HYBRID RICE

INTERNATIONAL RICE RESEARCH INSTITUTE

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The International Rice Research Institute (IRRI) was established in 1960 by the Ford and Rockefeller Foundations with the help and approval of the Government of the Philippines. Today IRRI is one of the 13 nonprofit international research and training centers supported by the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), and the United Nations Development Programme (UNDP). The CGIAR consists of 50 donor countries, international and regional organizations, and private foundations.

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The responsibility for this publication rests with the International Rice Research Institute.

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Foreword

After the People's Republic of China announced the successful development and use of F_1 rice hybrids in the late 1970s, hybrid rice technology attracted the attention of researchers around the world. The yield advantage of hybrid rice, currently about 20% higher than that of the best semidwarf commercial varieties, would help meet future rice demands of a growing world production.

China's initial success led IRRI to intensify research on hybrid rice, particularly on the potentials and problems of developing the technology for the tropics. Subsequent progress in China and at IRRI encouraged several other countries to take a critical look at the prospects for hybrid rice.

The International Symposium on Hybrid Rice was held 6-10 October 1986 at the Hunan Hybrid Rice Research Center, Changsha, China, where Prof. Yuan Longping and his coworkers developed hybrid rice. Participants assessed the status of hybrid rice technology in China and explored the prospects for hybrid rice in other countries. This book documents the information exchanged, and presents for the first time, in English, much of the Chinese literature on hybrid rice.

I am grateful to our cosponsors, the Hunan Provincial Association for Science and Technology and the Hunan Hybrid Rice Research Center, for their contributions to the technical program. Financial support from the Government of Italy to IRRI's Hybrid Rice Project for cosponsorship of the symposium and publication of the proceedings is gratefully acknowledged.

The organizing committee was composed of Yuan Longping, M. S. Swaminathan, Huang Chungrong, and Ren Zhi, cochairmen; and committee members D. L. Umali, E. Simura, J. N. Rutger, Min Shaokai, M. Mahadevappa, H. P. Moon, and B. Suprihatno. They were ably assisted by Mao Changxiang and Bai Jianwei, executive secretaries, whose logistical talents contributed immeasurably to the success of the Symposium. I especially want to recognize the efforts of Yuan Longping and Sant S. Virmani, whose duties as technical secretaries involved Symposium planning and organization, and technical review of the published papers.

This proceedings volume was edited by W. H. Smith, editor, and Lloyd R. Bostian, visiting editor, with the assistance of Emy Cervantes, assistant editor.

Klaus Lampe
Director General

Recommendations

The First International Symposium on Hybrid Rice, held at Changsha, Hunan, China, 6-10 Oct 1986, was attended by about 220 delegates and observers from 17 countries. Nine technical sessions over the 4-d meeting covered many aspects of hybrid rice, emphasizing 1) heterosis, 2) male sterility and fertility restoration, 3) outcrossing mechanisms and hybrid seed production, 4) breeding procedures, 5) disease and insect resistance, 6) grain quality, 7) cultural management of hybrids, and 8) physiological and genetic research in hybrids.

The current status of hybrid rice research in China, India, Indonesia, Italy, Japan, Malaysia, Mexico, Republic of Korea, and the U.S. was reported. Regional prospects of hybrid rice for Asia and the Pacific Region were also discussed.

Three task forces were arranged to make recommendations on

- target environments,
- directions for future research, and
- mechanisms for collaborative research.

Target environments

In China, hybrid rice is a cost-reducing, yield-increasing innovation. It has contributed to national food self-sufficiency in the short run and provides a long-run perspective for Asia to feed itself as population pressures on arable land increase.

The major success of hybrid rice has been in subtemperate China, but varieties adapted to the tropics are rapidly being developed. These materials must be widely tested in situations including farmer-realistic conditions.

Hybrid rices can increase efficiency of rice production in times of low and stagnant rice prices. Therefore, adoption of hybrid rices might reduce total rice area, leading to opportunities and pressures to diversify into other crops, livestock, and household industry to increase the earning capacity of rice households. Thus, research and policies that encourage agricultural diversifcation should be promoted by national programs together with hybrid rice research.

Focus by rice culture type

1. China is emphasizing development of early-duration (<115 d) hybrids suitable for the first crop season in double rice cropping areas.
2. Outside China, irrigated and favorable rainfed areas would be the primary ecological target for hybrid rice for these reasons:
   a. Yields are already high in these areas, so F_1 hybrids could further raise yield ceilings.

   b. Infrastructure in these regions is best developed to aid hybrid seed production and distribution.
   c. Farmers in irrigated areas probably are most able to meet the cost of hybrid seed purchases each crop season.
3. With their vigor, hybrid rices have a largely unquantified potential in adverse rice environments (drought, problem soils). Major constraints to the spread of hybrids in these environments may include
   a. lack of research and institutional infrastructure to promote or justify hybrid seed production and distribution;
   b. less capacity among upland rice farmers to purchase hybrid seed each year.

   Brazil is an exception to this general position.
4. Research management strategies to maximize the combined benefits of conventional and hybrid rice improvement and management were not discussed in the symposium.
5. Delegates recognized that the viability of hybrid rice technology would be conditioned by technical (gains in yields and yield stability), economic (profitability, marketability), organizational (hybrid seed production, infrastructure), and policy (seed regulation) determinants. These factors tend to be country specific. Thus, their possible impact needs to be assessed on a country-by-country basis.

Quality

The first developed hybrids had poor grain quality because of the narrow range of parents available. Now, breeding for quality is becoming more manageable as the range of parent materials increases. The conference recognized that grain quality is critical to ensure producer and consumer acceptance of hybrid rices.

Hybrid seed production

The success of hybrid rice will depend on establishment of viable hybrid seed production systems. (This point is considered in greater detail later.) The participants believed that hybrid rice would be best served by government continuing to accept major responsibility for upstream research in breeding and crop management, with the commercial sector accepting major responsibility for market-specific hybrid seed production, distribution, and marketing. Thus, the public and private sector may be partners in hybrid rice research, production, and extension.

In addition to upstream research, a major government responsibility would be legislation to guide the hybrid seed industry. The hybrid seed industry would provide self-regulation to ensure farmers get quality seed at equitable prices.

Distribution of benefits

1. The principal beneficiary of hybrid rice will be consumers able to buy rice at lower prices than would prevail without this innovation. Hybrid rice will therefore contribute to future reductions in poverty-induced malnutrition. The technology may also contribute to human welfare through job creation, particularly in labor-intensive hybrid seed production.

2. Hybrid rice programs are likely to benefit the favorable rice environment most, further increasing the gap between favorable and unfavorable rice ecologies. Stress-tolerant hybrids may technically allow productivity increases in unfavorable environments, but farmers there, with given institutions, market, and infrastructure, may be least able to benefit from these technologies.
3. The distribution of benefits of hybrid rice technology should be carefully studied to identify opportunities to promote equitable benefit-sharing between producers and consumers. Such studies are encouraged in China, the only country where hybrid rice is produced commercially. These insights would be most valuable to other nations as they consider the broader implications of a hybrid rice program as a component of their national development strategies.

Directions for future research

Spectacular progress in the development and use of F_1 hybrid rice has been achieved in China in the last decade. These successes have stimulated research on hybrid rice by IRRI and several national programs to explore the potentials of hybrids outside China. This symposium has provided an excellent forum for discussion of current research areas.

On the basis of the discussions, the following were identified as priority areas for future research.

Methodology of hybrid development

Although three-line breeding is expected to dominate over the next 10 yr, the two-line method shows great promise. The one-line method, however, is considered the most worthwhile long-term breeding goal.

Three-line method. In the long run, three-line breeding will likely be replaced by simpler (2-line or 1-line) systems. Meanwhile, research on the 3-line system is needed:

1. Diversification of CMS systems to reduce potential genetic vulnerability due to widespread use of a single (WA) system.
2. Adoption of standard nomenclature for CMS sources.
3. Development of improved CMS and restorer lines possessing improved disease and insect resistance and improved grain quality.
4. Testing elite lines coming from interspecific crosses. These lines may have unique gene blocks which may result in greater heterosis.
5. Evaluation of CMS sources for positive or negative effects on disease and insect resistance and on agronomic characters.
6. Determination of photoperiod and thermoperiod effects on seed production characteristics of CMS lines.

Two-line method. The two-line method shows great promise for the next decade.

Efforts should be intensified to identify photoperiod-sensitive genetic male steriles. The value of the two-line system is that any normal line can serve as a restorer. Such material should be tested under different environments. Male sterile mutants should be evaluated under different photoperiods.

Chemical emasculation offers several advantages. First, any two lines can be used as parents. Second, chemical emasculation can be used as a breeding tool to produce enough hybrid seeds to conduct combining ability and preliminary yield trials. This would allow breeders to identify promising combinations before embarking on line conversion.

Chemicals can also be used to modify the floral characters of rice which affect outcrossing.

One-line method. Apomixis, or asexual seed production, is the ultimate genetic tool for developing true breeding hybrids with permanently fixed heterosis. Appropriate application of apomixis in rice would provide the breakthrough needed for more of the world's rice farmers to economically capture the increased yields of hybrids. Development of apomictic rice will require biotechnological research.

Other basic research needs

1. Determination of molecular basis of CMS.
2. Use of anther culture for purifying R lines and to produce gametoclonal variation for converting R lines to B lines.
3. Root characteristics of hybrid rice.
4. Use of hybrids to identify physiological and morphological factors, such as large embryo size, that are responsible for faster growth, more biomass, and higher yield. Once identified, these factors can be used to develop better hybrids or conventional varieties.

In these basic research needs, biotechnology can help in

- apomixis,
- molecular basis of CMS,
- development of new sources of CMS via somatic hybridization/protoplast fusion, and
- somatic embryogenesis for hybrid seed production, as an alternative to large-scale seed production in the field.

Raising the level of heterosis

1. For intervarietal heterosis, identify new combinations through combining ability studies including chemical emasculation.
2. Develop improved techniques for prediction of heterosis. For example, isozyme diversity among potential parents may aid in choosing heterotic combinations.
3. Use of heterosis from indica/japonica hybrids. The wide compatibility genes should aid this.
4. Use hybrid vigor from wide crosses.

Development of practices for growing hybrids

1. Fertilization—investigate whether hybrids require less nitrogen.
2. Canopy arrangement and plant spacing.
3. Planting methods, including direct sowing.

Field tolerance

Use of hybrid vigor to impart field tolerance for diseases and insects.

Economics of hybrid rice production

Use of economic modeling of different production systems to determine the best use of expensive hybrid seed.

Mechanisms for collaborative research

The symposium noted the excellent progress in hybrid rice research and development in China spearheaded by the Hunan Hybrid Rice Research Center (HHRRC) and intensive efforts to further strengthen the program. The symposium also noted the coordinating role that IRRI has been playing in promoting hybrid rice research. It further recognized that hybrid rice programs in rice-growing countries other than China are, by and large, in their infancy. It was appreciated that China, IRRI, and other organizations are willing to make available technical assistance to supplement efforts of countries in developing their hybrid rice programs.

The symposium recognized that several constraints to hybrid rice are common in most countries. The symposium also recognized that similar approaches may be used to solve the constraints. Therefore, the symposium recommended establishing a collaborative program to ensure effective participation of countries interested in hybrid rice and to increase their overall capability for research and development in hybrid rice.

Constraints

The common constraints identified are

1. Lack of outstanding and stable male sterile lines, especially in the tropics.
2. Lack of strikingly superior and stable hybrid varieties.
3. Lack of effective restorer lines in japonica rices.
4. Inadequate infrastructure and governmental support for hybrid rice research and development.
5. Inadequate and sometimes poor arrangements for production and distribution of hybrid rice seed.
6. Lack of trained manpower for undertaking hybrid rice research.
7. Inadequate information on relevant genetic resources, A, B, and R lines and hybrid combinations, as well as on research on hybrid rice in different countries.

The network

The symposium suggested that a collaborative technical network among rice-growing countries should provide the necessary framework for alleviating these constraints.

National level

1. In close linkage with its programs on inbred rice, each participating country needs a multidisciplinary national unit on hybrid rice research and development with scientists working full time on hybrid rice.

2. Hybrid rice seed production programs should be initiated simultaneously with hybrid rice breeding programs to avoid time lag in hybrid rice production in farmers' fields.

International level

1. Following the pattern of international rice testing programs, international cooperative trials of A, B, and R lines and F_1 hybrid combinations should be undertaken. A monitoring tour of the international trials should be organized.
2. Workshops, seminars, and symposiums should be organized periodically to facilitate exchange of information and critical examination of prospects and problems of heterosis breeding in rice.
3. Relevant germplasm stocks including wild rices in countries should be catalogued and made available to interested scientists to facilitate exchange of germplasm. Genetic resources conserved at the International Rice Germplasm Center at IRRI should be used to augment breeding material in countries. Germplasm exchange among countries should be promoted.
4. Centers of excellence on specific components of hybrid rice research should be identified, such as hybrid rice breeding methodology in China; development of A, B, and R lines with multiple disease and insect resistance at IRRI; use of anther culture in development A, B, and R lines in China; and genetic manipulation for strengthening the japonica hybrid program in Japan (use of wide compatibility gene, $S\text{-}5^n$). Such centers should lead research on identified topics; information and material they generate should be shared with all cooperating countries. This approach will avoid duplication of efforts.
5. Collection, collation, and dissemination of information are vital network activities. The symposium recommended that IRRI, in close collaboration with the HHRRC, publish a newsletter on hybrid rice. The scientists were urged to regularly feed information to the clearinghouse.
6. To solve the lack of trained manpower, training courses at national, subregional, regional, and international levels should be organized on different aspects of hybrid rice research and development. A training manual on hybrid rice should be prepared jointly by Yuan Long Ping and S. S. Virmani and made available to interested scientists.
7. The network should maintain close linkages with other relevant international programs and networks such as those of FAO as well as through bilateral programs. This will avoid duplication and ensure judicious use of scarce resources.
8. The symposium recommended that Yuan Long Ping of HHRRC and S. S. Virmani of IRRI coordinate the proposed cooperative technical network on hybrid rice.

Status of hybrid rice research and development

L. P. Yuan and S. S. Virmani

China's commercially produced hybrid rices yield about 20% more than the best conventional varieties. This has encouraged rice breeders at IRRI and in some national programs to explore the potentials of hybrid rice, especially under irrigated conditions. Initial results indicate prospects of increasing rice yields by about 1 t/ha.

Genetic tools (cytoplasmic male sterile [CMS], maintainer, and restorer lines) essential to develop rice hybrids are available; however, one cytosterility source, wild abortive (WA), is used mostly, making hybrid rice potentially vulnerable to a disease or insect epidemic. Chinese and IRRI scientists are developing new CMS lines with diverse cytosterility systems. CMS lines developed in China are not suitable for the tropics because of their susceptibility to major diseases and insects. Therefore, IRRI scientists are developing new CMS lines adapted to the tropics. The frequency of restorer lines in indica rices is fairly high (20%) but japonica rices have practically no restorers.

Hybrid seed production techniques have been well developed in China and seed yields of 1-3 t/ha are being obtained. These techniques are being adopted in countries outside China.

Grain quality of rice hybrids is receiving attention.

Major problems of hybrid rice are high seed cost, the need to change the seed every crop season, continuous dependence on off-farm sources for seed supply, and the need for an efficient infrastructure to produce and supply good-quality seeds. The spread of hybrid rice in China indicates these problems have not constrained its development. However, outside China, adoptions of this technology would depend on yield advantage of hybrids compared to the best available conventional varieties and a country's capacity to organize efficient seed production, processing, certification, and distribution.

Jones (1926) was the first in USA to report heterosis in rice which has been exploited by Chinese scientists in developing hybrid rice. Subsequently, other reports indicated significant heterosis for various agronomic traits in rice (Chang et al 1973, Davis and Rutger 1976, Virmani et al 1981, Virmani and Edwards 1983). Suggestions of developing F_1 rice hybrids to exploit heterosis commercially have been made by rice scientists in India (Richharia 1962, Swaminathan et al 1972), China (Yuan 1966), U.S. (Stansel and Craigmdes 1966, Craigrmiles et al 1968, Carnahan et al 1972), Japan (Shinjyo and O'mura 1966), and IRRI (Athwal and Virmani 1972). However, difficulties anticipated in hybrid seed production discouraged most of these researchers, except the Chinese, from continuing their efforts.

China is the first country to commercially exploit heterosis in rice. Research on hybrid rice began in 1964. The genetic tools (cytoplasmic male sterile [CMS], maintainer, and restorer lines) essential to develop F_1 rice hybrids were successfully developed in 1973. Hybrids with strong heterosis were identified in 1974 and seed production techniques were primarily established in 1975. In 1976, hybrid rice was released to farmers. Since then, the area of hybrid rice in China has increased rapidly each year.

IRRI revived research on hybrid rice in 1979 to explore its yield potential, an essential focus because of the anticipated increase in demand for rice by the end of this century. National rice improvement programs of India, Indonesia, Republic of Korea, Japan, USA, Brazil, Mexico, and Vietnam are researching hybrid rice. Also, several private seed companies, including Ring Around Products, Inc. in the U.S. and Cargill Seeds in the Philippines, are testing and evaluating rice hybrids from China and developing hybrid seed production technology.

This paper highlights current status of hybrid rice research and development.

Hybrid rice research and development in China

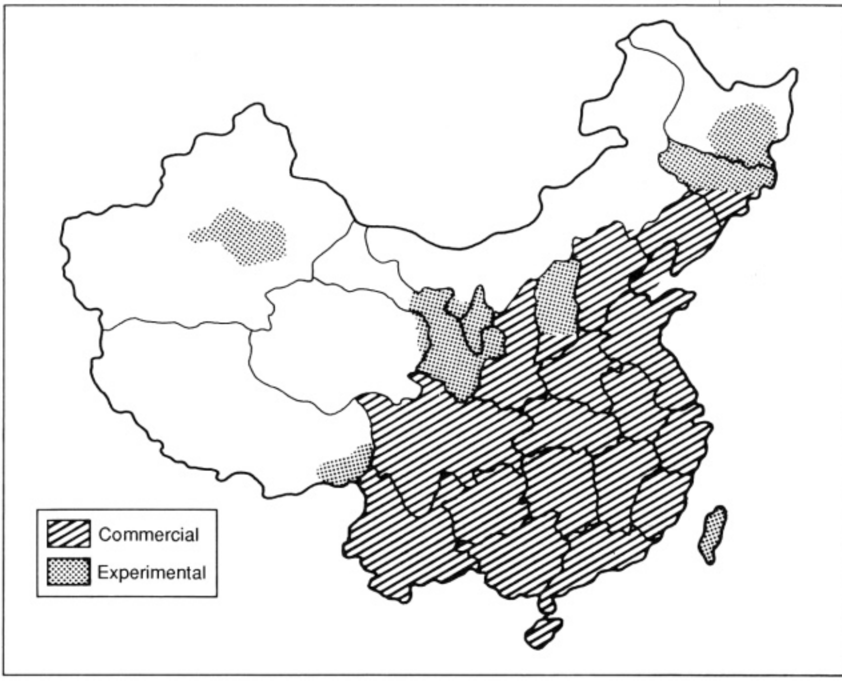
Area and distribution

About 33 million hectares are growing rice in China, and of these, more than 8 million hectares grow hybrid rice (Table 1), planted in a wide range of agroclimatic conditions all over the major rice-growing areas. Hybrids are grown from Liaoning Province (cold temperate region) at latitude 43°N to Hainan Island (tropic region) at 18°N, and from Shanghai at longitude 125°E to Yangnan Province at 95°E (Fig. 1). Different types of hybrid rice fit the various climates and soils.

1. Photoperiod-sensitive indica hybrids with long growth duration, such as Zhen-Shan 97 A/IR30, are used as late-season crops in South China.
2. Medium-duration indica hybrids, such as Zhen-Shan 97 A/IR24 and V20 A/IR26, are planted as early- and late-season crops in southern China. In the middle south (Yangtze valley) they are widely cultivated as the late-season crop in the two-rice crop regions, or as a medium-season crop in one-rice crop areas.

Table 1. Harvested areas of hybrid rice in China (1976-85).

Year	Area (million ha)
1976	0.15
1977	2.13
1978	4.33
1979	5.07
1980	4.93
1981	5.10
1982	5.60
1983	6.75
1984	8.84
1985	8.43
Total	51.33



1. Hybrid rice in China in 1983.

3. Early-maturing indica combinations, such as V20 A/26 Zhai-Zao and V20 A/IR9761-19-1-64, are used as the first crop in two-rice crop regions south of the Yangtze River.
4. Early-maturing japonica hybrids are distributed north of the Yellow River.
5. Japonica hybrids with medium or long growth duration are grown as the second crop in the Yangtze River basin.

Yield potential of rice hybrids

Hybrid rices yield 20-30% more than conventional varieties with adequate management. In 1985, for example, the yield increase from planting hybrid rice is shown in Table 2.

Table 2. China rice area and yield in 1985. [a]

	Area (million ha)	Production (million t)	Yield (kg/ha)
Total rice	31.8	166.9	5248
Conventional rice	23.4	112.3	4799
Hybrid rice	8.4	54.6	6474
Hybrid rice/total rice (%)	26.4	32.7	123.4
Hybrid rice/Conventional rice (%)	35.9	48.6	134.9

[a] Statistics from the China Ministry of Agriculture, Husbandry and Fisheries in 1985.

In recent years, 1.9 and 0.8 million hectares were planted to medium-duration hybrid rice in Sichuan and Jiangsu Provinces, respectively, with average yields of about 7.5 t/ha. Hunan Province had about 1.0 million hectares of hybrid rice under second crop season, averaging 6 t/ha, while the average yield of conventional varieties (planting area roughly equal to that of hybrids) was 4.5 t/ha. The yield of japonica hybrids in Liaoning Province ranged from 7.5 to 8 t/ha. Yields can be very high in small areas. For example:

I. The yield of 24,000 ha of second-crop hybrid rice Wei-You 6 in Lilin county of Hunan Province was 7.6 t/ha in 1983.
2. The average yield of 62 ha of medium-season hybrid rice Shan-You 2 grown in an army farm south of Beijing in 1983 and 1984 was 11.3 t/ha.
3. The maximum yield of japonica hybrid Li-You 57 was 13.7 t/ha in Liaoning Province.
4. The maximum yields of indica hybrid were 14.4 t/ha in Jiangsu Province for medium-crop and 22.6 t/ha for double hybrid rice cropping in Fujieng Province.

The cumulative yield increase due to cultivation of hybrid rice from 1976 to 1985 is more than 94 million tons, indicating hybrid rice's strategic significance in increasing food production in China.

Three-line breeding

Since the first sets of CMS (A), maintainer (B), and restorer (R) lines of the WA, Gam, and BT types were developed in the early to mid-1970s (Lin and Yuan 1980), other types of CMS lines, their maintainers, and restorers have been found. However, only a few are in commercial production. This is because many CMS lines from other cytoplasm sources have shortcomings; either their flowering behavior is abnormal, or no effective R line could be found, or the male sterility is unstable over environments. Seven types of CMS are used in the rice hybrids grown commercially (Table 3).

China's experience in developing three-line breeding can be summarized as follows:

1. The effective way to breed a cytogenic system of CMS lines is through distant hybridization, using primitive types as cytoplasmic sources and advanced breeding lines as nuclear sources.
2. Screening elite varieties or lines by testcrossing with CMS lines is a convenient way to obtain restorer lines for indica rice.
3. Restorer genes and male sterile inducing cytoplasm can be transferred to any variety through breeding.

Hybrid seed production

Hybrid rice seed production involves multiplication of CMS lines and production of F_1 hybrid seeds.

At present, the field area ratio between CMS line multiplication, hybrid seed production, and commercial production is about 1:50:2500.

Table 3. CMS lines from cytoplasmic sources being used commercially.

Type	Cytoplasmic source		Representative CMS line	Area (thousand ha)	Remark
	Species	Strain or variety			
1. WA	*O. sativa* f. *spontanea*	Male sterile wild rice	Zhen-Shan 97 A, V20 A	8,100	Indica, sporophytic
2. GA	*O. sativa* L.	Gambiaca	Chao-Yang 1 A	70	Indica, sporophytic
3. Di	*O. sativa* L.	Dissi	Di-Shan A	70	Indica, sporophytic
4. DA	*O. sativa* f. *spontanea*	Dwarf wild rice	Xie-Qing-Zao A	50	Indica, sporophytic
5. HI	*O. sativa* f. *spontanea*	Common wild rice	QingSi-Ai A	30	Indica, gametophytic
6. BT	*O. sativa* L.	Chinsurah Boro II	Li-Ming A	110	Japonica, gametophytic
7. TI	*O. sativa* L.	E-Shan-Ta-Bai	Liu-Qian-Xin A	10	Japonica, gametophytic
a. Others				10	

Currently, land in hybrid rice seed production in China is about 0.15 million hectares and average yield is 1.6 t/ha. In Hunan Province, the average yield of 20,000 ha is 2.1 t/ha, and the maximum reached almost 6 t/ha in 1985. The seed rate of commercial hybrid rice is only about 30 kg/ ha compared to 110 kg/ha for conventional rice varieties.

Before 1981, the average yield of seed production was low (only about 0.75 t/ha).

The striking increase of seed yield in recent years is attributable to the constant improvement of seed production techniques. The salient points (Lin and Yuan 1980) are

1. Transplanting two seedlings per hill for CMS lines instead of one as practiced before.
2. Expanding row ratio between male and female parents from 1:6-2:8 to 1:8-2:12 or even larger.
3. Using higher dosage of GA_3 (75 g/ha) to make the whole panicle grow taller than the flag leaf to avoid leaf clipping.
4. Special field management to promote vigorous growth in the early and middle stages but to inhibit flag leaf growth in the late stage.

Recent progress

More recently, Chinese rice scientists have further improved hybrid rice in these aspects.

- Developing early and very early hybrids possessing high yield potential. Indica hybrids used in China mainly are medium- and long-duration varieties which cannot be cultivated as the first crop for double rice cropping in Yangtze Valley. Yangtze Valley has 7 million hectares of early rice and its yield level (6 t/ha) has stagnated. Development of early and very early hybrids began in the mid-1970s and achieved good results recently (Table 4). Several new early hybrids which mature in less than 110 d and can yield 8-9 t/ha in large scale have been released to farmers for first crop in Yangtze Valley. For instance, a 70-ha demonstration for V20 A/Ce 49 was held this year in Shuang-feng county, Hunan Province. It yielded 8.2 t/ha while nearby conventional varieties yielded 6.5 t/ha.

Table 4. Yield potential of an early hybrid V20 A/Ce 49 in Hunan Province regional trial.[a]

Year	Line/Variety	Growth duration (d)	Yield (kg/ha)	% of check	Sites
1984	V20 A/Ce 49	116	7.5	114.4	4
	Guang-Liu-Ai (Ck)	114	6.6		
1985	V20 A/Ce 49	117	7.3	104.4	18
	Xiang-Zao 1 (Ck)	117	7.0		

[a] Data from Hunan Province regional trial. *Significant at 1% level.

- Developing CMS lines with new cytoplasm sources. Currently, more than 95% of CMS lines used in production belong to WA system. This unitary cytosterility system could, in the long run, make hybrid rice vulnerable to destructive diseases. So hybrids must be derived from elite CMS lines of other cytoplasm sources. CMS lines with new cytoplasm, such as DA and Di types, have been developed and are being gradually released (Table 3).
- Breeding CMS lines with higher outcrossing potential. The high price of seed limits hybrid rice adoption. The best way to enhance hybrid seed yield and reduce seed production costs is to develop CMS lines with high outcrossing rate. For example, some new CMS lines with good flowering behavior, larger stigmas, and higher exsertion rate of stigmas have been developed recently. Their natural outcrossing rate is 30-50% higher than that of CMS lines used today.
- Hybrid rices produced commercially by the farmers in China yield well but lack good grain quality. To improve the quality of hybrid grain, both male and female parents must have good, uniform grain. A new hybrid with excellent grain quality (No. 1 grade in the U.S.) was developed last year. The hybrid, L301 A/R29, has long grains, alkali value of 2.0, 23% amylose content, milled rice yield of 70%, head rice yield of 57%, and 0-1 chalkiness.
- Some leading hybrid rices are gradually losing their resistance to diseases and insects in certain regions because of the change of physiological races of pests. In Sichuan Province, for example, about 7,000 ha of Shan-You 2 (resistant to G group race) were seriously damaged by blast in 1985, because the B group race emerged and became epidemic. Fortunately, most resistant characters are controlled by dominant or partially dominant genes. Therefore, with appropriate selection of parents, especially using IRRI's latest breeding lines possessing multiple resistance, hybrids with both high yield and multi-resistance can be developed. Wei-You 64 is such a new hybrid combination. It has not only high and stable yield potential, but also resistance to five major diseases and insects—blast,bacterial blight, yellow stunt, brown planthopper, and green leafhopper (Table 5).

Hybrid rice research outside China

Outside China, hybrid rice research is exploratory. These areas of research are being investigated:

1. Heterosis and combining ability for yield and other agronomic traits.
2. Cytoplasmic-genetic male sterility and fertility restoration.

Table 5. Disease resistance of Wei-You 64 V20 A/IR9761-19-1.[a]

	Blast	Leaf blight	Sheath blight	Yellow dwarf	PBH
1982 late season		S	MR	MR	
1983 late season	MR	MR	LS	R	MR
1984 late season	R	S	LS		

[a] Data from national region trial

3. Natural outcrossing on CMS lines and hybrid seed production techniques.
4. Grain quality in hybrid rice.

In addition, basic studies support these areas of research.

Heterosis and combining ability studies

Evidence of significant positive heterosis and heterobeltiosis for yield and other agronomic traits was reviewed by Rutger and Kim (1988). IRRI and national programs of India, Indonesia, Korea, and USA are actively involved in research on this subject.

Three leading F_1 hybrids from China (Shan-You 2, Shan-You 6, and Wei-You 6) when evaluated at IRRI in 1979 did not outyield the best available conventional rice varieties (IR36 and IR42) because the hybrids lacked disease and insect resistance for the tropics. Since then, IRRI has developed experimental hybrids derived from intervarietal crosses and CMS lines from China and restorer lines from IRRI, Indonesia, India, and Korea.

Of more than 400 hybrid combinations tested at IRRI, several significantly outyielded the best check varieties (Table 6). The best hybrids outyielded the best check varieties by 22%.

Experiments elsewhere showed 13-23% yield advantages of experimental F_1s over the best check varieties in 1980-85 trials (Table 7). V20 A/Milyang 46, V20 A/ IR54, Zhen-Shan 97 A/ IR2307-247-2-3, and IR45831 A/IR54 showed superior performance at more than one location. However, their disease/ insect susceptibility and/or poor grain quality (inherited primarily from the female parents) has discouraged promotion on farmers' fields. Recently, some new F_1 combinations derived from new CMS lines at IRRI have shown encouraging results (Table 8).

Table 6. Yields of best experimental hybrids compared with those of the best check varieties in trials at IRRI, 1980-86.

Hybrid	Year	Yield (t/ha)	% of check
Wet season			
IR11248-242-3/IR15323-4-2-1-3	1980	5.9	122
Zhen-Shan 97 A/IR13420-6-3-3-1	1981	6.2	123
Zhen-Shan 97 A/IR54	1982	4.4	113
IR46828 A/IR54	1983	5.3	112
IR46828 A/IR54	1984	4.5	140
IR54752 A/IR13419-113-1	1985	5.1	107
	Mean	5.2	120
Dry season			
IET3257/IR2797-105-2-2-3	1981	10.4	132
IET3257/IR2797-105-2-2-3	1982	8.9	135
IET3257/IR42	1983	9.6	124
IR29799-17-3-1-1A/IR2797-125-3-2-2	1984	7.2	108
IR46828 A/IR13524-21-3-3-3-2-2	1985	5.4	123
IR54754 A/IR46 R	1986	7.4	119
	Mean	8.2	123
Overall	Mean	6.7	122

Table 7. Yield of best experimental hybrids and superiority over best check varieties in trials in Indonesia, Korea, and India, 1980-85.

Country	Trials (no.)	Yield of the best hybrid (t/ha)		% of the best check	
		Range	Mean	Range	Mean
Indonesia	11	4.2- 8.9	6.2	102-170	123
Korea	9	8.1-11.5	9.0	97-142	113
India					
Subtropics	5	6.2- 9.8	8.1	103-130	120
Tropics	14	3.3- 7.3	5.6	91-132	112
Overall	39	3.3-11.5	7.1	91-170	116

Table 8. Growth duration and yield of promising rice hybrids derived from new IRRI CMS lines and check varieties, IRRI, 1986 DS.

Hybrid/check	Growth duration (d)	Yield (t/ha)	% of check
Group 1			
IR54754 A/IR46 R	126	7.4	119*
IR64 (check)	126	6.2	100
Group 2			
IR54754 A/ARC11353 R	133	7.9	141*
IR54753 A/IR46 R	126	7.0	125*
IR54752 A/IR46 R	126	7.0	125*
IR54752 A/ARC11353 R	131	6.8	121*
IR64 (check)	126	5.4	96
IR54 (check)	130	5.6	100

*Difference statistically significant at 5% level.

In the U.S., some commercially grown hybrids from China were introduced in 1983 and 1984 as proprietary varieties of Ring Around Products, Inc., and evaluated in multilocation trials in Texas (Rutger and Bollich 1985, unpubl.) and Mississippi, Louisiana, Arkansas, and Florida (Calub and Norcio-Locker 1985, unpubl.). In 1984, the hybrids yielded 14% more than the indica varieties and 19% more than the 4 southern varieties. However, grain quality was unacceptable for the U.S. market.

Kim (1985) reported yield advantage of F_1 rice hybrids at different fertilizer levels (Table 9).

Heterosis in rice has also been reported in several other agronomic traits which influence yield. These include dry matter production, harvest index, leaf area index, crop growth index, crop growth rate, chlorophyll content, grains per panicle and per m^2, and grain weight (Ponnuthurai et al 1984, Kim 1985, Yamauchi et al 1985). Heterosis has also been reported for early maturity (Virmani et al 1982), root length, root weight (OToole and Soemartono 1981, Wang and Yoshida 1984), and root pulling resistance (Ekanayake et al 1986). The heterosis for root characteristics should be helpful in adapting F_1 hybrids to rainfed environments. Hybrids generally

Table 9. Heterosis, heterobeltiosis, and standard heterosis in yield in four F_1 rice hybrids under different nitrogen levels, 1983 and 1984 (Kim 1985).

Hybrid	N level (t/ha)	Yield (t/ha)		Heterosis (%)	Heterobeltiosis (%)		Standard heterosis (%) Suweon 294	
		1983	1984	1984	1983	1984	1983	1984
V20 A/Suweon 287	120		9.2	56**	–	22**	–	17**
	180	10.0	9.6	64**	16**	23**	-4	20**
	240	10.0	8.8	70**	17**	32**	-2	20**
	Mean	10.0	9.2	63	17	26	-3	19
V20 A/Milyang 46	120	–	10.7	86**	–	48**	–	36**
	180	12.0	11.5	92**	21**	43**	16**	43**
	240	12.0	9.9	84**	12*	40**	17**	34**
	Mean	12.0	10.7	87	17	44	17	37
Zhen-Shan 97 A/Suweon 287	120	–	9.0	55**	–	19**	–	14**
	180	10.8	9.6	63**	26**	22**	4	19**
	240	10.8	8.8	67**	25**	31**	5	19**
	Mean	10.8	9.1	62	26	24	5	18
Zhen-Shan 97 A/Milyang 46	120	–	10.4	84**	–	43**	–	32**
	180	11.0	10.9	81**	11*	36**	6	35**
	240	10.8	9.9	82**	1	41**	5	34**
	Mean	10.9	10.4	82	6	40	6	34

* and **: Significant at 5 and 1% levels, respectively.

produce more grain per day per hectare, indicating their higher physiological efficiency.

Studies of combining ability (Table 10) indicate significant general combining ability (GCA) and specific combining ability (SCA) effects for yield. Variance due to SCA was sometimes greater than to GCA, suggesting predominance of dominant gene action. Some studies have shown equally important additive gene action. In a study at IRRI (J. Y. Peng and S. S. Virmani, unpubl.) most combinations showing high SCA had at least one parent possessing high GCA effects; the other parent had high, medium, or low GCA effects (Table 11). Only in a few cases were high SCA effects obtained from low-GCA lines. It is, therefore, logical to select at least one parent with high GCA.

Cytoplasmic genetic male sterility and fertility restoration

The role of cytoplasm in causing male sterility in rice was first reported by Weeraratne (1954) and Sampath and Mohanty (1954). The first cytoplasmic male sterile line in cultivated rice was developed by Shinjyo and O'mura (1966) in Japan from an indica source of cytoplasm (Chinsurah Boro II) in the genetic background of a japonica variety Taichung 65. Later, Erickson (1969) and Carnahan et a1 (1972) developed CMS lines in japonica varieties (Calrose and Caloro) of California, USA, using another indica source of cytoplasm Birco (PI 279120). At IRRI, Athwal and Virmani (1972) developed a CMS line in an indica variety derived from the cytoplasmic source of Taichung Native 1 and nuclear source of variety Pankhari 203. Cheng and Huang (1979) also developed CMS line in the genetic background of variety Taichung 65 with a cytoplasmic source of *Oryza rufipogon.*

Among these CMS lines, the one most stable for complete pollen sterility was developed by Shinjyo and O'mura (1966). This line has been used extensively in China to develop japonica hybrids. Other CMS lines developed outside China have not been used for lack of stability or effective restorer lines.

After CMS lines were developed in China from *Oryza sativa* f. *spontanea,* designated as WA cytoplasm (Lin and Yuan 1980, Yuan 1972), IRRI and several national rice improvement programs developed new CMS lines by transferring the cytosterility system of the WA CMS lines such as V20 A, Zhen-Shan 97 A, Er-Jiu-Nan 1 A, and V41 A, into elite lines by backcrossing. Table 12 lists some of these new CMS lines. Some appear well adapted to the tropics, possess acceptable grain quality, are good general combiners, and show satisfactory outcrossing rate. Therefore they hold promise for developing rice hybrids for countries outside China.

Restorer lines have been identified outside China for the cytosterility systems BT, WA, and Gam (Shinjyo 1969, 1972a,b, 1975; Virmani et a1 1981; Virmani 1986). Effective restorers for cytosterile Pankhari 203A (Athwal and Virmani 1972) have not yet been identified. Effective restorer varieties are mainly from the tropics where indica rices are exclusively grown. About 20% of rice varieties and breeding lines developed at IRRI possess restoration ability of WA cytosterile lines. The frequency of restorer lines among japonica varieties is negligible (Shinjyo 1975). A number of Tongil (indica/japonica derivates) varieties from Korea are effective restorers (Virmani et a1 1986), perhaps due to gene(s) inherited from the indica parents.

Table 10. Summary of studies on combining ability analysis for yield in rice reported in the literature.

Mating design[a]	Combining ability effects		Variance		s^2 SCA/s^2 GCA	Reference
	GCA	SCA	GCA	SCA		
4 × 4 D_r	–	–	**	NS	–	Sivasubramanian and Menon (1973)
5 × 5 D	***	***	321.9	133.3	2.4	Rahman et al (1981)
4 × 4 D	**	**	40.5	143.7	3.5	Mohanty and Mohapatra (1973)
6 × 6 D_r	**	NS	–	–	–	Ranganathan et al (1973)
6 × 6 D	**	**	5.2**	12.5**	2.4	Singh and Nanda (1976)
8 × 8 D	NS	**	8.9	22.4	2.5	Singh et al (1977)
6 × 6 D_r	**	**	118.0***	118.0***	1.0	Singh (1977)
7 × 7 D	**	**	1.5**	53.5**	35.7	Maurya and Singh (1977)
7 × 7 D	**	**	–	–	1.0	Rao et al (1980)
5 × 5 D	**	NS	–	–	1.0	Haque et al (1981)
6 × 6 D_r	**	**	23.34	26.09	1.1	Zhao and Rui (1982)
6 × 6 D	***	***	295.47***	736.70***	2.5	Singh et al (1980)
15 × 15 D	**	**	144.11***	39.49***	0.27	Shrivastava and Seshu (1983)
6 × 6 D	***	***	361.24***	315.49***	0.87	Kurnar et al (1975)
8 × 2 L	**	**	49.23***	31.47**	0.64	Anandakumar and Rangasamy (1983)
10 × 10 D	***	***	91.88***	11.58***	0.13	Subramanian and Rathinam (1984)

[a] D = diallel mating without reciprocal, D_r = diallel mating with reciprocal, L = line × restorer. ** = significant at 5% level, *** = significant at 1% level.

Table 11. Number and percent frequency (in parentheses) of cross combination generated from parents with different types of GCA effects and their corresponding SCA effects for yield in rice, IRRI, 1985 DS.

GCA of parents	No. of crosses showing SCA effects			Total crosses
	+	0		
+/+	6 (55)	2 (18)	3 (27)	11
+/– or –/+	8 (36)	– (0)	14 (64)	22
+/0 or 0/+	5 (26)	3 (16)	11 (58)	19
–/0 or 0/–	5 (42)	4 (33)	3 (25)	12
0/0	- (0)	2 (50)	2 (50)	4
–/–	1 (14)	1 (14)	5 (72)	7
Total	25	12	38	75

Table 12. New CMS lines developed at IRRI and national rice improvement programs by transferring WA cytosterility into the genetic background of elite breeding lines and varieties.

CMS line	Developed at	Elite line/variety converted	Origin of elite line/variety
IR46826 A	IRRI	IR10154-23-3-3	IRRI
IR46827 A	IRRI	IR10176-24-6-2	IRRI
IR46828 A	IRRI	IR10179-2-3-1	IRRI
IR46829 A	IRRI	IR19792-15-2-3-3	IRRI
IR46830 A	IRRI	IR19807-21-2-2	IRRI
IR46831 A	IRRI	Jikkoku Seranai 52-37	India
IR48483 A	IRRI	MR365	India
IR54752 A	IRRI	IR21845-90-3	IRRI
IR54753 A	IRRI	IR19657-87-3-3	IRRI
IR54756 A	IRRI	Iri 356	Korea
IR54757 A	IRRI	Suweon 310	Korea
IR54758 A	IRRI	PAU269-1-8-4-1-1-1	India
Madhu A	Cuttack, India	Madhu	India
CRMS1	Cuttack, India	?	India
CRMS2	Cuttack, India	?	India
TNMS 31 A	Tamil Nadu, India	?	India
TNMS 37 A	Tamil Nadu, India	?	India
TNMS 47 A	Tamil Nadu, India	?	India
HR7017 A	Iri, Korea	Samkangbyeo	Korea
HR7019 A	Iri, Korea	Hankangchalbyeo	Korea

Table 13 lists some effective restorer lines identified at IRRI and elsewhere. Reports from China suggest that restorer lines have been more frequent among late-maturing than among early-maturing varieties, perhaps because late-maturing indicas are primitive and relatively closer to the wild rice. However, considering the volume of hybridization between rice varieties of different growth durations, this correlation does not hold true for improved rice varieties and breeding lines.

