS), and genes for wide compatibility. Studies showed that fertility restoration of WA CMS is controlled by at least two independent dominant genes *(RF-1* and *RF-3)*. The chromosomal locations of these two genes need to be resolved further. Single-locus inheritance has been observed for all TGMS mutants. The TGMS genes of two independently derived mutants were mapped to two different chromosomes. Studies demonstrated a relatively simple mode of inheritance of PGMS. But the chromosomal locations of the PGMS genes were complicated. A single wide compatibility gene having a large effect on the fertility of indica/japonica F_1 hybrids has been confirmed in a range of indica/japonica crosses based on the results from studies on inheritance and mapping. Another locus modifying hybrid fertility was also detected in the presence of the wide compatibility gene. Future efforts should be directed to reconciling the discrepancies between the results of mapping and inheritance studies in some of the systems and identifying markers tightly linked to the genes of interest to facilitate marker-aided selection.

Hybrid rice breeding has advanced rapidly in recent years. In China alone, for example, the total area planted to hybrid rice is 15–17 million ha yr^{-1} (Yuan 1992a). The hybrid rice program started with wild abortive (WA) cytoplasmic male sterility (CMS)-based three-line indica hybrids. In the past few years, two-line hybrids making use of both intra- and intersubspecific heterosis have also been developed and released for commercial rice production. Several other Asian countries, such as India, Japan, Korea, the Philippines, and Vietnam, also have active hybrid rice breeding programs (Virmani 1994).

Most breeding programs emphasize yield, stress tolerance, and adaptation. Hybrid rice breeders have to breed for a number of additional fertility-related traits that are essential for hybrid seed production or hybrid fertility. These include cytoplasmic male sterility and fertility restoration in breeding for three-line hybrids, environmentally induced male sterility in developing two-line hybrids, and wide compatibility to produce normally fertile hybrids in intersubspecific rice hybrids. Breeding for these traits is laborious and time-consuming. For example, environmentally induced male sterility is usually controlled by recessive genes and can only be observed in homozygous states, whereas the presence of fertility restoration and wide compatibility genes has to be confirmed by test crosses.

Marker-aided selection has now technically matured following the development of high-density molecular linkage maps and various types of molecular markers (Causse et al 1994, Kurata et al 1994). Using marker-aided selection in hybrid rice breeding programs will greatly expedite breeding processes.

There are two prerequisites for practicing marker-aided selection in rice breeding programs: (1) a good understanding of the genetic basis of the targeted genes, and (2) availability of tightly linked molecular markers that can be used reliably as selection criteria for the targeted traits at a reasonable cost. This chapter summarizes current progress in mapping and molecular marker-based genetic analysis of several important fertility-related traits. The feasibility of applying marker-aided selection is also discussed in the context of breeding for these traits.

Fertility restoration of wild abortive cytoplasmic male sterility

Hybrid varieties developed by making use of WA CMS account for approximately 90% of the hybrid rice produced in China. These varieties have significantly outyielded conventional pure line cultivars. The WA CMS system has also been used extensively in hybrid rice breeding programs of other countries to produce hybrids in various genetic backgrounds (Virmani 1994).

The inheritance of fertility restoration in the WA CMS system has been extensively investigated. All the studies have demonstrated that two independent loci control fertility restoration in this system (Zhou 1983, Young and Virmani 1984, Li and Yuan 1986, Govindaraj and Virmani 1988, Bharaj et al 1991), except the study made by Huang et al (1986), who reported that a single dominant gene controlled fertility restoration.

Attempts have been made to assess the chromosomal locations of the fertility restorer genes in the WA CMS system. Bharaj et al (1995) crossed a whole set of primary trisomic lines (Triplo 1 to 12) of IR36 (restorer) to IR58025B (maintainer), and test-crossed a number of selected disomic and trisomic plants from the F_1s to IR58025A (CMS). Results indicated that fertility restoration was controlled by two independent dominant genes. The two fertility restorer genes were located on chromosomes 7 and 10. Based on the amounts of deviation from the expected segregation ratio, Bharaj et al (1995) further inferred that one gene was stronger than the other.

The stronger gene, designated as *Rf-WA-1,* was located on chromosome 7 and the weaker gene, designated as *Rf-WA-2,* was on chromosome 10.

Molecular marker studies have also been employed to determine the map positions of the fertility restorer genes. Zhang et al (1996) surveyed DNA polymorphism using a randomly amplified polymorphic DNA (RAPD) analysis in a set of near-isogenic lines (NILs) developed by introgressing the fertility restorer genes from IR24 into the genetic background of Zhen Shan 97. From a survey of 720 random primers, six RAPD markers were identified in association with a fertility restorer gene, which we referred to as *Rf-3.* Three of the six RAPD markers, OPK05-800, OPU10-1100, and OPW01-350, were mapped to chromosome 1. Two F_2 populations were used for mapping *Rf-3.* One F_2 population was from the cross between Zhen Shan 97A and ZSR21, a near-isogenic restorer line of Zhen Shan 97 containing *Rf-3.* The other F_2 population was from the cross between Zhen Shan 97A and IR24. Based on the F_2 data, the three RAPD markers and three random fragment length polymorphism (RFLP) markers (RG532, RG140, and RG458) were found to be closely linked to *Rf-3.* The same location of *Rf-3* was also found in a BC_1 population from the cross of IR58025A/IR36//IR58025B.

In another study, Yao et al (F. Yao, C. Xu, Y. Gao, and Q. Zhang, unpublished data) conducted an RFLP analysis of fertility restoration in the cross between Zhen Shan 97A and Minghui 63, the combination of an elite hybrid, Shan You 63. Spikelet fertility in the F_2 population segregated in a 15 fertile: 1 sterile ratio, thus proving the involvement of two independent dominant genes. Yao et al subsequently surveyed the whole genome for linkage between fertility and RFLP markers using a bulked segregant analysis (Michelmore et al 1991) with 150-plus well-distributed polymorphic markers across the genome. The survey detected a number of RFLP markers showing significant linkage to the restorer genes. All of these markers fell into two chromosomal blocks. One block was located on chromosome 1, which apparently corresponded to the *Rf-3* locus, as the linked markers were the same as some of the RFLP markers identified by Zhang et al (1996). The other block was located on chromosome 10, but its correspondence with the *Rf-WA-2* locus was uncertain. A marker-based analysis using an additional large sample from the same F_2 population showed that the locus on chromosome 10 had a much stronger effect on fertility restoration than the one on chromosome 1 (Table 1).

Most genetic studies consistently showed a digenic mode of inheritance for fertility restoration of WA CMS. But the mapping studies conducted thus far have not produced consistent results with respect to chromosomal locations of the restorer genes. The causes for such discrepancies may involve differences in the sources of the restorer genes (IR36 vs Minghui 63), background effects of the different experimental populations, and the methods used to locate genes on rice chromosomes.

Table 1. One-way analysis of variance of the effect on fertility restoration (measured by spikelet fertility) of two loci based on the genotypes of each RFLP locus [a].

Locus	Chromosome	MS effect	df error	MS error	F	P
C668	1	1,746	157	385	4.53	.01
G359	1	1,810	157	384	4.71	.01
RG532	1	3,027	157	369	8.21	.00
G4003	10	7,549	157	311	24.26	.00
C234	10	6,968	157	319	21.87	.00
C677	10	7,587	155	314	24.14	.00

There are two degrees of freedom for the effect of each marker locus. The order of appearance of the markers in the table is according to the order of their locations in the RFLP linkage map (data not shown).

Environmentally induced male sterility

In environmentally induced male sterile rice, pollen fertility is regulated by environmental conditions. Under certain conditions (permissive), the male is fertile but becomes sterile under a different environmental condition (stressed). Thus, environmentally induced male sterile rice can be used to propagate itself under permissive conditions. Hybrid seeds can also be produced by interplanting it with normal fertile lines under stressed conditions. Such environmentally induced male sterile rice may therefore provide an opportunity to replace the widely used three-line system with a two-line system that would reduce labor costs, time, and resources in hybrid rice seed production. Two types of environmentally induced male sterility systems have been found useful in hybrid rice breeding: photoperiod-sensitive and thermosensitive male sterility. Both have been studied extensively.

Photoperiod-sensitive male sterility (PGMS)

Data from inheritance studies have established that male sterility of PGMS rice is controlled by a relatively simple genetic system (Jin and Li 1991). When Nongken 58S was crossed to its wild-type progenitor (normal Nongken 58), fertility in the progenies segregated in a typical single-locus Mendelian ratio. A two-loci segregation ratio was typically obtained in progenies of Nongken 58S crossed with many other japonica varieties.

Zhang and his coworkers conducted a series of studies to determine the locations of the PGMS loci in the rice molecular marker linkage map. They extended the bulked segregant analysis to a two-step approach for mapping the PGMS genes: (1) using bulked DNA from extreme plants to identify the chromosomal segments likely to carry sterility vs fertility alleles, which avoided classifying individuals into a fertile or a sterile class in a more or less continuously distributed population, and (2) determining the map locations of the genes using only the extreme sterile individuals. As pointed out by Zhang et al (1994a), this approach in gene mapping had several advantages

over routine segregation population analysis, including higher efficiency and reduced probability of misclassification.

In the first experiment, Zhang et al (1994a) made a cross between two indica lines, 32001S (a PGMS line developed by transferring the PGMS genes from Nongken 58S) and Minghui 63 (a normal rice cultivar), that demonstrated a relatively high level of RFLP in a preliminary study. Large F_2 populations were planted annually in the field during the 1991-93 growing seasons under natural long-day conditions for fertility examination. A digenic ratio was consistently observed in all the years studied.

Two DNA bulks, F (fertile) and S (sterile), were made by selecting extreme individuals from the F_2 population of about 1,500 plants. These two bulks and the two parents were digested with 6–21 restriction enzymes, probed for RFLP with a total of 368 probes, and assayed using the 10 SSR (single sequence repeats) markers. These markers covered more than 90% of the rice molecular marker linkage map. The survey of bulked extremes identified positive markers from three regions, located respectively on chromosomes 1, 3, and 7, that were probably linked to PGMS loci.

All available polymorphic probes surrounding the positive markers in the RFLP linkage map were added to the survey. Highly sterile plants from the F_2 population were assayed individually with all the positive markers to assess linkage: The results from the bulked extremes and recessive class analysis were confirmed using an additional large sample of 224 individuals from the same F_2 population and from data collected in different years. RFLP genotypes of these 224 individuals were determined using positive markers from chromosomes 1, 3, and 7. When a three-way analysis of variance was performed using the marker showing the largest effect from each of these three regions, all the effects involving the locus on chromosome 1 became insignificant. These analyses established the existence of two loci, designated *pms2* on chromosome 3 and *pms1* on chromosome 7.

The recombination frequency between a positive marker and a target PGMS locus was calculated, assuming that all the highly sterile plants were homozygous for the recessive (sterility) allele at the PGMS locus. The recombination values were then converted to map distances (Fig. 1). Genetic effects were estimated for these two loci based on the marker genotypes using a two-locus model (Table 2). The effect of *pms1* was two to three times larger than that of *pms2,* and dominance was almost complete at both loci. The data also suggested that alleles of these two loci interacted more or less like alleles of duplicated loci, and highly sterile individuals were apparently homozygous for recessive alleles at both loci. Various genotypes containing at least one allele from the normal parent (Minghui 63) appeared to produce highly fertile individuals.

The next question was whether the same two loci caused male sterility in the original Nongken 58S. To address this question, Zhang and his coworkers (Q. Zhang, M. Mei, and F. Wang, unpublished data) crossed Nongken 58S with several japonica varieties. Gene mapping was done essentially following the bulked segregant analysis. After a whole genome screening, they detected linkage of the PGMS loci with markers from chromosomes 7 and 12 in F_2s of crosses Nongken 58S/1514 and Nongken

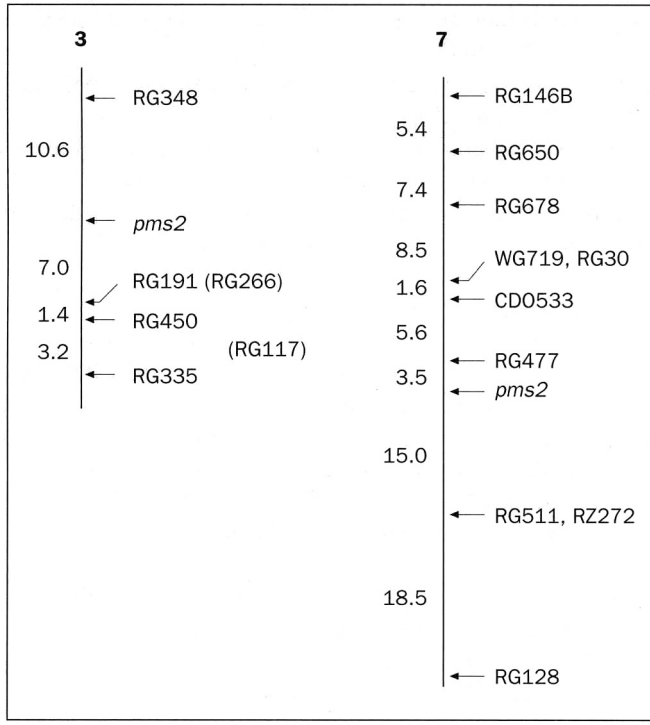

Fig. 1. Locations of the two loci for photoperiod-sensitive male sterility *pms1* and *pms2* in the rice RFLP linkage map adapted from Zhang et al (1994a). Mapping is based on data from the F_2 population of 32001S/Minghui 63 using the bulked extremes and recessive class approach.

Table 2. Spikelet fertility (in %) of each of the two-loci genotypes in the F_2 of 32001S/Minghui 63 as marked by the two RFLP loci on chromosomes 3 and 7. Allele 1 is from 32001S and allele 2 is from Minghui 63. The data were adapted from Zhang et al (1994a).

RFLP marker		RG477 11	RG477 12	RG477 22	Average
RG191	11	12.9	61.6	72.1	52.0
	12	35.9	73.1	75.3	64.4
	22	50.6	72.7	74.5	67.6
Average		33.8	70.1	74.3	

58S/Lunhui 422. In the cross of Nongken 58S/Nongken 58, linkage was detected between fertility and a marker from chromosome 12, but not for markers from chromosome 7. Nongken 58S therefore differed from other japonica varieties by alleles at two independent loci controlling male fertility. The normal Nongken 58 must have already mutated once at the locus on chromosome 7 before it became PGMS rice.

Zhang et al (1990) examined linkage between male sterility and morphological markers in progenies from Nongken 58S crossed with a set of morphological marker lines. They found that one of the two loci segregating for male sterility was located on chromosome 5.

These results demonstrated a contrast between the relatively simple mode of inheritance and the complexity of chromosomal locations of PGMS genes. The chromosomal locations of the loci causing an apparently similar digenic segregation are not necessarily the same in different populations. It is particularly interesting that the original PGMS locus of Nongken 58S, located on chromosome 12, was not involved in fertility segregation in the cross of 32001S/Minghui 63, perhaps because PGMS is controlled by a biochemical pathway involving enzymes encoded by a number of different genes. Many mutations, mapped to different locations of rice chromosomes, have been accumulated in different genetic backgrounds.

Thermosensitive genic male sterility (TGMS)

There is considerable interest in using TGMS to develop two-line hybrid rice. TGMS may be more useful than PGMS, especially in the tropics where the seasonal difference in daylength is much less. Three TGMS mutants, 5460S, H89-1, and IR32364TGMS, were identified, respectively, in China (Sun et al 1989), Japan (Maruyama et al 1991), and at IRRI (Virmani and Voc 1991); all three were obtained through irradiation mutagenesis. Unlike the complicated genetics in PGMS rice, a single recessive gene controlled the thermosensitive male sterility in each of the three mutants (Maruyama et al 1991, Yang et al 1992, Borkakati and Virmani 1996).

Molecular marker analyses have been performed to determine the map locations of the TGMS genes in two of the mutants. Wang et al (1995) used a bulked segregant analysis to survey RAPD polymorphism of the fertile and sterile bulks made from the F_2 population of a cross between the TGMS mutant, 5460S (found in Fujian, China), and Hong Wan 52 (a normal variety). Among the 400 primers screened, four detected polymorphism and one fragment represented a single-copy sequence and showed linkage with male sterility in this segregating population. These researchers subsequently mapped this fragment on chromosome 8 using a different population, which allowed them to infer that the TGMS gene was located on chromosome 8 near RFLP markers RZ667 and RG648. But the exact map location has yet to be determined.

In another study, Subudhi et al (1997) used the F_2 population from a cross between TGMS mutant line IR32364TGMS and IR68 to determine the map location of the TGMS gene. Fertile and sterile bulks were constructed following the classification of F_2 plants into true-breeding sterile, fertile, and segregating fertile plants based on F_3 family studies. From the survey of 389 arbitrary primers in bulked segregant analysis, four RAPD markers were identified. Of these, three (OPF18-2600, OPB19-

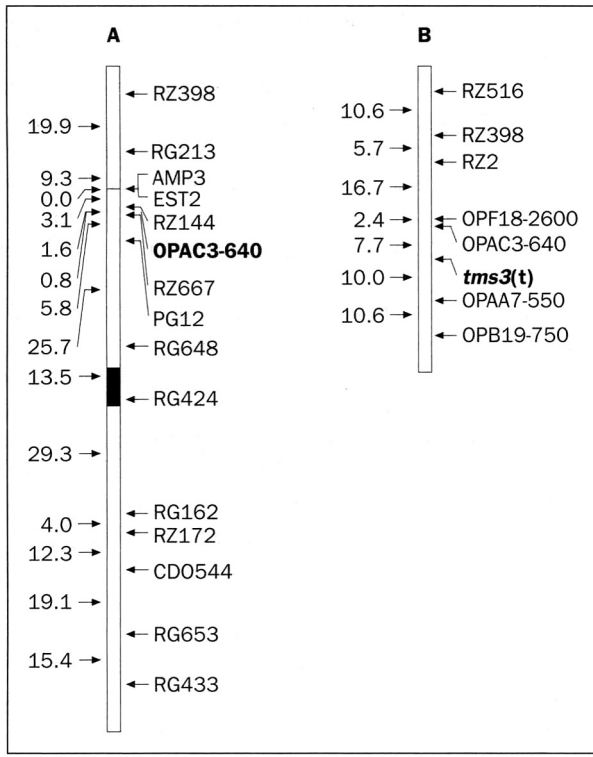

Fig. 2. Location of *tms3*(t) on the molecular marker linkage map. (A) A linkage map of chromosome 6 constructed from the doubled haploid population of IR 64/Azucena, where OPAC3-640 was placed between RFLP markers RZ144 and RZ667. The approximate centromere position is indicated as a solid bar based on Singh et al(1996). (B) Partial linkage map of the region surrounding tms3(t) on chromosome 6 derived from the cross IR32364TGMS/IR68, adapted from Subudhi et al (1997).

750, and OPAA7-550) were linked to the TGMS locus *tms3*(t) in the repulsion phase, whereas OPAC3-640 was linked to *tms3*(t) in the coupling phase. The *tms3*(t) locus was flanked by OPF18-2600 and OPAC3-640 on one side, and by OPAA7-550 and OPB 19-750 on the other side (Fig. 2). Hybridization of the amplified DNA products with genomic blots showed that all four PCR fragments were low-copy sequences and two of them (OPF18-2600 and OPAC3-640) detected polymorphism between the parents. OPAC3-640 was mapped on the short arm of chromosome 6 using a mapping population available at IRRI. But RFLP markers from this region in the molecular marker linkage map could not detect polymorphism between the parents. The precise location of the *tms3*(t) locus on the molecular marker linkage map remains to be determined.

Wide compatibility

Hybrid sterility in intervarietal crosses of rice is well known (Kato et al 1928). Most of the sterility was demonstrated to be caused by reproductive barriers between indica and japonica rice groups, often referred to as subspecies, although low fertility has also been observed in crosses other than indica by japonica parents (Oka 1988). Such intersubspecific hybrid sterility has attracted renewed interest in recent years with the discovery of wide compatibility varieties (WCVs) that produce normal fertility hybrids when crossed with both indica and japonica varieties (Ikehashi and Araki 1985). This finding has brought hope for bridging the fertility barriers between indica and japonica subspecies, and makes it possible to exploit intersubspecific heterosis in hybrid rice breeding programs.

Ikehashi and Araki (1986) proposed a genetic model, known as the allelic interaction model, to account for the wide compatibility phenomenon. This model assumes the presence of three alleles at the S_5 locus, s^i_5 (indica), S^j_5 (japonica), and S_5^n (neutral). Zygotes formed with the S_5^n allele in any three combinations, such as, $S_5^nS_5^j$, $S_5^nS_5^j$, and $S_5^nS_5^n$, have normal fertilily, whereas $S_5^iS_5^j$ zygotes are partly sterile. Thus, the S_5^n allele is compatible with both indica and japonica varieties, and this model is also known as the wide compatibility model. Genetic analyses and mapping studies using both morphological and molecular markers conducted by several groups indicated the existence of such a wide compatibility gene, located on chromosome 6 (Ikehashi and Araki 1986, Liu et al 1992, Zheng et al 1992, Yanagihara et al 1995). Data from a number of crosses support this wide compatibility model (Gu et al 1993, Liu et al 1996).

Inspection of the large volumes of data also showed that wide compatibility varieties differ in their abilities to influence the fertility of their progeny. Fertility in the progeny of the same wide compatibility variety crossed to different indica or japonica varieties also varies. There are quite a few loci at which allelic interactions can cause various degrees of hybrid sterility in indica-japonica hybrids (Kinoshita 1995). Thus, an obvious possibility for the variable levels of fertility observed in the progeny carrying the wide compatibility gene is that alleles of other loci that cause hybrid sterility may have been involved in the crosses, in addition to the wide compatibility gene.

To assess such a possibility, Liu et al (K. Liu, J. Wang, H. Li, C. Xu, A. Liu, and Q. Zhang, unpublished) conducted a genome-wide analysis of hybrid sterility in a triparent cross of Nanjing 11 (indica)/02428 (WCV)//Balilla (japonica) using molecular markers. From this triparent cross population, spikelet fertility was examined for a total of 360 plants. These plants showed approximately 1:1 segregation for high and low fertility, suggesting a single-locus inheritance, From this population, 240 random plants were assayed for RFLP, with more than 180 probes covering the entire rice molecular marker linkage map. The whole genome was scanned for quantitative trait loci (QTLs) controlling hybrid fertility using Mapmaker/QTL. Two loci showing significant effects on hybrid fertility were identified. The major locus, with a log likelihood (LOD) score of 33.5, accounted for 5 1.9% of the fertility variation and corresponded well with the S_5 locus. The minor locus, with a LOD score of 3.8, ex-

Table 3. Spikelet fertility (in %) of each of the two-loci gene types in the triparent cross of Nanjing 11/02428//Balilla as marked by the two RFLP loci on chromosomes 6 and 12 (K. Liu, J. Wang, H. Li, C. Xu, A. Liu, and Q. Zhang, unpublished data). Allele 1 is from the wide compatibility variety 02428 and allele 2 is from the indica tester Nanjing 11.

RFLP marker		RG138 (Chrom 6) 1	RG138 (Chrom 6) 2	Average
G1112A (Chrom 12)	1	71.6	45.2	58.4
	2	62.2	37.2	49.7
Average		66.9	41.2	

plained 8.1% of the fertility variation and was located on chromosome 12. No significant interaction between the two loci was detected. The amount of fertility change caused by allelic substitution at the major locus was three times larger than that at the minor locus (Table 3). Fine mapping of the S_5^n region resolved the position of this locus at 1.5 cM from RFLP locus RG138 on chromosome 6.

This analysis indicated that an additional locus can indeed modify the fertility of indica-japonica hybrids in crosses involving the wide compatibility gene. It is therefore quite likely that different loci for hybrid sterility are involved in crosses between different parents. Varying levels of modifications thus result in varying levels of hybrid fertility.

Perspectives of applying marker-aided selection

A good understanding of the genetics of traits of interest and the availability of tightly linked molecular markers are the two prerequisites for efficient application of marker-aided selection to breeding programs. Among the systems discussed, the wide compatibility gene may be the closest to fulfilling the two prerequisites. Both the wide compatibility gene (S_5) and its chromosomal location have been confirmed by repeated testing in the past 10 years. The linkage (1.5 cM) between the S_5 locus and RFLP marker RG138 on its side is also tight enough for practicing marker-aided selection. Marker-aided selection for this trait should be very beneficial to plant breeders, as the presence of this gene in selections can be easily detected by test crosses to both indica and japonica varieties, which take two rice-growing seasons to complete. In this system, efforts should be made to convert the RFLPs to PCR markers to facilitate fast genotyping of the selected individuals. In addition, attention should also be given to the locus on chromosome 12, which, based on the available evidence, may be a locus for stability of fertility in indica-japonica hybrids (Li et al 1996).

The genetic basis of TGMS is apparently simple and in all three cases segregated in single-locus Mendelian ratios. The two reported molecular marker studies mapped the TGMS genes of two different mutants, 5460S and IR32364TGMS, to chromosomes 8 and 6, respectively, indicating the distinctness of these two genes. The linkages between the RAPD markers and the TGMS gene given by Subudhi et al (1997)

may be useful to generate more specific markers. This may be used as a starting point for marker-aided selection, but we need to identify more closely linked markers to make this selection tool routinely useful.

Data from large numbers of genetic analyses of fertility restoration have consistently demonstrated a system in which two independent dominant genes control fertility restoration. The results from mapping studies may need more discussion. Zhang et al (1996) and Yao et al (unpublished) detected the same locus *(Rf-3)* on chromosome 1. Bharaj et al (1 995) and Yao et al detected another locus on chromosome 10 (*Rf* · WA-2). Interestingly, Bharaj et al (1995) and Zhang et al (1996) used IR36 as a restorer, IR58025A as a male sterile, and IR58025B as a maintainer line in their studies. The differences between these two studies may be reconciled with a marker-based analysis using markers from the concerned chromosomal regions. In developing a system for marker-aided selection, emphasis should be given to identifying molecular markers tightly linked to the fertility restorer genes.

The PGMS system appears to be more complex, especially with regard to the chromosomal location of PGMS genes. The results from many studies confirmed that fertility induction is conditioned by a complex interaction between photoperiod and temperature (Zhang et al 1992). In certain genetic backgrounds, photoperiod plays the leading role, whereas in other cases, the effect of temperature may be more pronounced for sterility induction. The loci conditioning photoperiod and temperature responses are numerous among various rice varieties and breeding lines. The expression of male sterility, therefore, may be caused by a diversity of gene combinations that may not necessarily involve the original mutant allele of Nongken 58S, although the male sterile line was developed by transferring the male sterility gene from Nongken 58S. Whether this is a correct explanation for such observations remains to be tested. The difficulty of working in this system, however, is obvious as we are not certain which loci will function in given breeding materials. It is therefore difficult to decide on the loci to be targeted for marker-aided transfer. Nonetheless, such a complex system may provide enough challenge to attract further studies.

It is often critical in heterosis breeding that allelic substitution has to be precise so that only the targeted gene and the shortest possible segment of the linked chromosome are transferred from the donor parent to the recipient parent, which is usually a variety with very good combining ability. In this case, a three-marker system, with the three marker loci located on a chromosome block of a few (£ 5) cM, will be desirable. The marker in the middle, preferably cosegregating with the gene, will be used to indicate the presence of the targeted gene (positive selection) in the selection process. The marker on each side will be used to indicate the absence of the chromosome segment from the donor parent (negative selection), that is, selection for recombination between the targeted gene locus and the marker locus. Such a selection system has been made available for bacterial blight resistance gene *Xa21* (Williams et al 1996). It is desirable but not necessary to have such a three-marker system if the goal is to convert a heterotic cross to a hybrid as it is, or to transfer single genes to male sterile or restorer lines, unless proven genetic drag is associated with the introgressed segment.

A further need for making hybrid rice breeding more efficient is for molecular marker-based characterization of the genetic basis of hybrid performance and heterosis. In recent years, data have been generated in assessing the relationship between molecular marker diversity and hybrid performance (Zhang et al 1994b, 1995) and characterizing the genetic basis of heterosis (Xiao et al 1995). Knowledge from such studies will facilitate hybrid rice breeding by three possible means: (1) predicting heterotic crosses, (2) transferring QTLs for high heterozygous performance from other varieties to hybrids, particularly genes showing relatively large additive and nonadditive effects, and (3) transferring paired chromosomal blocks with large heterotic effects to the two parental lines if dominance effects are pronounced.

Marker-mediated modification of hybrid rice has already begun in some breeding programs in China. With the knowledge generated by molecular marker-based studies and from genome research in general, we anticipate an improvement in the efficiency of hybrid rice breeding programs.

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CHAPTER 20

Diversification of cytoplasmic male sterility systems through somatic cell hybridization

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Cytoplasmic male sterility (CMS) occurs widely in higher plants and is due to interactions between nuclear and cytoplasmic (mitochondrial) genome transcripts. Most hybrid rice currently grown is based on wild abortive cytoplasm. Using sexual crossing, CMS sources from closely related A genome *Oryzae* have been transferred into the nuclear background of cultivated rice. Somatic hybridization offers the ability to speed up the process of hybrid production and to produce novel nuclear-cytoplasmic combinations not possible by sexual crossing. In Japan, asymmetric protoplast fusion has been employed for the transfer of CMS to a wide range of japonica cultivars. The aim of our work is to develop, via somatic hybridization, alloplasmic lines between a range of cultivated rice and *Oryza* species other than A genome ones. Cell suspension cultures of *O. australiensis, O. granulata,* and *O. latifolia* have begun. Japanese studies have already produced two novel lines based on BT-type CMS and have demonstrated that somatic hybridization has considerable potential for transferring a well-established source of CMS into a broad range of cultivars. At Nottingham, a more speculative approach is being employed, which will produce more diverse CMS lines for use in rice breeding for the next millennium.

Cytoplasmic male sterility (CMS) occurs widely in higher plants and is due to interactions between nuclear and cytoplasmic (mitochondrial) genome transcripts. CMS plants produce nonfunctional pollen and, consequently, are unable to self-fertilize. Normal seed set can be obtained by crossing with plants that produce functional pollen. Because CMS eliminates the possibility of self-pollination, it has been used to develop the three-line system for exploitation of heterosis for yield enhancement in commercial rice cultivars.

In the three-line system, breeders cross a CMS line (A line) with a maintainer line (B line, isonuclear with the A line) to propagate the A line for distribution to

hybrid seed producers. In hybrid seed production plots, the A line is grown alongside a restorer line, and is thus pollinated by the restorer line selected for its ability to give heterosis for yield. The best hybrids outperform the best inbred varieties by about 20% under optimal conditions (Virmani et al 1991).

In 1985, more than 95% of the CMS lines in commercial indica rice hybrids cultivated in China were of the CMS-wild abortive type (CMS-WA). This makes hybrid rice potentially vulnerable to disease or insect pest epidemics related to susceptibility of the WA system. It is therefore desirable to widen the genetic background of CMS and introduce foreign mitochondrial genomes.

Additional sources of male sterility-inducing cytoplasms have been detected in several species of the genus *Oryza*. These cytoplasms are considered to be particularly suitable for use in CMS systems because they possess a basic compatibility with *Oryzae* nuclei. Wide hybridization studies based on sexual crossing have been employed to develop alternatives to the WA system by using these *Oryzae* cytoplasms and identifying suitable restorers. For hybrids that demonstrated CMS, repeated backcrossing with *O. sativa* varieties was necessary to eliminate nuclear elements from the wild parent and develop usable CMS lines. To date, it has only been possible using sexual crossing to transfer cytoplasms from the closely related A genome *Oryzae* into *O. sativa*. For most CMS sources developed so far, restorers have been found within the indica lines that are cultivated throughout the tropics.

Somatic hybridization as used at the laboratories of Mitsui Toatsu Chemicals Inc. and the Plant Genetic Manipulation Group, University of Nottingham, offers the ability to speed up the process of hybrid production and to produce novel nuclear-cytoplasmic combinations not possible via sexual crossing. Protoplast fusion can be used to overcome sexual incompatibility at both the pre- and postzygotic levels to generate interspecific hybrids at the inter- and intrageneric levels. When applied to CMS transfer, in which the ability to induce (and direct) chromosome elimination is desirable, the donor-recipient protoplast fusion technique is most commonly employed. In this process, the nuclear genome of one parent, the cytoplasmic donor, is inactivated prior to electrically or chemically induced protoplast fusion. Asymmetric somatic hybrids produced in this way therefore demonstrate uniparental inheritance of nuclear genomes and biparental inheritance of cytoplasmic genomes. At the whole-plant stage, the chloroplasts of one partner usually dominate, resulting in the elimination of plastids of the other partner. DNA recombination is common between mitochondrial genomes. This ability to produce novel nuclear-mitochondrial combinations is of particular relevance to the development of alternative CMS systems. Kumar and Cocking (1987) considered the theoretical aspects of organelle genetics and somatic hybridization.

Methods for inducing asymmetry have included enucleation of donor protoplasts by centrifugation through a density gradient (Maliga et al 1982), production of subprotoplasts and microplasts (Bilkey et al 1982), the use of mutants and anti-metabolites (Aviv and Galun 1986), and lethal or sublethal dose irradiation of cells or protoplasts with either g - or X-rays. The first successful production of asymmetric hybrids came from fusions of X-irradiated (9 krad) leaf mesophyll protoplasts of

Petroselinum hortense (parsley, 2n = 2x = 22) with cell suspension protoplasts of a nuclear albino mutant of *Daucus carota* (2n = 2x = 18) (Dudits et al 1980). Results indicated an extensive elimination of parsley chromosomes. Somatic segregation of albino tissues and plants from the callus lines generated in these experiments also indicated an unstable integration of parsley chromosomes carrying the complementary gene. All of the regenerated asymmetric hybrid plants were sterile.

One of the first groups to report asymmetric hybridization in rice was Yang et al (1988). Irradiated protoplasts of male sterile rice line A-58 CMS were electrofused with iodoacetamide-treated protoplasts of the fertile (normal) rice cultivar Fujiminori to produce cybrids. These cybrids differed from those of *Daucus* in two ways: they produced identical mitochondrial DNA digestion patterns and there were no novel (i.e., nonparental) mitochondrial DNAdigestion profiles, indicating limited mitochondrial DNA recombination.

In Japan, asymmetric protoplast fusion has been employed to transfer established types of CMS to a wide range of japonica rice cultivars. In initial experiments (Akagi et al 1989), an aryl acylamidase I-deficient mutant of cultivar Norin 8 (Akagi et al 1995c) was fused with strain MTC-5A, the cytoplasmic donor, which carried a cytoplasm derived from indica cultivar Chinsurah Boro II (BT-type CMS). In order to be able to select exclusively for heterokaryons, the recipient protoplasts were treated with 30 mM iodoacetamide before fusion, while the cytoplasmic donors were pretreated by X-ray irradiation (125 krad). Shoot regeneration was observed 3 wk after transfer of the hybrid calli to a plant regeneration medium.

Restriction digestion analysis of mitochondrial DNA revealed that all regenerated plants possessed a fragment specific to the donor, as well as fragments specific to the recipient. Thus, these plants were cytoplasmic hybrids (cybrids) of MTC-5A and the mutant of Norin 8. Aryl acylamidase I was assayed to determine the source of the nuclei of these cybrid plants. All of the cybrid plants analyzed, like the recipient variety, lacked aryl acylamidase I activity and contained either 24 or 48 chromosomes derived from the recipient parent. Thus, it was demonstrated that asymmetric protoplast fusion was capable of transferring cytoplasms from donor to recipient partners in rice.

In subsequent experiments, the fertile cultivar Sasanishiki was used as the recipient because it had been employed previously in conventional breeding programs. A male sterile version of Sasanishiki has been produced by recurrent backcrossing. Asymmetric fusion experiments were performed with lines MTC-5A (BT-type CMS) and MTC-9A (Liao-type CMS); 142 cybrids were regenerated with chromosome numbers and mitochondrial characteristics similar to those in the previous experiment (Akagi et al 1995b). Among these 142 regenerants, 72 plants were identified as having normal morphology and were assessed for their fertility. Of these 72 cybrids, more than 80% were unable to set seed by self-fertilization. It was assumed that in the remaining 20% of the plants, elimination of the CMS trait had occurred after interparental recombination of mitochondrial genomes (Akagi et al 1994, 1995b). The sterile plants were able to set seed when pollinated by wild-type plants of cultivar Sasanishiki, indicating male sterility but female fertility. All of this backcross popula-

tion (BC_1) was male sterile. Line MTC-10R carries the gene *Rf-1,* which restores fertility for both BT-and Liao-type CMS. Crossing BC_1 plants with pollen from MTC-10R produced progenies that had panicles with 75–95% seed set in all cases except one. A similar rate of fertility restoration was observed when the sexually produced CMS derivative of Sasanishiki was pollinated by MTC-10R. The CMS traits introduced from both MTC-5A (BT-type) and MTC-9A (Liao-type) into the cybrids were stable through seven generations following backcrossing of the cybrids with Sasanishiki.

To convert fertile elite cultivars to CMS, BT-type CMS has been transferred to 40 Japanese cultivars by asymmetric protoplast fusion (Akagi et al 1995a). In all cases, the diploid progenies were male sterile, except for crosses involving Hoshiyutaka, an indica/japonica sexual hybrid. The *Rf-1* restorer gene is widely distributed throughout the tropics among indica varieties and it is conceivable that Hoshiyutaka contains this gene. As a result of these studies, the BT-type CMS trait has been introduced into 40 Japanese cultivars, which provide useful germplasm for hybrid rice breeding programs.

The work being carried out at Nottingham involves collaboration among the Indian Council for Agricultural Research (ICAR), the Department of Life Science at the University of Nottingham, UK, and the International Rice Research Institute (IRRI), Philippines. The aim is to develop, by somatic hybridization at Nottingham, alloplasmic lines between a range of cultivated rice that is grown in India and *Oryza* species other than A genome ones.

Protocols for protoplast culture and plant regeneration have been established for several promising rice varieties such as Pusa Basmati I (Jani et al 1995), Gayati and Saviti (Azhakanadam et al 1996), Swarna, and two elite breeding lines, IR65597 and IR65598.

For all of the rice cell suspension cultures, the protoplast isolation procedure described by Abdullah et al (1 986) gives reproducible yields (in excess of 10^6 protoplasts g^{-1} fresh weight of suspension-cultured cells). Immediately before protoplast isolation, the cytoplasmic donor cell suspension (the wild rice species) was irradiated by exposure to 180 krad using a Gammacell- 1000 Elite (Nordion International, Ontario, Canada). Three to 5 d after subculture, 1 g fresh weight of cells was transferred to a Petri dish and incubated for 16 h in the presence of 10 mL of enzyme solution (0.25% w/v Cellulase RS [Yakult Honsha Co. Ltd., Nishinomiya, Japan], 0.025% w/v Pectolyase Y23 [Seishin Pharmaceutical Co. Ltd., Tokyo, Japan], and 1.25 mM 2-[M-morpholino]ethanesulphonic acid [MES] dissolved in CPW13M solution [Frearson et al 1973] at pH 5.8). After passage through 64- and 30-mm pore-size nylon meshes (Wilson Sieves, Nottingham, UK), the protoplasts were centrifuged (100 x g, 20 min) and resuspended in liquid KPR medium (Abdullah et al 1986).

Protoplasts of Pusa Basmati I were cultured using fast-growing cell suspensions of *Lolium multiflorum* (5-yr-old, nonembryogenic) as nurse cultures. The cell suspensions of *L. multiflorum* were obtained from Dr. E. Guiderdoni (IRAT-CIRAD, Montpellier, France). Cell suspensions of *L. multiflorum* were maintained by weekly

subculture in N_6 medium (Chu et al 1975), essentially as described for rice cell suspensions.

For all of the rice cultures, cryopreservation using procedures developed at Nottingham (Anthony et al 1997) is being used to ensure a readily available supply of material throughout the program. Ampoules (more than 100) of each cell suspension culture are stored in liquid nitrogen immediately after the cultures become suitable for protoplast isolation. Ampoules are removed from the storage dewar as required and, after thawing, the cells are used to reinitiate cell suspensions.

The ability of rice lines to regenerate was considered to be genome-dependent (Narayanan and Virmani 1996) and was stated to impose a limitation on the use of somatic hybridization. As a result of advances in rice protoplast culture during the past few years, especially the use of nurse cells (Guiderdoni and Chair 1992, Torrizo and Zapata 1992, Jain et al 1995), success in plant regeneration is being reported for an increasing range of rice varieties. Many workers have now reported plant regeneration from indica (Lee et al 1989, Torrizo and Zapata 1992, Jain et al 1995, japonica (Fujimura et al 1985, Abdullah et al 1986, Lynch et al 1995), and javanica (Coulibaly and Demarly 1986) varieties. It would therefore appear that, essentially, the previously assumed constraints to the use of somatic hybridization for developing hybrid rice lines have been overcome.

Cell suspension cultures of *O. granulatu, O. australiensis,* and *O. latifolia* have been initiated at Nottingham using germplasm supplied by IRRI. For these three species, dehusked seeds were surface-sterilized and germinated by incubation for 5 d in the dark at 28 °C on an MS-based medium supplemented with 30 g L^{-1} sucrose and semisolidified by the addition of 8 g L^{-1} agar (Sigma), pH 5.8. The coleoptile was dissected away from the endosperm and radicle, trimmed to a length of 25 mm, transferred to an MS medium supplemented with 50 g L^{-1} sucrose and 2 mg L^{-1} 6-benzylaminopurine, and made semisolid by the addition of 2.5 g L^{-1} Phytagel, pH 5.8. Multiple shoots developed at the bases of the explants (Finch et al 1992). After 21 d (14 d in the case of *O. austruliensis),* these shoots were separated and transferred to a fresh medium to encourage branching at the explant base.

Ninety days after germination, segments (2 mm thick) were cut from the firm white tissue at the base of the explants and transferred to 25 mL of LS2.5 medium with 4 g L^{-1} SeaKem agarose, pH 5.8, in 9-cm plastic Petri dishes. These cultures were maintained in the dark at 28 °C for callus induction. *O. australiensis* formed pale yellow calli from which those with a dry and friable appearance were selected and transferred to a fresh medium every 28 d. In the case of *O. latifolia* and *O. granulatu,* two types of calli were observed—namely, a gray-colored wet callus (80% of the explants) and a gold-colored, dry friable callus (20% of *O. latifolia* explants, 2.5% of *O. granulata* explants). The remainder of the calli were intermediate in appearance. Segments (5 mm in diameter) of the friable gold-colored callus of *O. latifolia* were transferred to a fresh medium and subcultured at 21-d intervals. Sixty-three d after callus induction, the individual calli of *O. latifolia* were transferred *en masse* to a 55-mm-diameter piece of filter paper on the surface of an LS2.5 medium with 4 g L^{-1}

SeaKem agarose, pH 5.8, in 9-cm plastic Petri dishes. The friable gold-colored callus of *O. granulata* was cultured *en masse* from the time of the first subculture.

Cell suspension cultures were initiated by transferring a dry friable callus to the appropriate medium in a small Erlenmeyer flask that was incubated in the dark at 28 °C on a rotary shaker (120 rpm). For *O. australiensis,* 0.5 g of callus was inoculated into 3 mL of NR medium (R2 medium of Ohira et al [1973], modified by the addition of 560 mg L^{-1} L-proline and 10 g L^{-1} maltose) after being subcultured 4 times. Suspension cultures of *O. granulata* were initiated by transferring 1.5 g of fine yellow callus to 18 mL of AA2 medium containing 2 mg L^{-1} 2,4-D (Abdullah et al 1986). In the case of *O. latifolia,* culture of the callus for 9 mo was necessary before suspension cultures could be successfully initiated by the transfer of 1.5 g of gold-colored callus to 18 mL of NR liquid medium.

The medium was replaced for all suspension cultures at 3- or 4-d intervals and the volume gradually increased to 30 mL in accordance with the growth of the cells. When a suspension of small callus aggregates had formed, the subculture procedure was changed. Cells (1.5-mL packed cell volume, pcv) were transferred to a 100-mL flask together with 5 mL of the old medium and 16 mL of fresh medium. After 28 d, the suspensions of *O. latifolia* became darker in color and small cream-colored areas developed on the surface of each piece of callus. These small groups of cells were continually released into the medium as individual calli and replaced by new cream-colored areas. These calli were collected by sedimentation and were transferred to a flask containing 8 mL of NR medium, together with 2 mL of spent medium. Once rapid growth of these new suspensions had been achieved, the older dark-colored suspensions were discarded and subculture of the new suspensions was carried out every 7 d. A 100-mL flask was inoculated with 1.5 mL pcv of cells, 5 mL of old medium, and 16 mL of fresh medium. After approximately 10 wk (depending on individual cultures), the small pieces of callus in the suspension cultures had been reduced in size to less than 1 mm in diameter. Subculture was then carried out by transferring 1 mL pcv of cells and 12 mL of spent medium into 42 mL of fresh medium in a 250-mL Erlenmeyer flask at 7-d intervals.

For electrofusion of protoplasts, each well of a 25-well square plastic Petri dish was filled with 750 mL of both cytoplasmic donor and recipient protoplast suspensions (1.5 x 10^6 protoplasts mL^{-1} in electrofusion solution; 110 g L^{-1} mannitol, 0.5 mM $CaCl_2$). The electrofusion apparatus developed by the Plant Genetic Manipulation Group (Jones et al 1994) was used to supply an AC field (250 V cm^{-1}, 1 MHz) for alignment and 4 DC pulses (1,150 V cm^{-1}, 1 msec, pulse separation 2 sec) to induce fusion (Blackhall et al 1994). Fusion frequencies in the range of 5–10% have been routinely obtained. Fusion-treated protoplasts were cultured using protocols developed for the appropriate cytoplasmic recipient species.