Natural outcrossing on CMS lines and hybrid seed production techniques

Floral structure, anthesis, and anther dehiscence patterns in rice make this crop strictly self-pollinating. The extent of natural outcrossing in cultivated varieties varies from 0 to 6.8% (Sahadevan and Namboodiri 1963). In wild rice forms of *O. perennis* Moench., 16.5-100% outcrossing has been observed (Sakai and Narise 1959, Oka and Morishima 1967). Natural outcrossing on male sterile plants is reported to vary between 0 and 44% (Athwal and Virmani 1972, Carnahan et al 1972, Stansel and Craigmiles 1966). Azzini and Rutger (1982) observed 5-32% outcrossing in Birco CMS line. At IRRI, we have observed natural outcrossing on CMS lines ranging from 3 to 43%. In India, 10-25% outcrossing rate has been observed on some CMS lines (M. Mahadevappa and P. J. Jachuck, pers. comm.).

Rice flowers reach peak blooming between 0900 and 1130 h on a sunny day. Species differ in time of peak blooming. About 60% of the spikelets of *O. glaberrima* flowered at 0900 h but less than 5% of spikelets of *O. sativa* cultivar IR36 reached anthesis at this time (IRRI 1978). Varietal differences in floral behavior and floral traits influencing outcrossing in rice have been well documented (Parmar et al 1979, Virmani and Athwal 1973, Virmani et al 1980, IRRI 1983). Parmar et al (1979) found direct correlation between growth duration of rice variety and duration of floret opening. Delay or failure in pollination prolongs flowering (Grist 1953) and consequently, CMS plants have a longer duration of floret opening than do fertile maintainer plants (Virmani 1986). High temperatures delayed anthesis and slightly increased the period of floret opening (IRRI 1983).

Virmani and Edwards (1983) published the range of variation for floral traits influencing outcrossing in rice and sources of desirable floral traits. Inheritance studies (Virmani and Athwal 1974) for floral traits such as anther length, stigma length, and stigma exsertion in rice indicated that these traits were governed by polygenes. Both additive and nonadditive effects were important for their inheritance. IRRI researchers are transferring large stigmas and large anthers from *Oryza longistaminata* into cultivars with improved plant type (IRRI 1983).

The hybrid seed production techniques developed in China have been tested in experimental seed production plots at IRRI and in Indonesia and Korea. Seed yields ranged from 10 to 1,578 kg/ha at IRRI and from 600 to 1,800 kg/ha in Indonesia (Suprihatno, pers. comm.). Korean rice scientists obtained 750-1,530 kg seed yields per ha during 1985 (C. H. Kim, pers. comm.). Seed yields depended on the synchronization of flowering of male and female parents, season, and agronomic and floral traits of parental lines. Generally, seed yields increase with experience of seed producers.

Table 13. Some elite restorer lines identified at IRRI and in Korea, India, and Indonesia.

Restorer	Origin	Restorer	Origin
IR24	IRRI	Suweon 294 R	Korea
IR26	IRRI	Suweon 332 R	Korea
IR36 R	IRRI	Suweon 333 R	Korea
IR42 R	IRRI	Iri 347	Korea
IR48 R	IRRI	Iri 371	Korea
IR50 R	IRRI	Sadang	Indonesia
IR52 R	IRRI	Sumeru	Indonesia
IR54 R	IRRI	Krueng Aceh	Indonesia
IR56 R	IRRI	CO 39	India
IR62 R	IRRI	CR 1009	India
IR64 R	IRRI	Intan mutant	India
IR9761-19-1 R	IRRI	Pankaj	India
IR13146-243-2-3 R	IRRI	Radha	India
IR19661-63-1-2-3-1-3 R	IRRI	Vani	India
IR28228-119-2-3-1-1 R	IRRI	Pusa 33	India
IR29723-143-3-2-1 R	IRRI	ADT36	India
IR3304346-1-3 R	IRRI	Krishna	India
Milyang 46 R	Korea	PR106	India
Milyang 54 R	Korea	PAU102-8-3-10	India
Suweon 287 R	Korea	PAU50-9	India

Grain quality in hybrid rice

Some experts have been concerned that cooking quality of commercial F_1 hybrid (F_2 grains) might be impaired because of genetic segregation for chemical characteristics of the grains. Because researchers outside China are developing F_1 hybrids for yield superiority, they may not be emphasizing grain quality problems. It seems that genetic segregations for chemical characteristics of rice grains borne on the commercial F_1 crop do not impair its cooking and eating quality and hybrids with desired grain quality can be produced with appropriate selections of parents. Khush et al (1988) have comprehensively discussed grain quality problems of hybrid rice.

Conclusion

Hybrid rice brings us closer to achieving the physiological yield potential of rice. Rice hybrids have increased yields 15-20% (about 1 t/ha) beyond the limits set by the improved semidwarf varieties. China has capitalized on this technology extensively, and prospects outside China look bright especially in irrigated areas. The stronger and more active root systems and early seedling and vegetative vigor of rice hybrids may help them adapt to rainfed lowlands and adverse soils and climates. Brazilian rice scientists are exploring prospects of developing hybrid rice for upland.

The genetic tools (male sterile, maintainer, and restorer lines) essential to develop hybrid rices are available; parental lines adapted to rice-growing countries will also be available soon.

More than 90% of the hybrid rice area is currently planted to hybrids derived from 1 source of cytosterility (WA), because it is the most stable over environment and reasonably easy to restore. It is being transferred to various genotypes to develop suitable CMS lines for other countries. This makes hybrid rice potentially vulnerable to diseases and insects which may be associated with WA cytosterility. China and IRRI are identifying diverse sources of cytosterility and using them in developing new combinations. An effective chemical hybridizing agent is useful here; some chemicals have been identified in China but their use is very limited because they are not safe and their effectivity is inconsistent.

Hybrid seed production techniques have been developed fairly well in China and are being improved continuously. These techniques appear adaptable for outside China. Although labor-intensive, these techniques should be practical where labor is abundant and available at reasonable cost. In fact, introduction of hybrid rice technology could generate employment opportunities. Development of parental lines with desirable floral traits influencing outcrossing could also help to increase cross-pollination rate on male sterile parents and consequently hybrid seed yield per unit area.

The major problems of hybrid rice are high seed cost, necessity of changing seed every crop season, and continuous dependence on the ex-farm source for supply of seed. China has established an efficient infrastructure for seed production, processing, certification, and distribution. However, it is uncertain how other countries can organize such an infrastructure. Hybrid rice technology will not be successful without an efficient seed industry. Adoption of this technology outside China, would depend on 1) the extent of pressure to produce more from a piece of land, 2) yield advantage shown by hybrids over best available conventional varieties, and 3) the country's capacity to organize an efficient seed production, processing, certification, and distribution program in the public or private sector.

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Prospects for hybrid rice in the Asia-Pacific Region

R. B. Singh

Rice production, area planted to rice, and yields in countries of the Asia-Pacific Region are reviewed. The impact of hybrid rice in China is used to explore the prospects for hybrid rice in other countries of the region, including cropping intensification and constraints to hybrid rice technology. Strategies for expanding hybrid rice technology are suggested, and biological and economic studies are proposed.

Rice is the foremost food of the developing world. It provides about four-fifths of the calories for the more than 2 billion people of Asia and one-third the caloric intake of the nearly 1 billion people in Africa and Latin America.

Rice occupies one-third of the area planted to cereals in developing countries, 50% more than the area under wheat, the second most important crop. There are 36 countries with more than 100,000 ha of rice, and half fall in the lowest income group, with annual income less than US$300 per capita. More than 95% of the world's rice area is in the developing countries, mostly in Asia.

The Asia-Pacific Region produces and consumes more than 90% of the world's rice. In 1984, the region produced 426 million tons of rice, out of 465 million tons worldwide. Twenty of 27 countries studied in the region produced rice. In these 20 countries, rice constituted 57% of the cereals produced (Table 1). Cereals accounted for 70% or more of total agricultural output and 85 to 90% of the daily food intake.

The proportion of rice to total cereal production in countries varies considerably. In Bangladesh, Burma, Kampuchea, Japan, Laos, Malaysia, Republic of Korea (ROK), Sri Lanka, and Vietnam, rice accounted for 90% or more of cereals produced. In Fiji, Indonesia, and Thailand, its share ranged between 70 and 90%. In China, Democratic People's Republic of Korea (DPRK), India, Nepal, and the Philippines, the proportion ranged from 50 to 69%.

Rice production

Rice production in the region reached an all-time high of 435 million tons in 1985, 110 million tons more than in 1975 (Table 2). In the rest of the world, production increased only 5 million tons, from 34 to 39 million tons. China, producing 179 million tons, and India producing 93 million tons, accounted for almost three-fifths of the world's production.

Table 1. Cereal crops produced in the Asia-Pacific Region, 1985.

Crop	Production (t × 10^6)	% of total cereals
Rice	435	57
Wheat	165	22
Coarse grains	157	21
Total cereals	757	100

Table 2. Rice production, yield, and area in the Asia-Pacific Region and world, 1975-85.

Geographic entity	1975	1982	1983	1984	1985	Average annual growth rate (%) 1975-85	1981-85
			Production (million metric tons)				
Asia-Pacific Region	325	384	416	432	435	3.3	4.4
Rest of world	34	40	35	38	39	1.4	- 0.9
World	359	424	451	470	474	3.1	3.9
			Yield (t/ha)				
Asia-Pacific Region	2.5	3.1	3.2	3.3	3.3	3.1	3.7
Rest of world	2.3	2.5	2.4	2.5	2.6	1.4	1.4
World	2.5	3.0	3.1	3.3	3.2	3.0	3.6
			Area (million ha)				
Asia-Pacific Region	127	126	129	129	131	0.2	0.6
Rest of world	15	14	14	15	15	0.0	- 2.3
World	142	140	143	144	146	0.2	0.3

During 1975-85, the average annual compound growth rate for production in the region was 3.3%, against 1.4% in the rest of the world. In 1981-85, production in the region grew annually at 4.4%, but declined annually by 0.9% in the rest of the world.

Regional yield progressed satisfactorily. Between 1975 and 1985, it increased from 2.5 to 3.3 t/ha, or 0.8 t/ha. In the rest of the world, the average yield moved up marginally by 0.3 t/ha, increasing from 2.3 to 2.6 t/ha. In the region, thus, yields annually grew 3.1% against only 1.4% for the rest of the world. In 1981-85, regional yield further accelerated, with annual growth rate of 3.7%, while it leveled off in the rest of the world.

The regional area under rice increased only marginally, from 127 to 131 million ha, growing annually at only 0.2%. In the rest of the world, it remained unchanged.

From 1975 to 1985, about 90% of the increase in rice production occurred due to increase in productivity.

Performances of countries varied considerably. China increased its rice production by 50 million tons from 1975 to 1985, from 129 to 179 million tons, a 3.8% annual growth. India increased its rice production from 73 to 93 million tons, a 2.7% annual growth. Indonesia, the third major rice-producing country, went from 22 million tons in 1975 to 39 million tons in 1984, an impressive annual growth of 6.3%.

In relative increases during the past 10 yr, Sri Lanka's production grew 7.8%, closely followed by Australia, Indonesia, Burma, and Laos with rates from 5.7 to 6.5%. DPRK, Thailand, and Vietnam increased production from 3 to 4.2%. Bangladesh, Bhutan, Kampuchea, Fiji, Nepal, and ROK showed low growth rates of 0 to 2%. Japan and Malaysia showed declines.

China and India increased production 5 to 5.4% during 1981-85. Kampuchea rehabilitated its rice production, growing 14.2%. Production accelerated in Nepal and Japan in 1981-85 but decelerated in Burma, Sri Lanka, and Australia.

Yields differed widely from country to country. Australia, DPRK, Japan, and ROK averaged 6.2-6.7 t/ha. China registered 5.3 t/ha and Indonesia averaged 4 t/ha. Yields in Burma, Malaysia, and Sri Lanka were about 3 t/ha. Yields in other countries ranged from 1.1 t/ha (Kampuchea) to 2.6 t/ha (Vietnam). Yield increases during the past 10 yr were highest in Burma, China, Indonesia, Laos, and Sri Lanka, with average annual growth from 4.7 to 6.6%. In 1981-85, China, Laos, India, and Nepal further accelerated their yield growth rates.

Rice area decreased in Burma, China, Malaysia, Philippines, and Japan, and remained stagnant in Laos and ROK. In 13 countries, rice area increased, with fastest annual growth in Australia (4.4%), Kampuchea (4.3%), and Sri Lanka (3%). In absolute terms, India gained 1.6 million ha, followed by Thailand (1.5 million ha) and Indonesia (1.3 million ha). China decreased rice hectarage 1.8 million ha between 1974 and 1984.

Production vs population growth

Rice production growth was higher than population growth in 13 of 18 countries. In Bangladesh, Japan, ROK, Malaysia, and Nepal, population growths outstripped rice production growths. In Japan, cutback was deliberate. In Malaysia, plantation crops like rubber and oil palm received priority attention, and Malaysia aimed for 60-70% self-sufficiency in rice.

Rice yields are already very high in ROK, but there is a considerable gap between farmers' yields and demonstration plot yields. The government is striving to bridge the yield gap through extension activities and improved soil, water, fertilizer, seed, and pest management. Cropping intensity can be further increased in irrigated areas by the development of suitable varieties and cropping patterns. Marineland reclamation is expected to increase rice area by about 400,000 ha by the year 2000.

Malaysia and ROK are close to being classed as developed countries, and can meet food demands through imports. Contrarily, the lag of growth in rice production in Bangladesh and Nepal is of concern. While all-out effort should be made to decelerate population growth, there is considerable scope for increasing productivity and cropping intensity in these countries.

Impact of hybrid rice in China

In the late 1960s, Yuan Long-Ping of the Hunan Academy of Agricultural Sciences and his associates developed the male sterile version of the long-grained, non-glutinous rice, Yebai. Based on the cytoplasmic male sterile (CMS), maintainer, and restorer system, Chinese breeders demonstrated the feasibility of large-scale cultivation of hybrid rice.

From 1972 to 1974, the Hunan Academy used CMS lines as female parents to make 53 crosses. At present about 100 CMS lines and 200 restorer lines are being used to produce hybrids. The most promising hybrids are Shan-You 6, Shan-You 2, Wei-You, Wei-You 35, Wei-You 64, Wei-You 30, and Si-You. These hybrids represent distinct crop duration, yield ability, fertilizer responsiveness, physiological efficiency, disease and pest resistance, grain type, protein content, and adaptability to soil types. Scientists are searching for new sources of male sterile and restorer types to diversify the genetic base and reduce genetic vulnerability. Chemical male gametocides are also being used on limited scale.

Large-scale adaptive trials of hybrids were laid out in 1974. The first commercial hybrid was released to Chinese farmers in 1976, when the area under hybrid varieties was only 0.15 million ha. It jumped to more than 2.1 million ha in 1977, 6.75 million ha in 1983, and about 8 million ha in 1984.

Wherever hybrid rice is grown, its yield may be 20-30% more than that of conventional varieties. When planted under special conditions, such as in saline-alkali fields and in fields soaked with cold underground water or deep muddy soil, hybrid rice may outyield conventional varieties by 100% or more.

In 1983, hybrid rice production was estimated to be more than43 million tons. The average yield increased from 5.8 t/ha in 1982 to 6.4 t/ha in 1983. In Jiangsu Province, 0.8 million ha were under medium-maturing hybrids with yields averaging 7.4 t/ha, and as high as 13.5 t/ha in small areas. In Hunan Province, 1 million ha of late-maturing hybrids yielded 6.0 t/ha.

The yield advantages of hybrids varied from province to province and over agroecological conditions. Table 3 shows that the superiority of hybrids over conventional varieties was greater in Hunan Province compared with Fujian Province, although absolute yields were higher in Fujian.

Because of high productivity of hybrid rice, China has reduced its rice area from 34 to 32 million ha and intends to maintain it with an average national yield of 6.5 t/ha.

Prospects for hybrid rice in other countries

Hybrid rice as a path for rice intensification

The Asia-Pacific Region accounts for 55% of the world's population but has less than 30% of the world's arable land. The region houses 70% of the world's farming households and about three-fourths of the world's malnourished 400 million people. Increased and sustained food production in the region is fundamental to food security.

Demand for rice in the region is expected to increase by about 3%/ yr during the next decade. Production increases will have to come primarily through increases in yield.

Development of semidwarf, high-yielding, and photoperiod-insensitive conventional varieties was pivotal to improved production of rice during the past two decades. Now, heterosis has been exploited in rice to elevate the production ceilings

Table 3. Average yield (kg/ha) in Fujian and Hunan Provinces, China, 1982 late season crop.

	Fujian		Hunan	
	Hybrid	Conventional	Hybrid	Conventional
Rough rice	8.4	7.2	7.0	5.8
Straw	6.6	5.7	7.1	5.4

attained by the semidwarf varieties. So far, China is the only country growing hybrids commercially. Under intensive management, hybrids in China gave a yield advantage of 1-1.5 t/ha.

Chinese hybrids and female lines were unsuccessful when tested in other Asian countries because they were not adapted to tropical conditions. Led by IRRI, work on production of suitable male-sterile lines and F_1 combinations of A/ R lines was initiated in India, Indonesia, ROK, Thailand, and Japan. Preliminary trials showed about 20% yield advantage in favor of hybrid varieties in favorable environments.

However, economic superiority of hybrids under various agroecological conditions is yet to be analyzed. In Japan, Australia, DPRK, and ROK, where average yields using conventional semidwarf high-yielding varieties (HYVs) have reached high plateaus, a yield advantage of 1-1.5 t/ha by adopting F_1 hybrids seems attractive. But in areas where less than half of the yield potential of conventional HYVs has been exploited, hybrid rice may not be the most appropriate choice. However, in high-yielding areas such as Punjab Province of India, hybrids could increase yields.

Using the WA cytosterility system, Thai scientists developed a male sterile version of RD21, a popular high-yielding good-grain-quality variety. Certain combinations of this female line (RD21 A-23), particularly the hybrid RD21 A-23/RD7, in an evaluation trial gave up to 70% higher yield than the best local check (RD23), and outyielded all foreign hybrids or hybrids derived from exotic male sterile lines. The Thai hybrids were superior in grain quality, matured somewhat earlier than traditional varieties, and showed greater resistance to yellow orange leaf virus.

Hybrids are also superior in disease and pest resistance, earliness (sometimes), and quality (sometimes). For double-cropping schemes, early-maturing rice varieties are a prerequisite. It is possible to develop early-maturing and high-yielding varieties through heterosis breeding. In China, hybrids occupy almost 65% of the double-cropped area.

Constraints to hybrid rice technology

CMS lines introduced into tropical countries from China were usually susceptible to major diseases and insects. To remove this drawback, tropical countries have started testing their local superior agronomic lines for their ability to function as maintainer lines and have reconstituted some promising local male steriles. The Thai female line RD21 A-23 is a locally adapted male sterile with high combining ability.

Lack of production and distribution of hybrid seed quality are constraints to the adoption and spread of hybrids. In several countries, the seed production system is weak, and certified seed of even standard conventionals is not adequately available. Furthermore, the cost of hybrid seed is out of reach of most resource-poor farmers in developing countries. Therefore, to popularize hybrid rice, it is essential to establish an effective hybrid seed production and distribution system which delivers reasonably priced certified seed.

Large-scale production of hybrid seed is highly complex, but countries can learn from the Chinese experience. In China, original seed is usually produced by the breeder or in the experimental station. The foundation seed is usually produced on foundation seed farms. The multiplication seed is usually produced on seed multiplication farms and released to growers for commercial hybrid seed production. In China each province has one or two farms to multiply and purify foundation seed; some prefectures and counties have multiplication farms.

Sterile lines are used as female parents and restorer lines as male parents to produce hybrid seed. An isolation strip of more than 100 m between seed production fields and common ricefields is needed to avoid outside pollination on the sterile line (female).

The hybrid seed production area in China is now about 150,000 ha, producing about 200 million kg. Price has declined to yuan 3-4/ kg, greatly helping spread the hybrid varieties.

Future strategies and approaches

Diversification of male sterile and restorer lines. More than 90% of the male sterile material in China is based on the WA cytosterile source. This narrowness renders the genetic base of hybrids highly vulnerable to pests, diseases, and other stresses. There is an urgent need to diversify the cytoplasm of male steriles. Furthermore, finding efficient restorer combiners will help confer high heterosis in F_1 combinations.

Male sterility inducing factor(s) in the cytoplasm of available male steriles should be probed by restriction endonuclease fragment analyses of organelle DNAs. This will help in identifying sources of cytoplasmic male sterility as well as restorer lines. The more diverse the male sterile cytoplasm donor from the recipient line, the greater will be the chances of producing stable CMS systems. Using protoplast fusion techniques, it should be possible to produce distant hybrids.

Testing for combining ability. A pool of male sterile/ maintainer and restorer lines should be created in different countries and selected ones exchanged among interested scientists. Line-tester analyses could quickly identify promising parental lines to be tested using diallel systems for identifying most promising hybrid combinations.

Increasing outcrossing under hybrid seed production. Genes that alter floral morphology of male steriles should be discovered and transferred to female parents to induce greater percentage of outcrossing and seed setting. Further, improved agronomic practices should be developed including clonal multiplication for increasing rate of F_1 seed production.

Economics of hybrid rice production. Large-scale trials of hybrid varieties along with best local checks should be conducted under different agroecological conditions with varying management practices for assessing degree of economic heterosis. Cost-benefit ratios of hybrid rice production vs conventional rice varieties should be computed. This analysis will be extremely useful for breeders, extension workers, policy formulators, and executors of plans and programs.

Notes

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Organization of a hybrid rice breeding program

L. P. Yuan and S. S. Virmani

The paper outlines the procedures adapted to develop F_1 rice hybrids using cytoplasmic genetic male sterility and fertility restoration system. It provides guidelines for organizing a hybrid breeding program so that it supplements rather than competes with an inbred breeding program.

Procedures for developing F_1 hybrids of self-pollinated crops for commercial cutivation are strikingly different from those used for inbred varieties. Inbred line breeding accumulates productivity genes that perform well under homozygous condition, while hybrid breeding assembles genes that perform well under heterozygous condition (F_1).

Outside China and IRRI, only a few countries are exploring the potentials of breeding hybrid rice. When other countries begin, they may take advantage of experience in China and IRRI and adopt the model of breeding procedure described here.

The model

The model is based on the use of cytoplasmic male sterility and fertility restoration, the most effective genetic tool for developing F_1 hybrids in rice, a strictly self-pollinating species (Lin and Yuan 1980, Virmani et al 1981).

Because lines used to produce F_1 hybrids are inbreds, it is always advisable to have a close link between inbred breeding and hybrid breeding. Improved lines developed by inbred breeding can be used to produce heterotic hybrids with additional genetic improvement. Therefore, hybrid breeding should be organized so that it supplements rather than excludes the inbred breeding program.

Procedure

Hybrid rice breeding procedures of this model are divided into two phases: (1) development of parental lines, and (2) evaluation of heterosis.

Development of parental lines

This phase involves developing male sterile (A), maintainer (B), and restorer (R) lines. In China this phase is called three-line breeding. The essential features of the parental lines are (1) adaptability to local conditions, (2) good general combining ability, and

(3) desirable floral characteristics that will increase cross-pollination and seed production. A number of cytoplasmic male sterile (CMS) lines, their maintainers, and many more restorers are available in China and at IRRI. Initially, these can be tested under local conditions for adaptability, combining ability, and floral characteristics for direct use in developing hybrids.

Elite lines or varieties possessing good agronomic characteristics such as high yielding ability, pest and disease resistance, and good grain quality are selected from the ongoing inbred program. These are then testcrossed as single plants with the best available CMS lines. These testcross F_1s, their corresponding male parents, and best local varieties (checks) are grown in rows, 1 seedling/ hill (10-20 plants/row).

If a testcross shows 95-100% pollen sterility, its male parent can be considered a prospective maintainer. If so, the testcross F_1 is backcrossed to the corresponding male parent and the completely male sterile plants in the backcross F_1 progenies are repeatedly backcrossed on a single plant basis to develop a new CMS line. Usually, a completely pollen sterile and stable line in the genetic, background of the original male parent is obtained after 5-7 backcrosses. The male parent becomes the maintainer of the new CMS line.

If an F_1 combination shows normal pollen and/ or spikelet fertility (>80%), its male parent can be considered a prospective restorer for that CMS line. Such restorers must be reconfirmed and purified genetically by re-testcrossing on a single plant basis.

In the same test, fertile hybrids that show superiority over check varieties must be identified and their respective A and R lines evaluated for combining ability and later for heterosis in the hybrid.

Testcross F_1 combinations that show partial pollen and spikelet sterility are discarded because their male parents cannot be converted into a CMS line and are difficult to develop into a restorer line.

Combining ability evaluation of selected A and R lines

When several A and R line combinations are available, it is essential to evaluate them for combining ability. F_1 hybrids are made employing a line/tester mating scheme. The F_1s are evaluated along with their male parents in a replicated trial. General and specific combining ability effects are then estimated using the procedure of Kempthorne (1957). A and R lines with good general combining ability and hybrids showing high specific combining ability are thus selected for further evaluation.

Heterosis evaluation

On the basis of their per se performance and general combining ability, promising A and R lines are selected to make experimental hybrids. These hybrids are evaluated against the best available varieties in sequential yield trials: preliminary yield trial(s), advanced yield trial(s), and regional yield trial(s) where F_1 hybrids significantly superior to checks are selected and advanced to the higher order yield trial(s). The F_1 hybrids found consistently superior to check varieties can be tested on a large scale for selection and release.

Organization of nurseries

The breeding procedure to develop A, B, and R lines can be handled more efficiently if breeding materials are organized in the following nurseries:

source nursery,
testcross nursery,
re-testcross nursery, and
backcross nursery.

The source nursery includes the CMS lines and prospective parental lines developed locally or introduced from other national or international rice improvement programs. The testcrosses made between the CMS lines and parental lines included in the source nursery are included in the testcross nursery. This nursery includes the testcross F_1s and their corresponding male parents. The best available check varieties are also included for selecting heterotic combinations showing normal spikelet fertility.

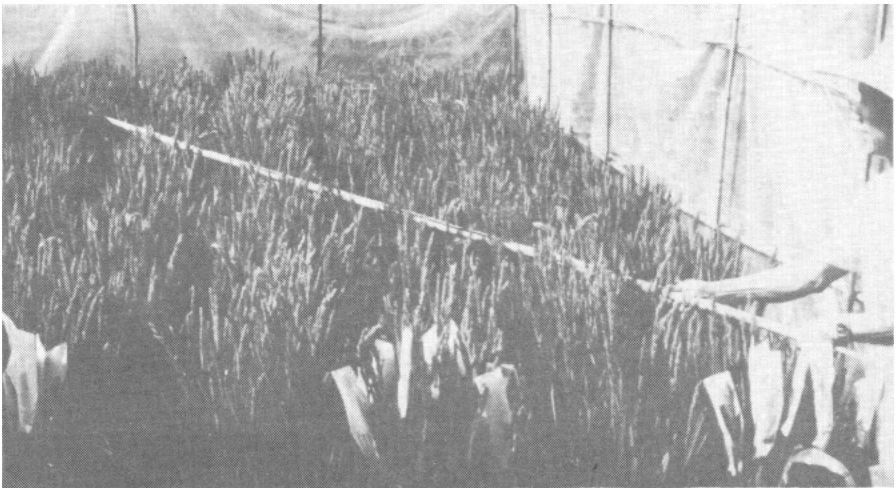
The re-testcross F_1s and the progeny of single plants of restorer lines used for retest crossing are included in the re-testcross nursery, which aims to reconfirm and purify the restorer lines. Check varieties are included to identify heterotic crosses.

The backcross F_1s and progenies of their male parents are included in the backcross nursery, which aims to develop suitable CMS and maintainer lines.

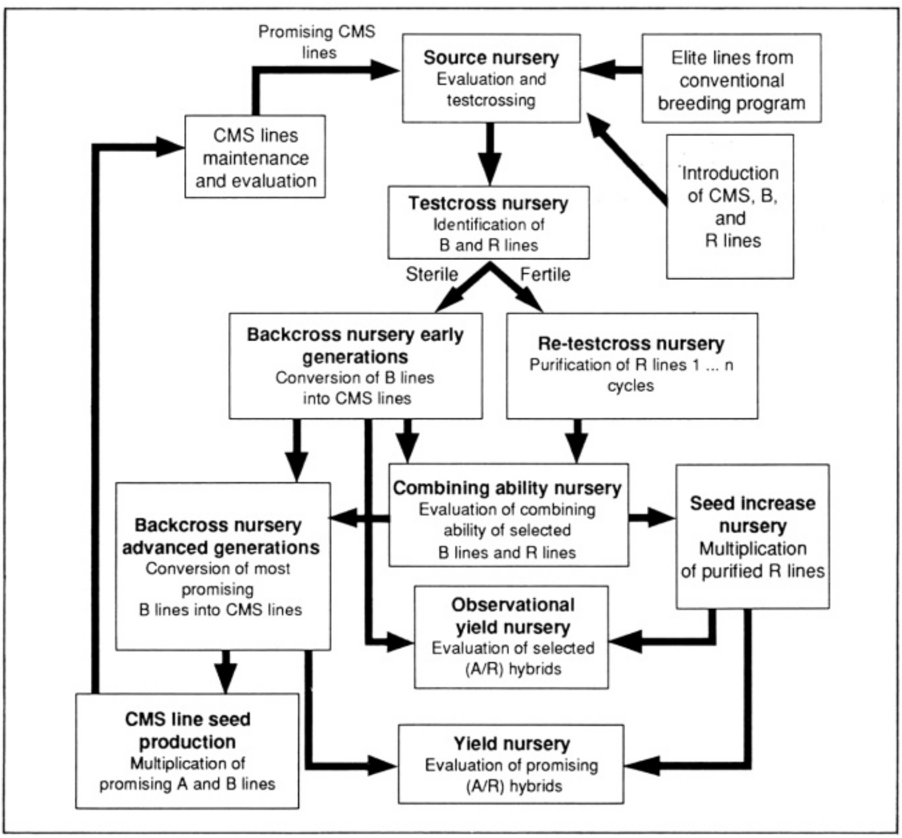
Seed production for yield trials

Heterosis evaluation needs to be backed up by organized seed production. Hybrid seed for combining ability and observation yield nurseries can be produced by handcrossing or by enclosing synchronously flowering A and R lines in chimneylike isolation plots (Fig. 1). Hybrid seed for replicated yield nurseries and regional yield

1. Chimney like isolation plots.



2. Small-scale isolation plot to produce hybrid seed for replicated yield nursery and regional yield nursery.



3. Organizational plan of hybrid rice breeding program used in China and IRRI.

nurseries is produced in small- to medium-scale isolated seed production plots (Fig. 2). The relation between hybrid nurseries is illustrated in Figure 3; it differs considerably from those used in pedigree breeding.

These described procedures are general guidelines and should be practiced with flexibility. A hybrid combination found excellent may skip any subsequent step. For example, if a combination performs exceptionally well in re-testcross nursery, the plant breeder may advance it in replicated yield nursery or even regional yield nursery. Thus, the breeding cycle can be shortened and good hybrid combinations are developed faster for commercial production.

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Heterosis in rice

Chong Ho Kim and J. Neil Rutger

Overwhelming evidence of the existence of heterosis in rice comes from some 8 million ha of hybrid rice being grown in China and from the research literature. Heterotic F_1 hybrids yield about 10 t/ha in both tropic and temperate regions. Heterosis for grain yield appears to be 20-30% under favorable conditions, but seasonal variations are wide in the tropics; hybrids have lower grain yield heterosis at lower temperatures. The yield advantage of F_1 hybrids is due primarily to more spikelets and higher grain weights. With relatively low nitrogen, the grain yield potential of F_1 hybrids is higher than it is in conventionally bred semidwarf varieties. Reports on gene action and combining ability indicated that both general and specific combining ability effects are important. General combining ability effects often are larger than specific combining ability effects, but enough exceptions exist to make further use of hybrids worthwhile.

Semidwarf varieties have increased yields dramatically in many rice-growing countries. High-yielding varieties have been developed to meet the food demands of an increasing world population with decreasing arable land.

However, yield plateaus have been reached in many areas. Hybrids offer opportunity to break through the yield ceilings of semidwarf rice varieties. Hybrids have already been successfully used in maize, pearl millet, and sorghum.

The discovery of cytoplasmic male sterility (CMS) in rice (Athwal and Virmani 1972, Erickson 1969, Shinjyo 1969) suggested that breeders could develop a commercially viable F_1 hybrid, but little serious interest was paid until China reported, in 1977, successful production of F_1 hybrids. Those hybrids yielded 20-30% higher than conventionally bred varieties (Anonymous 1977, Lin and Yuan 1980, Shen 1980). This was the first example of a hybrid rice which used cytoplasmic-genetic male sterility and fertility restoration systems for seed production being commercially grown in farmers' fields. The 20-30% higher yield potential in the F_1 hybrid over high-yielding semidwarf varieties is worth considering, even though many problems involved in F_1 hybrid seed production need to be solved.

Heterosis for grain yield and other characters

Hybrid vigor in rice was reported by Jones in 1926. Chang et al (1973), Davis and Rutger (1976), Virmani et al (1981), and Virmani and Edwards (1983) have reviewed the research since then. Several experiments on heterosis in rice were conducted during the 1970s at Davis, California. All trials were conducted in the field, at 30- × 30-cm plant spacing, 7-10 plants/plot, with 34 replications/experiment (Davis and Rutger 1976, Rutger and Shinjyo 1980). Eleven of the 153 F_1 hybrids tested showed significant standard heterosis in grain yield (Rutger and Shinjyo 1980). Heterosis ranged from 16 to 63%, with a 41% average. While these levels seemed exciting, they were tempered by the fact that only small plots transplanted with wide spacing were used; in the U.S., the commercial crop is broadcast.

Virmani et al (1981) presented data on the large variation in heterosis and heterobeltiosis for yield and yield components. Heterobeltiosis ranged from 369 to −91% for yield, 55 to −70% for grains per panicle, 14 to −31% for grain weight, and 505 to −45% for panicles per plant. Virmani et al (1981) and Virmani and Edwards (1983) noted that those experiments usually had been conducted with non-commercial densities in the field or in pots in the greenhouse. Unproductive varieties or lines frequently had been used as parents and heterosis was evaluated in limited populations. Significant heterosis, heterobeltiosis, and standard heterosis have been reported (Carnahan et al 1972, Chang et al 1971, Cheema and Awan 1985, Davis and Rutger 1976, Devarathinam 1984, Kim and Heu 1979, Mallick et al 1978, Maurya and Singh 1978, Mohanty and Mohapatra 1973, Murayama 1973, Murayama et al 1974, Nijaguna and Mahadevappa 1983, Panawar et al 1983, Parmar 1974, Pillai 1961, Rao 1965, Saini and Kumar 1973, Saini et al 1974, Sivasubramanian and Menon 1973, Singh et al 1980, Singh et al 1984, Yoshida and Fujimaki 1984). Other studies reported almost negative heterosis or heterobeltiosis in grain yield (Chang et al 1973, Karunakaran 1968, Khalique et al 1977).

Grain yield. For grain yield, positive heterosis ranged from 1.9 to 157% and positive heterobeltiosis ranged from 1.9 to 367%. However, most of the data showed heterosis ranging from about 20% over the midparent to 70% over the better parent and heterobeltiosis ranging about 20 to 40%.

China has shown 20-30% higher yield potential for hybrids in large-scale production plots, with wider adaptability than conventionally bred varieties (Anonymous 1977, Hunan Provincial Paddy Rice Heterosis Scientific Research Coordination and Cooperation Group 1978, Li 1977, Lin and Yuan 1980, Wu et a1 1980). Virmani et al (1981) reported that the highest hybrid rice yield obtained in China was 12.8 t/ha; the highest conventional yield is 10.0 t/ha. Virmani et a1 (1981) also presented data collected in 1977 and 1979 from Hunan Province, China; yields of 6 F_1 hybrids ranged from 6.1 to 6.5 t/ha. In experiments by Lin and Yuan (1980), hybrid Nan-You 2 produced 10.1 t/ha. The hybrids had fewer effective panicles per m^2 but showed more filled grains per panicle and larger seeds. Wei (1980) reported that the best hybrids outyielded check varieties by 24-55%. Deng (1980) reported a highest experiment plot yield of 16.5 t/ha. Virmani et a1 (1981, 1982) showed up to 73% heterosis, 57% heterobeltiosis, and 34% standard heterosis in hybrid yield, and obtained yields up to 5.9 t/ha (22% standard heterosis) in the wet season (WS) and 10.4 t/ha (34% standard heterosis) in the dry season (DS). Reddy et al (1984) reported a hybrid with 68% heterosis, 33% heterobeltiosis, and 24% standard heterosis in grain yield.

Hung et a1 (1984) reported F_1 hybrids that yielded 27% more than the check variety in the first crop (9.5 t/ha) (Table 1).

Four hybrids studied at Iri, Korea, in 1984 showed significant positive heterosis (up to 92%), heterobeltiosis (up to 48%), and standard heterosis (up to 43%) in grain yield under 3 different nitrogen levels (120, 180, and 240 kg N/ha) (Table 2). The 2-yr average of four F_1 hybrids showed positive heterosis (74%), heterobeltiosis (25%), and standard heterosis (17%) in grain yields; hybrids yielded an average 10.4 t/ha. Yoshida and Fujimaki (1985) showed higher standard heterosis in grain yield of F_1 hybrids.

Table 1. Grain yield and yield components of hybrid Shan-You 6 and controls (Huang et al 1984).

Variety	Year and season	Grain yield (t/ha) Single plant/hill	Grain yield (t/ha) 3-5 seedlings/hill	Panicle no./hill	Grain no./panicle	1000-grain weight (g)
Shan-You 6	1981, 2d crop	5.4	5.9	14	97	27.2
	1982, 1st crop	8.5	9.5	22	101	27.3
Taichung Sen 3 (check 1)	1981, 2d crop	5.3	5.8	15	89	26.1
	1982, 1st crop	6.8	7.5	24	104	26.3
Taichung 67 (check 2)	1981, 2d crop	5.4	5.5	13	89	26.6
	1982, 1st crop	7.3	7.8	23	89	25.2

Table 2. Heterosis, heterobeltiosis, and standard heterosis in grain yield of 4 hybrids under 3 N levels at 30 × 15 cm spacing (Kim 1985).[a]

Hybrid	Nitrogen level (kg/ha)	Grain yield (t/ha)		Heterosis (%)	Heterobeltiosis (%)		Standard heterosis[b] (%)	
		1983	1984	1984	1983	1984	1983	1984
V20 A/Milyang 46	120	–	10.7	87**	–	48**	–	36**
	180	12.1	11.5	92**	21**	43**	16**	43**
	240	12.0	9.9	84**	12*	40**	17**	34**
	Mean	12.1	10.7	87	17	44	17	37
V20 A/Suweon 287	120	–	9.2	56**	–	22**	–	17**
	180	10.0	9.6	65**	16**	23**	4	20**
	240	10.1	8.8	70**	17**	32**	2	20**
	Mean	10.1	9.2	63	17	26	3	19
ZhenShan 97 A/ Suweon 287	120	–	9.0	55**	–	19**	–	14**
	180	10.9	9.6	63**	26**	22**	4	19**
	240	10.8	8.8	67**	25**	31**	5	19**
	Mean	10.9	9.1	62	26	24	5	18
Zhen-Shan 97 A/ Milyang 46	120	–	10.4	84**	–	43**	–	32**
	180	11.0	10.9	81**	11	36**	6	35**
	240	10.3	9.9	82**	1	41**	5	34**
	Mean	10.9	10.4	82	6	40	6	34

[a]* = significant at 5% level, ** = significant at 1% level. [b]Over Suweon 294.

In general, F_1 hybrids based on a cytoplasmic genetic male sterile system have shown as much heterosis as F_1 hybrids between conventional cultivars/lines.

Yield components. In some studies, increased yields in most heterotic F_1 hybrids were due to heterosis in panicle number and spikelet number (Chang et a1 1971, 1973; Carnahan et al 1972; Devarathinam 1984; Hsu et a1 1969; Murayama 1973; Namboodri 1963; Saini et a1 1974). Heterosis in 1,000-grain weight did not contribute much toward yield. In other studies, panicle number contributed the most to increasing heterosis in grain yield (Devarathinam 1984; Dhulappanavar and Mensikai 1967; Karunakaran 1968; Murayama 1973; Palaniswamy and Palaniswamy 1973; Ramiah and Ramaswamy 1941; Rao 1965; Singh and Singh 1978, 1979). In still other studies, high heterosis in grain yield was due to simultaneous heterosis in one or more yield components (Amandakumar and Sreehangasamy 1984; Carnahan et al 1972; Chang et al 1971; Devarathinam 1984; Hsu et al 1969; Maurya and Singh 1978; Mohanty and Mohapatra 1973; Murayama 1973; Nijaguna and Mahadevappa 1983; Panawar et al 1983; Purohit 1972; Saini et al 1974; Singh et al 1980; Subramanian and Rathinam 1984; Virmani et al 1981, 1982).

However, Virmani et al (1981,1982) reported heterosis in yield was mainly due to more spikelets per panicle, as did Pillai (1961), Singh and Singh (1978), Murayama (1973), Nijaguna and Mahadevappa (1983), Panawar et al (1983), and Yoshida and Fujimaki (1984); and heavier 1,000-grain weight, as did Chang et al (1971), Murayama (1973), Saini et al (1974), Nijaguna and Mahadevappa (1983), and Reddy et al (1984). Similar results were found by the Hunan Provincial Paddy Rice Heterosis Scientific Research Coordination and Cooperation Group (1978), Deng (1980), Lin and Yuan (1980), Wei (1980), Wu et al (1980a), Xu and Wang (1980), Huang et al (1984), and Ponnuthurai et al (1984).

Other studies at Iri, Korea, in 1984 showed the same trend in yield components, indicating significant positive heterosis, heterobeltiosis, and standard heterosis in spikelet per plant and 1,000-grain weight, but almost negative or nonsignificant heterosis, heterobeltiosis, and standard heterosis in panicles per m^2 and filled grain ratio (Virmani et al 1981, 1982) (Table 3). Usually, panicles per unit area and filled grain ratio did not contribute significantly to yield heterosis. Standard heterosis in spikelets per plant decreased with increased nitrogen (120-240 kg/ ha). This suggests that the hybrid has a greater potential sink size (attained with less N than conventionally bred semidwarf varieties).

Kim (1985) found significant positive relation between grain yield and spikelets per plant (r = .783**) as did Chang et a1 (1971), and Murayama (1973); between yield and 1,000-grain weight (r = .566**) and between yield and panicles per plant (r = .344**) with 180 kg N/ha. A higher relation was observed between grain yield and spikelets per plant. Chang et al (1971) and Murayama (1973) observed higher correlations between grain yield and panicles per plant (r = .4206**, .619**) than between yield and 1,000-grain weight (r = .1569**, .462**).

Kim (1985) found higher direct effects of spikelets per plant and 1,000-grain weight on grain yield than of panicles per plant.

Research indicates positive heterotic values predominantly in spikelets per plant and 1,000-grain weight and high correlations between grain yield and spikelets

Table 3. Heterosis (Ht), heterobeltiosis (Hb) and standard heterosis (Sh) in yield components of 4 hybrids for 3 N levels at 30 x 15 cm spacing in 1984 (Kim 1985).[a]

Hybrid	Nitrogen level (kg/ha)	Panicles/m* (%)			Spikelets/plant (%)			Field grain ratio (%)			1,000-grain Wt (%)			Harvest index (%)		
		Ht	Hb	Sh	Ht	Hb	Sh	Ht	Hb	Sh	Ht	Hb	Sh	Ht	Hb	Sh
V20 A/Milyang 46	120	3	-1	20**	101**	65**	67**	5	1	1	9**	3**	31**	6**	0	11**
	180	10	8	32**	100**	54**	64**	2	1	2	8**	3**	38**	9**	6**	15**
	240	-2	-3	5	71**	29**	21**	6	3	1	8**	2**	34**	8**	6**	12**
V20 A/Suweon 287	120	-1	-3	16**	63**	27**	44**	3	-3	1	5**	-6	19**	7**	2*	11**
	180	4	2	24**	75**	35**	48**	-1	-3	-1	2*	-11	21**	5**	2**	11**
	240	17	16	22**	97**	40**	43**	3	-1	-2	3*	-8	21**	4**	2*	8**
Zhen-Shan 97 A/Milyang 46	120	7	1	12*	99**	51**	54**	5	1	2	11**	9**	29**	11**	8**	14**
	180	6	1	16**	90**	45**	50**	3	1	2	9**	9**	31**	12**	10**	15**
	240	9	1	10**	81**	43**	34**	-2	-3	-5	11**	9**	31**	6**	0	12**
Zhen-Shan 97 A/Suweon 287	120	-4	-11	0	68**	21**	38**	4	-1	2	5**	-3	14**	10**	6**	12**
	180	-5	-10	4	66**	23**	35**	1	0	2	7**	-1	18**	7**	4**	10**
	240	9	-4	6	78**	29**	33**	0	-3	-3	4**	-3	17**	3**	-4	9**

[a]Ht over midvalues of B line and corresponding pollinator, Hb over pollinator Sh over Suweon 294.

per plant, and yield and 1,000-grain weight. Therefore, highest grain yields should result from hybrids of parents which contribute increased spikelet numbers and higher grain weight.

Plant height. Most studies have reported higher heterosis in plant height in the hybrid than in the midparent; some have reported higher heterosis than the high parent and check variety. Other studies showed negative heterosis for plant height of F_1hybrids (Pillai 1961, Singh and Singh 1978). Chang et al (1971) pointed out that heterosis in plant height might introduce the problem of lodging and noted the importance of parental selection. Disadvantages of positive heterosis for plant height have been reported (Hunan Provincial Paddy Rice Heterosis Scientific Research Coordination and Cooperation Group 1978, Murayama 1973, Ponnuthurai et al 1984). Ponnuthurai et al (1984) reported that taller plants may have better plant canopy for photosynthesis.

Virmani et al (1982) noted that the height of F_1 hybrids was almost equal to or slightly taller than the parents and/ or check varieties in the DS. Because plant height is negatively correlated with lodging resistance (Chang 1967), positive heterosis for plant height in hybrids would not be desirable with higher N rates.

Days to flowering. Most data have indicated negative heterosis in days to flowering in hybrids (Chang et al 1971,1973; Dhulappanavara and Mensikai 1967; Fujimaki and Yoshida 1984; Mallick et al 1978; Namboodri 1963; Purohit 1972; Singh et al 1980). Some research has reported both positive and negative heterosis in flowering duration.

Most hybrids have long growth duration (Deng 1980, Lin and Yuan 1980, Tian et al 1980, Wu et al 1980). Xu and Wang (1980) found that days to maturity in hybrids depends on the male plant. Ponnuthurai et al (1984) noted hybrid growth duration similar to that of the shorter growth duration parent; hybrids had higher productivity in the DS.

Dry matter production. Rao (1965) reported that hybrids that gave high grain yields also produced high straw yields. Jennings (1967) found that extremely vigorous vegetative development of the hybrid during early growth stages resulted in excessive shading and produced no heterosis in yield. Kawano et al (1969) showed that promoting vegetative vigor in F_1 hybrids may not be useful in increasing yield. Certain hybrids showing too much vegetative growth had lower grain-straw ratios (Hunan Provincial Paddy Rice Heterosis Scientific Research Coordination and Cooperation Group 1978). Higher heterosis in leaf area was observed in F_1 hybrids (Chang et al 1971, Kawano et a1 1969, Kim 1985, Wang and Yoshida 1984).

Hybrid superiority to conventional varieties in dry matter (DM) production was reported in China (Anonymous 1977). High-yielding hybrids also showed signifcant heterosis and heterobeltiosis in total dry matter and harvest index (HI) (Kim 1985, Ponnuthurai et al 1984, Virmani et al 1981). Hybrids have shown higher vegetative growth rates than the parents and larger green leaf area at earlier growth stages, which contributed to photosynthetic efficiency (Lin and Yuan 1980, Wang and Yoshida 1984, Wang et al 1983, Wu et al 1980). However, Yamauchi and Yoshida (1985) reported that hybrid vigor was not reflected in net photosynthetic rate.

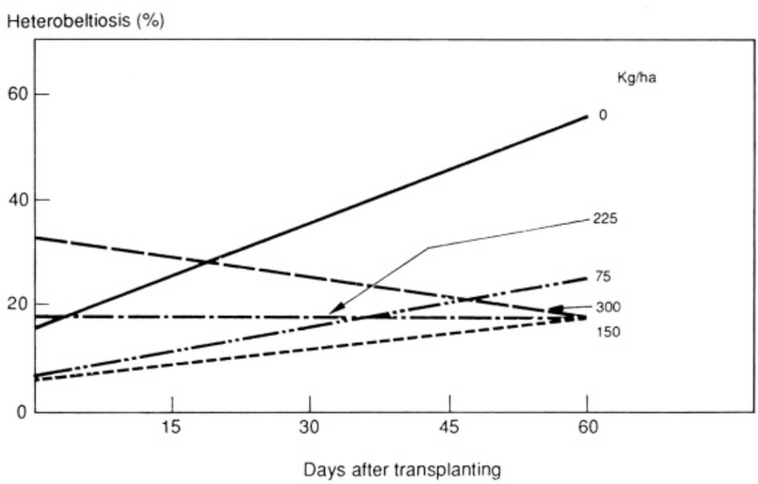
A study of hybrid growth under 5 N levels at Iri, Korea, showed that positive heterobeltiosis in plant weight increased linearly from no N to 150 kg N/ha (Fig. 1). The highest heterobeltiosis in plant weight was found with 0 N, followed by 75 kg N and 150 kg N/ ha. This suggested that hybrids attain higher DM at less N than do conventional semidwarf varieties.

Hybrids differed consistently from their parents and from conventionally bred semidwarf varieties in growth rate, leaf area index, leaf area ratio, plant weight, and HI.

Photosynthesis and respiration. Hybrid superiority has been reported for such physiological traits as higher photosynthetic area, chlorophyll content per unit area, photosynthetic efficiency, and lower respiration intensity (Hunan Provincial Paddy Rice Heterosis Scientific Research Coordination and Cooperation Group 1978, Lin and Yuan 1980) and elsewhere (Deng 1980, Kim 1985, McDonald et al 1971, Wang and Yoshida 1984, Wang et al 1983, Wu et al 1980). However, in other studies no superiority in photosynthetic rate of F_1 hybrids was reported (Hunan Agricultural College, Department of Chemistry 1977, Kabaki et al 1976, Yamauchi et al 1985).

Root development. Hybrids showed higher heterosis in number of roots, root thickness, root dry weight, number of adventitious roots per plant, number of fibrils, and root activity (Anonymous 1977, Hunan Provincial Paddy Rice Heterosis Scientific Research Coordination and Cooperation Group 1978, Lin and Yuan 1980, Tian et al 1980, Wu et al 1980, Kim 1985).

Temperature and other stresses. Hybrids showed higher heterosis for cold tolerance at seedling, but negative heterosis at grain ripening (Deng 1980, Kaw and Khush 1985). Hybrids were more sensitive to extreme temperatures at flowering, especially at lower temperatures, than conventionally bred varieties (Huang et al 1984, Lou 1979, Tian et al 1980). Hybrids tended to be superior in drought tolerance



1. Change in heterobeltiosis on dry weight 60 d after transplanting under five N levels at 30 × 15 cm spacing (Kim et al 1985, unpublished data).

(Tian et a1 1980), salt tolerance (Akbar and Yabuno 1975), ratooning ability (Chauhan et a1 1983), and deep water tolerance (Singh 1983).

Heterosis in yield in relation to environment

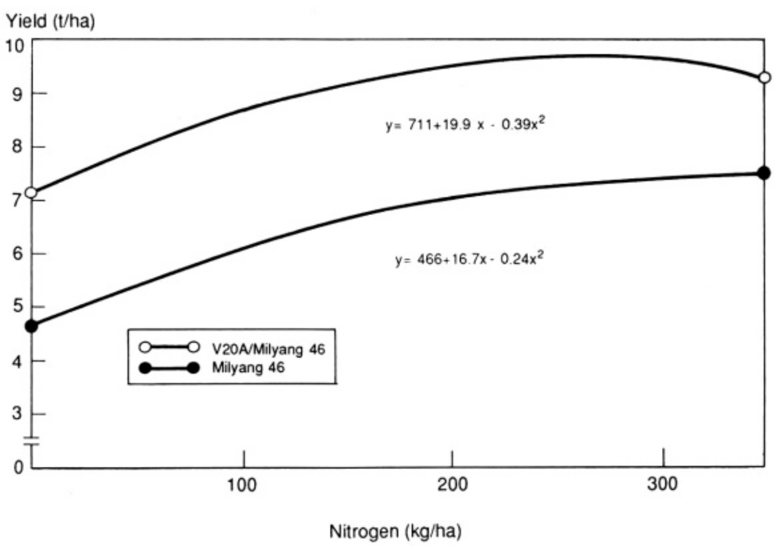
Positive heterosis in grain yield at noncommercial wide spacing (Jennings 1967) and negative heterosis in grain yield under economic spacing have been found (Kawano et a1 1969). However, Murayama et a1 (1974) observed that heterosis did not differ much under different spacings and soil fertility. Table 4 shows higher heterobeltiosis in grain yield under 5 N levels, although the values vary. Grain yield of F_1 hybrid was higher with lower N levels (Deng 1980). The highest yield was with 200 kg N/ha in the hybrid, compared to more than 250 kg N/ha in the conventionally bred variety (Fig. 2).

One reported advantage of F_1 hybrids is wider adaptability with high yield (Anonymous 1977, Deng 1980, Lin and Yuan 1980). Virmani et a1 (1982) pointed out the benefit of hybrids during the DS and their disadvantage in the WS.

Table 4. Heterobeltiosis in grain yield under 5 N levels of hybrid V20 A/Milyang 46 in 30 × 15 cm spacing at Iri, Korea, 1985 (Kim et al 1985, unpublished data).

	0 N	75 kg/ha	150 kg/ha	225 kg/ha	300 kg/ha
Heterobeltiosis (%)	45 b	61 a	32 bc	31 bc	30 c
Grain yield (t/ha)	7.0 c	8.8 b	8.8 b	9.8 a	9.5 a

Means followed by the same letter in a row are not significantly different at 5% level.



2. Effect of N on yield of F_1 hybrid at 30 × 15 cm spacing in Iri, Korea, 1985 (Kim et a1 1985, unpublished data).

Grain yield of F_1 hybrids tended to vary considerably across seasons in the tropics (Ponnuthurai et al 1984, Virmani et al 1982) and subtropics (Chang et al 1971, Huang et al 1984). Grain yield of F_1 hybrids also has shown instability under lower temperature (Tian 1980, Wu et al 1980). Chang et al (1971) observed lower heterosis in grain yield under unfavorable conditions and higher heterosis in grain yield under favorable conditions.

However, Yab and Chang (1976) observed that hybrids performed better under dryland conditions. Kim (1985) showed nonsignificant yearly variation in grain yield of F_1 hybrids in contrast to significant yearly variation in yield of the parents. Huang et al (1984) suggested that semidwarf indica rice cultivars show more yield potential under favorable conditions, More research is needed to understand the effect of environments on adaptability and heterosis for grain yield.

Gene action and combining ability

Interest in gene action and combining ability in relation to heterosis lies in determining whether heterosis is fixable and/or predictable. Heterosis can result from partial to complete dominance, overdominance, epistasis, and combinations of these (Comstock and Robinson 1952). If partial to complete dominance predominates, it is theoretically possible to develop homozygotes with fixed heterosis. If overdominance or overdominant types of epistasis predominate, then the highest yielding lines must be heterozygotes (Sprague and Eberhart 1977).

Studies of gene action on yield and yield components in rice are rare. Most suffer from small population sizes and wider spacings than is normal for rice, and in only one location or year. Here, we review research on gene action and combining ability for yield and other agronomic characters (Table 5).

Davis studies. Several hybrid experiments at Davis involved diallels, yielding information on gene action and combining ability (Davis and Rutger 1976, Rutger and Shinjyo 1980). However, each Davis diallel included crosses between indica and japonica types, so several combinations exhibited large levels of F_1 sterility. Thus, any conclusions on combining ability and gene action for yield per se would be meaningless, and would even cast doubt on the validity of yield components. Nevertheless, gene action and combining ability data were collected on important agronomic characters thought to be unaffected by sterility.

Kramer (1973) studied inheritance of leaf angle and length and width of the top 2 leaves in a 7-parent diallel. Four parents were of indica-japonica origin and had erect leaves; three were japonicas and had more horizontal leaves. Variation in leaf angle was due largely to additive effects, with some partial dominance. Overdominance was observed in length of both leaves, especially for longer leaves. Epistasis seemed minor.

Foster and Rutger (1978) investigated inheritance of plant height in a 10-parent diallel that included 2 tall japonicas and 8 semidwarf lines of varying japonica and indica-japonica backgrounds. Most height variation was accounted for by three major genes. The Hayman-Jinks diallel analysis demonstrated significant additive and dominance effects, but epistasis was not detected.

Li and Rutger (1980) studied nature of gene action for cool-temperature seedling tolerance in the F_1 and F_2 generations of a 9-parent diallel. Both additive

Table 5. Reports of diallel crosses indicating gene action and combining ability for yield and other agronomic characters.

Authors	Parents (no.)	Characters studied		
		Yield	Yield components[a]	Other
Li and Chang (1970)	4		×	Heading, height
Chang (1971)	4	×	×	Heading, height
Murayama (1972)	7	×	×	Height
Kramer (1973)	7			Leaf angle, length, width
Mohanty and Mohapatra (1973)	4	×	×	
Chang et al (1973)	4	×	×	Heading, height
Li (1975)	6	×	×	Height
Singh and Nanda (1976)	6	×	×	
Maurya and Singh (1977)	7	×	×	Heading, height,grain length, leaf length, etc.
Singh (1977)	6	×	×	
Foster and Rutger (1977)	10			Height
Haque et al (1981)	5	×		Heading, height, sterility
Singh et al (1980)	6	×	×	Heading, height, leaf length, and width
Chang (1980)	4		×	Height
Rao et al (1980)	7	×	×	Heading
Li and Rutger (1980)	9			Seedling vigor
Rahman et al 1981	5	×	×	Heading, height
Devarathinam (1984)	12	×	×	Heading
Murai and Kinoshita (1986)	5		×	Height, grain width, plant weight
Moon and Rutger (1986)	9			Cold-induced sterility

[a]Traditional yield components refer to panicles per plant, grains per panicle, and individual grain weight. Several authors measured one or two of these traits.

and dominance effects were significant, with additive effects being more consistent than dominance effects. Varying degrees of dominance and overdominance were observed for seedling vigor.

Other reports. Murayama (1972) studied heterosis in yield and yield components in a 7-parent diallel, with reciprocals, among Japanese varieties grown in the field. Significant general combining ability (GCA) and specific combining ability (SCA) variances were found for all characters.

Mohanty and Mohapatra (1973) studied yield and yield components in a 4-parent diallel among two tall and two semidwarf varieties, with plants grown in pots. Highly significant GCA and SCA variances were found.

Chang et al (1973) studied heterosis in yield and yield components in a 4-parent diallel among diverse varieties grown over 2 seasons. Heterobeltiosis was not observed. Lack of heterobeltiosis and of inbreeding depression was attributed to additive gene action for yield components. Spikelet sterility in three of the crosses also reduced heterosis potential.

Li (1975) studied heterosis in yield and yield components in a 6-parent diallel grown under 2 nitrogen levels. For yield per plant, additive variance was small and dominance variation predominated, with indications of overdominance. For other traits, most variance was due to additive effects.

Singh and Nanda (1976) studied heterosis in yield and yield components in a 6-parent diallel during 1 season in the field. GCA effects were larger than those for SCA, indicating predominance of additive gene action.

Singh (1977) studied yield and panicle characters in a 6-parent diallel in the field. Both GCA and SCA effects were significant for all characters.

Maurya and Singh (1977) studied combining ability for yield and fitness in a 7-parent diallel grown 1 season in the field. Both GCA and SCA variances were highly significant, with GCA variances higher than SCA.

Singh et al (1977) studied yield and quality components in an 8-parent diallel in the field. The combining ability analysis indicated that nonadditive gene action predominated for yield and protein.

Singh et al (1980) studied yield and other characters in a 6-parent diallel in the field. Both nonadditive and dominance effects were important for yield.

Rao et al (1980) studied combining ability for 10 characters, including yield, in a 7-parent diallel grown in the field. Both GCA and SCA effects were significant for all characters.

Rahman et al (1981) studied combining ability for yield and yield components in a 5-parent diallel grown in the field. Both GCA and SCA effects were significant, with GCA the highest, indicating predominance of additive genes.

Devarathinam (1984) studied combining ability in a 12-parent diallel grown in the field. Both GCA and SCA affected yield and other characters. The hybrids showing a high degree of heterosis also had high performance.

Murai and Kinoshita (1986) studied yield components, but not yield per se, in a 5-parent diallel of Hokkaido rice varieties grown in the field. Additive effects generally predominated over dominance effects.

Gene action summary. In general, the studies on types of gene action in rice seem more viable than those for maize and wheat. For maize, Sprague and Eberhart (1977) concluded that "..the genetic variability of the more important traits is due largely to additive genetic variance ..." Similar conclusions seem to apply to wheat (Johnson and Schmidt 1968, Virmani and Edwards 1983). However, very few studies on gene action in rice have been conducted compared to maize and wheat. More and larger studies are needed to know whether rice shows more variability for types of gene action than do other crops.

Prediction of heterosis

Successful prediction of heterotic combinations would be of great value to breeders. In maize, numerous early studies concentrated on determining correlations between parent lines and hybrids (Sprague and Eberhart 1977). Rice breeders also have studied correlations between parent lines and F_1 hybrid performance (Kim 1985, Virmani et al 1981). Some correlations have enabled breeders to suggest which types of parents would be most appropriate for use in hybrids.

Performance of F_1s could be predicted more precisely if genetic effects, including additive, dominance, and epistatic effects, were known. Despite estimating these effects in diallel analyses, most diallels involve limited amounts of handcrossed seed, which leads to small plots with low planting rates.

Bailey et al (1980) presented a technique for predicting heterosis of hybrid wheat combinations without field testing the F_1 itself. The F_1 means were closely predicted from information obtained from the parent, the F_2 generation, and three-way F_1 means. In 2 test years, highly significant correlations of r =0.97 and r= 0.89 were obtained between predicted and observed means. A limitation was that the technique still depended on producing large amounts of handcrossed seed of the three-way F_1s. When the three-way F_1s were omitted from the prediction analysis, leaving only data from the parent and the F2 generations, correlations between actual and predicted F_1 means dropped to r= 0.76 and 0.49. However, the authors noted that other generations not used in their study, such as backcross, F_3 or backcross-selfed means, could be used to improve the predictions.

Some techniques suggested by Bailey et al (1980) might be used to select heterotic combinations for hybrid rice, especially if advanced generations F_2, F_3, or backcross F_2 where large quantities of seed are readily obtained are used.

Will use of hybrids expand?

China's successes with hybrid rice and the reports of heterosis from other countries indicate that further use of hybrids is warranted. Some breeders might draw the opposite conclusion, on the basis of studies that often indicate the importance of additive gene actions. However, the occurrence of occasional specific heterotic combinations, which may result from partial to complete dominance, overdominance, epistasis, and combinations make further pursuit of hybrids worthwhile. If the cost of hybrid seed production could be reduced to levels comparable to standard highquality seed, hybrids probably would be adopted not only in rice but also in other normally self-pollinated cereals. The inherent variety protection offered by hybrids stimulates private investment in plant breeding and could lead to greater overall crop improvement than would be attained if breeding were left solely to public agencies.

Asexual seed production would be an interesting alternative that could reduce hybrid seed costs in situations in which heterozygotes with nonfixable gene action are the highest-yielding. Apomixis has the potential to provide hybrids with permanently fixed heterosis that breed true (Bashaw 1980). Identifying and applying apomixis could provide the breakthrough needed if more of the world's rice farmers are to economically capture the increased yield of hybrids. Research designed to find and/or transfer apomixis into rice is under way (Rutger et al 1986).

Heterosis and genetic diversity

Heterosis in grain yield showed wide variability, depending on the cross combination. Some were positive and some were negative in expression of heterosis. Hybrid yield in experiment plots appears to be about 10 t/ha both in the tropics and the temperate regions. Maximum yields in conventionally bred varieties in the temperate region already are approaching more than 10 t/ha. For commercial use of hybrids in the future, a hybrid variety must yield at least 25% more than the best commercial variety (Swaminathan et al 1972).

Heterosis in hybrid yield should be continuously sought. One challenge in hybrid breeding is selecting suitable parental (cytoplasmic male sterile/maintainer and restorer) lines to develop heterotic combinations (Julfiquar et al 1985).

Chang et al (1971) observed that hybrids exhibited higher heterosis than the midparents. Saini et al (1974) observed that positive standard heterosis for F_1 hybrids were derived from a parent selected for improved plant type. Virmani et a1 (1982) reported F_1 hybrids that correlated significantly with parental traits. They suggested selecting parents from among elite breeding lines based on yield performance. Diverse origins of resistance to insects and disease should give heterotic combinations.

But Khalique et al (1977) found no association between parental and hybrid yield performance. Anand and Murty (1968) argued that the more diverse the parents, the greater the chances of heterotic value in the F_1. Heterosis higher than the better parent showed a significant positive correlation with the intercluster distance between the parents, in several crosses (Maurya and Singh 1978). But when divergent parents were crossed, heterosis did not always occur (Cress 1977). Maurya and Singh (1978) suggested that the estimate of intercluster distance has to be reviewed carefully.

Cross combinations involving parents of diverse origin showed high heterosis in yield (Singh et al 1984, Subramanian and Rathinam 1984). However, some crosses which showed significant heterosis for yield involved parents of the same geographical origin. Pedigree and geographical diversity are both important for occurrence of heterosis (Hunan Provincial Paddy Rice Heterosis Scientific Research Coordination and Cooperation Group 1978, Xu and Wang 1980). However, there were no indications of a relation between geographical diversity and genetic diversity (Julfiquar et al 1985, Srivastava 1980). More heterosis may be achieved in specific combinations using indica varieties that have genetically dissimilar traits with higher yield potential and resistance to disease, insects, and other environmental stresses.

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Biochemical basis of heterosis in rice

Deng Hongde

Growth and yield are the results of a series of reactions, and phenotypic expression ultimately is due to genetically controlled biochemical mechanisms. The causes of the expression of significant heterosis in F_1 rice hybrids are reviewed from the biochemical point of view.

Hybrid vigor or heterosis is the basis for the yield advantage of hybrid rice over conventional varieties. To more fully understand heterosis, Hunan Agricultural College undertook a program in 1977 to determine the biochemical basis of heterosis in rice. The research program included studies of amylase and α-amylase activity, RNA content in young roots, the ability of roots to synthesize amino acids, the ability of hybrids to absorb and synthesize assimilates, photosynthesis and transpiration of assimilates to panicles, soluble sugar and total N, grain filling ability, the isoenzyme basis of heterosis, and the nucleohistone in hybrid rice.

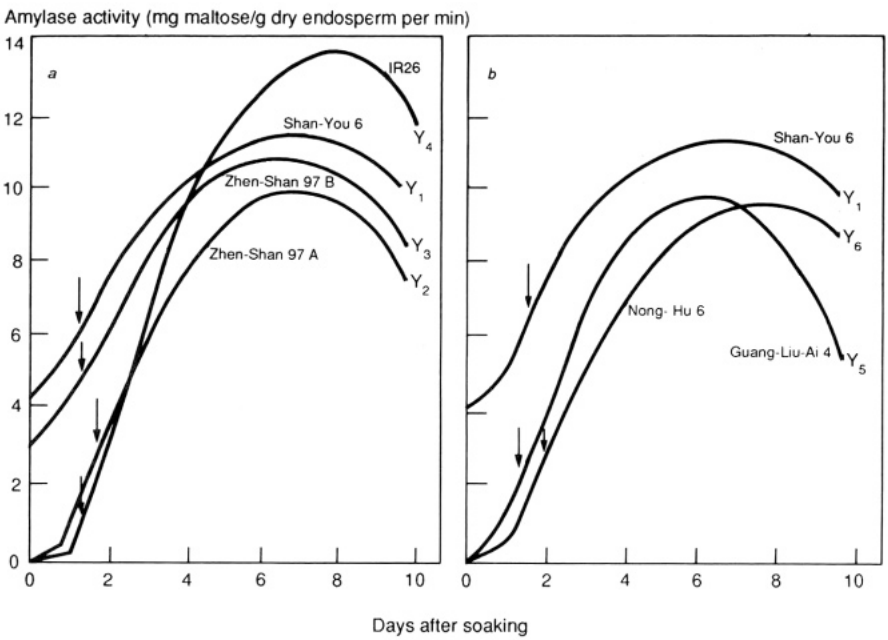
In all studies, hybrid rice was superior to the conventional variety checks. This paper presents the results of our studies at Hunan Agricultural College, and explains how each of the characters measured contributes to the yield advantage of hybrid rice.

Amylase and α-amylase activity

Hybrid rice has a higher initial growth rate than conventional varieties. This growth advantage is expressed as early as in seed gemination. The germination speed of Nan-You 2 and its three parental lines is in the order of hybrid > R line > B line > A line (Li et al 1982). A similar order is seen in the activity of their α-amylases.Theactivity of α-amylase in hybrids is 20% higher than the mean value in 3 parental lines (Hunan Agricultural College 1977a). During initial germination (0-3 d), hybrid rice showed obvious heterobeltiosis (202%) in amylase activity and 433% in α-amylase activity. It is generally considered that α-amylase and amylase are synthesized after germination begins. Further study has shown that amylase and α-amylase are conserved in the endosperm of dry seed of hybrid rice and its B line, and that their activity is higher in hybrid rice than in its B line (Table 1, Fig. 1, 2). The higher α-amylase and amylase contents reserved and their higher activities make it possible to rapidly hydrolyze the starch in the endosperm, providing energy and assimilates for embryo development.

Table 1. Activity of α-amylase in the endosperm of dry seed of hybrid rice and its B line determined twice in each of 3 replications (Liu 1986).

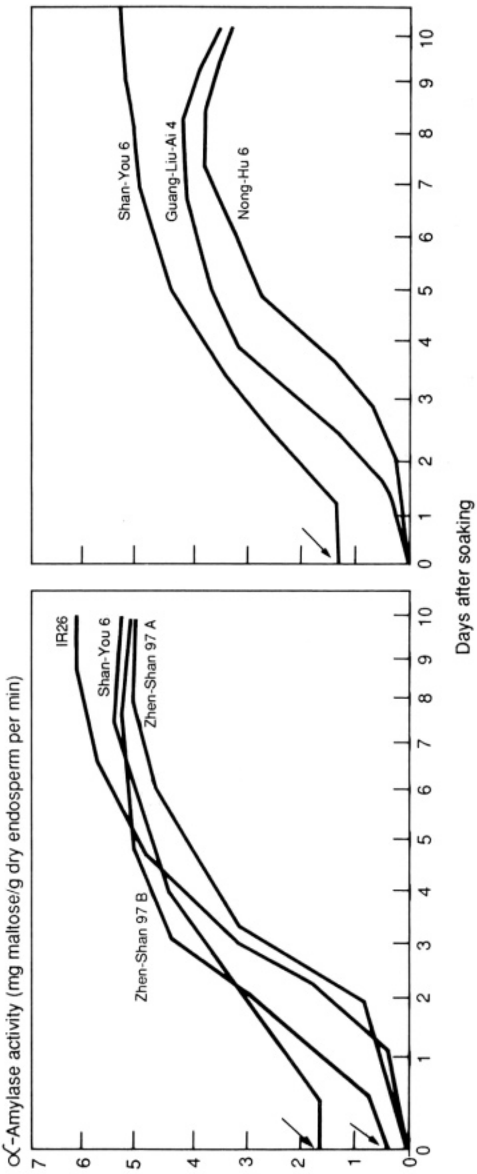
Variety	mg meltose/g dry endosperm per min						X	*t*	t = 0.01
	Replication 1		Replication 2		Replication 3				
	1	2	1	2	1	2			
Shen-You 6	0.6661	0.7484	0.6201	0.6201	0.9280	0.9280	0.7500	4.4026	3.1690
Zhen-Shan97 B	0.2439	0.2220	0.4963	0.4963	0.4689	0.4689	0.3994		



1. Change in total amylase activity after seed soaking (Liu 1986). *a*) $Y_1 = 3.90 + 2.14X - 0.15X^2$, $r_1 = 0.98^{**}$; $Y_2 = 3.41 + 3.78X - 0.27X_2$, $r_2 = 0.99^{**}$; $Y_3 = 1.79 + 2.69X - 0.20X^2$, $r_3 = 0.98^{**}$; $Y_4 = 6.00 + 4.95X - 0.31X^2$, $r_4 = 0.97^{**}$. *b*) $Y_1 = 3.90 + 2.14X - 0.15X^2$, $r_1 = 0.98^{**}$; $Y_5 = -2.21 + 3.85X - 0.30X^2$, $r_5 = 0.98^{**}$; $Y_6 = -3.27 + 3.25X - 0.20X^2$, $r_6 = 0.99^{**}$.

RNA content of young roots

The change of RNA content of the root tips of Nan-You 2 and its three parental lines was studied histochemically (Hunan Agricultural College 1977a). There were more cells containing RNA in the tips of sprouting roots of Nan-You 2 than in those of its three parental lines. Nan-You 2 continued to have higher RNA content in the tips of its new roots during tillering, early panicle differentiation, and booting (Table 2). RNA synthesis in roots was closely correlated with their metabolism and nutrient-absorbing ability and, in particular, with increased K absorption. The K content in the sheath of



2. Change in a -amylase activity after seed soaking (Liu 1986).

Table 2. Relative RNA content in the tip of new roots of Nan-You 2 and its three parental lines (Hunan Agricultural College 1977a).

Development stage	Relative RNA content			
	Nan-You 2	R line	B line	A line
5 d after germination	++++	+++	++	+
Active tillering	+++	++	++	+
Early panicle differentiation	++	++	+	+
Booting	+	+	-	-

functional leaves was 6,250 ppm in Nan-You 2, 5,050 ppm in IR24, 4,250 ppm in Er-Jiu-Nan 1 A, and 3,550 ppm in its B line. The value for hybrid rice was much higher than that of its parents.

Ability of roots to synthesize amino acid

A paper chromatography-ninhydrin method was used for microanalysis of the exudate in roots during flowering. The roots of Nan-You 2 synthesized and transported to the aboveground plant parts 13 kinds of amino acids, but the roots of the conventional variety, Zhen-Zhu-Ai 11, synthesized only 7 kinds (Table 3). Total amount of amino acids synthesized and transported in hybrid rice during flowering and the milk stage was 33.5%, 9 times that of the check.

Liu (1984) studied the physiological and biochemical bases of the high yield of Shan-You 6. They identified 23 free amino acids in the exudate of Shan-You 6,22 of which they quantitatively determined. Three unknown free amino acids were also

Table 3. Relative amino acid absorption ability of roots of hybrid rice Nan-You 2 and conventional Zhen-Zhu-Ai II (Guangxi Agric. College 19771.

Amino acid	Relative amino acid absorption ability	
	Nan-You 2	Zhen-Zhu-Ai II
Cystine	++	+
Glycine	+	+
Histidine	++	
Serine	+	+
Leucine	+	
Lysine	++	
Phenylalanine	++	
Alanine	++	+
Valine	++	
Glutamic acid	++	+
Aspartic acid	+++	+
Asparagine	++++	+
Tyrosine	++++	

isolated, but their concentration was not determined. The calculation of the 22 free amino acids showed that total amino acid in the exudate reached 808.7 mg/liter, which was 36.5, 3.6, and 75% more than that in the 3 parental lines, and 31.4 and 27.4% more than that in the 2 conventional varieties. The necessary amino acids made up 228.1 mg/ liter of the total free amino acid content in Shan-Y ou 6, which was 44.8, 39.7, and 114.9% more than those in the 3 parental lines, and 49.6 and 92.8% more than those in the 2 conventional varieties. This indicated that hybrid rice was superior in the synthesis of organic matter (Table 4) (Liu 1984).

The changes of some biochemical components such as total N content, starch in the leaf, and free amino acid in the sheath of Nan-You 2 and its R line IR24 during the seedling, tillering, booting, and milk stages were studied in the Hunan Agricultural College in 1977. The results showed that hybrid rice was superior to the R line in the synthesis of assimilates (Table 5) (Hunan Agriculture College 1977a).

When the extract from roots of a hybrid rice variety (Hong-Lien-Hua-Ai/Gu-Xuan) was added to a culture medium, the induction rate of rice calli and the rate of green seedling regeneration significantly increased (Shu 1978). Those results indicated there may be hormones in the roots of hybrid rice.

Glycolic acid oxidase

Besides the demonstrated ability of hybrid rice to absorb and synthesize assimilates, its low assimilate consumption is the main cause of its superior growth and yield. The activity of glycolic acid oxidase in hybrid rice, which is closely related to photorespiration, is 10-50% lower than that in its parents and conventional varieties. The difference is most obvious after booting. A 1977 study (Shanghai Plant Physiology Research Institute 1977) showed that glycolic acid oxidase activity in the hybrid Nan-You 3 was 34.1% lower than that in its male parent IR661 (Fig. 3).

Accumulated photosynthates were 4.53 mg/dm^2 per h in hybrid rice and 2.40 mg/dm^2 per h in conventional varieties. The correlation of glycolic acid oxidase activity to the low compensation point of CO_2 and the high photosynthetic intensity in hybrid rice is shown in Table 6 (Shanghai Plant Physiology Research Institute 1977). The conclusion that hybrid rice has low glycolic acid oxidase activity, high photosynthetic intensity, and a low percentage of unfilled spikelets is supported by a 1978 study (Hunan Agricultural College 1977b).

Catalase and peroxidase

Accumulated hydrogen peroxide in the cells of a plant may be removed by catalase, which leads to a toxic effect. Peroxidase is a common oxidase. Catalase activity in hybrid rice from early panicle differentiation to heading was higher than that in its male parent (Table 7), which favors photosynthesis and transmission of assimilate to panicles (Wuhan University 1977). A study of peroxidase activity in hybrid Nan-You 2 and the conventional variety Guang-Yu 73 showed that enzyme activity in the flag leaf and the panicle of both varieties tended to decline, but the enzyme activity in

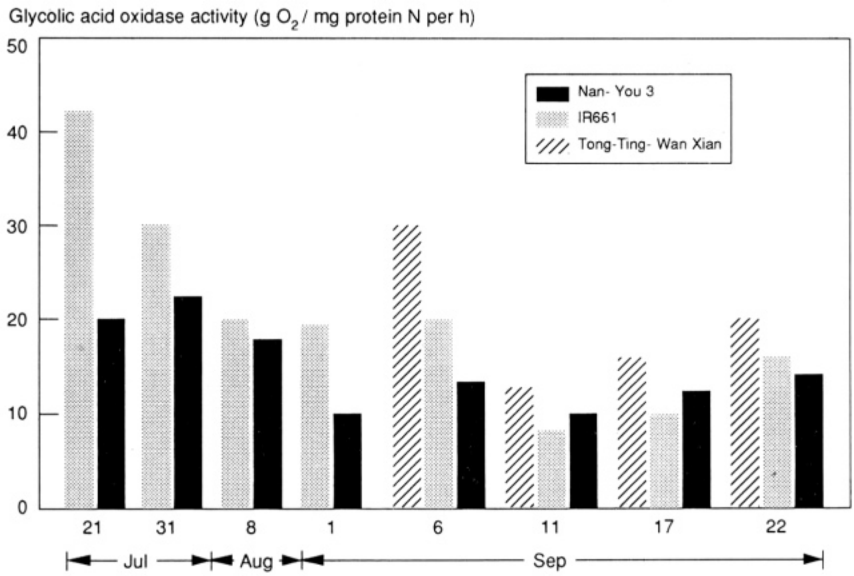
Table 4. Concentration of 23 free amino acids in the exudates of 3 parental lines, a hybrid rice, and 2 conventional lines (Liu 1986).

Amino acid [a]	Concentration (mg/liter)					
	Parental lines			Hybrid	Conventional	
	Zhen-Shan 97 A	Zhen-Shan 97 B	IR26	Shan-You 6	Guang-Liu-Ai 4	Nong-Hu 6
Aspartic acid	63.2	81.6	60.9	90.8	68.4	67.7
Asparagine	46.1	42.0	38.2	60.8	32.0	28.8
Threonine	23.7	22.6	13.6	26.0	22.3	16.9
Serine	49.3	36.8	36.8	66.4	57.9	61.3
Glutamic acid	30.2	34.2	25.4	37.1	32.7	40.9
Glutamine	125.2	171.4	87.0	152.0	135.7	140.9
Proline	7.9	11.9	6.6	15.7	11.9	11.3
Glycine	5.2	10.1	3.7	6.6	3.8	3.9
Alanine	13.9	43.7	15.6	26.0	22.6	31.8
Cystine	0.9	1.2	0.5	0.6	1.3	0.9
Valine	37.5	36.3	24.9	49.9	39.9	31.6
Methionine	2.2	2.2	2.0	3.4	2.7	3.3
Isoleucine	21.8	23.2	14.5	31.6	19.3	14.5
Leucine	20.7	22.0	12.3	32.9	18.9	12.2
Tyrosine	10.2	13.3	11.6	20.0	11.1	9.3
Phenylalanine	11.0	11.6	6.8	22.1	12.4	7.5
ß-alanine	2.1	4.6	2.5	4.3	4.9	5.9
g-aminobutyric acid	27.7	53.2	21.9	26.6	31.4	59.0
Histidine	8.7	10.4	7.6	15.6	8.6	6.3
Lysine	31.9	34.9	26.2	46.6	23.4	26.0
Ammonia	3.2	9.3	2.5	3.4	4.3	5.3
Tryptophan [b]	P	P	P	P	P	P
Arginine	42.8	43.2	32.4	57.6	39.2	44.4
Ornithine	10.2	11.1	9.3	16.1	10.1	10.5
Total	592.4	744.9	460.3	808.7	615.5	634.7

[a] Ammonia excluded. [b] Qualitatively determined.

Table 5. Some biochemical components in hybrid Nan-You 2 and its R line at different stages (Hunan Agricultural College 1977a).

Variety	Seedling stage (40 d)		Active tillering	Booting		Milk stage	
	Total N (mg/g)	Starch (mg/g)	N in leaf (%)	N in leaf (%)	Amino acid in sheath (ppm)	N in leaf (%)	Amino acid in sheath (ppm)
Nan-You 2	15	125	4.4	3.2	140	3.1	152
IR24	9.6	103	3.2	3.4	140	2.9	95



3. Activity of glycolic acid oxidase in hybrid rice (Shanghai Plant Physiology Research Institute 1977).

Table 6. The activity of glycolic acid oxidase in hybrid rice and its relation to compensation point of CO_2 and photosynthetic intensity (Shanghai Plant Physiology Research Institute 1977).

Variety	Activity of glycolic acid oxidase at transplanting (%)	Compensation point of CO_2 at heading (ppm)	Photosynthetic intensity at flowering (mg dry wt/dm^2 per h)
Nan-You 3	62.2	76	15.6
IR661	96.3	80	10.1
Er-Jiu-Nan 1 A	79.0	88	11.2

Table 7. Catalase activity in two hybrid rices and a conventional variety (Wuhan University 1977).

Variety	Catalase activity (mg/g fresh wt per h)			
	Early panicle differentiation (27 Jun)	Booting (11 Jul)	Heading (23 Jul)	Milk ripe (10 Aug)
Ai-You 3	0.428	0.530	0.502	0.480
Nan-You 3	0.346	0.470	0.424	0.554
IR661	0.285	0.420	0.431	0.554

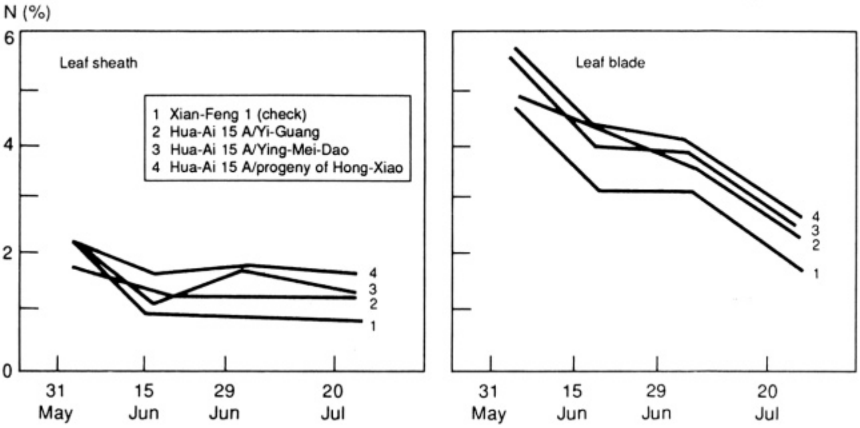
Table 8. Peroxidase activity in flag leaf and panicle of a hybrid rice and a conventional rice at 3 development stages (Hunan Agricultural College 1977a).

Variety	Peroxidase activity (mg/g fresh wt per h)					
	Booting		Full heading		Milk ripe	
	Flag leaf	Panicle	Flag leaf	Panicle	Flag leaf	Panicle
Nan-You 2	163	188.5	59.2	41.3	44.8	31.1
Guang-Yu 73	191	150.8	70.0	44.2	37.1	41.6

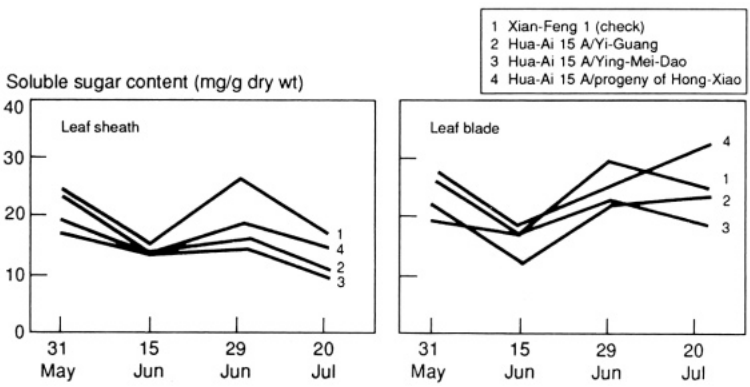
Nan-You 2 was lower than that in Guang-Yu 73 (Hunan Agricultural College 1977b) (Table 8). This makes it possible to lower metabolism of hybrid rice to postpone its senescence.