Studies being carried out both in Japan and at Nottingham represent an alternative approach to conventional breeding to develop new lines for hybrid rice breeding. The Japanese studies have already produced two novel lines based on BT-type CMS and have demonstrated that somatic hybridization has considerable potential for transferring a well-established source of CMS into a broad range of cultivars. At Nottingham,

a more speculative approach is being employed, which will produce more diverse lines for use in rice breeding for the next millennium.

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CHAPTER 21

Use of anther culture in hybrid rice breeding

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This chapter outlines improvements in techniques of anther culture in indica rice, including the optimal stage for inoculation of pollen, media, and physical and chemical pretreatments. It analyzes the genetic stability and diversity of pollen progenies and the feasibility of using anther culture in hybrid rice breeding on the basis of results from many studies. It summarizes advances and achievements in hybrid rice breeding via anther culture, including purification and development of parental lines for hybrid rice. The chapter also discusses the tendency of anther culture research and prospects for its application in hybrid rice breeding.

Diploids derived from pollen plantlets are uniform in both genotype and phenotype. This can accelerate the breeding process and shorten the breeding cycle. Since the pioneering work on the development of haploid embryoids via anther culture in *Datura innoxia* by Guha and Maheswari (1964) in the 1960s, anther culture has received much attention worldwide. After Niizeki and Oono (1968) produced pollen plantlets in rice, scientists in China began research in rice anther culture in 1970. In the past two decades, the anther culture technique has been greatly improved, and many pollen-derived cultivars, mostly japonica, have been bred and released for commercial use. Since the mid-l970s, hybrid rice has become increasingly popular in China and elsewhere because of its expression of heterosis for yield.

Scientists undertook anther culture in hybrid rice to take advantage of the hybrid's genetically rich background and breed elite varieties of cereals (Yang et al 1978, Ling et al 1978,Tang 1978, Picard 1989, Wenzel et al 1995, Alejaret al 1995, Balachandran et al 1996, Bicar and Darvey 1997). By increasing the green pollen plantlet induction frequency (based on inoculated anthers) to 1.7%, Zhu et al (1983) made a systematic analysis of inheritance of the regenerant progenies derived from hybrids. Therefore, they evaluated and verified the applied value of the pollen progenies from three-line hybrid rice. Several registered and commercially used varieties were then developed

through anther culture of three-line hybrids. One hybrid rice was developed through the conventional method combined with anther culture. As a result, more and more breeders realized that anther culture has a significant role and provides a particular advantage in three-line hybrid rice breeding.

For breeding a wide compatibility restorer (WCR) to cytoplasmic male sterile (CMS) lines, wide compatibility (WC) lines, and photoperiod-sensitive (thermosensitive) genetic male sterile—P(T)GMS—rice, programs were begun in the mid-1980s and carried out with excellent progress. Anther culture has now become an important approach for breeding hybrid rice. With the increasing induction frequency of green pollen plantlets, anther culture will have bright prospects for improving indica hybrid rice breeding and genetic studies.

Improving the anther culture technique

Improving anther culture efficiency is a prerequisite for anther culture breeding. Anther culture breeding in indica rice, compared with japonica rice, is less efficient because it is difficult to obtain a large pollen plantlet population for breeding purposes. This challenge has motivated many scientists to investigate and increase the efficiency of anther culture in indica rice.

Microspores at the mid-late uninucleate stage are optimal for inoculation (Mercy and Zapata 1986, Datta et al 1990a). At this stage, the glume color is light green and the anthers extend to about two-fifths of glume length. Chen (1977) cultured anthers at different developmental stages of microspores. Callus induction frequency for the tetrad, early uninucleate, middle uninucleate, late uninucleate, and binucleate stages was 0%, 5.6%, 35.7%, 10.5%, and 0%, respectively.

Genotypes respond differently to media (Guha et al 1970, Aruna and Reddy 1988, Mandal and Gupta 1995). Many researchers have demonstrated that basal media such as MS (Murashige and Skoog 1962) and LS (Linsmier and Skoog 1965) are suitable for callus induction. M8 (Mei et al 1988), N6 (Chu et al 1975), and general medium (Yang et al 1980) are acknowledged to be more adaptive for callus formation. For pollen callus induction, auxin is known to be a major element for dedifferentiation. Indica usually requires a higher concentration of auxins than japonica. For indica and indica/japonica hybrids, media supplemented with 1–2 ppm of 2,4-D and 2–5 ppm of NAA have a better effect on pollen callus induction. For differentiation, indica needs a lower concentration of auxin than japonica. Some naturally active substances such as potato extract, bleaching sap of towel gourd, coconut juice, and maize extract and some organic substances such as casein hydrolysate, yeast extract, and proline significantly improved anther culture efficiency in indica rice. Sun et al (1993) and Datta et al (1990a) found that for indica rice maltose was better than sucrose for pollen callus formation and plant regeneration. Abscisic acid has also been found to increase plant regeneration (Torrizo and Zapata 1986).

Suitable physical and chemical treatments can improve physiological activities in pollen, resulting in increased induction frequency. The most-used method is low-temperature pretreatment. Sunderland (1978) pretreated young inflorescences at

6–10 °C for 48–160 h and found that the induction frequency increased by 40–100% over the control following such pretreatment. Different genotypes also differ in optimal low-temperature requirements and duration of exposure to low temperature (Datta and Wenzel 1987,1988, Zhu et al 1995). Zhao (1983) reported that pretreatment at 6–8 °C for 3 d was most effective for Guang Lu Ai 4, whereas an average of 8 d cold pretreatment showed an enhanced indica anther culture response (Datta et al 1990a). Centrifugal pretreatment can likewise stimulate microspores toward sporophytic development. Zhu and Wang (1982) pretreated inflorescences of Shan You 3 at 2,000 rpm for 10 min before inoculation. The result proved that the induction frequency was 1.65 times higher than the control following centrifugal pretreatment. Other pretreatments such as constant temperature, g radiation (Zapata et al 1986, Aldemita and Zapata 1991), and ultraviolet rays also have some positive effect on anther culture.

The proper choice of parents also determines anther culturability (Quirino and Zapata 1990). For callus induction, the effects of genotype and genotype × medium interaction were significant, while genotype and ABA level were likewise found to significantly affect plant regeneration.

Analyzing the potential use of anther culture

Genetic stability of pollen progenies

Theoretically, diploids derived from microspores via anther culture are in a homozygotic state and are genetically stable. Studies by Chinese scientists indicate that 80–90% of pollen-derived diploids are homozygotic and do not degenerate in vitality with the advance in generations.

An analysis of agronomic characters in 577 pollen-derived strains from Shan You 2 showed that about 88% of them were stable; the remaining strains segregated for only a few characters such as total growth duration, plant height, palea tip color, and grain shape (Zhu et al 1983). To investigate the inheritance of five main characters, one pollen strain was randomly sampled in the H2 to H5 populations. Coefficients of variation (CVs) for these characters in samples drawn from different generations were similar to those of the parents and hybrids, indicating that pollen strains in different generations are uniform and stable in characters. Investigating the productivity of one stable pollen strain from Shan You 2 in the H4, H5, and H6 generations indicated no degeneration in plant height, panicle length, spikelets panicle $^{-1}$, seed set, and 1,000-grain weight. Yin et al (1983) analyzed the CVs for plant height, panicle length, and 1,000-grain weight in 184 pollen strains derived from Shan You 2, Shan You 6, Wei You 2, Ai You 2, and Nan You 2 and concluded that 83% of the total pollen strains were stable. In a pollen strain from Ai You 2, the populations from H3 to H8 showed no degeneration in major agronomic traits such as panicle length, plant height, number of panicles, spikelets panicle^{-1}, and 1,000-grain weight.

Yin et al (1993) analyzed segregation and expression of sterility in pollen plantlets from hybrids of six indica P(T)GMS rice lines such as W6154S and others. The results showed that:

1. P(T)GMS pollen plants in H1 averaged 22.4%;
2. Variants with an ideal response to photo- and thermo-conditions can be produced via anther culture; and
3. P(T)GMS in pollen plants can be inherited stably.

From the research on anther culture of seven PGMS lines and their hybrids, Li et al (1 995) concluded that both callus and green pollen plantlet induction frequencies are higher for PGMS lines than for conventional varieties. In addition, the frequency of haploids, diploids, and polyploids in such pollen plant populations was similar to that of pollen plants derived from conventional varieties.

Studies showed that anther culture can be used to purify CMS lines provided their pollen abortion occurs after the mid- or late uninucleate stage, and the pollen-purified CMS lines are stably heritable (Ge et al 1985, 1989, Chen et al 1994, Zhang et al 1994b).

At IRRI, anther culture of four crosses involving TGMS parents was done to evaluate the possibility of transferring TGMS traits to indica rice and to a high-yielding new plant type.

Diversity of pollen-derived plants

The key to success in anther culture breeding is whether ideal recombinants exist in pollen progenies. As a result, anther culture breeders' greatest concern is how recombinants are distributed among pollen-derived strains.

Xu et al (1983) and Zhu et al (1983) compared differences in CVs, variation ranges, and recombinant types for growth duration, plant height, spikelets primary panicle^{-1}, unfertilized spikelets panicle^{-1}, seed-setting rate, and 1,000-grain weight between pollen progenies from two hybrids and their respective F_2 populations. Results showed that the values for all the parameters studied were-basically similar, indicating that the variability of recombinants produced through the doubled-haploid method and conventional breeding is the same. Ling et al (1978) and Liang et al (1983) reported that fertility restorer genes in pollen plants of wild-abortive-type three-line hybrids were expressed and segregated as expected.

All the results discussed so far demonstrate clearly that anther culture can be used efficiently to extract inbred varieties from superior hybrids, purify parental lines, and breed hybrid rice.

Extracting high-yielding inbred varieties from superior hybrid rice

Hybrid rice has a rich genetic diversity. Anther culture can be used to obtain from hybrid rice excellent homozygotes that combine parental advantages (Tang 1978, Zhu et al 1989, Zapata et al 1991, Ba Bong and Swaminathan 1995). Since 1975, Chinese breeders have developed and put into commercial use a number of inbred varieties by using anther culture methods.

From hybrids Shan You 2, Shan You 5, and Yin You 1, many lines with significantly higher yield potential or quality than local commercial varieties were bred and released for trial production (Li 1983, Anonymous 1983a, b, Wu 1985, Zhu et al

Table 1. Elite varieties developed from superior hybrid rice via anther culture breeding in Jiangxi Province, China[a].

Variety[a]	Donor	Year regional test was conducted	Year registered	Cumulative area occupied (ha)[b]	Remarks
Gan Zao Xian 11 (original, Shan Hua 369)	Shan You 2	1984-85 (provincial)	1990	166,000	
Gan Zao Xian 31 (original, F_4)	(Zhen Shan 97A// 36 Tian Hui/IR24) F_1	1991-94 (provincial and national)	1993	233,000	
Guan 18	(Shan You 2 H2/ IR661) F_1	1985			High quality
Lp1	(H092s///GIIA//8504/ 02428) F_1	1995-96		On-farm production trial	

[a]All are suitable for early planting, [b]The varieties are grown commercially in Jiangxi Province.

1990). Several varieties have been bred via anther culture of hybrid rice (Table 1). Zhu et al (1990) successfully bred Gan Zhao Xian 11 (originally named Shan Hua 369) through anther culture of hybrid Shan You 2. This variety passed the regional adaptability test of Jiangxi Province in 1985 and was registered in 1990. It showed uniformity in characters, early maturity, high yielding ability, and strong resistance to diseases and cold tolerance at the seedling stage, and was awarded the prize for "Remarkable Achievements in Science & Technology Invention & Innovation" by the United Nations. The area planted with this variety has reached 166,000 ha in China.

Gan Zhao Xian 31 was bred from pollen progenies of ShanA/IR24 × Tian Hui 63 (Ding et al 1995). This variety has uniform characters, high yield potential, and good resistance to diseases and pests. In regional tests in Jiangxi between 1991 and 1994, this variety outyielded the control by 12.5%. It was registered in Jiangxi in 1993 and listed as a major early season variety for double cropping by the Agricultural Ministry of China and Jiangxi Province in 1994. It has been released for commercial use and its cumulative planted area now exceeds 230,000 ha.

Anther culture of five heterotic crosses was made in the 1992 wet season at IRRI and 144 anther culture lines were regenerated. Five promising anther culture lines with excellent phenotypic acceptability (based on the 1993 wet-season screenhouse evaluation) were selected. The selected promising anther culture-derived lines are now being evaluated in the field.

Anther culture has also been used in the production of doubled-haploid plants for adverse environments. At IRRI, the single cross IR51500 (IR5657-33-2/IR4630-22-2-5-1-3) was made in the 1985 dry season. The cross was primarily intended for breeding of salinity-tolerant varieties, a feat which at that time was far from being

realized through conventional breeding. Several lines from this cross were evaluated in-house (Zapata et al 1991). From the 1990 wet season to 1993 dry season, anther culture-derived line IR51500-AC11-1 was included in tests at five different locations in the Philippines by the National Cooperative Test Trials. Because of its salinity tolerance and comparatively higher average grain yield than the other test lines and checks, this line was approved in 1993 by the Rice Varietal Improvement Group of the Philippine Seed Board as one of the two prerelease varieties for saline soil conditions (Alejar et al 1995).

Purification of parental lines for hybrid rice

Since the first complete set of three-line hybrid rice was formed in the early 1970s, hybrid rice has been used in production for more than 20 yr. Because of gene drift and mutation, artificial and biological confounding, and exposure to unfavorable natural stresses, the purity of parental lines used in developing hybrid rice has strongly decreased. Such a decrease during the long period of propagation and production results in reduced yield potential and grain quality. It is therefore necessary to take strict and effective measures to purify these parental lines. The conventional technique of purification is overelaborate and selection is made on phenotype; this cannot guarantee that selected materials will be genetically homozygotic and stable. Because anther culture can make genes highly homozygotic, it is a more effective method for purification. Xu et al (1983) successfully purified CMS line V20A using anther culture by generating different types with varying stages of pollen abortion and a small proportion of fertile pollen recovered in the normal state of mononucleate abortion. Investigating Zhen Shan 97A purified via anther culture showed that the typical abortion rate and sterility were 11.l% and 14%, respectively, higher than those of the donor. Its hybrid rice Shan You 6 yielded 19.1% higher than the control (Ge et al 1985). Bai et al (1991) reported that hybrids derived from restorer line anther culture-purified Minghui 63 were significantly improved in purity, seed-setting rate, yield potential, and resistance. Ge et al (1989) purified Zhen Shan 97A, Zhen Shan 97B, and Minghui 63 (a restorer line) via anther culture. They reported that the purity of the hybrid increased when the parental lines were purified via anther culture without excluding the restorer line.

Zhang et al (1994a) and Chen et al (1994) obtained some normal sterile or fertile pollen diploids through anther culture of the male sterile lines of II-32A, You IA, and Xie Qing Zhao A and their respective maintainer lines. These purified CMS lines were screened for use through test crosses, sterility tests, and heterosis trials.

In the 1990 dry season at IRRI, 95 anther culture lines were regenerated from CMS line IR54752A. From these, 10 lines were identified possessing 100% male sterility.

Two-line hybrid rice derived from P(T)GMS lines is another approach for exploiting heterosis in rice. Most P(T)GMS lines currently used are not stable enough. Anther culture can also be used to purify P(T)GMS lines. Three P(T)GMS lines—ZaXi30S, 1286S, and 1356s—were purified via anther culture. These purified lines

were stable in sterility performance and were similar to the donors in major characters.

Use of anther culture in breeding parental lines

Significant achievements have been obtained in breeding CMS restorer lines via anther culture (Table 2). Zhou (1996, unpublished) inoculated the anthers of an F_1 hybrid derived from two highly disease-resistant parents and successfully developed a disease-resistant restorer line, DT Ai. A hybrid derived from DT Ai and Wei 20A had excellent disease resistance and high yield potential. Zhu et al (1993a) developed CMS restorer line 2374 with strong combining ability by improving a pollen strain derived from Shan You 2 by the conventional method. Hybrid Xie You 2374, which has excellent adaptability, has been registered in Jiangxi Province and released for commercial use; its cumulative planted area is 300,000 ha. Zhu et al (1996, unpublished) also bred a CMS restorer line (1044) via anther culture of (Ce64 × 1050) F_1. The hybrid Jiang You 1044 underwent the regional adaptability test in 1995. Wang et al (1994) adopted the cross irradiation anther culture procedure to create new restorer lines for CMS. Young panicles of (Minghui 63/Zi Gui) F_1 underwent anther culture and 19 clusters of green plantlets were regenerated. From the anther culture lines, restorer line Chuan Hui 802 was selected for strong restorability. Hybrid II You 802 (II 32A/Chuan Hui 802) was registered in 1996 and grown on 66,667 ha.

Breeding widely compatible restorers (WCRs) for indica CMS is now an available channel of intersubspecific heterosis use. Some WCRs resulting in highly heterotic hybrids have been developed via the conventional technique, but this method requires a long process to stabilize and subsequently test the WC and CMS restoring ability. By using the anther culture method, however, we can develop WCR lines expeditiously and simultaneously screen them for WC and restoring ability.

Li et al (1992) reported the use of anther culture in intersubspecific heterosis breeding for the first time. Zhu et al (1993b) created a good-quality WCR named HR1004 through anther culture of the F_1 hybrid (Cps1o17 × Skybornnet) in 3 yr or 5 cropping seasons from parent selection to the identification of a WCR. The WC and restoring ability were identified by crossing, respectively, with the four test varieties—IR36, Nanjing 11, Balilla, and Akihikari—and with CMS lines Xie Qing Zhao A, Chang Fei 22A, and You IA. The fertile pollen rate and seed-setting rate of the F_1 hybrids were above 75%, showing that these materials were widely compatible. The seed-setting of F_1 hybrids with CMS was more than 70%, suggesting that these had strong restoring ability.

Zhang et al (1994b) reported that 33 of 120 pollen strains from single hybridization combinations of WCV 02428 and CMS restorer lines and 15 of 35 of the composite hybridization combinations could restore the fertility of CMS. A WCR named H8801-3 was singled out by further crossing with the WC test varieties. Yin et al (1994) obtained 20 WCRs and 3 intersubspecific hybrids with strong heterosis from pollen-derived progenies of WCV 02428 and Cpslo l7 crossed with CMS restorer lines. Anther culture as an effective technique was demonstrated to have an important

Table 2. Use of anther culture to develop restorer lines for CMS in China.

Name	Planting season	Donor	Representative hybrid	Year of regional adaptability test	Year registered	Area occupied (ha)	Province	Remarks
2374	Late	Shan You 2 H2/ Jian 30	Xie You 2374	1988-91	1990	333,000	Jiangxi	Through regional test
Chuan Hui 802	Middle	(Minghui 63/ Zi Gui) F_1	II 32A/Chuan Hui 302, Shan You 802	1994-95	1996	66,667	Sichuan	
1044 (original name 7024)	Late	(Ce 64/1050) F_1	Jiang You 1044	1993-94		10,000	Jiangxi	Through regional test
HR1004	Early and late	(Cpslo17/ Skybonnet) F_1	Xie You, HR1004	1994-95			Jiangxi	Awarded prize for high quality. Restorer line with WC and high quality.

position in intersubspecific heterosis breeding to overcome the unusual segregation found in crosses between indica and japonica lines. Yan and Xue (1995) developed WCRs such as TG7 and others by anther culture of the hybrid of Cps1o17/Minghui 63. When further crossed with several types of CMS, they displayed a wide spectrum of restoration.

Does cytoplasm influence anther culture?

Among the several factors known to affect anther culture response, genotype seems to be the most important. Whether cytoplasm has any role in anther culturability has been studied by the Directorate of Rice Research group (Balachandran et al 1996). It was found from a study of F_1 hybrids involving a cytosterile line (A), its isonuclear maintainer (B), and the restorer (R) that sterility-inducing cytoplasm influences anther culture response. Five sets of hybrids involving A × R and B × R lines (IR58025A/IR9761R, IR58025B/IR9761R, IR58025A/IRl0198R, IR58025B/IR10198R, IR58025A/IR29723R, IR58025B/IR29723R, IR58025A/IR46R, IR58025B/IR46R, IR58025A/Swarna, and IR58025B/Swarna) underwent anther culture. The hybrids varied in their degree of response to anther callus induction and plant regeneration. Callus induction frequencies among A × R hybrids ranged from 2.8% (IR58025A/IR46R) to 10.7% (IR58025A/IR9761R) and, in the case of B × R combinations, from the lowest, 0.5% (IR58025B/IR46R), to the highest, 3.5% (IR58025B/Swarna). The mean callus induction frequency of all the A × R hybrids was significantly higher than that of the corresponding B × R hybrids. Plant regeneration also showed a similar trend. The promotive effect of male sterility-inducing cytoplasm on anther culture response on the one hand and the reported negative influence on yield heterosis evident from the performance of A × R and B × R hybrids on the other hand suggest the differential influence of cytoplasm on anther culturability in rice (Balachandran et al 1996).

Anther culture has been reported to provide advantages such as accelerating the breeding process and increasing selection efficiency in the breeding of P(T)GMS rice over conventional techniques (Ling et al 199 1, Zhu et al 1988, Yin et al 1993). Several experiments with indica P(T)GMS were carried out to study anther culture and selection techniques, and the characteristic inheritance of pollen plants (Xie et al 1993, Xiang et al 1993, Li et al 1994, 1995). Based on a series of practices in searching for natural mutants, cross-breeding, mutation breeding, selecting in segregating generations, anther culture, and high-intensity screening, Pan et al (1993) outlined an effective breeding procedure for developing indica P(T)GMS, which involves crossing, anther culture, screening for fertility/sterility reaction at high elevation, sterility identification, and testing for heterosis. By this procedure, two TGMS lines (named 6442S and 1286S) were developed in 3 yr or 5 cropping seasons and tested in Jiangxi Province in 1994. One P(T)GMS line, Lu Guang 2S, and two TGMS lines, HS- 1 and HS-5, have been bred via anther culture and tested in Sichuan and Fujian provinces, respectively (Table 3). Anther culture of four crosses involving TGMS parents was done in the 1994 dry season at IRRI to evaluate the possibility of transferring TGMS

Table 3. Use of anther culture to develop photoperiod- and thermosensitive genic male sterile lines in China.

Name[a]	Sterility response type [b]	Achievement appraisal time	Use	Province
6442s	T-sensitive	Dec 1994	For crossing and production trial	Jiangxi
H1286S	Interaction of T and P	Dec 1994	For crossing and production trial	Jiangxi
Lu Guang 2S	Interaction of T and P	Sept 1995		Sichuan
HS-1	T-sensitive	Sept 1995		Fujian
HS-5	T-sensitive	Sept 1995		Fujian

[a] All lines are indica. [b] T =temperature, P = photoperiod.

traits to indica rice and to a high-yielding new plant type. In the 1995 wet season, 136 anther culture lines were evaluated in the field and 10 promising lines possessing very good male sterility were selected. One anther culture line showed consistent male sterility in both phytotron and field evaluations carried out in the I996 dry season.

Low seed set caused by semisterility due to incompatibility between indica and japonica lines can be increased to nearly normal by using WC genes. An alternative for using indica/japonica heterosis is to breed CMS lines with WC.

In collaboration with biotechnologists, breeders at the China National Rice Research Institute successfully bred a CMS line with WC. The F_1 anthers of 02428 (japonica WC) x Peiai 64 (javanica) were cultured in 1991. By test-crossing 80 doubled-haploid (DH) lines with Zhen Shan 97A, one DH line with high quality and maintaining capacity for CMS was selected and named 064B. Seed set in F_1 hybrids between 064B and indica or japonica testers indicated that 064B was a WCR. Continuous backcrosses were made with 064B after crossing it with Zhen Shan 97A. A new CMS line with cytoplasm of Zhen Shan 97A and the nucleus of 064B was obtained in I995 (BC_6 F_1) and named 064A; it has fairly good agronomic characters. Its rate of protruding stigma reaches 74%, with prolonged glume opening. Abortive pollen in 064A accounted for more than 99.8% and bagged seed set was zero. It has a strong combining ability with restorer lines. Hybrids with 064A displayed large panicles with more grains, high seed set, and short growth duration. Hybrids using 064A as a CMS parent are now being tested under different environments.

Procedure for three-line hybrid rice breeding via anther culture

Efficiency in breeding via anther culture relies on adopting the proper procedure, especially for indica. Figure 1 summarizes the available procedure for three-line hybrid rice breeding via anther culture (based on experiences of Chinese scientists: Zhu and Ding 1992).

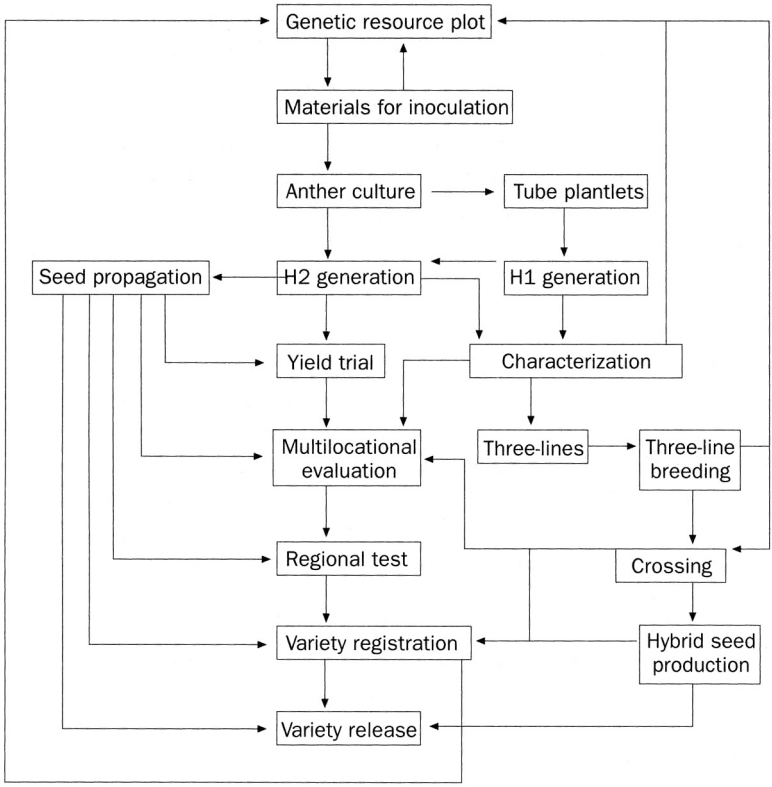

Fig. 1. Procedure for indica hybrid rice breeding through anther culture.

Genetic engineering approach in developing hybrid rice

Male sterility is used in many crop species as a means to develop hybrid seeds with superior characters. Cytoplasmic and nuclear mutations that prevent normal pollen development, which results in male sterility, have been identified. The genes responsible for male sterility may be nuclear (NMS), cytoplasmic (CMS), or a combination of both. Excellent progress has been made in describing many of the gene expression events that occur during androgenesis as well as genes controlling stamen primordia (Goldberg et al 1993). Genetically engineered tobacco and rapeseed have been reported based on the NMS system (Mariani et al 1990). The RNase genes were placed under the control of tobacco-derived tapetum-specific promoter *pTA29* and introduced into tobacco and rapeseed. Expression on the chimeric *pTA29:RNase* genes resulted in selective destruction of the tapetal cell layer and male sterility. The sterility encoded by the *pTA29:barnase* system can be restored by simultaneous expression in the tapetum of the chimeric *pTA:barstar* gene (Mariani et al 1990). Barstar inhibits barnase activity, which results in restored fertility (Goldberg et al 1993).

So far, three pollen-specific genes have been isolated and characterized: maize (*Zm13*), tomato (*LAT52,* Twell et al 1989), and rice (*PS1,* Zou et al 1994). The *PS1-GUS* chimeric gene was introduced into tobacco and it was expressed in specific tissues that resulted in male sterile plants (Zou et al 1994). We are working on *PS1-barnase* and *PS1-barstar* genes in rice to engineer male sterility for use in hybrid rice. We have reported recently that transgenic rice can be developed with the *PS1-barnase* gene, resulting in male sterile plants (Ling et al 1997). Detailed transmission genetics is now in progress.

Problems and prospects

The low in vitro induction rate is a key problem for indica rice breeding via anther culture. The first important task is to establish a rational anther culture system by integrating available individual techniques. Further research on the following aspects should also be intensified to enhance anther culturability in indica rice:

1. Investigate the nature of differences in anther culturability, such as the influence of endogenous hormones and amino acid levels on genotypes to help adjust the amount and proportion of exogenous hormone added to the culture medium.
2. Locate the anther culturability genes through molecular techniques based on the study of quantitative genetics and inheritance of the frequencies of callus formation and green plantlet and albino regeneration, and establish a germplasm bank of high anther culturability for parental selection to enhance the culture success rate.
3. Ascertain in vitro gamete selection effects in the process of dedifferentiation and differentiation.
4. Develop practical techniques to prevent necrosis, maintain the regenerative potential of embryogenic calli in subculture and suspension culture, and diploidize pollen-derived haploids.

Many problems still exist in pollen progeny selection; the most important one is how to increase selection efficiency. The current selection method is based mainly on the phenotype of pollen plants and cannot reach the expected efficiency for in vitro cell culture. An effective selection technique based on genotype instead of phenotype needs to be established after a thorough investigation on the relationship between calli and regenerants. Thus, selection could be conducted at the cell level rather than at the plant level, which would greatly enhance efficiency. Microspore culture could be used to achieve this objective (Cho and Zapata 1990, Datta et al 1990a). Key work would be to develop methods for screening cell culture-derived lines against biotic and abiotic stresses to breed highly resistant parental lines for hybrid rice.

Anther culture can be used extensively for hybrid rice breeding in the following ways:

1. By deploying the anther culture system for haploid protoplast culture, which is essential for genetic engineering. Plants regenerated from microspore-de-

rived cell suspensions (Datta et al 1990b, Chair et al 1996, Datta 1996) would allow the prompt recovery of homozygous diploid transgenic plants.

2. By analyzing the genetic mechanism governing some important characters. such as P(T)GMS, WC, and CMS restoring ability using anther culture-derived doubled-haploid lines, which can effectively eliminate gene interaction existing in the heterozygous genotypes derived from conventional breeding procedures. DH lines also avoid interference from the environment on gene expression.
3. By incorporating WC genes expeditiously into CMS or restorer lines for developing indica/japonica rice hybrids.
4. By developing bridge materials from the pollen-derived progenies of intervarietal and intersubspecific crosses, which can be used as parental lines of heterotic rice hybrids to develop diverse CMS lines.
5. By facilitating the genetic engineering approach to develop diverse and improved CMS lines possessing novel genes transferred from the transformed maintainer lines (Alam et al 1998).

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Notes

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CHAPTER 22

Apomixis in crop improvement: traditional and molecular approaches

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Apomixis is a method of reproduction that allows a chromosomally unreduced egg cell to develop into an embryo without fertilization by a sperm. It is controlled by qualitative genetics and found mainly in polyploid species in the tertiary gene pools. The apomictic mechanism is present in a number of fruit and nut crop species and in the wild relatives of some important agronomic crops. The main advantages of apomixis are that it would simplify hybrid seed production, provide the opportunity to develop and fix the genotype of unique and superior hybrids, and make hybrid production possible in crops without good male sterility systems. Apomixis can be identified by progeny tests, test crosses, cytological methods, and molecular methods. Progress is being made in transferring this mechanism from wild to cultivated species by the backcrossing method. Molecular methods are being used to develop molecular markers linked to the gene(s) controlling apomixis and to map it(them). Ultimately, the gene controlling apomixis will have its greatest value if it can be cloned and made to express itself stably when inserted into an alien genome. The gene controlling apomixis could have a major impact on food, feed, and fiber production around the world.

Apomixis can be used to effectively harness hybrid vigor because any obligate apomictic plant, regardless of its heterozygosity, will breed true for its genotype. This reproductive mechanism allows a chromosomally unreduced egg cell in the ovary to develop into an embryo without fertilization by a sperm, which allows for vegetative reproduction through the seed. The egg cell in an apomictic ovule is usually derived from a chromosomally unreduced megaspore mother cell or somatic cell in the nucellus (to be discussed later). But microsporogenesis does occur on the male side in apomicts and produces pollen with chromosomally reduced sperm cells in the pollen. Chromosomally reduced gametes in the pollen allow for the transfer of this reproductive mechanism, especially if apomixis is controlled by a dominant gene(s).

Apomixis was described as early as 1841 (Asker and Jerling 1992). It has been reported in more than 300 species in at least 35 different plant families (Hanna and Bashaw 1987). In general, apomixis has not been used extensively to produce true cultivars in the past. One reason for this is that it was assumed that apomixis resulted in a dead end in breeding programs. Second, usable apomixis has not been found in the "important" agronomic crops and, if it is found in the genus, it is in a tertiary gene pool polyploid relative. Third, obligate types of apomixis are usually found in species with little direct economic value. The potential of apomixis for developing true-breeding superior hybrids is being increasingly recognized as sexual plants are discovered in apomictic species, as more information is known about its genetics and cytology, as apomictic cultivars are released, and as traditional and molecular methods are demonstrated for manipulating the mechanism.

Advantages of apomixis

The most exciting advantage of apomixis is that any apomictic plant with desirable characteristics from a sexual × apomictic cross has the potential to become a cultivar. The genotype of the superior obligate apomictic hybrid is fixed in the embryo of the seed that produced the plant and can be increased indefinitely. Advantages of apomixis in breeding and seed production have been discussed previously (Hanna and Bashaw 1987, Hanna 1995). We discuss a few general advantages below.

Pyramiding genes

Apomixis would allow breeders and geneticists to easily pyramid genes and blocks of genes for specific characters. An apomictic plant with a superior gene combination could be crossed as the male parent with another plant having a desirable gene(s). An apomictic plant from such a cross with the new desired gene combination would breed true and eliminate the need to stabilize the gene combination by further breeding and selection. Two crosses of the apomictic plant to the plant with the desired gene(s) may be needed for the expression of recessive characters if the apomictic pollinator does not already have one recessive allele.

Exploiting the potential of germplasm pools

Once the gene(s) controlling apomixis is introduced and made to function in the species of interest, all germplasm in the species (and potentially cross-compatible related species) acquires the potential to immediately contribute to the development of a cultivar regardless of its heterozygosity. Superior individual apomictic plants can be selected from sexual × apomictic crosses in the first generation and would be ready for performance testing. Apomixis would eliminate concerns about maintainer and restorer genes and cytoplasms in the germplasm for producing hybrids.

Simplifying hybrid seed production

The breeding process can be greatly shortened. But the testing and evaluation phase may not be much different than that used for stabilized sexual hybrids. There would

be no need to develop and maintain inbreds, which would be a great savings in time. resources, and space. Selfing and progeny testing to stabilize a genotype would be eliminated. A progeny test to confirm the level of apomictic reproduction estimated by cytological and/or molecular methods, however, would be needed for apomicts.

Apomictic mechanisms

Bashaw (1980) discussed various apomictic mechanisms and presented photomicrographs of cytological development. Classification of each mechanism is based on the origin and development of the cell from which the embryo develops. Adventitious embryony is the most frequent apomictic mechanism in plants. Two mechanisms, apospory and diplospory, however, seem to have the most potential in Gramineae (Hanna and Bashaw 1987).

Embryo sacs of diplosporous apomicts look identical to sexual sacs, having an egg, two polar nuclei, two synergids, and three antipodals (or a proliferation of antipodals). The difference is that the nuclei are chromosomally unreduced in a diplosporous apomict. Diplosporous apomicts can be identified by the absence of both meiosis and a linear tetrad of megaspores.

Apospory is more easily identified in mature ovules because of the lack of antipodals in the embryo sac and, usually, the presence of more than one embryo sac in the ovule. Orientation and shape of the embryo sacs can also be used to distinguish aposporous and sexual embryo sacs. In this mechanism, all four megaspores or products of meiosis degenerate and one or more nucellar cells enlarge to produce embryo sacs with one or more unreduced nuclei. In adventitious embryony, embryos develop through mitotic division of the somatic cells of the ovule, integuments, or ovary wall and begin as bud-like structures. If endosperm is needed for seed development, it is derived from the fertilization of polar nuclei in sexual embryo sacs of the same ovule. This mechanism is common in citrus.

Levels of apomixis

Apomixis can be obligate, in which a plant reproduces only by an apomictic mechanism, or facultative, in which a plant reproduces by both an apomictic mechanism and sexual reproduction (in the same ovule or in different ovules on the same plant). We agree with Asker and Jerling (1992), who questioned whether any plant or species can be classified as an obligate apomict, because, if we look at enough plants, one or more offtypes can be found. Technically, a few offtypes may prevent a plant from being classified as obligate, but, practically, such a plant could function as an obligate apomictic with few to no detrimental effects on a cultivar.

In a breeding program in which cultivar uniformity is needed, obligate apomixis is usually considered desirable, especially if a sexual counterpart is available, because stable true-breeding genotypes are more likely to be produced. This would apply to sexual species in which a gene controlling apomixis is introduced or to apomictic

species in which sexual plants have been identified. Facultative apomixis is useful in apomictic species for which sexual genotypes have not been identified.

Facultative apomixis may also be used effectively in situations where both hybrid vigor and genetic diversity are needed. A facultative apomictic cultivar would not maximize hybrid vigor for the cultivar, but a certain amount of vigor would be maintained depending on the level of apomixis, whereas sexual reproduction in the cultivar would allow for recombination and the production of new genotypes.

Sources of genes controlling apomixis

In sexual crops, the ideal situation is to find apomixis in the species or in a cross-compatible species. Apomixis is known to exist in a number of tertiary gene pool species related to pearl millet, *Pennisetum glaucum* (L.) R. Br. A number of these *Pennisetum* species can be crossed with pearl millet. Apomixis has also been reported in *Elymus rectisetus,* a wild relative of wheat (*Triticum aestivum* L.) (Carman and Wang 1992), and in *Tripsacum dactyloides* (L.), a wild relative of maize (*Zea mays* L.) (Dewald et al 1992). Facultative apomixis has been discovered in sorghum, *Sorghum bicolor* (L.) Moench, but a usable level of apomixis has not been stabilized (Schertz 1992). A search for apomixis in 13 species of rice (*Oryza sativa* L.) has not revealed any apomictic species for this genus (Rutger 1993). A similar search among the wild rice species in the IRRI International Rice Genebank has not revealed any apomictic reproduction (G. Khush, personal communication, 1995). Wilson (1993) summarized much of the research on apomixis in rice conducted by scientists in the People's Republic of China. Although some interesting abnormal reproductive behavior has been observed in rice there, more documentation is needed to confirm apomictic reproduction.

Apomixis is usually found in polyploid species. But this reproductive mechanism has also been reported in diploids (Asker and Jelling 1992, Nogler 1984). Obligate apomictic reproduction was maintained in polyhaploids of an interspecific *Pennisetum* hybrid (Dujardin and Hanna 1986) and an interspecific maize-*Tripsacum* hybrid (LeBlanc et al 1996). Two apomictic mutants have been induced with radiation and chemical mutagens in pearl millet (Hanna et al 1993a). One of the mutants is facultative and unstable with varying levels of apomictic reproduction. Another mutant shows a high level of aposporous embryo sac development but is female sterile. Both mutants are controlled by recessive genes (Morgan et al 1997). A mutagenesis program has also been initiated to induce apomixis in *Arabidopsis thaliana* (Peacock et al 1995).

Another source is a gene cloned by molecular techniques from an apomictic species (to be discussed in more detail later). This gene could theoretically be introduced into any sexual species regardless of taxonomic relationship. The value of such a gene will depend on whether it will be stable and express itself in an alien genome.

Identifying apomixis

A number of characteristics are indicators of apomixis. If a plant or progeny has one or more of these characteristics, efforts should be made to document whether the response is due to apomixis or another mechanism.

Usually, more than one test is needed to document apomixis and the level of it. Characteristics that may indicate apomixis are:

1. A lack of hybrids or a lower number of hybrids than expected in controlled crosses. One way to document apomixis or the level of it is to pollinate a suspected apomict with a dominant marker. Other factors, however, such as cleistogamy, improper emasculation, and poor-quality pollen, could also produce a lower than expected number of hybrids.
2. Uniform progenies from plants expected to be heterozygous for morphological characteristics. A lack of hybrids and uniform progenies are two characteristics that researchers should be aware of in traditional breeding and genetics programs. Of course, uniform progenies could be due to factors unrelated to apomixis, such as nondistinct morphological variation in plants that reproduce sexually.
3. Cytological observations can be used to detect and distinguish apomictic mechanisms. This technique may require special training and equipment or cooperation with someone familiar with it. Apospory and adventitious embryony are the easiest to distinguish cytologically. All three mechanisms, however, can be distinguished cytologically by ovule characteristics, as discussed earlier under "apomictic mechanisms."
4. Molecular markers linked to apomixis can be used to detect apospory at the seedling stage. Markers such as UGT 197 (Ozias-Akins et al 1993, Lubbers et al 1994) that are closely linked to apomixis can be used to detect apomictic reproduction (Miles et al 1994).

Progress in transferring apomixis to cultivated crops

Backcrossing methods are used to transfer genes controlling apomixis from wild tertiary gene pool species to the cultivated species in maize, wheat, and pearl millet. Progress is being made in this transfer, but sterility and facultative apomixis are being encountered. High sterility of the F_1 interspecific hybrids has hampered progress in the transfer to wheat (Carman and Wang 1992). High male sterility has slowed progress in gene transfer from *Tripsacum* to maize (LeBlanc et al 1996, Y. Savidan, personal communication, 1996). Progress has been made in transferring the apomictic mechanism (apospory) from *P. squamulatum* Fresen to tetraploid (2n = 4x = 28) pearl millet. Obligate apomixis was maintained to the BC_3 generation. In the BC_4 generation, apomictic plants produced 89% maternal types (Hanna et al 1993b). Apomictic BC_6 plants have been recovered that produce greater than 95% maternal types (Hanna, unpublished results, 1996). Pollen viability on most of the advanced-generation pearl millet-like plants is greatly reduced but good enough to effect seed set. A major prob-

lem encountered is that up to 90–95% of the seeds on both sexual and apomictic pearl millet-like plants abort at about 10 d postpollination. Efforts are under way to introduce new germplasm into the apomictic genotypes in an attempt to eliminate seed abortion. A similar problem was encountered in tetraploid pearl millet. Hanna et al (1992) have summarized the procedures used to transfer apomixis in *Pennisetum*.

Using apomixis in cultivated crops

Apomixis is beginning to make contributions to cultivar development. The discovery of a sexual plant in buffelgrass (*Cenchrus ciliaris* L.), an apomictic species, renewed interest in the potential of apomixis in cultivar development. The sexual plant allowed for the production of hybrids in buffelgrass and the subsequent release of forage cultivars (Bashaw and Hussey 1992). The discovery of facultative apomixis in sorghum (Schertz 1992) and progress made in the transfer of apomixis in *Pennisetum* (Hanna et al 1992) showed that it may be possible to use apomixis in cultivated crops.

Bashaw and Funk (1987) have outlined breeding procedures for using apomixis. Hanna (1995) recently discussed the use of apomixis in cultivar development. We would like to comment on a couple of aspects of breeding.

It is not necessary to convert a genotype to apomixis before it can be used. The "conversion" occurs automatically when we make a sexual × apomictic cross because the cross produces sexual and apomictic progenies. Superior apomictic genotypes or plants are ready for performance testing regardless of the heterozygosity of either parent. In fact, diversity in the parents (both sexual and apomictic) increases the diversity of the progeny and the likelihood of producing a new unique genotype with potential to become a cultivar.

Obligate apomixis is probably most effective if crop uniformity (maturity, height, etc.) is desired. In many parts of the world, crop uniformity is not needed and in some cases is undesirable. In these situations, facultative apomixis can be used effectively to increase (although not maximize) yields, especially if obligate apomixis is not available. In areas of the world where hybrids are not being used because of high costs or lack of availability, any increase in yield or vigor would be welcome.

In cross-pollinated species, a gene controlling apomixis could be introduced into a diverse population or landrace. Apomictic plants would pollinate sexual plants to produce sexual and apomictic plants with new gene combinations. Superior apomictic genotypes adapted to a particular environment should have a selective advantage to improve a population. A dominant gene introduced into a population would fix genotypes at a more rapid rate than a recessive gene.

Genetic vulnerability

Hanna (1995) discussed some aspects of genetic vulnerability and concluded that there may be less vulnerability if apomixis is used in cultivar development because hybrids would not depend on specific cytoplasms and inbreds. Potentially, a superior hybrid or single genotype could dominate a production area. This would probably not

happen because numerous superior apomictic hybrids could be easily produced because the gene pools could readily be incorporated into cultivars. In developing countries, genetic diversity in production fields could be increased by blending apomictic hybrids. The gene controlling apomixis could be introduced into landraces and local ecotypes and allowed to randomly mate in the population. This would constantly produce new hybrids, the best of which would have a competitive advantage. At the same time, apomictic plants would constantly be recombining with sexual plants to produce new genotypes, both sexual and apomictic.