Soluble sugar and total N

The change of C and N metabolism in rice plants affects the relation between vegetative and reproductive growth, and is the main basis of high yield. The N content and the metabolic level in the leaf blade and sheath of hybrid rice throughout the life of the plant is higher than that of ordinary rice (Fig. 4) (Li et a1 1982). That is why the leaves of hybrid rice are dark green and do not yellow early at the late growth stage. The change in soluble sugar content peaks twice (at tillering and at initial heading) and drops markedly at early panicle differentiation and at ripening (Fig. 5) (Li et a1 1982). The drops in soluble sugar content are closely associated with the decomposition and transmission of nutrients. It is important that the soluble sugar content in the leaf blade and leaf sheath of hybrid rice during its entire growth is lower than that of ordinary rice. This indicates that hybrid rice is superior to ordinary rice in transmitting assimilates at the late growth stage, a trait that leads to the large panicles and high yields of hybrid rice. Until 20 Jul, the leaves of the two cross combinations Hua-Ai 15 A/progeny of Hong-Xiao and Hua-Ai 15 A/Yi-Guang remained dark green. Note that soluble sugar content tended to continually increase (Fig. 5).



4. Change in total N content in hybrid rice (Xiao 1979).



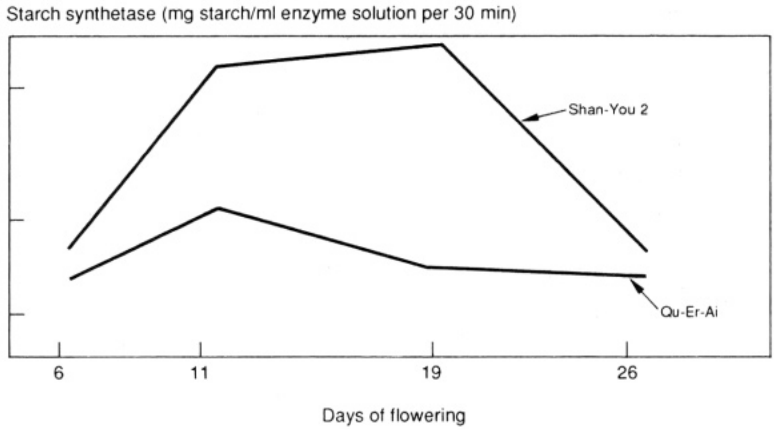
5. Change in soluble sugar content in the flag leaf and leaf sheath of hybrid rice (Xiao 1979).

Starch synthetase

The Guangdong Plant Physiology Institute (1978) studied the superior grain filling ability of hybrid rice. Starch synthetase activity in hybrid rice from the 6th day of flowering to the 26th day after flowering was much higher than in ordinary rice; the enzyme activity in ordinary rice started to decline the 11th day of flowering (Fig. 6).

Isoenzyme basis of heterosis

Isoenzyme refers to multiple forms of a single enzyme, identical in properties and catalytic action, but different in molecular structure of protein. The enzyme is genetically controlled and is a bridge between genes and characters. Since Schwartz (1960) first used isoenzymes to study heterosis in maize, isoenzymes have been widely applied in genetics and biochemistry.



6. Starch synthetase activity in hybrid rice and ordinary rice (Guangdong Plant Research Institute 1978).

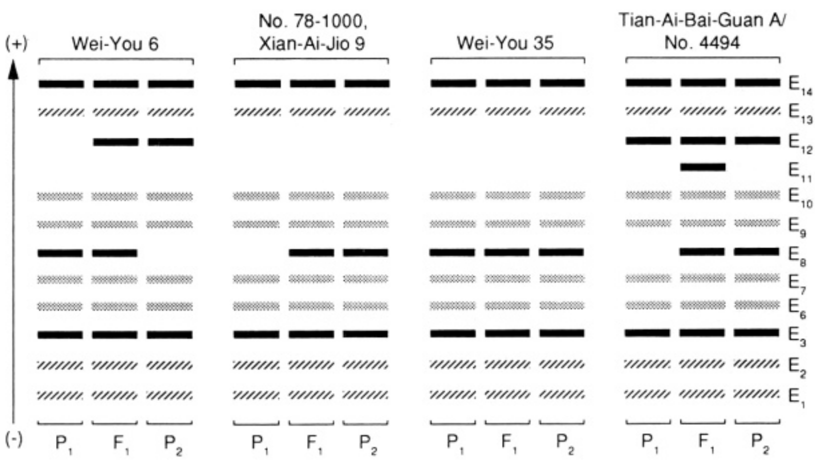
Many scientists in China have studied the relation between isoenzymes and heterosis in rice. They found that the hybrid combination whose F_1 had dominant complementary bands in the esterase isoenzyme was closely associated with expression of heterosis (Li et al 1982). That is, the zymogram of the F_1 of a strong hybrid combination is predominantly the complement of extraordinary enzyme bands of both parents. The zymogram is characterized by numerous enzyme bands, high enzyme activity, dark color, and wide band width. The complementary enzyme bands may be used as one of the biochemical indicators for predicting heterosis. As the esterase isoenzyme of the F_1 has complementary enzyme bands, which differ from the zymogram of its parents, this characteristic has been used in China to do preliminary evaluation of the purity of hybrid seeds. Yi (1981) showed that biological heterosis and economic heterosis are caused by the respective complementary bands at different loci (the former is a complement of 3A with 6A, and the latter is a complement of 5A with 6A). Many scientists studied the correlation of the peroxidase isoenzyme with heterosis in hybrid rice. In the F_1 of some combinations with good heterosis, a number of complementary enzyme bands with high activity were observed. Meanwhile, new bands were found in the F_1 of other combinations with good heterosis. They considered this phenomenon as another zymogram indicator (besides the complementary bands) for expression of heterosis (Li et al 1982).

Studies of the relation between the isoenzyme spectra of hybrid rice and heterosis show that complementation is an important biochemical indicator for heterosis, but not all combinations of complementary band type exhibit heterosis (Table 9). Heterosis is also expressed in the combinations of noncomplementary band type. There are multiple zymogram indicators for heterosis in rice: complementary band type, dominant band type, heterozygous band type, and distinctive band type (Fig. 7). The complementary band type, however, is considered the most important. The genetic explanation for each band type is shown (Deng and Wang 1984).

Table 9. Correlation of complementary and noncomplementary bands of esterase isozyme with heterosis in 160 hybrid rice combinations (Deng and Wang 1984).

Complementary					Noncomplementary				
Heterosis (A)		No heterosis (B)		Total	Heterosis (C)		No heterosis (D)		Total
no.	%	no.	%		no.	%	no.	%	
76	85.4	13	14.6	89	27	38.0	44	62.0	71

$R_1 = \frac{(A) + (D)}{160} = 75\%$, $R_2 = \frac{(B) + (C)}{160} = 25\%$.



7. Four patterns of the isoenzyme spectra correlated with heterosis in rice (Deng and Wang 1984).

The dominance hypothesis, which is based on the accumulation and complementation of favorable dominant genes, and the overdominance hypothesis, which is based on the interaction between heterozygous alleles, are both used to explain the mechanism of heterosis. These two hypotheses seem further confirmed by isoenzyme spectra (Deng 1986).

The nucleohistone in hybrid rice

He et al (1980) found no difference in the nucleohistone in Wei-You 6 and in its three parental lines. However, the H_1 content of the nucleohistone in the F_1 of Wei-You 6 exhibited heterobeltiosis, which might be closely associated with heterosis in characters (He et al 1980).

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Physiological bases of heterosis in rice

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The physiological bases of heterosis in F_1 rice hybrids were reviewed, with these conclusions:

1. F_1 hybrids exhibit vigor in initial seedling growth and maintain that growth advantage until heading. Heterosis in crop growth rate at the ripening stage was minimal. Therefore, the physiological mechanism involved in heterosis at initial growth needs further analysis.
2. Little heterosis in net assimilation rate (NAR) and relative growth rate after transplanting was observed, consistent with the negligible heterosis in photosynthetic activity in F_1 hybrids obtained by using cytoplasmic male sterile (CMS) lines. In other crosses, heterosis in photosynthetic and respiration rates can be an additional advantage. Thus, further analysis of NAR, using more crosses, is necessary.
3. Recent F_1 hybrids obtained by using CMS lines showed better canopy structure and better partitioning of photosynthate to grain at later growth stage. The higher number of spikelets per unit land area of recent F_1 hybrids was not due to the amount of absorbed N.

Grain yield and vegetative growth of some F_1 hybrid plants are higher than those of their parents. However, grain yields in populations under practical management are more important than yields of single plants. Before 1970, the use of heterosis at single plant level in improving grain yield seemed impractical and not promising based on the following physiological observations: conventional genotypes showing high yield ability at single plant level yielded lower in the field at economic planting density and fertilizer level (Jennings and de Jesus 1968, Jennings and Herrera 1968, Kawano and Tanaka 1969, Tanaka 1964); little heterosis in agronomic grain yield of F_1 hybrids was observed with rice grown in economical spacing (Jennings 1965); higher degree of positive F_1 heterosis was significantly correlated with hybrid sterility (Kawano et al 1969). These observations were based on limited crosses.

F_1 hybrids obtained using cytoplasmic male sterile (CMS) lines and tested in farmers' fields in China yielded 20-30% more than the best conventional cultivars (Lin and Yuan 1980). At IRRI, the yield of promising F_1 hybrids bred using CMS lines showed high-parent heterosis of 59% and standard heterosis (heterosis to the best check variety) of 35% (Virmani et al 1982). F_1 rice hybrids seem to have significant yield advantage over conventional commercial cultivars.

Heterosis in plant weight and grain yield was highly variable depending on crosses, cultural management, and climatic conditions (Murayama et al 1974, Virmani et al 1982, Virmani and Edwards 1983). This paper reviews the growth characteristics of F_1 hybrids observed in field experiments when they show significant heterosis in agronomic grain yield, and discusses heterosis of plant characteristics for different growth stages.

Growth of F_1 hybrids in field experiments

Ponnuthurai et al (1984) compared growth and yield of six F_1 hybrids in the wet season and four in the dry season under normal agronomic practices. F_1 hybrids and parents yielded higher in the dry season than in the wet season due to higher solar radiation. In the dry season, F_1 hybrids significantly outyielded their better parent, attributed to their greater plant weight and increased leaf area index (LAI).

A significantly higher crop growth rate (CGR) of F_1 hybrids was observed at late vegetative and reproductive stages after topdressing of nitrogen. After heading, little heterosis in CGR was observed. Blanco et al (1986) compared growth and grain yield of two F_1 hybrids in the dry season using two N levels. Both hybrids showed higher heterosis in plant weight at both N levels. When hybrids showed higher heterosis in plant weight, CGR at the initial stage was always higher than the high parent but this heterosis in CGR decreased with growth. A similar trend was observed on the heterosis in LAI. However, net assimilation rate (NAR) and relative growth rate (RGR) of F_1 hybrids after transplanting did not significantly differ from those of parents.

Significant heterosis in some yield components was observed but higher heterosis was noted in spikelet number per unit land area (Blanco et al 1986, Ponnuthurai et al 1984, Virmani et al 1982), due to higher dry matter production of F_1 rice hybrids at heading (Blanco et al 1986). In most cases, grain weight showed slight positive heterosis. However, heterosis in other yield components such as panicles per plant, spikelets per panicle, and spikelet fertility varied highly among crosses and cultivation conditions due to yield component compensation.

In recent F_1 hybrids, higher harvest index (HI) was reported (Virmani et al 1982). High HI was chiefly due to higher heterosis in spikelets per unit land area and comparatively better grain filling. Shorter growth duration aided higher HI of F_1 hybrids. Generally, F_1 hybrids gave negative heterosis at maturity (Virmani and Edwards 1980).

These results indicate F_1 hybrids grow faster at the vegetative stage and maintain this advantage until heading. Similar characteristics were observed in F_1 hybrids of other crops (Ashby 1930, Donaldson and Blackman 1973, Sprague 1936, Whaley 1939).

Heterosis in physiological characteristics during vegetative growth

Several approaches have been reported to identify the physiological characteristics responsible for vigorous vegetative growth of F_1 hybrids. Kawano et al (1969) compared the physiological characteristics of 33 F_1 hybrids and their 12 parental cultivars grown individually for 7 wk. Most F_1 hybrids showed mid-parent heterosis and more than half showed high-parent positive heterosis in plant weight, total absorbed N, root to total dry weight ratio (R:TDW), and tiller number. They showed negative heterosis in N content. Plant weight, showing the highest heterosis, originally was considered responsible for heterosis in vegetative growth, being transmitted to other physiological characteristics such as LAI, N uptake, and tiller number.

Another approach was to identify heterosis in photosynthesis and respiration activity, because these are the most fundamental physiological reactions for plant growth. The results were not consistent because the crosses, growth stages, and growing conditions were varied.

At the earlier vegetative stage of F_1 hybrids, higher heterosis in photosynthetic activity was reported (Lin and Yuan 1980), but not at the late vegetative stage. Kabaki et al (1976), Lin and Yuan (1980), Po et al (1984), Sunoharaet al (1985), and Yamauchi and Yoshida (1985) observed little heterosis and McDonald et al (1974) and Murayama et al (1982, 1984) reported higher positive heterosis (Table 1). Murayama et al (1984) and Lin and Yuan (1980) reported negative heterosis in respiration rate. Kabaki et al (1976) reported little heterosis in photosynthetic rate with 45 crosses (Fig. 1), while Lin and Yuan (1980) indicated lower photosynthetic rate in F_1 hybrids, although data were not shown.

In other crops, heterosis in photosynthetic activity of F_1 hybrids was similar to that of F_1 rice, even though rice is self-pollinating and the degree of heterosis in photosynthetic activity may be lower than that of the heterotroph as suggested by Monma et al (1971). Ojima (1972) and Akita et al (1986b) observed little heterosis at the later vegetative growth stage in soybean and maize. Higher heterosis was reported at earlier vegetative growth stages in maize (Akita et al 1986b, Heichel and Musgrave 1969). Heichel and Musgrave (1969) and Akita et al (1986b) reported that F_1 maize hybrids obtained by crosses between genetically distant parents showed heterosis higher than those of F_1 hybrids obtained from genetically closer parents. Kawano et al (1969) observed more heterosis in dry weight and less heterosis in leaf area of F_1 rice hybrids obtained from crosses between genetically distant indica and japonica parents. This may indicate higher heterosis in photosynthetic activity and/or lower respiration activity in these F_1 rice hybrids.

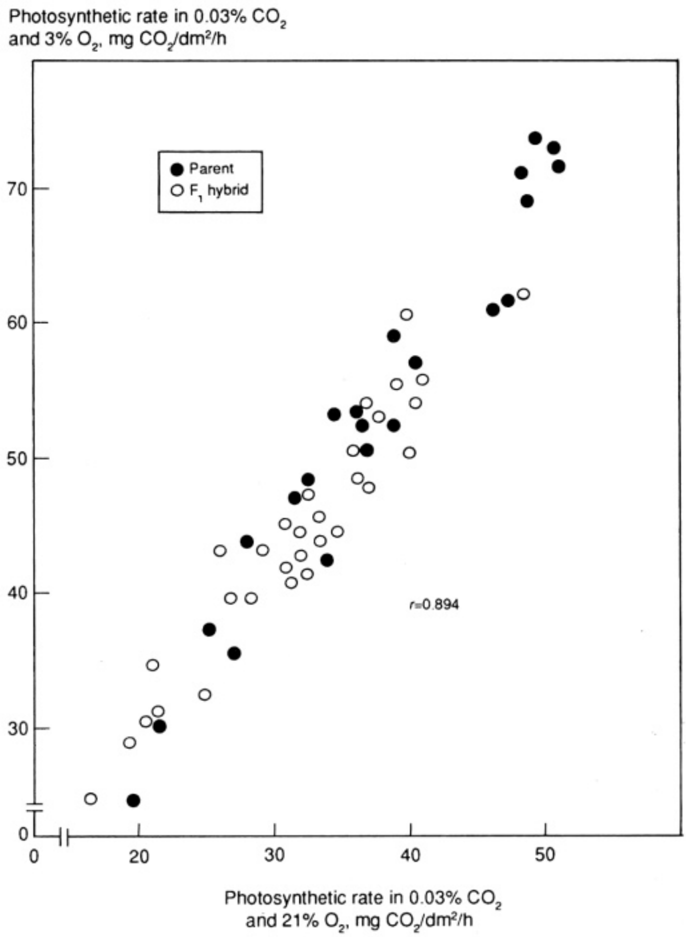
One reason for these inconsistent results in heterosis may be the different N content in plant tissue. N content was determined by the balance between absorbed N and rate of expansion of plant tissue. Additionally, the rates of these physiological processes were closely related with N content (Akita 1980). Higher vegetative growth of F_1 rice hybrids often decreased N (Kawano et al 1969, Murayama et al 1984) but in some cases N increased (Murayama et al 1982). Actually, higher heterosis in photosynthetic activity (McDonald et al 1974; Murayama et al 1982, 1984) was observed with positive heterosis in N content. Furthermore, materials used to measure photosynthetic and respiration rates were usually grown in the pot for convenience, and not in the field. This could cause fluctuation in tissue N content.

These facts suggest that heterosis in these characteristics can be seen in some crosses or conditions. However, the recently observed little difference in NAR (Blanco et al 1986) and little heterosis in photosynthetic activity with similar materials crossed using CMS lines (Yamauchi and Yoshida 1985) strongly suggest

Table 1. Distribution of heterosis in physiological characteristics (Murayama et al 1984).

Heterosis (%)	Photosynthetic rate				Dark respiration		N content		Chlorophyll content		Specific leaf area	
	60 klx		30 klx									
	F_1/MP	F_1/HP	F_1/MP	F_1/HP	F_1/MP	F_1/HP	F_1/MP	F_1/HP	F_1/MP	F_1/HP	F_1/MP	F_1/HP
40					9	16						
41-50					3	5				1		
51-60					4	2				2		
61-70				1	3	3		3	2	5		
71-80		5	2	3	2		3	5	5	4		
81-90	6	6	4	12	1	1	5	8	8	7		3
91-100	5	8	5	6	2		11	8	7	4	10	12
101-110	4	5	8	5	2		9	4	3	5	10	10
111-120	8	3	6	3	1	1	1	2	3	1	10	5
121-130	3	3	2				1		1	1		
131-140	4		3		1				1			
141-150												
151-160												
161-170												
171-180												
Total	30	30	30	30	30	30	30	30	30	30	30	30

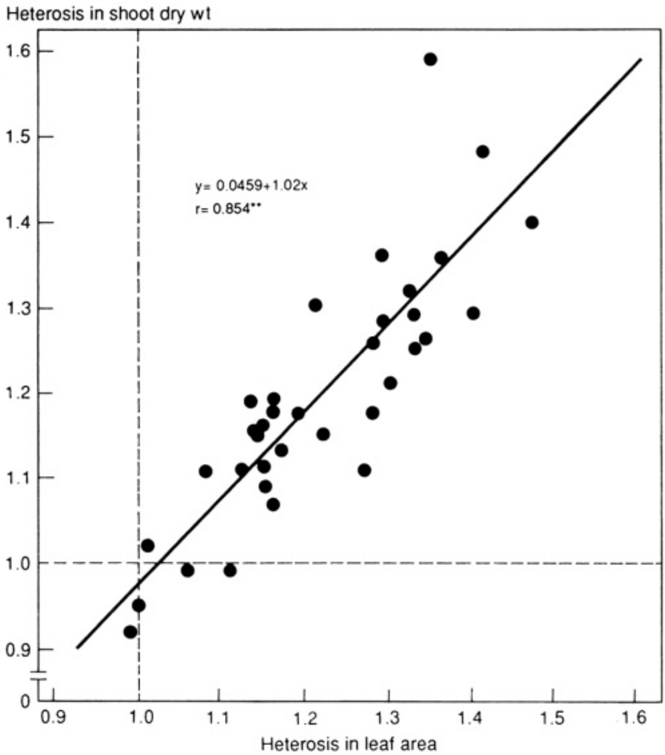
Notes: MP = mid-parent, HP = higher-parent.



1. Relation between photosynthetic rates of parents and F_1 hybrids in 3 and 21% O_2 (Kabaki et al 1976).

that heterosis in photosynthetic activity and respiration rate is not necessarily related to vigorous growth of F_1 hybrids at the vegetative stages. Further studies on growth analysis of F_1 hybrids, especially on NAR, will give a better understanding of heterosis in these characteristics. Evaluation per unit of N content of field-grown materials also will give valuable information.

Another key to heterosis at the vegetative stage is faster leaf area development. Yamauchi and Yoshida (1985), Sunohara et a1 (1985), and Blanco et a1 (1986) found similar degree of heterosis in leaf area development and shoot dry weight and a high correlation between them at the early vegetative growth stage (Fig. 2). High heterosis was observed in CGR, but little heterosis in NAR after transplanting (Blanco et a1 1986) (Table 2). This indicates that heterosis in leaf area development during seedling stage would be the key to vigorous growth after transplanting. These results indicate that vigorous growth of F_1 hybrids at the early vegetative stage was due to their high ability for leaf area development. High heterosis in leaf area was also



2. Relation between heterosis in leaf area and shoot dry wt in 35 F_1 hybrids (Yamauchi and Yoshida 1985).

reported in other crops (Ashby 1930, Duarte and Adams 1963, Ghildyal and Sinha 1973, Rao and Venkateswarlu 1971).

Higher heterosis in R:TDW (Kawano et al 1969) at the midvegetative stage and in root weight and root activity at the reproductive stage of F_1 rice hybrids (Lin and Yuan 1980) were reported. Heterosis in root growth was reported in other crops (Ashby 1932, Whaley 1952). This may partly be attributed to the transmission of heterosis in plant weight. Higher root growth and activity may not trigger heterosis but may help explain higher increase in CGR in F_1 hybrids after N topdressing (Ponnuthurai et al 1984) and higher tolerance and homeostasis of F_1 rice hybrids to adverse environment such as salinity and drought (Lin and Yuan 1980).

Heterosis in physiological characteristics in embryo and seedling growth

What are the physiological characteristics responsible for F_1 vigor at seedling stage?

The initial growth stage is embryo development, stopping temporarily when seeds mature but starting again after sowing. Embryo growth does not involve photosynthesis, so the existence of heterosis in F_1 embryo will help elucidate the physiological mechanism of heterosis. Ashby (1930, 1932) observed positive

Table 2. Net assimilation rate (g/dm^2 per wk) of F_1 hybrids and parents at 2 N levels from 3 to 11 wk after transplanting (WAT). IRRI, 1985 DS.

Cultivar	N assimilation (g/dm^2 per wk)							
	40 kg N				150 kg N			
	3-5 WAT	5-7 WAT	7-9 WAT	9-11 WAT	3-5 WAT	5-7 WAT	7-9 WAT	9-11 WAT
IR747-B	0.66	0.67	0.45	–	0.68	0.43	0.49	–
IR50	0.66	0.46	0.38	0.27	0.68	0.36	0.35	0.23
IR747 A/IR50	0.77	0.51	0.46	0.18	0.62	0.36	0.38	0.32
MR365B	0.80	0.55	0.46	0.58	0.67	0.36	0.39	0.37
IR36	0.73	0.60	0.26	0.36	0.67	0.39	0.35	0.32
MR365_A/IR36	0.77	0.55	0.31	0.32	0.76	0.38	0.38	0.41

heterosis in embryo weight and little heterosis in RGR of F_1 maize hybrids. He concluded that heterosis is the maintenance of a growth advantage in the embryo, and proposed the so-called "initial capital theory." This theory is controversial. Inconsistent results on heterosis in F_1 embryo weight could be due to experimental conditions such as sample size and the identification of true F_1 seeds in the heterotroph. In rice, embryo weight varies highly even within one cultivar. The difference within cultivars or lines becomes significant only when sample size is more than 110 seeds (Akita et al, unpubl. data). In many previous reports, results were based on fewer embryos.

Akita et al (1986a) examined heterosis in F_1 and F_2 rice embryo weights using 200 seeds from each hybrid obtained by CMS lines and parents. All 15 F_1 hybrids tested showed higher embryo weight than the mid-parent, and 9 F_1 hybrids showed larger embryos than the high parent. F_2 embryos of two crosses tested showed mid-parent values. However, F_1 seed setting was extremely low when CMS lines were used.

Therefore, the effect of low seed setting percentage of F_1 rice seeds on embryo weight was examined in two experiments. The embryo weights of the plants subjected to artificial thinning treatment was 5-6% higher than those of control plants. Embryo weights of male sterile lines were 10% higher than those of isogenic fertile lines (Akita et al, unpubl. data). Low seed setting of F_1 seeds affected embryo weight to some extent. When a maximum of 10% increase of embryo weight by low seed setting was subtracted from mid-parents' heterosis, 10 of 15 F_1 hybrids still showed higher mid-parent heterosis. Thus, the effect of low seed setting on embryo weight was not large enough to explain the higher heterosis. Besides, the mid-parent heterosis in F_1 embryo weight was closely related with that of F_1 seedling weight at 16 d after seeding (Akitaet al 1986a) (Table 3). The negligible heterosis in F_2 embryo was consistent with lower heterosis observed in F_2 generation (Davis and Rutger 1976, Kim and Heu 1979). However, these results were from F_1 hybrids obtained by using CMS lines and both parent genotypes have relatively small genetic differences.

Observing F_1 hybrids crossed between genotypes having contrasting seedling growth characteristics, such as indica and japonica, will help explain this because seedling growth is highly related to embryo weight in cultivars having similar ability

Table 3. Mid-parent heterosis in embryo, endosperm, and seedling weight.

Cultivar	Embryo	Endosperm	Seedling growth
IR21845/IR54	116	100	117
IR46830/IR9761	109	98	107
IR46830/IR50	124	98	122
T97/M54	114	99	119

of leaf expansion. However, the difference in seedling growth among genotypes from different geographic origin is rarely related to embryo weight (Akita et al, unpubl. data). In other crops, Yamada (1985) reported significant heterosis of F_1 maize embryo and reconfirmed Ashby's observation by using a marker gene to identify true F_1 seeds.

These results suggest that heterosis in embryo weight may exist even in self-pollinating species such as rice. Heterosis in embryo weight could be used to indicate heterosis in vegetative growth, especially in F_1 hybrids obtained from indica parents having relatively similar plant characteristics.

Matsuba et a1 (1986) observed dormancy of primary tiller primordia, which is high in conventionals. In three F_1 hybrids, it was less than in parent genotypes. This would explain the faster emergence of strong tillers in F_1 hybrids. Urano and Sakaguchi (1950) observed more leaf primordia in F_1 maize embryo, showing significant heterosis in embryo weight. Faster germination of F_1 seeds was also reported (Ashby 1930, Hageman et a1 1967, Sarkissian et a1 1964, Whaley 1952).

These findings suggest hybrid vigor at the embryo stage. On the other hand, Cooper and Brink (1940) and Groszmann and Sprague (1948) reported larger endosperms of F_1 seeds in alfalfa and maize and claimed they were the main cause of heterosis. However, the endosperm is just a reservoir of energy and substrate for plant growth. In rice, the endosperm weight of F_1 seeds was not always more than that of parents, and seedling growth was hardly related to endosperm weight (Akita et a1 1986a).

Leaf area development ability is a major determinant of seedling growth among cultivars (Kawano and Tanaka 1969). However, little information is available on F_1 hybrids. In other crops, McDaniel and Sarkissian (1966) reported heterosis in mitochondrial activity. Woodstock and Grabe (1967) observed high correlation between respiration rate and seedling growth. However, Van Gelder and Mediema (1975) and Sen (1981) countered these results. Sarkissian et a1 (1964) reported higher photosynthetic activity of F_1 maize hybrid in seedling.

Thus, heterosis in respiration and photosynthesis in seedling growth is still controversial.

Discussion

Vigor in initial growth is considered the principal trigger for heterosis of F_1 rice hybrids. Further analysis of the physiological mechanism involved in seedling growth rate would help explain heterosis of F_1 hybrids.

Vigor in initial growth brings higher CGR in vegetative stages. Dense planting can also increase CGR. However, increasing planting density within an economic range hardly changes final dry matter production and grain yield in a cultivar. Another way is to apply more N, but excess N to promote initial growth will cause slower CGR at later growth stages due to more shading and lodging.

Because the high initial CGR does not carry through to later growth stages or to higher yields, in modern high-yielding cultivars early vigor is sacrificed to ensure a better canopy structure at late growth stages and better partitioning of photosynthate to grain.

On the other hand, the yield increases with recent F_1 hybrids involve the combination of high CGR in initial growth and less deterioration in canopy structure and partitioning of photosynthates to grain. This is attributed to the higher yielding ability and good plant type of parent genotypes selected from elite breeding lines. Reported lower CGR at later growth stages in some F_1 rice hybrids showing higher CGR at earlier stages (Jennings 1965, Sunohara et al 1986) may be related to poor performance at late growth stages of the parent genotypes used.

These facts indicate that to successfully use heterosis to improve grain yield, parent genotypes need to be selected from modern high-yielding cultivars.

Another reason for improved grain yield of recent F_1 hybrids is their relatively short growth duration. Two early-maturing F_1 hybrids showed positive correlations between yield and dry weight at heading and spikelets per unit land area (Blanco et al 1986).

On the other hand, these correlations are low in late-maturing cultivars in the tropics. In the tropics, the spikelet number per unit land area cannot be increased beyond a certain level even if the amount of absorbed N or dry weight at heading is increased (Yoshida et al 1972). The higher spikelet number per unit land area of two recent F_1 hybrids tested was not due to increased amounts of absorbed N. This indicates that, in the tropics, the increased dry matter of F_1 hybrids at heading may not be related to increased spikelet number per unit land area. Whether F_1 rice hybrids can produce more spikelets per unit land area per absorbed N needs further analysis.

Another physiological advantage of F_1 rice hybrids is better performance in adverse environments. Virmani et al (1982) reported that more F_1 hybrids showed higher heterosis in grain yield in the wet season when solar radiation was lower. Lin and Yuan (1980) observed better performance of F_1 hybrids in salinity and drought. F_1 rice hybrids could be more effective than conventional varieties in adverse environments such as low solar radiation, rainfed, saline conditions, and where improved agronomic management cannot be introduced.

Heterosis is a highly cross-specific phenomenon and the available crosses using CMS lines are still limited. By developing methods to give enough F_1 seeds, growth analysis in field level can be intensified to identify the physiological mechanism of heterosis.

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Role of wide-compatibility genes in hybrid rice breeding

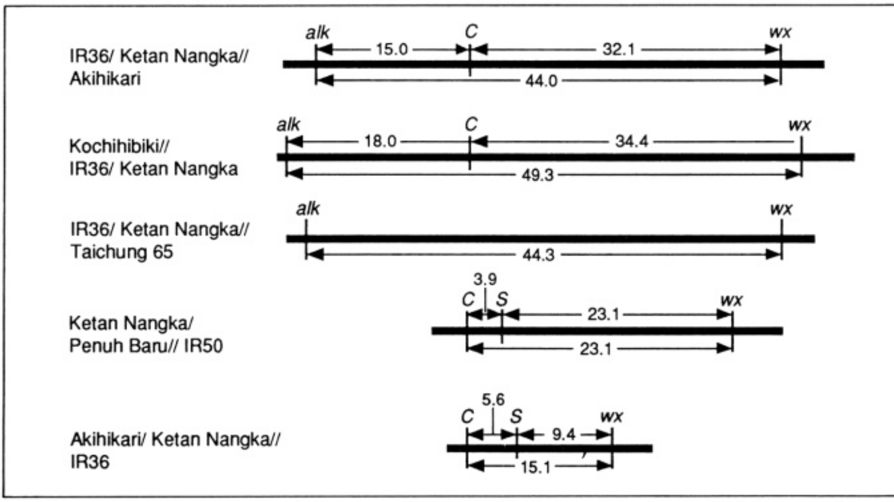
H. ARAKI, K. TOYA, AND H. IKEHASHI

The genetic base for F_1 sterility and wide compatibility is analyzed, and a wide-compatibility gene, *S-5*^{n}. which can overcome sterility in indica/ japonica crosses, is identified. The authors suggest that incorporating the *S-5*^{n} wide-compatibility gene into indica or japonica breeding lines could improve hybrid seed production because such lines show high heterosis without F_1 sterility. Indica/japonica hybrids, which are fertile due to *S-5*^{n}, still show pollen semisterility, but that does not effect seed fertility. This indicates a need for yet another gene of *S-5*^{n} type.

The F_1 hybrids of indica/japonica crosses show semisterility. That sterility continues in early generations. Some studies have shown that some wide-compatibility varieties do produce fertile F_1 plants when crossed with indica as well as with japonica varieties (Terao and Midzushima 1939, Heu 1967). We screened some rice varieties for such wide compatibility (Ikehashi and Araki 1984), and analyzed the genetic base of wide compatibility. The genetic base for F_1 sterility and wide compatibility followed a simple rule (Ikehashi and Araki 1985). Because that evidence of the genetic mechanism for F_1 sterility has been published elsewhere, here we cite some empirical evidence to support the new view and discuss applying the new understanding of F_1 semisterility to breeding practices, specifically to hybrid seed production.

Genetic bases for sterility

The genetic base for F_1 sterility and wide compatibility has been analyzed: a set of multiple alleles appears between the *C* (chromogen for apiculus color) and *wx* (waxy endosperm) loci; *S-5*^{n} for wide-compatibility varieties, *S-5*^{i} for indica, and *S-Y*^{j} for japonica varieties. The genotypes of *S-5*^{n}/*S-5*^{i} and *S-5*^{n}/*S-5*^{j} and the homozygous genotype of all the alleles are fertile, but *S-5*^{i}/*S-5*^{j} was semisterile due to partial abortion of female gametes carrying *S-5*^{j}. So far, some javanica varieties and derivatives of javanicas (Ketan Nangka, Calotoc, CPSLO 17, etc.) have been found to possess *S-5*^{n} and both of the complementary genes for apiculus color, i.e., *C* and *A*. Many high-yielding indica or japonica types do not have *C*. Because the *C* locus is closely linked with the *S-5* locus (Fig. l), the progeny of indica or japonica wide-compatibility varieties showing apiculus color are likely to possess *S-5*^{n} (wide-compatibility gene). Incorporating this allele is easy, if the marker is appropriately traced.



1. Intensity of linkage among some markers in Linkage Group I and assumed locus of *S*-alleles and the allelic interaction of which causes semisterility of spikelet (Ikehashi and Araki 1985).

The empirical fact that indica/japonica crosses do not produce wide-compatibility variety types supports this hypothesis. Female gametes carrying the allele $S\text{-}5^j$ from the japonica type in the hybrid population are aborted. The empirical fact that the level of fertility of japonica/indica//japonica hybrids is generally lower than that of japonica/indica//indica hybrids can also be explained by the abortion of female gametes carrying the japonica allele, $S\text{-}5^j$ in the first crosses. If the surviving gametes from the F_1, the majority of which carry $S\text{-}5^i$, are fertilized by pollen with the $S\text{-}5^j$ of a japonica recurrent parent, the backcrossed progeny would be mostly $S\text{-}5^i/S\text{-}5^j$ genotype, reproducing the semisterility. If the female gametes from the first cross are fertilized by pollen with $S\text{-}5^i$, the backcrossed progeny are mostly $S\text{-}5^i/S\text{-}5^i$, showing a high fertility level.

Our genetic analyses are in accordance with experiences with indica/japonica crosses. It should be noted that lines with wide compatibility would not be produced, even by repeated trials with indica/japonica crosses. To overcome the semisterility in indica/japonica crosses, systematic utilization of the $S\text{-}5^n$ allele is needed in wide crosses.

Initial results of japonica lines that carry $S\text{-}5^n$

We have developed some breeding lines that carry the $S\text{-}5^n$ allele from Ketan Nangka and obtained several japonica-type lines from the F_5 population of Nihonmasari (japonica)/Ketan Nangka. One of those is designated as NK4. We developed one series of breeding lines from Akihikari (japonica)/NK4, and another series of lines from Akihikari//Akihikaril/NK4. Although these lines are morphologically japonicas, the F_1 hybrids between these lines and some indica varieties were fertile (Table 1, 2). The F_2 population of the cross between the japonica line carrying the $S\text{-}5^n$ and an indica variety produced only a few semisterile plants (Table 3). We

Table 1. Characteristics of some promising lines with wide-compatibility gene ($S\text{–}5^n$) (1985).

Line	Cross or variety[a]	Generation	Flowering period	Plant height (cm)	F_1 fertility	
					Tester	(%)
A1	Akihikari/NK4	F_5	20 May	94	IR50	89.4
A9	Akihikari/NK4	F_5	22 May	87	IR50	85.4
B5	Akihikari//Akihikari/NK4	F_4	20 May	80	IR36	90.7
B20	Akihikari//Akihikari/NK4	F_4	18 May	92	IR36	86.9
C1	IR50//1R36/Ketan Nangka	F_5	29 May	92	Akihikari	83.5
C2	IR50//1R36/Ketan Nangka	F_5	29 May	98	Akihikari	90.3
	Ketan Nangka		15 Jun	158	IR36	88.2
					Akihikari	93.4
	Toyonishiki (check)		21 May	88		96.1
	IR36 (check)		4 Jun	81		89.7
	IR50/Akihikari F_1 (check)		(Test in 1983)			42.8

[a]NK4 is an F_6 line from Nihonmasari/Ketan Nangka.

Table 2. Pollen and spikelet fertility of the F_1 hybrids of Shinkei 8544/indica varieties.

Cross[a]	Fertility (%)	
	Pollen	Spikelet
Shinkei 8544/Milyang 23	81.9	94.7
Shinkei 8544/Suweon 258	65.2	85.6
Shinkei 8544/Nangjin 11	42.2	88.6
Shinkei 8544/IR50	43.5	72.0

[a]Shinkei 8544, an experimental line from Akihikari/NK4 has $S\text{–}5^n$ gene from Ketan Nangka instead of $S\text{–}5^j$.

Table 3. Level of spikelet sterility in F_2 hybrids of indica tester IR50/japonica lines with the $S\text{–}5^n$ gene from Ketan Nangka.

Cross[a]	Plants (no.)	Plant ratio in each fertility class							
		–30	–40	–50	–60	–70	–80	–90	–100
Shinkei 8544/1R50	143	1.4	1.4	1.4	2.1	0.7	9.1	33.6	50.3
N8M/IR36	152	53.3	5.3	10.5	9.2	4.6	7.2	5.3	4.6

[a]Shinkei 8544, a line from Akihikari/NK4, has $S\text{–}5^n$ gene instead of $S\text{–}5^j$. N8M is a dwarf mutant from Norin 8, a typical japonica.

have also selected several lines from indica/Ketan Nangka crosses. The progeny of IR50//IR36/ Ketan Nangka, when crossed with japonica testers, showed indica-like plant type as well as good fertility in the F_1 hybrids (Table 1). Introducing the $S\text{-}5^n$ allele from some wide-compatibility varieties proved to be a good solution to sterility in the indica/japonica crosses.

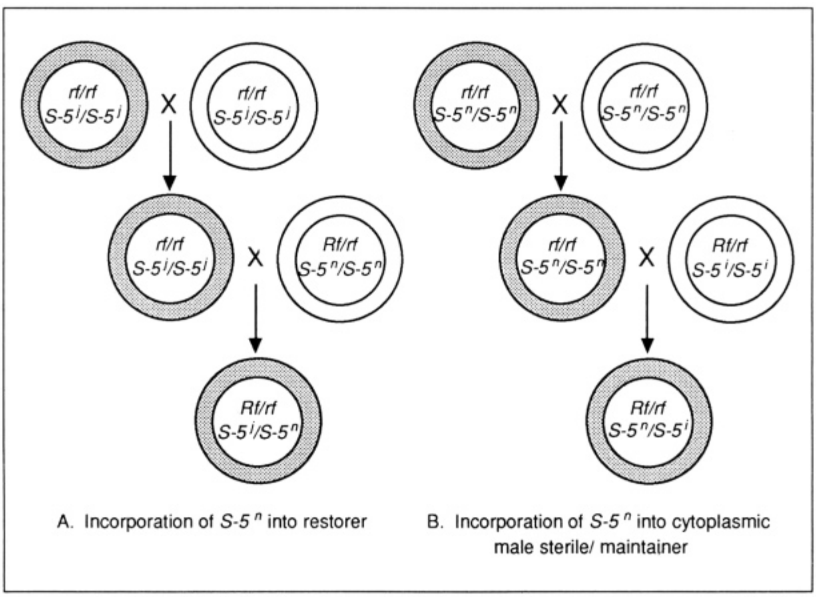
Using *S-5*^{n} allele in hybrid seed production

Incorporating the *S-5* ^{n} allele into indica or japonica breeding lines is expected to overcome the sterility problem in indica/japonica crosses. Specifically, those lines with *S-5*^{n} can be used in indica/japonica crosses for hybrid seed production, because such lines show high heterosis without F_1 sterility.

To produce hybrid seed from an indica/japonica cross, it is necessary to consider the components of hybrid seed production into which the *S-5*^{n} allele is to be incorporated (Fig. 2). Many high-yielding indica varieties, including IR36 and IR50, are known to possess a restorer gene *Rf* for cytoplasmic male sterility (CMS) from a tropical rice variety Chinsurah Boro II. If any indica lines with the *S-5*^{n} are developed, they can be used directly as the restorer in hybrid seed production (Fig. 1-A).

Another way to use *S-5*^{n} is to breed japonica lines with *S-5*^{n}, and then develop CMS lines by backcrossing the *S-5*^{n} line into a CMS donor. That way, it is possible to develop japonica CMS lines and japonica maintainers, both of which have *S-5*^{n}. The F_1 hybrids of those two, fertilized by any indica pollen donor, would produce fertile indica/japonica hybrids, which could exhibit high heterosis (Fig. 2-B).

From the experiences in China, developing good components from japonica CMS lines for hybrid seed production appears to be difficult. One reason could be inadequate varietal diversity for attaining high heterosis within japonica varieties. Perhaps the sterility problem is even more important. To use male-sterile japonica lines having sterility-causing cytoplasm of indica origin, the restorer gene should be introduced from indica varieties through indica/japonica crosses. However, most of



2. Components of hybrid seed production using the sterility-neutralizing gene *S-5*^{n}.

the progeny of indica/japonica crosses would show semisterility when crossed with japonica lines. Development of good restorer lines for japonica male sterile lines becomes extremely difficult, unless a means is devised to overcome the sterility of indica/japonica crosses. Using $S\text{-}5^n$ could be a solution.

Points for further studies

In developing a system to produce fertile indica/ japonica hybrids, it should be noted that the locus for pollen sterility in ordinary indica/japonica crosses is not yet known. The indica/japonica hybrids, which are fertile due to $S\text{-}5^n$, still show pollen semisterility. In our experiment, pollen semisterility did not decrease the seed fertility of indica/japonica hybrids with the $S\text{-}5^n$ allele (Table 2). However, pollen fertility in the use of cytoplasmic male sterility would not be perfect when the restorer gene functions as a gametophytic gene. The concurrent effect of inadequate pollen fertility due to gametophytic *Rf* and semisterility of pollen due to an indica/japonica cross should be clarified to establish a sound seed production system.

According to our studies, the $S\text{-}5^n$ allele is effective for most indica varieties, which seem to carry $S\text{-}5^i$. But some other loci also give semisterility. For example, the hybrids of some aus and japonica varieties show semisterility based on an allelic interaction at a locus near A (Antochianin activator). Also, the hybrids of many aus varieties and many javanica varieties, including wide-compatibility varieties, show semisterility due to allelic interaction at a locus near *Rc* (red pericarp). Evidently, the $S\text{-}5^n$ allele does not solve semisterility in all kinds of hybrids in the wide cross of rice. There is a need for some other gene of S^n type.

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Rice male sterile cytoplasm and fertility restoration

LI ZEBING AND ZHU YINGGUO

This paper summarizes status of research in rice male sterile (MS) cytoplasm and fertility restoration in China. Hundreds of MS lines, including indica and japonica rices, glutinous rice, early-, medium-, and late-maturing rices, have been developed. Twenty-two types of MS lines have been derived from nucleus substitution backcrosses between ecotypic strains of wild rice and cultivars. About 20 types of japonica MS lines have been derived from substitutional backcrosses between varieties of indica and japonica rice.

According to genetic properties, morphology of sterile pollens, and relations between restorers and maintainers, the MS lines are classified into three groups: 1) MS lines of wild abortive (WA) and similar types, 2) MS lines of Hong-Lien types, and 3) MS lines of Dian 1, Dian 3, and BT types. Hybrid rice derived from MS lines of WA types have been used in commercial production in China for 12 yr on nearly 60 million hectares. The MS lines of WA, Gam, D, Hong-Lien, Dian 1, Dian 3, and BT type currently are used in hybrid rice production.

Some investigations indicated that the genetic characteristics of WA type sterile cytoplasm were rather stable. And the studies on 8 sterile cytoplasms including WA, Liuye, Gam, Hong-Lien, Dian 1, Dian 3, and BT type indicated that sterile cytoplasm reduced the productive capacity of hybrids in contrast to normal cytoplasm of the maintainers. The restorers of the MS lines used in production were obtained mainly by screening and cross breeding.

Varieties possessing restoring ability for WA type MS lines are concentrated in low latitudes of Southeast Asia and South China. For the MS lines of WA type which belong to indica sporophytic sterile type, their restorer variety IR24 contains two pairs of dominant genes ($R_1R_1R_2R_2$). R_1R_1 comesfrom Cina, a late indica variety in China, and R_2R_2 comes from SLO 17. The restorers of MS lines of Dian 1 type, which belong to japonica gametophytic type, contain a pair of dominant restoring genes allelic with those of the BT type. Different genetic backgrounds of parents and varied growing conditions of hybrids have some effects on the restoring ability of restorer genes.

Since commercial hybrid rice production began in China in 1975, the planted area of hybrid rice has grown to 60 million hectares, with grain yield increase of 45 million tons. The three-line system based on cytoplasmic male sterility is the current chief way to exploit heterosis in rice. We herein present research developments in rice male sterile cytoplasms and fertility restoration.

Research on male sterile cytoplasms in rice

Sources of sterile cytoplasms

The earliest reports of cytoplasmically induced male sterility in rice were made by Weeraratne (1954) and Sampath and Mohanty (1954), cited in Virmani et a1 (1986). Katsuo and Mizushima obtained completely male sterile plants in the first backcross of *Oryza sativa* f. *spontanea/O. sativa* cultivar Fujisaka 5. Outside China, 9 cytoplasms that may induce male sterility have been reported: 1) Chinese red-awned wild rice *Oryza sativa* f. *spontanea,* 2) Chinsurah Boro II, 3) Lead rice, 4) Birco, 5) *Oryza glaberrima,* 6) Taichung Native 1, 7) *Oryza fatua (O. perennis* or *O. rufipogon),* 8) Tadukan, and 9) Akebono. In addition, Virmani et a1 (1986) reported that ARC13829-26 might also have sterile cytoplasm, because highly sterile plants were observed in the BC_4F_1 of ARC13829-26/IR10179-2-3-1. The distribution of male sterile cytoplasms in *O. rufipogon* was researched by Rutger and Shinjyo (1980).

About 300 strains of *O. rufipogon* were crossed as nonrecurrent male parents to a japonica rice, Taichung 65, as the recurrent male parent. The cytoplasm type of every tested strain was determined according to the fertility of its progenies in the B_4 or more advanced generations. The investigation showed that 62 had male sterile cytoplasms, including 8 gametophytic and 54 sporophytic types, and the remaining 68 had normal cytoplasms. The frequency of male sterile cytoplasms in Asian and American strains was about 64 and 4%, respectively (Shinjyo 1975).

In China, the research on male sterility in rice was initiated in 1964 when Yuan Longping found several male sterile plants in a field of Dong-Ting-Wan-Xian, a late-maturing indica rice. And a breakthrough was made in 1970 when Li Bihu found a natural male sterile wild rice plant (designated WA) on Hainan Island. Then in 1973, some elite indica MS lines of WA type (Er-Jiu-Nan 1 A, Zhen-Shan 97 A, and V41 A), their maintainers, and restorers were successfully developed by the cooperative research of Hunan, Jiangxi, Fujian, and other provinces (Yuan 1977). Also, in 1965 Li Zhengyou et al (1980) found some MS plants in the field of Taibei 8 and later developed the japonica MS lines of Dian 1 type, such as Hong-Mao-Ying A. In 1972, Zhang and Zhu developed indica MS lines of Hong-Lien type, in which the sterile cytoplasm was from red-awned wild rice (Wuhan University 1977). Zhou et a1 developed Gam type sterile lines by using Gambiaca, an indica variety of Western Africa, as the source of sterile cytoplasm (Sichuan Agricultural College 1973). More than 600 MS lines including indica and japonica rices, glutinous rice, and early-, medium-, and late-maturing rices, have been developed in China. They represent 60 types of sterile cytoplasm (Table 1). Most belong to WA, Dian 1, Dian 3, and BT types.

The basic method for breeding genetic-cytoplasmic MS lines with new source of cytoplasm is to use a distant nucleus-substitution backcross. In such crosses, wild rice, semiwild rice, and late indica rice are generally used as the nonrecurrent female parents. These types represent relatively lower evolutionary stages and are supposed to have sterile cytoplasms. Japonicas and early- or medium-maturing indicas are often used as the recurrent male parent. They are in the more advanced evolutionary

Table 1. Types of sterile cytoplasm from which MS lines were developed in China.

Nucleus-substitution type	Cytoplasm source	Nucleus source		CMS type	Pollen		Origin of MS line
		Variety	Kind		Reaction to I-KI	Morphology	
O. glaberrima X *O. sativa* L.	Danbotus	Hua-Ai 15	Indica	Guang-Fu	Individuals stained	Irregular or spherical	Hubei
Wild rice *(O. sativa* f. *spontanea)* X Cultivar (*O.sativa* L.)	Male-sterile wild rice	Er-Jiu-Nan 1	Indica	WA	None	Irregular	Hunan
		Zhen-Shan 97	Indica	WA	None	Irregular	Jiangxi
		V20	Indica	WA	None	Irregular	Hunan
		V41	Indica	WA	None	Irregular	Fujian
		Jing-Ying 66	Japonica	WA	None	Irregular	Hunan
		Du 129	Japonica	WA	None	Irregular	Xinjiang
	Dwarf MS wild rice	Xie-Qing-Zao	Indica	Aibai	None	Irregular	Anhui
	Ya county red-awned wild rice	Guang-Xuan 3	Indica	Yahonqye	A few stained	Majority irregular	Guangxi
	Yanglan wild rice	Ke-Liu-Er	Indica	Yangye	None	Irregular	Guangdong
	Tengqiao wild rice	Er-Jiu-Qing	Indica	Tengye	None	Irregular	
		Gui-Wei	Japonica	Tengye	5% stained	Irregular	Hubei
	He-Pu wild rice	Guang-Xuan-Zao	Indica	Heye	None	Irregular	Hunan
		Jing-Nan-Te 43	Indica	Heye	None	Irregular	Guangxi
		Li-Ming	Japonica	Heye	None	Irregular	Hubei
	Tiandong wild rice	IR28	Indica	Tianye	None	Irregular	Hunan
	Tiandong awned wild rice No. 1	Zhen-Shan 97	Indica	Tian-mangye 1	None	Irregular	Guangxi
	Tiandong red kernel wild rice No. 1	Zhen-Shan 97	Indica	Tian-hongye 1	None	Irregular	Guangxi
	Tiandong wild rice No. 2	Zhen-Shan 97	Indica	Tian-ye 2	None	Irregular	Guangxi
	Liuzhou wild rice	Zhen-Shan 97	Indica	Liuye	None	Irregular	Hunan
	Liuzhou red-awned wild rice	Jing-Nan-Te 43	Indica	Hongliuye	None	Irregular	Guangxi
	Liuzhou white-awned wild rice	Jing-Nan-Te 43	Indica	Bai Liuye	None	Irregular	Guangxi
	Heng county yunbiao straight wild rice	Zhen-Shan 97	Indica	Hengye	None	Irregular	Guangxi

continued on next page

Table 1 continued.

Nucleus-substitution type	Cytoplasm source	Nucleus source		CMS type	Pollen		Origin of MS line
		Variety	Kind		Reaction to I-KI	Morphology	
	Straight white-awned wild rice	Zhen-Long 13	Indica	Zhiye	None	Irregular	Fujian
	Wild rice No. 16	Zhen-Shan 97	Indica	Ye 16	None	Irregular	Guangxi
	Southwest wild rice	IR24	Indica	Dian 9	None	Irregular	Yunnan
	Junniya wild rice	Chao-Yang 1	Indica	Junye	None	Irregular	Guangxi
	Indian wild rice	JingNan-Te 43	Indica	Yinye	None	Irregular	Guangxi
	Sanya red-awned wild rice	Jing-Yu 1	Japonica	Sanhongye	None	Spherical	Beijing
	Red-awned wild rice	Lian-Tang-Zao	Indica	Hong-Lien	5% stained	Spherical	Hubei
		Xiao	Japonica	Hongxiao	5% stained	Spherical	Hubei
		Jing-Ying 59	Japonica	Hongye	A few stained	Spherical	Shanghai
Cultivar (*O. sativa* L.) X Wild rice (*O. sativa* f. *spontanea*)	Ping-Ai 58	South China wild rice	Wild rice	Zaiye	–	No pollen	Jiangxi
	Indian 8	Indian wild rice	Wild rice	Zaiye	–	No pollen	
		South China wild rice	Wild rice	Zaiye	–	No pollen	Jiangxi
	Jing-Nan-Te 43	Hainan wild rice	Wild rice	Jinhaiye	Stained	Spherical	Guangxi
indica × japonica	Yunnan high altitude indica rice	Hong-Mao-Ying	Japonica	Dian 1	Some stained	Spherical	Yunnan
	E-Shan-Da-Bai-Gu	Hong-Mao-Ying	Japonica	Dian 3	Some stained	Spherical	Yunnan
	Chinsurah Boro II	Li-Ming	Japonica	BT	Some stained	Spherical	Liaoning[a]
	Bao-Tao-Ai	Hong-Mao-Ying	Japonica	Dian 5	A few stained	Spherical	Yunnan
	Chun 190	Hong-Mao-Ying	Japonica	Dian 7	A few stained	Spherical	Yunnan
	Sheng-Li-Xian	Xin-Xi-Lan	Japonica	Lanxin	None	Irregular	Hunan
		Jing-Ying 83	Japonica		A few stained	Spherical	Jiangsu
	Tian-Ji-Du	Teng-Ban 5	Japonica	Tian-Teng	A few stained	Spherical	Hubei
	IR24	Xiu-Ling	Japonica	Liao	A few stained	Spherical	Liaoning
	Jing-Quan-Xian-Nuo	Nan-Tei-Jing	Japonica	Jing	5% stained	Spherical	Fujian
	Lian-Tang-Zao	Li-Ming	Japonica		Majority stained	Spherical	Hubei
	Shen-Qi	Nong-Ken 8	Japonica				Fujian
	Ai-He-Shui-Tian-Gu	An-Nong-WanJing	Japonica	Ai	A few stained	Spherical	Hunan

	Indonesia 7	An-Nong-Wan-Jing	Japonica	Yinni	A few stained	Spherical	Hunan
	Qiu-Gu-Ai 2	An-Nong-Wan-Jing	Japonica	Qiu	A few stained	Spherical	Hunan
	Tai-Guo-Dao-Xuan	An-Nong-Wan-Jing	Japonica	Tai	A few stained	Spherical	Hunan
	Gui-Lu-Ai 8	An-Nong-Wan-Jing	Japonica	Gui	A few stained	Spherical	Hunan
	Indonesia 6	Ping-Rang 9	Japonica		A few stained	Spherical	Hunan
japonica X indica	Ke-Qing 3	Taichung 1	Indica	Dian 8	A few stained	Spherical	Yunnan
indica X indica	Gambiaca	Chao-Yang 1	Indica	Gam	None	Irregular	Sichuan
	Rao-Ping-Ai	Guang-Er-Ai	Indica	228	None	Irregular	Guangdong
	Dissi	Zhen-Shan 97	Indica	D	None	Irregular	Sichuan
indica X japonica	Indonesia 6	Ping-Rang 9	Japonica		A few stained	Spherical	Hunan
japonica X indica	Ke-Qing 3	Taichung 1	Indica	Dian 8	A few stained	Spherical	Yunnan
indica X indica	Gambiaca	Chao-Yang 1	Indica	Gam	None	Irregular	Sichuan
	Rao-Ping-Ai	Guang-Er-Ai	Indica	228	None	Irregular	Guangdong
	Dissi	Zhen-Shan 97	Indica	D	None	Irregular	Sichuan
	Gu Y-12	Zhen-Shan 97	Indica	Gu			Hunan
	Indonesia 6	Zhen-Ding 28	Indica	Yinni	None	Spherical	Hunan
	Lian-Tang-Zao	Vasvasiatata	Indica	Lianyin	None	Irregular	Guangxi
	Jing-Nan-Te 43[b]	Chao-Yang 1	Indica	Jinye	None	Irregular	Guangxi
	Chao-Yang 1[c]	Chao-Yang 1	Indica	Chao 5	None	Irregular	Guangxi
	Qiu-Gu-Ai 2	Zhen-Ke	Indica	Qiuguai			Hunan
	Sha-Xian-Feng-Men-Bai	Jun-Xie	Indica	Sha-Xian-Feng-Men-Bai			Hunan
	IR665	Zhu-Xuan-Zao	Indica	IR665			Hunan
	Qiu-Tang-Zao 1	V41	Indica	Qiu-Tang-Zao			Hunan
	Ping-Jian	Zhen-Ke	indica	Ping-Jian			Hunan
japonica X japonica	Zhaotong-Beizigu	Ke-Qing 3	Japonica	Dian 4	A few stained	Spherical	Yunnan
	Ke-Qing 3	Zhaotong-Beizigu	Japonica	Dian 6			Yunnan
	Ma-Zao-Gu	Nong-Tai-Chi	Japonica	Dian 2			Yunnan

[a]Derived from BT-C, an MS line developed in Japan. [b]Substitution backcrossing combination: ([Jing-Nan-Te 43 X Hainan wild rice] B6 X Chao-Yang 1). [c]Substitution backcrossing combination: ([Chao-Yang 1 X Junniya wild rice] F_1 X Chao-Yang 1).

stages and are supposed to contain recessive nuclear sterile genes. Many distant nucleus-substitution backcrosses i.e., interspecies crosses, including wild rice (*O. perennis,* etc.)/cultivar (*O. sativa* L.) and cultivar of *O. glaberrima/*cultivar of *O. sativa;* intersubspecies crosses of cultivars; and intervarietal crosses between distantly related varieties have been conducted in China since the early 1970s. Some results are discussed below.

Interspecies nucleus-substitution backcrosses. In China (not including Taiwan), wild rice (*O. sativa* f. *spontanea,* or *O. perennis,* or *O. rufipogon*) of various strains were found sparsely distributed in the vast area of 111 counties, ranging from 100°44'(Jinghong county, Yunnan Province) to 117°08' east longitude (Zhangpu county, Fujian Province), and 18°09' (Ya county, Hainan Island, Guangdong Province) to 28°14' north latitude (Dongxong county, Jiangxi Province) (Cooperative Team of Wild Rice Resources Survey and Exploration of China 1984, Wu 1980).

When wild rices were used as female parents to cross with cultivar rices, as recurrent male parents, the sterile plant percentage was rather high in the progeny of such nucleus-substitution crosses. Particularly in wild rice/japonica rice, all crosses produced sterile progeny. In the crosses of wild rice/indica rice, sterile progeny rate was about 85% (Yuan 1985).

Twenty-two cytoplasms of wild rice have been used successfully in developing male sterile lines in China (Table 1). Of these, 8 originated on Hainan Island, 9 in Guangxi, and 1 in Yunnan. All 22 are sporophytic except Hong-Lien and Hongye, which are gametophytic sterile types. The MS lines developed through the nucleus-substitution between wild rice and cultivar rice are most abundant in China. However, with few exceptions, such as Hong-Lien, Tianye, and Dian 9 types, most MS lines have similar relationships between maintainers and restorers as do the MS lines of WA type.

Most progeny are fertile in adverse nucleus-substitution crosses, i.e., cultivars as nonrecurrent female parents and wild rice as recurrent male parents. Exceptions include four types of sterile lines (Table 1).

Intersubspecies nucleus-substitution backcross between indica and japonica. In these crosses, usually indicas are used as female parents and japonicas as recurrent male parents. About 20 types of indica cytoplasm MS lines have been developed in China (Table 1). Most cytoplasms came from relatively primitive native indica varieties or geographically distant indica varieties. Almost all japonica MS lines developed this way are gametophytic. Exceptions are MS lines of Nan-Xin which are sporophytic.

Also, indica MS line Taichung Native 1 A (Dian 8 type) was developed by substitution backcrossing of the genome of indica variety Taichung Native 1 into the cytoplasm of japonica variety Ke-Qing 3. However, rarely are MS lines developed by nucleus-substitution backcross of japonica/ indica.

Intervarietal nucleus-substitution between distantly related indica varieties. More than 10 types of MS lines with sterile cytoplasms have been developed by nucleus-substitution between distantly related indicas (Table 1). The parents of most crosses either have relatively distant genetic relations, or originated in distant areas, or belong to different ecotypes.

Exceptions are MS lines Chao-Yang 1 A of Jin-Ye type and Chao-Yang 1 A of Chao-Wu type. Their sterile cytoplasms originated from two early-maturing dwarf indicas, Jing-Nan-Te 43 and Chao-Yang 1, which appear closely related and may have the same origin with their recurrent male parent Chao-Yang 1. In the two instances, the female parents (Jing-Nan-Te 43 and Chao-Yang 1) were the recurrent male parent (Chao-Yang 1). Possibly the normal cytoplasm of the female parents turned sterile under the influence of nuclear genes of wild rice before backcrossing with the recurrent male parent (Guangxi Agricultural Sciences Academy 1985).

In addition, MS lines have been developed through nucleus-substitution between distantly related japonicas, including the MS lines of Dian, Dian 4, and Dian 6 types.

Classification of MS lines

The MS lines in China can be classified into three basic groups according to their genetic properties, morphology of sterile pollens, and relations between restorers and maintainers.

Group 1. MS lines of WA and similar types. The MS lines of WA type were developed by crossing the wild abortive rice (WA) as the female parent with recurrent male parents, mainly early-maturing indicas such as Er-Jiu-Nan 1, Zhen-Shan 97, V20, and V41. Their sterility was controlled by two pairs of recessive nuclear genes and sterile cytoplasm (Gao 1981, Hu and Li 1985, Lei et al 1984, Li 1985, Liang 1980, Liu et al 1982, Lu et al 1983, Wang 1983, Yang et al 1984, Zhou 1983). MS lines of WA types are sporophytic. The pollen sterility was determined by the genotype of sporophyte, without reference to the genotype of pollen itself. The pollen sterility of F_1 hybrids between WA type MS lines and their restorers was more than 90%. Fertility segregation appeared in F_2 generations, producing sterile plants. The pollens of WA type MS lines were typical abortion type. Most pollens were irregularly shaped and unstainable with I-KI solution (Table 2). Pollen abortion occurred relatively early, mainly around the nuclear division stage of uninucleate microspore, i.e., from the late uninucleate stage to the early binucleate stage (Jiangxi University 1977; Xu 1980, 1982).

The maintainers of WA type MS lines were usually early-maturing dwarf indicas cultivated in China. Some varieties such as SLO I7 originated in Southeast Asia. Peta, Tai-Ying 1, IR24, IR26, Indonesia 6, and late-maturing indicas such as Cina, Xue-Gu-Zao, and Zhu-Ai which originated in South China, possessed strong restoring ability. More than 95% of the hybrid rice area in China is occupied by hybrids derived from MS lines of WA type (Li and Yuan 1985).

In addition to WA type, group 1 also includes indica MS lines derived from crosses of wild rices, such as Ai-Bai, Ten-Ye, He-Ye, Liu-Ye, and In-Ye types, and some MS lines derived from crosses between indicas such as Gam, D, Gu Y-12, Indonesia 6, and No. 228 types. All these are sporophytic, possess typical aborted pollens, and have maintainer-restorer relationships similar to those of WA types. Hybrids of elite combinations of Gam and D types of MS lines are commercially produced in some districts of Sichuan Province. And some combinations derived from MS lines of Ai-Bai type, Gu Y-12, and Indonesia 6 types are being tested in the field.

Table 2. Pollens of different types of MS lines stained with I-KI solution (Xu 1982).

Type	MS line	Total observed (no.)	Pollens							
			Typical aborted		Spherical aborted		Lightly stained aborted		Deeply stained aborted	
			No.	%	No.	%	No.	%	No.	%
WA	Zhen-Shan 97	1523	913	60.0	604	39.7	4	0.3	2	0.1
	Hua-Ai 15	5191	2821	54.3	1572	30.3	361	7.0	437	8.4
Hong-Lien	Hua-Ai 15	3947	59	1.5	2071	52.5	1778	45.0	39	1.0
Dian 1	Hong-Mao-Ying	4490	80	1.8	371	8.3	1558	34.7	2481	55.3

In all these sporophytic MS lines, pollens abort relatively early, most at the uninucleate stage, and have irregular shapes. A few abort at the binucleate stage and are spherical. All aborted pollens are unstainable with I-KI solution. The sterility of these MS lines is rather stable under varied environments. Most have short ear stems so their panicle bases usually remain somewhat enclosed within their leaf sheaths when heading. The restoration spectrum of these MS lines is relatively narrow.

Group 2. MS lines of Hong-Lien type. The first MS lines of Hong-Lien type were developed by backcrossing red-awned wild rice as the female parent with the recurrent male parent Lian-Tang-Zao, an early-maturing, tall indica. The sterility of Hong-Lien type MS lines was controlled by a pair of recessive nuclear genes and some modifying genes as well as sterile cytoplasm (Hu and Li 1985, Zhu et a1 1985). Pollen fertility was directly determined by the genotype of pollens (gametophyte) and has no relation with the genotype of sporophyte. Although the pollen fertility of F_1 hybrid derived from Hong-Lien MS lines was only 50%, their seed set was normal. No fertility segregation occurred in the F_2 generation. Pollen abortion mainly occurred during the binucleate stage. The pollens were spherical and unstainable or lightly stainable with I-KI solution (Xu 1980, 1982) (Table 2).

The relation between restorers and maintainers of Hong-Lien MS lines tended to be contrary to that of WA MS lines. For example, some early-maturing dwarf indicas that originated in the Yangtze Valley (Zhen-Shan 97, Er-Jiu-Ai, Jing-Nan-Te 43, Long-Zi, Wen-Xian-Zao, Boli-Xian-Ai, and Xian-Feng 1) and were maintainers of WA MS lines had strong restoring ability to Hong-Lien MS lines. And some restorers of WA MS lines such as Peta, Tai-Ying 1, Indonesia 6, and Xue-Gu-Zao were good maintainers of the Hong-Lien MS lines. The restoration spectrum of the Hong-Lien type was wider than that of the WA type.

In research, 630 varieties were crossed with WA MS lines and 614 with Hong-Lien MS lines. Results showed 41.2% of the varieties were strong restorers of Hong-Lien type but only 10.5% of WA type MS lines (Zhu et a1 1985). Elite combinations of Hong-Lien MS lines are commercially produced in several districts of Guangdong Province.

Group 3. MS lines of Dian 1, Dian 3, and BT types. MS lines of Dian 1 and Dian 3 were developed by substitution backcrossing the genome of japonica Hong-Mao-Ying into the cytoplasm of an unknown indica growing in a high mountain area of Yunnan Province and an indica variety E-Shan-Ta-Bai-Gu. The BT KS line was derived from nucleus substitution backcrossing between an Indian spring indica Chinsurah Boro II as the female parent and a Chinese japonica variety Taichung 65 as the recurrent male parent. Some japonica MS lines such as Li-Ming and Feng-Jin were developed in China by using sterile cytoplasm of BT type MS line. The sterility of BT MS lines is controlled by a pair of recessive nuclear genes and sterile cytoplasm (Hu and Li 1985, Shinjyo 1975).

All MS lines of Dian 1, Dian 3, and BT types are gametophytic. The F_1 hybrids derived from them have normal seed set but only about 50% pollen fertility. No sterile plants occur in F_2 generation.

The pollens of Dian 1 MS lines developed until the binucleate stage but most microspores aborted before the trinucleate stage. The pollen of BT MS lines aborted slightly later than that of Dian 1; most developed into the trinucleate stage. The pollens of BT MS lines were stainable with I-KI solution (Xu 1980, 1982; Shinjyo 1975) (Table 2). Sterile genes of Dian 1 and BT types were allelic with each other. The two types had a common restoring gene. So the relations between restorers and maintainers of two types were identical (Hu and Li 1985). Japonicas from Japan and China were good maintainers. Restoring genes were mainly from indicas of high mountain areas of Yunnan and Southeast Asia, plus some indicas bred at IRRI.

Elite japonica hybrids derived from BT and Dian 1 MS lines are commercially produced in northeast and north China, and some combinations are being field-tested in east, southwest, and central China. Indica MS lines derived by substitution backcrossing the genome of indicas into the sterile cytoplasm of Dian 1 and BT types had the same relation between restorers and maintainers as did Hong-Lien type MS lines (Zhu et al 1985).

In these gametophytic MS lines, pollen abortion occurred at a relatively late stage, i.e., binucleate or trinucleate microspore stage. Pollens were spherical and unstainable or stainable with I-KI solution. Normally, anthers did not dehisce. However, at high temperature and low humidity, some anthers dehisced and a few had selfed seed set. Generally, panicles stretched out of flag leaf sheaths. The fertility restoration of these MS lines was relatively easy, and their restoring spectrum was relatively wide.

Genetic stability of sterile cytoplasm and the genetic effects of sterile cytoplasms on F_1 generation

In China, hybrids derived from MS lines of WA types have been commercially produced for 12 yr. Are the genetic characteristics of sterile cytoplasms stable? What genetic effects do sterile cytoplasms have on their F_1 progeny? Chinese researchers are studying these problems.

Yang and Lu (1983) studied the genetic stability of WA sterile cytoplasm. They used WA MS line V41 A (B_{10}), maintainers V41 B and Zhen-Shan 97 B, and restorer IR24. The genome of IR24 was introduced into the cytoplasm of V41 A by substitution backcrossing (SB). Then, in the generation of SB_5 (wIR24 SB_5 fertile line with WA sterile cytoplasm) and SB_7 (wIR24 SB_7), restorational backcrosses (RB) were made by using V41 B and Zhen-Shan 97 Bas recurrent parents. Restored V41 A (RB-V41 A) and restored Zhen-Shan 97 A (RB-Zhen-Shan 97 A) were obtained in RB_2 and RB_3, respectively.

The restored MS lines (RB-V41 A and RB-Zhen-Shan 97 A) showed no significant differences in fertility from the original MS lines (WA type V41 A and Zhen-Shan 97 A). Also similar were frequency distributions of the fertility of segregative generations of V41 A (B_{18})/IR24 and wIR24(SB_7)/V41 B and the percentages of dark stained pollens among generations from B_{10} to B_{20} of WA type V41 A.

Therefore, the researchers concluded that inheritance of the characteristics of WA type sterile cytoplasm was stable even after some generations of coexistence with allonucleus (maintainer or restorer). Their results suggest that purification of maintainers helps maintain MS line purity.

The effects of sterile cytoplasms on their F_1 progeny were successively studied by Yang et al (1980, 1984), Pan (1982), and Shen et al (1982). Several MS lines of 8 cytoplasms (WA, Liuye, Gam, Hong-Lien, Shenqi, BT, Dian 1, and Dian 3) and their maintainers were crossed with their restorers to create an A-F_1 (F_1 of MS line/restorer) and a B-F_1 (F_1 of maintainer/restorer). Iso-cytoplasm restorers (same sterile cytoplasm as that of the MS line) were crossed with maintainers to create an R-F_1 (F_1 of iso-cytoplasm restorer/maintainer).

These hybrids were compared on 12 agronomic characters. Values for A-F_1 and R-F_1 were lower than for B-F_1 on most characters including grain weight per plant. The same trend occurred on all 8 sterile cytoplasms studied.

These results indicated that the sterile cytoplasms reduced the productive capacity of hybrids compared with the normal cytoplasms of the maintainers. Shen et al (1982) found that indica sporophytic sterile cytoplasms had the largest negative effects, the japonica gametophytic sterile cytoplasms next, and indica gametophytic sterile cytoplasms the smallest negative effects.

The heterosis indexes, however, were the reverse. For example, in grain weight per plant, the heterosis indexes of the A-F_1s of indica sporophytic MS lines and japonica gametophytic MS lines were 1.39 and 1.19, respectively. But the changes of the heterosis indexes due to the effects of sterile cytoplasms (A-F_1-B-F_1) were –0.31 and –0.19, respectively. Thus, the negative effect of sterile cytoplasms is related to heterosis. For combinations having proper parents and possessing high combining and restoring abilities, the negative effects of sterile cytoplasm are not strong enough to significantly alter the direction or degree of heterosis.

Although most economic characters of hybrid rice are controlled by nuclear genes and the genetic effects of cytoplasm are much less than those of the nucleus, the effects of cytoplasm on hybrid should not be neglected. This is especially true because the hybrid rice currently commercially produced in China is derived from only a few sterile cytoplasms. Breeders must learn to minimize the negative effects of sterile cytoplasm and develop new sterile cytoplasms, which can improve economic characters such as yield, disease and insect resistance, and grain quality.

Research on fertility restoration

Source and classification of restorer genes

The effective restorer lines for WA, BT, Hong-Lien, Dian 1, and Gam MS lines used in China in commercial hybrid rice production were identified or developed mainly by screening, cross breeding, and mutation breeding.

Screening for restorers by testcross. A basic way to obtain restorers is to screen by making many testcrosses between MS lines and other varieties, including native and new varieties. The first restorer lines for WA MS lines, including Tai-Ying 1, IR24, IR661, Gu 154, Gu 155, and IR26, were identified by testcrosses in China in 1973 (Li and Yiao 1982). More than 1,000 testcrosses for screening restorers are made in China and IRRI each year. New restorer lines IR54, IR9703-41-3-3-1, and IET5103 have been identified. The origin, evolution, and genetic relations of rice should be considered in making testcrosses. Restorer genes mainly exist in wild rice and indica varieties growing in tropic and subtropic zones.

Selection of restorers by cross breeding. Both MS line BT-C and its restorer BT-A were developed from the cross of Chinsurah Boro II/ Taichung 65. The partial fertiles and complete steriles of the first backcrossed progeny were selected to cross with the recurrent male Taichung 65. Then, BT-C was developed and maintained by successive backcrossing with Taichung 65, while BT-A was selected from the selfed progeny of partial male steriles after repeated backcrosses. When BT-A was crossed with BT-C, the F_1 progeny was completely fertile. The cytoplasm of BT-A came from Chinsurah Boro II. Of 12 pairs of chromosomes, 11 were from Taichung 65 and 1 a chromosome fragment of Chinsurah Boro II containing the restorer genes (Shinjyo 1984). The restorer line for Dian 3 MS lines was developed through pedigree selection from the three-way cross of E-Shan-Da-Bai-Gu/ Ke-Qing 3//Hong-Mao-Ying by Li (1980). The restorer gene came from E-Shan-Da-Bai-Gu.

The use of restorer genes in indica varieties by cross breeding is important particularly in developing japonica restorer lines. For example, the restorers C55 and C57 were developed from a three-way cross between indica and japonica rice (IR8/ Ke-Qing 3//Jing-Ying 35) by Yang et al (Agricultural Science Academy of Liaoning 1980). Restorers Fan-Wu 1 and Fan-Wu 2 were derived from the multiple cross Fan-Xiu 1 A/ / /IR24/Ai-Ke-Qing/ /C55 by Li and Yiao (1982).

Some restorers for WA MS lines were developed by cross breeding, using two methods: 1) selection of iso-cytoplasm restorers, i.e., by crosses between MS lines and restorers and, 2) selection of allo-cytoplasm restorers, i.e., by crosses between restorers and elite varieties. Although pedigree selection was used, allo-cytoplasm restorers should be further identified by testcrosses with MS lines. Restorers Ming-Hui 63 and Er-Liu-Zhai-Zao were developed thusly (Li and Yiao 1982, Wu 1980).

Selection of restorers by mutation breeding. Gamma rays could be used in developing new restorers. Restorers IR24 and IR26 were treated with 30 Krads γ rays. Twenty-seven mutants were selected in M2 of IR24 and 22 in IR26. Then the 49 mutants were tested with Er-Jiu-Nan 1 A and V20 A of WA type. Four were identified as maintainers and 45 restorers. Restorer mutants varied as early-maturing, dwarf, narrow-leaf, round-grain, large-panicle, and big-grain. Fujian Agricultural Academy has developed some restorer lines by radiation treatment of the seed of hybrid Si-You 2, restorer Tai-Ying 1, and some semirestorer varieties (Li and Yiao 1982).

Classification of restorer genes. About one hundred restorer lines have been selected in China, IRRI, and Japan. To classify restorer genes, Zhu et al (l985) tested 14 restorers with 18 MS lines of differing cytoplasm and divided the restorers into four groups. Group 1, including IR24, has effective restorers for WA and Gam MS lines and weak restorers for Hong-Lien, BT, and Dian 1 MS lines. Group 2, including Tai-Ying 1, Peta, IR8, Indonesia 6, and Xue-Gu-Zao has restorers for WA and Gam MS lines but maintainers for Hong-Lien, BT, and Dian 1 MS lines. Group 3, including Zhen-Shan 97 and Long-Zi 1, are maintainers for WA MS lines but restorers for Hong-Lien, BT, and Dian 1 MS lines. Their restorer genes belong to another type. And Group 4, such as No. 5350, No. 75 P12, No. 85661, and No. 300 developed by cross breeding, are restorers for WA, Gam, Hong-Lien, BT, and Dian 1 MS lines, but are relatively weak restorers (Zhu 1984).

Wang (1983) divided restorer genes for WA MS lines into 3 kinds: strong restorer R^s, weak restorer R^w, and recessive restorer gene *r*.

Geographical distribution of restorer genes

The geographical distribution of restorer genes for BT MS lines was studied by Shinjyo (1972). One hundred fifty Japanese varieties and 153 varieties from other countries were tested for fertility restoration. Of the 150 Japanese varieties, 131 had complete male sterile F_1 progeny and 19 had partial fertile F_1 progeny, ranging from 25 to 53% in pollen fertility and 1.7 to 11.5% in seed setting rate. These weak restorers were concentrated in southern Japan (Shinjyo 1975). Of the 153 varieties from 15 countries, progeny of 54 varieties had some fertile pollens and seed setting rates of 70% or higher. These varieties were considered to have effective restorer genes. Twenty-eight varieties showed lower seed setting rates. The remaining 71 showed complete male sterility and were considered to lack restorer genes.

In the three ecotypic varieties–aman, aus, and boro–of indica, all aman and boro varieties tested possessed effective restorer genes, but aus varieties had weak or no restorer genes. Of the two ecotypic varieties on Java Island, bulu and tjereh, bulu contained no effective gene while tjereh had one.

In Asia, effective restorers were mainly in southern countries and South China, while nonrestorers were concentrated in northern countries. Effective and weak restorers, and nonrestorers were found in European countries. In the U.S., no effective restorer was found in California, where japonica varieties were cultivated exclusively, but three effective restorers were detected in Louisiana and Texas, where indica is the main type. In the three grain types, A, B, and C, the frequency of effective restorer genes was 8.5, 27.6, and 62.9%, respectively. The effective restorer genes were mainly distributed in the tropics while the nonrestorers were concentrated in temperate countries (Shinjyo 1975).

In China, more WA type MS restorers were found in low-latitude tropical and subtropical areas than in high-latitude temperate areas. Many restorers came from Southeast Asia and South China, but varieties from Japan, Korea, USSR, eastern European countries, and northwestern, northeastern, and northern China generally possessed no restoring ability for WA type MS lines. Most varieties introduced from Mediterranean countries, North America, Latin America, and Africa also had no restoring ability. Hunan Agricultural Academy tested 721 varieties with WA type MS lines; 35 (33%) of the 107 varieties from Southeast and South Asia were restorers. Of southern and southwestern Chinese varieties, 20% were restorers. Of 433 varieties tested from Yangtze Valley, 7.5% were restorers. No tested japonica variety from northern China had restoring ability (Li and Yiao 1982, Cooperative Research Group for Hybrid Crop Breeding of Guangdong Province 1978). Research at Wuhan University (1977) on Hong-Lien MS lines showed that most early-maturing indicas in Yangtze Valley possessed restoring ability, while most varieties from Southeast Asia had none.

Zhu (1984) studied geographical differences in restorer genes in native varieties by testing them with Zhen-Shan 97 A of WA type and Hua-Ai 15 A of Hong-Lien type. The native varieties were selected from six geographical areas: A (Guangxi, Guangdong, and Fujian), B (Yunnan), C (Sichuan, Guizhou, and Hanzhong district of Shanxi), D (Yangtze Valley, including Hubei, Hunan, Jiangxi, and Anhui), E (Jiangxu, Zhejiang, Shanghai), and F (northeast, northwest, and north China).

Of 630 varieties tested with Zhen-Shan 97 A of WA type, the frequency of effective, weak, and nonrestorers was 10.5, 7.6, and 81.9%, respectively. The effective restorers were 15.9% in A (South China) and 13.5% in B (Yangtze Valley). All restorers were indica, while all japonicas tested were nonrestorers. Of the 614 varieties tested with Hua-Ai 15 A of Hong-Lien type, the frequency of effective, weak, and nonrestorers was 41.0, 16.5, and 42.5%, respectively. Restorer genes were found in all areas but concentrated in A (68.9%), C (45.7%), and D (52.6%). Thus, there were more restorers for Hong-Lien type than for WA type MS lines (Zhu et al 1985). Hong et al (1985) studied the geographical distribution of japonica restorers for japonica MS lines. The japonica MS lines of BT, Dian 1, Lead, Yinje, and WA sterile cytoplasmic types were crossed with japonicas collected from Yunnan, Tai Lake Valley, and Japan, Italy, America, Africa, and Australia. The results showed genes possess different restoring abilities in japonicas. Of 595 F_1 progeny derived from varieties of Yunnan, 6.7% F_1 progenies had a fertility restoring rate of 50% or more. For 1,146 crosses with varieties of Tai Lake Valley and 717 crosses with varieties of foreign countries, the fertility restoring percentages were 3.1 and 5.0%, respectively (Hong et al 1985).

Inheritance of restorer genes

Criteria for assessing fertility. When Shinjyo (1969) stadied fertility inheritance of BT type, pollen fertility was a main criterion. In studying inheritance of the sterility of MS lines of WA type, Hu and Li (1985) used three indexes: percentage of fertile pollen, percentage of bagged seed set, and percentage of natural seed set. Percentage of fertile pollen was the most reliable criterion of fertility (Hu 1983). These three indexes plus morphological characteristics such as degree of panicle exsertion, anther shape, and anther color were used by Yang et al (1984) to appraise fertility. They suggested that the percentage of stained pollen or the percentage of typical aborted pollen should be used as an essential index for determining plant fertility. Most research confirms that pollen fertility could be a main criterion for assessing fertility.

Sporophytic inheritance. Gam type MS lines of WA are sporophytic.

Gao (1981) analyzed the bagged seed setting rate of F_1 and F_2 plants of WA type Er-Jiu-Nan 1 A/IR24 and BC_1 population of Er-Jiu-Nan 1 A/F_1 hybrid. He concluded that Er-Jiu-Nan 1 A sterility was controlled by two pairs of independent recessive genes, while the restorer IR24 had two pairs of independent dominant genes with obvious dosage or additive effects. Zhou (1983) studied the plant fertility of male sterile, maintainer, restorer lines, hybrid F_1, F_2, (MS line × hybrid F_1) F_1, (hybrid F_1 × maintainer) F_1, (maintainer × hybrid F_1) F_1, F_2, and (restorer × hybrid F_1) F_1, F_2 of WA type Nan-You and Shan-You system. He considered that the sterility of indica MS lines with WA type cytoplasm was sporophytic and that the genotypes of MS line, maintainer, and restorer were S$(r_1r_1r_2r_2)$, N$(r_1r_1r_2r_2)$, and N$(R_1R_1R_2R_2)$, respectively (Zhou 1983). Yang et al (1984) analyzed the restorer genes in restorer line IR24 and pointed out that IR24 had two pairs of dominant restorer genes $R_1R_1R_2R_2$, with R_1 appearing more important than R_2.

Hu and Li (1985) investigated fertility segregation in F_1, F_2, and F_3 hybrids descended from crosses between WA type Zhen-Shan 97 and IR24, IR26, and WA type V20 A; Liuye type Zhen-Shan 97 A and IR24; as well as BC_1 of WA type Zhen-Shan 97//WA type Zhen-Shan 97 A/IR26. Fertility frequency distributions of F_2 and BC_1 populations appeared to have double-peaked curves. There were more parental types and fewer intermediate ones in the segregating populations and the distribution of fertility frequency was continuous. Results suggested there were two pairs of major fertility genes in the same linkage group and the average recombination frequency was 34%, (Hu and Li 1985).

Li (1985) analyzed the pedigree of IR24 and pointed out that IR24 possessed two pairs of major restorer genes ($R_1R_1R_2R_2$). One pair (R_1R_1) came from Cina, a late indica variety in China, while another pair (R_2R_2) was from SLO 17, inherited to IR127 through CP-SLO, R_1R_1 was then combined with R_2R_2 to form the strong restorer line IR24 ($R_1R_1R_2R_2$) by crossing IR8 with IR127.

But Wang (1980) considered that the fertility restoration of WA type Zhen-Shan 97 A was controlled by one pair of genes. Research on the inheritance of fertility restorer genes for WA type MS line has also been done by He (1985), Cai et al (1983), and Huang et al (1986).