Genetics

Apomixis is usually reported to be controlled by one or a few genes (Asker and Jerling 1992, Nogler 1984). Both dominant and recessive gene action have been reported. The qualitative inheritance of this reproductive trait enhances its manipulation by both traditional and molecular methods. Varying degrees of facultative apomixis in some species indicate that modifying genes or the genetic background may affect its expression. In the *Pennisetum* genus, several genetic studies demonstrate that apospory is inherited as a single dominant gene or a tightly linked cluster of genes (P. Ozias-Akins and W. Hanna, unpublished results).

Emerging molecular work on apomixis in plants

An international conference ("Harnessing Apomixis: A New Frontier in Plant Science") held 25-27 September 1995 at College Station, Texas (USA), showed new trends of research on apomixis but also the relative paucity of these efforts, considering the impact that such research could have on world agriculture. We will refer to some of the work that was presented on posters at this conference as personal communications along with a limited amount of work already published. Among dicotyledonous species, work on "pinpoint" inactivation of apomictic gene(s) by transposon tagging is currently under way in New Zealand on *Hieracium pilosella,* a tetraploid aposporous Compositeae (Koltunow et al 1995). In a diplosporous crucifer, *Arabis holboellii,* work is beginning on the molecular characterization of "a putative cytoplasmic apomixis factor" (B. Lehnhardt, Humboldt Univ., Berlin, Germany, personal communication). In a nonapomictic species, *Arabidopsis thaliana,* work by mutagenesis is under way to generate apomictic mutants able to produce seeds without fertilization. A few mutants, *fis* for fertilization-independent seed, have been found in which there is partial development of the embryo and endosperm up to the point of cellularization for the latter (A.M. Chaudhury, CSIRO, Australia, personal communication).

For monocotyledonous species, most molecular work has been conducted on genotypes generated by the introgression of wild apomictic genotypes into their domesticated related species, such as *Tripsacum* sp. with maize (LeBlanc et al 1996) and *Pennisetum* sp. with *P. glaucum* or pearl millet (Ozias-Akins et al 1993). Molecular mapping of diplospory and apospory, respectively, is currently under way in maize

and *Pennisetum* sp. Comparable work is being conducted with apospory in *Brachiaria* sp. (N. Palacios, CIAT, Cali, Colombia, personal communication). Research with more emphasis on gametophyte development in aposporous genotypes with subtractive hybridization between pools of RNA from sexual and apomictic ovaries is being conducted on *Brachiaria* sp. (V.T.C. Carneiro, EMBRAPA/CENARGEN, Brasilia, Brazil, personal communication) and *Pennisetum ciliare* (J.-Ph. Vielle-Calzada, Texas A&M, College Station, Texas, USA, personal communication).

We intend to present here the status of our progress in molecular mapping of apospory in the *Pennisetum* genus as well as our strategy to achieve cloning of the gene(s) for apospory.

Searching for apospory-linked molecular markers in *Pennisetum*

The *Pennisetum* genus has several apomictic wild relatives of cultivated pearl millet. Work in our laboratory has been pursued for more than a decade to introgress the apomictic trait from *P. squamulatum* into pearl millet (Dujardin and Hanna 1986). It therefore appeared logical to search for molecular markers linked to the aposporous trait in apomictic BC_3 plants as well as within progenies of BC_4 plants. This effort yielded two molecular markers that are coinherited with the apomictic mode of reproduction. It also suggested that apospory is transmitted by a single chromosome (Ozias-Akins et al 1993).

Further mapping resolution of the apomictic trait, however, seemed likely to be hampered by the alien nature of the *P. squamulatum* chromosome bearing the aposporous trait, with the following consequences: no recombination with the *P. glaucum* genome, very low frequency of transmission of the apomictic trait through the pollen (Dujardin and Hanna 1986), and unknown potential for chromosome breaking, rearrangement, and deletion. Meanwhile, analysis of a few interspecific F_1 hybrids between tetraploid pearl millet and *P. squamulatum* (used as the pollen donor) demonstrated that the latter species is heterozygous for its mode of reproduction with apospory as a dominant trait. That single finding led to the possibility of mapping the trait in a polyploid population in a comparable manner to the analysis by single-dose restriction fragments (SDRF) (Wu et al 1992). An SDRF "is equivalent to a simplex allele in an autopolyploid or to an allele at one heterologous locus in a diploid genome in allopolyploids" (Wu et al 1992).

Because in our subsequent mapping efforts we have pursued PCR-generated molecular markers such as sequence-tagged sites (STS from RFLPs), RAPDs, and sequence-characterized amplified regions (SCARs from RAPD-cloned bands), it would be more appropriate to refer to our work as an analysis of single-dose amplified fragments (SDAF). We are using these amplified fragments to locate gene(s) conferring apospory. Segregation/recombination for these alleles occurs during male meiosis in *P. squamulatum,* the pollen donor in the interspecific cross. *P. squamulatum,* being an obligate apomict, can only be used as a pollen donor to generate a segregating progeny for the apomictic trait.

In the past three growing seasons, a mapping population of 400 individuals from tetraploid pearl millet x *P. squamulatum* crosses has been characterized for mode of reproduction as well as by RAPD analyses. Five hundred sets of operon primers were tested. The mode of reproduction in the 400 progenies was determined cytologically by an ovule-clearing technique (Young et al 1979) and by progeny testing. We have now found and sequence-characterized I1 single-dose molecular markers linked to the aposporous trait in *P. squamulatum.* All informative RAPD bands were successively cloned and end-sequenced, primers were generated for SCAR analysis, and linkage to the aposporous phenotype was confirmed. Genetic recombination between all these markers is apparently very low (Ozias-Akins et al, in preparation).

The quest for genes for apospory in *Pennisetum*

Several challenges lie ahead before the gene for apospory is cloned. First, there is no available genetic map of *P. squamulatum.* Furthermore, the lack of recombination hampers the potential use of classical approaches of genetic/physical mapping and chromosome walking. Therefore, rapid progress toward map-based cloning is not expected soon if we limit our strategy to these conventional means.

We intend to overcome these obstacles by considering several innovative approaches: (1) genetic mapping by a linkage disequilibrium approach involving many *Pennisetum* spp., (2) physical mapping by the RARE (RecA-assisted restriction endonuclease) method, (3) irradiation-induced deletions within the chromosome-bearing gene(s) for apospory, (4) making a large DNA insert in the bacterial artificial chromosome (BAC) library, and (5) making a transcriptional map of the apospory DNA contig by direct selection. The details are discussed below.

Genetic mapping using a linkage disequilibrium approach among 29 accessions of *Pennisetum*

Earlier work demonstrated that two molecular markers found to be linked to the aposporous trait in *P. squamulatum* are also present in other species of *Pennisetum* that reproduce by apospory (Lubbers et al 1994). Among 11 SCAR markers whose linkage to the gene(s) for apospory has been confirmed within the mapping population, three are found in two other species of *Pennisetum—P. ciliaris* (buffelgrass) and *P. massaicum* (D. Roche et al, in preparation). Two other markers are found in *P. ciliaris, P. massaicum, P. squamulatum,* and *P. orientale.*

Most of the remaining markers are found in apomictic *Pennisetum* species, but some also appear in certain sexual species. This may provide a way to tag molecular markers to the gene(s) for apospory by a linkage disequilibrium analysis (Hastbacka et al 1994) based on the following principle. Among a collection of species from the same genus, markers nearest the gene(s) controlling apospory will have recombined and mutated the least and should show the best allelic association with the studied trait. This approach is also substantiated by the segregation of several molecular markers linked to apospory in *P. squamulatum* in two mapping populations of buffelgrass kindly provided to us by Drs. R. Sherwood and D. Gustine (USDA-ARS, College

Park, Maryland, USA). It appears that some of these markers are tightly linked to the aposporous phenotypes, while the remaining could be found in both sexual and apomictic phenotypes (D. Roche et al, in preparation).

Physical mapping by the RARE cleavage method

Fine physical mapping of molecular markers (i.e., knowing the distance between each one, as well as ordering them) could be troublesome without a genetic map available. RARE cleavage allows for the selection of only one restriction site in 200–300-kb lengths of DNA for specific cleavage (Ferrin and Camerini-Otero 1991). Using this technique, physical mapping of human chromosomes and especially of their telomeric regions has been enhanced (Macina et al 1995, Reston et al 1995). We intend to measure by pulse field gel electrophoresis physical distances between linked markers in which we identified unique restriction sites.

Irradiation-induced deletions within the chromosome bearing the gene(s) for apospory and our informative molecular markers

Irradiation-induced deletions have been useful in mapping DNA segments with clustered genes for which recombination was very low (Anderson et al 1996). We intend here to mutate or delete a single-dose dominant gene(s) controlling apospory in a polyploid apomict genotype to produce plants that reproduce sexually. We are currently producing 5,000 seeds from an obligate apomict F_1 interspecific hybrid (tetraploid pearl millet × *P. squamulatum),* which will be irradiated with fast neutrons at the International Atomic Energy Agency (Vienna, Austria). Plants will be established from irradiated seeds and subsequently crossed with pollen from a tetraploid pearl millet carrying a dominant plant color marker. Plants whose progenies indicate some level of reversion to sexuality will be investigated for the presence or absence of all informative molecular markers.

Making large DNA inserts in bacterial artificial chromosome (BAC) libraries

BAC libraries are a recent improvement over yeast artificial chromosome (YAC) libraries in regard to ease of producing. We are currently developing a BAC library from a full obligate polyhaploid of an interspecific *Penneisetum* hybrid (Dujardin and Hanna 1986). Modifications of cloning procedures in order to yield a vast majority of 200–400-kb clones are currently being tested (D. Roche, unpublished results). Larger DNA insert libraries will positively limit the size of the BAC library as well as facilitate research on DNA contigs and chromosome walking.

Transcriptional map of the apospory DNA contig

With the above mentioned approaches (genetic/physical mapping and deletion studies), we should be able to establish a DNA contig containing the gene(s) for apospory with several BAC clones. Another major challenge is to identify genes within that region. We intend to hybridize and select in vitro against these BAC clones and cDNAs (or cloned expressed genes) from libraries made from floral tissues of apomicts at different stages of megagametogenesis. This technique of hybridization of cDNAs to

large genomic regions is called direct selection (Lovett et al 1991) and it has been vital to the recent cloning of human genes (Simmons et al 1995). Candidate cDNAs will be studied for their expression at the transcriptional level in both apomictic and sexual genotypes. They will be used to isolate genomic clones from BAC clones that include both promoters and protein-encoding regions.

Transforming pearl millet and other cereal crops

After isolating candidate genomic clones, we intend to transform a tetraploid sexual pearl millet to an apomict by high-velocity coated microprojectiles. The transgenic expression of the aposporic gene(s) should be as straightforward as in the apomict BC_3, a tetraploid sexual parent of pearl millet with a single alien chromosome of *P. squamulatum* (Dujardin and Hanna 1989). Further transformation of a diploid sexual pearl millet should provide valuable insights into the penetrance of the trait at lower ploidy levels. Finally, transformation of major crops, including rice, will be investigated.

The cloning of a gene(s) controlling apospory whose selective fitness has been demonstrated *in natura* and conserved by evolution in many species of Gramineae (Reddy 1977) could be rewarding. The breeding and propagation of cereal crops should greatly benefit from apomixis. Transformation of these crops with a gene for apospory from a Gramineae will likely ease recombinant expression of the trait as well as experimental interpretation and trouble-shooting of transgenic studies.

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Notes

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CHAPTER 23

Apomixis in rice and prospects for its use in heterosis breeding

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Apomixis is being explored as a new frontier to exploit hybrid vigor and to develop true-breeding hybrid rice varieties. Apomixis is common in grasses and in several polyploid plant species and is controlled by one or a few genes. Among the major cereals, maize, wheat, and pearl millet have apomictic relatives. But there is no clear evidence of apomixis in rice. A-genome diploid wild relatives have been examined based on studies of crosses with dominant marker stocks. Reports on cyto-embryological studies also lack genetic evidence for the occurrence of apomixis in rice. We proposed three strategies to develop apomictic rice: (1) screening germplasm of tetraploid wild species as a source of apomixis and transferring the apomictic trait to rice cultivars, (2) inducing apomictic mutants in rice through mutagenesis, and (3) developing apomictic rice using molecular ap proaches. We have screened 108 accessions of tetraploid *Oryza* species for apospory (multiple embryo sac development) and 86 accessions for diplospory (based on callose detection), including five related genera. But none of the accessions showed any evidence of apomixis. We have also undertaken the second approach to induce apomictic mutants. Mutagenized populations derived from treating seeds and fertilized egg cells with gamma rays, ethyl methane sulphonate, and N-methyl-N-nitrosourea are being screened. We have selected a dominant purple leaf mutant of rice for identification of the apomictic mutants following mutagenesis. IRRI is collaborating with advanced laboratories to develop apomictic rice through molecular approaches. Molecular markers linked to the apomictic mode of reproduction have been identified in progenies of maize *(Tripsacum)* and in crosses of sexual and apomictic wild species of *Pennisetum.* Cloning of the gene(s) for apomixis is under way from apomictic plant species such as *Tripsacum, Pennisetum, Brachiaria,* and *Cenchrus.* Once such genes become available, they will be introduced into elite breeding lines of rice using transformation technology. We are also exploring the possibility to identify dividing nucellar cells capable of forming adventitious embryos in a transgenic rice line (HSK-1). Apomixis will increase the efficiency of heterosis breeding in producing many true-breeding hybrids compared with those

produced by using three-line or two-line hybrid breeding systems. The availability of a large number of hybrids will increase genetic diversity and reduce genetic vulnerability. Moreover, the possible vulnerability to pests and diseases because of narrow cytoplasmic male sterility sources will also be eliminated. The development of apomictic rice would enable resource-poor farmers in developing countries to adopt high-yielding hybrid rice technology. This would lead to an increase in area planted to hybrid rice, resulting in higher productivity and production.

During the past 25 years, major increases in rice production have occurred because of the large-scale adoption of high-yielding semidwarf varieties and improved technology. World rice production doubled from 257 million t in 1965 to 518 million t in 1990. The population of rice-consuming countries is increasing faster than that of the rest of the world and the number of rice consumers will probably double in the next 30 years. By the year 2025, we need to produce 70% more rice to meet the growing demand. But major increases in the area planted to rice are unlikely to occur. In fact, we will have less land, water, and labor for rice production. Thus, further increases in rice production will have to come from increased yields per unit land area. Therefore, we need rice varieties with higher yield potential and better management practices. In its strategy document for 2000 and beyond, the International Rice Research Institute (IRRI) gave the highest priority to increasing the yield potential of rice (IRRI 1989).

Two strategies have been adopted to develop rice germplasm with a higher yield potential. One strategy aims at developing the new plant type to increase the harvest index to 0.6 instead of 0.5 (Khush 1993). Such a plant type is expected to have a yield potential of 12–12.5 t ha^{-1} under tropical conditions. The second strategy aims at developing hybrid rice by exploiting heterosis in indica/indica and indica/tropical japonica (new plant type) crosses. Indica hybrids yielding 15–20% higher than indica inbreds are already available (Virmani, this volume, Chapter 4). Hybrids involving the new plant type are expected to have even stronger (20–25%) heterosis or a yield potential of 15 t ha^{-1} (Khush and Aquino 1994).

Hybrid rice has been successfully developed and used in China and approximately 50% of China's rice area is now planted to hybrid rice (Yuan et al 1994). Recently, some rice hybrids have been released in India, Vietnam, and the Philippines. The constraint to the wide-scale adoption of hybrid rice is the complexity of seed production based on male sterility systems. Moreover, rice hybrids produced through these male sterility systems do not breed true and lose the yield advantage in subsequent generations. Therefore, farmers have to buy seed for each crop. The cost of hybrid seed is generally 10–20 times the cost of seed of true-breeding varieties. Resource-poor farmers will therefore be unable to purchase the costly seed and take advantage of the hybrid technology.

Apomixis is being explored as a new frontier project to exploit hybrid vigor and develop true-breeding hybrid rice varieties. Apomixis refers to asexual reproduction

through seed. It is a method of reproduction in which the embryo (seed) develops without the union of the egg and sperm. Apomictic seed is genetically identical to the maternal parent. Apomixis will also increase the efficiency of hybrid rice breeders in producing many new true-breeding hybrids compared with those produced by using the three-line or two-line hybrid breeding system. The availability of a large number of hybrids will help increase genetic diversity and reduce genetic vulnerability. Vulnerability caused by cytoplasm would be virtually eliminated because a specific cytoplasmic-nuclear male sterility-inducing cytoplasm would not be needed to release a hybrid commercially.

Apospory, diplospory, and adventitious embryony are the most common types of apomixis (Asker and Jerling 1992). Various cytological, histological, and genetic tests are available to screen germplasm for apomixis. Apomixis is widespread and more than 300 plant species are apomictic. It occurs mainly in polyploid species. Genetic studies indicate that the switch between sexual reproduction and apomixis is controlled by one or two genes. Koltunow (1993), Khush et al (l994), and Hanna (1995) have reviewed different aspects of apomixis, such as occurrence, types, inheritance, screening techniques, and potential for exploiting hybrid vigor and cultivar development.

Approaches for developing apomictic rice

A number of attempts have been made to screen rice germplasm for apomixis. Hongde (1993) has reviewed progress made in China to screen rice germplasm for the occurrence of twin seedlings. More than 40 varieties with twin seedlings were found after screening thousands of varieties at the Hunan Hybrid Rice Research Center. Cyto-embryological analysis indicated the occurrence of adventitious embryony. Certain rice genetic stocks (SAR-1, 84-15, C1001) have been reported to show apomictic characteristics (Kaida 1993). Cai et al (1993) also reported a high frequency of apomixis in HDAR00I, selected from the progeny of a cross of a twin seedling and a japonica variety following radiation treatment. All these studies have been based on cyto-embryological analysis. Further investigations based on genetic analysis, however, are needed to verify the occurrence of apomixis in these lines.

Khush et al (1994) proposed three approaches to obtain apomictic rice: (1) searching for apomixis in the wild *Oryza* germplasm, (2) mutagenesis to induce apomixis, and (3) molecular approaches to engineer apomixis.

Screening wild *Oryza* germplasm for apomixis

As discussed earlier, apomixis has been reported in the wild relatives of several crop species. Besides its two cultivated species, the genus *Oryza* has 18 wild species. IRRI has a collection of more than 2,700 accessions of wild species. Rutger (1992) screened 547 accessions of closely related wild species of rice with AA genomes through the pistil clearing technique. Results were negative. Apomixis is rare in diploid species but is common in polyploid relatives of crop plants. Therefore, we started screening

the germplasm of tetraploid wild species of *Oryza* and other related genera using two techniques: (1) pistil clearing and (2) callose detection using fluorescence microscopy.

About 200 accessions of tetraploid species are available in the IRRI Genetic Resources Center. We have screened 108 accessions for apospory using the pistil clearing technique, which is simple and fast and is widely used to examine embryo sacs (Young et al 1979, Crane and Carman 1987). Pistil clearing methods using aromatic esters greatly reduce the time needed to prepare samples for examination. The method consists of fixing pistils at different stages of development in formalin-acetic acid-alcohol (FAA) consisting of 70% ethanol, glacial acetic acid, and 37% formaldehyde (18:1:1), and passing pistils through alcohol series, clearing them with methyl salicylate, and examining them under a phase-contrast microscope. The contents of the entire ovule are examined by changing the focal level of the microscope. This technique has been used extensively in *Pennisetum* to screen for aposporous embryo sac development. We examined several accessions of diploid and tetraploid wild species of rice, including related genera (Table 1), for the possible occurrence of apospory based on multiple embryo sac development. None of the accessions, however, showed any evidence of apospory (Brar et al 1995).

We have also examined 22 accessions of diploid and 86 of tetraploid wild species of *Oryza* and five accessions of related genera for diplospory through callose detection using fluorescence microscopy (Table 1). None of the accessions of *Oryza* species and related genera examined so far, however, showed a diplospory type of apomixis. The callose fluorescence technique is used in combination with the pistil clearing technique to detect diplosporous embryo sac development. A sucrose clearing solution (2.46 M sucrose, 136 µM aniline blue, 50 mM K_2HPO_4, pH 9.5) induces excellent callose fluorescence of embryo sac walls. Callose is deposited in the cell walls of megaspore mother cells during megasporogenesis in sexual species, but this deposition is nearly absent in the cell walls of apomictic embryo sacs. Carman et al (1991) compared the embryo sacs of sexual *Elymus scabrus* and diplosporous *E. rectisetus.* Callose accumulated in and around the cell walls of embryo sacs of *E. scabrus* but was absent in those of *E. rectisetus.* Peel and Carman (1992) combined the pistil clearing technique with callose fluorescence to rapidly screen for apomixis. This technique is also used at the International Maize and Wheat Improvement Center (CIMMYT) to screen diplosporous embryo sac development in *Tripsacum* germplasm and backcross progenies from maize - *Tripsacum* crosses.

We are also examining the histological sections of ovules of tetraploid species through the standard paraffin sectioning method to detect apomixis. In this technique, embryo sac development is examined by histological serial sections of ovules. Female florets at different stages of maturity are collected and fixed in FAA for 24 h and are then transferred to 70% ethanol. Pistils are dissected and dehydrated using the method of Young et al (1979). The pistils are then embedded in paraffin, sectioned at 10 µm, and stained with safranin-fast green.

Once apomictic germplasm is identified, the gene(s) for apomixis will be transferred to cultivated rice through the standard backcrossing procedures outlined by

Table 1. Wild species of *Oryza* and related genera screened for apospory (multiple embryo sac development) and diplospory (callose fluorescence) types of apomixis.

Species		Genome	Accessions (no.) analyzed for:	
			Apospory	Diplospory
Diploid *Oryza* species				
0. sativa (cultivated)		AA	3	3
0. nivara		AA	4	4
0. longistaminata		AA	1	1
0. barthii		AA	6	0
0. glumaepatula		AA	1	1
0. punctata		BB	2	2
0. officinalis		CC	5	3
0. australiensis		EE	6	3
0. brachyantha		FF	3	3
0. granulata		Unknown	4	2
Total			35	22
Tetraploid *Oryza* species				
0. punctata		BBCC	16	15
0. minuta		BBCC	32	28
0. malampuzhaensis		BBCC	3	2
0. alta		CCDD	9	8
0. latifolia		CCDD	30	21
0. grandiglumis		CCDD	8	4
0. ridleyi		Unknown	8	5
0. longiglumis		Unknown	2	3
Total			108	86
Related genera				
Porteresia coarctata	(2n=48)	Unknown	1	1
Hygroryza aristata	(2n=24)	Unknown	1	1
Leersia perrieri	(2n=24)	Unknown	1	1
Rhynchoryza subulata	(2n=24)	Unknown	1	1
Chikusichloa aquatica	(2n=24)	Unknown	1	1
Total			5	5

Savidan et al (1994) for the maize- *Tripsacum* program. The feasibility of producing wide-cross hybrids of rice through embryo rescue (Jena and Khush 1984) has already been demonstrated. Moreover, gene transfers from wild species into cultivated rice have been obtained in the wide-cross progenies (Jena and Khush 1990. Multani et al 1993. Ishii et al 1994).

Mutagenesis to induce apomixis

A defined sequence of events in the ovules leads to the development of haploid eggs, which, when fertilized by male gametes, result in sexual embryos. These steps involve (1) differentiation of a megaspore mother cell, (2) megasporogenesis through meiosis, leading to the development of a haploid megaspore, and (3) gametogenesis

resulting in an embryo sac with an egg apparatus, polar nuclei, and antipodal cells. In meiotic diplospory, meiosis fails, resulting in an unreduced megaspore, In mitotic diplospory, the megaspore mother cell does not enter meiosis—it undergoes gametogenesis directly. The end result in both cases is the production of embryo sacs with 2n chromosomes in the eggs. Furthermore, these eggs are prevented from fertilizing and they develop into embryos with a maternal chromosome number.

In apospory, nucellar cells adjacent to the megaspore mother cell differentiate and give rise to an unreduced embryo sac by the mitotic process. The aposporous embryo sacs develop faster than the sexual embryo sac because they do not have to go through meiosis. The development of the sexual embryo sac is terminated and the aposporous embryo sacs take their place at the chalazal end. Like the diplosporous embryo sacs, the unreduced eggs start to develop into an embryo without fertilization.

It appears that the switchover from sexual to apomictic reproduction involves simple steps. The rather simple inheritance of apomixis as discussed earlier supports this conclusion. The occurrence of apomixis in more than 300 species belonging to 35 families of higher plants suggests that such mutations have occurred frequently in nature. The appearance of sexual plants in species that are predominantly obligate apomicts suggests that even reverse mutation at the apomixis locus can occur.

If mutations for the apomictic mode of reproduction can occur in nature, it should also be possible to induce such mutations through mutagenesis. Many physical and chemical mutagens are known to induce mutations for various plant characteristics and processes. Attempts to induce mutations for apomixis in higher plants have been halfhearted. One of the first apomictic strains of grain sorghum was selected from irradiated progenies (Hanna et al 1970). Hanna and Powell (1973) obtained facultative apomicts of pearl millet following treatment of seed with thermal neutrons and diethyl sulfate. Analysis of female sterile mutants in pearl millet obtained through mutagenesis showed evidence of apospory (Arthur et al 1993).

At IRRI, we have started a program of mutagenesis using physical and chemical mutagens to explore the possibility of inducing mutations for apomixis in rice. Identifying mutants with the asexual mode of reproduction in a large population of mutagenized individuals is problematic. We are employing a genetic male sterile line (msms) of rice variety IR36. Male sterile plants are pollinated with pollen from fertile (MsMs) plants. Fertilized egg cells (Msms), 16–18 h after pollination, are treated with 1.5 mM of N-methyl-N-nitrosourea (MNU) for 1 h. We are in the process of screening mutagenized progenies. M_1 progenies (Msms) breeding true for fertility in the M_2 would be further examined as a possible source of dominant mutation for apomixis. Recessive mutations can be identified in the M_3 generation. Treatment of fertilized egg cells with MNU has been found to be effective for inducing a wide spectrum of mutations involving chlorophyll deficiencies and panicle and embryo mutations in rice (Satoh and Omura 1986, Kitano et al 1993, Hong et al 1995).

We are using a dominant marker—purpleleaf mutant of rice—for identifying apomictic mutants following mutagenesis. Two approaches are being followed: (1) emasculated panicles of IR36 are irradiated with gamma rays and are immediately pollinated with the pollen of purple leaf mutant, and (2) fertilized eggs from the cross

of IR36 × purple leaf mutant are mutagenized with the MNU treatment. M_2 progenies breeding true for purple leaf are examined further for dominant mutations for apomixis.

Molecular approaches to engineering apomixis in rice

In addition to searching for apomixis in wild rice germplasm or inducing it by mutation, IRRI is also interested in achieving apomixis in rice through genetic engineering. Two principal approaches of this type are envisioned: (1) transferring isolated apomixis genes into rice from known apomicts, and (2) synthesizing a switch from sexuality to apomixis in rice based on genes isolated from nonapomicts. These two approaches present formidable challenges in terms of the isolation and modification of appropriate genes and promoters, but it is unlikely that rice transformation per se will pose significant difficulties.

Genetic engineering is now well established in rice

Three major protocols are available for the introduction and stable integration of genes into rice (Table 2): (1) DNA uptake by protoplasts (Shimamoto et al 1989), (2) microprojectile bombardment (Christou et al 1991), and (3) *Agrobacterium* - mediated DNA transfer (Hiei et al 1994). Cultivars from four of the six groups of Asian rice described by Glaszmann (1987) have already been transformed, including the two major groups (I and VI) and the two minor groups (II and V). The two satellite groups (III and IV) remain to be studied from this perspective. Experiments on apomixis may well focus on those cultivars and protocols that give the highest frequency of transformation, but the unique features of apomixis may be more restrictive. For example, if large segments of the genome of an apomict are to be transferred instead of individual genes, preference will be given to the protocol that can best accomplish large-fragment transformation.

Isolation of genes for apomixis from apomicts is under way

Several laboratories are attempting to map and isolate the genetic loci thought to be responsible for the apomixis trait. Although IRRI is following closely the develop-

Table 2. Progress in transforming the six groups of rice defined by Glaszmann (1987).

Glaszmann group	Direct DNA delivery to protoplasts	Microprojectile bombardment	*Agrobacterium* mediated transformation
I Indica	+	+	+
II Aus	+	+	nd
III Deepwater	nd[a]	nd	nd
IV Deepwater	nd	nd	nd
V Basmati	+	+	nd
VI Japonica	+	+	+

[a] nd = not yet determined.

Table 3. Comparison of the haploid genome sizes and largest RFLP map lengths of rice, five other cereals, and *Arabidopsis*.

Grass	Genome size (Mbp/1C)[a]	RFLP map length (cM)[b]	Map resolution (Mbp/cM)
Rice	415	1,491	0.28
Sorghum	748	950	0.78
Maize	2,292	2,300	1.00
Barley	4,873	1,403	3.47
Wheat	15,966	3,500	4.56
Oats	11,315	2,410	4.70
Arabidopsis	145	512	0.28

[a] Smallest value quoted by Arumuganathan and Earle (1991) for species. [b] Longest map distance reported by articles in Phillips and Vasil (1994).

ments in any apomictic species, we are most interested in the progress being made in the apomictic grasses, including *Tripsacum* (Savidan et al 1994), *Pennisetum* (Ozias-Akins et al 1993, Sherwood and Gustine 1994), and *Brachiaria* (Thomé et al, personal communication). By far the most common form of apomixis in grasses is apospory. For this reason, we expect that apospory will be the principal focus of attempts to engineer apomixis by gene transfer from cereals.

Segregating populations have been constructed between apomicts and their sexual relatives to allow the mapping of apomixis genes. Mapping will be followed by isolation of the genes by chromosome walking. Progress has been rapid in *Pennisetum, Tripsacum,* and *Brachiaria,* where linkage to markers has already been achieved. One impediment to these endeavors is the large genome size of the genera that contain apomictic species (Table 3). In comparison, the rice genome is very small and much more easily studied by the above molecular techniques. In particular, the mean number of base pairs per map unit in rice is only 0.28 Mbp/cM. similar to that of *Arabidopsis* and less than a third that of maize (1 Mbp/cM). One way in which IRRI could contribute to the isolation of apomixis genes is to exploit the synteny of the cereal genomes to provide markers for segregation analysis in apomictic genera, or facilitate the physical analysis of relevant genomic regions.

A different molecular approach is being taken with buffelgrass (Sherwood and Gustine 1994). In this case, a subtractive cDNA library has been made between ovule tissue of apomictic and sexual species of *Pennisetum.* The first cDNA clone to arise from this study (Gustine et al 1995) encodes a protein with an ankyrin repeat, named after the cytosolic protein that binds to proteins of the erythrocyte plasma membrane. Although ankyrin repeats have a diverse range of roles in protein-protein interactions, one class of ankyrin proteins are regulatory factors that transduce signals from the cell membrane to the nucleus (Grumont et al 1994). The cDNAisolated by Gustine et al (1995) has homology with random rice cDNAs and the homology includes the ankyrin repeats. Comparisons of this sort may help to identify rice genes expressed in

the ovule and whose manipulation may allow a switch to apomictic reproduction to be accomplished.

Synthetic apomixis

Like the mutational and apomixis gene approaches, the synthetic apomixis approach (Peacock 1992) has, as its starting point, the notion that the switch between sexual and apomictic modes of reproduction is controlled by only one or two genes. But the synthetic approach considers that genes isolated from nonapomicts can also induce apomixis, if they are suitably modified to switch reproduction from the normal sexual pathway.

Given the existence of several distinct forms of apomixis (Koltunow 1993), which is the most appropriate for the synthetic approach? Because all cultivated rice is diploid, yet only adventitious embryony is seen in diploid plants (Asker and Jerling 1992), this form of apomixis may be the most appropriate for introduction into rice. It is not clear, however, whether diplospory and apospory are intrinsically incompatible with diploidy or have become associated with polyploidy for ecological or evolutionary reasons. At present, therefore, we favor two synthetic routes to apomixis in rice. The first route is to develop adventitious embryony, in which the embryo arises directly from a diploid cell of the maternal nucellus, whereas the endosperm arises from the fusion of a sperm cell from the pollen with the nuclei of the central cell of the sexual embryo sac. The zygotic embryo should either fail to form or should abort at a very early stage. The second route is to develop the *Panicum*-type of apospory that is common in grasses (Sharma and Thorpe 1995). The embryo should arise by parthenogenesis from the diploid egg cell of an unreduced 4-nucleate aposporous embryo sac, whereas the endosperm should arise by fusion of a sperm cell with the diploid polar nucleus in the same aposporous embryo sac.

Cell cycle control in the nucellus

Introducing apomixis in rice by either of these approaches will require a much greater understanding of the regulation of gene expression in the ovary than we now possess. For immature rice spikelets, we propose to develop an in vitro culture system similar to that reported for involucres of buffelgrass by DeGroote and Sherwood (1984). We shall use this system to deliver to the nucellus various molecules that may trigger mitosis or even the formation of aposporous embryo sacs or adventitious embryos. Among the molecules of greatest interest are plant hormones, inhibitors of hormone synthesis/action, cell cycle inhibitors, elicitors, ions, and chemicals known to stimulate callose deposition, including Ca^{2+} ions, UDP glucose, b -furfuryl- b -glucoside, syringomycin, etc. Preference will be given to spikelets in which archesporial cell differentiation has occurred but embryo sac maturity has not been reached.

We are particularly interested in exploiting a transgenic line (HSK1) of rice in which expression of the *gus* reporter gene is switched off (apparently by methylation of the CaMV promoter). Expression is restored by treating tissue with the methylation inhibitor 5-azacytidine (Kohli et al 1996), but only in cells undergoing cell division. This transgenic line may provide clear information on the distribution within the

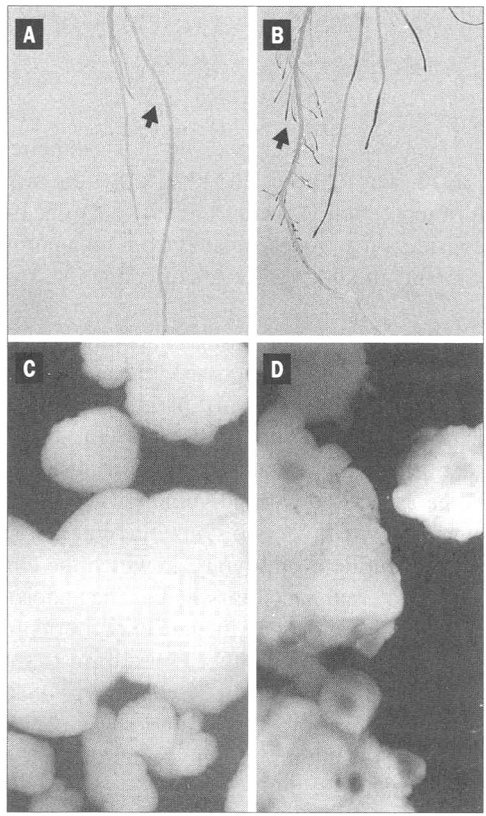

Fig. 1. Reactivation by 5-azacytidine of a silenced CaMV35S-*gus* construct in line HSK1 of rice (*Oryza sativa* L. cv. Anjungbyeo). Roots (A,B) and embryogenic calli (C,D) were stained with X-glquc for 16 h after 48 h pretreatment without 5-azacytidine (A,C) and with 30 µM 5-azacytidine (B,D). Staining followed the method of Kosugi et al (1990).

nucellus of cells undergoing mitosis and on how that distribution changes in response to the abovementioned chemicals.

Our step-wise plan to exploit line HSKl is to study reactivation of the gene in rice roots (Fig. 1A, B) and then its reactivation in calli-forming somatic embryos (Fig. 1C, D). The third step will be to reactivate *gus* expression in the nucellus via the formation of adventitious embryos. The first two steps have been achieved, but the third step remains to be accomplished. The arrows in Fig. 1 point to lateral roots that are unstained in the absence of 5-azacytidine (Fig. 1A) but stained after 48 h in its presence (Fig. 1B). The apical root tips are also stained only after treatment with 5-azacytidine. Embryogenic calli from line HSK1 also require 5-azacytidine for staining (Fig. 1C, D); the stain is localized initially in the embryogenic region. We look forward to being able to induce adventitious embryos in the nucellus of line HSKl and to identify embryo initials by this simple GUS staining procedure.

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CHAPTER 24

Hybrid rice technology in India: current status and future outlook

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The commercial success of hybrid rice in China has clearly demonstrated its potential to meet the increased demand for rice. Efforts to develop and use this technology in India began in the 1970s and were systematized and intensified in December 1989 with the launch of a mission-oriented project. Within seven years, more than 12 hybrids from the public and private sectors were made available for commercial cultivation. During the 1996 wet season, more than 60,000 ha were planted to hybrid rice in India. Some more promising hybrids with better grain quality, resistance to pests and diseases, and a higher magnitude of heterosis are in the final stages of evaluation. Cytoplasmic male sterile lines with diversified sources of sterility-inducing cytoplasm have been developed. Research on two-line heterosis breeding and the development of intersubspecific indica × tropical japonica hybrids has begun and the results attained appear promising. A seed production technology has been developed and its adaptability demonstrated on a large scale. Seed yields of 1.0–1.5 t ha^{-1} can be obtained. During the 1996 dry season, 1,300 t of F_1 seed were produced by private- and public-sector seed agencies. Crop production and protection practices for the successful cultivation of hybrids in the target areas are being standardized. Future research and development strategies for hybrid rice technology are briefly discussed. Prospects for the large-scale adoption of this technology in India appear to be bright.

The requirement of rice by the year 2000 is estimated to be around 150 million t at the current rate of population growth in India. Total production during 1995 was around 125 million t. Thus, within the next few years, a production increase of 25 million t has to be achieved to sustain self-sufficiency in rice. The task is challenging, but the options available are limited. In high-productivity areas, the plateauing trend of yield because of the decreasing availability of land, water, labor, and other inputs is strikingly visible. Hybrid rice technology as the most feasible and readily adoptable

option for increasing production has been demonstrated in the People's Republic of China during the past two decades.

Efforts to develop and use hybrid rice technology in India began in the 1970s. A few scientists were trained in this technology in China and at the International Rice Research Institute (IRRI) in the Philippines. To investigate heterosis breeding, some infrastructure facilities were developed at a few research centers. Preliminary work was carried out on floral biology and the evaluation of introduced parental lines and hybrids. Only when the Indian Council of Agricultural Research (ICAR), New Delhi, identified hybrid rice as a top priority did a time-bound, goal-oriented network project begin in December 1989 in collaboration with IRRI. This project was further strengthened with assistance from the United Nations Development Programme and Food and Agriculture Organization of the United Nations starting in September 1991.

This project operates as a national research network with 12 centers in different states coordinated by the Directorate of Rice Research (DRR). It has two lead centers, Kapurthala in Punjab for northern India and Mandya in Karnataka for southern India. The three strategic research centers, primarily for conducting basic research, are the DRR, Hyderabad; the Central Rice Research Institute (CRRI), Cuttack; and the Indian Agricultural Research Institute (IARI), New Delhi. The seven associate centers responsible for conducting region-specific adaptive research are located in Coimbatore (Tamil Nadu), Maruteru (Andhra Pradesh), Karnal (Haryana), Pantnagar and Faizabad (Uttar Pradesh), Chinsurah (West Bengal), and Karjat (Maharashtra). The major objectives are to develop hybrids with a 15–20% yield advantage over the highest-yielding inbred check varieties, optimize the seed production method, standardize cultivation practices for hybrids, and conduct relevant basic research. This chapter briefly discusses the current status of and future outlook for hybrid rice research in India.

Development and evaluation of experimental hybrids

Release of hybrids

About 800 experimental hybrids have been evaluated so far. During the wet season (*kharif*), they were evaluated at all 12 centers, whereas in the dry season (*rabi*) they were evaluated at seven centers in southern and eastern India. As a result of the time-bound coordinated efforts, four rice hybrids—APHR-1, APHR-2, MGR-1, and KRH-1—were released for commercial cultivation in 1994 by the State Variety Release Committees. Later, CNRH-3, DRRH-1, and KRH-2 were released (Siddiq and Ilyas Ahmed, this volume, Chapter 5). Other hybrids marketed by private seed companies include 6201 (from Hybrid Rice International), MPH-516, and MPH-517 (from Mahyco Seed Company). At present, hybrid rice seed has a good demand in India. Private seed companies market rice hybrids at Rs. 70–105 (US$2–3) kg^{-1} seed.

During the 1996 wet season, 60,000 ha were planted to hybrid rice in the country. Intensive efforts are under way to identify suitable hybrids for the northwestern part of the country (Punjab, Haryana, and Uttar Pradesh). The lower magnitude of heterosis, average grain quality, and the lack of an adequate and timely availability of quality F_1 seed at a reasonable price are the major constraints.

Table 1. Experimental hybrids evaluated in national trials in India.

Year	Hybrids (no.)		
	From India	From IRRI	Total
1989	7	7	14
1990	20	20	40
1991	93	20	113
1992	110	16	126
1993	55	58	113
1994	68	52	120
1995	73	64	137
1996	76	40	116
Total	502	277	779

National hybrid rice trials

The experimental hybrids have been systematically evaluated on a large scale for yield and yield components. This work has been carried out for 8 yr under the national research network for hybrid rice (Table 1). Nearly 65% of the hybrids were nominated indigenously (including those from the network research centers); the rest came from IRRI. So far, 112 hybrids have been nominated by six private seed companies—Pioneer Overseas Corporation, Hybrid Rice International, Mahyco Seed Company. Vikki's Agrotech Limited, ITC-Zeneca, and Hindustan Lever Limited. From network centers, the most hybrid rice nominations were from DRR (166), Kapurthala (68), Maruteru (43), and Mandya (34).

Thirty-five promising hybrids have recorded an increase in yield advantage of 1 t ha^{-1} over that of the check at three or more locations or seasons. APRH-2, UPRH-632,NDRH-2, MTURH-2020, DRRH-5, DRRH-8, UPRH-27, CNRH-3, KMRH-3, HKRH-1005, PHB-71 (ORI161), 3RI086, 3RI160, PA103, AH802, VRH-l, MPH-516, MPH-517, MPH-518, and PA 112 were developed in India. IR62829A/IR10198, IR58025A/IR9761, IR58025A/IR34686, IR58025A/IR54742, IR58025A/IR29723, IR58025A/IR72, IR58025A/IR40750, IR58025A/IR633, IR58025A/IR13419, IR58025A/IR21567R, IR58025A/IR32809, IR58025A/IR55838, PMS 10A/IR48725-B-B, IR58025/IR48751, and PMS 10A/BR 827-35 are nominations from IRRI. Some of these have been released for commercial cultivation, others are in extensive on-farm evaluation. The nonavailability of adequate restorer seed and lack of requisite isolation facilities for the large-scale production of F_1 seeds have slowed progress.

Hybrids for the rainfed lowland ecosystem

The favorable rainfed lowland rice ecosystem occupies more than 10 million ha of rice area in India. The experimental hybrids developed for the irrigated ecosystem were evaluated for the rainfed lowlands and some were found to be promising (Table 2). A specific breeding program has been organized to generate the requisite parental lines for the development of hybrids suited to this ecosystem at CRRI.

Table 2. Promising hybrids identified for the rainfed (shallow) lowland ecosystem at CRRI, Cuttack, India.

Hybrid	Duration (d)	Plant height (cm)	Yield (kg ha^{-1})		
			Hybrid	Check	Increase over check
IR58025A/IR29723	136	98	6,181	5,083[a]	1,098
IR62829A/IR54742	131	89	6,131	5,083[a]	1,048
IR62829A/Swarna	132	82	6,131	5,083[a]	1,048
IR62829A/Vajram	133	89	6,117	5,083[a]	1,034
IR58025A/IR54742	136	100	5,604	3,379[a]	2,225
ORI 161	133	110	5,465	3,379[a]	2,086
IR58025A/IR40750	132	89	5,130	3,379[a]	1,751
IR58025A/Vajram	136	100	4,961	3,379[a]	1,382
2RI 075	132	128	5,233	4,133[b]	1,200
PMS 8A/IR46	134	120	5,712	4,422[b]	1,290
IR58025A/RP 1057	131	117	5,069	4,422[b]	702

[a]Jaya. [b]Swarna.

Evaluating hybrids for grain quality characteristics

Consumer acceptance of hybrids depends primarily on cooking and eating quality characteristics. These factors also determine the price of commercial produce. The first Chinese hybrids introduced were totally unadapted to Indian conditions and possessed poor grain quality. Therefore, parental lines and hybrids were developed specifically for the tropics at IRRI and in national agricultural research systems (NARS). Fortunately, all the hybrids released in India, which are based on two CMS lines developed at IRRI (IR58025A and IR62829A), and the promising prereleased hybrids possess acceptable grain quality. IR58025A has long slender grains with a slight aroma and the cooked rice is slightly sticky in hybrids based on this line. IR62829A has medium slender grains. Head rice recovery is reported to be low in hybrids based on these CMS lines.

The physical and chemical quality characteristics of several promising hybrids have been studied (Table 3). MPH-517, MTURH-2015, 2RI- 158, MTURH-20202, and 3RI 086 have head rice recovery of >60%. URH-I, IR58025A/IR54742, IR58025A/IR34686, and IR58025A/IR32809 have a kernel length of >7 mm. Hybrids IRI 023, KMRH-3,3Rl-160, IR58025A/IR34686, and PMS 10A/IR48725 have a volume expansion ratio of >5. PA-112, UPRH-833, HKRH-1002, IR58025A/IR29723, IR58025A/IR32809,IR58025A/IR55838, IR58025A/IR34686, IR58025A/IR48751, IR58025A/IR48749, IR62829A/IR10198, and IR58025A/IR21567 have an intermediate amylose content of 20–25%. Hybrids IRI 023, HKRH-1002, 2RI 158, IR58025A/IR55838, AH-802, PMS 10A/IR48725, IR58025A/IR48749, and IR58025A/IR21567 have a very occasionally translucent grain appearance. Hybrids 3R1 160, MPH-517, IR58025A/IR48715, and IR58025A/IR21567 have a very occasionally attractive grain appearance. Due emphasis is being given to quality considerations in the heterosis breeding program in India.