Gametophytic type. The inheritance of fertility restoration in BT, Dian 1, and Hong-Lien types is gametophytic. In studying the inheritance of BT system, Shinjyo (1972) found the genotypes of BT system were S*(rr)* MS line BT-C, N*(rr)* maintainer, S*(RR)* restorer BT-A, and N*(RR)* restorer BT-X. Results from some crosses are listed below:

1) The genotype of F_1 progeny of BT-C/ BT-A was S(R_r). Its pollen fertility was 50% although the seed setting rate was more than 90%. The F_2 progeny segregated into a nearly 100% fertile pollen class and a 50% fertile pollen class in a 1:1 ratio. Although all plants had a seed setting rate of more than 90%, no segregation occurred in seed set.
2) When the restorer S*(RR)* was used as a female parent to cross with maintainer N*(rr),* the genotype of F_1 progeny was also S(R_r) and the corresponding pollen fertility and seed setting rate of F_1 and F_2 progeny were the same as that in 1.
3) When maintainer N*(rr)* was crossed with restorer N*(RR),* F_1 progeny were completely fertile and the F_2 progeny revealed no segregation in both pollen fertility and seed set.

Thus, Shinjyo (1972) concluded that the inheritance of fertility restoration in BT system was controlled by a pair of restorer genes whose effect in the sterile cytoplasm was gametophytic. Linkage analysis showed the restorer gene was located on the seventh chromosome.

Hu and Li (1985) studied F_1, F_2, and B_1 derived from crosses between 4 MS lines (Dian 1 type Hua-Jing 14 A, Dian 1 type Tu-Dao 4 A, BT type Nong-Jin 2 A, and Hong-Lien type Hua-Ai 15 A) and their restorers. All 4 MS lines were gametophytically sterile. The fertility of Dian 1 type Hua-Jing 14 A, Tu-Dao 4 A, and BT type Nong-Jin 2 A was controlled by a pair of recessive genes (rr) and the genes in Dian 1 and BT types were allelic. The fertility of Hong-Lien type Hua-Ai 15 A was controlled by a pair of major genes and possibly also some modifying factors.

Zhu (1984) studied the fertility of the F_1 and F_2 derived from Hong-Lien type hybrid Qing-You-Zao. Pollen fertility was about 50% and the average seed setting rate of F_1 hybrid was 77%. The F_2 progeny segregated out a complete normal pollen class and a 50% normal pollen class in a 1:1 ratio, whereas the distribution of selfed seed setting rate appeared to be a single-peaked continuous curve, with 1.2% sterile plants. Fertility restoration was considered controlled by a pair of major genes with gametophytic effects in Hong-Lien type sterile cytoplasm, plus some modifying genes (Zhu et al 1985).

Main factors affecting fertility restoration

Genetic diversity. Genetic diversity includes differences in sterile cytoplasm and backgrounds of maintainers and restorers.

Isogenic MS lines with different sterile cytoplasms may belong to different sterile types and have different restorers. For example, the cytoplasms of WA and Hong-Lien types, although both coming from wild rice plants of Hainan Island, belong to different sterile types. WA lines are sporophytic, while Hong-Lien types are gametophytic. When both WA type Hua-Ai 15 A and Hong-Lien type Hua-Ai 15 A were tested with IR24, the F_1 progeny of WA were completely fertile but progeny of Hong-Lien were partially fertile (Zhu 1984).

The genetic background of maintainers influences the fertility of F_1 hybrids having the same sterile cytoplasm. For example, fertility restoration of WA type MS line Zhen-Shan 97 A was easier than of WA type Er-Jiu-Nan 1 A (Li and Yiao 1982).

The genetic background of restorers apparently influences fertility restoration. When restorers IR24, IR28, and Gu 154 were crossed with the same MS line, the fertility of F_1 hybrids revealed that the restorers differed in fertility restoring ability. Especially under unfavorable climate, the seed setting rate of hybrids derived from IR28 and Gu 154 was lower than that of the hybrid derived from IR24.

Environmental variation. Hybrid rices are more sensitive to environmental variations than most conventional varieties. Environmental factors, particularly temperature, greatly influence fertility restoration. Seed setting rate may drop when unfavorably high or low temperature occurs during the pollen mother cell meiosis stage or heading stage.

Joint IRRI-China research indicated that 15% of Chinese elite breeding materials tested were effective restorers, and 24% of IRRI materials were; only 6% were effective at both sites. Thus, the frequency of restorer genotypes was higher in the tropics than in the subtropics (Virmani and Edwards 1983).

K. Govinda Raj tested WA type MS lines V20 A and Zhen-Shan 97 A with restorer ADT33. The F_1 hybrid was cultivated at Delhi in the rainy season and at Aduthurai from July to October. Hybrids at Delhi appeared completely fertile but partially fertile at Aduthurai.

Chinese research showed that temperature and moisture affect fertility restoration. The most sensitive stage of hybrid rice to temperature is the flowering stage. If unfavorably high or low temperature occurs during that stage, many florets will not flower, few anthers will dehisce, and the germination rate will decrease, with low seed setting. Hybrids derived from different MS lines and restorers differ in their reactions to environmental variations (Li and Yiao 1982).

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Development of CMS lines in hybrid rice breeding

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Cytoplasmic and genetic diversity can protect F_1 rice hybrids against vulnerability to diseases or insects. Of cytoplasmic male sterile (CMS) sources developed in rice, only wild abortive(WA) is used widely. Similarly, only a few CMS lines are used extensively in developing commercial F_1 rice hybrids in China, and they lack genetic diversity. They do not possess multiple disease and insect resistance and, therefore, cannot be used as such to develop F_1 rice hybrids for the tropics where disease and insect pressure is high. Their grain quality is also poor.

IRRI has been developing new CMS lines adapted to the tropics and possessing genetic and cytoplasmic diversity. CMS lines IR54752 A, IR54753 A, and IR54754 A appear suitable for developing F_1 rice hybrids for the tropics.

Although CMS systems often weaken agronomic characteristics of F_1 hybrids derived from them, appropriate restorers can prevent this. Breeders need to diversify CMS sources to avoid disease or insect susceptibility in F_1 hybrids. We propose cooperative search for and analysis of new CMS sources so that diverse cytoplasmic and nuclear genotypes will be continuously available for developing F_1 hybrids in and outside China.

Cytoplasmic male sterility is the most effective genetic tool for developing F_1 rice hybrids (Lin and Yuan 1980, Virmani et al 1981, Virmani and Edwards 1983). Practically all hybrid rice area (8.5 million ha) in China is planted to hybrids derived from cytoplasmic male sterility systems. Only about 10,000 ha is planted to hybrids derived from chemically induced male sterility. About 95% of the area under cytoplasmic male sterile (CMS)-derived hybrids is occupied by hybrid: from wild abortive (WA) cytosterility system (Yuan and Virmani 1988). Outside China, WA cytosterility system is mostly used to develop suitable CMS lines.

This reliance on a narrow genetic base makes hybrid rice cultivation potentially vulnerable to disease or insect susceptibility. Crop breeders know of the extensive damage caused by the epidemics of southern corn blight in the US. and pearl millet downy mildew in India. Both these diseases were associated with the cytosterility systems used to develop the F_1 hybrids.

Although no disease or insect susceptibility in rice has yet been associated with WA or any other cytosterility system, hybrid rice breeders must be alert to the possibility. Cytoplasmic and genetic diversity must be ensured while choosing parents to develop commercial hybrids.

This paper highlights the current status and future prospects of research on development and use of genetically diverse CMS lines in hybrid rice breeding.

Sources of cytoplasmic male sterility in rice

Virmani and Edwards (1983) listed 19 cytoplasmic sources identified until 1981 to induce male sterility in rice. These and other sources of CMS are given in Table 1. Some CMS sources (WA, CMS-boro or BT in China, and Gambiaca) have been transferred in different nuclear backgrounds. Similarly, nuclear genotypes Zhen-Shan 97, Jing-Nan-Te 43, Fu-You 1, and IR10179-2-3-1 have been substituted into CMS sources.

Studies reported from China (Lin and Yuan 1980) and IRRI (Young et al 1983, Virmani and Dalmacio 1986) suggest that

- WA cytosterility system is different from CMS-boro (or BT), Taichung Native 1, Gambiaca, and ARCl3829-16.
- Chinsurah Boro II and Taichung Native 1 system appear similar (Young et al 1983).
- Gambiaca CMS system differs from Chinsurah Boro II.
- The cytosterility system of MS577 A (CMS source developed in Korea) differs from WA, CMS-boro, and Gambiaca/(Young et al 1983).

Based on maintenance-restoration behavior in F_1 testcrosses, Chinese scientists identified 7 CMS systems among 36 CMS lines developed from various cytoplasmic sources (Table 2). The CMS systems were designated sporophytic or gametophytic depending on the effect of their restorer gene(s). Five of seven CMS systems were sporophytic and two gametophytic. Gam CMS had the most restorer lines followed by Indonesian paddy (IS), WA, and dwarf wild (DW). Although the WA and long-awned wild (LW) systems had similar spectra of restoration, they were differentiated because some restorers of WA were maintainers of LW and vice versa.

Of the gametophytic CMS systems, CMS-boro BT had more restorers than Hong-Lien (HL).

Cytoplasmic diversity of CMS sources needs comprehensive and systematic study. A uniform system for designation of cytosterility sources needs to be followed to avoid confusion among rice geneticists and breeders.

Cytoplasmic male sterility sources suitable for developing F_1 rice hybrids

To be suitable for developing F_1 rice hybrids, a CMS source should

- be stable for complete pollen sterility over environments,
- be easy to maintain so that diverse genotypes can be converted into CMS lines,
- be easy to restore so that diverse genotypes can be selected as male parents,
- not weaken agronomic characteristics of the hybrids, and
- enhance flowering behavior and characteristics influencing outcrossing, so that bulk hybrid seed can be produced economically.

Pollen grains of CMS lines derived from sporophytic CMS systems abort at the uninucleate stage, hence, they are more stable than CMS lines derived from a gametophytic system in which pollen grains abort at the binucleate or trinucleate

Table 1. Cytoplasmic sources for inducing male sterility in rice.

Cytoplasm donor		Nuclear donor	CMS line developed at	Year released
Name	Designated symbol			
Wild rice				
Oryza sativa f. *spontanea*				
O. sativa f. *spontanea*		Fujisaka 5	Japan	1958
Wild rice with abortive pollen	CMS—WA	Zhen-Shan 97	Jiangxi, China	1973
Ya Cheng	CMS—YC	Guang-Xuan 3	Guangxi, China	1975
Tiandong	CMS—TD	Zhen-Shan 97	Guangxi, China	1985
Liuzhou	CMS—LZ	Zhen-Shan 97	Guangxi, China	1985
Indian	CMS—IN	Jing-Nan-Te 43	Guangxi, China	1985
Dong Pu	CMS—DP	Jing-Nan-Te 43	Guangxi, China	1985
Jun Ni Ya	CMS—JNY	Chao-Yang 1	Guangxi, China	1985
He Pu	CMS—HP	Li-Ming	Hubei, China	1982
Teng Qiao	CMS—TQ	Er-Jiu-Qing	Hubei, China	1982
Hainan, red awned	CMS—HL	Lian-Tang-Zao	Wuhan, China	1979
San Ya	CMS—SY	Jing-Ying 1	CAAS, China	?
Rao Ping	CMS—RP	No. 6964	South China Agric. Univ.	?
Guangzhou	CMS—GZ	No. 6964	South China Agric. Univ.	?
Dwarf, abortive pollen	CMS—DA	Xue-Qin-Zhao	Anhui, China	?
Oryza fatua				
O. fatua	CMS—OF	Fujisaka 5	Japan	1958
O. fatua		Fujisaka 5	Korea	1970
Oryza rufipogon				
KR7	CMS—KR	Taichung 65	Taiwan, China	1979
Oryza perennis				
W1080	CMS—W18	Taichung 65	Japan	1981
W1092	CMS—W19	Taichung 65	Japan	1981
Cultivar				
Oryza glaberrima				
O. glaberrima	CMS—OG	Colusa	USA	1972
Danboto	CMS—DBT	Hua-Ai 15	Hubei, China	1979

continued on next page

Table 1 continued.

Cytoplasm donor		Nuclear donor	CMS line developed at	Year released
Name	Designated symbol			
Oryza sativa				
Chinsurah Boro II	CMS–bo	Taichung 65	Japan	1966
Lead Rice	CMS–ld	Fujisaka 5	Japan	1968
Birco (PI 279120)	CMS–Bi	Calrose Caloro	USA	1969
Taichung Native 1	CMS–TN	Pankhari 203	IRRI	1972
Akebono	CMS–ak	*O. glaberrima*	Japan	1977
E-Shan-Ta-Bai-Gu	CMS–STB	Hong-Mao-Ying	Yunnan, China	1975
Tian-Ji-Du	CMS–TJD	Fujisaka 5	Hubei, China	1982
IR24	CMS–IR24	Xiu-Ling	Liaoning, China	1980
Jing-Chuan-Nuo	CMS–JCN	Nan-Tai-Geng	Fujian, China	1977
Shenqi	CMS–SQ	Nong-Ken 8	Fujian, China	1977
Nan-Guan-Zhan	?	New Zealand	Hunan Agric. College, China	?
Li-Up	CMS–LU	Jing-Ying 83	Jiangsu, China	?
Zao-Jin-Feng	CMS–ZJF	Lanbery	Fujian, China	?
Gambiaca	CMS–AM	Chao-Yang 1	Sichung, China	1977
Zhao-Tong-Bei-Zi-Gu	CMS–ZTB	Ke-Qing 3	Yunnan, China	1980

Table 2. CMS systems in rice as classified in China.

CMS system	CMS lines (no.)	Restorer lines (no.)
Sporophytic		
Gam	2	Most
IS	2	< Gam
WA	19	< Gam and IS
DW	2	< Gam, IS, and WA
LW	5	= to WA but some restorers of WA are maintainers of LW and vice versa
Gametophytic		
CMS-boro or BT	3	Most
HL	3	< than BT

stage (Chaudhary et al 1981, Lin and Yuan 1980). Experience in China indicates that developing sporophytic and stable CMS lines is more likely from wider crosses where the female is a primitive line and the male parent is an advanced line, e.g., CMS lines developed from WA system. A closer relation between the cytoplasmic and nuclear donor parents produces gametophytic male sterility, making it harder to obtain a stable CMS line. Sporophytic CMS lines have a narrower spectrum of restorer lines than do gametophytic lines.

Yuan and Virmani (1988) listed CMS sources used in China to develop commercial F_1 rice hybrids. These include WA, Gambiaca (Gam); CMS-boro (BT), Dissi (Di), HL, and dwarf wild rice with abortive pollen (DA).

CMS lines for developing commercial F_1 rice hybrids in China

To be used widely in a hybrid breeding program, a CMS line should possess

- complete pollen sterility to avoid self-fertilization in hybrid seed production plots,
- stable pollen sterility over environments,
- good adaptability to cultural conditions for which hybrids are to be developed,
- good agronomic potential,
- fair to good general combining ability, and
- agronomic and floral traits to allow sufficient cross-pollination in seed production plots.

At IRRI about 8% of improved breeding lines screened each year are able to maintain the sterility of WA CMS system. The frequency is reported to be even higher among indica rices developed in China. Therefore, genotypes are being routinely converted to CMS lines in China and IRRI. The leading CMS lines used for developing commercial F_1 rice hybrids in China are listed in Table 3. These are developed from modern semidwarf high-tillering and early-maturing varieties which have wide adaptability in China.

Table 3. List of important CMS lines used in China to develop commercial rice hybrids.

CMS line	Source of cytoplasm
V20 A	WA
Zhen-Shan 97 A	WA
V41 A	WA
Er-Jiu-Nan 1 A	WA
Zhen-Eai A	WA
Chang-Fu A	WA
Li-Ming A	CMS-boro or BT
Nong-Fu A	CMS-boro or BT
Jing-Nan-Te 43 A	WA
Xiu A	CMS-boro or BT
Feng-Jin A	CMS-boro or BT
Zhong-Dan 2 A	CMS-boro or BT
Nan-Zhao A	WA
Ke-Mei A	WA
Jun-Xie A	WA
No. 691 A	WA
Yar-Ai-Zhao A	Gam

Strengths and weaknesses of cytoplasmic male sterile lines in China

The CMS lines V20 A, Zhen-Shan 97 A, and V41 A used widely in developing commercial rice hybrids in China possess wide adaptability, but lack genetic diversity.

They possess good general combining ability and their floral behavior and characteristics are suitable for outcrossing in hybrid seed production plots especially V41 A with its high frequency of exserted stigmas.

These lines do not possess multiple resistance to diseases and insects and have poor grain quality (medium bold chalky grain with high amylose and hard gel consistency). Therefore, they cannot be used to develop F_1 rice hybrids for the tropics where disease and insect pressure is higher. Similarly, these CMS lines are not preferred in countries such as USA where consumers demand high grain quality.

Development of CMS lines for countries outside China

The leading CMS lines developed in China were introduced at IRRI in 1979 for developing F_1 rice hybrids for tropical and subtropical countries outside China.

IRRI 1) transferred the WA cytosterility system of Chinese CMS lines into lines adapted to the tropics and subtropics, and 2) crossed several wild species, land races, and traditional varieties with advanced breeding lines.

A total of 14 CMS lines (Table 4) have been developed so far. Nine lines are based on elite lines bred at IRRI and five on the genetic background of elite lines from Korea (Iri 356 and Suweon 310) and India (Jikkoku Seranai 51-37, MR365, and PAU269-1-8-4-1-1-1). MR365 and PAU269-1-8-4-1-1-1 possess good grain quality. CMS lines IR54752 A, IR54753 A, and IR54754 A appear well adapted to

Table 4. CMS lines developed at IRRI.

Line	Parentage		Year designated	CMS system
	Female parent	Male parent		
IR46826 A	Zhen-Shan 97 A/7*IR10154-23-3-3	IR10154-23-3-3	1983	WA
IR46827 A	Zhen-Shan 97 A/6*IR10176-24-6-2	IR10176-24-6-2	1983	WA
IR46828 A	Zhen-Shan 97 A/6*IR10179-2-3-1	IR10179-2-3-1	1983	WA
IR46829 A	Zhen-Shan 97 A/6*IR19792-15-2-3-3	IR19792-15-2-3-3	1983	WA
IR46830 A	V20 A/6*IR19807-21-2-2	IR19807-21-2-2	1983	WA
IR46831 A	V20 A/G*Jikkoku Seranai 52-37	Jikkoku Seranai 52-37	1983	WA
IR48483 A	Zhen-Shan 97 A/6*MR365	MR365	1984	WA
IR54752 A	Zhen-Shan 97 A/8*IR21845-90-3	IR21845-90-3	1986	WA
IR54753 A	Zhen-Shan 97 A/8*IR19657-34-2-2-3-3	IR19657-34-2-2-3-3	1986	WA
IR54754 A	V20 A/8*IR19657-87-3-3	IR19657-87-3-3	1986	WA
IR54756 A	Zhen-Shan 97 A/8*Iri 356	Iri 356	1986	WA
IR54757 A	Zhen-Shan 97 A/7*Suweon 310	Suweon 310	1986	WA
IR54758 A	V20 A/4*PAU269-184-1-1-1	PAU269-1-8-4-1-1-1	1986	WA
IR54755 A	ARC13829-16/6*IR10179-2-3-1	IR10179-2-31	1986	ARC

the tropics, are good general combiners, and show satisfactory outcrossing rate. They are being used to develop F_1 rice hybrids for the tropics. CMS lines IR46826 A, IR46827 A, IR46828 A, IR46829 A, and IR46830 A are better adapted than Chinese lines to the tropics but are poor general combiners and possess low outcrossing rate, restricting their use in hybrid development.

CMS lines IR46831 A, IR48483 A, and IR54758 A have been bred for India and IR54756 A and IR54757 A have been bred for Korea.

IRRI's experience in converting elite maintainer lines into CMS lines (Virmani et al 1986) has shown that

- certain genotypes tend to increase pollen sterility with successive backcrossing with occasional decline in sterility in a BC generation;
- certain genotypes are difficult to sterilize because even after 7-9 backcrosses, the frequency of completely pollen sterile plants is less than 100%; and
- certain genotypes are easy to sterilize, since completely pollen sterile plants occur at high frequency (90-100%) after 1-3 backcrosses.

Lack of complete pollen sterility even after five to nine backcrosses may be due to some minor gene for fertility restoration present in the maintainer genotypes.

To develop CMS lines from different sources, we crossed wild species and traditional cultivars with elite breeding lines at IRRI. Some crosses showed high pollen sterility from F_1 up to several BC generations. In the first set of 32 crosses, only 1 cultivar (ARC13829-16) of *Oryza sativa* L. was a source of cytoplasmic male sterility. This line was crossed with IR10179-2-3-1 and high frequencies of completely pollen sterile plants were observed in BC generations (Table 5).

A stable CMS line from this cross has been developed in BC_6 generations and designated IR54755 A. The maintainer of this line is also the maintainer of the WA CMS line. A preliminary cytogenic analysis at IRRI (Virmani and Dalmacio 1986) showed that ARC CMS source differs from the WA CMS source. Additional crosses at IRRI to develop new CMS lines indicated that two accessions of *Oryza breviligulata,* Casamance V6 and Casamance V8 B, and one accession of *O. glaberrima,* Zakpale III, introduced from West Africa, are prospective CMS sources.

Effect of cytoplasmic male sterility on agronomic traits

Shinjyo (1975) reported that CMS-boro cytoplasm reduced plant height but did not affect leaf number, growth duration, and panicles per plant. Zhu (1979) found that CMS cytoplasm reduced plant height (through shorter first internode), panicle exsertion, and grains per panicle but did not significantly affect width and length of flag leaf and panicle length. Wan (1980) also observed delayed heading, reduced plant height, reduced panicle exsertion, and more panicles in CMS lines compared to maintainer lines.

Lu et a1 (1980) studied the effects of WA cytoplasm on the agronomic traits of F_1 hybrids and found that WA cytoplasm delayed growth duration and reduced plant height, productive tillers, grains per panicle, spikelet filling, and 1,000-grain weight.

Table 5. Pollen fertility in generations of the cross ARC13829-16/IR10179-2-3-1 resulting in the stable CMS line IR54755 A at IRRI.

Generation no.	Plants observed (no.)		% of plants in pollen fertility class[a]				
	Row basis	Total population	CS	S	PS	PF	F
BC_6	10		100.0	0	0	0	0
BC_6		113	96.5	3.5	0	0	0
BC_5	10		100.0	0	0	0	0
BC_5		46	87.0	6.5	6.5	0	0
BC_4	9		88.9	11.1	0	0	0
BC_4		48	79.2	14.6	6.2	0	0
BC_3	9		100.0	0	0	0	0
BC_3		45	98.8	2.2	0	0	0
BC_2	10		70.0	30.0	0	0	0
BC_2		17	41.2	35.3	11.7	11.7	0
BC_1	4		0	50.0	50.0	0	0
BC_1		10	0	30.0	50.0	0	20.0
F_1	10		10.0	20.0	50.0	20.0	0
F_1		15	6.7	13.3	14.6	26.7	6.7

[a]CS = completely sterile (0% pollen fertility), S = sterile (1-10% pollen fertility), PS = partially sterile (11-30% pollen fertility), PF = partially fertile (31-60% pollen fertility), F = fertile (61-100% pollen fertility).

Virmani et al (unpublished) observed at IRRI that WA CMS lines were generally shorter and possessed more tillers and had poorer panicle exsertion than the corresponding maintainer lines. These traits were, therefore, influenced by male sterility or sterility-inducing cytoplasm. No significant differences in seedling vigor and flag leaf width were found between CMS and maintainer lines.

These studies indicated that CMS cytoplasm does weaken certain agronomic characteristics. The negative effect of gametophytic CMS systems is weaker than that of sporophytic systems (Wan 1980). Choosing an appropriate restorer can reduce the negative effects of CMS cytoplasm. Thus, a suitable CMS system for hybrid development should be selected after assessing the performance of hybrids involving diverse restorer genotypes.

Effect of cytoplasmic male sterility on disease and insect resistance in rice

At Guanxi Academy of Agricultural Sciences, researchers evaluated 55 CMS lines (developed from 13 cytoplasmic sources) and their maintainers for reactions to neck blast and bacterial blight. Disease reaction did not differ between F_1 hybrids derived from A/ R (male sterile/restorer) and from B/R (maintainer/ restorer) crosses. Thus, resistance to these diseases was controlled entirely by genes in the nucleus without cytoplasmic control.

Preliminary studies at IRRI (Hibino and Virmani, unpublished) did not indicate a relationship between susceptibility to viral diseases (tungro, grassy stunt, and ragged stunt) and the CMS source, since both A and B lines showed similar susceptibility or resistance.

In 1985, blast seriously damaged hybrid rice in Sichuan Province in China but this could not be related to the WA cytosterility system because some nonhybrid rices were equally damaged.

Resistance, or susceptibility to major insects is controlled by nuclear genes. No data indicate any relationship between a CMS source and susceptibility to an insect pest of rice.

The absence of any relationships between CMS sources and disease/insect susceptibility does not ensure that this will continue. Hybrid rice breeders must be vigilant and diversify CMS sources in rice hybrids to avoid potential vulnerability.

Development of new sources of cytoplasmic male sterility

Research to identify new CMS sources in China and IRRI has used interspecific, intraspecific, and intervarietal crosses. However, differences among new and old sources have not been thoroughly substantiated. We suggest these techniques be systematically used to establish difference among CMS sources: 1) substitution backcrossing, 2) use of cytoplasm-differentiating genes, 3) interaction of restorer genes with cytoplasms, 4) study of pollen abortion patterns, and 5) restriction endonuclease fragment analysis of organelle DNAs. This needs cooperative research among scientists from China, Japan, and IRRI to develop and collect CMS sources and analyze their genetic differences.

Outlook

Genetic diversity for developing commercial F_1 rice hybrids is needed both for nuclear genotypes and for cytoplasmic factors inducing male sterility. Quite a few elite rice lines and varieties have been found to maintain WA, Gam, and CMS-boro cytosterility systems, therefore, it is easier to develop CMS lines with diverse nuclear genotypes. This can be done effectively by closely linking the hybrid breeding program with the inbred breeding program. This will help ensure that diverse elite breeding lines and varieties are continuously available for identifying effective maintainers and converting them into CMS lines using recurrent backcrossing. The hybrid rice breeding program at IRRI uses this approach.

Diversification for cytoplasmic factors inducing male sterility is cumbersome to achieve. Many interspecific, intraspecific, and intervarietal crosses have to be made. It is unpredictable which crosses will result in a line representing a new CMS system. Once rice scientists understand the molecular basis of cytoplasmic male sterility, they can screen wild and cultivated rices for specific organelle DNA responsible for inducing male sterility. The prospective donors of cytoplasmic male sterility can then be crossed with elite lines (preferably restorers or partial restorers of the available CMS lines). Some of these crosses can produce male sterile lines after 5-6 backcrossing generations. The new CMS lines should possess different cytoplasmic male sterility systems because the maintainers are the restorers or partial restorers of the previously available CMS lines.

Cytogenic analysis and restriction endonuclease fragment analysis of organelle DNA would be useful for determining cytoplasmic diversity among old and new CMS lines. To avoid confusion in naming cytoplasmic male sterility systems, we suggest that a uniform system of designation be followed by rice geneticists and breeders.

Protoplast fusion and regeneration techniques, once perfected in rice, would also substitute for the recurrent backcrossing. Then the entire nucleus of the maintainer line could be substituted into the cytoplasm of the CMS donor in one step, developing the CMS lines faster than through recurrent backcrossing.

Because the CMS lines have to possess several traits (specified earlier in the paper) before being usable for developing commercial F_1 rice hybrids, they should be evaluated extensively by hybrid rice breeders around the world.

The identification of a wide compatibility gene in rice (Ikehashi and Araki 1984, 1986) opens up new possibilities for crossing indica and japonica parents to develop indica/japonica F_1 hybrids possessing high levels of heterosis. Therefore, it would be extremely useful to transfer this gene into the CMS lines so they would become easy to restore by diverse restorer genotypes. This would indirectly diversify the genetics of commercial F_1 hybrids.

In searching for new and diverse CMS sources, we should select sources that do not weaken the agronomic traits of the hybrids. Similarly, CMS and maintainer lines should be continuously evaluated for disease and insect reaction so that any association between a CMS source and disease and insect susceptibility can be detected and preventive measures taken to avoid an epidemic.

Some chemicals and growth regulators induce male sterility in rice. Their use is limited because either they induce only partial male sterility or the induced sterility is not consistent across environments. It would be worthwhile to test the efficacy of chemicals to induce complete pollen sterility on partially sterile CMS lines. This would also enable the use of CMS sources which cannot be used now because of partial male sterility.

Chinese scientists have identified a male sterility gene closely linked with the gene for photoperiod sensitivity (Shi 1985). The male sterile mutant shows complete pollen sterility under long daylength and normal pollen fertility under short daylength. This enables the use of genic male sterility in developing F_1 hybrids. We should explore developing CMS lines in which the cytosterility system is associated with daylength sensitivity. This may give pollen fertility in CMS lines grown under certain daylengths. Thus, the selfed seed collected from a CMS line grown under one daylength would give male sterile plants in another daylength. This would make hybrid seed production more convenient than the current use of two steps, A/B multiplication and A/R seed production.

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Cytohistology of cytoplasmic male sterile lines in hybrid rice

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By 1985, 36 male sterile lines from 14 cytoplasmic sources and 6 male sterile materials from pollen-free types had been cytologically studied in China. Based on pollen abortion pattern, male sterile rice can be classified into four types; pollen-free, uninucleate abortive, binucleate abortive, and trinucleate abortive. Pollen-free types abort before the uninucleate stage and have no pollen. In uninucleate and binucleate abortion types, nuclei and cytoplasms dissolve, forming wrinkled irregular or spherical empty pollen. The trinucleate abortion types have normal shaped pollen, but form abnormal sperm during reproductive division.

In some male sterile species, a special pattern of pollen abortion occurs: sterility for all factors from sporogenesis through germination of pollen grains. This results in few mature pollen grains, low percentage of split anthers, and low pollen germination. Among the variant types, the vascular bundles of filaments and connectives develop abnormally, and are not identical. The vascular system of a wild abortive male sterile line was more poorly developed and differentiated than other male sterile lines. The abnormal dehiscent structure in the anther of male sterile are shown mainly at the splitter in the fracture cavity and by irregularly developed anther "springs."

Little cytohistological work was done on cytoplasmic male sterile (CMS) rice before the 1970s. Laser and Lersten (1972) enumerated the crops on which the process of pollen abortion in male sterile lines had been cytologically studied between 1925 and 1972; rice was not included. Shinjyo (1972) published a cytological study of pollen abortion in male sterile rice that same year. China began research on pollen abortion in male sterile rice in 1971, when breeding of CMS lines, maintainer lines, and restorer lines was under way. By 1985 an estimated 36 male sterile lines of rice from 14 different cytoplasmic sources had been studied. In addition, male sterile materials of six pollen-free types of rice had been analyzed.

The patterns of pollen abortion in cytoplasmic male sterile lines and their morphological features disclose different genetic backgrounds, conducive to hybrid rice production.

Microscopy is used to check pollen fertility or infertility and to estimate the degree of sterility. Three technical terms have been accepted to describe pollen abortion: typical abortion type, spherical abortion type, and stained abortion type. Typical abortion occurs at the uninucleate stage, spherical abortion at the anaphase of the uninucleate stage or at the binucleate stage, and stained abortion at the anaphase of the binucleate stage or at the trinucleate stage.

To further explore the linkages of pollen abortion, we studied various filaments and anthers in male sterile lines and found degrees of abnormal development and differentiation in the filaments and anthers of different male sterile lines. That explained the mechanisms of dehiscence in rice and indicated that the abnormal development of the splitter and fission cavity in the dehiscent anther is the main reason male sterile anthers fail to dehisce normally.

Cytological studies of pollen abortion

The patterns of pollen abortion in male sterile rice vary. The most important character is the stage at which pollen abortion occurs. Most of the male sterile material tested can be grouped into four types: pollen-free, uninucleate abortive, binucleate abortive, and trinucleate abortive.

Pollen-free types

Pollen abortion occurs at a stage before the uninucleate. In four kinds of male sterile material (Hunan Teachers' College 1972), three patterns were identified:

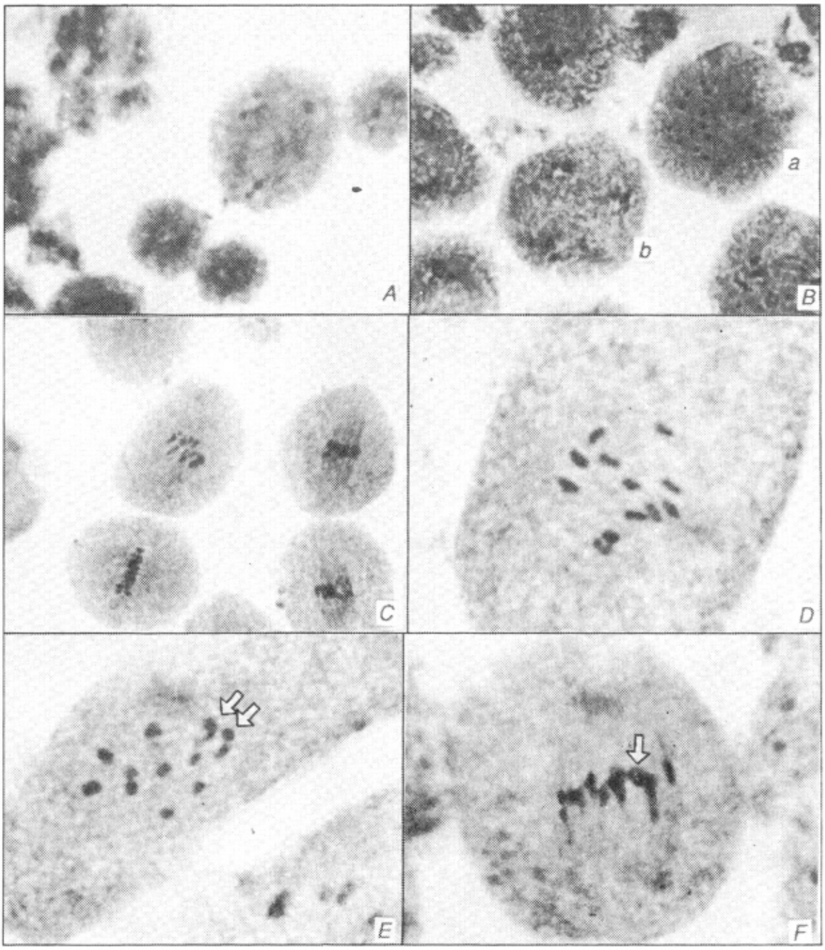
- Abnormal development of sporogenous cells. Sporogenous cells fail to develop into normal pollen mother cells. They reproduce by amitosis in nucleolar budding, forming new cells to divide by pare-off fission into many irregular, bladelike, tiny cells of different sizes. These tiny cells become more and more slender until they disappear.
- Abnormal development of pollen mother cells. The pollen mother cells developed by sporogenous cells vary in size and shape. No typical mutation occurs at the prophase of the primary meiosis and there is no distinction of pollen meiosis between the metaphase and the anaphase. The two lunar dyad cells formed are linked at the ends. They do not form tetrads but, through mitosis, turn into smaller and smaller cells until they disappear.
- Abnormal development after formation of tetraspores. Some pollen mother cells form tetraspores but have a tendency to nucleolar budding, changing into smaller and smaller cells until they disappear. Others enter the second contraction stage, but remain wrinkled. The protoplasm gradually dissolves until only remnant cell walls of different sizes and shapes remain.

Pan et al (1981) discovered another pattern of pollen abortion in male sterile material of abortive pollen types 424 and 131. On the whole, meiosis is normal in the pollen mother cells, which can be transformed into microspores but not into pollen exines. No germ pores (apertures) can be seen after microspores have taken shape. The inner parts of the cells develop normally. Some cells pass through the binucleate stage but fail to engage secondary mitoses. Mature anthers have different sized pollen grains with no pollen exines and remnant cell components.

Abnormal chromosome behavior of pollen-free types is shown in Figure 1.

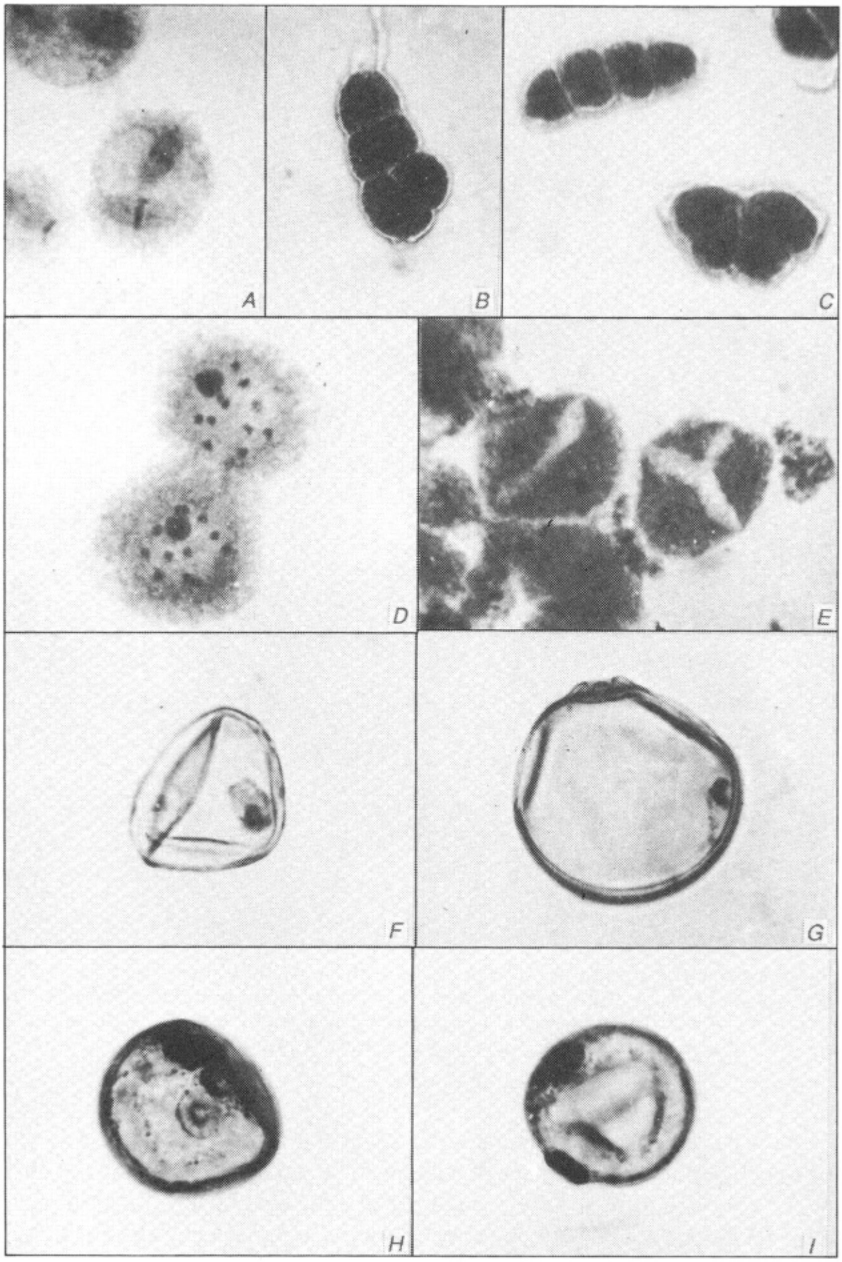
Uninucleate abortive types

Pollen abortion occurs mainly at the uninucleate stage. Empty pollens that look extremely irregular are formed. The male sterile lines of wild abortive (WA) types are typical (Fig. 2).



1. Photomicrographs of pollen-free abortion showing abnormal chromosome behavior; *A,* pollen mother cells vary in size and shape; *B,* chromosomes fail to pair at the meiosis stage (*a*), dividing into three poles *(b); C* and *D,* cells fail to form normal nuclear plates at metaphase I; *E*, the cells form two trivalents (arrows); and *F,* a tetravalent probably formed by two bivalents (arrow).

Material from the early generation (B_1F_1-B_3F_1) and advanced generation ($B_{15}F_1$-$B_{17}F_1$) of male sterile lines of wild abortive type primary material and wild abortive type Er-Jiu-Nan 1 were analyzed in 1972 and 1978 (Hunan Teachers' College 1972,1978). The WA type primary material is remarkably abnormal. A pair of chromosomes is unable to form a bivalent in some cells at the diakinesis of the primary meiosis, but is able to form a trivalent with two other bivalents. In some cases, two bivalents integrate to form a tetravalent. Most cells fail to form normal nuclear plates at metaphase 1. Chromosome arrangement is loose and disordered. A pair of homologous chromosomes may lead or lag at anaphase I. The two division phases of some dyads are not parallel. Some dyads take the shape of a T to form tetrads, others link in a straight line.



2. Photomicrographs of uninucleate abortive types in primary material of WA type: *A,* two division phases of some dyads are not parallel at the second meiotic division; *B* and *C,* abnormal tetrads; *D,* pollen cells cohere in Er-Jiu-Nan 1 A (B_3F_1); *E,* triads formed by abnormal meiosis in Er-Jiu-Nan 1 A (B_3F_1); *F* and *G,* pollen of Er-Jiu-Nan 1 A abort at the uninucleate stage; *H* and *I,* pollen of V20 A abort at the uninucleate stage.

The early generation material of WA male sterile lines has proved to be more abortive at the prophase. But in B_3F_1, reduction divisions tend to be normal. Most pollen grains abort at the uninucleate stage, but when they are at an advanced generation, they appear to be similar to the uninucleate abortive type.

Most pollen aborts before or after the second contraction stage, with three patterns of protoplasmic degeneration:

1. The nucleolus divides into two or three parts, which transfer to the border when the nuclear envelopes begin to dissolve. This causes the nuclear contents to decentralize. The whole protoplasm turns into different sized particles, which spread over the pollen grains.
2. The whole protoplasm appears to be withered and wrinkled, with the cell nucleus enveloped inside. The nuclear membrane begins to dissolve, then the nucleolus, and finally the whole protoplasm.
3. The cell nucleus adheres to the pollen wall. First the nuclear membrane dissolves, then the nucleolus becomes smaller and smaller as the nuclear contents slowly reduce. Now the nucleolus has reduced ability for staining. The mass of protoplasm attached to the pollen wall gets thinner and thinner until it disappears. As a result, the pollen grains are empty and irregularly shaped.

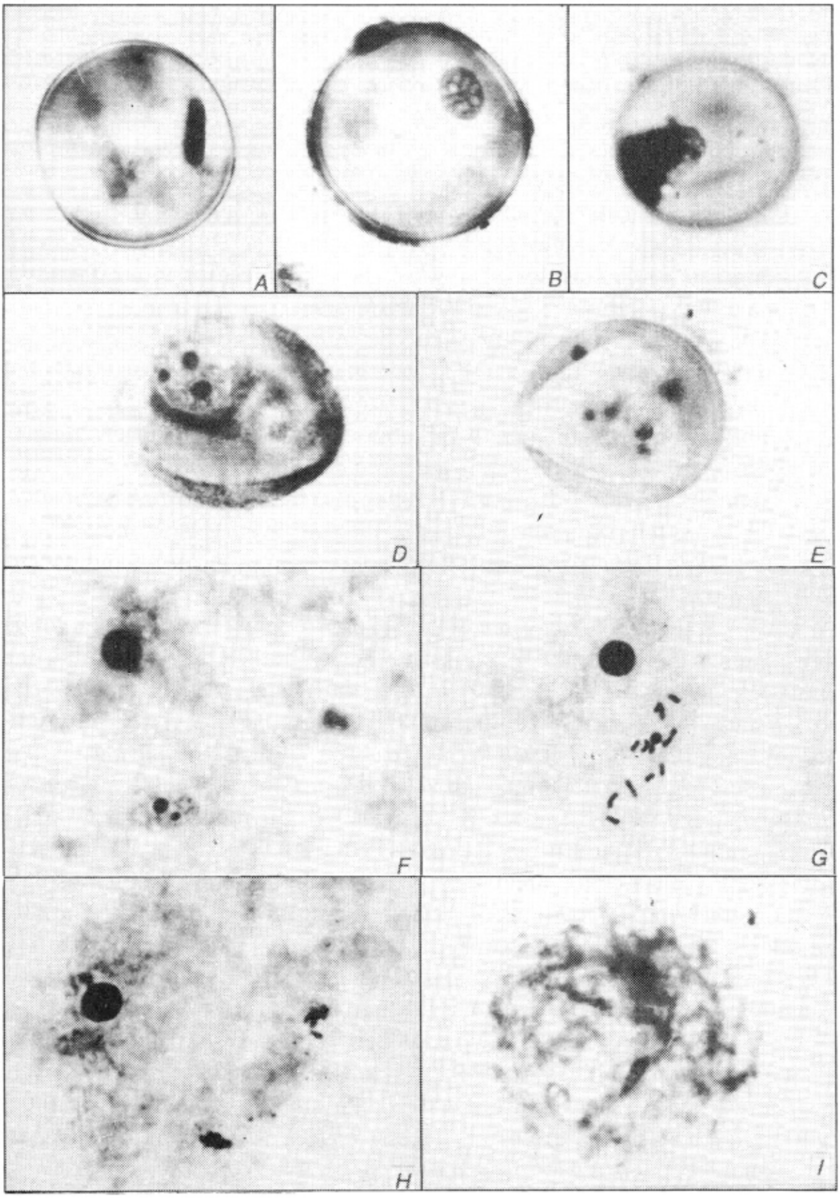
Chiang et al (1981) discovered that in V20 A, used in rice cultivation for years, most pollen grains do not abort until the binucleate stage. Only 26% abort at the uninucleate stage, but 96.2% abort at the trinucleate stage. In studying pollen abortion in V20 A offspring of anther culture, Rao et a1 (1983) found a V20 A pollen abortion rate of 96.7% at the uninucleate stage and of 99% at the binucleate stage. At the trinucleate stage, pollen in the anthers were vacuous with 100% abortion.

Biological genetics researchers at Sun Yat Sen University, Kwangchou, discovered that a few pollen mother cells of Er-Jiu-Ai 4 A had abnormal meiosis; only some of the abnormal microspores resulting from abnormal meiosis develop into mononucleate pollen grains of different sizes. Most of them abort at the late uninucleate stage. The pollen grains of Zhen-Shan 97 A cohere to form several masses on the anthers at the late uninucleate stage. It is difficult to make the pollen grains dehisce out of the anthers; most adhere to tapetal cells. At the junction where cellular walls are fused, degeneration of pollen walls (including intines and exines) can be seen. Thicker intines and exines are observed where cellular walls are not fused. A few pollen grains are fragmentary and thin and the germination aperture looks vague. The pollen grains are mostly empty. A few contain degenerated micronuclei and nucleoli, but their nuclear membranes collapse.

Binucleate abortive types

Most pollen grains in male sterile lines of the binuclear abortive type pass through the uninucleate stage; their reproductive nuclei and nutritive nuclei do not collapse until they have entered the binucleate stage. The Hong-Lien-type male sterile line is typical (Fig. 3A to 3E).

Studies of five generations (B_2F_1-B_7F_1) of wild rice with red awns/ Lian-Tang-Zao (Wuhan University 1977) show that abortive pollen grains are mostly spherical.



3. Photomicrographs of binucleate (*A* through *E*) and trinucleate (*F* through *I*) abortive types: *A* and *B,* pollen of Hong-Lien A ($B_{26}F_1$) abort at the binucleate stage, the nucleoli become deformed and vacuolated; *C* and *D,* pollen of Hong-Lien Hua-Ai 15 A abort at the binucleate stage, their nuclear contents leak into the cytoplasm tending to form chromatin masses, the nucleolus divides into three nucleoli while the reproductive nucleus collapses and dissolves; *E,* pollen of Hong-Lien 1024 A abort at the binucleate stage, the nucleolus turns into several small nucleoli; *F,* pollen of Hong-Lien A ($B_{26}F_1$) abort at the trinucleate stage, the male nuclei formed vary in shape and size with the large one having two nucleoli and the small one a single nucleolus; *G,* in BT type Nong-Zhin 2 A, 12 chromosomes are seen in the prophase of reproductive karyokinesis; *H,* in Nong-Zhin 2 A, several chromatin grains are thrown out at anaphase of reproductive karyokinesis; *I,* abnormal male nuclei (sperm) are formed in the trinucleate pollen of Tianfu type Zeyechin A.

Only a few appear to be irregular. When they are stained with KI, 98.2% do not turn blue. Pollen abortion occurs mainly at the anaphase of the uninucleate stage. Only a few pollen abort at the binucleate stage. In abnormal conditions during meiosis, part of the pollen mother cells vacuolize. Cell nuclei are damaged and some pollen mother cells do not have marked cell walls. Some cells form protoplasmic masses. Two or three pollen mother cells connect at their nucleoli in an irregular manner. Multinucleolar phenomenon is found in some pollen mother cells. Prophase I chromosomes are haphazardly arranged on the equatorial plate. Lag chromosomes are seen at anaphase I and telophase I. The chromosomes and micronuclei outside the nucleus are visible when the dyads form and the tetrads separate. The pollen grains produced vary in size.

At the uninucleate stage, pollen cell walls are thin and wrinkled. Sometimes pollen protoplasm contracts, the nuclear membrane disappears, and the nuclear contents permeate into cytoplasm and concentrate to form masses similar to plasmolysis. After that, the protoplasm degenerates. Some nuclear contents change from thick to thin, into reticulated substances, and then collapse, resulting in empty pollen.

Chiang et al (1981) studied Hong-Lien male sterile line ($B_{26}F_1$). They discovered that 80% of the pollen grains abort at the binucleate stage and 12.8% at the uninucleate stage. Nucleoli become deformed when binuclear pollen abortion takes place. Subsequently, the nuclear membrane collapses and the nuclear contents leak out. Some nucleoli start to sprout, turning into many micronucleoli, while the nuclear membrane dissolves and the nuclear contents spread over the cytoplasm and collapse. Some reproductive nuclei collapse before the nutritive nuclei, then abort after the nutritive nuclei become deformed. As a result, spherical empty pollen grains are formed.

In Hong-Lien (HL) type Hua-Ai 15 A, B_8F_1 and B_9F_1, most pollen abort at the binucleate stage (Xu 1979). The reproductive nucleus is the first to collapse. A lot of chromatin grains appear around the nucleus and tend to form chromatin masses. Then the nutritive nucleus collapses and turns into chromatin masses. These masses are gradually absorbed. Meanwhile, the cytoplasm collapses. Finally, only one spherical, empty pollen with a sprouting aperture remains.

Observations of HL 1024 A (Rao et al 1985) show pollen abortion is 80% at the binucleate stage and 95% at the early trinucleate stage.

When the nutritive nucleus is drawn to the reproductive nucleus, the reproductive nucleus and the nutritive nucleus collapse and dissolve. In some cases, the two nuclear membranes mix. As the nuclear contents cohere, it is impossible to distinguish the nutritive nucleus from the reproductive nucleus. Then the nuclear membrane collapses, causing cytoplasmic dissolution. About 5% of the trinuclear pollen grains contain male nuclei of different shapes and sizes that are abnormally located. In some pollen grains, two sperm merge. All these pollen grains are abortive.

Trinucleate abortive types

The male sterile Chinsurah Boro II (BT) type is typical (Fig. 3*F* to 3*H*). Observations of BT Taichung 65 A (Sun Yat Sen University 1976) found no distinguishable abnormality in pollen abortion before the trinucleate stage. At the binucleate or trinucleate stage, size of reproductive nucleoli is reduced in only a few cells. Some nutritive nuclei degenerate at the trinucleate stage, with reduced nucleoli size, giving rise to dissolved nuclear membrane. Only a few micropollen grains remain intact.

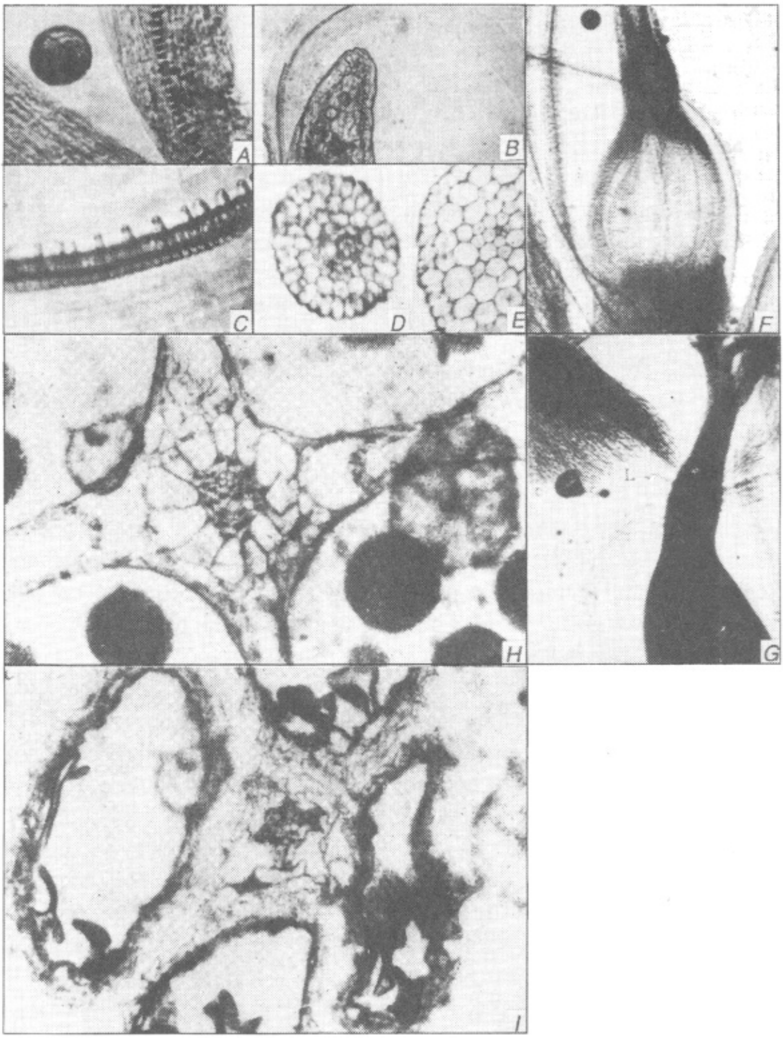
At the trinucleate stage, 88% of the pollen of Nong-Zhin 2 A and 93% of the pollen of Fu-You 1 A remain intact (Teng 1982). The pollen abortion pattern is: At the anaphase of reproductive karyokinesis in Nong-Zhin 2 A, several microscopic chromatin grains are thrown out. These disappear, with an abnormal division phasal rate of 39-59% of the conventional division rate. In Fu-You 1 A, many micronucleoli form after some pollen reproductive nucleoli have sprouted at the anaphase of the binucleate stage. They scatter in the cytoplasm and gradually dissolve. At the trinucleate stage, some pollen nutritive nuclei possess two equal-sized nucleoli each. Some nucleoli sprout for growth and some male pollen nuclei vary in shape and size. These phenomena are rare in maintainer lines.

In Zeyechin A transferred from Tianjitu type, pollen development is not abnormal before the trinucleate stage (Fig. 3*I*) (Rao et al 1983). Abortion occurs mainly at the anaphase of reproductive karyokinesis, when movement of chromosomes toward the two poles is loose. Tiny chromosome grains are usually thrown out. In some cases, some lag chromosomes are visible. At the metaphase, some chromosomes remain stagnant for a long time. Abnormal male nuclei form at abnormal locations. Some of these abnormal sperm collapse and dissolve during development.

One or two male sterile lines are hard to classify. One very specific abortive pattern is of Tienrei 409 A (Pan et al 1982). It is characterized by an unsynchronized fission process. Different stages of the division phase are visible in the same anther, exerting direct influence on the synchronization of pollen abortion. In the same anther cell, vacuous, uninuclear, binuclear, spherical, half-filled, and filled pollen grains have been found. Filled spherical fertile pollen grains make up 21.68%, but unfilled pollen grains are as high as 97.48%. The anther fission rate in male sterile lines is only 66.67%; the pollen sprouting rate is 18.03%. Some pollen grains may sprout, but only abnormally. Two hours after the flower blooms, the anther begins to dehisce. Nearly all the pollen grains are infertile because of the comprehensive effects.

Histological studies of stamen

Pollen abortion and the development of filaments and connective vascular bundles. Male sterile lines of the wild abortive type and pollen-free abortive types show completely degenerated filament vessels (tracheae) (Pan 1979). The degree of degeneration is related to the backcross filial generations. Generally, more degeneration occurs first in the middle part of the filament in B_1F_1, in half of the B_2F_1 filaments, and in most of the B_3F_1 filaments. The level of degeneration is directly proportionate to the number of fertile and abortive pollen grains in the anther cells of the related stamen. Normal and abnormal development of filaments and connective vascular bundles of wild abortive and pollen-free types are shown in Figure 4.



4. Photomicrographs of the development of filaments and connective vascular bundles; *A,* filament vessels of Zhen-Zhu-Ai; *B,* broken and discontinuous filament vessels of WA type Er-Jiu-Nan A (B_2F_1); *C,* filament vessels of wild rice; *D,* filament vessels of Zhen-Zhu-Ai (transverse); *E,* filament vessels of Zhen-Zhu-Ai A (transverse); *F,* filaments of cultivated Zhen-Zhu-Ai with normally developed connective tissues; *G,* degeneration in conducting tissues of filaments of WA type Zhen-Zhu-Ai A; *H,* vascular bundle sheath cells are large, round, and orderly when connective vascular bundles of Er-Jiu-Ai Bare well developed at the trinucleate stage; *I,* vascular bundle sheath cells of WA type Er-Jiu-Ai A are degenerate and disordered when vascular bundles are poorly developed at the uninucleate stage.

In HL Hua-Ai 15 A, fusion occurs at the base, where glumal flowers are close to each other (Xu 1980,1984). The filaments fuse in two's or three's. No abnormality can be seen in the development of conducting tissues in the filaments. Abnormal development takes place in the connective vascular bundles. Before the onset of pollen abortion, the vessels of the connective vascular bundles develop poorly, leaving an enlarged annular space. The annular lines look sparse, broken, and scattered. In the middle part of the connective, only one vessel is poorly developed and incapable of differentiation. In the upper part, great differences in number, width, and annular space exist.

The tracheary cells in the different anther cells also differentiate poorly. The vessels extend abnormally. Vessel cells are damaged and cannot enlarge or thicken. The vessel cavity is damaged. The junctions at which the vessel cells meet become disconnected. The vessel cells grow fibrillous, sometimes thicker, sometimes thinner, with loose connectives, disorderly arrangement, and degenerated function. Frequently the connective vascular bundles in WA Hua-Ai 15 A develop poorly. The thin-walled cells are also disorderly. Some may be suspended or lost.

In WA Er-Jiu-Nan 1 A connective vascular bundles are underdeveloped (Hunan Teachers' College 1975). Only one bundle of tracheid contains two or three cells, with two or three sieve cells around it. The vascular bundle sheath cells are large.

Researchers at Sun Yat Sen University (1976) found that the connective vascular bundles in CMS lines of WA type degenerate at the uninucleate and at the binucleate stages, and their cells wrinkle and contract. The phloem and the xylem do not differentiate well. The vascular bundle sheath is not highly visible because its cells have degenerated and are disordered. At the early stage of pollen abortion, the vascular bundles do not degenerate much. But when remnant pollens are seen, the vascular bundle sheath is extremely degenerated. The connective vascular bundles in BT Taichung 65 A have poorer development than the maintainer lines. The inner vascular bundle cells and the vascular bundle sheath cells are not well arranged. The phloem and the xylem are visible and pollen abort rather late.

Tapetal development and pollen abortion. In WA Er-Jiu-Nan 1, uninucleate pollen grains draw close to the tapetum, resulting in the formation of a big vacuole in the center of the anther cell and the diffusion of tapetal nuclear contents into the cytoplasm (Hunan Teachers' College 1975). The tapetum degenerates with the pollen.

In Kwang-Shian 3 A, WP type, when pollen comes to the trinucleate stage, tapetal cells and their nucleoli remain intact; pollen development remains at a certain stage to abortion (Kwangxi Teachers' College 1975). Xu's (1979) observations of HL Hua-Ai 15 A show that the abnormal proliferation of tapetal cells causes tapetal periplasmodia to form, pushing pollen mother cells to the center of the anther cells, ending their dissolution. In some anthers of WA Hua-Ai 15 A, the fibrocytes of the dermal layer suddenly become larger and deformed in a radial manner, damaging the tapetum and pushing the cells toward the center of the anther cells to make the tapetum dissolve rapidly. Pollen abortion results.

In Er-Jiu-Nan and other male sterile lines of WA type, vacuolization of the cells in intercellular layers at the uninucleate stage damaged tapetal cells (Pan 1979, Pan and He 1981). Their disappearance brings about pollen abortion. Radial thickness and vacuolization of the intercellular cells along the anther increase, pushing tapetal cells toward the center of the anther cell. The anther cell appears to be distinctively thin with many vacuoles. The tapetal cells are damaged and disappear, followed by uninucleate pollen abortion in the anther. With the growth of anthers, the middle lamella cells grow larger, but the radial thickness of the cells does not increase and the cells remain withered. In traverse section, the cells are no longer square but appear to be oblong, with visible nuclei. At this moment, the secondary tapetal walls on the intercellular cells can be easily seen.

At the early uninucleate stage in sterile material of pollen-free types such as Type 424, a layer of ubisch body membrane, consisting of exceptionally large, closely arranged ubisch bodies (0.8-0.9 µm diam), appears on the tapetal cells. These abnormal ubisch bodies cannot be used to form pollen exines by microspores, which remain in a one-wall state. At the binucleate stage, the other cellular components also cease to develop. The unused materials of the ubisch bodies do not dissolve, but adhere to the tapetal cytoplasmic membranes and deposit outside the intercellular cells, which have been vacuolatedly enlarged. As a result, an exceptionally thickened layer of secondary tapetal wall is formed.

Dehiscent structure in anthers and MS lines (system). Studies by Chou (1978) and Pan and He (1981) disclosed the dehiscent structure and mechanisms of rice anthers as well as abnormal dehiscent structure of anthers in CMS lines. In WA Bo-Li-Zhan-Ai A, the anther “spring”is not strong enough, so the dehiscent cavity is not often formed or is formed on only one side. The pulling force of the “spring” is too weak to open the dehiscent cavity, causing the anthers to fail to dehisce. Although a strong “spring” may be formed in an anther of WA Nan-Tai 13 A, no dehiscent cavity is formed on either side of the anther. The pulling force of its “spring” is not strong enough to draw open the anther cell to bring about dehiscence. Although a strong “spring” may be formed in BT Li-Ming A, no cavity can take shape in the anther. The cavity may be on one side or on both sides.

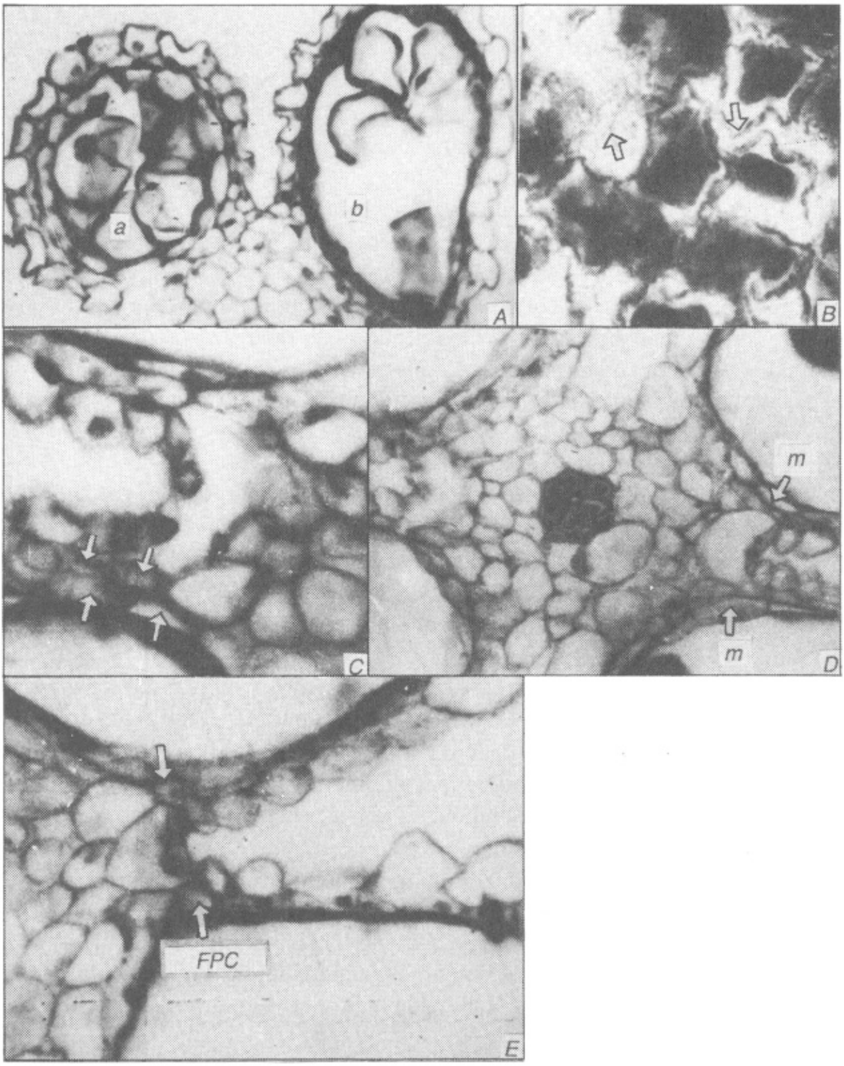
WA Er-Jiu-Ai A is characterized by an absence of splitters and dehiscent cavities, poor differentiation, and no anther dehiscence. In Zhen-Shan 97 A, the dehiscent cavity poorly differentiates, with two or three layers of cells on both sides. Its anthers may dehisce, but with great difficulty. Male sterile lines of WA type have an enlarged vacuolation in the middle lamella cells of anther walls. In some stamens, a certain degree of hypertrophy exists even at blooming, causing more difficulty to anther dehiscence.

The abnormal conditions of anther dehiscence in WA male sterile often affect those of the F_1 hybrid. For example, in some anthers of Nan-You 2, vacuolation occurs in the middle lamella cells on both sides of the dehiscent cavity. Other anthers have two layers of cells on both sides. Ai-You 2 has fibrous parenchyma cells on both sides of the anther. These cells do not wither, but raise the degree of hypertrophy in vacuolation, so that resistance is added to anther dehiscence.

Some normal and abnormal anther structures are shown in Figure 5.

Summary and discussion

Pollen abortion, a key characteristic of male sterility in rice, varies in pattern and phase. Abortive pollen can be observed at various stages of pollen development in



5. Anther structures in some male-sterile lines: *A,* uninucleate pollen is about to abort, intercellular layer is vacuolized and large, tapetum has disappeared (*a*), normal development of uninucleate pollen grains and intercellular cells with tapetal cells dissolved normally (*b*); *B,* ubisch bodies and ubisch body membrane (arrows) of pollen free type 131 A; *C,* in Zhen-Shan 97 A, splitters and fracture cavities have differentiated, but two or three layers of cells lie on both sides of the fracture cavity (arrow); *D,* anther of hybrid Nan-You 2 with vacuolized cells in the middle lamella on both sides of the fracture cavity (*m*); *E,* anther of hybrid Ai-You 2 with fibrous parenchyma cells (*FPC*) on both sides of the fracture cavity, cells do not wither but increase the degree of hypertrophy in vacuolation.

male sterile lines. Statistical analysis shows that a particular line will abort at a particular nucleate stage. Pollen abortion can be classified into four types: pollen-free, uninucleate abortive, binucleate abortive, and trinucleate abortive.

Improved male sterile lines are all pollen abortive types. On the whole, they correspond to restorer and maintainer lines: WA type—uninucleate abortive, HL type-binucleate abortive, and BT type—trinucleate abortive. In genetic characteristics, the interaction between cytoplasm and nucleus in male sterility is: sporophytic male sterile system—uninucleate abortive; gametophytic system—binucleate and trinucleate abortive.

The patterns of pollen abortion are related to the affinity of the hybrid parents. If parents are distantly related, abortion will occur at an earlier stage. For example, Er-Jiu-Nan 1 belongs to the Ai-Jiao-Nan-Te system; Zhen-Shan 97 belongs to the Ai-Zhi-Zhan system. Ai-Jiao-Nan-Te originated in the Yangtse Valley; its morphological characteristics are different from the WA type. Ai-Zhi-Zhan originated in Southeast Asia; its morphological characteristics are similar to the WA type. Male sterile lines, such as Er-Jiu-Nan 1, are characterized by pollen abortion at an early nucleate stage. But Zhen-Shan 97 aborts rather late.

Similarly, the relation between indica rice and sinica rice is closer than that between wild rice and cultivated rice. The male sterile system, developed by crossing an indica rice and a sinica rice, mostly aborts at the binucleate and trinucleate stages. The development of filaments and connective vascular bundles is normal in the male sterile lines of some crops, such as wheat. But the filaments and connective vascular bundles in male sterile lines of wild abortive-type rice have degenerated. The degree of abnormal development is low in the vascular bundles of other male sterile lines, which might explain why there are several abortive pollen.

Tapetum cells provide nourishment for the developing pollen. Abnormal conditions can be found in the tapetal layers of pollen-free types, uninucleate abortive types, and binucleate abortive types. Accelerating or delaying degeneration directly influences pollen development. But the male sterile tapetum of trinucleate abortive types is similar to that of the maintainer system, causing little difference in the morphological character of their pollen. The only possible identification is to distinguish their reproductive division processes at the trinucleate stage. Because the anther wall development is abnormal in male sterile rice lines, these abnormal conditions can be found in the dehiscent structure of the anther:

- No differentiation in the splitter and dehiscent cavity.
- No differentiation or poor differentiation in the dehiscent cavity.
- Dehiscent cavity found on only one side.
- Locule “spring” of the anther develops poorly.

These conditions are related to the flowering habits of a male sterile system.

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Notes

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Fertility transformation and genetic behavior of Hubei photoperiod-sensitive genic male sterile rice

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From 1982 to 1985 we studied the stability of fertility and the genetic behavior for Nong-Ken 58 of Hubei photoperiod-sensitive genic male sterile rice (HPGMR) (MSR58 A(B)). Under the daylength in Wuchang, HPGMR possesses a marked fertility-transformation character, which converts a male sterile line to male fertile. The critical period of fertility transformation is 1 or 2 Sep, i.e., the plant is male sterile if heading occurs before 1 or 2 Sep; male fertile if heading occurs after those dates. The pollen of the male sterile period resembles "typical aborted type" in the late monokaryotic stage: the rate of male steriles is 100% in the population; degree of male sterility is 99.8-100%. On the contrary, in the fertile stage, the percentage of fertile plants is 100%; the degree of spikelet fertility is 10-40%. Fertility transformation is not affected by seed storage or by planting in another region. The sterility of the sterile stage is stable, but the degree of fertility is unstable in the fertile stage. Degree of fertility varies by region, and in different years at the same place, and at different heading stages of the same fertility stage at the same location. HPGMR fertility is probably controlled by a pair of recessive major genes in the mesoplast, which are also modified by minor genes. Preliminary investigation indicates the potential of using the fertility genes of HPGMR in rice breeding.

HPGMR was discovered in a field of Nong-Ken 58, a strain of single late rice, by Shi Mingsong in 1973. Its original male sterile parent is japonica. It appears male sterile when the plants are heading under long daylength, and male fertile under short daylength. Further research proved that daylength is critical to fertility transformation, i.e., the degree of male sterility is 99-100% at heading under artificial light of more than 14 h; but male fertile at heading under artificial light less than 13 h 45 min. The sensitive stage of fertility transformation, subsequently induced by the length of light, is from the first and second branches during primordial division until the pollen mother cell formation stage. We began studying this material in 1982, exploring the possibility of using it in rice breeding.

Materials and methods

Using the initial plant MSR58 A(B), we selected plants with 100% sterile pollens; selfed seeds were also harvested from fertile plants whose panicles had been covered with transparent bags.

Fertility was identified by staining the pollens with I-KI solution and examining them under the microscope, and by determining spikelet fertility.

MSR58 A(B) was crossed (with partial reciprocal crosses) with indica and japonica varieties (strains), to investigate the fertility of F_1. MSR58 A(B) was also topcrossed or backcrossed with the F_1 of japonica varieties. We compared the segregation condition of fertility of the F_1 and the topcross or backcross F_1, and identified the male sterile plants of the segregative generation.

Experimental design, results, and analysis

Fertility-transformation stage of MSR58 A(B) under natural daylight

MSR58 A(B) was sown successively on 20 Apr, 5 May, 20 May, 5 Jun, and 20 Jun in 1983 and 1984 in Wuchang (30°27'N). On each date 40-60 seedlings were individually transplanted with heavy fertilization to promote tillering. The planting dates were separated by 15 d so that the late heading stage of one planting would coincide with initial heading of the next. At initial heading (emergence of the panicle 1 or 2 cm out of the flag leaf sheath) we marked the tops of the heads of 10-15 plants with paper labels.

On the same day we took 5-10 anthers from spikelets at the tops of the panicles and fixed them in 3: 1 glacial alcohol and then stained them with I-KI solution. We examined the pollen under the microscope and counted sterile and fertile pollens in two fields of vision. At the same time, all single heads of the initial heading were covered with Na_2SO_4 paper bags to investigate the filled spikelet percentage after maturity.

MSR58 A(B) appeared fertility-transforming from sterile to fertile in both years. The rate of pollen sterility was 99.5-100% before 1 Sep (Table 1). From 2 to 5 Sep 1983, it dropped from 98.3 to 74.5%, dropping gradually thereafter to 20% after 6 Sep. The degree of fertility increased gradually to about 40%. Based on the rate of sterile pollens, the initial stage of fertility transformation of MSR58 A(B) came about 2 Sep. Based on filled spikelet percentage, the initial stage of fertility transformation was about 4 or 5 Sep. We used pollen stainability to identify fertility and obtained more reliable results than with filled spikelet percentage on the bagged panicles.

Fertility stability of MSR58 A(B)

Sterility and fertility of male fertile stage. We sowed MSR58 A(B) on 13 May 1983, 1 May 1984, and 18 Apr 1985 in Wuchang and individually transplanted 500-1,000 plants. We identified sterile and fertile plants by the morphology of pollens from heading to flowering of the male sterile stage and the male fertile stage of the ratoon rice. After the ratoon rice matured, we investigated the degree of sterility and the degree of fertility by microscopic examination of the pollen grains.

Results

In all 3 yr the rate of sterile plants in the field at the sterile stage was 100%; the rate of typical abortive pollens was higher than 93.1%. Because both the rate and the degree

Table 1. Fertility appearance of MSR58 A (B) at various heading Stages in 1983 and 1984, Wuchang, China.

Initial heading date	Sterile pollens (%)[a]		Seed-setting percentage	
	1983	1984	1983	1984
August				
5	100	–	0	–
7	99.5	–	0	–
9	100	–	0	–
10	100	–	0	–
11	100	–	0	–
13	100	100	0	0
14	100	–	0	–
15	-	100	–	0
16	99.7	–	0	–
17	-	100	–	0
19	100	99.7	0	0
21	100	100	0	0
23	100	100	0	0
24	100	–	0.1	–
25	–	100	–	0
26	100	–	0	–
27	–	100	–	0
29	100	–	0	–
30	100	99.5	0	0
31	99.5	100	0.2	0
September				
1	100	100	0	0
2	89.0	98.3	0	0.3
3	86.5	98.5	0.5	0
4	74.5	85.4	2.1	0
5	74.7	75.5	6.5	3.0
6	57.2	60.4	8.0	7.5
7	–	45.0	–	19.9
8	24.5	33.2	21.7	31.4
9	22.6	–	30.4	–
10	–	21.5	–	29.4
11	21.3	–	34.5	–
13	–	19.9	–	41.7
14	18.9	–	41.3	–
15	–	15.2	–	51.3
17	20.2	18.6	40.2	38.9
20	20.4	–	37.7	–
21	–	20.3	–	37.7

[a] Typical abortive pollens in the late monokaryotic stage were counted as sterile pollens.

of sterile plants were 100%, the sterility of the MSR58 A(B) population in the sterile stage matched the Chinese seed production standard.

The results in Tables 2 and 3 indicate a rate of stained pollens in the fertile stage above 50.1%, concentrated in a range of 75.1-90%. When the rate of stained pollens reached 50%, the filled spikelet percentage was normal according to previous research (Li 1984). Although the rate of stained pollens of MSR58 A(B) was above

Table 2. Distribution of stained pollen of MSR58 A (B) in the fertile stage.

Sampling date	Total plants (no.)	Stained pollen rate (%)									
		95.1 to 100	90.1 to 95.0	85.1 to 90.0	80.1 to 85.0	75.1 to 80.0	70.1 to 75.0	65.1 to 70.0	60.1 to 65.0	55.1 to 60.0	50.1 to 55.0
13 Sep 1983	96	3	4	25	36	22	2	2	0	1	1
12 Sep 1984	99	0	8	21	39	24	1	4	0	1	1
13 Sep 1985	71	1	2	16	27	17	1	5	2	0	0

Table 3. Fertility appearance of MSR58 A (B) ratoon population in the fertile stage.

Total plants (no.)	Fertility (%)	Natural seed-setting percentage								Initial heading date
		<10	10.1 to 20.0	20.1 to 30.0	30.1 to 40.0	40.1 to 50.0	50.1 to 60.0	60.1 to 70.0	Total plants (no.)	
511	100	8	21	25	16	16	4	0	101	10 Sep 1983
588	100	11	27	31	23	14	9	0	115	8 Sep 1984
494	100	6	18	13	12	12	6	1	68	12 Sep 1985

50% in 3 successive yr, the highest filled spikelet percentages of single plants were only 57.7, 56.6, and 68.9%. According to our investigation, male fertility of MSR58 A(B) in Lingshui, Hainan (18°30’N) averaged 50.9% in 1983 (complete heading 13 Mar); 79.2% in 1984 (complete heading 19 Mar), and 83.5% in 1985 (complete heading 12 Mar). The highest filled spikelet percentages for single plants were 59.5% in 1983, 96.5% in 1984, and 88.5% in 1985. In Lingshui, Hainan, the filled spikelet percentage in mid-March 1983 generally approximated the normal level. Therefore, we conclude that the degree of male fertility was unstable—mainly among regions, secondarily among single plants, and among heading stages (as shown in Table 1).

Effect of seed storage and cultivation on fertility transformation. In autumn 1982, we divided MSR58 A(B) selfed seeds harvested in the fertile stage into two groups. Half the seeds were stored in a desiccator containing $CaCl_2$; the others were planted in Hainan the same winter. The daylength of less than 12 h in March in Lingshui, Hainan, forced heading and flowering in isolation. We harvested the selfed seeds and similarly divided them into two groups, preserving one-half in the desiccator and isolating and reproducing the other under the short daylength in the fall in Wuchang. Seeds produced each season were treated with this method. Each seed from the various treatments was sown and each plant was observed for the fertility-transformation character. We also compared them with the isolated reproductive seeds (ratooned from the stubble of MSR58 A(B)) that had been preserved in the greenhouse in successive years as the check. The rate of sterile plants in all field treatments was measured at heading in the male sterile period, taking anthers of 10 single plants at random and examining the pollen grains under the microscope. Beginning 31 Aug, we randomly sampled at intervals about 5 spikelets from the top 1 or 2 panicles of 5 single plants. We then stained the pollen grains with I-KI solution and examined them under the microscope.

The results in Table 4 show a sterility rate of 100% for all treatments in the sterile stage, and a 99.9-100% rate of sterile pollens. All treatments had conspicuous fertility-transformation character. Because treatments were synchronized with the check treatment, we concluded that storage and planting at various sites did not affect fertility or the fertility-transformation character of MSR58 A(B).

Outcrossing in fertile stage. On 20 Jun 1983 in Wuchang, selfed seeds of MSR58 A(B) that had been harvested from isolated sites were sown and transplanted into four plastic pots. After initial heading on 7 Sep, two pots were placed where they could receive pollen from neighboring plants. The other two pots were returned to the isolated site. We then determined the filled spikelet percentage of the whole plants during maturity and preserved the original seeds.

MSR58 A(B) from the same source was sown on 7 Dec 1984 in Lingshui, Hainan, with half the seedlings transplanted in the isolated site for heading and

Table 4. Effect of seed storage and planting site on fertility transformation.[a]

Seed source[b]	Sterile pollen (%) at various initial heading dates											
	August			September								
	17	24	31	1	2	3	5	7	9	10	13	15
Wuchang 1984												
Wuchang Aut 1982	–	100	100	–	99.5	90.5	77.7	42.5	–	17.5	15.5	18.7
Lingshui Spr 1983	–	100	100	–	99.0	88.7	71.8	48.3	–	21.2	20.0	20.5
Wuchang Aut 1983	–	99.9	100	–	96.5	84.5	69.5	45.6	–	15.5	14.5	19.0
Lingshui Spr 1984	–	100	100	–	94.5	90.5	72.5	50.7	–	19.0	20.5	12.5
Check	–	100	100	–	95.0	89.5	75.2	51.0	–	22.5	13.5	18.6
Wuchang 1985												
Wuchang Aut 1982	100	–	100	100	–	98.5	80.5	45.5	21.5	–	18.5	21.5
Lingshui Spr 1983	100	–	99.9	99.9	–	99.0	83.6	45.5	24.6	–	20.0	14.0
Wuchang Aut 1983	100	–	100	100	–	89.5	79.3	42.6	19.5	–	20.5	15.0
Lingshui Spr 1984	100	–	100	99.9	–	97.5	76.7	40.5	23.0	–	17.5	17.5
Wuchang Aut 1984	100	–	100	100	–	97.5	85.4	41.5	25.5	–	20.0	15.5
Lingshui Spr 1985	100	–	100	100	–	99.0	80.5	50.0	18.5	–	15.5	15.5
Check	100	–	100	100	–	96.5	78.5	46.5	20.7	–	14.3	15.0

[a]100% of the sterile population was in the sterile stage on sampling dates; [b]Aut = autumn, spr = spring.

flowering and the other half transplanted in a selected field plot to receive pollen by natural pollination. We determined filled spikelet percentage during maturity and preserved the seeds. Seeds of the two treatments were sown in Wuchang in 1985. We individually transplanted about 500 single seedlings, and investigated the purity of plant populations after complete heading in the field.

The results in Table 5 indicate MSR58 A(B) has certain outcrossing ability in the fertile stage. Although fertility reached 87.2% of normal, outcrossing was 0.6%. As fertility degree declines, outcrossing percentage increases. These results suggest that when the male sterile line was reproduced in the spring in Hainan, it should not have been planted in an isolated plot. It should have been isolated when it was grown in the fall in Wuchang.

Genetic behavior of HPGMR male sterility

Fertility of F_1 hybrid with the normal variety. We investigated the F_1 of MSR58 A(B) crossed with 97 indica and japonica varieties or lines (partial reciprocal). We used the partial combination of F_1 for sowing and transplanting at intervals and for ratooning, investigating the rate of spikelet fertility of single panicles visible before and after 5 Sep. The results are shown in Tables 6 and 7.

Although the filled spikelet percentage of F_1 hybrid combinations of MSR58 A(B) with various varieties differed significantly, all had various degrees of spikelet fertility. The filled spikelet percentage of japonica F_1 hybrids was higher than that of indicas. The filled spikelet percentage of reciprocal F_1 crosses did not differ significantly, showing that filled spikelet percentage was not affected by cytoplasm. All reciprocal F_1 crosses headed in the sterile stage before 5 Sep or headed in the fertile stage after 5 Sep in Wuchang. This indicates that daylength could not induce the fertility factor of the heterozygous stage. Perhaps the fertility of MSR58 A(B) is recessive nuclear hereditary.

Table 5. The outcrossing setting percentage of MSR58 A (B) in the fertile stage.

Seed source	Seed-setting percentage	Plants studied (no.)	Natural hybrids (no.)	Natural hybrids (%)
1983, Wuchang (free)	56.5	503	11	2.2
1983, Wuchang (isolated)	48.7	497	0	0
1985, Lingshui (free)	87.2	485	3	0.6
1985, Lingshui (isolated)	88.5	488	0	0

Table 6. Seed-setting percentage in 97 single F_1 crosses.

Seed-setting percentage	>80.1	70.1 to 80.0	60.1 to 70.0	50.1 to 60.0	40.1 to 50.0	30.1 to 40.0	20.1 to 30.0	10.1 to 20.0	<10.0
Self pollinated	27	31	14	12	9	3	1	0	0
Open pollinated	17	25	23	13	6	7	4	1	1

Table 7. Seed-setting percentage of single cross F_1 MSR58 A (B) with japonica varieties at various heading stages.

Cross	Seed-setting percentage						
	August			September			
	13	22	31	5	1–	15	17
MSR58 A/IRAT1-1	–	77.7	81.4	–	–	–	73.9
IRAT1–1/MSR58 B	–	78.5	77.8	–	–	–	69.5
MSR58 A/M1-1	84.7	88.7	–	77.6	82.3	79.4	–
M1-1/MSR58 B	80.6	85.9	–	80.5	85.7	81.8	–
MSR58 A/Li-Ming	65.6	86.3	–	82.4	80.2	–	84.5
Li-Ming/MSR58 E	87.6	89.6	–	79.9	82.3	–	79.9

Fertility in F_2 and topcross and backcross F_1. We investigated fertility segregation of 18 japonica varieties with MSR58 A(B) F_2 and the topcross and backcross F_1 of partial F_1 with MSR58 A(B) in the sterile stage to identify sterile plants. The results are shown in Tables 8 and 9.

We randomly sampled the sterile plants (determined by the morphological character of anthers in the field), and examined the pollen under the microscope before and after the critical stage of fertility transformation to identify sterile plants. In the field, 71 male sterile plants of the topcross and backcross F_1 were identified; all showed fertility transformation by microscopy, which was 100% reliable. In the field, of 207 sterile plants of F_2, 204 plants (98.6%) showed fertility transformation by microscopy. Therefore, the data listed in Tables 8 and 9 are credible.

Table 8. Fertility segregation in F_2 of MSR58 A (B) japonica variety.