Table 3. Quality characteristics of promising hybrids.

Hybrid	Head rice recovery (%)	Kernel length (mm)	Volume expansion ratio	Amylose content (%)	Grain type [a]
IR58025A/IR34686	54.6	7.2	5.3	23.6	LS
IR58025A/IR29723	60.3	6.8	3.5	25.0	LS
IR58025A/IR55838	55.0	6.2	4.0	24.8	LB
IR58025A/IR21567	56.0	6.9	4.3	23.3	LS
MPH-517	63.9	5.2	4.3	–	MS
3RI 160	58.0	6.3	5.3	–	LS
PMS 10A/IR48725	58.9	6.6	5.3	–	LS

[a] LS = long slender, LB = long bold, MS = medium slender.

Table 4. Cooking quality characteristics of some recently developed basmati hybrids and restorer lines.

Basmati hybrids/ restorers	Kernel length (mm)		Kernel breadth (mm)		L/B ratio		Aroma
	Before cooking	After cooking	Before cooking	After cooking	Before cooking	After cooking	
Hybrid							
Pusa 3A/PRR-69	6.50	12.10	–	–	–	0.86	–
Pusa 3A/PRR-71	6.10	12.60	–	–	–	2.07	–
Restorer							
SPS 95-694-1	6.27	12.48	1.87	2.18	0.68	5.87	Present
SPS 95-694-2	7.07	12.00	1.67	2.33	4.23	5.15	Present
SPS 95-694-3	6.73	12.20	1.74	2.26	3.87	5.40	Present
SPS 95-693-1	7.00	12.88	1.80	2.56	3.89	5.03	Absent
SPS 95-693-2	6.67	13.11	1.74	2.60	3.83	5.04	Absent
SPS 95-81-1	7.60	12.60	1.87	2.50	4.06	5.04	Present
SPS 95-81-3	7.80	13.80	1.44	2.33	5.42	5.92	Present
SPS 95-81-4	7.33	12.14	1.44	2.44	5.09	4.97	Present
SPS 95-81-5	7.33	13.33	1.47	2.40	4.99	5.55	Present
SPS 95-81-6	7.20	12.00	1.40	2.47	5.14	4.86	Present
SPS 95-167-1	7.60	11.74	1.80	2.87	4.22	4.09	Present
SPS 95-768	7.07	12.40	1.40	2.00	5.05	6.20	Present
Pusa Basmati-1	6.07	12.62	1.60	1.94	3.79	6.50	Present
Karnal Local	6.20	12.26	1.80	2.28	3.44	5.38	Present

Development of basmati hybrids is also under way at the New Delhi and Kapurthala centers, where the requisite parental lines with basmati characteristics have been identified. In the preliminary evaluation stage in observational yield trials at IARI, two basmati hybrids have outyielded check variety Pusa Basmati by 30–40% (Table 4). These hybrids will be evaluated extensively in larger trials during the ensuing seasons. The two basmati CMS lines, Pusa Basmati-1A and Pusa 3A, have been developed at Delhi and basmati-like restorers have also been identified. Table 4 shows cooking quality characteristics of some basmati hybrids and restorers.

Table 5. Promising hybrids with multiple resistance to diseases and pests.

Hybrids	Blast	Sheath rot	False smut	Brown plant-hopper	Stem borer	Gall midge
KMRH-3	R[a]	R		R	R	
IR58025A/IR21567	R	R	R	R		
IR58025A/IR29723	R	R		R	R	
IR58025A/IR55838	R	R	R			
IR58025A/IR59656	R	R	R			
IR58025A/IR34686	R	R	R			R
IR58025A/IR58100	R	R		R		
IR58025A/IR40750	R					R
IR62829A/IR46	R					R
IR58025A/IR54742	R					R
IR58025A/Vajram	R			R		

[a]R = resistant.

Resistance to diseases and pests

For the large-scale adoption of hybrid rice technology, released hybrids should have a distinct yield advantage over popular varieties. They should also possess a fair degree of resistance to major diseases and pests in the target area. Promising hybrids are therefore evaluated regularly for resistance to major diseases and pests, both in the glasshouse and field. Some hybrids have been found with resistance (Table 5).

Studies conducted so far have clearly shown that the hybrids are resistant if the parents are resistant. If one parent is susceptible, the hybrids are either resistant or susceptible, depending on whether the gene(s) imparting resistance is dominant or recessive. If the genes conferring the resistance are recessive, then both parents must have these genes in the recessive condition for the hybrid to be resistant. It is much easier to incorporate resistance in hybrids if it is controlled by a single dominant gene.

During the early phase of heterosis breeding, the emphasis was justifiably on yield heterosis. In the second phase, additional emphasis is given to developing hybrids with good grain quality and resistance to diseases and pests.

Preliminary evaluation of two-line intersubspecific hybrids

There are wide differences in temperature across latitudes and altitudes in India. Therefore, work on two-line heterosis breeding using thermosensitive genic male sterile (TGMS) lines began recently. TGMS lines received from IRRI, as well as those identified and isolated from germplasm collections, mutated populations, and farmers' fields, were evaluated under controlled conditions of temperature and photoperiod in a growth chamber and in the field by sowing at 15-d intervals. Some of these TGMS lines have been characterized for their critical sterility or fertility point.

Among the TGMS lines developed in India, ATG- 1, ATG-4, and ATG-8 appear to be promising for use in the two-line heterosis breeding program. ATG-8 is a reverse TGMS type that is sterile below 24 °C and fertile above 28 °C (Table 6).

Table 6. Characterization of promising TGMS lines.

TGMS line	Critical sterility point (°C)	Critical fertility point (°C)	Max. seed set under fertile phase (%)	Source
ATG-1	26	22	75	Mutated population at DRR [a]
ATG-2	26	20	15	Mutated population at DRR
ATG-3	28	24	18	Segregating population at DRR
ATG-4	27	24	75	Germplasm maintained at DRR
ATG-7	25	20	23	Germplasm maintained at DRR
ATG-8	24	28	80	Germplasm maintained at DRR
IR32364	25	20	18	Mutated population at IRRI
IR68945	32	28	80	TGMS gene transferred from Norin PL-12 at IRRI
IR68949	32	28	80	TGMS gene transferred from Norin PL-12 at IRRI
IR70118		28	80	TGMS gene transferred from Norin PL-12 at IRRI

[a] DRR = Directorate of Rice Research.

The four TGMS lines received from IRRI were evaluated in fields at Hyderabad, Maruteru, Cuttack, and Faizabad. At Hyderabad, all these lines are sterile if flowering takes place during April-July but fertile if flowering occurs during September-February (Table 7). Among the four lines received from IRRI, IR68949 appears to be promising. Using this line, hybrid seed production can be taken up in the late rabi and multiplication in kharif.

The problem of sterility encountered in indica × tropical japonica crosses can be overcome by having the wide compatibility (WC) gene(s) in one of the parents. A few experimental hybrids were produced by using TGMS lines and tropical japonica genotypes having WC genes. In a preliminary evaluation, the magnitude of heterosis in these hybrids ranged from 50% to 60% over that of check variety Jaya (Table 8). The identification of appropriate locations or seasons for large-scale seed production of these hybrids is under way.

Economics of hybrid rice cultivation

During the past three seasons in on-farm trials, in a commercial farmer's crop under good management, an additional profit of Rs 3,500 (US$100) ha^{-1} was obtained by cultivating hybrids. The only additional cost involved was the extra cost of seed.

Development of parental lines

The choice of appropriate parental lines possessing good combining ability, high yield potential, good grain quality, and resistance to diseases and pests is a prerequisite for developing hybrids. In addition to developing new improved parental lines possessing desirable characteristics and evaluating them, maintainers and restorers have been identified from elite breeding lines and germplasm through test crossing for three-

Table 7. Sterility-fertility behavior in IRRI TGMS lines at Hyderabad (1994-95).

TGMS line	Sterility-fertility behavior during the month of flowering												Max seed set (%)
	Jun 1994	Jul	Aug	Sep	Oct	Nov	Dec	Jan 1995	Feb	Mar	Apr	May	
IR68945	S[a]	S	F	F	F	F	F	F	F	S	S	85 S	
IR68949	S	S	F	F	F	F	F	F	F	F	S	85 S	
IR68294	S	S	S	F	F	F	F	F	F	S	S	65 S	
IR32364	S	S	F	F	F	F	F	S	S	S	S	20 S	

[a]S = sterile, F = fertile.

Table 8. Performance of two-line intersubspecific hybrids.

Cross	Spikelet fertility (%)	Panicle length (cm)	Plant height (cm)	Yield (kg ha^{-1})	Heterosis (%)
ATG3/9310	77	24.3	88	9,148	57
ATG6/IR65598	78	25.8	134	7,785	34
ATG5/BPI 176 P	74	26.7	151	8,463	45
ATG4/Pakisan	80	26.1	134	9,563	64
ATG8/IR65598	73	24.0	120	6,000	3
ATG2/N22	81	22.4	102	8,175	40
Jaya (check)	68	22.3	101	5,826	–

line heterosis breeding. During the past 6 yr, about 15,000 test crosses have been evaluated at the 12 network research centers. The frequency of restorers identified was high at Hyderabad and Mandya, whereas the frequency of maintainers was higher at Cuttack and Coimbatore. The frequency of restorers was low at Faizabad and New Delhi, whereas the frequency of maintainers was low at Chinsurah, Karjat, Faizabad, New Delhi, Kapurthala, and Maruteru. The overall frequency was 22% for restorers and 14% for maintainers. Thus, improved female and male parents needed for three-line heterosis breeding are readily available.

Evaluating CMS lines

The CMS lines developed at the network centers (>60) and those received from IRRI (50) were evaluated for their stability of sterility and other desirable traits. Five CMS lines—PMS 2A, PMS 3A, PMS 10A, IR58025A, and IR62829A—were found to be good for large-scale commercial hybrid seed production. Though IR62829A was a very good combiner, at higher temperatures it showed 1–2 plump and yellow anthers in the spikelet. A few darkly stained pollen were seen under the microscope. Surprisingly, there was no seed set because of selfing. The hybrids produced by using this line were very uniform. Perhaps this is a case of functional male sterility.

At many locations, for PMS 2A, PMS 3A, and PMS 10A developed at Kapurthala, the outcrossing percentage was low (15–25%). Presently, only IR58025A is used extensively in released hybrids. An evaluation of CMS lines at network research centers (Tables 9) helped identify promising CMS lines. Some good hybrid combinations based on these CMS lines are in observational yield trials.

Developing restorer and maintainer lines

To enlarge variability and combine good characteristics, specific breeding programs for restorers and maintainers were started. Crosses made involved B × B, R × R, and A × R. Desirable segregates were selected from the F_2 generation onward. This material is now in the F_6 generation. Desirable restorers selected have been used to make test crosses. The backcross breeding program to convert some of the desirable maintainers selected into CMS lines is in progress. The genetic male sterility-facilitated recurrent selection program has begun to develop populations possessing restoration genes from which desirable restorers can be isolated.

The two most-used CMS lines are IR58025A and IR62829A, which do not possess specific resistance to any of the major diseases or pests. Therefore, at present, resistance in the hybrids is to be incorporated from the restorer lines only. In this context, some restorers identified in NARS possess multiple resistance to diseases and pests in addition to the elite IRRI-bred restorers such as IR46R and IR72R, which have good combining ability and are useful for incorporating resistance in hybrids. A large number of restorers identified in the national program are being evaluated critically for their multiple resistance to major pests and diseases (Reddy et al, this volume, Chapter 13).

Table 9. Promising CMS lines identified through evaluation at network research centers in India.

CMS lines	Days to 50% flowering	Plant height (cm)	Panicle exsertion (%)	Stigma exsertion (%)	Grain type[a]	Glume opening duration (h)	Acceptability score[b]
Developed in India							
RR 2A	95	75	70	55	MS	2:05	4
PMS 2A	112	83	65	50	MS	2:30	5
PMS 3A	110	85	72	52	MS	1:40	5
Pusa 5A	105	70	71	48	LS	2:00	3
CRMS 6A	95	80	92	45	SB	3:00	5
CRMS 21A	85	70	65	46	MS	3:20	4
APMS 1A	96	75	60	54	MS	2:00	4
APMS 2A	93	75	58	52	MS	1:50	4
PMS 8A	113	80	66	45	MS	1:45	4
PMS 10A	116	80	70	50	MS	1:55	4
Developed at IRRI							
IR58025A	105	80	60	52	LS	1:16	2
IR62829A	95	75	70	60	SS	2:30	3
IR64607A	90	80	60	50	SS	2:10	4
IR67684A	108	85	80	50	LS	2:40	2
IR68281A	108	90	75	50	LS	1:50	3
IR68887A	110	90	81	60	MB	2:50	4
IR68888A	95	75	78	60	MS	2:40	3
IR68891A	100	70	72	50	MS	1:20	4
IR68894A	100	75	72	50	MS	1:35	4
IR68901A	98	70	72	60	MS	2:30	2

[a]MS = medium slender, LS = long slender, SB = short bold, SS = short slender, MB = medium bold. [b]On a 1-9 scale, where 1 = most suitable and 9 = least suitable.

Developing CMS lines with a diversified source of sterility-inducing cytoplasm

New sources of cytoplasm such as Kalinga 1 and V20B at CRRI, Cuttack, and *O. nivara* and *O. rufipogon* at DRR, Hyderabad, have been identified. Using these sources, several new CMS lines have been developed (Tables 10 and 11). These cytoplasmic sources could help diversify the genetic background of hybrids.

All six CMS lines developed at DRR using *O. nivara* and *O. rufipogon* sources are highly stable and show almost complete panicle exsertion. These lines also possess other desirable floral traits such as higher stigma exsertion and a higher outcrossing rate. Efforts are now being made to identify restorers for these new CMS lines and develop good heterotic combinations.

Parental lines for the rainfed lowland ecosystem

The available parental lines and hybrids identified or developed for the irrigated ecosystem were evaluated for their suitability to favorable rainfed lowlands. Specific efforts were also made during the past 5 yr to develop the parental lines best suited to this ecosystem. As a result, 15 CMS lines have been developed (Table 12). For all

Table 10. CMS lines with new sources of sterility-inducing cytoplasm developed at CRRI, India.

CMS line	Genotype	Cytoplasmic source	Plant height (cm)	Days to 50% flowering
CRMS 20A	Zhunghua A	V20B	52	74
CRMS 21A	Krishna A	Kalinga 1	60	90
CRMS 32A	Mirai A	Kalinga 1	79	100
CRMS 33A	Blazin A	Kalinga 1	70	95

Table 11. CMS lines with new sources of sterility-inducing cytoplasm developed at DRR, India.

CMS line	Genotype	Cytoplasmic source	Spikelets (no.)	Panicle exsertion (%)	Outcrossing (%)
RPMS 1-1	IR66	*O. rufipogon*	139	97	52
RPMS 1-2	V20B	*O. rufipogon*	111	94	41
RPMS 1-3	IR70	*O. rufipogon*	155	99	54
RPMS 1-4	PMS 2B	*O. rufipogon*	175	99	58
RPMS 2	IR66	*O. nivara*	146	98	58
RPMS 4	IR66	*O. nivara*	147	98	56

Table 12. Stable CMS lines developed for rainfed (shallow) lowland ecosystem.

CMS line	Genotype	Cyto-plasmic source[a]	Plant height (cm)	Days to 50% flowering
CRMS 7A	Pragati A	WA	91	108
CRMS 16A	Deepa A	WA	91	110
CRMS 24A	Moti A	WA	90	127
CRMS 25A	Padmini A	WA	119	119
CRMS 26A	IET 11350 A	WA	95	97
CRMS 27A	IET 11668 A	WA	93	96
CRMS 28A	Kalashree A	WA	95	134
CRMS 29A	IET 10428 A	WA	104	98
CRMS 30A	BKS 64 A	WA	85	94
CRMS 31A	Manipur A	WA	82	100
CRMS 34A	IET 10983-IA	WA	75	100
CRMS 32A	Mirai A	Kalinga 1	79	100
CRMS 33A	Blazin A	Kalinga 1	70	95
CRMS 35A	IET 10983-IIA	*O. perennis*	65	98
CRMS 36A	Tharrangsong A	*O. perennis*	71	88

[a]WA = wild abortive.

these CMS lines, more than 50 restorers have been identified for the rainfed lowland ecosystem. Some promising restorers are Gayatri, Vajram, Mahsuri, Johinga, Salivahana, NDR 30030, NDR 30077, NDR 40032, NDR 40013, TCA 88-89, TCA 88-68, CR 310-10, CR 580-5, CR 662-2211, and CN 1035-42. All these restorers are semitall or tall and mature in 120–135 d.

Seed production

For the commercial viability of hybrid rice, developing an efficient and economical seed production method is a prerequisite. The extent of adoption depends primarily on the magnitude of realizable heterosis and availability of pure seed at a reasonable cost. Seed production is the most crucial link between hybrid rice breeders and farmers. Several technical intricacies have to be managed satisfactorily to obtain an acceptable hybrid seed yield of 1.5–2.0 t ha^{-1}. For different hybrid combinations and locations, many aspects needed to be standardized. such as proper synchronization of flowering between parental lines, optimum row ratio, critical outpollination-promoting factors, the appropriate dosage and stage for application of GA_3, and the frequency and timing of supplementary pollination. During the past 5 yr, extensive trials have been conducted on these aspects of seed production technology.

Based on growth duration, effective accumulated temperature (degrees), and leaf counts, methods have been designed to obtain synchronization of flowering in parental lines used for hybrid seed production at each of the network research centers. The optimum row ratios of pollinating parents to seed parents in the initial years were found to be 2:6 to 2:8. But with the experience in obtaining better synchronization of parental lines, the optimum ratio is now fixed at 2:10 for CMS multiplication and 2:12 for hybrid seed production. Application of GA_3 is essential for obtaining higher seed yields. The most economically viable dose is 45 g GA_3 ha^{-1}. At the 5–10% panicle emergence stage, applying GA_3 on two successive days using 18 g ha^{-1} on the first day and 27 g ha^{-1} on the second day gave the best performance. If an ultra-low-volume sprayer instead of a knapsack sprayer is used, the quantity of GA, needed can be reduced to 30 g ha^{-1}, retaining the same efficiency. During the initial years, the seed yield obtained in the experimental plots was less than 0.5 t ha^{-1}. Recently, the average hybrid seed yield has been around 2 t ha^{-1}. Considering the results obtained from several experiments over the years, optimum practices have been identified for increased seed production (Table 13). But it is most important to work out the finer details, particularly on the synchronization of parental lines for every hybrid combination and location.

Within a short period of 5 yr, hybrid rice seed production has achieved yields of 1.5-2.0 t ha^{-1}. Hybrid rice seed production has been taken up on a large scale by some public-sector and several private-sector seed agencies in India. These seed agencies were supplied with promising parental lines and seed production personnel were trained on all aspects of seed production technology at DRR and some network centers. The average seed yield obtained over a large area today is around 1.0-1.5 t ha^{-1}. During

Table 13. Optimum cultural practices for hybrid rice seed production and CMS multiplication.

Activity	Practice
Seed rate	Seed parent—15 kg ha^{-1}; pollen parent—5 kg ha
Nursery	Sparse seeding to ensure more tillers, 4–5 $seedling^{-1}$ in 25 d
Row ratio	2B:10A for CMS multiplication, 2R:12A for hybrid seed production
Transplanting	2 $seedlings\ hill^{-1}$ for seed parent, 3 $seedlings\ hill^{-1}$ for pollen parent
Spacing	B/R to B/R 30 cm, B/R to A 20 cm, A to A 15 cm
GA_3 application	At 5% heading in two split doses on consecutive days with 18 g on the first day and 22 g on the second day
Supplementary pollination	Twice a day at peak anthesis during flowering phase
Roguing	Twice during vegetative phase based on morphological characters and twice during and after floweing based on floral characters, sterility, etc.
Seed yield	1.5–2.0 t ha^{-1}

the 1995-96 dry season, more than 1,300 t of F_1 seeds were produced (Siddiq and Ilyas Ahmed, this volume, Chapter 5).

In large-scale seed production plots, the outcrossing percentage presently observed is 25–35. This needs to be improved to 50%. To organize research on hybrid seed production technology, interproject linkages have been established with the National Seed Project centers. Maintenance breeding programs have begun at a few network centers to produce and supply pure seed of promising parental lines. A number of training programs on hybrid rice are being conducted at most of the research centers and nongovernmental organizations such as Krishi Vigyan Kendras (Farm Science Centers). They train a large number of seed production personnel from public- and private-sector seed agencies, progressive farmers, women laborers, and others in seed production technology.

Future outlook

For the next 5 yr, well-defined objectives and priorities have been set for developing and using hybrid rice in India. They include the expeditious and effective transfer of the technology already generated; developing hybrids with desirable grain quality, resistance to diseases and pests, and adaptability to a specific ecosystem; enhancing the magnitude of heterosis by developing two-line hybrids or intersubspecific indica × tropical japonica hybrids; and further refining hybrid seed production techniques and cultivation practices.

Notes

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CHAPTER 25

Hybrid rice research and development in Vietnam

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The commercial success of hybrid rice in China has encouraged Vietnam to introduce and develop this technology to increase rice yields in the country, especially in irrigated areas of the Red River Delta. Some Chinese rice hybrids were introduced and found to yield significantly higher than local check varieties. F_1 seeds of these hybrids were imported from China and distributed to rice farmers in the Red River Delta covering an area of 86,000 ha in 1996. Difficulties faced with this strategy included total dependence on China for hybrid seeds, the high cost of seeds, and susceptibility of hybrids to brown planthopper and bacterial blight. Collaboration with IRRI has been strengthened to overcome these difficulties. Several IRRI hybrids and parental lines have been introduced, some of which have been found to be suitable in both the Red and Mekong River deltas of Vietnam. Seed production technology developed in China and at IRRI has also been adapted. This chapter discusses results of adaptive research on hybrid rice technology introduced from China and IRRI.

Vietnam uses about 4.3 million ha of land for rice cultivation. Rice is grown on 6.7 million ha every year as a result of increased cropping intensity. About 80% of the country's rice area is planted with modern varieties. Annual rice production is 24.9 million t, with an average yield of 3.68 t ha^{-1}. The major rice ecologies are irrigated (40%), rainfed lowland (31 %), deepwater (14%), floating (8%), and upland (7%).

To meet the consumption requirement of the country in the year 2000, the target of the Vietnamese government for food grain is set at 30–32 million t. Rice production must increase to 26–27 million t to achieve this target because there is no scope for expanding the area under rice. The yield of improved varieties reached a plateau during the past 25 yr. Therefore, hybrid rice is considered to be a readily available approach for increasing yields on 0.5–1.0 million ha of irrigated rice area, where farmers harvest 6 t ha^{-1} in winter and 4 t ha^{-1} in summer.

The successful development and use of hybrid rice in China has impressed farmers and scientists in Vietnam. Hybrid rice yields an average 6.66 t ha^{-1} compared with 4.53 t ha^{-1} for conventional rice (Lou and Mao 1994). China and Vietnam have similar climatic conditions. Therefore, Vietnam has imported F_1 hybrid rice seeds from China for commercial production at the early stage of the national hybrid rice research program. This chapter reports on the use of hybrid rice and results from hybrid rice research in Vietnam.

Identifying hybrid combinations for direct introduction in Vietnam

Several hybrid rice varieties or promising combinations have been introduced from China for field testing. After testing at 27 locations, three hybrid varieties—Shan you 63, Shan you Gui 99, and Bo you 64—were selected for commercial hybrid rice production (Tables 1 and 2). Shan you 63 and Shan you Gui 99 have been used for spring and summer crops. Bo you 64, a photosensitive variety, was used for the summer crop only in rainfed lowlands.

Table 1. Yield evaluation of Chinese rice hybrid Shan you in Namha Province, Vietnam. Fertilizers were 10 t of farm manure, 120 kg N, 80 kg P_2O_5, and 60 kg K_2O.

Season and varieties	Yield in irrigated area (t ha^{-1})			Yield in lowlands (t ha^{-1})		
	1993	1994	1995	1993	1994	1995
Spring crop						
CR203 (check)	5.57	5.69	5.87	4.12	4.45	4.05
Shan you 63	8.21	8.15	8.02	7.21	7.15	6.75
Summer crop						
CR203 (check)	5.02	4.37	5.07	3.95	1.38	4.02
Bo you 64	6.25	5.57	6.38	5.55	3.88	5.55
Standard heterosis (%)						
Spring crop	47.3	43.2	36.6	75.0	57.1	65.7
Summer crop	24.5	27.4	25.6	40.5	81.1	38.6

Source: Agricultural Department, So Wong Nghien, Namha Province 1995.

Table 2. Yields of rice hybrids introduced for commercial production in northern Vietnam (1992–96).

Hybrid	Years	Mean yield range (t ha^{-1})	Standard heterosis (%)	Ecosystem
Shan you 63	1992-95	6-11	20-30	Sprlng and summer, irrigated
Shan you Gui 99	1992-95	6-10	20-25	Spring and summer, irrigated
Bo you 64	1995	6-8	20	Summer rainfed lowland
Jin you Gui 99	1996	6-8	20	Spring and summer, irrigated
Te you 63	1996	6-11	20–30	Spring and summer, irrigated

The area covered under hybrid rice in Vietnam increased from 1,300 ha in 1992 to 86,000 ha in 1996 (Table 3). On average, hybrid rice yielded 1.5–2.0t ha^{-1} higher than the best conventional varieties (An and Nhu 1995). But dependence on China for F_1 seeds, the high cost of F_1 seeds, and problems of pest susceptibility of the hybrids restricted any further increase in area in Vietnam. Because of damage from brown planthopper and bacterial leaf blight in China, hybrid rice is not accepted by farmers in southern Vietnam. It has limited use as a summer crop in the north.

To overcome these difficulties, we sought to identify suitable combinations among hybrids introduced from IRRI. During the summer season in northern Vietnam, six IRRI hybrids—IR58025A/IR54791-19, IR58025A/BR827-35, IR58025A/Rp1057-393-1, IR58025A/BR1356, PMS8A/IR46, and PMS10A/BKN6486-108—gave increased yield (20–30%) compared with the check (CR203) (Table 4). The most promising hybrid was IR58025A/Rp1057-393-1. This hybrid matured in 126 d, showed medium resistance to bacterial leaf blight, and possessed good grain quality. During

Table 3. Area and yield of hybrid rice in Vietnam, 1992–95.

Year	Spring-season crop		Average for 2 crops yr^1	
	Area (ha)	Yield (t ha^{-1})	Area (ha)	Yield (t ha^{-1})
1992	1,317	5.76	11,137	6.66
1993	17,205	6.94	34,828	6.71
1994	45,430	6.25	60,007	5.84
1995	39,598	6.34	73,503	6.14
1996	—	—	86,000	—

Table 4. Performance of promising hybrids introduced from IRRI in northern Vietnam, 1994–95.

Hybrid/inbred	Year	Duration (d)	Bacterial leaf blight (score)[a]	Yield (t ha^{-1})[b]	Standard heterosis (%)
Summer season					
IR58025A/Rp1057-393-1	1994	126	3–5	5.36 a	30.0
IR58025A/BR827-35	1994	122	5	5.20 a	26.0
PMS10A/BKN6486-108	1994	130	3–5	5.08 a	23.0
CR203 (check)	1994	116	3	4.10 c	0
Winter-spring season					
TG20 (Chi You Huong)	1995	167	–	4.00 c	–24.2
TG5 (Z97A/Gui 99)	1995	168	–	4.43 c	–19.1
TG1 (Z97A/Minghui 63)	1995	168	–	5.48 b	+3.8
IR58025A/Rp633-76	1995	176	–	6.29 a	+19.1
CR203 (check)	1995	156	–	5.28 b	0

[a]On a scale of 1–9, where 1 = resistant and 9 = highly susceptible. [b]Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

the winter and spring crop seasons, IR58025A/Rp 633-76 had a maturity duration similar to that of the check, but produced 19% higher yield.

For the southern part of Vietnam, the most promising hybrids—IR58025A/IR29723-143-3-2-IR and IR62829A/IR29723-143-3-2-IR—were named UTL1 and UTL2, respectively. These two hybrids produced significantly higher yields compared with the checks MTL58, MTL61, and IR64 in four seasons. At the Cuu Long Delta Rice Research Institute, some more promising hybrids were identified in the winter and autumn crop seasons of 1994 and 1995 (Table 5).

Cytoplasmic male sterile (CMS) lines and restorer lines and hybrids from China were susceptible to brown planthopper and other insects and diseases (Table 6). Sev-

Table 5. Performance of IRRI hybrid rice combinations in Cuu Long River Delta, Vietnam, 1990-95.

Hybrid	Season, year of testing	Yield ($t\ ha^{-1}$)	Standard heterosis (%)
IR58025A/IR29723-143-3-2-IR [a]	1990-92	4.6–7.6	0–43
IR62829A/IR29723-143-3-2-IR [b]	1990-92	4.9–6.7	12–32
IR58025A/IR34686R	Winter and autumn 1994	7.83	32.7
IR58025A/IR32419R	Winter and autumn 1994	7.07	19.8
PMS 10A/IR48725	Winter and autumn 1995	5.96	46.7
IR62829A/IR13139-43	Winter and autumn 1995	5.93	46.0
IR58025A/IR48751-13-13	Winter and autumn 1995	5.93	46.0
IR58025A/Rp1057-393-1	Winter and autumn 1995	5.67	46.5
IR58025A/Rp44962-7-6-2-3	Winter and autumn 1995	5.30	36.9
IR58025A/IR53479-B-B-2-1-1	Winter and autumn 1995	6.10	27.0

[a]UTL1. [b]UTL2.

Table 6. Reaction [a] of parental lines and hybrids to major diseases and insects in the northern province of Vietnam, 1992-95.

Parental lines or hybrid	Brown planthopper	Whorl maggot	Gall midge	Bacterial leaf blight	Blast	Sheath blight
Zhen Shan 97A	HS	HS	HS	S	S	S
Zhen Shan 97B	HS	HS	HS	MS	S	S
Gui 99	HS	S	HS	HS	HS	S
Minghui 63	S	S	S	MS	–	S
AMS 24A	S	S	S	MS	–	S
AMS 24B	S	S	S	MS	–	S
AMS 23A	S	S	S	MS	–	S
AMS 238	S	S	S	MS	–	S
AMS 30A	S	S	MS	MS	–	S
AMS 30B	S	S	MS	MS	–	S
Shan you 63	S	S	MS	S	MR	S
Shan you Gui 99	HS	S	MS	S	S	HS
Bo you 64	S	S	MS	S	S	HS
CR203 (check)	R	MS	MS	MR	HS	HS
C70 (check)	S	MS	MS	MR	R	MS

[a]R = resistant, MR = moderately resistant, S = susceptible, and HS = highly susceptible. Screening for brown planthopper biotype II in 1992, 1993, 1994, and 1995. Reaction recorded on whorl maggot and gall midge from field observations in spring-season crop 1993, 1994, and 1995. Reaction for bacterial leaf blight, blast, and sheath blight evaluated at Plant Protection Institute in 1993, 1994, and 1995.

Table 7. Reaction of hybrid rice combinations to brown planthopper (BPH) and bacterial leaf blight (BLB) in Vietnam, 1994.

Hybrid/inbred	Origin	Reaction to BPH		Reaction to BLB	
		Score [a]	Level	Score	Level
IR58025A/BR 827-35-2-1-1-1R	IRRI	8	HS	5	MS
IR58025A/IR42686-C2-118-6-2	IRRI	5	MS	5	MS
IR58025A/IR48725-B-B-141-2	IRRI	5	MS	5	MS
IR58025A/IR37721-90-3-3-3-2	IRRI	7	S	5	MS
IR58025A/IR54056-64-2-2-2	IRRI	5	MS	5	MS
IR58025A/Rp1057-393-1	IRRI	9	HS	3–5	MS
IR58025A/IR34686-179-1-2-IR	IRRI	7	S	7	S
IR58025A/IR21567-18-3R	IRRI	7	S	5	MS
IR58025A/IR39323-182-2-3-3	IRRI	7	S	9	HS
IR58025A/IR54791-19-2-3	IRRI	5	MS	7	S
IR58025A/IR48563-44-1-2-2R	IRRI	5	MS	3–5	MS
IR58025A/IR49461-128-3-3-3R	IRRI	5	MS	5	MS
IR58025A/IR50404-57-2-2-3	IRRI	5	MS	7	S
IR58025A/IR58232-86-2-1-2R	IRRI	5	MS	7	S
IR58025A/IR58773-35-3-1-2R	IRRI	6	S	7	S
IR58025A/IR59606-119-3R	IRRI	7	S	7	S
IR58025A/IR59566-157-1-3R	IRRI	7	S	7	S
IR58025A/OM576	IRRI	5	MS	5	MS
IR58025A/Rp633-76-IR	IRRI	9	HS	5	MS
IR58025A/Taichung Shan you 85	IRRI	7	S	7	S
PMS 10A/BKN6486-108-2R	IRRI	9	HS	3–5	MS
Shan you 63 (TG 1)	China	8	HS	7	S
Shan you Gui 99 (TG 5)	China	8	HS	9	HS
Shan you Quang 12 (TG 6)	China	7	S	7	S
Bo you 64 (TG 4)	China	7	S	3	R
CR203 (check)	Vietnam	3	R	4	MR
C70 (check)	Vietnam	7	S	4	MR
Zhen Shan 97A	China	7	S	9	HS

[a] Score on a 0–9scale. R = resistant, MR = moderately resistant, HS = highly susceptible, S = susceptible, and MS = moderately susceptible.

eral hybrid rice combinations from IRRI, however, were found to be less susceptible to brown planthopper and bacterial blight (Table 7).

Evaluating CMS lines

In all, 77 introduced CMS lines have been evaluated in Vietnam. In southern Vietnam, because of the high pressure of diseases and insects, Chinese CMS lines such as V20A, Zhen Shan 97A, V41A, etc., were not adapted. CMS lines from IRRI, India, and Thailand were found to be better. After evaluation from 1992 to 1995 in the Cuu Long River Delta, eight CMS lines—IR58025A, IR62829A, IR66707A, IR67684A, IR64607A, IR64608A, IR68888A, and PMS 10A—were found to be stable for male sterility. For IR66707A, the CMS source is *O. perennis* and, for this, restorer lines are not yet available. The rest are wild abortive (WA) types. All these CMS lines have the improved plant type developed at IRRI except PMS 10A, which was developed in

Table 8. Characteristics of promising CMS lines in the northern part of Vietnam (summer-season crop).

CMS line	Days to 50% heading		Plant height (cm)		Maximum tillers hill^{-1}		Panicles hill^{-1}		Panicle exsertion (score)		Grain type
	A[a]	B	A	B	A	B	A	B	A	B	
Z 97A	58	56	70	85	10	8	6	5	5	3	Bold
AMS 24A	56	54	60	65	10	8	8	8	2	2	Bold
AMS 23A	82	80	84	99	9	10	6	8	6	7	Bold
AMS 30A	53	51	58	60	7	6	5	5	3	2	Long
IR58025A	78	76	62	77	9	7	9	7	7	2	Long
IR62829A	77	75	60	68	8	10	7	7	4	3	Long
IR68893A	68	66	67	75	15	10	10	8	7	4	Long
PMS 10A	85	83	67	77	11	9	8	7	7	1	Long
PMS 1A	87	85	79	70	13	12	11	10	1–2	3	Long

[a]A = A line, B = B line.

India. They are promising for hybrid rice breeding in southern Vietnam (Luat and Bong 1995). For northern Vietnam, nine CMS lines having stability for male sterility and good floral characters have been selected for use in hybrid rice breeding programs (Table 8).

Developing locally bred rice hybrids

Stable CMS lines such as Zhen Shan 97A, IR58025A, and IR62829A were used as female parents and crossed with local high-yielding varieties and IRRI-bred restorers. The Hybrid Rice Research Center at the Vietnam Agricultural Science Institute (VASI) made nearly 1,800 crosses while Agricultural University No. 1, Hanoi, made 202 crosses. The stable CMS lines were AMS 23A, Zhen Shan 97A, AMS 24A, AMS 30A, IR58025A, IR62829A, PMS 8A, PMS 10A, and AMS 38A (Hoan 1995).

In all, 138 F_1 crosses having a yield advantage of 20% or more compared with the check (CR203) were selected. Among these, 13 hybrids have a yield advantage of more than 50% (Table 9). A cross combination—AMS 24A/R7—was released as HR 1 and was tested in three crop seasons. HR 1 produced yields of 7.5–10t ha^{-1} (Qui et al 1995).

These hybrids were also evaluated for bacterial leaf blight resistance. Among 658 crosses, 137 hybrids were resistant, 157 hybrids were moderately susceptible, and 483 hybrids were susceptible. The remaining 20 crosses were found to be highly susceptible.

Table 9. Growth characteristics and yield potential of the most promising hybrid combinations identified at Ankhanh-Hoaiduc-Hatay in Vietnam (summer 1995).

Hybrid	Duration (d)	Plant height (cm)	Productive height (cm)	Filled grams panicle $^{-1}$	1,000-grain weight (g)	Yield advantage (%) over hybrids and check in sample, 5 hills cross $^{-1}$		
						Hybrids		inbred
						Shan you 63	Shan you Gui 99	CR203 (check)
AMS 24A/R5	95	106	352	100	25	50	48	76
AMS 24A/R10	116	100	408	90	25	50	48	76
AMS 24A/R228	113	101	496	90	23	65	63	94
AMS 24A/R7 [a]	95	101	360	110	27	65	64	95
AMS 24A/R14	111	105	408	110	24	51	48	77
AMS 24A/R232	102	95	352	120	24	63	61	91
Z 97A/R26	97	102	328	80	27	51	48	77
Z 97A/R148	102	100	280	120	28	51	48	77
Z 97A/R190	99	101	370	100	28	63	61	92
AMS 30A/R134	118	96	324	80	28	50	48	76
AMS 30A/R254	99	101	352	110	27	61	59	90
IR62829A/R112	102	95	360	100	28	53	51	80
AMS 23A/R44	114	106	232	150	28	56	54	83

[a] Released as HR 1.

Two-line hybrid rice breeding

From IRRI, 17 thermosensitive genic male sterile (TGMS) lines were introduced. In addition, 17 TGMS lines were developed through mutation breeding and 29 TGMS were selected from a crossing program. The TGMS lines were studied in the nethouse and field conditions near Hanoi (Gam and Hoan 1995). Pollen sterility was studied every 5 d by staining with I-KI solution at 1% concentration and the pollen was viewed under a microscope. Changes in sterility-fertility and seed-setting capacity in some promising TGMS lines developed in Vietnam were studied in the Red River Delta (Table 10).

From early January to the end of March, low temperature (min 13.9 °C and max 19.9 °C) caused complete sterility of the TGMS lines. But from 10 to 30 April (min 21.4 °C and max 27 °C), pollen fertility increased (from 89.5% to 99.2%). leading to a high selfed seed set rate ranging from 42.8% in TGMS 10 to 69.2% in TGMS 11. From 1 May to 30 September (min 24.5 °C and max 31 °C), pollen sterility was found to be 100% in VN TGMS 86, VN TGMS 7, VN TGMS 8, and VN TGMS 11. During October (min 24 °C and max 28 °C) and November (min 18.4 °C and max 25 °C), temperatures were ideal, leading to a high selfed seed set rate in all TGMS. The results indicated that, in the Red River Delta, two seasons can be used to multiply TGMS lines. Every year, the first crop will flower in April and the second crop will flower in October-November.

F_1 seed production of two-line hybrids using TGMS as the female can be arranged for the crop to flower from May to the end of September. This means that two

Table 10. Changes in sterility-fertility and seed-setting capacity in some promising TGMS lines developed in Vietnam, 1995.

Period for flowering (day/month)	Fertility capacity (%)	VN TGMS 3	VN TGMS 6	VN TGMS 7	VN TGMS 8	VN TGMS 10	VN TGMS 11
1-I–30-III	Stained pollen	15.3	18.2	10.0	15.5	26.6	22.8
	Seed set	0	0	0	0	0	0
1-IV–10-IV	Stained pollen	56.8	65.7	65.2	76.5	66.1	86.5
	Seed set	6.6	4.8	18.5	28.5	0	20.5
10-IV–30-IV	Stained pollen	99.2	95.7	99.4	96.5	89.5	98.5
	Seed set	62.5	56.8	62.7	55.6	42.8	69.2
1-V–15-V	Stained pollen	0	0	0	0	0	0
	Seed set	0	0	0	0	0	0
15-V–27-V	Stained pollen	0	0	0	0	0	0
	Seed set	0	0	0	0	0	0
15-VIII–30-IX	Stained pollen	20.5	0	0	0	2.5	0
	Seed set	8.2	0	0	0	0.5	0
1-x–30-XI	Stained pollen	92.1	68.3	84.6	85.3	98.2	92.7
	Seed set	86.7	48.8	57.5	75.8	65.5	78.5

Table 11. Selfed seed set rate in TGMS lines in natural and artificial conditions (Haihung, Vietnam, 1995).

TGMS	Selfed seed set (%)							
	Temperature under artificial conditions			Temperature under natural conditions and date of panicle initiation				
	22 ± 1°C	25 ± 1°C	30 ± 1°C	18°C 20-II	21.5 °C 5-III	26°C 20-III	29°C 5-IV	31°C 20-IV
T4	63.6	30.5	0.5	19.8	36.8	22.4	1.5	0.5
T8	51.4	25.2	0.6	16.5	39.6	19.8	1.7	0.6

crops per year can be harvested and used for F_1 seed production. The first crop will flower from 10 to 20 May and the second crop will flower in the first week of September. The climate during this period is also favorable for the rice crop to flower in the Red River Delta.

The TGMS lines were screened for selfed seed set under artificial and natural conditions. Lines TGMS 4 and TGMS 8 in Haihung also gave similar results (Table 11). These two TGMS lines had the highest selfed seed set (36.8–39.6%) when the temperature reached at the panicle initiation stage was 21.5 °C and the lowest selfed seed set (0.5–0.6%) when it reached 31 °C. These results were confirmed in phytotron studies at air humidity of 75% ± 2 with 20-d treatments from panicle initiation. TGMS 4 and TGMS 8 had the highest selfed seed set (51-63%) at 22 °C ± 1 and the lowest selfed seed set (0.5–0.6%) at 30 °C ± 1 (Tuan et al 1995).

Studies on growth characters showed several important variations (Table 12):

- In all of the TGMS lines belonging to the short-duration group, flowering occurred 50–89 d from sowing.

Table 12. Growth characters of promising TGMS lines in Vietnam.

TGMS line	Days to 50% heading	Plant height (cm)	Tillers hill^{-1}	Leaves plant^{-1}	Spikelets panicle^{-1}	1,000-grain weight (g)
VNTGMS 3	75	81.5	4.2	14.0	102	25.0
VNTGMS 6	80	82.6	6.3	15.0	106	24.0
VNTGMS 7	86	85.5	6.0	15.5	99	25.0
VNTGMS 8	82	90.5	5.8	15.5	112	25.0
VNTGMS 9	72	65.8	4.8	13.5	79	24.0
VNTGMS 10	75	105.2	5.5	13.5	115	24.0
VNTGMS 11	70	95.5	5.2	13.0	114	25.0
TGMS 8	89	83.2	8.4	15.2	176	23.4
TGMS 3	85	75.2	9.1	13.8	132	22.6
TGMS 1	84	74.9	6.8	13.4	213	24.1
TGMS 7	75	78.3	7.3	13.7	134	22.4
TGMS 5	50	61.2	6.5	12.1	114	23.1
CR203	82	87.3	5.2	14.0	147	23.0
1S	76	92.6	4.5	15.5	309	–
15S	65	71.9	3.9	13.1	152	–

Table 13. Some promising two-line hybrid rice combinations identified in Vietnam (1995-96).

Hybrid	Plant height (cm)	Duration (d)	Panicles hill^{-1}	Grains panicle^{-1}	Yield (kg m^{-2})
1S/R6	115	113	7.6	294	1.6
15S/R18	110	108	10.9	212	1.5
1S/R4	115	115	7.6	276	1.2
1S/R50	104	107	7.0	204	1.2
15S/R9	113	120	13.2	245	1.4
TGMS 1/J1		115–120			1.2–1.4
TGMS 2/T1		115–120			1.2–1.4
TGMS 2/4		115–120			1.0–1.2
TGMS 2/411		95–105			0.8–1.0
TGMS 2/Jasmine		115–121			0.8–1.0

- Plant height was short, ranging from 66 to 105 cm.
- Flag leaf angle was almost erect (30–45° to the main culm) in most lines, except VN TGMS 4 and VN TGMS 12, which had horizontal flag leaves.
- Grain shape ranged from bold to long type.
- Flowers in most lines opened from 0845 to 1045 h and closed at about 1300 h. In some TGMS lines, however, flowers closed at 1515 h.

Almost all TGMS lines had one or two exserted stigma. Some TGMS lines that showed a high percentage of exserted stigma are VN TGMS 6 (32.5%), VN TGMS 8 (41.7%), and VN TGMS 10 (41%).

At this stage of research in Vietnam, the best TGMS lines for two-line hybrid breeding are TGMS 1,TGMS 3,TGMS 5,TGMS 7,TGMS 8,T4, T8, VN TGMS 6, VN TGMS 7, VN TGMS 8, VN TGMS 11, 1S, and 15S.

Detailed studies are now under way to confirm the critical level of temperature in the phytotron for these TGMS lines. Several test crosses were made involving TGMS as the female and some hybrid combinations have been identified (Table 13).

In the summer 1996 season, about 1,500 test crosses involving TGMS lines as female parents were evaluated in the field for yield heterosis. Simultaneously, four hybrids of the two-line system were demonstrated in farmers' fields.

Genetics of the TGMS Trait

Luong and Qui (1995) made some genetic studies using TGMS lines. The F_1 from TGMS 1/TGMS 5 and TGMS 3/TGMS 5 were all fertile at high temperature, suggesting that the genes causing thermosensitive genic male sterility in TGMS 1 and TGMS 3 are different from the genes of TGMS 5. Studies on the fertility of F_2 and F_1BC_1 progenies of TGMS 1, TGMS 3, TGMS 5, and TGMS 8 with high-yielding inbred varieties at high temperature showed a segregation ratio of 3 fertile: 1 sterile in the F_2 generation (Table 14) and 1 fertile: 1 sterile in the F_1BC_1 (Table 15). This clearly demonstrated that thermosensitive genic male sterility is controlled by a nuclear recessive gene.

Table 14. Segregation ratio for fertility/sterility in the F_2 generation of crosses involving TGMS lines as female parents. The heading period was from 15 May to 10 June 1995.[a]

Cross	Sterile plants	Fertile plants	Plants observed	Segregation ratio fertile:sterile	X^2 value
TGMS 1/CR203	15	50	65	3:1	0.127
TGMS 1/A20	17	61	77	3:1	1.912
TGMS 1/DT10	31	87	118	3:1	0.101
TGMS 1/DT40	34	116	150	3:1	0.180
TGMS 1/C70	49	137	186	3:1	0.178
TGMS 1/C71	21	72	93	3:1	0.290
TGMS 3/CR203	34	120	154	3:1	0.233
TGMS 3/DT10	29	105	134	3:1	0.302
TGMS 5/CR203	31	88	119	3:1	0.026
TGMS 5/DT10	54	170	224	3:1	0.035
TGMS 8/CR203	36	132	168	3:1	0.424
TGMS 8/DT10	41	139	180	3:1	0.196

[a] *P* value was 0.01 in each case.

Table 15. Segregation ratio for fertility/sterility of F_1BC_1 generation involving TGMS lines as recurrent female parents.[a]

Combination, F_1BC_1	Sterile plants	Fertile plants	Plants observed	Segregation ratio fertile:sterile	X^2 value
TGMS 1//TGMS 1/CR203	12	15	27	1:1	0.333
TGMS 1//TGMS 1/DT10	21	19	40	1:1	0.100
TGMS 3//TGMS 3/CR203	18	21	39	1:1	0.115
TGMS 3//TGMS 3/DT10	23	29	52	1:1	0.346
TGMS 5//TGMS 5/CR203	24	26	50	1:1	0.040
TGMS 5//TGMS 5/DT10	23	27	50	1:1	0.160
TGMS 8//TGMS 8/CR203	19	24	43	1:1	0.290
TGMS 8//TGMS 8/DT10	23	29	52	1:1	0.346

[a] *P* value was 0.01 in each case.

Developing hybrid seed production technology

Planting density of restorer and CMS lines

Based on experiences in China and at IRRI with F_1 seed production, studies were made to find the best ratio of R:A (restorer to A lines) in relation to density of the A line population. In adopting the method of single sowing for the male parent, a ratio of 1R:8A and density of A lines at 15 × 10 cm hill^{-1} gave the highest yield. A combination density of 15 × 13 cm hill^{-1} (1R:8A) and 15 × 10 cm hill^{-1} (lR:10A), however, also gave a high seed yield in the F_1. By using the method of single sowing the male parent, the F_1 seed yields obtained for Shan you Gui 99 were 1,053 kg ha^{-1} in the spring crop and 1,462 kg ha^{-1} in the summer crop.

Flowering synchronization based on maturity duration

Almost all restorer and A lines have been studied in the spring and summer seasons. It was found that the maturity duration of R and A lines depended on the date of sowing for different season crops. In general, in the summer crop, duration from sowing to flowering in 10% of the population was 59–62 d for Zhen Shan 97A, 72–76 d for Gui 99, and 82–86 d for Minghui 63. The flowering duration of each variety was almost stable. In the spring or winter-spring seasons, however, because of low temperature, all varieties showed a longer flowering duration. In F_1 seed production plots in the summer-season crop, male parent Gui 99 should be sown in the nursery 14–16 d before Zhen Shan 97A, and Minghui 63 should be sown 23–24 d before Zhen Shan 97A. In the spring, however, the differences in sowing dates between the male and female parents are based on the emergence rate of the first seven leaves. We use the following formula:

$$Y = [\, Rn - (An - 3.5) \,] \times X$$

where Y = number of days between sowing the R lines and A lines, Rn = total number of leaves of restorer lines, An = total number of leaves of A lines, X = rate of leaf emergence, and 3.5 = constant.

According to this formula, the appropriate time identified for sowing Zhen Shan 97A was when Gui 99 reached the 5.5 leaf stage or when Minghui 63 reached the 6.4 leaf stage.

Techniques to control heading time

Even though the sowing dates of R and A lines were determined, because of the fluctuation in climatic factors, especially changes in temperature, agronomic techniques, and damage by diseases and insects, synchronization in the heading of parental lines in each season's crop was prevented. To overcome this in seed production plots, experiences in China and at IRRI are exploited in Vietnam. If, in the first 3 leaf stage, panicle development of the R line is earlier than that of the A line by one stage, their heading will be synchronized. If the heading day of the two parents is predicted

to differ by about 7 d, the following methods are used to synchronize heading in the two parents:

- Draining water, cutting plant roots, or applying 30–40 kg urea ha^{-1} is used to delay flowering time in restorer lines.
- Applying 110–150 kg urea ha^{-1} or MET (a coded compound obtained from China) at 40–100 g ha^{-1} is used to delay flowering in A lines.
- Applying 70 kg K_2O ha^{-1} for A lines and 15–20 kg K_2O ha^{-1} for restorer lines at the third stage of development of the panicle, or spraying 7.5 g GA_3 + 1.5 kg KH_2PO_4 ha^{-1} for A lines 4–5 d before heading when they are late in development, can synchronize their flowering.

Seed yields in Vietnam

At the beginning of the program in 1992, F_1 seed yields in seed production plots (0.5–3.0 ha) were very low (300 kg ha^{-1}). In the seed production demonstration plots conducted by the Hybrid Rice Research Center, F_1 seed yield increased in spring 1995 to 884 kg ha^{-1} for the two-time seeding method of the male parent, and to 1,053 kg ha^{-1} for the single seeding method of the male parent. In the summer, F_1 seed yield increased, reaching 2,200–3,000 kg ha^{-1} for Bo you 64 (Table 16). At the Cuu Long Rice Research Institute in southern Vietnam, seed yields of 1,400–2,150 kg ha^{-1} were reported in 1991–92 from two IRRI hybrids designated as UTL 1 and UTL 2.

Table 16. Results of F_1 seed production at research institutes in Vietnam, 1992-96.

Institute[a]	Hybrid combination	Area (ha)	Season and year	Yield (kg ha^{-1})	Remarks
PPI	Z 97A/Gui 99	3.0	Summer 1992	350	
VASI	Z 97A/Minghui 63	0.5	Spring 1993	200–680	
CLRRI	UTL 2	0.1	Winter-spring 1991	2,150	
CLRRI	UTL 2	0.1	Autumn 1992	1,400	
FCI	Z 97A/Gui 99	0.1	1993-1995	750–850	
HRRC (VASI)	Z 97A/Gui 99	0.1	Spring 1995	1,053	Seeding male parent once
HRRC	Z 97A/Gui 99	0.5	Summer 1995	1,068–1,462	Seeding male parent once
HRRC	Z 97A/Gui 99	1.2	Summer 1995	1,200–1,420	Seeding male parent once, then transplanted twice
HRRC	Z 97A/Gui 99	0.3	Spring 1995	884	Seeding male parent twice
HRRC	Z 97A/Gui 99	0.5	Summer 1995	1,142–1,503	Seeding male parent twice
HRRC	Bo you 64 (AMS 24A/IR9761)	0.3	Spring 1996	2,200–3,000	Seeding male parent once

[a] VAS1 = Vietnam Agricultural Science Institute, PPI = Plant Protection Institute, CLRRI = Cuu Long Delta Rice Research Institute, FCI = Food Crop Institute, and HRRC = Hybrid Rice Research Center.

Agronomic studies on hybrid rice

Planting method

In northern Vietnam, hybrid rice is planted using the following methods :

1. Wet seeding in the nursery followed by deep transplanting.
2. Wet seeding in the nursery followed by shallow transplanting.
3. Direct seeding in rows.

Studies showed that wet seeding followed by shallow transplanting was found to be the best crop establishment method; the ideal density of transplanting was 40 hills m^{-2} with 2 seedlings $hill^{-1}$. For transplanting, seedlings at the 2.5 to 3 leaf stage (15 d old) for spring and 20–30-d-old seedlings for the summer-season crop were used.

In the Cuu Long River Delta, hybrid rice UTL2 (IR62829A/IR29723R) gave the highest yield (5.78 t ha^{-1}) when transplanted at a spacing of 25 x 30 cm. Transplanting at 20 x 15 cm or 20 x 20 cm between rows and hills gave significantly lower yields. In a direct-seeded experiment at 20, 30, 40, and 50 kg of F_1 seeds ha^{-1}, hybrid rice yield ranged from 5.32 to 5.89 t ha^{-1}. There were no significant differences between the different seeding methods and F_1 seed rates used.

Fertilizer requirement for hybrid rice

In the Red River Delta, Dinh (1995) studied nitrogen requirement using 10 t of farm manure, 90 kg P_2O_5, and 60 kg K_2O ha^{-1}. The highest yield in conventional rice was obtained at 100–120kg N ha^{-1}. Hybrid rice yielded the highest at 180 kg N ha^{-1} in the spring season and 150 kg N ha^{-1} in the summer season (Table 17). But the most economically beneficial response to N was attained with 15.1 kg rice kg^{-1} N at 150 kg N ha^{-1} in spring versus 17.5 kg rice kg^{-1} N at 120 kg N ha^{-1} in summer. In the degraded

Table 17. Amount of nitrogen application and yield of F_1 hybrid rice in Red River Delta. [a]

Season	N applied (kg ha^{-1})	Yield (t ha^{-1})	Efficiency (kg rice kg^{-1} N)
Spring	0	4.83	
	60	5.71	14.7
	90	6.14	14.6
	120	6.51	14.0
	150	7.09	15.1
	180	7.34	13.9
	210	7.26	11.6
Summer	0	4.72	
	30	5.05	11.0
	60	5.55	13.8
	90	6.09	15.2
	120	6.82	17.5
	150	6.87	14.3
	180	6.37	9.3

[a] All treatments received 10 t of farm manure ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 60 kg K_2O ha^{-1}.

soils, the maximum yield of hybrid rice was obtained at 150 kg N ha^{-1}; 120 kg N ha^{-1} proved to be the most economical. For hybrid rice, applying 50% of the N as a basal dose, 25% as a first topdress, and the remaining 25% as a second topdress gave the highest yields (Table 18).

In the alluvial soils of the Red River Delta having a high potassium content, applying potassium (60–90 kg K_2O ha^{-1}) for hybrid rice as well as conventional rice gave increased yield only when farm manure was not used. In degraded soils that are poor in potassium content, however, applying potassium increased yield (Table 19). Studies also indicated that applications of 150–210 kg K_2O ha^{-1} to hybrid rice or conventional rice still gave higher yields. In terms of economic benefit, however, the application of 120 kg K_2O ha^{-1} is the best for hybrid rice in this soil. The results also

Table 18. Stage of nitrogen application for hybrid rice in fertile (alluvial) soil of the Red River Delta.[a]

Fertilizers used (%)			Yield	N efficiency
Basal application	Tillering stage	Panicle initiation	(t ha^{-1})	(kg rice kg^{-1} N)
0	50.0	50.0	5.20	–
25	37.5	37.5	5.65	5.0
50	25.0	25.0	6.00	8.9
75	12.5	12.5	5.87	7.4
75	25.0	0.0	5.45	2.8
75	12.5	12.5	5.55	3.9
100	0.0	0.0	5.61	4.6

[a]All treatments received 8 t farm manure ha^{-1}, 120 kg N ha^{-1}, 90kg P_2O_5 ha^{-1}, and 60kg K_2O ha^{-1}.

Table 19. Efficiency of potassium fertilizer for hybrid rice (t ha^{-1}), averaged yield of two crops.

Season	Treatment	Alluvial soil[a]				Degraded soil[b]			
		Hybrid rice		CR203		Hybrid rice		CR203	
		Yield	Yield increase	Yield	Yield increase	Yield	Yield increase	Yield	Yield increase
		(t ha^{-1})							
Spring	NP	6.18		4.71		3.59		3.02	
	NPK	6.85	0.67[c]	5.06	0.35[d]	5.09	1.50[c]	4.15	1.13[c]
	PC + NP	7.07		5.76		4.33		3.31	
	PC + NPK	7.21	0.14	5.81	0.05	5.41	1.08[c]	4.21	0.90[c]
Summer	NP	5.97		4.91		2.83		2.63	
	NPK	6.35	0.38[d]	5.14	0.23	4.25	1.42[c]	3.28	0.65[c]
	PC + NP	6.27		5.64		4.10		3.64	
	PC + NPK	6.56	0.29[d]	5.65	0.01	4.90	0.80[c]	4.22	0.58[c]

[a]Fertilizer dose was N = 120–150, P_2O_5 = 90, K_2O = 60–90. [b]Fertilizer dose was N = 90–120, P_2O_5 = 90, K_2O = 120. [c] Significant at 1% level using LSD. dSignificant at 5% level using LSD.

indicated that hybrid rice gave increased yields compared with conventional rice when potassium fertilizer was applied.

The relationship between N and K in nutrition of hybrid rice was also studied. In the Red River Delta soil, potassium fertilizer showed a low efficiency in soils that received a low level of N. But its efficiency increased significantly in the soil with an increased amount of applied N (150 kg N ha^{-1}). This increased potassium efficiency was more clearly demonstrated in soils with poor K content. The results showed that, in these soils, without potassium application, hybrid rice yielded 4.21 t ha^{-1} and nitrogen efficiency was 4–8 kg of rice kg^{-1} N. With a potassium application of 120 kg K_2O ha^{-1}, yield increased to 6.03 t ha^{-1} and N efficiency increased to 7–13 kg of rice kg^{-1} N.

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CHAPTER 26

Hybrid rice in the Philippines: progress and prospects

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In June 1994, the National Seed Industry Council approved the commercial release of the promising hybrid IR64616H. Registered as PSB Rc26H and named Magat hybrid, IR64616H became the first hybrid rice variety in the Philippines. An upcoming hybrid, IR68284H, showed standard heterosis of 16.4% across seasons (dry and wet) and 26.9% during the dry season in the National Cooperative Trials for rice. More heterotic hybrids from PhilRice, IRRI, and Cargill are now being evaluated in test nurseries. Hybrid rice research at PhilRice has been strengthened through international collaboration. In the Cagayan Valley region, farmers were trained in on-farm F_1 hybrid seed production. In 1994, the National Rice Seed Production Network facilitated the nationwide dissemination of new hybrid rice varieties. This chapter discusses the challenges facing hybrid rice breeding research and technology development in the Philippines.

The higher yield potential of F_1 rice hybrids relative to inbred cultivars has been known to plant breeders in the Philippines for at least three decades (Umali and Bernardo 1959). But the spectacular increases in rice yields achieved by developing modem high-yielding semidwarf varieties in the 1960s made plant breeders in the Philippines focus on the relative technical simplicity of inbred rice breeding. Hybrid rice breeding and seed production achieved in China during the late 1970s clearly demonstrated the usefulness of the technology. Faced with projected increases in rice demand from a burgeoning population and stabilization of yield levels in modern inbred varieties, the rice variety improvement program of the Philippine Rice Research Institute (PhilRice) conceived and implemented the project "Development of F_1 Rice Hybrids and Related Technologies" in 1989. The major objectives of this project were to develop F_1 hybrids with a yield advantage of at least 15% over the best pure lines, and to develop and introduce technologies that would make hybrid rice production commercially profitable. The project also aimed to collaborate with the International Rice Research Institute (IRRI) and a private seed firm, Cargill Seeds.

PhilRice has actively pursued collaboration with other research institutions having strong hybrid rice research and technology development programs. Already, strong collaboration has been established with IRRI and the Rice Research Institute (RRI) of Yunnan Agricultural University (YAU), Yunnan Province, China. The latter was formerly the Dian-type Hybrid Rice Research Center of China and is now the center for japonica hybrid rice breeding research in that country. In 1995, a collaborative research agreement on hybrid rice was also signed with the Jiangxi Academy of Agricultural Sciences. These joint research undertakings will help strengthen PhilRice's hybrid breeding program through the exchange of technical expertise and germplasm. In addition, they will facilitate local efforts to develop hybrid rice-related technologies by introducing and testing mature technologies from other countries.

Cytoplasmic male sterile materials

The cytoplasmic genetic male sterility (CMS) or three-line system has been the main system used for hybrid rice breeding in the Philippines. Several CMS lines were evaluated initially at PhilRice for adaptability and stability of their pollen sterility trait. These lines were developed at IRRI during 1980-89 through the transfer—by backcrossing into elite lines—of the cytosterility system of CMS wild abortive (WA) lines V20A, Zhen Shan 97A, Er-Jiu-Nan 1A, and V41A (Yuan and Virmani 1988). The more stable and adapted CMS lines that also possessed acceptable grain quality, good combining ability, and satisfactory outcrossing rates were used immediately in the PhilRice hybrid breeding program. Among the usable and highly satisfactory CMS lines were IR58025A and IR62829A. In addition, CMS lines from India were evaluated in the Philippines through IRRI and some were found to be commercially useful (Virmani 1994), such as PMS 8A, PMS 10A, and Pragathi A. PhilRice plant breeders are now using these CMS lines to develop heterotic combinations.

In 1993, several Dian-type CMS lines were introduced to PhilRice through its collaborative project with YAU, including CMS lines with cytoplasms derived from E-shan-ta-bei-gu (STB type) and Zhao-tong-bei-zi-gu (ZTB type). Test crosses with high-yielding local cultivars led to the identification of maintainer varieties that could be converted through backcrossing into cytoplasmically diverse CMS lines with typical indica grain features and plant type. STB-type CMS line 913A, for example, is being used in the CMS conversion of recommended inbred variety PSB Rc4 (Table 1). Promising restorers for 913A have already been identified among elite breeding lines (PR23531-13-2-2, PR23373-61, and MRC23519-1497) and recommended variety PSB Rc12 (Xu et al 1995).

Restorability-maintainability patterns of Dian-type CMS lines 28A, 913A, and Ginante A have also been investigated. CMS line 913A has many restorers, 28A has many maintainers, and Ginante A has the same restorer-maintainer pattern as WA-type CMS lines. Pollen abortion in ZTB-type CMS line Ginante A is similar to that of WA-type CMS lines IR58025A, IR62829A, PMS 8A, PMS 10A, and PRlA (Xu et al 1995). On the other hand, the pollen abortion patterns of STB-type CMS lines 913A

Table 1. Maintainer variety conversion to new Dian-type CMS lines in the BC_3F_1 generation.

Donor parent	Maintainer variety
28A	IR56
28A	IR65
28A	IR68
28A	IR72
913A	PSB Rc4
28A	PSB Rc12
28A	PSB Rc10
28A	PSB Rc20
28A	PSB Rc22

and 28A were different from those of WA types. The WA and STB CMS systems appeared to be distinct, based on anther inspection, pollen fertility, and percentage seed setting in A x B crosses between WA and STB types, and in crosses between restorers of STB and WA CMS lines (Xu et al 1996). Molecular genetic studies are under way to assess the diversity of about 24 CMS lines developed by IRRI, YAU-RRI, and PhilRice that are currently being used, or will be used, in our breeding program.

Evaluating F_1 hybrids

Advanced yield trials (AYT) of very promising hybrids have been conducted in collaboration with IRRI since 1991. In the 1994 wet season, PhilRice also joined in the testing of more F_1 hybrids entered in the International Hybrid Rice Observational Nursery (IRHON), a multicountry trial coordinated by IRRI's International Network for Genetic Evaluation of Rice (INGER).

In the 1996 dry season alone, 102 F_1 hybrids were evaluated at PhilRice: 27 in the observational nursery, 6 in the preliminary yield trial, 36 in the AYT, and 33 in the IRHON. In the 1995 dry-season IRHON, only the hybrid IR70400H yielded better than the check varieties. Restorer lines IR55722-B-B-6-2-2R, IR53466-B-149-B-B-5R, IR54791-19-2-3R, IR51078-33-2-1-1-3R, and IR57298-31-2-2R, however, were selected for inclusion in our source nursery. Superior combinations developed by the PhilRice breeding program, relative to check varieties, were also identified (Table 2). Heterosis for most of these combinations exceeded that of Magat, the only released hybrid in the country. The CRR restorer lines were introduced from YAU, indicating the usefulness of a genetically diverse germplasm base in heterosis breeding.

Heterosis for yield in promising hybrids

Table 3 summarizes the general performance of hybrids in the 1995-96 AYT. Because the PhilRice hybrid breeding program is relatively new, all of the entries in the AYT are bred at IRRI but evaluated by PhilRice researchers at PhilRice-Maligaya, Nueva Ecija, and at PhilRice-San Mateo, Isabela. On average, the hybrids performed better than the check cultivars in San Mateo, but not in Maligaya. Although some hybrids outyielded the check varieties in Maligaya during the 1995 tests, a more consistent

Table 2. Promising hybrids in the PhilRice breeding program, 1996 dry season.

F_1 hybrid combination	Yield (t ha^{-1})	Advantage over check (%)	Check variety[1]/ hybrid[2]
Observational nursery			
IR58025A/CRR126	6.04	19.7	Magat[2]
IR58025A/CRR120	6.61	38.5	Magat[2]
28A/CRR102	5.85	15.8	Magat[2]
PMS 8A/CRR107	6.00	18.9	Magat[2]
913A/Yongjubyeo	6.85	35.8	Magat[2]
913A/BPI Ri-10	7.32	45.1	Magat[2]
Pragathi A/IR30	7.67	51.9	Magat[2]
Preliminary yield trial			
IR58025A/CRR157	6.31	17.3	PSB Rc4[1]
		12.5	Magat[2]

superiority of hybrid performance was observed in San Mateo across groups and seasons. Standard heterosis observed in the San Mateo trials ranged from 5% to 23% based on upper yield range comparisons. It is interesting to note that although heterosis for yield was better expressed in San Mateo, the trials at Maligaya generally gave higher yields. To fully exploit the production potential of high-yielding environments, such as Nueva Ecija and Isabela provinces, hybrids with a stable yield performance across sites will be necessary.

From the advanced breeding nursery, elite hybrids are ultimately evaluated in the multilocational national cooperative tests (NCT) for rice. In the 1995 wet-season national trials, hybrids CXRH 05 and CXRH 07 from Cargill and IRRI-bred IR70965H recorded the highest mean yields among very early maturing entries for the irrigated lowland ecosystem (4,580 kg ha^{-1}, 4,553 kg ha^{-1}, and 4,447 kg ha^{-1}, respectively). Check variety IR72 yielded 4,071 kg ha^{-1}. In the 1995 dry season, the performance of hybrids with various growth durations in the irrigated lowland NCT, however, was not as promising (Table 4). Although the average yields of some hybrids across the nine test sites were better than those of the check cultivars, specifically in the early and medium-maturity groups, the top-ranked entries in all groups were all inbred cultivars. At some sites, however, yields of hybrid entries surpassed those of both the check variety and the top-ranked entry. As in the case of the Magat hybrid, a location-specific recommendation may be possible for these hybrids, pending the collection of additional NCT data.

Although current NCT data show that some inbreds continue to perform better than hybrids at most test sites, they also imply that these outstanding inbreds could be useful in developing new hybrid combinations, either as lines for CMS conversion or as restorers for existing CMS lines. It is therefore apparent that the output of the inbred breeding program is an input to the hybrid rice breeding program. Interestingly, it is possible that the use of similar germplasm bases in both inbred and hybrid breeding programs may lead to a limited gain in phenotypic performance. A genetic

Table 3. Performance of F_1 hybrids in advanced yield trials at PhilRice research stations.

Test and season	Yield (kg ha^{-1})		Days to maturity	
	Maligaya	San Mateo	Maligaya	San Mateo
	1995 dry season			
Maturity group I				
Mean (F_1 hybrid entries)	6,046	4,858	117	109
Mean (checks)	6,669	4,463	116	110
Mean (yield of group I)	6,145	4.795	117	109
Range (F_1 hybrids)	5,132–7,334	3,841–5,558	113–122	106–115
Range (checks)	6,161–6,966	4,193–4,582	107–121	103–117
Maturity group II				
Mean (F_1 hybrid entries)	5,675	4,371	123	120
Mean (checks)	5,675	3,932	129	120
Mean (yield of group II)	5,691	4,290	124	120
Range (F_1 hybrids)	3,986–6,842	3,341–5,219	118–136	108–128
Range (checks)	4,852–6,445	3,485–4,241	122–139	112–131
	1995 wet season			
Maturity group I				
Mean (F_1 hybrid entries)	2,562	3,248	116	115
Mean (checks)	3,025	4,037	116	104
Mean (yield of group I)	2,634	3,365	116	113
Range (F_1 hybrids)	1,449–3,657	1,487–5,339	109–125	106–119
Range (checks)	2,026–4,124	3,028–4,792	107–121	97–109
Maturity group II				
Mean (F_1 hybrid entries)	3,375	3,574	124	117
Mean (checks)	3,715	3,545	125	115
Mean (yield of group II)	3,413	3,571	124	117
Range (F_1 hybrids)	1,927–4,430	2,512–4,469	119–134	103–129
Range (checks)	3,231–4,207	3,377–3,670	123–126	106–123
	1996 dry season			
Maturity group I				
Mean (F_1 hybrid entries)	4,998	4,364	115	123
Mean (checks)	5,341	4.203	113	115
Mean (yield of group I)	5,061	4,334	115	121
Range (F_1 hybrids)	3,414–5,870	3,575–5,124	108–120	111–130
Range (checks)	4,221–6,415	3,923–4,880	109–116	113–117
Maturity group II				
Mean (F_1 hybrid entries)	4,745	4,539	121	128
Mean (checks)	5,182	4,491	126	127
Mean (yield of group II)	4,825	4,530	122	127
Range (F_1 hybrids)	4,119–5,331	2,862–5,345	117–130	122–137
Range (checks)	4,887–5,592	4,066–5,015	117–131	120–131

study by de Leon (1994) based on pedigree analysis indicated that the Magat hybrid is similar by genetic descent and therefore closely related to current check varieties and popular cultivars (Table 5). The relatively high coancestry values of Magat with these popular materials that are founded on the same genetic base as most semidwarf genotypes could be a contributory factor to the generally unimpressive heterosis levels being observed in test hybrids. The use in test crosses of CMS and restorer lines that are genetically related to each other and to most of the current high-yielding

Table 4. Performance (in kg ha^{-1}) of F_1 hybrid entries in PhilRice-coordinated national trials in the Philippines, 1995 dry season.

Test entries	Pedigree	Maturity (d)	Best	CMU[a]	CPU	CVES	DES	PRRI	MES	UPLB	USM	Mean
IR69674H	IR62829A/IR46	112	5,263##	4,992	3,606	4,983	2,376##	5,970##	4,093##	5,425**	4,433##	4,571
IR70965H	IR58025A/IR4221	112	6,296	5,309	3,560	4,433##	2,409##	6,148#	4,089##	5,040	4,874#	4,684
IR59606-119-3		103	6,085	5,466*	3,864*	4,973	2,610#	7,061	5,703	5,089	5,588	5,160
PSB Rc4 (check)		107	6,081	4,691	3,224	4,967	3,208	7,039	5,445	4,798	5,680	5,015
IR72 (check)		114	6,336	5,151	3,196	4,800	2,335	6,513	4,715	5,102	5,284	4,826
IR68877H	IR58025A/IR21567	112	6,541	5,380	3,840	4,633	2,897	6,478*	4,324	4,951	4,787	4,870
IR71016H	IR62829A/IR13149	115	6,931	5,845	3,405	4,500	2,356	5,774	4,834**	4,743	5,724**	4,901
C3559-B-18-2-3-1-1		116	6,777	6,179*	3,866*	5,057**	1,926	5,871	5,709**	5,436**	5,590*	5,157
IR72 (check)		116	6,499	5,126	3,409	4,383	2,357	5,760	3,959	4,610	4,567	4,519
IR65488H	IR58025A/IR54742	123	4,674	5,936	3,540	5,117	2,057	4,350	4,292**	3,290	4,156	4,156
IR68879H	IR58025A/IR32809	112	6,439	5,055	3,860	5,317	3,048**	6,215**	4,799**	4,096	4,874	4,874
IR71017H	PMS 10A/IR29341	120	5,996	4,505#	3,675	5,367	2,070	4,685	5,186**	4,590*	4,613	4,613
IR61979-138-1-3-2-2		122	6,637	5,375	4,066	5,200	2,931*	8,957**	4,488**	4,590*	5,116	5,116
IR68 (check)		122	5,725	5,315	3,021	5,017	2,419	4,238	3,654	3,800	4,077	4,077
IR70967H	IR58025A/IR34686	124	6,624		3,705**	4,333**	1,728	4,008	4,931	4,666**	3,491	4,186
IR62079-67-1-2-1-2		124	6,523		3,459	4,100*	2,585	5,325	6,363**	3,978**	4,930	4,658
IR74 (check)		128	7,023		2,949	3,733	2,006	3,591	4,988	2,863	3,981	3,892

*,** Significantly higher than the check at 0.05 and 0.01 probability levels, respectively. #,## Significantly lower than the check at 0.05 and 0.01 probability levels, respectively. [a]CMU = Central Mindanao University, CPU =Central Philippine University, CVES = Cagayan Valley Experiment Station, DES = Dingras Experiment Station, PRRI = Philippine Rice Research Institute (Branch Station in Midsayap), MES = Midsayap Experiment Station, UPLB = University of the Philippines Los Baños, USM = University of Southern Mindanao.

Table 5. Coancestry of the Magat hybrid with commonly used inbred check varieties and popular inbred cultivars.

Inbred check/ variety	Coancestry with Magat	Inbred check/ variety	Coancestry with Magat
IR72	0.277	PSB Rc20	0.202
IR68	0.164	PSB Rc22	0.120
IR74	0.326	IR60	0.226
PSB Rc2	0.234	IR64	0.155
PSB Rc4	0.182	IR66	0.214
PSB Rc18	0.140	PSB Rc14	0.102

varieties results in increased inbreeding, thus reducing the genetic diversity on which the expression of a high heterosis level depends. This argues for the development of' hybrids using parental lines with wider genetic bases relative to the pedigrees of current materials in the breeding program. Although high-yielding inbreds could be used either as CMS lines after conversion or as restorer lines, they may have to be paired with parents derived from a different germplasm base to increase heterosis levels. The pedigrees of promising entries in our current observational nurseries lend support to this possible strategy.

Thermosensitive genic male sterile materials

Thermosensitive genic male sterile (TGMS) lines of indica and tropical japonica types were developed by IRRI using the TGMS gene from the japonica donor variety Norin PL12. Preliminary yield-trial results at IRRI showed standard heterosis reaching 1–1.6 t ha^{-1} for some of the two-line test-cross hybrids (Lopez and Virmani 1996). At PhilRice, some TGMS lines, tested by IRRI at Los Baños and by IRRI and PhilRice in Nueva Vizcaya since 1993, were selected for the development of two-line experimental hybrids. Test crosses with some of these selections were made in the 1996 dry season and are included in our 1996 wet-season test-cross nursery for evaluation. The 26 TGMS lines used in test crosses with leading varieties were also grown in the greenhouse for sterility observation. Spikelet sterility ranging from 14.5% to 93.5% has been observed. Thermosensitive lines capable of reverting to male sterile plants at critical temperatures lower than 31 °C, such as Norin PL12-derived thermosensitive lines, will probably be more useful for two-line hybrid rice development in the Philippines.

The Magat hybrid

IRRI-bred hybrid IR64616H (IR62829A/IR29723-143-3-2-1R) was formally registered in June 1994 as rice hybrid PSB Rc26H, or the Magat hybrid. Approved for release by the National Seed Industry Council (NSIC, formerly the Philippine Seed Board), this hybrid matures in 112 d. Table 6 summarizes the yield performance of the Magat hybrid in multilocational irrigated lowland trials in the Philippines. Standard heterosis for yield of the Magat hybrid was higher during the dry season. Be-

Table 6. Summary of yield performance of the Magat hybrid in national cooperative tests.

NCT	Magat yield (kg ha^{-1})	Check variety	Standard heterosis (%)
Phase I—PSB Rc4			
Dry season	6,039	5,544	8.9
Wet season	5,166	4,919	5.0
Across seasons	5,608	5,147	8.8
Phase I—IR50			
Dry season	5,558	4,073	36.5
Wet season	5,023	3,990	25.9
Across seasons	5,275	4,029	30.9
Phase II—PSB Rc4			
Dry season	5,626	4,799	17.2
Wet season	4,607	4,092	12.6
Across seasons	4,994	4,357	14.6

cause of its pronounced location-specific performance, the Magat hybrid was released for the Cagayan Valley region, a major rice-growing area north of Manila.

Promising hybrid IR68284H

Another hybrid, IR68284H (IR58025A/IR34686-179-1-2-1R), has been evaluated as very promising in recent NCT. Standard heterosis for this hybrid was 16.4% across seasons (dry and wet) and 26.9% during the dry season alone. A potential rice hybrid in the Philippines must have a yield advantage of 1.5% over the check variety and over the best inbred entry. This promising hybrid will be analyzed by the NSIC and may be considered for release in 1997.

Hybrids from Yunnan

During the 1996 dry season, some hybrids were introduced from Yunnan, China, and tested for adaptation and popularization in large demonstration plots at PhilRice-Maligaya as well as in Ilocos and Benguet provinces. Indica hybrid Lian yu 258 had the highest yield at Maligaya (8.2 t ha^{-1}) and indica/japonica hybrid Yuza 29 had the highest yields in both Ilocos (6.4 t ha^{-1}) and Benguet (10.4 t ha^{-1}) using 150 kg organic fertilizer ha^{-1}, all applied as basal fertilizer. The yield of Yuza 29 in Benguet is a new record for the Cordilleras and indicates the suitability of some Yunnan hybrids for the Philippines' cool-elevated rice-growing areas. This hybrid also holds the record (above 1.5 t ha^{-1}) for the highest yield in Yunnan Province, China, and its growth duration is more than 180 d (Li Z, personal communication). Traits related to grain quality and resistance to Philippine rice diseases and insect pests, however, still need to be incorporated into introduced hybrids to increase their commercial acceptability.

Technology demonstration and dissemination and seed production

Technology demonstration and training

Development of the Magat hybrid facilitated the demonstration of hybrid rice technology and training of farmers on F_1 hybrid seed production. In Isabela Province, a target area for Magat, orientation seminars were conducted for participating farmers, technicians, and researchers. Fifteen farmers trained in producing hybrid seeds had average top yields of 1.9 t ha^{-1} over four seasons. Standard yield heterosis of 28–35% was obtained in trials using farmer-produced F_1 seeds. The 15 seed growers in Isabela were later accredited to commercially produce hybrid rice starting in the 1995 dry season (Lara et al 1996).

Rice production technology demonstration trials at PhilRice during the 1995 dry season showed the Magat hybrid yielding 11.8 t ha^{-1} using 180-60-60 NPK fertilizer applied in three splits. The popular inbred variety PSB Rc 14, on the other hand, yielded 9.9 t ha^{-1} in the experiment. A high yield potential of 11.2 t ha^{-1} for Magat was also observed in Isabela in the 1996 dry-season technology demonstration trials of the revitalized nationwide rice production program (Gintong Ani). Although these results indicate that high yields may be obtained through heterosis breeding, the generally lower yields of the Magat hybrid compared with inbred varieties entered in the same trials indicate the need for better hybrids to expedite the popularization of hybrid rice technology nationwide.

Seed production in farmers' fields

In the 1995 dry season, 10 of the 15 accredited hybrid rice seed growers from Isabela produced hybrid seeds of Magat on 0.2–0.5-ha plots. Their F_1 seed harvest ranged from 800 to 1,600 kg ha^{-1}, and the additional harvest from the restorer parent was about 1,500 kg ha^{-1}. PhilRice bought about 800 kg of these hybrid seeds at P60 kg^{-1} (about $2.40) for technology promotion purposes. A total of 75 technology demonstrations were established in the 1996 dry season with Magat as an entry. Based on a price of P60 kg^{-1} for hybrid seeds and P8 kg^{-1} for seeds of the restorer line, gross income derived by seed growers ranged from P63,000 to P110,000 ha^{-1}, or $2,520 to $4,440 ha^{-1} (Lara and Miranda 1995). Economic studies are needed, however, to compare these income levels with those earned by seed growers of pureline varieties.

Seed production and purification of parental lines at PhilRice

In 1995, significant amounts of seed for CMS lines IR58025A and IR62829A and their maintainer lines were produced at PhilRice. Purification and multiplication of CMS line IR62829A and restorer line IR29723R, the parents of Magat, were also undertaken during the 1995 wet season using paired crosses. Good maintainer and restorer plants were used to produce nucleus parental seeds.

Likewise, seed production of the restorer line (IR34686R) for the most promising NCT entry (IR68284H) started in the 1995 dry season. Beginning in the 1996 dry season, the Seed Production and Health Division of PhilRice formally engaged in producing Magat hybrid seed. An area of 0.8 ha planted to the A and R lines of this

hybrid produced-306 kg of F_1 hybrid seed and 1,530 kg of R line seed. In this initial attempt, flag leaf clipping and gibberellic acid application were not practiced.

Seed production and certification

The good performance of Magat in the NCT and on-farm experiments before its official release prompted the NSIC to formulate guidelines for hybrid rice testing, seed production, and seed certification. Purity standards for hybrid parental lines and F_1 hybrid seeds are currently under review by the Technical Working Group (TWG) on Seed Certification and Seed Standards of the NSIC. Establishing these general policies will facilitate seed production and certification for hybrid varieties that may be released in the future.

National testing of promising hybrids

Current NCT guidelines are being revised by the TWG for Rice of the NSIC. Hybrids will be tested separately from inbreds and testing will be limited to progressive rice-growing areas and will include different fertilizer levels. This strategy will facilitate the adoption of released hybrids by progressive farmers in major rice-growing areas while identifying nutrient management and related technologies necessary for the maximum expression of heterosis in future rice hybrids.

National Rice Seed Production Network (NRSPN)

The organization of the NRSPN by PhilRice in 1994 (Malabanan et al 1996) has strengthened the seed production infrastructure of the country's public sector. Some network members could be trained and subsequently accredited as parental and hybrid rice seed growers in order to take advantage of their established technical know-how and farmer clientele, thus facilitating the production, certification, and distribution of hybrid rice seed in the future.

Challenges facing hybrid rice

The successful commercialization of F_1 hybrids to increase rice production is the ultimate goal of heterosis research. It is worth noting that in countries where hybrid rice programs have attained substantial success, the programs were launched either as a cooperative research project, as in China, or as a research network, as in India and Vietnam. Thus, implementing hybrid rice programs in these countries involved significant increases in manpower investment as well as strong and sustained government support. These countries also had the capability to produce, process, and distribute high-quality hybrid seeds through established seed production and distribution systems.

For hybrid rice technology to be popularized and adopted in the Philippines, greater manpower involvement will be necessary. The national rice research and development (R&D) network, already established by PhilRice, will have to be involved in hybrid rice research as well as in technology development and promotion. The participation of local government units and nongovernment institutions, particularly

in technology promotion, needs to be encouraged. To support this increase in manpower involvement, financial support will have to be mobilized, both locally and externally.

In the area of breeding research, superior hybrids that surpass the 5–10 t ha^{-1} yield technology currently being demonstrated using inbred cultivars will be necessary. The success of hybrid rice in other countries was mainly triggered by the development of hybrids with a clearly superior performance relative to existing inbred cultivars. In current nationwide technology demonstration trials, however, pure-line varieties continue to attain the highest yields. Hybrid rice breeding research will therefore be a critical determinant for the success of hybrid rice technology in the Philippines. At PhilRice, the strategy we are following involves a tight linkage between the hybrid and inbred rice breeding programs. Outstanding inbred cultivars will form the basis of hybrid rice breeding. New tools in genetics and biotechnology will be used to ensure diversity of our germplasm base in order to attain higher levels of heterosis. The stability of yield performance across sites will have to be addressed to facilitate the release of hybrids to key rice-growing areas of the country. Although an exceptional performance in specific regions and in a particular season may be valid bases for varietal recommendations (Redoña and Sebastian 1996), a stable performance across seasons and locations will greatly simplify hybrid seed production and related activities. This simplification is particularly important, especially over the short term, when farmers are still familiarizing themselves with the new technology.

In the area of seed production, certification, and distribution, seed quality standards have to be set properly and reasonably. Seed certification personnel need to be introduced to parental-line and F_1 hybrid cultivation practices. Prospective hybrid rice seed growers in key rice production areas need to be trained in preparation for the recommendation and release of improved hybrid rice varieties. Seed production and distribution schemes for hybrids, tailored to local seed industries, must be established and refined. Policies on price support and private-sector participation also need to be clearly defined.

Training and technology promotion activities also need to be intensified through greater participation of technical and extension personnel from government as well as nongovernment units. Training of seed producers to acquaint them with parental and hybrid seed production technologies has to be undertaken even before outstanding hybrids are released. Identifying institutions in major rice-growing areas of the country that will work on hybrid rice research and seed production will be an excellent initial step toward bolstering the country's hybrid rice R&D agenda. The establishment of a strong national hybrid rice R&D network will be in consonance with the "strategic rice area approach" of Philippine rice R&D planning. The activities reported in this chapter are indicative of the increased efforts of PhilRice to develop hybrid rice technology in the Philippines and of the government's resolve for the country to attain rice self-sufficiency and sustainable food security.

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Notes

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CHAPTER 27

Developing hybrid rice technology in Malaysia

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The Malaysian Agricultural Research and Development Institute (MARDI) began hybrid rice research in the country in 1984. Local cytoplasmic male sterile (CMS) lines MH805A, MH813A, MH821A, and MH841A were developed through a backcrossing program. More than 100 new CMS lines are presently in the BC_2 and BC_3 generations. To date, more than 130 restorer lines have been identified for producing hybrid seed. More than 530 F_1 hybrids have been evaluated in yield trails from 1991–92 to 1995–96 in the main season for identifying heterotic hybrids. At Bumbong Lima in the 1995–96 main season, IR62829A/IR46R significantly outyielded MR84 by 26%. During the same season, MH841-IA/MR167 significantly outyielded MR167 by 24% at Bertam. Efforts are under way to produce large quantities of seeds of male sterile lines and hybrids to extend heterotic hybrid rice cultivation in farmers' fields.

The total area under rice cultivation in Malaysia ranged from 0.69 to 0.7 1 million ha during 1991-94. Physical area devoted to rice cultivation is only 0.47 million ha. This area is exceeded only by rubber (1.75 million ha) and oil palm (2.36 million ha). Modern varieties were planted on 90% of the rice area in West Malaysia in 1990. Milled rice production reached 1.3 million t in 1993, which was about 65% of the national requirement. To meet the full national requirement, rice is imported from other countries. The foreign exchange spent on rice imports was equivalent to RM 389 million in 1993 and RM 340 million in 1994 (US$1 = RM 2.50). Per capita consumption of milled rice is expected to decrease from 88 kg in 1988 to 75 kg in 2000. But Malaysia's population is projected to increase from 20 million in 1995 to 32 million in 2025. Production in the country therefore needs to be increased to meet the increasing demand for rice. The yield potential of conventionally bred varieties has reached a plateau in Malaysia. Hybrid rice technology offers scope for further increasing rice yields. In late 1984, heterosis studies began (Mohamed et al 1987).

Heterosis for yield

The yields of hybrids must significantly exceed the yields obtained from the best conventionally bred varieties. This yield increase is known as standard heterosis (Virmani and Edwards 1983). To successfully develop hybrid rice varieties, sufficient heterosis must be achieved.

Although IR58025A/IR29723- 143-3-2-1R was identified as a potential experimental hybrid (Guok 1994), the unstable pollen sterility observed in IR58025A prevented its release. Its performance was inconsistent. This hybrid did not perform well even in the 1991-92 main season (Table 1). Another hybrid, IR62829A/IR29723-143-3-2-IR, produced the highest yield, but was not significantly different from the check (MR84).

We evaluated 253 experimental hybrids in the 1995 off-season and 264 in the 1995-96 main season. Table 2 lists some of the high-yielding hybrids. Bronzing of leaves occurred in some experimental plots at the Bertam station and this severely affected all conventional inbred varieties. Leaf bronzing in the hybrids was not severe and standard heterosis was high. A yield trial involving six local hybrids using MH841-1A indicated that MH841-1A/MR167 produced a significantly higher yield (24%) than MR167 (Table 3). In the yield trial conducted at Bumbong Lima, one IRRI hybrid, IR62829A/IR46R, produced a significantly higher standard heterosis (26%) than

Table 1. Results of a hybrid yield trial at Bumbong Lima, 1991-92 main season.

Hybrid/variety	Yield (kg ha^{-1})		Standard heterosis (%)
IR62829A/IR29723-143-3-2-IR	5,628	a*	18.3
IR58025A/MR24	5,066	ab	6.6
IR62829A/MR24	5,066	abc	5.3
IR62829A/MR51	4,860	abcd	2.2
IR58025A/MR81	4,796	abcd	0.9
IR58025A/IR29723-143-3-2-1R	4,795	abcd	0.9
IR62829A/MR81	4,792	abcd	0.8
MR84	4,754	abcd	–
IR62829A/MR25	4,689	abcd	-1.4
IR58025A/MR59	4,461	bcd	-6.2
IR58025A/IR28238	4,431	bcd	-6.8
IR58025A/IR54742	4,423	bcd	-7.0
IR58025A/MR25	4,384	bcd	-7.8
IR62829A/IR35366	4,101	bcd	-13.7
IR58025A/PL20	4,062	cd	-14.6
MR123	3,981	d	
MR103	3,905	d	
CV (%)	8.91		

* Means with the same letter within a column are not significantly different based on Duncan's multiple range test at the 0.05 probability level. Significance was determined over MR84.

Table 2. Performance of IRRI hybrids tested at Bertam, 1995 off-season (OS) and 1995-96 main season (MS).

Hybrid	Year/season	Days to 50% flowering	Yield (t ha^{-1})		Yield increase over check (t ha^{-1})
			Hybrid	Check (MR84)	
IR58025A/IR52256-5-2-2-IR	1995 OS	90	5.35	2.76	2.59 (94)[a]
IR58025A/IR54969-41-2	1995 OS	90	5.16	2.76	2.40 (87.0)
IR62829A/IR46R	1995 OS	91	5.15	2.76	2.39 (86.6)
IR58025A/BR827-35-2-1	1995 OS	95	5.05	2.76	2.29 (82.8)
IR58025A/IR54791-19-2-3R	1995-96 MS	88	4.86	3.10	1.76 (56.8)
IR58025A/Taichung Sen Yu 85	1995-96 MS	90	4.81	3.10	1.71 (55.3)
IR58025A/IR25912-81-2-IR	1995-96 MS	92	4.67	3.10	1.57 (50.6)

[a] Numbers in parentheses are percentage values.
Sources: Dr. Kato, personal communication, 1995, Kato et al 1996.

Table 3. Performance of local experimental hybrids at Bertam, 1995-96 main season.

Hybrids/varieties	Yield (kg ha^{-1})	Check (%)
MH841-1A/MR 167	4,576 a*	24
MH841-1A/Imp. Mahsuri	4,300 ab	17
MH841-1A/MR71	4,056 abc	11
MH841-1A/MR159	4,055 abc	10
MH841-1A/IR29723-143-3-2-IR	3,934 bc	7
MH841-1A/MR184	3,865 bc	5
MR167	3,687 c	–
MR159	3,512	
CV (%)	8.93	

* Means with the same letter within a column are not significantly different based on Duncan's multiple range test at the 0.05 probability level. Significance was determined over MR167.

MR84 (Table 4). But none of the experimental hybrids could be released for general cultivation because of unstable pollen sterility in the cytoplasmic male sterile (CMS) lines, IR62829A and IR58025A.

Breeding for locally adapted CMS lines

Most Chinese and IRRI CMS lines mature too early (<120 d) in Malaysia. There is a need to develop locally adapted CMS lines because of unstable pollen sterility in the introduced CMS lines. Although a few CMS and B pairs, such as MR83A & B, MR112A & B, Ru 2340A & B, and MR118A & B, were developed earlier (Guok 1994), they could not be used for seed production because of low outcrossing potential (in MR83A) and unstable pollen sterility (in the other CMS lines).

Another set of seven newly developed A & B lines has been produced (Guok and Ku Yahaya 1994), which included MH805A & B, MH813A & B, and MH841A & B. These are currently at BC_7F_1 for the A lines and F_{10} for the B lines. Both MH805B and

Table 4. Yield of hybrids in Bumbong Lima in the 1995–96 main season.

Cross combinations/varieties	HD[a]	SS	CL	PL	NP	GY	HT	BRW
IR69672H (IR58025A/ IR25912-81-2-IR)	98	76.5	64.0	27.6	19.1	5.34	0.5	4.47
IR69677H (IR58025A/ IR42686-C2-118-6-2)	90	58.6	61.4	27.5	18.6	5.44	2.2	4.04
IR69679H (IR58025A/ IR49461-128-3-3-3R)	92	78.7	67.1	27.2	17.3	5.42	1.8	4.25
IR69682H (IR58025A/ IR54791-19-2-3R)	92	64.9	61.9	26.7	23.3	5.63	5.8	4.63
IR69684H (IR58025A/ IR54969-41-2-2R)	92	71.5	62.6	26.5	17.3	5.72	7.5	4.92
IR69685H (IR58025A/ IR58110-114-2-2-2R)	98	63.9	67.8	26.9	17.9	5.50	3.4	4.08
IR69687H (IR58025A/ IR59566-157-1-3R)	98	73.5	66.6	26.6	19.2	5.38	1.0	4.42
IR69690H (IR58025A/ BR827-35-2-1-1-1R)	101	85.3	76.3	27.0	18.6	6.21	16.7	5.17
IR69694H (IR58025A/ Taichung Sen Yu 85)	91	88.3	60.6	26.0	17.7	6.07	14.1	4.92
IR69692H (IR58025A/ RP633-76-IR)	93	82.0	56.0	26.6	17.0	6.42	20.6	5.33
IR65488H (IR58025A/ IR54742-22-19-3R)	105	56.0	74.1	29.6	17.5	6.11	14.9	4.04
IR62829A/IR46R	89	87.8	57.0	23.8	20.6	6.71	26.1	5.50
IR62829A/ IR40750-82-2-2-3R)	91	71.4	59.7	26.1	21.9	6.17	15.9	4.75
IR65488H (IR58025A/ IR54742-22-19-3R)	100	79.5	74.9	28.4	17.1	5.34	0.3	3.95
IR70967H (IR58025A/ IR59601-301-3-6R)	102	59.6	69.2	28.1	18.6	5.50	3.4	4.42
Chenyou22/HCh3	81	95.6	60.2	26.1	14.7	5.99	12.5	5.19
MH841-1/H92-5	86	80.3	59.7	23.3	18.3	6.00	12.8	5.08
MH841-1A/Iri359	93	78.2	62.2	25.3	18.3	5.50	3.4	4.75
MH841-1A/Jangswong	93	74.9	60.5	25.5	19.6	5.85	9.9	4.92
MR64/Guang er ai 5	93	76.2	63.7	24.6	14.7	5.50	3.4	4.42
IR68275A/BR827-35-1-1-1R	101	80.1	71.3	25.3	18.9	5.83	9.7	4.42
MR84	104	80.3	68.8	22.6	17.9	5.32		3.97
MR167	100	79.9	63.6	24.2	19.2	5.25		4.08
LSD = 0.05						1.12		

[a] Determined over MR 84; HD = heading date (d), SS = seed set (%), CL = culm length (cm), PL = panicle length (cm), NP = number of panicles plant^{-1}, GY =grain yield (t ha^{-1}), HT = standard heterosis (%), BRW = brown rice weight (t ha^{-1}).
Source: Kato et al 1996.

MH813B were derived from the cross RU 5257 (or IR62829/MR112//L3/MR83// IR62829). MH 841B was derived from the cross RU 5263 (or IR62829/MR83//L4/ MR83//IR62829///IR58025). These CMS lines were found to be nearly stable for male sterility, and only 0.5% selfed seeds were found in bagged panicles of MH841 A. These lines are being multiplied for use in large-scale seed production plots.

To diversify the sources of male sterility-inducing cytoplasm, F_2 populations involving MH841B and other B lines were used to develop new CMS lines. For this, we used TR66707A (derived from *Oryza perennis*) and *O. barthii* as female parents. They

are currently in the early stage of a conversion program. IR66707A (or IR64A) could not be used earlier because of the absence of an R line. After using 50 lines/varieties in test crosses, Mahsuri and IR21820-38-2 were identified as effective R lines (Pradhan and Jachuck 1995). We hope that the pollen sterility of these wild species of rice will be more stable than the WA-CMS system as in IR62829A and IR58025A. More than 100 other breeding lines have been used to produce new CMS lines, some of which are in the BC_3F_1 generation.

Developing restorer lines

Breeding lines and cultivars developed locally were crossed with ZS97A, V20A, and/or MR83A. Setanjung, AYT 43, YKK 52, MR 8, 12, 13, 14, 15, 16, 18, 23, 26, 27, 31, 32, 43, 54, 55, 67, 68, 70, 72, 82, 87, and 88, Y 615, 635, 833, 837, 839, 840, 841, 860, 866, and 869 were identified as strong restorers (Mohamed et al 1987). Other important R lines included MR 71 and 81, Y 961 and 964, Seberang, Mahsuri Mutant, Malinja, Mahsuri, Ria, Bahagia, Murni, and Jaya SM II. More than 130 strong restorer lines have been identified. Many varieties and breeding lines that originated in China, Korea, and Japan were introduced recently to increase the genetic diversity of R lines.

Multiplication and hybrid seed production

The seed yields in male sterile seed multiplication of IR62829A and IR58025A showed seasonal variation from 1.32 to 1.56 t ha^{-1} during the 1990-91 main season; the seed yield was only 0.20 t ha^{-1} in the 1990 off-season (Guok 1994). Attempts are being made to produce male sterile and hybrid seeds using isolated plots and newly developed local CMS lines MH 805A, 813A, 821A, and 841A.

Problems of and future outlook for hybrid rice in Malaysia

The major constraint to the development and adoption of hybrid rice technology in Malaysia is the dearth of stable CMS lines. Contrary to initial expectations, breeding a stable CMS system for use proved to be as difficult as in Indonesia (Trinh 1994). Although four new pairs of CMS lines—MH 805A, 813A, 821A, and 841A—have been developed, they must be evaluated critically for pollen sterility over seasons and for outcrossing potential. There is also a need to develop CMS lines based on other male sterility-inducing cytoplasm such as IR66707A, *O. rufipogon,* and *O. nivara* (McWilliam et al 1996).

To fetch a premium price for the rice produced, long-grain hybrid rice varieties should be developed and evaluated. The specific requirement that both parents have long grains (Khush et al 1988), however, severely limits the choice of parents available in making heterotic hybrids.

Hybrid rice produces a 20–40% yield advantage over conventionally bred varieties in Malaysia. When hybrid rice was planted under special conditions, such as in

deep muddy soil, saline alkaline fields, and fields soaked with cold underground water, the yield increase exceeded 100% compared with conventional varieties (Singh 1988). Such highly heterotic hybrids need to be identified to meet location-specific requirements in Malaysia.

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CHAPTER 28

Developing intersubspecific hybrid rice in the DPR of Korea

Lee Ul Byong and Mun Jong Won

The commercial cultivation of hybrid rice is currently restricted to areas south of 35°N latitude in the DPR of Korea. This suggests that prevalent unfavorable conditions in high-latitude areas limit the exploitation of hybrid rice. Yield of an inbred variety in the DPR of Korea is high. Yield of hybrids must be 30% more than that of an inbred variety to use hybrid rice for commercial production in the country. Yield of hybrid rice seed produced via natural outcrossing must also be more than 1.5–2.0 t ha^{-1}. To achieve these targets, development of intersubspecific hybrid rice began in 1983. Research has focused on solving problems such as nuclear sterility, lodging caused by transgressive heterosis for height, cold damage, poor grain quality, faster senescence in the F_1 generation, and low seed yield. Results are reported in this chapter.

Testing newly bred CMS lines

The development of, testing of, and breeding procedure for cytoplasmic male sterile (CMS) lines have been reported earlier (Lee and Kim 199 1). All cultivars in Korea are grown at high latitude and are maintainers for boro-type sterile cytoplasm. To increase the outcrossing rate, the character of stigma exsertion commonly found in wild rice was transferred to CMS lines. To obtain semidwarf intersubspecific (japonica/indica) hybrids possessing resistance to lodging, CMS lines with stigma exsertion were bred (Table 1) through the introduction of the *sd-1* gene from indicas.

The optimum heading time for rice varieties is around 15 August in the southern region with the longest period of growth in Korea. The heading time of newly bred CMS lines ranged from 29 July to 10 August, which is ideally suited to all regions. The culm length of these CMS lines was 45–60 cm, whereas that of conventional variety Pyongyang 15 was 74 cm. The rate of stigma exsertion in the newly bred CMS lines varied between 33% and 50%.

Table 1. Flowering date, plant height, and stigma exsertion in some Korean cytoplasmic male sterile and maintainer lines.

Line[a]	Heading (day-month)	Culm length (cm)	Rate of stigma exsertion (%)
129A	5-VIII	46.3	42.3
129B	5-VIII	47.7	40.0
237A	5-VIII	43.9	51.9
237B	5-VIII	44.0	50.4
120-2A	31-VII	56.1	40.6
120-2B	31-VII	62.5	33.6
133-20A	29-VII	46.1	46.3
133-2B	29-VII	50.4	38.4
150-6A	10-VIII	46.8	42.5
150-6B	10-VIII	50.0	40.7
Pyongyang 15	1-VIII	74.0	6.2

[a] A = sterile line, B = maintainer. Planting date was 29-III, transplanting took place 15-VI.

Table 2. Seed fertility in F_1 in test for WC gene in newly developed Korean sterile lines.

Korean × indica combination	Total grains panicle^{-1} (no.)	Filled grains panicle^{-1} (no.)	Fertility rate (%)
129A × M-23 [a]	139	109	79
237A × M-23	179	142	80
120-2A × M-23	132	100	76
133-20A × M-23	119	90	76
150-6A × M-23	152	140	93
Pyongyang 15 × M-23	113	76	67

[a] M-23 = indica tester with fertility-restoring gene.

To overcome problems of japonica/indica hybrid sterility, the wide compatibility (WC) gene must be used to develop intersubspecific hybrids (Ikehashi and Araki 1984, 1987, Ikehashi et al 1994). The WC gene *S-5^n* is located in chromosome 6 (Ikehashi et al 1991). Lee and Kim (1991) bred maintainer lines with the WC gene and subsequently developed CMS lines through the method of nuclear substitution using these maintainers. The maintainer lines were tested for their allelic constitution (*j* or *i*) at the *S5* locus. Only the japonica maintainer lines possessing the *S5^i* allele were used to breed CMS lines.

In Korea, the seed setting rate in all conventional and general cultivars is about 85%. The rate of seed fertility tends to decrease if there is an increase in the number of grains panicle^{-1}. As a result of the increased number of grains panicle^{-1} in hybrid F_1, the general limit for seed fertility is about 75% (Table 2). Despite recording more grains panicle^{-1}, the seed setting rate in Pyongyang 15 × M-23 (check) was only 67%, mainly because of the absence of the WC gene.

Developing restorer lines

The male pollen parent in the intersubspecific hybrid combinations can be used directly after testing for the fertility-restoring gene on indica rice varieties already bred in the low-latitude tropics and subtropics (Yuan 1992). Most varieties grown in the temperate northern zone, however, do not possess the restorer gene for the CMS-Bo system. The restorer line must definitely be developed in the temperate northern zone with adaptability to high latitude (Yang et al 1989).

To develop intersubspecific hybrid combinations, the choice of the restorer line is important. The restorer must be an indica when using japonica or a tropical japonica sterile line, and it must be japonica or tropical japonica when using an indica sterile line (Khush and Aquino 1994). When using pure japonica and indica varieties for intersubspecific combinations, the WC gene has to be introduced to one of the parents (Ikehashi and Araki 1987). The proper choice of breeding materials such as IR38 for use as restorer lines would solve problems of nuclear sterility, shattering dominance, poor grain quality, and susceptibility to blast. IR38 is characterized by bold grains that are not easily shattered, and it is resistant to blast. The main factor determining the purity of the sterile line is the stability of sterile cytoplasm (Mao 1988). The sterile line must maintain total sterility even under different environmental conditions (Yuan 1994, Virmani 1992). The selfed seed set in the sterile plant in isolated conditions reduces the purity of the sterile line in multiplication plots. This selfed seed set also reduces the purity of hybrid seed production (Mao 1988).

The new CMS-Bo-type male sterile lines were found to set 0–0.5% seed even in an isolated condition (Table 3). This seed was progeny-tested to identify the effects of self-pollinated seeds on male sterile lines in the F_1 seed production plots. The self-pollinated seeds on CMS-Bo lines gave more or less male sterile plants (Table 4). Thus, the main drawback in this male sterile cytoplasm is the production of self-pollinated seeds on the sterile line. This problem can be overcome by clearly defining geographical areas where these male sterile lines show complete male sterility to produce pure hybrid rice seeds.

Table 3. Stability of sterility in CMS-Bo-type sterile lines at the Rice Research Institute, Korea, 1992.

Male sterile line	Grains (no.)		Sterility rate (%)	Selfed seed (no.)	Selfed seed rate (%)
	Total	Sterile			
129A	1,723	1,715	99.55	8	0.46
237A	2,996	2,987	99.70	9	0.30
152A	2,030	2,021	99.56	9	0.40
101A	2,890	2,890	100.00	0	0.00
131A	3,168	3,166	99.94	2	0.06
141A	3,704	3,703	99.97	1	0.03

Table 4. Test of self-pollinated seed set in boro-type male sterile lines at the Rice Research Institute, Korea, 1992.

Male sterile line	Grains (no.)		Sterility rate (%)	Selfed seed (no.)	Selfed seed rate (%)
	Total	Sterile			
237A	2,702	2,698	99.80	4	0.15
152A	3,189	3,186	99.99	3	0.09
131A	2,915	2,913	99.90	3	0.10

Table 5. Seed fertility in F_1 of R90 combination at the Rice Research Institute, Korea, 1992.

Female	Male	Cross combination	Seed fertility in F_1 (%)
R90	Korean	R90 × 1361-4-1	>80
R90	Korean	R90 × 1129-2	>80
R90	Korean	R90 × 1361-4-1	>80
R90	Korean	R90 × 1371-2	>80
R90	Korean	R90 × 667	>80
R90	Korean	R90 × 1129-2	>80
Korean	R90	Pyongyang 15 × R90	>80
Korean	R90	9181-8-1 × R 90	>80
Korean	R90	9508-6-1 × R 90	>80
Indica	R90	IR29 × R90	23
Indica	R90	D-23 × R90	47
R90	indica	R90 × IR29	22
R90	indica	R90 × D-23	45

The following breeding strategies can also overcome this problem:

- Selecting of a restorer line with high compatibility to pollinate male sterile line.
- Variation in the degree of fertilizing compatibility among restorer lines: japonica > tropical japonica > indica.
- Selecting a restorer line with stronger pollen viability and germination rate than the maintainer of the specific cross combinations. Pollen viability of the restorer line was generally stronger than the viability recorded in the maintainer line.
- Obtaining synchronized heading and flowering time of the sterile line and the restorer line.

We have developed nearly 300 CMS-Bo-type male sterile lines and are evaluating them for their natural outcrossing ability and combination ability to produce desired hybrids.

Analysis of a group of lines such as IR38 indicated that these compose a distinct third type (Pak CH, unpublished) belonging to neither pure indica nor japonica. The newly bred R90 is a restorer line developed from the cross IR38 × Hyangdo (Fig. 1). To study the effect of japonica or indica germplasm on seed fertility of F_1 combinations with R90, reciprocal crosses were made with japonica and indica varieties (Table 5). Seed fertility reached normal levels (more than 80%) when R90 was crossed with

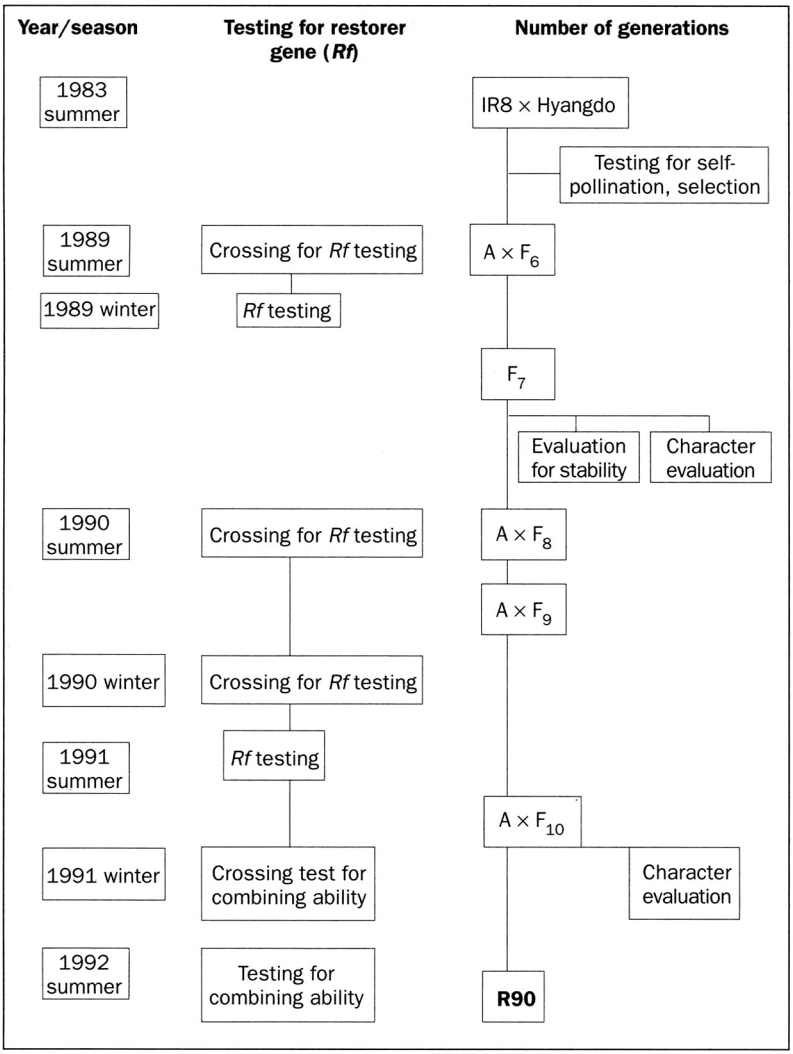

Fig. 1. Scheme used for breeding the R90 restorer.

a Korean variety in reciprocal crosses, but sterility occurred in all combinations when the F_1 involved an indica rice variety. These results confirm that, like IR38, R90 cannot be classified as either pure japonica or indica.

Tests were also made to determine the restoration ability of R90 with Korean male sterile lines. In most combinations, R90 improved the seed fertility of Korean sterile lines to more than 75% (Table 6).

Table 6. Test for restorer gene in R90.

Korean sterile line and R90 combination	Total grains panicle^{-1} (no.)	Filled grains panicle^{-1} (no.)	Fertility rate (%)
154A × R90	277	211	76.1
122-5A × R9O	205	157	76.5
133-24A × R90	184	147	79.8
222-2A × R90	169	140	82.5
120A × R90	179	132	73.7
82A × R90	180	128	71.0
135-24A × R90	178	136	82.7
151-4A × R90	156	123	78.8
152-8A × R90	137	106	77.3

Table 7. Yield components and other characters in rice hybrids at the Rice Research Institute, Korea, 1992.

Trait/characteristic [a]	Rice hybrids		Variety
	129A x R90	237A x R90	Pyongyang 35
Heading	12-VIII	17-VIII	17-VIII
Resistance to blast	R	R	R
Resistance to bacterial blight	M	M	M
Tolerance of cold	R	R	R
Resistance to shattering	R	R	R
Culm length	84.0	83.0	73.7
Resistance to lodging	R	R	R
Panicles plant^{-1} (no.)	10.1	10.3	11.3
Grains panicle^{-1} (no.)	163.1	163.4	101.1
Filled grains (no.)	121.6	130.1	84.5
Fertility rate (%)	74.6	79.6	83.6
1,000-grain weight (g)	34.0	32.6	29.5
Amylose content (%)	16.0	17.7	21.7
Senescence	Early	Early	Late

[a] Sowing date was 14-IV, transplanting date 18-V. N fertilizer was 170 kg ha^{-1}. Density was 100 plants 3.3 m^{-2}. R = resistant, M = moderate.

The value of male sterile and restorer lines can be determined from the performance of the A × R crosses. Results (Table 7) from two cross combinations of A × R90 showed nearly normal seed fertility, which indicated that these crosses performed better than the check variety Pyongyang 35 for yield components (e.g., number of filled grains and 1,000-grain weight). Culm length in the F_1 generation was only slightly more than that of the parents. Although the female parent was a Korean type and the male an indica, tolerance of cold was dominant in the F_1 generation. Therefore, the progeny can be grown safely in the high latitude of the northern temperate zone. Amylose content ranging from 15% to 18% in Korean cultivars is the main criterion for evaluating rice quality. In the F_1, amylose content ranged from 16% to 18%.

The shattering characteristic in the intersubspecific F_1 hybrid was easily solved by breeding for nonshattering female and male parents. These results suggest prospects for developing intersubspecific rice hybrids in the DPR of Korea. Information on outcrossing rate and seed yield of CMS lines is being collected.

Conclusions

The basic problems in developing intersubspecific hybrids are intervarietal hybrid sterility, lodging susceptibility because of increased culm length in intersubspecific crosses, cold damage, poor rice grain quality, and the dominance of grain shattering in the F_1 generation. All these problems have been solved as discussed.

Before these intersubspecific hybrids are used for commercial production, they need to be improved for early senescence of hybrid combinations, resistance to bacterial blight, natural outcrossing rate (>30%) in male sterile plants, and seed yield in hybrid seed production plots.

Efficiency of breeding intersubspecific hybrids can also be improved by using two-line hybrids and by fixing heterosis through apomixis.

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CHAPTER 29

Research and development for hybrid rice technology in Egypt

A.O. Bastawisi, I.R. Aidy, H.F. El-Mowafy, and M.A. Maximos

Research and development for hybrid rice in Egypt passed through two main stages: the evaluation of hybrids for heterosis, and the transfer of cytoplasmic male sterility and restoring ability to Egyptian rice. Starting in 1986, materials evaluated, which included hybrid varieties provided by the International Rice Research Institute and hybrid seed companies, were either comparable or inferior to local high-yielding varieties. Only in 1995 did some hybrids show standard heterosis of 5% to 16%. The transfer of the cytoplasmic male sterility (CMS) factor(s) and restoring-ability gene(s) to Egyptian rice began in 1995. Using 15 CMS lines received from IRRI, local varieties, and elite lines, 132 test crosses were made. Another 49 crosses were made to transfer the restoring-ability gene(s) to Egyptian varieties. Six thermosensitive genic male sterile lines were sown on two different dates to evaluate percentage sterility in Upper Egypt (New Valley). There the average temperature is above 30 °C during the reproductive stage. This two-line method for producing hybrid rice, if successful, might be used. Although the productivity in inbred rice is very high, some hybrids may produce still higher yields in Egypt.

Rice is grown on more than half a million hectares in Egypt. Productivity is very high, with an average yield of 8.2 t ha^{-1}. Rice is the second staple food after wheat. Rice is highly important in Egypt for farmers, because it provides high profits, and for consumers, because it is their cheapest food.

Following the release of variety Giza 175 in 1990, rice productivity in Egypt increased quickly and reached a plateau. Breeders made several improvements through the breeding program. They improved grain quality (Giza 178), shortened growth duration by 1 mo (Giza 177, 125 d), and incorporated and maintained a high level of resistance to diseases (blast and brown spot) and insects (leaf miner and stem borer). They also developed suitable production practices and widely disseminated the production technology to derive the maximum benefit from the high-yielding varieties.

This enabled agronomists and extension workers to narrow the gap between potential yield and national yield. To move the yield plateau in rice productivity to a higher level, one option is to develop super rice and hybrid rice, with which a 15-30% yield increase can be achieved (Khush and Aquino 1994, Virmani 1994, Yuan 1994).

Hybrid rice research in Egypt started in 1981-82. Cytoplasmic male sterile (CMS) lines were tested with Egyptian cultivars under both greenhouse and field conditions. Results showed that japonica cultivars had poor restoring ability (Maximos and Aidy 1994). Evaluation of hybrids began in 1986 and the hybrid rice breeding program started in 1995. This chapter summarizes the current status of research and development for hybrid rice technology in Egypt.

Evaluating F_1 hybrids

Several F_1 hybrids were introduced from IRRI and private seed companies starting in 1986 and were evaluated in Egypt. Until 1993, all F_1 hybrids showed yields comparable or inferior to those of high-yielding commercial inbred varieties, except in 1986, when the best check was an old commercial variety, Giza 171 (Table 1). Negative standard heterosis ranging from -2% to -23% in the hybrids was mainly due to their poor adaptability to Egypt's environment.

Since 1995, two more experiments have been conducted to evaluate some IRRI hybrids. The first experiment included six hybrids and seven local checks (Table 2). Only two hybrids showed similarity in yield to the best local checks. In addition, all the hybrids were tall in stature and matured late. The second experiment, received from IRRI through the INGER (International Network for Genetic Evaluation of Rice) program, was the second International Rice Hybrid Observational Nursery (IRHON). In all, 29 hybrids, 3 maintainers, and 26 restorers were studied in two sets using a Latin square design. The hybrids were compared with four international varieties and four local checks. The best hybrids showed a standard heterosis ranging from 5% to 16% (Table 3). In these tests, some hybrids, especially those with a short maturity duration, showed better adaptation.

Table 1. Yield performance and standard heterosis of the best F_1 hybrids tested in Egypt from 1986 to 1993.

Hybrids tested	Year	Yield (t ha^{-1})	Standard heterosis (%)	Best check
V20A/Milyang 54R	1986	10.5	13	Giza 171
Liming/4811 (seed company)	1987-90	10.3	-2	Giza 176
Chang Fei-22A/T230 (seed company)	1987-90	10.1	-4	Giza 176
IR64611H	1990	9.7	-5	Giza 181
IR58025A/IR66R	1991	7.3	-23	Giza 176
IR58025A/IR29723-143-3-2-IR	1993	7.8	-16	Giza 175

Table 2. Yield performance and ancillary characters of F_1 hybrids tested at the Rice Research Training Center, Sakha, Egypt, 1995.

Entry	Yield[a] (t ha^{-1})	Heading (d)	Height (cm)	Panicles m^{-2}	Blast reaction[b]		Grain test		Translucence[c] (score)
					Leaf	Neck	Grain type	Milling (%)	
IR69691H	6.90 c	138	121	440	2	R	Long	67.0	3
IR71116H	6.99 c	137	113	440	2	R	Long	50.2	7
IR71092H	9.88 a	122	113	320	2	R	Long	69.4	3
IR69692H	10.22 a	121	107	380	1	R	Long	69.2	3
IR71105H	8.25 b	127	101	280	1	R	Long	70.7	3
IR58025A/IR52774-B-B-6	7.60 bc	129	107	480	1	R	Long	71.0	3
Giza 171 (late maturing)	8.45 b	126	133	420	5	S	Short	72.2	3
Giza 176 (medium maturing)	10.24 a	116	101	484	6	S	Short	69.9	3
Giza 177 (early maturing)	9.66 a	96	98	440	2	R	Short	75.4	3
Giza 178 (medium maturing)	10.48 a	100	90	420	1	R	Short	71.9	3
Giza 181 (medium maturing)	9.87 a	119	92	320	1	R	Long	70.4	3
Gz 4596-3-4 (medium maturing)	10.56 a	108	87	382	1	R	Short	73.0	3
Gz 5379-22-2 (early maturing)	9.97 a	91	102	420	2	R	Short	73.0	3

[a] Numbers followed by a common letter are not significantly different according to Duncan's multiple range test. [b]On a 1–9 scale, where 1 = resistant and 9 = susceptible. R = resistant, S = susceptible. [c] On a 1–9 scale, where 1 = translucent and 9 = opaque.

Table 3. Yield performance, ancillary characters, and standard heterosis of the new tested F_1 hybrids compared with the best hybrids, international checks, and national checks evaluated at the Rice Research Training Center, Sakha, Egypt, 1995.

Entry	Yield[a] (t ha^{-1})	Heading (d)	Height (cm)	Panicles m^{-2}	Phenotypic acceptability[b]	Spikelet sterility (%)	Panicle weight (g)	Leaf blast[c]	Stem borer[d]	Standard heterosis (%)
New hybrids										
IR64619H	9.80	88	103	460	5.0	22	5.10	2	3	16
IR70396H	9.00	94	98	450	3.0	44	4.50	2	3	7
IR70397H	8.80	90	95	550	5.0	25	3.00	2	3	5
IR70409H	8.80	100	91	540	5.0	19	4.00	2	3	5
IR70410H	8.80	98	86	330	3.0	29	3.50	2	3	5
Hybrid checks										
IR64616H	8.40 a	96	93	533	4.6	44	2.54	2	3	
IR68284H	7.32 abc	101	98	487	5.8	24	4.50	2	3	
International checks										
IR50	7.00 cd	95	83	500	5.0	20	2.60	2	3	
IR72	8.30 abc	100	83	496	6.6	28	3.04	2	3	
PSBRC-2[e]	7.20 abcd	114	82	535	7.0	23	2.65	2	3	
IR42[e]										
Local checks										
Giza 177	7.80 ab	87	93	442	3.0	6	3.24	2	3	
Giza 178	8.42 a	94	84	485	3.0	12	4.04	2	3	
Giza 181	7.92 a	100	88	496	3.0	17	4.16	2	3	
Giza 171	6.88 abcd	97	123	368	3.0	15	4.26	4	3	

[a]In a column, means followed by the same letter are not significantly different according to Duncan's multiple range test. [b]On a 1–9 scale, where 1 = excellent and 9 = unacceptable. [c]On a scale of 0–9, where 0 = no lesions observed, 2 = small roundish to slightly elongated, necrotic gray spots, about 1–2mm in diameter, with a distinct brown margin; lesions are mostly found on the lower leaves, 4 = typical susceptible blast lesions 3 mm or longer, infecting less than 4% leaf area, 9 = more than 75% leaf area affected. [d]On a scale of 0–9, where 0 = no damage, 3 = 11-20% damage, 9 = 61% and above damage. [e]No data available because it did not flower.

Table 4. CMS lines provided by IRRI in 1995 and number of their maintainers, restorers, and test crosses planted at RRTC, Sakha, Egypt, 1996.

CMS lines	Maintainers (no.)	Restorers (no.)	Test crosses (no.)
Auto A	1	3	10
D2 97A	1	2	14
G 46A	1	2	8
IR67701A	1	–	13
IR68273A	1	–	5
IR68276A	1	–	10
IR68277A	1	–	13
IR68283A	1	–	7
IR68884A	1	–	5
IR68889A	1	–	–
IR79960A	1	2	5
IR70964A	1	1	3
Large stigma A	1	–	8
Pragathi A	1	2	3
Reimei A	1	–	18
Total	15	12	122

Hybrid rice breeding

CMS lines, maintainers, and restorers

In 1995, Egyptian scientists selected 15 CMS lines mainly possessing short grains from the IRRI nursery. All the CMS lines except one showed complete pollen sterility and no seed setting under bagged conditions. Only CMS line IR68273A showed 3% pollen fertility and 7% seed set during the two seasons in 1995-96.

Maintainers and restorers have been identified for these CMS lines (Table 4). In all, 132 test crosses were made with Egyptian rice varieties. Some local varieties, such as Giza 175 and Giza 178, showed good restoring ability because of the involvement of indica and japonica varieties in their parentage. The poor restoring ability observed in 1982, however, was caused by the use of Egyptian japonica varieties (Maximos and Aidy 1994). Recent results showed that some Egyptian japonica varieties and promising lines such as Giza 176, Giza 177, and GZ 5379-22-2 could be used as maintainers by transferring CMS factors. Forty-nine crosses were made in 1995 to transfer the restoring-ability gene(s) to local varieties.

Thermosensitive genic male sterility (TGMS)

Five TGMS indica-type lines were introduced from IRRI. Norin PL 12 from Japan was also introduced in Egypt. In 1995, the seeds of these six TGMS lines were increased at Sakha. They are now being evaluated in Upper Egypt in the New Valley, where the average daily temperature during summertime surpasses 30 ºC.

Future plans

Egypt is a unique place where both indica and japonica types can perform well. Some indica varieties, such as Giza 181 (IR1626-203) and IR25571-31, have attained grain yields of 13 t ha^{-1} in some national replicated yield trials. Our recent studies have shown that early maturing F_1 hybrids from IRRI also perform well under Egyptian conditions. Further evaluation of IRRI F_1 hybrids, especially the early maturing ones, may be rewarding. Attempts to transfer the CMS factor(s) and restoring-ability gene(s) and wide compatibility gene(s) to Egyptian varieties will also be continued. If the current evaluation of TGMS lines in the New Valley proves effective, the TGMS gene(s) will be transferred to local materials. Further research would involve conserving yield vigor in F_1 hybrids through the anther culture technique and improving production practices to maximize hybrid rice seed production.

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CHAPTER 30

Research on hybrid rice technology in the United States

D.J. Mackill and J.N. Rutger

The high cost of seed production as well as poor grain quality have hindered the commercial application of hybrid rice in the United States. Development of U.S.-adapted rice hybrids is carried out in the private sector. Research by USDA–ARS has focused on characterizing germplasm for genetic diversity and developing genetic mechanisms of hybrid seed production. U.S. cultivars have been classified via RAPD and AFLP markers. The maximum genetic diversity of U.S. cultivars occurs between long-grain (tropical japonica) and short/medium-grain (temperate japonica) cultivars. In the relatively cool environment of California, hybrids between temperate and tropical japonicas may be appropriate. In the southern U.S., where indica cultivars are adapted, indica-japonica hybrids may be feasible. Cytoplasmic male sterility, restorer genes, and wide compatibility are being transferred into California cultivars so that the potential for various hybrid combinations can be evaluated. Photoperiod-sensitive genetic male sterile (PGMS) mutants are being sought in U.S. cultivars. In addition, a program is being planned to transfer apomixis from *Pennisetum* species into rice by molecular techniques.

Despite its promise for increasing yields, hybrid rice is not currently grown commercially in the United States because of the high cost of seed production as well as the poor grain quality of high-yielding hybrids. Breeding efforts for hybrid rice are conducted in the private sector. Research conducted by United States Department of Agriculture–Agricultural Research Service (USDA–ARS) scientists focuses on genetic studies and developing mechanisms for hybrid seed production. Research progress on hybrid rice technology in the U.S. has been reviewed (Mackill and Rutger 1994). This chapter considers progress in hybrid rice research since 1992.

Genetic diversity heterosis of US. cultivars

Few studies have been conducted on heterosis under U.S. growing conditions (Davis and Rutger 1976, Gravois and McNew 1993). These reports indicated significant heterosis for grain yield among U.S. cultivars, although heterosis for milling yields was essentially zero in one study (Gravois 1994). These experiments were not conducted using realistic procedures for yield trials under direct-seeded culture. Accurate estimates of yield potential would require large enough F_1 seed samples to grow replicated, direct-seeded plots.

It is generally recognized that heterosis depends on the use of two genetically divergent parents. Molecular marker diversity has been used to estimate genotypic variation in rice (Wang and Tanksley 1989). Studies on the relationship between molecular marker diversity and heterosis in rice have yielded mixed results (Kato et al 1994, Zhang et al 1994, 1995, Xiao et al 1996). It appears, however, that heterosis should be related to molecular marker diversity, because it has been shown that the wider the cross, the higher the level of heterosis. For example, Yuan (1994) reported that the level of heterosis in various combinations followed the pattern indica/japonica > indica/javanica > japonica/javanica > indica/indica > japonica/japonica. This follows the same trend as molecular marker diversity.

Both random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers have been used to classify U.S. rice cultivars into groups (Mackill 1995, Mackill et al 1996). The results showed that all commercial U.S. cultivars evaluated belonged to the japonica subspecies, with the short- and medium-grain cultivars classified as temperate japonica and the long-grain cultivars as tropical japonica. The short-/medium- and long-grain classes therefore correspond to the level of maximum genetic diversity among U.S. cultivars. This genetic divergence between temperate and tropical japonicas does not appear to be distributed randomly throughout the genome. In a genetic map constructed in a temperate-tropical japonica cross, polymorphic RAPD markers were highly clustered on chromosomes 10 and 11, but were relatively sparse on chromosomes 1 and 2 (Redoña and Mackill 1996b).

Genetic diversity, and consequently heterosis, has been thought to be lower within japonica cultivars than within indicas. Maximum heterosis within japonica rice, however, would likely be achieved through a temperate × tropical cross. This may be an appropriate objective for the cooler California environment. Both tropical and temperate japonica cultivars have superior cold tolerance to indica cultivars (Mackill and Lei 1997). Although the tropical japonicas are more prone to shattering and have poor panicle exsertion, they surpass indica cultivars in these traits (Mackill and Lei 1997). One potential negative factor of temperate × tropical hybrids may be unacceptable grain quality: the medium- and long-grain cultivars are extremely different in grain characteristics, and a hybrid between the two would probably not meet the requirements for any of the normal market types.

Indica cultivars can be grown successfully and are highly productive in the southern U.S. But they lack the grain quality and high milling yields of tropical japonicas. Nevertheless, many indicas have a grain type similar to that of the southern long-

grain cultivars; therefore, indica-japonica hybrids would probably be feasible for the southern U.S. Fortunately, many of the U.S. long-grain types can be classified as wide compatible, and their hybrids with indica types should be fertile. Grain quality is still likely to be a problem, with the emphasis on very high milling recovery.

Gene mapping studies have identified quantitative trait loci (QTL) that are associated with yield components. One report suggested that heterosis was due to dominance effects and not overdominance (Xiao et al 1995), but there have been reports of QTL that appear to show overdominant action (Nair et al 1995). We have identified such loci for seedling vigor traits in both an indica-japonica cross (Redoña and Mackill 1996a) and a tropical-temperate japonica cross (Redoña and Mackill 1996b). Seedling vigor traits have been related to the superior performance of hybrid rice (Akita et al 1990).

Developing male sterile lines

Cytoplasmic male sterility

Commercial interest in hybrid rice has largely focused on the southern U.S. and the three-line method is well established in long-grain cultivars. The CMS and R lines required for the three-line method have not been previously developed in California cultivars. The CMS trait has recently been transferred from IRRI indica lines into California medium-grain lines, which are maintainer lines. Because R genes are not present in these materials, they are also being introduced from indica sources. Heterosis would probably be too low in hybrids within the medium-grain gene pool, so the R sources are being crossed mostly with long-grain cultivars. By using just one or two backcrosses, we hope to retain enough genetic diversity for higher heterosis.

Photoperiod-sensitive genetic male steriles

Because temperatures during the reproductive phase fluctuate widely in U.S. rice-growing regions, the two-line system has emphasized photoperiod-sensitive genetic male sterility (PGMS). Early attempts to develop PGMS mutants in California rice were documented by Oard et al (1991) and Rutger and Schaeffer (1994). The PGMS mutants previously isolated from California germplasm have not shown optimum sterility patterns for hybrid seed production. The mutant PI543851 (Rutger and Schaeffer 1994), for example, shows fairly high pollen and spikelet fertility even under long-day conditions (S.A. Han, unpublished data). Current efforts seek to isolate new mutants in both a California medium-grain and southern long-grain background.

In California, seeding rates of 200 kg ha^{-1} or more in farmers' fields ensure that most panicles are borne on plants with one or two culms, and that the number of plants m^{-2} is very high (>500). It is possible for one person to observe several million plants in a day by walking through commercial rice fields. Male sterile panicles are easily recognized because they remain erect during grain filling. In 3 d of work, four workers isolated more than 600 putative male sterile mutants in 1994. Seed from the male sterile panicles was harvested for 1995 field plantings, and the plants were brought

to the greenhouse and reestablished in pots. Out of the 1995 plantings from seed of the male sterile panicles, approximately half of these lines were highly sterile, indicating that they were probably "leaky" steriles (i.e., seeds on the sterile plants were selfed, and the plants were not completely male sterile). Most of the plants that gave fertile progeny rows in the 1995 field plantings maintained their sterility upon replanting, indicating that they were true genetic steriles. From this large collection of mutants, we have identified a few that are potentially PGMS, and these are being tested further (S.A. Han, unpublished data).

In Arkansas, male steriles were sought in M_2 populations of southern U.S. varieties. Panicles from random M_1 plants, grown from seeds irradiated at 20 krad, were planted panicle-to-row in the 1995 Arkansas summer nursery (34°N, long days). Among some 5,000 M_2 rows, 141 were observed to segregate for sterility in an apparently recessive fashion, in which the sterile plants showed at least some seed set, presumed to be from outcrossing. Rows in which the steriles were completely barren were ignored because such steriles were not likely to have value for outcrossing studies. One panicle from each of at least four fertile plants was planted panicle-to-row in the Puerto Rico winter nursery (18°N, short days). These four presumably were homozygous-fertile and heterozygous-fertile. In a sample of four plants in such a population, the probability of recovering at least one heterozygous plant, which would show progeny segregation for sterility, was $1 - (1/3)^4 = 0.98$.

Forty-one of the 141 lines failed to show segregation for sterility in the Puerto Rico winter nursery, the desired reaction for PGMS types. Eleven panicles were taken from each of four rows of the 41 lines that did not segregate. Under the PGMS hypothesis, progeny tests of 11 plants, which presumably represented segregation for 1/4 homozygous-fertile: 1/2 heterozygous-fertile: 1/4 homozygous-sterile, were conducted in the 1996 Arkansas summer nursery by planting 1 panicle $hill^{-1}$. With a sample of 11 plants from such a population, the probability of observing segregation for sterility in these progenies was $1 - (1/4)^{11}$ (i.e., approximately 1). The highest evidence of PGMS would be segregation of the all-sterile hills ($P = 1 - (3/4)^{11} = 0.04$).

In the 1996 progeny tests. only one line segregated for homozygous-sterile hills—line 1388, which segregated 5 fertile hills: 4 segregating hills: 2 sterile hills ($0.25 < P$ 1:2:1 < 0.50). Appropriate progeny tests of line 1388 under short days and long days are under way.

Apomixis

In California in 1985, a search began for apomixis in rice (Rutger et al 1986). This search involved screening for aberrant segregation ratios in hybrid populations, embryo-sac screening of 547 A-genome entries of weedy *Oryza* species, and attempted rice × apomictic *Pennisetum* hybridization, but it failed to confirm the existence of apomixis in rice (Rutger 1992).

In Arkansas, the junior author is restarting the apomixis search, with short-term plans to again attempt rice × apomictic *Pennisetum* hybridization, and long-term plans involving molecular genetic techniques.

Conclusions

Interest in developing hybrid rice in the commercial sector remains strong and advanced hybrids are undergoing intense evaluation in the southern U.S. The success of hybrid rice in other countries has stimulated interest from public-sector breeding programs, which are responsible for the vast majority of commercially grown cultivars. Research on genetic approaches to improving hybrid rice will continue. Despite many obstacles, the most serious of which are the economics of seed production and the need for premium cooking quality and high milling recovery, hybrid rice technology is seen as one of the most promising approaches for significantly raising U.S. rice yields.

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CHAPTER 31

Developing hybrid rice in Brazil: methodology, highlights, and prospects

E.P. Guimarães, V. dos A. Cutrim, and J.A. Mendonça

EMBRAPA-CNPAF began to explore the prospects for and problems of hybrid rice in 1984 via two areas: the introduction of allogamic traits to develop A and B lines and reciprocal recurrent selection. The methodological steps involve identifying maintainer and restorer lines, introducing allogamic traits into maintainer lines, and transferring the cytoplasmic genetic male sterility system to derived F_4 maintainer lines. Development of line 0461 with allogamic traits and good agronomic behavior and several hybrids is the most significant result. Future plans are directed toward developing an economically viable methodology to produce hybrid seeds, locating partners in the private sector, identifying new cytoplasm sources to produce genetically diverse A lines, and evaluating the economic need to use the allogamic traits.

The hybrid rice program at the Brazilian Enterprise for Agricultural Research–National Research Center for Rice and Beans (EMBRAPA–CNPAF) began in 1984 as a joint project with the Institute for Research in Tropical Agriculture (IRAT, today CIRAD–CA, Centre de coopération internationale en recherche agronomique pour le développement, departement des cultures annuelles). Because the yield plateau experienced with conventional varieties in the irrigated environment turned out to be the main issue, farmers pressured researchers to generate new technology. Farmers expressed their willingness to invest in the rice crop to obtain higher yields. A detailed study indicated that the possibility of success was in developing hybrid rice for the irrigated environment and not for the upland ecosystem.

This hybrid rice project followed Chinese technology. The three-line breeding method involved cytoplasmic genic male sterile lines (A lines). maintainer lines (B lines) to pollinate and produce seeds on A lines carrying the male sterile gene, and restorer lines (R lines) carrying genes to restore fertility in A lines and produce hybrid seed. The two-line method (thermosensitive or photoperiod-sensitive genic male ste-

rility) currently pursued vigorously to develop hybrids may be one alternative that will make hybrid rice more attractive to countries with little or no investment in this technology.

The prospects for developing three-line breeding to produce hybrids and the problems encountered in achieving this goal have been described already (Lin and Yuan 1980, Yuan and Virmani 1988). In countries with an agricultural system different from that of the Chinese, the key point for this technology is the hybrid seed production technique as described by Virmani and Sharma (1993). An important component for obtaining more than 4 t ha^{-1} of hybrid seed is the amount of hand labor required in this process.

As in other countries (Guok 1994, Moon et al 1994), labor-intensive hybrid seed production is a major constraint to the development of the seed industry in Brazil. If practical solutions are not found for this component, the hybrid seed cost will increase to levels that are commercially unaffordable to farmers. Experience in the Philippines demonstrated that if proper adjustments are made in seed production technology, it can provide incentives to seed growers and make hybrid seeds available at a price that farmers can afford (Lara et al 1994).

Thus, the basic idea of this project was to incorporate in hybrid rice development a factor that would reduce hand labor requirements. The way found was to introduce in the cultivated species *Oryza sativa* allogamic traits from the wild species *O. longistaminata;* the targeted trait was large stigma. The project drew inspiration from the French experience (Taillebois 1983). A few lines with the genetic background of cultivated species and allogamic traits were developed and tested in Brazil. The results observed by Taillebois and Guimarães (1988) and Breseghello and Neves (1995) indicated that lines with large stigma ensured higher outcrossing rates than normal lines.

This chapter attempts to describe the strategy adopted by the EMBRAPA–CNPAF/CIRAD–CA project (from 1994 onward EMBRAPA–CNPAF took full responsibility for the project). We also present some results obtained during the period and discuss future prospects.

Methodology

Identifying germplasm with the restoration gene

To identify B lines and R lines, the first step is to introduce or develop locally adapted cytoplasmic male sterile lines (CMS lines). The next step is to evaluate and identify lines that maintain the sterility of the CMS line or restore the fertility in the F_1s. To achieve this goal, it is necessary to screen germplasm from the target area for these genes.

It is important to have an ongoing conventional program in association with the hybrid development project. Advanced breeding lines or lines ready to be released, with resistance genes for diseases and pests, and genes for good grain quality, can be found easily. This will help identify restoration and maintenance ability in test crosses made with the best breeding lines available and the CMS line.

Guimarães et al (unpublished) tested 1,046 lines in this project during 1984 to 1995. Of these, 378 (36%) showed strong restoring ability and 43 (4%) possessed good maintaining capacity. The results clearly demonstrate the difficulty in finding maintainer lines; of the 43 identified, 18 came from EMBRAPA–CNPAF's germplasm screened in 1984. Most of these lines have no agronomically desirable characteristics. In the past three years, the best available breeding lines in the country coming from the National Rice Breeding Network (CTArroz) as an observation nursery have also been evaluated for this purpose.

The major constraint in breeding hybrid rice for Brazil is the low frequency of maintainer lines among Brazilian rice cultivars evaluated. So far, progress has been made in transferring the allogamic trait to some lines and some cytoplasmic male sterile lines have been developed. It may be difficult to find good combinations from the narrow genetic base available in the Brazilian (Rangel et al 1996) and Latin American varieties (Cuevas-Pérez et al 1992) to produce higher heterosis. Therefore, we need to increase the number of maintainer lines to make it possible to develop numerous good A lines. Because hybrids are a product of A and R lines, a larger genetic distance between these lines would result in higher heterosis. The nonavailability of genetically diverse germplasm in Brazil may limit the identification of heterotic rice hybrids.

The two-line hybrid breeding approach may open up new possibilities for achieving the desired heterosis.

Steps to develop A and B lines

Figure 1 shows the germplasm flow chart followed to develop A and B lines in Brazil. Selected maintainer lines were crossed with an allogamous line (#24Z) developed by EMBRAPA–CNPAF and CIRAD–CA from a cross between *O. longistaminata* A. Chev. and *O. sativa*. The most commonly used allogamous line was #2RI (032G-98-1-5-1-1–1), which was obtained from the cross IR13540-56-3-2/#24Z.

Initially, we planned to backcross (BC) the F_1 plants twice with the maintainer. Because only a limited number of plants with allogamic traits were observed in the segregating generations, it was necessary to backcross only once. Besides, linkage drag also resulted in the transfer of several undesirable wild traits with the targeted allogamic characteristics in the segregating populations, which always presented a high degree of grain shattering, lodging, and poor grain type.

The best maintainers were crossed with the allogamous donor and one backcross was made. The BC_1F_1 plants were selfed and the BC_1F_2 seeds were planted at the Palmital experiment station, under irrigated conditions, to exercise selection for allogamic traits. The BC_1F_3 seeds were also planted at the Palmital experiment station, but under rainfed lowland conditions to expose plants to increased disease pressure. In the F_3 generation, selection was targeted not only for allogamic traits but also for other desired agronomic traits.

The BC_1F_4 generation was also planted at the Palmital experiment station under irrigated conditions. At this stage, all plants already exhibited large stigma and selection pressure basically targeted agronomic traits. The best plants were selected to

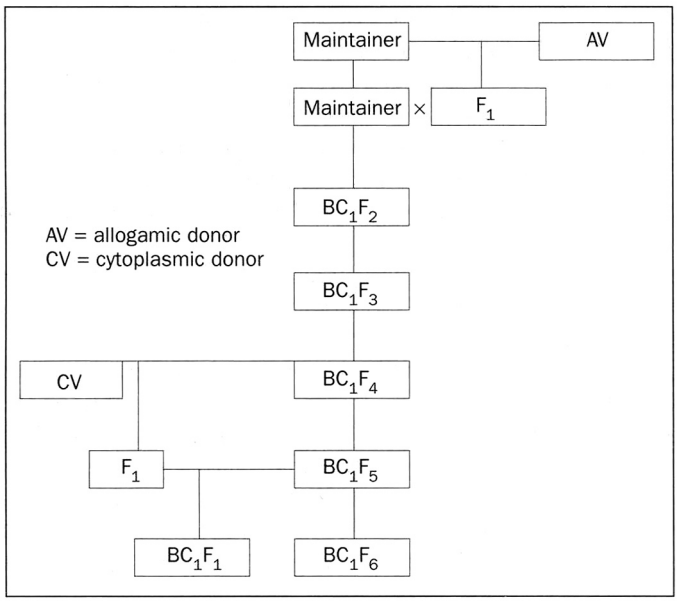

Fig. 1. Flow chart to develop A and B lines to produce hybrid rice in Brazil.

test-cross with a cytoplasmic male sterile donor to begin sterilization of the best maintainer lines. Between 20 and 50 BC_1F_4 plants were chosen and their panicles were clipped at the blooming stage and taken to the Capivara experiment station for hand crossing to cytoplasmic male sterile line Zhen Shan 97A. Recent emphasis, however, has shifted to 046IA, an improved CMS line developed locally.

The procedure involved a large number of backcrosses in each generation to incorporate the male sterile cytoplasm and to develop maintainer lines. The entire process was conducted at the Capivara experiment station under screenhouse conditions. The F_1 plants that originated from the combination of BC_1F_4 and the male sterile cytoplasm source were backcrossed to the BC_1F_5 (Fig. 1). The last backcross expression used in reality differed from the textbook meaning as the F_1s were combined to select BC_1F_5 plants. These plants were chosen and selfed when the BC_1F_4 generation was used to combine with the cytoplasmic donor, resulting in a similar genetic content. This backcross procedure was repeated several times (F_1s were crossed to BC_1F_6, the product was crossed to BC_1F_7, etc.). This step was repeated five or six times. Completely sterile plants were obtained from the backcrosses (A line) and plants with the capacity to maintain sterility were produced from the selfed generations (B line).

All this work was quite tedious. During each backcross generation, a large number of backcross progenies and plants in the selected progenies were discarded on the basis of absence of allogamic traits, a continuous high level of segregation for fertility in A line development, instability of sterility under different environmental conditions, and poor agronomic traits.

Following this methodology, we have been able to generate one cytoplasmic male sterile line (046IA) and 13 promising lines in advanced stages. Virmani et al (1991) reported that from 1980 to 1988, the International Rice Research Institute (IRRI) could generate 40 A lines, but only 3 had the required characteristics for commercially hybrid production. This experience has also helped to develop a well-trained staff and create satisfactory working conditions, including infrastructure.

Reciprocal recurrent selection

This reciprocal recurrent selection was targeted for mid- and long-term goals, as presented by Neves et al (1994). The strategy was to develop two heterotic populations for grain yield. One population was used as a source of A lines with male sterile wild abortive (WA) cytoplasm and from the same population attempts were made to obtain B lines for the cytoplasm. This population also carried allogamic traits. The other population was used as a source for R lines to restore fertility and produce hybrid seeds.

In the reciprocal recurrent selection method, the first step was to identify parents with a wide genetic base and to create two contrasting populations. Within the project, CNA 2M (source of A and B lines) and CNA 3R (source of R lines) were developed. To facilitate the pollination process, the large stigma trait was introduced in the first population.

In 1993-94, population CNA 3R/0/2 was planted and 700 S_0 male sterile plants were identified. From these, 300 were further selected based on seed production and plant height. During the 1994-95 cropping season, these S_1 lines were planted to select within and between rows; 150 lines were picked and two plants were chosen within each. A similar procedure was used with the CNA 2M/1/0 population. The selection criteria differed only in plant height. In this population, the plants had to be shorter to facilitate pollination.

In 1995-96, 300 S_2 plants were taken to the field. The male sterile plants in each line were marked, and at the blooming stage they were pollinated using pollen samples from the reciprocal population. A similar process was used with the reciprocal population also. This work resulted in the production of 300 half-sib families from each population.

The plan for 1996-97 includes evaluation and selection of all these half-sib families. General combining ability will be measured and the lines that show the best combinations will be selected to generate the base population for the next recurrent cycle. The remnant S_1 seeds will be used to recombine and generate new populations.

The hybrids will be produced through the combination of A and R lines coming from the two populations, CNA 2M and CNA 3R, respectively. Therefore, during each recurrent cycle, the best lines from each population will be extracted and advanced through pedigree. The large stigma trait will be taken into account in each generation and restorer capacity will be tested when the lines are fixed.

Results

Developing line 046IA

There are at least three ways to obtain A and B lines for a hybrid rice program: (1) introducing lines from another program, (2) transferring cytoplasmic male sterility to new materials, and (3) developing a line with new male sterile cytoplasm. EMBRAPA–CNPAF concentrated on the first two ways. Since 1985, it has introduced 18 lines with WA cytoplasm, mainly through IRRI (Table 1).

In 1988, line IR13540-56-3-2 was identified as a maintainer. In 1989, this line was crossed to the initial allogamous line (#24Z) and the combination was coded as 032G. The cross underwent selection for large stigma and agronomic traits from F_2 to F_4 (pedigree 032G-85-1-5). As mentioned earlier, we began to test-cross in the F_4 generation to incorporate cytoplasmic male sterility from Zhen Shan 97A. The F_1 was crossed to self-pollinated F_5 plants from the cross 032G. Sterile plants with allogamic and desirable agronomic traits were selected. The process was repeated five times and the 046IA and its maintainer *2RF were obtained in 1994.

In the initial stages of development, selection was for sterile plants. During the early generations, there was a wide range of segregation for sterility from fully fertile to completely sterile plants. As selection progressed, the percentage of completely sterile plants increased. In most crosses, most lines were discarded in this selection process because of a lack of stability, poor agronomic traits, and unsuitable grain type.

The efficiency of this work was improved by the check made for sterile pollen grains using a microscope. At the early stages, however, this check was done only in the field and, because of environmental conditions, this introduced an error compo-

Table 1. A and B lines introduced in the hybrid rice project by EMBRAPA–CNPAF.

Year	A lines	B lines	Origin
1985	Zhen Shan 97A	Zhen Shan 97B	China
1985	Er-Chiu-Nan 1A	Er-Chiu-Nan 1B	China
1985	V41A	V41B	China
1985	WU10A	WU10B	China
1985	Yar Ai Zhao A	Yar Ai Zhao B	China
1985	MS577A	MS577B	Korea
1985	MS519A	MS519B	Korea
1985	Pankari 203A	Pankari 203B	IRRI
1992	IR58025A	IR58025B	IRRI
1992	IR62829A	IR62829B	IRRI
1992	IR64608A	IR64608B	IRRI
1995	IR68886A	IR68886B	IRRI
1995	IR68888A	IR68888B	IRRI
1995	IR68891A	IR68891B	IRRI
1995	IR68887A	IR68887B	IRRI
1995	IR68275A	IR68275B	IRRI
1995	IR68890A	IR68890B	IRRI
1995	IR68281A	IR68281B	IRRI

Table 2. Yield (kg ha^{-1}) of rice hybrids during the 1995–96 cropping season at Goiás, Tocantins, and Rio Grande do Sul.

Treatment[a]	Goiás	Tocantins	Rio Grande do Sul	Mean
H348	6,668	11,000	6,044	7,904
H40	7,021	11,083	5,593	7,899
H29	6,399	10,500	5,517	7,472
H38	5,752	10,917	5,746	7,471
H37	6,258	9,833	5,968	7,353
H16	5,965	9,917	5,352	7,078
H349	5,794	9,583	5,815	7,064
H329	6,755	7,583	6,152	6,830
H39	5,479	8,583	6,361	6,807
H200	6,340	7,500	6,419	6,753
H518	5,935	9,250	4,825	6,670
BR-IRGA 419	5,976	8,500	4,419	6,298
Metica 1	6,763	9,583	3,421	6,589
H35	6,884	8,167	4,672	6,574
H347	6,406	9,000	4,146	6,517
Javaé	6,281	6,500	6,495	6,425
H512	6,065	5,833	5,149	5,682
H34	4,758	5,750	3,739	4,749
Mean	6,250	8,838	5,324	6,785
CV(%)	13.0	15.0	12.6	14.3

[a]Metica 1 and Javaé were check varieties in Goiás and Tocantins and BR-IRGA 419 was used as a check in Rio Grande do Sul.

nent that increased the amount of work. Later, this was corrected by bringing materials to the laboratory for a complete check on pollen sterility.

Evaluating hybrids

In 1994, the EMBRAPA–CNPAF project produced 660 hybrid combinations by using 10 A lines and 88 R lines. In the 1994-95 cropping season, these hybrids, their parents, and local checks were planted under irrigated conditions at the Palmital experiment station. From this effort, 30 high-yielding combinations were selected.

In the 1995 cropping season, only the best 15 hybrids were evaluated simultaneously under tropical (Tocantins and Goiás) and subtropical (Rio Grande do Sul) conditions. Yield data (Table 2) showed a marginal superiority of the hybrids (4%) in relation to the local checks at Goiás and Tocantins. But these hybrids outperformed the check at Rio Grande do Sul. The results demonstrated a potential for hybrid production, mainly in the subtropical region. The challenge was to develop a suitable and economically viable hybrid seed production technique. The project had already directed its efforts and resources to develop such a technique. During the 1996 winter season, massive seed production of CMS line 046IA was launched. Simultaneously, seed multiplication of several restorer lines for use as combiners with the cytoplasmic male sterile line was also being carried out.

Future plans

Future plans for hybrid rice in Brazil include the following:

- Develop a suitable and economically viable hybrid seed production technique for Brazilian conditions.
- Evaluate yield gains from hybrid seed production caused by the introduction of allogamic traits in male sterile lines and decide on the need to continue using this approach in the hybrid rice breeding program.
- Develop and deploy genetically diverse CMS sources.
- Once the hybrid rice technology is packaged, EMBRAPA will open financial participation to the private sector. EMBRAPA–CNPAF has already invested more than US$4 million in hybrid rice research since 1984. The proposal to develop hybrids that yield 30% more than commercial varieties would rely on strong participation by the private sector, with an annual investment of $300,000-400,000.

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CHAPTER 32

Research and development for hybrid rice technology in Colombia

D. Muñoz, P. Gutiérrez, and E. Corredor

Rice is the most important crop after coffee in Colombia and it is the second source of protein after meat. Area planted to rice is 300,000 ha. In 1995, the average productivity of the crop was 5.5 t ha^{-1} in irrigated lands and 4.6 t ha^{-1} in rainfed uplands. The development of 14 high-yielding semidwarf varieties has been pivotal to improved rice production during the past two decades. But average yields have reached a plateau. Rice hybrids offer an opportunity to break through the yield ceilings of semidwarf inbred varieties. At Saldaña (Tolima) in 1995, the highest-yielding hybrid was IR58025A/Oryzica Yacú-9. It produced an average yield of 7.1 t ha^{-1}, 16% higher than that of Oryzica Yacú-9, a commercial variety released in 1994. Of the 10 hybrids evaluated at Saldaña, seven showed less white leaf virus disease (hoja blanca virus) than their parents. In these tests, 82% of the hybrids recorded more grains $panicle^{-1}$ than their restorer parents. The results suggest that by using the female parent IR58025A, white center is increased in grains of the hybrids. Fortunately, the level of white center is still acceptable to rice millers and consumers. Currently, the hybrid rice program in Colombia has two major objectives: (1) to breed heterotic hybrids with a 15–20% yield advantage, good grain appearance, and good cooking quality, and (2) to ease the hybrid seed production process.

The average productivity of rice in Colombia is 5.5 t ha^{-1} in irrigated lands and 4.6 t ha^{-1} in rainfed uplands. The high production cost of the crop and the difficulty in overcoming the yield plateau made research necessary on new production methodologies, including the use of hybrids. Recent success in exploiting the heterosis exhibited in hybrid rice, primarily in China, has encouraged some Colombian rice breeders to explore the potential of hybrids to increase yields. Hybrid rice research began in 1983 in collaboration with the International Rice Research Institute (IRRI).

The hybrid rice program has two major objectives: (1) to breed heterotic hybrids with a 15–20% yield advantage, good grain appearance, and good cooking quality to

satisfy consumer demand, and (2) to ease the hybrid seed production process. These are discussed in this chapter.

The first study on hybrid rice was carried out in the Cauca Valley Department at the National Research Center (CNI-Palmira) of the Colombian Agricultural Institute (ICA) in 1983B (second-semester season) and 1984A (first-semester season) (Muñoz and Carvajal 1989). The five hybrids evaluated and their parents were introduced from IRRI. Hybrid V20A/Suweon 294 yielded 8.3 t ha $^{-1}$. This hybrid showed the highest heterobeltiosis (21%) and standard heterosis (10%) for yield. The other hybrids were not superior to Oryzica-1 , the most planted variety in Colombia, which yielded 7.5 t ha $^{-1}$.

Hand-pollinated hybrids

A study was conducted to determine the yield potential, yield components, and some agronomic characteristics of 15 hand-pollinated rice hybrids, four commercial varieties, and two experimental lines. The experiment was carried out at CNI-Palmira of ICA (Muñoz and Castellanos 1990). In general, the hybrids studied yielded more and also the yield components were superior to the average of the parents (heterosis) and to the best parent (heterobeltiosis). Hybrid Oryzica-1 x Cica-4 had the highest yield (8.5 t ha $^{-1}$) and the highest heterosis (49%) and heterobeltiosis (44%).

Restorers and maintainers of wild abortive cytosterile lines

Attempts were made to identify restorers and maintainers for WA cytoplasmic male sterile (CMS) lines. Using 42 different male parents, F_1 hybrids were obtained by crossing with CMS lines V20A and Zhen Shan 97A. These male parents included traditional standard varieties and elite breeding lines with varying heading time. Male parents of the F_1 that showed above 80% pollen fertility were designated as restorers. Among the male parents, 14% were found to be restorers and 45% to be maintainers (Muñoz and Lasso 1991).

Grain quality of some rice hybrids

Chalkiness is the principal determinant of the price producers receive for their rice. Therefore, a study was carried out with three F_1 hybrids to evaluate grain yield and some grain quality characteristics. The replicated trial was harvested at CNI-Palmira in 1988. All the F_1 hybrids showed heterosis for milling recovery grain yield. Hybrid IR46830A/IR9761-19-1 had a grain chalkiness score of 0.6 compared with 0.4 for Oryzica- 1. The higher chalkiness of the grains in the hybrids appeared to be inherited primarily from the female parents (Muñoz 1994). Recently, some F_1 hybrid combinations derived from new CMS lines of IRRI have shown encouraging results for grain quality, which are presented later.

Evaluating rice hybrids

During 1991-92, 12 rice hybrids received from IRRI were evaluated in Colombia and harvested in February 1992. The highest-yielding hybrid was IR62829A/IR40750-82-2-2-3R, with an average yield of 5.6 t ha^{-1} versus the 4.7 t ha^{-1} of Oryzica-1. The yield performance of most of the hybrids was quite similar at both test locations. The most remarkable characteristic in these hybrids was the low white center in grains, which varied between 0.4 and 1.8 (Muñoz 1994).

The F_1 rice hybrids obtained in Colombia were evaluated in both the first (A) and second semester (B) seasons of 1995. The replicated yield trials were direct-seeded at two different locations. The Saldaña area is located in the central part of the country and Bosconia is in the Atlantic coast area. At both locations, rice is grown under irrigated conditions.

The best adapted sterile line (IR58025A) was used to obtain hybrids with the most-planted Colombian varieties, Oryzica Caribe-8, Oryzica-1, and the last released (1994) commercial variety, Oryzica Yacú-9. The highest-yielding hybrid evaluated at Saldaña (Tolima) was IR58025A/Oryzica Yacú-9, with an average yield of 7.1 t ha^{-1}, which was 16% higher than that of Oryzica Yacú-9. The best hybrid in Bosconia (Cesar) was IR58025A/Oryzica Caribe-8, which yielded 6.7 t ha^{-1}, which was 16% higher than the yield of Oryzica Caribe-8 (Tables 1 and 2). Virmani (1994) reported yields of the best rice hybrids and compared them with those of the best inbred varieties evaluated at IRRI during 1980-90. In these evaluations also, the best hybrids outyielded the best improved variety by about 16% on average.

Grains per panicle

Increased yield in heterotic hybrids in rice has been reported to be caused by heterosis in panicle number and spikelet number (Virmani 1994). At Saldaña and Bosconia, 82% of the hybrids studied showed more grains $panicle^{-1}$ than their restorer parents.

Table 1. Yield of four hybrids and their parents evaluated in Saldaña, Tolima, 1995[a].

Hybrid/parent	Yield (t ha^{-1})[b]	
	1st semester	2nd semester
IR58025A/O. Yacú-9	6.9 ab	7.3 a
Oryzica Yacú-9	6.2 abc	6.1 bcdef
IR58025A/CT8008	6.9 ab	6.7 abc
CT8008-16-10-10P	6.4 abc	5.7 cdef
IR58025A/O. Caribe-8	4.9 cde	6.7 abc
Oryzica Caribe-8	5.7 bde	5.4 def
IR58025A/Oryzica 1	4.3 de	6.5 abcd
Oryzica 1	5.6 bcde	5.6 def

[a] In the first-semester season, 10 hybrids and their parents were evaluated, and in the second-semester season 15 hybrids and their parents were evaluated. [b] Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 2. White center in the kernels of four hybrids and their parents evaluated in Saldaña, Tolima, 1995 second-semester season.

Hybrid/check	White center[a]
IR58025A/O. Yacú-9	1.5
Oryzica Yacú-9	1.1
IR58025A/CT8008	1.6
CT8008-16-10-10P	0.6
IR58025A/O. Caribe-8	1.7
Oryzica Caribe 8	0.7
IR58025A/Oryzica 1	1.5
Oryzica 1	0.8

[a] On a scale of 0–5, where 0 = free from chalkiness and 5 = completely chalky.

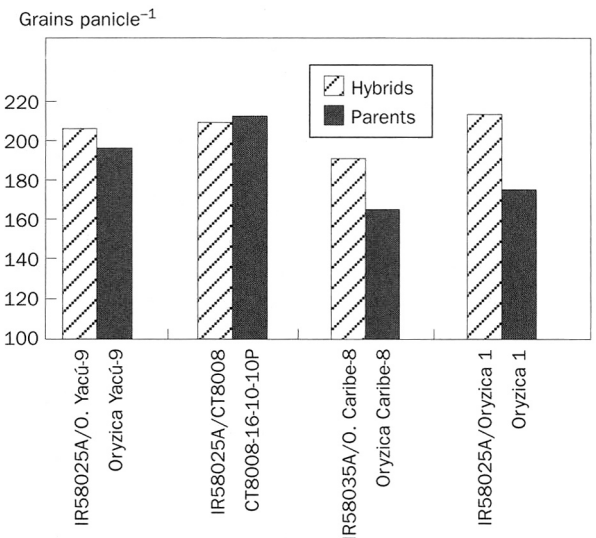

Fig. 1. Grains panicle^{-1} of four hybrids evaluated in Saldaña, Tolima, 1995 second-semester season.

Standard heterosis over commercial variety Oryzica-1 for grains panicle^{-1} was 17% and 19%, respectively, for IR58025A/Oryzica Yacú-9 and IR58025A/Oryzica Caribe-8. Details on grains panicle^{-1} in different rice hybrids evaluated at Saldaña and Bosconia in 1995 are given in Figures 1 and 2.

Grain quality

Kernel chalkiness (white center), translucency, and color are the major determinants of rice grain quality. Studies conducted at IRRI showed that chalkiness scores of the hybrids were intermediate between those of the parents. In this study, we found that in the kernels of hybrids, the white center was influenced by the sterile line. At Saldaña in 1995, 70% of the hybrids evaluated showed a higher white center in their kernels

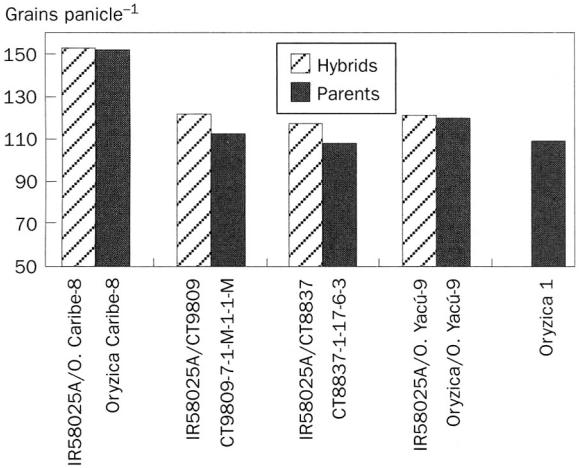

Fig. 2. Grains panicle^{-1} of four hybrids evaluated in Bosconia, Cesar, 1995 second-semester season.

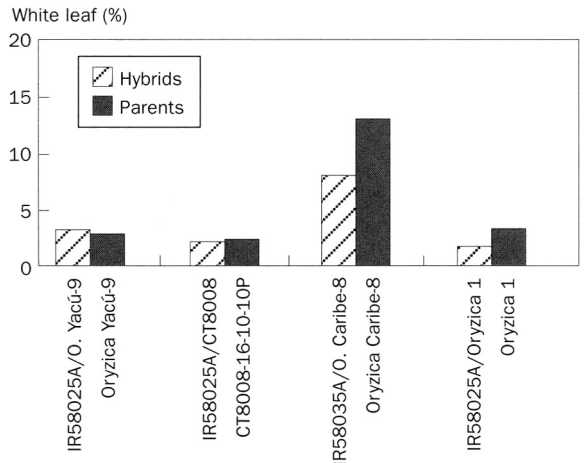

Fig. 3. Percentage of white leaf virus disease of four hybrids evaluated in Saldaña, Tolima, 1995 second-semester season.

than their restorer parents (Table 3). These results suggest that female parent IR58025A was instrumental in increasing white center in the kernels of the hybrids. Fortunately, the level of white center with kernels of these hybrids is acceptable to both rice millers and consumers.

White leaf virus disease

Disease and insect resistance of rice hybrids can be manipulated in the desired direction by the appropriate choice of parental lines. The CMS lines used should also be screened for their disease/insect resistance to test whether any susceptibility is found

Table 3. White center of four hybrids and their parents evaluated in Saldaña, Tolima, 1995 second-semester season. Twelve hybrids and their parents were evaluated in the trial.

Hybrid/parent	Yield (t ha^{-1})[a]
IR58025A/O. Caribe-8	6.7 a
Oryzica Caribe-8	5.8 cdef
IR58025A/CT9809	6.6 ab
CT9809-7-1-M-1-1-M	6.4 abc
IR58025A/CT8837	6.4 ab
CT8837-1-17-6-3	4.7 gh
IR58025A/O. Yacú-9	5.2 efg
Oryzica Yacú-9	5.4 defg
Oryzica 1	5.1 efg

[a]Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

associated with a CMS system. Seven out of 10 hybrids evaluated at Saldaña in the 1995B season showed less white leaf virus disease (hoja blanca virus) than their parents. It is important to point out the high reduction in white leaf virus of the hybrid IR58025A/Oryzica Caribe-8 compared with that of restorer line Oryzica Caribe-8 (Fig. 3).

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CHAPTER 33

Research and development for hybrid rice technology in Sri Lanka

S.W. Abeysekera, M.P. Dhanapala, and D.S. de Z. Abeysiriwardena

Sri Lanka has reached a stage where further expansion in rice area is not possible. With a per capita consumption of about 100 kg yr^{1} and a limited annual rice land area of about 830,000 ha, Sri Lanka must raise its present yield level of 3.4 t ha^{-1} to 4.5 t ha^{-1} within the next 5 yr to achieve self-sufficiency in rice. The low and stagnating yields of semidwarf inbred varieties presently cultivated in Sri Lanka limit the scope for increasing production. Sri Lanka's Rice Research and Development Institute began a research program on hybrid rice in collaboration with IRRI in 1980. The program concentrated mostly on evaluating promising genetic materials received from IRRI and other countries for adaptability in the target environments of Sri Lanka. Attempts were also made to identify cytoplasmic male sterile (CMS) lines suitable for Sri Lanka and transfer the CMS character from IRRI-developed CMS lines to promising Sri Lankan lines. During the past two seasons, nine CMS lines possessing wild abortive cytoplasm were tested for field performance and promising lines were selected. Pollen sterility in the selected CMS lines ranged from 92% to 99.5%. The heterotic combinations identified included 48 from CMS/IRRI restorer test crosses and five heterotic combinations selected from test crosses made between IRRI CMS lines and SL elite lines. Of the 32 test crosses made during the minor dry *(yala)* season of 1996, two, IR58025A/Ld 355 and IR68887A/Bg 2039, were found to have high pollen sterility. They are being backcrossed with repetitive male parents to develop locally adapted CMS lines.

Rice is the staple food of Sri Lankans and the most important food crop in the country. It is the livelihood of more than 1.8 million farm families. More than 30% of the total labor force in Sri Lanka is directly involved in rice or rice-related activities. During 1991-95, the average annual rough rice production was about 2.24 million t. At the present consumption rate (100 kg $capita^{-1}$ yr^{-1}), Sri Lanka would require about 3.5 million t of rice to feed an estimated population of 20 million by the year 2000

(DOASL 1995). Sri Lanka has reached a stage in which there is limited scope for further expansion in rice area. Therefore, the emphasis is on increasing the production potential per unit land area and finding ways to do this at the farmer level through appropriate technology.

Rice ecosystems

Sri Lanka has approximately 730,000 ha of land suitable for rice cultivation: 41% under major irrigation schemes, 25% under minor irrigation schemes, and 34% under rainfed conditions. With an average annual cropping intensity of 113%, the annual sown area for rice is around 830,000 ha. The average productivity under major and minor irrigation schemes and rainfed lands is 4.2, 3.2. and 2.4 t ha^{-1}, respectively. Hence, irrigated lands (under major and minor irrigation schemes) represent about 75% of the total rice production.

Sri Lanka's rice lands are found mostly in the inland valleys and, to a limited extent, on the coastal plains, floodplains, and terraced slopes. Variable seasonal rains, soil types, elevation, temperature, and drainage patterns create complex and diverse environments for rice production (Will 1989). The agroclimatic features of the country are strongly influenced by the interaction between the two monsoons—the northeast and the southwest—and the mountainous land mass in the south-central area of the country.

Rice in Sri Lanka is cultivated in two seasons: the wet main season *(maha)* from October to March and the dry minor season *(yala)* from April to September. The seasons are very distinct in the dry zone, but become increasingly less distinct toward the intermediate and wet zones. The northeast monsoon during October to January is comparatively stronger and produces rains throughout the island. The southwest monsoon during April to July provides rains mostly for the wet zone, the southwestern quarter of the country, and the central highlands. As a result, moisture is sufficient year-round in the wet zone but dry spells are frequent in the dry and intermediate zones.

Problems associated with rice production

At present, the main problems facing the rice sector are low and stagnating yield (3.5 t ha^{-1}), escalating production costs (\$175 t^{-1} of rough rice), diminishing profitability (US\$12.50 mo^{-1} irrigated ha^{-1}), poor grain quality. and the high market price of polished rice (\$0.30–0.40 kg^{-1}). The increase in the cost of inorganic fertilizers and labor and the declining labor force have had a negative impact on rice farmers who use improved crop management practices. The government has therefore developed a comprehensive plan to reduce the cost of production (below \$100 t^{-1} of rough rice), maintain the quality of the rice environment, increase national rice yields, and raise the professionalism of rice farming (DOASL 1995).

Potential for hybrid rice in Sri Lanka

Hybrid rice has a greater chance to solve the problem of stagnating rice yield and to increase the production potential per unit area. Therefore, the Department of Agriculture (DOASL) took steps to introduce a hybrid rice research and development program at the Rice Research and Development Institute (RRDI) in collaboration with IRRI. Results from preliminary work conducted are encouraging enough for the DOASL to continue and expand the program.

The hybrid rice research and development program in Sri Lanka

Early work on hybrid rice mostly involved the evaluation of cytoplasmic male sterile (CMS) lines introduced from IRRI and transfer of the CMS character to local varieties. During the 1980s, five CMS lines from China and five from IRRI were evaluated at RRDI (Pathinayake and Dhanapala 1985). Of these, five promising CMS lines—Yar Ai Zhao, V20, MS577, P203, and WU10—were selected for use as parents to transfer the CMS character to local cultivars. As a result, a number of CMS lines that were found to be comparatively more adaptable to local conditions were developed (Pathinayake and Dhanapala 1985). A few private organizations also evaluated hybrid rice varieties developed in India under Sri Lankan conditions.

The current hybrid rice program is confined to a few high-priority areas of research. The progress made up to early 1995 was not encouraging mainly because of the lack of required human resources and infrastructure. Therefore, in 1995, this program was reoriented to evaluate promising genetic materials from IRRI and other countries for adaptability in target environments and to identify suitable CMS lines for transfer of the CMS character to promising Sri Lankan cultivars.

Several CMS lines received from IRRI have been evaluated for their adaptability to Sri Lankan conditions. Test crosses have been made using IRRI-developed CMS lines adaptable to Sri Lankan conditions with promising rice cultivars developed in Sri Lanka. These materials have been evaluated for their field performance along with IRRI restorer lines. The program also undertook multiplication of IR62829A/B and IR58025A/B at the RRDI seed farm with recommended seed production practices.

Achievements

Pollen sterility of the nine IRRI-developed CMS lines ranged from 92% to 99.5% (Table 1). Of these lines, eight were uniform and adapted to local conditions. Outcrossing was very high in IR68886A and IR58025A.

During the past two seasons, we were able to identify 49 heterotic combinations from IRRI CMS/IRRI restorers and IRRI CMS/Sri Lankan elite line test crosses (Table 2). From these, 37 combinations were selected based on phenotypic acceptability.

During the 1996 minor season, 32 test crosses were made. Two test crosses, IR580258A/Ld 355 and IR68887A/Bg 2039, were found to show 88% and 98% pol-

Table 1. Performance of IRRI CMS lines at the Rice Research and Development Institute, Batalagoda, Sri Lanka, 1996 minor (dry) season.

CMS line	Pollen sterility [a]	Outcrossing rate (OCR) [b] (%)	Adaptability [c]	Remarks
IR68886A	92.0	36.2	Uniform, adapted	Vigorous, short
IR68888A	97.0	27.0	Uniform, adapted	
IR68891A	Unstable	8.0	Nonuniform	Poor
IR68887A	96.0	7.0	Uniform, adapted	
IR68275A	99.0	3.0	Uniform, adapted	
IR68890A	99.5	6.5	Uniform, adapted	Flowering late
IR68281A	97.0	6.0	Uniform, adapted	
IR62829A	94.0	26.5	Uniform, adapted	
IR58025A	97.0	37.0	Not very uniform but adapted	Some plants possible maintainers

[a] Ratio of the number of sterile pollens to the total number of pollens in 3 fields of each of 2 separate slides prepared using 10 plants. [b] OCR% = no. of fertile spikelets/total no. of spikelets x 100. [c] Based on visual observations.

len sterility, respectively. These are being backcrossed with the respective male parents to develop locally adapted CMS lines.

The performance of 38 IRRI restorer lines for their adaptability to local conditions was investigated (Table 3). Of these, eight IRRI lines were identified as excellent because they were comparable to local checks Bg 300 and Bg 94-1.

Of the 38 IRRI experimental hybrids (Table 4), the following 5 had the highest percentage standard heterosis: PMS 10A/IR58841-48-B-3-2 (HRSP 701), IR62829A/IR49461-129-3-3-3 R (HRSP 714), IR58025A/lR55838-B2-2-3-2-3 (HRSP 684), IR58025A/Sanghuanzan #2 (HRSP 687), and IR58025A/IR48749-5-3-2-2-1 (HRSP 708). The yields of these hybrids ranged from 1.5 to 3.4 t ha^{-1}. The reason for the very low level of yield in this hybrid rice nursery was the high incidence of rice bug damage caused by late planting. Because this damage was uniform among the test entries, this would not cause any serious bias for grain yield comparison among entries.

Discussion

These observations indicate the need to continue evaluating genetic materials in Sri Lanka to identify better adapted lines and to develop new CMS lines for use in the hybrid rice program. The following are some major constraints that are to be addressed:

- Sri Lanka needs to train resource personnel to handle the hybrid rice program and to improve facilities to produce the required quantities of hybrid rice seed on government seed farms. The present capacity of the government sector, which is mainly under DOASL, is limited to about 5-6% of the country's total seed requirement.

Table 2. List of test-cross F_1s selected or rejected based on phenotypic acceptability from test-cross nursery at the Rice Research and Development Institute, Batalagoda, Sri Lanka, 1996 minor (dry) season.

Test cross	Selected/ rejected	Phenotypic acceptability[a]
IR68891A/IR51078-33-2-1-1-3R	Rejected	Poor
IR68891A/BG1370	Rejected	Poor
IR6889IA/MRC19340-12-15	Rejected	Poor
IR68891A/RP22378-848	Rejected	Poor
IR58025A/IR54742-22-19-3R	Selected	Good
IR58025A/IR51078-33-2-1-1-3R	Selected	Excellent
IR58025A/BG1370	Selected	Good
IR58025A/Sanghuanzan #2	Selected	Good
IR58025A/IR56455-206-2-1-2	Selected	Excellent
IR58025A/IR49615-11-3-1-1-3	Selected	Good
IR58025A/MRC19340-1215	Selected	Good
IR58025A/IR60821-191-3-3-2-1	Selected	Good
IR58025A/AT354	Selected	Good
IR68281A/Sanghuanzan #2	Selected	Good
IR68281A/IR49615-11-3-1-1-3	Selected	Excellent
IR68281A/IR62030-83-1-3-2	Selected	Good
IR68281A/RP22378-848	Selected	Good
IR68281A/IR56455-206-2-1-2	Selected	Good
IR68281A/IR57312-119-2-1	Selected	Good
IR68281A/IR62030-54-1-2-2	Selected	Good
IR68281A/IR60966-119-3-3-2-1	Selected	Good
IR68281A/IR59656-113-1-2	Selected	Good
IR68281A/94-16-9-1	Selected	Good
IR68281A/95-22-11	Selected	Good
IR68281A/95-28-9	Selected	Excellent
IR68887A/MRC19340-1215	Selected	Good
IR68887A/BG1370	Selected	Good
IR68887A/IR51078-33-2-1-1-3R	Selected	Good
IR68890A/RP22378-848	Selected	Good
IR68890A/IR60966-119-3-3-2-1	Selected	Good
IR68890A/IR51078-33-2-1-1-3R	Selected	Good
IR68890A/94-16-182-1	Selected	Good
IR68890A/IR50404-57-2-2-3	Selected	Good
IR68275A/RP22378-848		Fair
IR68275A/Sanghuanzan #2		Poor
IR68275A/IR60821-191-3-3-2-1		Poor
IR68275A/IR34686-179-1-2-1R		Poor
IR68275A/RP22378-848		Fair
IR68888A/IR50404-57-2-2-3	Selected	Good
IR68888A/IR60821-191-3-3-2-1	Selected	Good
IR68888A/IR59656-113-1-2	Selected	Good

[a] Based on visual observations on plant growth, flowering, and spikelet fertility performance.

- Labor wages have increased during the past decade. As a result, the seed production cost has doubled for foundation (US$0.40 kg^{-1}), registered ($0.30 kg^{-1}), and commercial ($0.25 kg^{-1}) seed.
- Hybrid rice seed is expected to cost about 3-4 times more than inbred seed.

Table 3. Field performance of IRRI restorer lines at the Rice Research and Development Institute, Batalagoda, Sri Lanka, 1996 minor (dry) season.

Restorer line	Field performance[a]	Restorer line	Field performance[a]
IR29723-143-3-2-1R	Excellent	IR62030-97-3-2-2	Fair
IR46R	Excellent	RP22378-848	Fair
IR51078-33-2-1-1-3R	Good	IR44962-7-6-2-2	Excellent
IR21567-18-3R	Excellent	IR28228-28-3-3-2	Very good
IR54742-22-19-3R	Very good	IR56455-206-2-1-2	Fair
IR32809-26-3-3R	Fair	IR58841-48-B-3-2	Very good
IR34686-179-1-2-1R	Fair	IR33380-7-2-1-3	Very good
IR32419-28-3-1-3R	Fair	IR58100-97-2-1	Excellent
IR42221-14-1-3-1-2R	Very good	IR58100-79-1-3	Excellent
IR49615-11-3-1-1-3	Fair	BG1370	Very good
IR51672-62-2-1-1	Very good	IR49735-SRN-4B-1-4-1	Excellent
IR59601-301-3-6R	Fair	IR48749-5-3-2-2-1	Very good
MRC19340-1215	Very good	IR57312-5-3-2-2-1	Fair
IR48751-B-B-79-3	Very good	IR50404-57-2-2-3	Fair
IR55838-B2-2-3-2-3	Very good	IR57284-34-3-2	Fair
Sanghuanzan #2	Fair	IR50400-64-1-2-2-2	Very good, late
IR59656-113-1-2	Excellent	IR49461-129-3-3-3R	Fair
IR60821-191-3-3-2-1	Very good	BG300	Excellent
IR60966-119-2-3-1-2	Very good	BG94-1	Excellent
IR62030-54-1-2-2	Fair	BG379-2	Very good
IR62030-83-1-3-2	Fair		

[a] Based on adaptability and uniformity evaluated through spikelet fertility of each plot: >90% = excellent, 90-75% = very good, 74-50% = good, <50% = fair.

- The supply of hybrid seed for every crop season under the current seed production system is uncertain.

There is no clear government policy for the promotion of hybrid rice varieties in Sri Lanka. Even the private sector does not have large-scale farms or seed-processing units. This prevents the private sector from supplying hybrid rice seed to farmers in a big way. This calls for urgent improvement of DOASL seed farms to handle hybrid seed production. The private sector may also be encouraged to enter the hybrid seed production program. Steps must be taken to change farmers' crop management methods, especially stand establishment methods, to suit hybrid rice technology. The government should also consider the need to introduce policies to upgrade the development of hybrid rice and to promote its cultivation.

Table 4. Evaluation of IRRI-developed experimental hybrids at the Rice Research and Development Institute, Batalagoda, Sri Lanka, 1995-96 major seasons.

Entry no.	Yield (t ha^{-1})	Standard heterosis (%)
Age 3 mo		
IR67693H (HRSP 665)	2.92	34.40
IR69614H (HRSP 666)	2.27	4.71
IR68877H (HRSP 667)	2.67	22.87
IR65488H (HRSP 668)	2.62	20.81
IR69674H (HRSP 671)	2.74	26.20
IR70965H (HRSP 672)	2.75	26.58
IR62829A/IR49615-11-3-1-1 (HRSP 674)	2.38	9.66
IR70966H (HRSP 675)	2.71	24.83
IR70968H (HRSP 679)	2.85	31.26
IR58025A/IR48751-B-B-79-3 (HRSP 682)	2.69	23.78
IR58025A/IR55838-B2-2-3-2-3 (HRSP 684)	3.09	42.36
IR58025A/Sanghuanzan #2 (HRSP 687)	3.06	40.91
IR58025A/IR59656 (HRSP 688)	2.79	28.36
IR58025A/IR60821-191-3-3-2-1 (HRSP 689)	2.31	6.56
IR58025A/IR60966-119-2-3-1-2 (HRSP 690)	2.26	4.01
IR58025A/IR62030-54-1-2-2 (HRSP 691)	1.83	-15.76
IR58025A/IR62030-83-1-3-2 (HRSP 692)	1.86	-14.33
IR58025A/IR62030-97-3-2-2 (HRSP 693)	2.40	10.66
IR58025A/RP22378-848 (HRSP 694)	1.53	-29.27
IR58025A/IR44962-7-6-2-2 (HRSP 695)	2.38	9.42
IR62829A/IR28228-28-3-3-2 (HRSP 699)	2.64	21.62
IR58025A/IR56455-206-2-1-2 (HRSP 700)	2.60	19.86
PMS10A/IR58841-48-B-3-2 (HRSP 701)	3.41	57.19
IR58025A/IR33380-7-2-1-3 (HRSP 702)	1.67	-23.04
IR58025A/IR58100-97-2-1 (HRSP 703)	2.60	19.74
IR58025A/58100-79-1-3 (HRSP 704)	2.63	21.35
IR58025A/Bg1370 (HRSP 705)	2.25	3.86
IR62829A/IR49735-SRN4-B1-4-1 (HRSP 707)	2.27	4.54
IR58025A/IR48-749-5-3-2-2-1 (HRSP 708)	3.00	38.37
IR62829A/IR57312-119-2-1 (HRSP 709)	2.70	24.40
IR69680H (HRSP 711)	2.17	-0.02
IR58025A/IR57284-34-3-2 (HRSP 712)	1.91	-11.80
IR62829A/IR49461-129-3-3-3R (HRSP 714)	3.17	45.94
Bg 300	2.17	
Age 3 1/2 mo		
IR68879H (HRSP 669)	2.70	7.72
IR68284H (HRSP 670)	3.07	22.82
IR58025A/IR50400-64-1-2-2-2 (HRSP 713)	3.09	23.47
IR64615H (HRSP 664)	2.90	15.91
IR70967H (HRSP 678)	3.01	20.45

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CHAPTER 34

Hybrid rice breeding in Japan

R. Ikeda

Motivation for breeding and producing hybrid rice has been weak during the past decade because of a decline in the area under rice cultivation in Japan. Despite this discouraging situation, some basic research has been conducted on hybrid rice. Simultaneously, the private sector has continued to invest in hybrid rice breeding in conjunction with breeders in China, India, and other countries. Marketing of a small amount of hybrid rice seed has been announced recently by a private company. This chapter briefly outlines ongoing work in hybrid rice breeding in Japan.

Current status of rice production

Japan reduced its rice production because of the long-term decline in rice consumption and imports of other cereals. Total rice cultivation has fallen to 2.0–2.2 million ha from a potential area of 3 million ha. Japan's cost of rice production is the highest among rice-growing countries. To keep farm income at a comparable level with that of other domestic industries and to cope with international rice markets, reducing the production cost is the primary task for all rice-related sectors. In all farm operations, saving labor is required. But because hybrid rice demands more labor, farmers have not adopted it.

With increasing family income, the Japanese prefer and pay a higher price for quality rice. The price of quality rice is 20-30% more than that of ordinary rice. Because hybrid rice with acceptable quality is not yet readily available, a low priority is given to conducting trials to increase yield at the expense of quality.

Hybrid rice breeding in Japan

National or public sector

For the cytoplasmic male sterility (CMS) from Chinsurah Boro II used in developing hybrid rice, there is practically no restorer gene in japonica rice. Restorers for this CMS had to be bred by incorporating the *Rf* gene from indica rice. But in such a process, the genetic base for a high level of heterosis was lost. Alternative ways to achieve this have been to use CMS lines with the wide compatibility gene (S-5^n) and indica lines as restorers. Several attempts have been made in hybrid rice breeding. Some experimental lines have been developed (Ikehashi et al 1994, Kato et al 1994). Several CMS lines with the wide compatibility gene, such as MS Nekken 2 and MS H90-125, have been developed together with pollen donors, such as H87-53. Korean variety Gaya was found to be a promising pollen parent.

Recently, an improved japonica line, TML 1 with *S-5*^{n} from Suweon 258/Tainung 67//Nekken 2, has been developed in Japan with the cytoplasm of Chinsurah Boro II. The restorer is Habataki, a high-yielding indica line. The hybrid gave increased yields of 15% (in 1993) and 48% (in 1994) compared with the control. This hybrid also had a 14% higher yield than that of the high-yielding parent (Takita 1995). Despite such trials, an acceptable level of grain quality is not easily attained because of the poor acceptability of indica rice.

Indica-japonica hybrids that exhibit a high level of heterosis appear to be promising. But in these hybrids, yield stability has been low because of poor pollen fertility, especially in cool climates. So far, hybrid sterility expressed in pollen is not solved by the wide compatibility genes for spikelet fertility. In this regard, japonica varieties may be used, because many of them are known to possess the wide compatibility gene for hybrid sterility in pollen (Ikehashi and Araki 1987).

Private sector

The hybrid rice project of the Japan Federation of Agricultural Cooperatives (Zen Noh) was limited because of the unstable yield of hybrid rice during 1993, when yield was affected by cool weather in central to northern Japan. The program at Mitsui Chemicals Inc., however, has announced the marketing of hybrid rice seed. This company seems to have overcome the problems of yield level and quality by a large investment in research and by cooperative approaches with some programs in China. It seems to have identified many hybrid combinations that express a high level of heterosis. From Japan Tobacco Inc., which adopted the three-line method for the past several years, little information is available.

The hybrid rice program at Mitsui Chemicals Inc. has also used advanced breeding technologies, including cell fusion, to obtain cybrid and molecular markers for *S-5*^{n} and the *Rf* gene (Yokozeki 1995). According to some early press releases, Japan Tobacco Inc. may have research programs for molecular control of anther development, but details are unknown.

Research at the National Agriculture Research Center (NARC)

The program of NARC has adopted both the three-line and two-line methods. The two-line method depends on thermosensitive genetic male sterility (TGMS). The three-line method using cytoplasmic male sterility is more stable, but is cumbersome to use for seed multiplication.

With the two-line method, male sterility is slightly unstable depending on environmental conditions. Weather conditions occasionally change the expression of male sterility. In 1993, particularly, some unusually cool weather in the summer season in the northern part of Japan caused significant yield losses. Even at Tsukuba in the central region, some early varieties showed panicle sterility. The plants of TGMS lines showed total sterility to semisterility in the fields of NARC. It was therefore difficult to determine heterozygotes of TGMS lines in the field. The promising paternal line for hybrid rice, H90-125, derived from the cross of Milyang 23 and Akihikari, showed a high level of sterility under such conditions. Seed set in H90-125 was much lower than that of the parents, Akihikari and Milyang 23. This experience indicated the need for long-term and continued testing for hybrid rice materials.

Mechanization of seed increase

A bentazone-sensitive mutant was developed from Norin 8. This trait has been incorporated into male parents. By using male parents, which are sensitive to bentazone, a mixture of female and male parents in the field has been tested to multiply hybrid seeds. After pollination of the female parent, the male parent sensitive to bentazone can be selectively killed by spraying chemicals. Adoption of such a method is expected to lower the cost for hybrid seed.

IRRI-Japan shuttle breeding of TGMS

A TGMS line was induced from Japanese variety Reimei (Maruyama et al 1991). This male sterile mutant, designated as H89-1 (also known as Norin PL 12), exhibited no seed set under 3 1/24 °C (max/min temperature) and complete fertility under 25/15 °C. Pollen sterility in this mutant was not affected by daylength.

The IRRI-Japan Shuttle Breeding Project began in October 1990 to develop indica-type TGMS lines. So far, shuttle breeding for TGMS lines has been conducted 13 times between IRRI and NARC. The materials developed are now in the F_{12} or F_{13} stages.

Tagging of TGMS gene *tms2*

Norin PL12 showed complete sterility in the field, whereas Dular and the F_1 plants showed complete fertility (Table 1). The F_2 plants of Norin PL12 and Dular did not show any hybrid sterility, and bias in the segregation of the TGMS gene was avoided in the F_2 and later generations. TGMS is controlled by one recessive gene. The seed set percentage in F_2 plants from Norin PL12/Dular showed a continuous distribution

Table 1. Heading date and fertility in TGMS mutant Norin PL 12, pollen donor parent Dular, and their F_1 hybrid.

Variety	Heading date	Fertility (%)
Norin PL 12	14 August	1.2
Dular	11 August	95.7
F_1	31 July	90.5

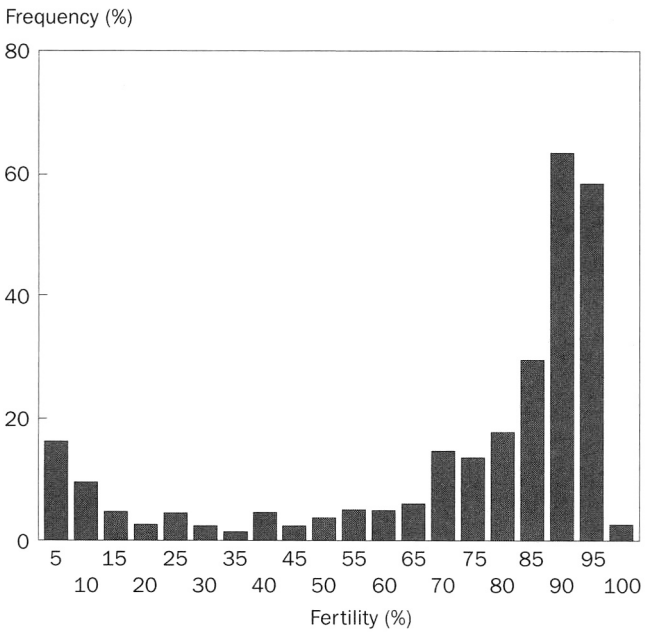

Fig. 1. Frequency distribution of fertility in an F_2 population (n = 250) from Norin PL 12/Dular.

(Fig. 1). Eighty-seven RFLP (random fragment length polymorphism) markers, which were polymorphic between the parents, were used to detect the TGMS gene, *tms2*. A linkage between *tms2* and an RFLP marker, R1440, was found on chromosome 7 (Fig. 2). Wang et al (l995), using RAPD (random amplified polymorphic DNA) and RFLP markers of Cornell University, reported that the TGMS gene was most likely located on chromosome 8. Some markers on chromosome 8 were also tested at NARC, but no linkage was detected. Therefore, the TGMS gene was designated as *tms2,* which may be different from that reported by Wang et al (1995).

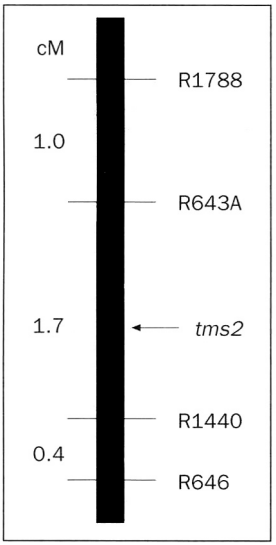

Fig. 2. Estimation of the locus of *tms2* on chromosome 7.

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CHAPTER 35

Hybrid rice in Thailand

S. Amornsilpa

Encouraged by experiences in China and at IRRI, rice breeders in Thailand have been exploring the prospects of hybrid rice technology for increasing yield potential in rice. IRRI-bred rice hybrids have not shown a consistent yield advantage over local checks although the grain quality of some hybrids was comparable with that of the checks. This chapter outlines strategies for developing hybrid rice technology in Thailand.

Rice is one of the most important cereal crops in the world because it provides food for more than two billion people. The population of rice consumers is now increasing at an exceptionally fast rate. It was predicted that demand for the crop would exceed production by the year 2000 (Lampe 1995). Therefore, the growth rate of rice production must be accelerated.

There are environmental concerns about the overuse of farm chemicals. But genetic improvement is a potential means to sustain a high growth rate of rice production. During the 1970s, semidwarf varieties played a crucial role in rice yield improvement. During the 1980s, much breeding work attempted to maintain yield potential through resistance breeding (David 199l).

Hybrid rice has brought a 15–20% higher yield potential than ordinary pure lines in farmers' fields in China, where about 16 million ha are cultivated under hybrid rice each year. In recent years, India and Vietnam have also started commercializing hybrid rice technology and several other countries are in different stages of developing this technology.

Hybrid rice has an advantage not only in yield but also in combining resistance to diseases and pests found in the parents. The dominant nature of resistance genes found in A or R lines appears to adequately confer multiple resistance in the hybrid. Hybrid rice possesses another important characteristic—adaptability to various environmental constraints, especially drought.

This chapter summarizes the current status of hybrid rice research in Thailand.

Development of CMS lines, maintainers, and restorers

Cytoplasmic male sterile (CMS) lines in Thailand were found to be unsuitable. The wild abortive cytosterility system was therefore transferred to several selected local varieties/lines. The new CMS lines now available are RD 21A, RD 25A, BS 4A, Sonalee A, IR21845A, IR17492A, KDML 105A, SPRLR 75001-68-2-2A, SPRLR 76102-26-1-1A, CNT 82001-3-2-1-1-2-1A, CNT 86003-2-1-1-1-2-1-1-1A, and BKN6-18-3-2A.

A large number of high-yielding local lines have been tested for use as restorers. Fifteen lines were identified as good restorers: RD 1R, RD 7R, RD 11R, RD 23R, SPR 60R, SPR 90R, ARC 11353R, Magali 35R, IR2797 - 125 - 3 - 3 - 2R, SPRLR 77205 - 3 - 2 - 1 - 4R, SPRLR 82058 - 19 - 1 - 1R, SPRLR 82216 - 26 - 1 - 1, SPRLR 82216 - 26 - 1 - 3, SPRLR 83136 - 14 - 2 - 2 - 1 - 1, and SPRLR 83260 - 143 - 1 - 1. Because grain quality is very important in Thailand, many of the CMS and R lines available currently do not meet the desired standards for grain quality.

Productivity of hybrids

In the 1993 wet season, 10 hybrid rice varieties derived from locally bred A and R lines were compared with four checks (SPR 60, SPR 90, RD 7, and RD 21) at Kasetsart University, Kamphangsaen, Nakhorn Pathom. The highest-yielding hybrids marginally outyielded the inbred checks SPR 90 (5.03 t ha^{-1}) and SPR 60 (5.01 t ha^{-1}). Most of the hybrid varieties performed poorly, although their grain quality was similar to that of the inbred check varieties. Later in the 1995 wet season, two trials were conducted at the Pathumthani Rice Research Center using IRRI-bred hybrids. In the first trial, one of the 22 hybrids—IR62829A/IR49735-SRN-4-B-1-4-l—significantly outyielded the best check variety by about 1 t ha^{-1} (Table 1). The grain quality of this hybrid was similar to that of the check varieties (Table 2). In the second trial, none of the 19 hybrids outyielded the check SPR 60 (4.26 t ha^{-1}) by more than 0.29 t ha^{-1}, although several hybrids were somewhat earlier in maturity; some of these hybrids compared well with the check variety for grain quality. Similar results were obtained from the yield trials of IRRI hybrids evaluated at Bangkhen. Only one of the hybrids—IR58025A/RP22378-848—significantly outyielded the highest-yielding check, SPR 60 (3.74 t ha^{-1}), by a margin of 0.8 t ha^{-1}. These results were not encouraging enough to introduce IRRI rice hybrids for direct use by Thai farmers. Therefore, it is essential to strengthen local rice breeding efforts to breed heterotic rice hybrids with acceptable grain quality using IRRI and Thai rice cultivars as parental lines. Both CMS and thermosensitive genetic male sterility (TGMS) systems will need to be deployed for the purpose, and research for developing hybrid rice seed production technology would also have to be carried out concurrently.

Table 1. Yield maturity duration and plant height of hybrids and local checks tested at Pathumthani in the 1995 wet season.

Hybrid/variety	Yield[a] (t ha^{-1})	Maturity (d)	Plant height (cm)
IR62829A/IR49461-129-3-3-3R	4.36 bcd	106	100
IR62829A/IR57312-119-2-1	4.11 b-e	105	97
IR58025A/IR50404-57-2-2-3	3.39 f-j	102	100
IR58025A/IR57284-34-3-2	3.67 d-g	105	104
IR58025A/IR42221-14-1-3-1-2R	3.91 b-e	106	106
IR58025A/IR62030-54-1-2-2	3.35 j-i	101	101
IR58025A/RP22378-848	3.95 b-e	113	107
IR58025A/BG 1370	4.44 bcd	117	116
IR58025A/IR32419-28-3-1-3R	3.64 d-h	107	106
IR58025A/IR60821-191-3-3-2-1	3.21 hij	102	100
IR58025A/IR60966-119-2-3-1-2	3.81 c-f	105	106
IR58025A/IR62030-83-1-3-2	3.81 c-f	102	107
IR62829A/IR28228-28-3-3-2	4.59 bc	112	96
IR58025A/IR33380-7-2-1-3	3.40 f-j	105	106
IR58025A/IR59656-113-1-2	3.55 e-i	102	103
IR58025A/IR62030-97-3-2-2-2	3.63 e-i	102	98
IR58025A/IR58100-97-2-1	3.08 ij	102	103
IR58025A/IR58100-79-1-3	3.66 d-g	106	103
IR62829A/IR49735-SRN-4-B-1-4-1	4.99 a	113	102
IR58025A/IR51078-33-2-1-1-3R	4.20 b-e	111	107
IR58025A/IR48751-8-8-79-31	4.52 bcd	118	113
PMS10A/IR58841-48-8-3-2	4.66 ab	125	118
RD 25 (check)	2.54 j	100	96
SPR 1 (check)	3.92 b-e	121	124
CV(%)	10.4		

[a] Values in a column followed by a common letter are not statistically different according to Duncan's multiple range test ($P = 0.05$).

Future plans

The agenda for future hybrid rice research includes the following:

- Developing A lines possessing long slender grains, intermediate amylose content, intermediate gel temperature, and soft gel consistency.
- Transferring TGMS into Thai rice cultivars to develop the two-line hybrid system.
- Developing and testing experimental hybrids derived from IRRI-bred CMS or TGMS lines and locally bred pollen parents.
- Developing hybrid rice seed production technology suited for Thai conditions.

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Table 2. Grain quality characteristics of IRRI hybrids and local checks tested at Pathumthani in the 1995 wet season.

Hybrid/variety	Physical analysis			Amylose content (%)	Chemical analysis		
	Length (mm)	Shape[a]	Chalkiness (%)		Gel consistency (mm)	Alkali test[b]	Elongation ratio[c]
IR62829A/IR49461-129-3-3-3R	6.84	SL	1.21	27	94	5.6	1.6
IR62829A/IR57312-119-2-1	6.64	SL	0.98	25	71	4.7	1.6
IR58025A/IR50404-57-2-2-3	7.03	SL	0.89	25	40	6.1	1.7
IR58025A/IR57284-34-3-2	6.91	SL	1.11	24	40	5.6	1.7
IR58025A/IR42221-14-1-3-1-2R	7.31	SL	0.60	23	65	5.0	1.6
IR58025A/IR62030-54-1-2-2	7.28	SL	0.55	22	60	4.9	1.6
IR58025A/RP22378-848	7.55	SL	0.54	26	53	7.0	1.6
IR58025A/BG1370	7.29	SL	0.95	25	70	5.2	1.6
IR58025A/IR32419-28-3-1-3R	7.03	SL	0.70	24	38	6.1	1.7
IR58025A/IR60821-191-3-3-2-1	7.31	SL	0.69	23	60	5.0	1.5
IR58025A/IR60966-119-2-3-1-2	7.49	SL	0.57	24	60	5.5	1.7
IR58025A/IR62030-83-1-3-2	7.49	SL	0.50	25	44	5.6	1.7
IR62829A/IR28228-28-3-3-2	6.88	SL	0.75	27	54	6.0	1.6
IR58025A/IR33380-7-2-1-3	7.46	SL	1.87	24	75	6.0	1.7
IR58025A/IR59656-113-1-2	7.68	SL	0.71	23	60	5.1	1.6
IR58025A/IR62030-97-3-2-2-2	7.49	SL	0.60	22	70	5.8	1.6
IR58025A/IR58100-97-2-1	7.20	SL	0.52	25	55	6.0	1.6
IR58025A/IR58100-79-1-3	7.12	SL	0.30	25	65	5.6	1.8
IR62829A/IR49735-SRN-4B-1-4-1	7.20	SL	0.72	29	95	5.4	1.6
IR58025A/IR51078-33-2-1-1-3R	7.80	SL	0.85	27	43	7.0	1.8
IR58025A/IR48751-B-B-79-3	7.46	SL	0.73	27	40	6.7	1.7
PMS10A/IR58841-48-B-3-2	6.79	I	1.08	30	95	6.3	1.6
RD25	7.26	SL	0.61	29	78	7.0	2.0
SPR1	7.26	SL	0.68	30	95	5.0	1.8

[a] SL = slender, I = intermediate. [b] On a scale of 1 to 7, where 1 = not affected but chalky and 7 = completely dispersed and clear. [c] Length to breadth.

CHAPTER 36

Research and development for hybrid rice technology in Indonesia

B. Suprihatno and dan Satoto

Hybrid rice research in Indonesia began in 1983, with the initial objective to explore the prospects and problems of using this technology. During the past 15 yr, although the yield advantage of hybrids over inbred rice has been established, no hybrid has been released and recommended for cultivation, primarily because of the nonavailability of a commercially usable cytoplasmic male sterile line. During the past few years, government support for the program has been reduced. Currently, some promising F_1 hybrids are in the pipeline and might qualify for release by 1998. Hybrid rice seed production still faces some problems, especially in the synchronization of flowering of parental lines. Seasonal variation within a location is believed to be one of the factors affecting the flowering behavior of parental lines. Therefore, identification of suitable locations having more stable weather conditions is needed.

Rice production in Indonesia increased steadily from 12.3 million t in 1970 to 25.8 million t in 1985. This tremendous production increase has made Indonesia self-sufficient in rice since 1984 (Suprihatno 1986). Rice production in 1993 was 47.8 million t. Table 1 shows the trend in rice production, harvested area, and productivity during the Fifth Five-Year Development Plan (1988-93). The implementation of a special intensification program and the use of improved high-yielding varieties are the major factors contributing to this success. In this special intensification program, farmers usually apply very high inputs to harvest a maximum yield. Using currently available rice varieties, increases in production can be achieved only up to a certain level until all rice area is covered by this program. A further increase in production may not be possible unless varieties with a higher yield potential are used.

It appears that the rice yield potential attained through conventional breeding methods and strategies has reached a plateau. Many rice breeding programs around the world continue to maintain and stabilize rice yield per unit area by incorporating

Table 1. Trends in rice production in Indonesia, 1988-93.

Year	Area harvested (000 ha)	Production (000 t)	Productivity (t ha^{-1})
1988	10,138	41,676	4.11
1989	10,531	44,726	4.25
1990	10,502	45,179	4.30
1991	10,282	44,688	4.35
1992	11,103	48,240	4.35
1993	10,930	47,885	4.38

Source: Ajid 1994.

desired characters such as resistance to pests and diseases and tolerance of environmental stresses. Crop management systems are concurrently developed to make a high-yielding rice variety reach its potential. These efforts, however, do not increase the yield potential of a variety. Experience from China and the International Rice Research Institute (IRRI) has indicated that the use of hybrid rice can help to push rice yields beyond the levels of semidwarf high-yielding inbred rice varieties. We have been exploring the prospects for hybrid rice to increase yields in irrigated lowlands and intensive farming systems in Indonesia. The current status of research on and development of hybrid rice technology in the country is reported in this chapter.

Recent progress

Evaluation of CMS lines

The first set of Chinese cytoplasmic male sterile (CMS) lines introduced into Indonesia in 1980 through IRRI included ZS 97A, V20A, V41A, Er Jiu Nan 1A, and Wu 10A. Subsequently, IRRI-bred CMS lines were periodically introduced. Most of the Chinese CMS lines were not suitable for developing hybrid rice in Indonesia. Although stable for pollen sterility, these lines were susceptible to major tropical pests and diseases, particularly sheath blight.

IRRI-bred CMS lines IR46826A, IR46827A, IR46828A, IR46829A, IR46830A, IR46831A, and IR48483A were evaluated in Indonesia. In these tests, IR46828A, TR46830A, and IR48483A showed broad adaptability. Virmani et al (1985 reported that pollen sterility of IR46828A and IR46830A was not affected by environment. But these lines showed poor combining ability and a low outcrossing rate. IR54752A, IR54753A, and IR54754A were also evaluated later. IR54752A was identified as a good female parent and was resistant to some major pests and diseases. Pollen sterility of this line, however, was not stable because hybrids derived using this male sterile line were nonuniform, possessed numerous sterile plants, and therefore showed negative heterosis.

Currently, the most promising IRRI-bred CMS lines are IR62829A, IR58025A, and IR29744A (Suprihatno et al 1994). IR62829A and IR58025A, which are genetically similar, are stable for pollen sterility, are early maturing, have semidwarf plant

height, and are uniform. IR62829A, however, was susceptible to bacterial leaf blight and tungro virus diseases and also had a low outcrossing rate (17%). But IR29744A, which was developed in Indonesia, was better than IR62829A in terms of its resistance. IRRI reported that IR58025A was stable, and was found to be segregating in Indonesia.

The next batch of CMS lines introduced from IRRI involved IR64607A, IR64608A, and IR66707A (origin IRRI), and Krishna A and Pragathi A (origin India). These CMS lines were evaluated at Sukamandi in the 1991 dry season. All these lines also showed high sterility and good agronomic traits (Suprihatno et al 1994). In 1994, some additional CMS lines were introduced from IRRI. These showed very high sterility: 99% for pollen and 100% for spikelets (Table 2). CMS lines Tondano A and M8601A were also developed in Indonesia (Suprihatno et al 1994).

Table 2. Performance of some new IRRI CMS and maintainer lines in Sukamandi, 1994 wet season.

Lines	CMS			Maintainer		
	Days to 50% flowering	Pollen sterility (%)	Spikelet sterility (%)	Days to 50% flowering	Pollen fertility (%)	Grains panicle^{-1} (no.)
IR19809	88	98	100	86	99	126
IR58025	96	99	100	97	98	121
IR62829	92	99	100	93	98	123
IR64607	99	99	100	97	99	132
IR67683	104	99	100	104	100	120
IR67684	97	100	100	98	98	129
IR68275	92	99	100	95	99	129
IR68279	106	98	99	103	97	132
IR68281	95	99	100	95	98	134
IR66282	84	98	100	84	99	146
IR68882	88	99	100	89	100	137
IR68886	91	99	100	87	100	143
IR68887	95	99	100	95	98	132
IR68889	97	99	100	111	99	129
IR68890	100	98	100	102	100	144
IR68891	95	98	100	96	98	139
IR68892	87	98	100	85	100	139
IR68893	91	99	100	89	99	125
IR68894	89	99	100	90	100	130
IR68895	90	99	100	90	99	116
IR68896	97	99	100	95	100	128
IR68897	92	99	100	91	100	139
IR68898	89	99	100	89	99	129
IR68899	103	99	100	100	100	129
IR68900	98	99	100	97	100	120
IR68901	84	99	100	84	99	116
IR68902	100	99	100	97	100	123
Tondano	98	97	99	97	97	107

Evaluation of F_1 hybrids

In most of the yield trials conducted, some F_1 hybrids always outyielded the best check (Suprihatno 1986, Suprihatno and dan Satoto 1986). Results (Table 3) from yield trials conducted during 1992-96 further confirmed these findings. Most of the hybrids derived from IR58025A produced grain yields higher than that of hybrids derived from IR62829A or other CMS lines. Therefore, it is evident that this CMS line is a good general combiner. Moreover, this CMS line also possesses good characters such as aroma and good grain quality, and moderate resistance to some major pests and diseases.

Results from the experiment conducted at Kuningan in I994 indicated that three F_1 hybrids—IR58025A/BR827, IR58025A/IR53942, and IR58025A/IR54852—yielded more than 7 t ha^{-1} of grain, thus outyielding the check IR64 by 30–40%. In 1995, hybrid IR62829A/BR736 yielded 8.1 t ha^{-1}, which was about 18% higher than IR64 at Kuningan. In 1996, IR58025A/IR53942 yielded higher than Memberamo, a newly released Indonesian improved inbred variety, by 17% and 27%, respectively, at two locations, Tegalgondo (Central Java) and Sukamandi (West Java).

In the test-cross nursery, out of 170 test crosses evaluated, six were highly sterile; therefore, their male parents—H 270-30-2-1-1 (5387), RP 2095-1-10-22 (5408), S 3115e-5, S 3066-3d-Pn-3-1, S 2824e-Kn-14, and IR54017-131-1-3-2—were classified as suspected maintainers and used as recurrent parents, converting them into CMS lines. The backcross nursery consisted of materials in the BC_1 to BC_8 generation (Table 4).

Table 3. Yields of promising experimental hybrids versus the best check varieties in replicated yield trials in Indonesia, 1992-96.

Hybrids	Year	Yield (t ha^{-1})	% of best check	Check	Location
IR29744A/Sadang	1992	5.20	140	IR64	Sukamandi
IR29744A/IR64	1992	4.90	132	IR64	Sukamandi
IR58025A/IR64	1992	4.70	127	IR64	Sukamandi
IR19774A/IR54	1992	4.80	129	IR64	Sukamandi
IR58025A/IR72R	1993	6.18	105	IR64	Sukamandi
IR58025A/BR 827	1994	5.89	131	IR64	Sukamandi
IR58025A/IR53942	1994	6.48	131	IR64	Batang
IR58025A/IR53942	1994	7.76	139	IR64	Kuningan
IR58025A/BR 827	1994	7.84	140	IR64	Kuningan
IR58025A/IR54852	1994	7.27	130	IR64	Kuningan
IR58025A/IR54852	1994	5.74	116	IR64	Batang
IR62829A/BR 736	1995	8.09	118	IR64	Kuningan
IR62829A/IR58100	1995	7.43	108	IR64	Kuningan
IR58025A/BG 1370R	1996	6.80	130	IR64	Kuningan
IR58025A/IR53942	1996	7.10	112	Cibodas	Tegalgondo
IR58025A/IR53942	1996	7.10	117	Memberamo	Tegalgondo
IR58025A/IR53942	1996	7.10	136	IR64	Tegalgondo
IR58025A/IR53942	1996	4.83	102	IR64	Sukamandi
IR58025A/IR53942	1996	4.83	116	Cibodas	Sukamandi

Table 4. Lines in various backcross generations to develop new CMS lines, Sukamandi, 1995 dry season.

Cross combination	Source	Backcross generation	Days to 50% flowering
IR62829A/Danau Tempe	TCN 5115	1	83
IR62829A/IR2936e-9	TCN 5145	1	83
IR62829A/S1304-1e-Pn-2	TCN 5147	1	91
IR29744A/RP1515-22-3-1	BCN 3709	3	83
IR29744A/Zhangyu 87-3	BCN 3711	3	83
V20A/S1304-1e-Pn-2	BCN 3717	4	86
IR62829A/IR29519-74-3-1-2	BCN 3707	5	86
V20A/S2150-lb-5	BCN 3721	7	84
V20A/IR36781-AC-1-1	BCN 3723	7	86
IR54752A/S992b-Pn-8-2	BCN 3727	7	84
IR54752A/New Bonnet	BCN 3731	8	83

Table 5. Performance of TGMS lines evaluated at Sukamandi (Ski) and Kuningan (Kng), 1993 dry season.

Lines	Pollen sterility (%)		Filled grains panicle^{-1} (no.)		Spikelet steriltty (%)		Days to 50% flowering		Plant height (cm)		Panicle number	
	Ski	Kng	Ski	Kng	Ski	Kng	Ski	Kng	Ski	Kng	Ski	Kng
IR32364	93.5	71.8	63.0	–	96.2	45.1	104	106	103	85	15.8	10.8
IR68293	83.7	70.1	28.0	–	72.2	38.1	73	75	109	73	8.6	16.2
IR68294	47.3	74.1	15.2	–	90.8	53.2	95	98	107	92	7.2	16.6
IR68295	34.1	64.9	25.5	–	20.3	16.1	67	70	86	65	12.0	19.4
IR68296	97.4	69.4	31.0	–	98.6	40.3	71	74	92	77	5.0	98.0

Evaluation of TGMS lines

Five IRRI TGMS lines were introduced into Indonesia in 1993. These TGMS lines were evaluated at Sukamandi and Kuningan in the 1993 dry season, assuming that the temperature at Sukamandi was high enough and at Kuningan low enough for inducing sterility and fertility, respectively. Observations were made on both pollen sterility and fertility. Two of the TGMS lines, IR32364 and IR68296, showed high pollen sterility at Sukamandi but partial sterility at Kuningan (Table 5). In the absence of temperature data during panicle initiation to flowering, however, no valid conclusion could be drawn on their thermosensitivity.

Government support

Hybrid rice breeding in Indonesia has made slow progress because of a lack of commercially usable CMS lines. CMS line IR62829A is good, stable, and highly uniform, but it is susceptible to bacterial blight and its outcrossing rate is still considerably low. IR58025A is also stable and more resistant to tungro than IR62829A, but seemed to be still segregating in height. Some newly introduced CMS lines have been found to be stable and phenotypically acceptable. The slow progress of hybrid rice

research during the past 15 years has discouraged the government of Indonesia from investing heavily in it. Since 1993, the budget for hybrid rice research has been reduced drastically. The extent of future support will depend on whether some promising hybrid combinations can be recommended soon. With the available promising hybrid combinations, we hope to recommend at least one hybrid in the near future.

Seed production

Experience in producing hybrid rice seeds has indicated that flowering behavior of the parental materials (CMS and restorer) is affected by seasonal variations and locations. The monsoon in regions south of the equator such as Java is erratic in some years. This erratic monsoon condition affects the flowering behavior of rice varieties. Consequently, synchronization of flowering between CMS and restorer lines in the same season but in different years is difficult to attain. This reduces seed set and hence lowers seed yield. Locations suitable for hybrid rice seed production need to be identified soon so that successful seed production can be planned.

To anticipate the development of hybrid rice and to fulfill the need for hybrid rice seeds, the Directorate of Food Crops Production has made an arrangement for some personnel of the Provincial Seed Farm of Cihea (West Java), Tegalgondo (Central Java), and Karangsuko (East Java) to receive some preliminary training on hybrid seed production techniques at Sukamandi. Six people from the three provinces visited Sukamandi for 5 days and studied hybrid rice seed production techniques. Now, with supervision by the Research Institute for Rice, they are also involved in the seed production of one or two hybrid combinations to gain experience.

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CHAPTER 37

Recommendations of the 3rd International Symposium on Hybrid Rice

The global requirement for rice by 2020 is expected to be around 800 million t compared with current production of 520 million t. With shrinking resources—particularly arable land area, irrigation water, and energy—the only option left is to increase production. Increasing rice production by 300 million t during the next 25 yr is a challenging task. Of the possible genetic approaches to meet this challenge, hybrid rice technology is an immediate option because it has been a proven technology over the past two decades in China and now has commercial prospects in India.

The theme of the 3rd International Symposium on Hybrid Rice was "enhancement and sustenance of hybrid rice technology." The symposium was cosponsored by the Indian Council of Agricultural Research (ICAR), the United Nations Development Programme (UNDP), and the International Rice Research Institute (IRRI). About 150 Indian delegates and 50 international delegates from 20 countries addressed various aspects and issues for improving this technology and making it available outside of China. These issues were discussed in six sessions (current scenario, increasing breeding efficiency and enhancing yield heterosis, sustainability of hybrid rice technology, tissue culture and molecular approaches in heterosis breeding, toward true-breeding hybrids, and status of development and adoption of hybrid rice technology in various countries) and at a meeting of the International Task Force on Hybrid Rice. During the sessions, the following major recommendations emerged.

Technology development

- The formation of a research network and effective collaboration with international agencies have been the main reasons for the remarkable success achieved in India. Use these approaches as models for similar achievements in other countries of South and Southeast Asia.
- Intensify efforts to develop hybrid rice technology in countries such as India, the Philippines, Vietnam, and Indonesia, where there is a demand for hybrid rice seeds and the capability to produce them.

- To meet consumer preferences, emphasize improving milling, head rice recovery, and other quality characteristics.
- Especially emphasize incorporating resistance to major pests and diseases in promising parental lines.
- To enhance the level of heterosis, the Chinese are successfully using two-line hybrids—photoperiod-sensitive genic male sterility (PGMS) and thermosensitive genic male sterility (TGMS)—and are developing hybrids through intersubspecific crossing of indicas and japonicas. Vigorously pursue these approaches in the tropics using indica/tropical japonica lines. Countries that grow japonica rice should be exploring the prospects for temperate japonica/tropical japonica hybrids.
- To fully exploit rice hybrids, develop special management practices that promote the efficient use of nitrogen, phosphorus, and other nutrients, and water.
- Improve parental lines by using random-mating populations—the private sector particularly should pursue this to minimize dependence on the public sector for the supply of improved parental lines.
- Develop rice hybrids adapted to different ecosystems—especially for the shallow lowlands, which are very similar to the irrigated ecosystem.
- Intensify research to develop rice hybrids for the boro season (India, Bangladesh) and the spring season (Vietnam, Myanmar).

Technology transfer

- To speed up large-scale adoption, create awareness and demand for hybrid rice by conducting extensive on-farm trials, front-line demonstrations, and training programs.
- Expeditiously develop mechanisms for the registration of parental lines so that they can be shared freely among collaborating countries.

Seed production

The higher cost of hybrid seed is a constraint to adoption of the technology. The following measures are recommended to reduce prices to affordable levels.

- Intensify studies on proper flowering synchronization of parental lines to get higher seed yields in the target areas. Low seed yield is a major problem faced by seed growers.
- Emphasize producing and supplying parental lines with only the highest purity; identify suitable agencies to perform this task.
- Strengthen the breeding of cytoplasmic male sterile (CMS) lines (possessing higher outcrossing potential) and restorers (providing high pollen load).
- Identify in each country locations and seasons that are most favorable for seed production.
- Economize the use of gibberellic acid (GA_3) and simultaneously intensify the search for cheaper alternatives.

- Develop special management practices to obtain higher seed yields.
- Wherever feasible, involve interested nongovernment organizations in producing hybrid rice seed.
- Encourage governments to develop policies that aggressively advocate private-sector participation in hybrid rice seed production and research.
- Conduct mass-scale in-country training on hybrid seed production.

Basic studies

- Initiate intensive work to identify molecular markers associated with quantitative trait loci (QTLs) for yield heterosis and subsequently incorporate these heterotic blocks into the parental lines.
- Speed up work on apomixis as a long-term strategy by using all possible tools, including genetic engineering.

International Task Force on Hybrid Rice (INTAFOHR)

The establishment of INTAFOHR was first proposed during the 2nd International Symposium on Hybrid Rice at IRRI in 1992 to promote the technology outside of China. Subsequently, this was endorsed by several countries in various international forums organized by FAO and IRRI. In October 1995, IRRI and FAO convened a joint meeting with country representatives and prospective donors (UNDP, Asian Development Bank, and MAHYCO Research Foundation of India). The participants concluded that establishing INTAFOHR involving IRRI-FAO and the national agricultural research systems (NARS) was an excellent idea and the prospective donors asked IRRI to submit a project profile for their consideration. After this was done, the donors asked IRRI to submit a detailed project proposal for possible funding. During the 3rd symposium, a panel discussion was held, attended by the representatives of 16 countries, during which a consensus was reached on the following points:

Goal

Improved food security and sustainable development through increased rice production using hybrid rice.

Objectives

- Promote the free exchange of registered germplasm, information, and data from ongoing research and development (R&D) programs on hybrid rice among interested partners.
- Strengthen national systems' capabilities for applied and strategic research so that they can develop hybrid rice technology expeditiously.
- Intensify collaborative strategic research on hybrid rice by establishing effective regional and interregional collaboration on hybrid rice R&D.
- Assist member countries in formulating appropriate policy incentives for hybrid rice development and use.

- Strengthen the hybrid seed industry and the linkage between it and hybrid rice research centers.

Participation

Participation in the task force is open to all interested countries.

Strategy

General framework. Development of hybrid rice technology in member countries, particularly the charter members, will be expedited by (1) establishing goal-oriented hybrid rice R&D programs aiming to strengthen human resources for R&D of hybrid varieties and hybrid seed production capacity, (2) establishing collaborative research linkages, and (3) freely exchanging breeding materials and information.

Technology transfer will be expedited by strengthening on-farm testing, promoting technology through appropriate channels, and identifying policy interventions, and by respective governments encouraging investment in hybrid rice research and seed production.

Phase-wise development. In recognition of the conditions governing rice production and the situations concerning research, development, and the use of hybrid rice in different countries as well as the need to obtain time-bound outputs to sustain the activities of the task force, it will initially have three categories of membership: (1) charter (or core) members, (2) observers, and (3) affiliate members. All members will gradually become core members as conditions permit.

The charter-member countries should have the following features:

- Rice is an important crop and planted on a large area so that an economically viable local hybrid seed industry is possible.
- Hybrid rice research, development, and use have been adopted as national priorities.
- Staff and facilities are adequate to make effective and positive contributions to the task force activities, especially in terms of exchange of germplasm, information, and data.

Bangladesh, India, Indonesia, the Philippines, and Vietnam agreed to participate as charter-member countries. China and Sri Lanka agreed in principle to serve as charter-member countries but first need to get approval from their respective governments. Brazil, Colombia, Egypt, and Thailand agreed to participate as observers. Australia, Japan, and the United States agreed to participate as affiliates.

Implementation. IRRI, possessing a strong multidisciplinary hybrid rice research program linked with several NARS, will be the executing agency that coordinates task force activities—especially for technical assistance in developing hybrid rice technology and establishing necessary collaboration with selected institutions that have advanced programs on strategic research for hybrid rice development.

FAO, having expertise in agricultural development, will provide guidance and technical backstopping for seed industry development and transfer of hybrid rice technology in member countries. FAO will also facilitate institutional linkage among agen-

cies dealing with hybrid rice research and seed production within and among member countries.

Member countries will designate respective national coordinators to work collaboratively with IRRI, FAO, and other member countries to implement the work plans as prepared and adopted in annual meetings of the task force.

Management. The task force should be a component of the Council for Collaborative Research in Asia (CORRA) and should be managed on the pattern of the rainfed and upland rice consortia already in operation at IRRI in collaboration with several NARS.

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