Combination	Total plants (no.)	Fertile plants (no.)	Sterile plants (no.)	c^2 3:1
MSR58 A/R7-1	591	441	150	0.045
MSR58 A/Ai/R66	432	328	104	0.197
MSR58 A/Li-Ming	563	419	144	0.100
Li-Ming/MSR58 B	400	298	102	0.053
MSR58 A/Nong-Hu 26	713	531	182	0.165
Nong-Hu 26/MSR58 B	580	433	147	0.036
MSR58 A/Nong-Ken 58	458	342	116	0.026
MSR58 A/C-cheng 1	501	397	104	4.807
MSR58 A/Milyang 49	771	583	188	0.156
MSR58 A/Fanny	418	321	97	0.717
MSR58 A/M101	504	379	125	0.010
MSR58 A/Han-Gu 4	506	405	101	6.853
MSR58 A/Bei-Zi-Gu	749	601	148	10.969
MSR58 A/japonica	790	589	201	0.082
japonica/MSR58 B	754	570	184	0.143
MSR58 A/19-5	416	317	99	0.320
MSR58 A/62-2	537	400	137	0.075
MSR58 A/IRAT101	519	386	133	0.108

Table 9. Fertility segregation of topcross and backcross F_1.

Combination	Total plants (no.)	Fertile plants (no.)	Sterile plants (no.)	c^2 1:1
MSR58 A/Nong-Hu 26*MSR58 B	20	11	9	0.200
MSR58 A/MSR58 A*Nong-Hu 26	20	12	8	0.800
MSR58 A/MSR58 A*IRAT101	37	18	19	0.027
MSR58 A/MSR58 A*C-cheng 1	26	15	11	0.615
MSR58 A/Li-Ming*MSR58 B	18	10	8	0.222
MSR58 A*Li-Ming/MSR58 B	27	13	14	0.037
MSR58 A*japonica/MSR58 B	9	5	4	0.111
MSR58 A/MSR58 A*Bei-Zi-Gu	20	17	3	9.800
MSR58 A/MSR58 A*R7-1	45	24	21	0.200
MSR58 A/MSR58 A*Nong-Ken 58	18	9	9	0
MSR58 A/Nong-Ken 58*MSR58 B	22	12	10	0.181

The results show the fertility segregation ratios of most combinations of F_2 and topcross and backcross F_1 conformed to the mendelian ratio, and the inheritance was controlled by a pair of genes. In two combinations, MSR58 A/Hangu 4 and MSR58 A/Bei-Zi-Gu, the frequency of sterile plants was less than expected.

In addition to breeding new indica and japonica sensitive nuclear lines, we investigated the fertility appearance of the progeny of selected sterile plants from the F_2 population. The results (Table 10) show that most F_3 progenies exhibited fertility segregation. We further selected male sterile plants from the F_3 progenies and found we could get completely sterile progenies in F_4 (Table 10).

We conclude that the fertility of HPGMR is controlled by a pair of recessive major genes in mesoplast and modifying minor genes. New indica and japonica photoperiod-sensitive sterile lines, homozygous for the fertility transformation gene, were identified among hybrid progenies.

Table 10. Fertility of F_3 and F_4 of two combinations.

Combination	F_3			F_4	
	Progeny designation	Total plants (no.)	Sterile plants (no.)	No. of progenies	No. of fully sterile progenies
MSR58 A/R7-1 B	173	60	42	12	5
	174	60	27	7	7
	175	60	53	10	0
	176	60	23	10	1
	177	60	25	16	2
Ai/R66//MSR58 A/R7-1	179	60	20	19	4
	180	60	31	20	15
	181	60	27	9	2
	182	60	13	8	4

Discussion

Determination of fertility-transformation stage of HPGMR

Daylength is the main factor inducing fertility transformation in HPGMR. The fertility-transformation phenomenon of male sterile to fertile is obvious.

However, initial sterility will vary by 1 or 2 d from year to year as will degree of sterility. Further study is needed to explain these differences. One possible reason is the differences in daylength at different latitudes at the crop growth stage when fertility transformation should be expected. Before extending the technology of HPGMR to different geographical regions, different sowing and transplanting dates should be tried for 2 yr or more to determine the initial stage of fertility transformation under daylengths at various sites.

Stability of fertility in HPGMR

In natural sunlight, HPGMR sterility in the male sterile stage, fertility transformation, and rate of stained pollens in the fertile stage are all stable. However, the degree of fertility, as determined by spikelet fertility, is unstable. Degree of fertility varies by region, and within region with year, heading stage, fertility stage, and by plant within the population. Temperature, humidity, and other factors, in addition to daylength, influence the degree of fertility. The influence of these environmental factors needs further study to determine whether there are differences between the morphological structures of the HPGMR male flower and those of normal rice.

In recent years we have tried to transfer the HPGMR fertility-transformation character to some photoperiod-sensitive indicas to achieve male sterile plants. Under normal temperatures after 5 Sep in Wuchang, their fertility is lower than that of japonica sterile lines at the same heading stage. Apparently, the instability of fertility of indica lines will distinguish them from japonica lines.

We need to study further the structures of male flower organs and the ecological factors influencing fertility. Furthermore, through crossing and selecting, we need to breed indica and japonica photoperiod-sensitive nuclear sterile lines that possess a higher degree of fertility and exhibit adaptability in wider regions. Obviously, that is important for breeding nuclear sterile lines.

Use of HPGMR

Shi (1981) proposed using HPGMR for heterosis. We suggest further ways to use HPGMR in rice breeding.

HPGMR heterosis vs three-line breeding. HPGMR heterosis is superior to three-line heterosis, which is currently used widely.

1. Because sterility can be retained by selfing, only a sterile line and a restorer line are required for hybrid seed production. By simplifying seed propagation and seed production, HPGMR heterosis will reduce cost.
2. Because fertility is controlled by recessive mesoplast genes, HPGMR fertility is not related to cytoplasm. Therefore, HPGMR can be bred into japonica-plasm-nuclear and japonica-plasm-indica-nuclear sterile lines or into indica-plasm-nuclear and indica-plasm-japonica-nuclear sterile lines. The F_1 of these sterile lines, when crossed with all conventional rice varieties, is

theoretically dominant fertile. Thus, the restorer-maintainer relation of the plasm-nuclear interaction for male sterility to restrict the fertility of F_1 can be avoided. Also, free combination not possible with the three-line method can be realized.

3. HPGMR can be bred homotypical to the male sterile line of a particular variety through continuous backcrossing to succeeding generations. It can also be adapted to compound hybridization of multiparents, crossing with two or more nuclear male sterile lines to breed new male sterile lines from each fertility segregative generation. This eliminates the need for the three-line breeding method with its strictly regulated backcrossing of the sterile line in pairs.

HPGMR use, however, does rely on the breeding experience gained from the three-line method. First, we must breed nuclear male sterile lines with good combining ability, optimum flowering, superior grain quality, and wide-spectrum insect and disease resistance. That is the key to heterosis in future hybrid combinations.

HPGMR in recurrent crosses. Because rice is strictly a self-pollinated crop, to combine high yield, multiple resistance, and superior grain quality in one strain, breeders use male sterile lines in recurrent crosses to improve populations. In addition, they constantly search for new germplasm sources (Frey 1982, Khush 1984).

In recent years, IRRI has used the IR36 mutation nuclear sterile line in population improvement. Its use is limited, however, because sterility cannot be maintained. Because HPGMR maintains its sterility by selfing, it is possible to carry on selection in recurrent crosses.

Rice breeders not only can select the desirable genetic recombinant in each recurrent cross, but breed the new variety by pedigree selection. They can select new nuclear sterile lines from each cycle of the same population in the same manner. By combining conventional breeding with heterosis utilization, a new system of rice breeding is possible in China.

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Notes

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Chemical emasculators for hybrid rice

Shao Kaoxiang and Hu Dawen

Chemicals that can sterilize the stamen, with little or no effect on the normal functioning of the pistil, can be used to produce female parents for hybrid rice production. The advantages are obvious, no special development of male sterile and restorer lines is required, and extensive varietal resources are available. Chemical emasculators such as male gametocide 1 (MG1) and male gametocide 2 (MG2) were developed in China and have been used in hybrid rice production. Two of the most popular hybrids, Gang-Hua 2 and Gang-Hua/Qing-Lan, were developed by chemical emasculation. Future emphasis will be placed on the development of more effective and nontoxic chemicals. Preliminary techniques for hybrid seed production by chemical emasculation began in China in the 1970s. Present methods for producing high yields of high-quality, low-cost hybrid rice seed are discussed.

Hybrid rice development in China has proceeded in two directions. One is utilizing A, B, and R lines (three-line breeding); the other is chemical emasculation, which has been applied successfully to hybrid rice production (Guangdong Cooperation Group 1978, Hu et al 1981, Jiang et al 1980, Yuan 1985, Zhou et al 1982).

In chemical emasculation, physiological male sterility is artificially created by spraying the rice plant with chemicals to induce stamen sterility without harming the pistil. In hybrid seed production, two varieties are planted in alternate strips, and one is chemically sterilized and pollinated by the other.

Chemical emasculation is a promising alternative to the development of sterile and restorer parents, and extensive resources of rice varieties are available as parental material.

Male gametocides

Since chemicals were first reported to induce male sterility in plants, significant progress with chemical emasculation has been made in many crops. Admittedly, not all chemicals that kill the stamen or retard pollen development qualify as ideal emasculators. An ideal male gametocide should

- sterilize the stamen without affecting the normal functioning of the rest of the plant to ensure the quantity and quality of hybrid seed set on the female parent,
- be safe to humans and animals,

- be stable and persistent and have some latitude in timing to allow for delays caused by unsuitable weather, and
- be easily obtainable, simple and convenient to use, and economical.

In 1971, the chemical male gametocide MG1 was identified as a successful chemical emasculator from among more than 150 chemicals tested by Jiangxi Agricultural University and the Guangdong Cooperation Group on Heterosis Utilization of Crops. The active ingredient of MG1 is CH_3AsO_3Zn, a component of the pesticide Dao-jiao-qing, which was found by the Guangdong Cooperation Group in 1970. Another male gametocide (MG2) was identified in 1977 by Guangzhou Chemical Institute of Guangdong Province and the Guangdong Cooperation Group. It is soluble in water and its active ingredient is $CH_3AsO_3Na_2$. MG1 and MG2 are used extensively to produce hybrid rice seeds in China. Practice proves that gametocides of the type $CH_3AsO_3=$ meet the criteria of an ideal male gametocide.

The residual toxicity of As in rice grain and straw, and in soil, is of some concern, although As occurs naturally in all foods (Anonymous 1980). Hybrid seeds (F_1) from rice plants that had been sprayed with MG1 or MG2 were analyzed for residual As by the South China Agricultural University, Zhejiang Agricultural University, and Jiangxi Agricultural University. Residual As ranged from 0.214 mg As_2O_3/ kg to 0.765 mg As_2O_3/ kg, well below world standards for acceptable levels.

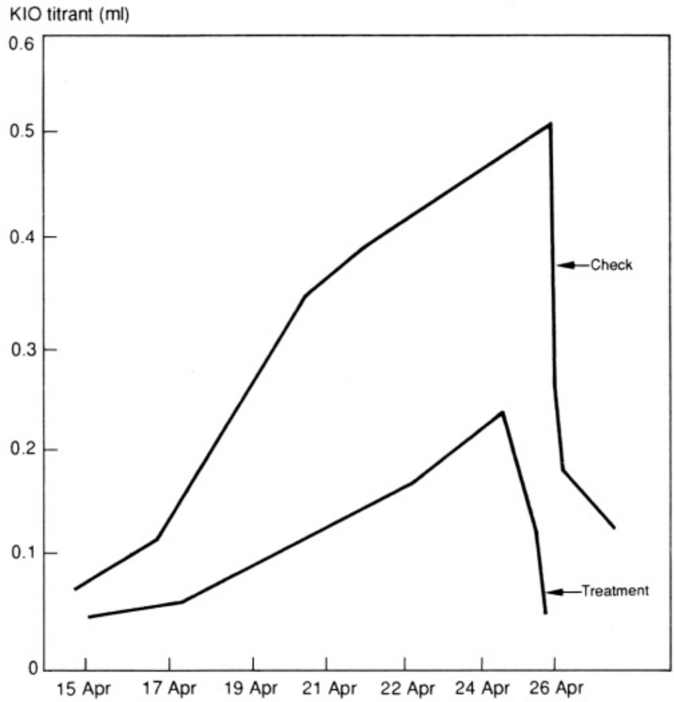
Mechanism of chemical emasculation

The mechanism of chemical emasculation has been studied with the MG1 and MG2 by the South China Agricultural University. Male gametocide labeled with ^{74}As was absorbed by leaves and transferred to panicles. Within 30 min of spraying, the male gametocide in panicles amounted to 0.001% of the total sprayed. The panicles accumulated more gametocide over time, and 6 h after spraying, the amount of male gametocide reached 0.01% of the total. At first, the male gametocide labeled by ^{74}As entered the spikelets and then distributed unequally in the various parts of the flower. MG1 or MG2 content, expressed by specific radioactivity in pistil, stamens, and lodicules within a spikelet, was in the proportion of 2:1:1.

The activity of sulfhydryl compounds group in anthers decreased significantly (Fig. 1). As the activity of enzymes, succinic dehydrogenase, and cytochrome oxidase decreased (Table 1), respiration intensity decreased to one-half to one-third of normal. The decrease was the result of small anther size, respiration inhibition, and disturbance of normal metabolism of pollen mother cells and microspores. Pollen did not develop normally, resulting in nongenetic sterility. When stamens in the meiosis stage and the uninucleate stage were treated with male gametocide, abnormal pollens developed (Fig. 2).

Chemical emasculation techniques

Where chemical emasculation is practiced over wide areas, hybrid seed yield is about 1.5 t/ha. Four guiding principles must be followed for a chemical emasculation program to successfully produce large quantities of pure hybrid seed.



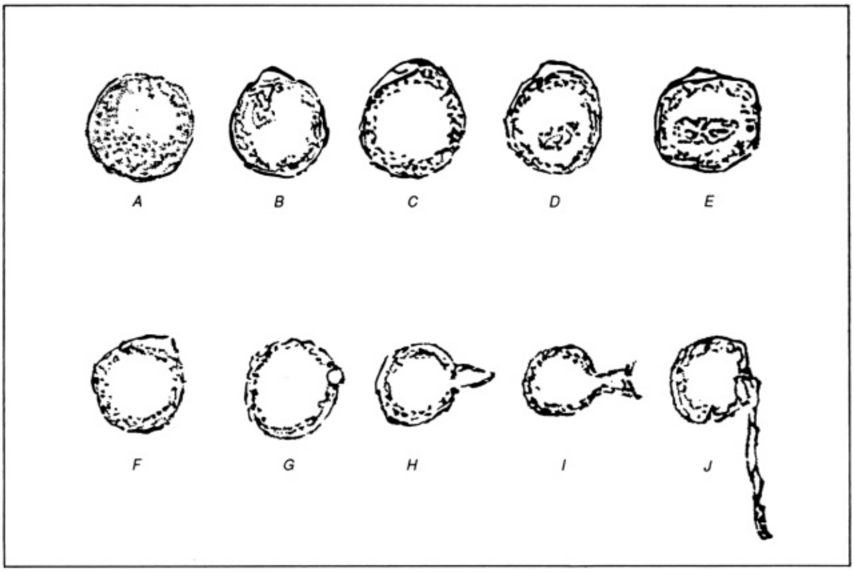
1. Changes in content of free sulfhydryl compounds during anther maturity in Guang-Liu-Ai 4 after treatment with a chemical emasculator at the pollen mother cell meiosis stage.

Table 1. Effect of MG1 on the activity of succinic dehydrogenase and cytochrome oxidase.

Sampling stage	Enzyme[a]	Sporogenesis stage at spraying[bc]					Check
		A	B	C	D	E	
Initial flowering	I	–	–	–	++	+++	+++
	II	–	–	–	++	+++	+++
Full bloom	I	–	–	–	–	–	+++
	II	–	–	–	–	++	+++
Late flowering	I	–	–	–	–	–	+++
	II	–	–	–	–	–	+++
End of flowering	I	–	–	–	–	–	+++
	II	–	–	–	++	++	+++

[a]I = succinic dehydrogenase, II = cytochrome oxidase; [b]A = secondary branchlet and glume primordium differentiation, B = pistil and stamen formation, C = pollen mother cell formation, D = pollen mother cell meiosis, E = uninucleate pollen; [c]+, - = enzyme activity in relation to check.

1. *Appropriate dosage.* The gametocide must emasculate the stamen without compromising pistil fertility.
2. *Even coverage.* Each plant in the maternal community must receive an equal amount of male gametocide during the effective emasculation penod (EEP) to ensure effective emasculation and to prevent overemasculation.



2. Behavior of pollen grain sprayed with chemical emasculator at the pollen mother cell meiosis stage and at the uninucleate pollen stage: *A* and *B,* disintegration of cytoplasm and nucleus; *C,* external wall of pollen remaining after disintegration; *D* and *E,* mass of cytoplasm contracted in the pollen; *F,* failure of pollen to germinate; *G,* bulge of pollen tube, *H,* abnormal pollen tube, *I,* broken pollen tube, *J,* normal development of pollen tube (control).

3. *Timeliness.* Application of the gametocide must be done during EEP, otherwise selfng will increase.
4. *Integration with overall program.* Chemical emasculation must be integrated with the total hybrid seed production program at the time parents are chosen and spraying schedules are determined.

In hybrid seed production, we consider 1) maternal varieties, 2) vigorous combinations, 3) EEP of the maternal community, 4) male gametocide dosage, 5) weather factors, and 6) management.

Suitable maternal varieties should have these characteristics:

- high rate of emasculation;
- pistil with high tolerance for male gametocide;
- long, exserted stigma;
- semidwarf;
- nonlodging; and
- high yield with excellent integrated performance.

In testing combinations, we look for

- easily controlled flowering date of both parents,
- high rate of hybridization and low rate of selfing, and
- heterosis and many panicles with relatively fewer panicles of selfing maternal plants.

The EEP of most varieties is from the pollen mother cell stage to the monokaryotic stage. However, varieties differ in the developmental stage at which effective emasculation occurs. The number of days from initial effective emasculation to terminal effective emasculation of maternal varieties is the EEP. It may vary from 1 to 3 d or from 7 to 10 d. Maternal plants that have more uniform development of young panicles have the longest EEP. Generally, the best time for chemical emasculation is 1-3 d before the end of EEP.

The effects of sunlight, temperature, wind, rain, leaf surface humidity, and leaf density all affect the concentration of male gametocide applied to the maternal community. In indica varieties, the optimum concentration is 0.01-0.02%. About 2.3-2.6 t solution/ha is applied with a single spray nozzle at 5 kg/cm^2 when the ratio of paternal to maternal rows is 2:8.

Standard management practices for uniform panicle development, erect leaves, moderate leaf density, normal leaf color, and vigorous growth should be followed.

However, in hybrid rice production, the leaves of parent plants and maternal panicles that head within 72 h of spraying should be clipped. If it rains within 4 h after spraying, other remedial measures are required. Except for these differences, management practices are similar to those for growing three-line hybrid rice.

Status of chemical emasculation

The area planted to hybrid rice covered more than 40,000 ha in Guangdong Province in the 1970s. Average yields were about 6 t/ha, a 10% increase compared with conventional varieties.

For some combinations in which the parents had the same maturity and plant height, the F_2 with heterosis was also used for rice production (Anonymous 1983).

The most popular hybrids produced by chemical emasculation are Gang-Hua 2, Gang-Hua/ Qing-Lan, and Qing-Hua/Fu-Gui.

Gang-Hua 2, bred with MG1 by the Jiangxi Agricultural University, has high yield potential and good grain quality. It is suitable as a single cropped rice in the middle and lower regions of the Yangtze River in Jiangsu and Jiangxi Provinces. The highest recorded hybrid rice yield of 13.5 t/ha was recorded for Gang-Hua 2, but average yields are 9-10 t/ha. It is now planted on 30,000 ha.

Gang-Hua/ Qing-Lan and Qing-Hua/Fu-Gui and some other F_1 hybrids were bred with MG2 by the Guangdong Academy of Agricultural Sciences, and so far cover 25,000 ha in Guangdong Province.

Gang-Hua/Qing-Lan has good grain quality and is moderately resistant to whitebacked planthopper. It is suitable for Guangdong Province and southern China as a second-crop rice.

Qing-Hua/Fu-Gui is blast resistant and is suitable for southern China as a first-crop rice. Average yields are 12 t/ha.

Yields of Gang-Hua/ Qing-Lan and Qing-Hua/Fu-Gui should increase significantly if seed purity can reach 80%. Seeds from the F_1 of the Gang-Hua/Qin-Lan combination might be used in rice production (Table 2).

The yield performance of some high-yielding hybrid rices with strong heterosis bred at the Guangdong Academy of Agricultural Sciences by chemical emasculation is shown in Table 3. Yield data from 1982 to 1985 show that yield increases over the check three-line hybrid rice ranged from 7.8 to 18.2%.

Problems and perspectives

Although hybrid seed production now approaches 1.5 t/ha, chemical emasculation is not popular because of 3 major problems that can reduce yield and lower purity of hybrid seed: weather, spraying techniques, and spraying equipment.

Unfavorable weather such as high winds or rain during EEP disrupts pollination because the male gametocide and gibberellin cannot be applied evenly and effectively. Spraying techniques are labor intensive and inefficient, partly because present spraying equipment is not suited to the precision required for equal coverage of the maternal community.

Table 2. Yield of Gang-Hua/Qing-Lan F_1 and F_2 in Guangdong Rice Research Institute.

Year	Check yield (t/ha)[a]	F_1			F_2		
		Yield (t/ha)	Increase (t/ha)	%	Yield (t/ha)	Increase (t/ha)	%
1984	7.2	8.4	1.2**	17	7.9	0.7*	10
1985	7.0	8.1	1.1**	16	7.7	0.7*	10

[a] Check combination: Shan-You 30. *Significant at 5% level using t test, **Significant at 1% level using t test.

Table 3. First crop and second crop yields of chemically emasculated hybrid rice in Guangdong hybrid rim testing program 1982-85.

Year	Check yield (t/ha)[a]	Combination	Yield (t/ha)	Order of yield	Yield increase	
					(t/ha)	(%)
		First crop				
1983	7.2	Qing-Hua/Gui-Shao	7.6	2	0.4*	5
1984	6.4	Qing-Hua/Fu-Gui	7.4	1	1.0**	16
1985	6.5	Qing-Hua/Fu-Gui	7.1	1	0.6**	9
		Second crop				
1982	6.0	Gang-Hua/Qing-Lan	6.6	1	0.6**	10
1983	5.4	Gang-Hua/Qing-Lan	5.8	1	0.4**	8
1984	5.7	Gang-Er-Hua/Shu-Ang-Qui	6.7	1	1.0**	18
1985	4.9	Gang-Hua/Qing-Hua 6	5.6	1	0.7**	16

[a] Check combination of three-ilne hybrid rice: Shan-You 2. *Significant at 5% level using t test, **Significant at 1% level using t test.

These problems, although difficult, are not insurmountable. Some possible solutions are

- growing hybrid seed in areas where there is little likelihood of rain, and temperature and sunlight are moderate during EEP;
- designing simple, efficient spraying machines that reduce the chances for operator error;
- strengthening the tolerance of the pistil to male gametocides;
- breeding hybrids by chemical emasculation that may produce less pure seed than three-line breeding, but that yield more seed (This could be accomplished by using the F_1 and F_2 for seed production.); and
- developing improved male gametocides that are efficient and less likely to damage the pistil of maternal plants.

If we can realize these objectives, hybrid seed production will likely reach 2 t/ha with a seed purity of more than 85% at a cost less than $1/kg.

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Effects of a new nontoxic chemical gametocide on rice

LUO ZEMIN, ZHONG AIPING, AND ZHOU QUIXUAN

Defects of current gametocides, including their toxicity to seeds and the environment, are enumerated. Plants treated with a new nontoxic gametocide N-312 were labeled with ^{32}P and analyzed for P, B, protein, proline, and soluble sugar content, and enzyme activity was measured. N-312 blocked P absorption and disturbed P translocation to leaves and panicles, inhibited proline biosynthesis in leaves and anthers, and reduced the B content of anthers, which reduced succinic dehydrogenase, polyphenol oxidase, and peroxidase activities. Optimum dosage and application times were determined. The authors suggest further studies on the mechanism of emasculation to increase gametocide efficiency, reduce plant damage, increase selectivity, and develop standard application techniques for field use.

Chemical emasculation aids in the production of male sterile lines for hybrid rice breeding. Research on rice emasculation was reported as early as the late 1940s (Stanley and Linskens 1947). During the last 20 yr, research on emasculation has been carried out in several provinces of China, including Guangdong, Hunan, Shandong, Zhejiang, and Jangxi. Several gametocides have been used, including zinc methyl arsenate and sodium methyl arsenate. Researchers have attempted to elucidate the mechanism of emasculation through studies in genetics, cytology, anatomy, biochemistry, and physiology. Results show that the mechanism of chemical emasculation shares some similarities with that controlled by genetic factors.

Studies on the mechanism of male sterility in rice plants sprayed with zinc methyl arsenate showed that the gametocide immediately translocates to the panicle (Kiangsi Communist University 1977, South China Agricultural College 1977, Nickell 1982). Male sterility results from a reduction in the activity of SH and succinic dehydrogenase, cytochrome oxidase, and ATP in the anther, and the disturbance of the normal respiration and energy metabolism of the stamen. The gametocide also decreases proline and protein content in the anther, preventing the pollen tube from elongating and pollen from germinating.

There are about 40 kinds of gametocides, all of which have some defects. Defects of some most commonly used gametocides are

- poor emasculation selectivity,
- difficulty in determining timing of spraying and dosage,
- instability of purity and seed set in the field, and
- gametocide toxicity, which pollutes both the environment and the seeds.

Considering these factors, we began to develop nontoxic gametocides in 1982. After many trials in Hunan, Changsha, Changde, Hanshow, Pingjiang, and on Hanian Island, two kinds of nontoxic gametocides were developed, N-312 and HAC-123. Emasculation efficiency reached 90-100% and the outcrossing seed set rate was as high as 70-80%, with a maximum of 90% for 40 varieties after 3 yr of trials. More studies on the mechanism of emasculation are required to increase gametocide efficiency in more varieties, reduce plant damage, increase selectivity of emasculation, and develop standard technology for applying gametocides in the field.

Materials and methods

Varieties and treatment

Varieties No. 79-1163 and Er-Jiu-Feng 305 were used in a pot experiment and Hong-You-Zao-Xian was used in a field experiment to determine the timing of spraying and dosage of gametocide N-312.

Pot experiment. In the pot experiment, the first spraying was done at the end of the third stage or early in the fourth stage of panicle differentiation (according to the standard described by Ding Ying) at 0.2 ml/seedling for No. 79-1163 and 0.15 ml/ seedling for Er-Jiu-Feng 305. The second spraying was done at the sixth stage of panicle differentiation with the same dosage as the first spraying.

Field experiment. There were 6 treatments in the field experiment at spraying rates of 0.10, 0.15, and 0.20 ml/seedling in each treatment. The treatment schedule was

- one spraying late in the 3d stage or early in the 4th stage of panicle differentiation,
- one spraying late in the 5th stage or early in the 6th stage,
- one spraying late in the 7th stage or early in the 8th stage,
- two sprayings, one late in the 3d stage or early in the 4th stage *and* the other late in 5th stage or early in the 6th stage,
- two sprayings, one late in 3d stage or early in the 4th stage *and* the other late in 7th stage or early in the 8th stage, and
- two sprayings, one late in 5th stage or early in the 6th stage *and* the other late in the 7th stage or early in the 8th stage.

Observations. We observed changes in the color of leaves; heading and flowering habit; and the shape of stamen, pistil, and caryopsis of treated plants. After flowering, some pollen was stained with either KI-I2 or TTC and examined under the microscope to determine its vigor. Spikelets from the treatment and control were examined separately to investigate the emasculation efficiency of N-312.

The biochemical analysis

At the same time of the second spraying of N-312, plants from treatment and control were cultured in water and labeled with ^{32}P. The initial radioactive intensity of ^{32}P in each bowl was 0.15 mCi. On the second day, the acid-soluble ^{32}P, ester-soluble ^{32}P, RNA-^{32}P, and DNA-^{32}P were extracted and examined. This was done once every other day, four times in total, and ^{32}P activity was measured.

1. Acid-soluble ^{32}P. One hundred mg fresh weight of leaves, stem, and panicle were ground in a chilled mortar with 1 ml 10% trichlorethanoic acid. The homogenate was centrifuged at 3,000 rpm for 10 min. This was repeated and the supernatants combined.
2. Ester-soluble ^{32}P. The precipitate was immersed in solvent containing an equivalent amount of ethanol and ether for 1 h and centrifuged at 1,000 rpm for 10 min. The process was repeated and the supernatants combined.
3. RNA-^{32}P. The precipitate was added to 0.3N KOH at 37 °C for 90 min and centrifuged. A suspension of the precipitate and 1 ml 0.3N KOH was formed, centrifuged, and the supernatants combined. One ml chilled 2.4N $HC1O_4$ was added to the supernatant, refrigerated for 1 h, then centrifuged. The supernatants of repeated suspensions were combined.
4. DNA-^{32}P. The two kinds of precipitates in step 3 were combined and 2 ml $HC1O_4$ was added. This was exposed to 70 °C for 20 min, then centrifuged repeatedly, and the supernatants were combined.

Protein content. Material was drawn every 3 d to examine the protein content, beginning on the 3d day of the second treatment. One hundred mg fresh wt of leaves or anther were ground in a mortar with a solution containing ethanol and alkali. Protein content was determined by UV absorption.

Boron content. Material was drawn on the 4th, 8th, and 12th day of the 2d treatment of N-312. Fifty mg fresh wt of anther plus C_aCO_3 was carbonized in an electric heater. The ashes were dissolved in 0. 1N HCl, and turmericine was added. The mixture was exposed to a constant-temperature water bath, vaporized, and dried. The precipitate was dissolved with 95% ethanol and filtered, then examined with a spectrophotometer.

Proline content. Material was drawn on the 2d day after treatment with N-312, and once every 4 days thereafter until 4 samples had been collected. Fifty mg anther or 1 g leaves were cut into pieces and extracted with 3% sulphonic salicylic acid in a tank of boiling water for 10 min. Acidic indene and triketone were added to a sample drawn from the solution, which was reacted at 100°C and then examined with a spectrophotometer.

Soluble sugar. Material was drawn on the 2d day after the 1st treatment with N-312 and once every 2 d thereafter until 6 samples had been drawn. One hundred mg fresh wt of leaves, stem, or panicle were cut into pieces and extracted with water at 100°C for 10 min. Then 0.9 ml solution was taken and added to 0.1 ml 2N NaOH, 1 ml triphenol, and 3 ml 10N HCl, reacted for 10 min, and examined with a spectrophotometer.

Histochemical test of enzyme activity. The anthers from similar parts of both treated and control spikelets were taken at 0900 h to test the activity of peroxidase and succinic dehydrogenase according to the method described by the Phyto-physiology Institute of Shanghai (1985).

Results and analysis

Emasculation efficiency of N-312

With N-312 treatment, plants were slightly dwarfed and exhibited slightly deformed leaf blades with chlorotic stripes and unfilled spikelets. Pistil and flower developed normally. Parts of the stamen and 2-3% of the caryopses were distorted. Pollen examination by 2 methods (see Table 1) showed that more than 90% of pollen in the control could be stained, 7% could be half stained, and only about 1% could not be stained. Only 2-3% of treated pollen could be stained.

Sensitivity to N-312 differs by variety, with Er-Jiu-Feng 305 being more sensitive. Although it received a lower total dose of N-312, emasculation efficiency was higher (94%) and the outcrossing rate was 87%. No. 79-1163 is less sensitive; emasculation rate was 87%, with more serious dwarfed plants, more plant damage, and more unfilled spikelets.

The field test on Hong-You-Zao-Xian showed that emasculation efficiency is increased by

- increased dosage,
- earlier treatment, and
- double spraying.

More plant damage occurs as the dose of N-312 increases.

Taking these findings into consideration, plus emasculation efficiency and plant damage, the best treatment for Hong-You-Zao-Xian is a double spraying late in the 3d or early in the 4th stage with a dose of 0.15 ml or 0.20 ml/seedling.

Absorption, translocation, and transformation of P

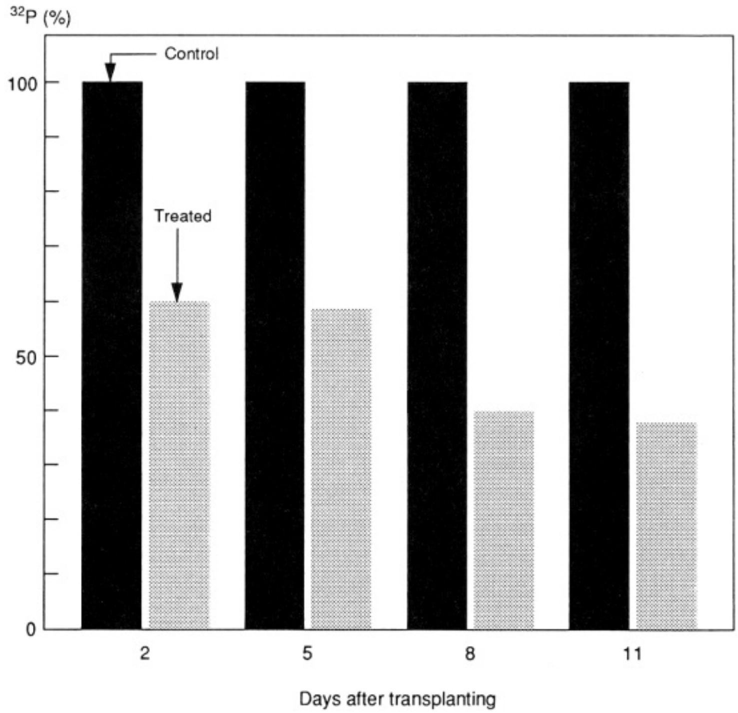
The results suggest that N-312 gravely reduces ^{32}P absorption of rice and results in serious P deficiency (Fig. 1). On the second day after treatment, ^{32}P radioactive intensity of treated plants was 55.8% as high as that of the control. The content of ^{32}P was reduced continuously, and on the 11th day after treatment, total ^{32}P content was only 38.4% that of control.

Table 2 indicates that ^{32}P translocation in plant parts is seriously disturbed. ^{32}P in the control accumulated mostly in the panicle. When treated with N-3 12, little ^{32}P translocated to the panicle with most of it accumulating in the stem. The average ^{32}P content in the panicle was about 30% of the total ^{32}P content of the plant. Reduced P content in the panicle deters panicle development and differentiation.

The incorporation percentages of ^{32}P into the four phosphorate compounds in leaves, stems, and panicles of treated and control plants are given in Table 3. The

Table 1. Results of pollen staining on rice varieties No. 79-1163 and ErJiu-Feng 305 treated with gametocide N-312.

Stain	Treated pollen (%)			Control (%)		
	Stained	Half-stained	Stainless	Stained	Half-stained	Stainless
TTC	3	53	44	92	7	1
KI-12	2	23	75	91	8	1



1. ^{32}P content in treated and control plants.

Table 2. Effect of gametocide N-312 on translocation of ^{32}P to different plant parts at 2, 5, 8, and 11 days after treatment (DAT).

Plant part	Control ^{32}P (%)				Treated ^{32}P (%)			
	2 DAT	5 DAT	8 DAT	11 DAT	2 DAT	5 DAT	8 DAT	11 DAT
Leaf	23	21	22	19	38	29	11	14
Stem	38	40	30	26	41	50	50	48
Panicle	39	38	46	55	21	22	40	38

Table 3. Incorporation percentage of ^{32}P into phosphorate compounds.

Plant part		Acidic-soluble	Ester-soluble	RNA-^{32}P	DNA-^{32}P
Leaves	Control	55	29	11	5
	Treated	67	22	7	4
Stem	Control	83	11	4	2
	Treated	82	10	5	3
Panicle	Control	34	46	17	2
	Treated	46	36	14	4

ratio of ^{32}P incorporation into each plant organ was not materially disturbed. We conclude that N-312 did not block the biosynthesis of these compounds. However, the content of those compounds in treated plants decreased (Table 4). The content of RNA and DNA underwent the greatest change. The lowest content of RNA in treated panicles was 26% of that of control 5 d after treatment (DAT); lowest DNA content was 18% of that of control 2 DAT.

Boron content of anther

Boron plays an important role in germination of pollen and elongation of the pollen tubule. Boron content in treated anthers was half that of control (Table 5), suggesting that one effect of N-312 treatment is reduction of B content in the anther.

Proline in leaves and anthers

Proline content in treated anthers was 30-50% more than that in control (Table 6). The first and second examinations were at the 6th and 7th stages of panicle differentiation. The proline content after treatment with N-312 increases until it reaches the 8th stage. It is 50% more than that in control, indicating that a great quantity of proline accumulates in sterile anthers after treatment. Proline content in leaves is quite low and is not influenced by N-312 treatment.

Protein content

N-312 inhibits the biosynthesis of protein. Figure 2 shows that protein in leaves and anthers is less than that in control, and that anthers, especially, are affected by N-312 treatment.

Table 4. The radioactivity of ^{32}P (cpm) incorporated in phosphorate compounds.

^{32}P compound	DAP[a]	Control			Treated		
		Leaf	Stem	Panicle	Leaf	Stem	Panicle
Acidic-soluble	2	3632	6758	3900	3600	4170	1022
	5	2424	6827	2708	2240	4769	1180
	8	1922	4348	7698	429	3286	2330
	11	1130	2714	6832	312	2056	1397
Ester-soluble	2	1228	1390	2940	1090	812	1140
	5	1248	927	3628	745	600	913
	8	1270	668	786	295	530	595
	11	986	523	591	261	432	447
RNA	2	415	850	2410	330	450	715
	5	480	320	1377	246	293	362
	8	390	243	250	87	133	163
	11	218	230	210	80	110	112
DNA	2	320	360	435	182	160	80
	5	217	182	192	130	176	112
	8	506	342	386	114	135	161
	11	372	293	373	109	87	152

[a]DAT = days after treatment.

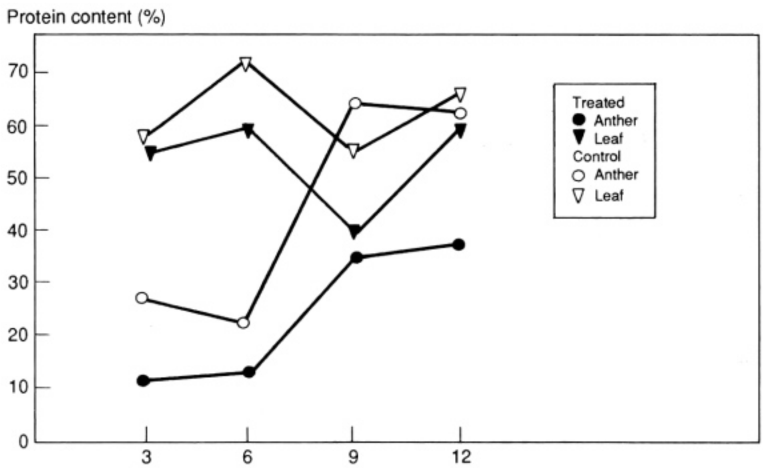
Table 5. Boron content in anthers of plants treated with gametocide N-312.

Treatment	B content (µg/50 mg fresh wt)	
	Control	Treated
1	0.45	0.20
2	0.20	0.10
3	0.40	0.20

Table 6. Proline content in anthers and leaves.

Sample	Control		Treated	
	Leaf[a]	Anther[b]	Leaf[a]	Anther[b]
1	0.36	7.1	0.32	11.7
2	0.28	15.6	0.26	22.4
3	0.30	12.3	0.28	24.2

[a]Sample unit µg/1 mg fresh wt. [b]Sample unit µg/50 mg fresh wt.



2. Protein content in leaves and anthers of rice plants treated with gametocide N-312.

Soluble sugar content

Soluble sugar was greater in leaves and stems of treated plants than in control (Table 7). In treated panicles, however, soluble sugar decreased slightly from the 4th to the 6th stage compared with control, and was slightly higher than that of control from the 7th stage onward. N-312 treatment disturbed the transfer and metabolism of sugar.

Table 7. Soluble sugar content (µg/100 mg fresh wt) in different plant parts at several stages of panicle differentiation.

Panicle stage	Control			Treated		
	Leaf	Stem	Panicle	Leaf	Stem	Panicle
Late 4th	3.79	5.53	5.25	5.28	10.23	4.13
5th	2.31	3.96	6.60	4.87	10.23	6.61
Early 6th	3.14	4.87	4.87	3.69	6.19	4.70
Late 6th	2.15	3.47	1.16	2.39	7.43	2.72
7th	2.46	4.88	0.79	3.43	5.37	1.32
Early 8th	1.47	1.37	0.18	1.72	2.24	0.62

Influence on enzyme activity in anthers

Histochemical analysis showed that the activities of succinic dehydrogenase, polyphenol oxidase, and peroxidase in anthers nearly disappeared when they were treated with N-312.

Conclusions

Results indicate that gametocide N-312 influences many aspects of rice metabolism.

Phosphorus metabolism

N-312 greatly reduces P metabolism in treated rice plants. Total P content in treated plants was only 38-56% as high as that in control, and more accumulated in stems than in panicles. During panicle development, especially at the division stage of pollen mother cells, a large amount of P is needed to synthesize RNA and DNA. Treatment reduced P distributed to panicles, decreased P content of DNA and RNA, and retarded development and formation of pollen.

Soluble sugar

Table 7 shows more soluble sugar in treated stems than in control, but less soluble sugar in treated panicles. This indicates that N-312 blocked the transfer of soluble sugar from stem to panicle. As panicles develop, however, soluble sugar at the 7th and 8th stages of panicle development is higher than that in control. This may indicate that the panicle is unable to convert soluble sugar into starch, resulting in low starch accumulation and abundant pollen, which reduces pollen fertility.

Proline in pollen

Research on proline in pollen is useful for understanding the response of plants to stress and the relation between proline content and genetic sterility (Banga and Labna 1984). Proline, which was thought to be related to male sterility, decreased in sterile pollen (Academia Sinica 1976, Xie 1985, Kwangsi Teachers College 1977, Zhu et al 1984, Zhu and Cao 1984, Zhu et al 1985, Zhu and Cao 1985). Other research (Tang 1984) showed that proline markedly increased in stressed plants leading

Biochemical basis of heterosis in rice

Deng Hongde

Growth and yield are the results of a series of reactions, and phenotypic expression ultimately is due to genetically controlled biochemical mechanisms. The causes of the expression of significant heterosis in F_1 rice hybrids are reviewed from the biochemical point of view.

Hybrid vigor or heterosis is the basis for the yield advantage of hybrid rice over conventional varieties. To more fully understand heterosis, Hunan Agricultural College undertook a program in 1977 to determine the biochemical basis of heterosis in rice. The research program included studies of amylase and α-amylase activity, RNA content in young roots, the ability of roots to synthesize amino acids, the ability of hybrids to absorb and synthesize assimilates, photosynthesis and transpiration of assimilates to panicles, soluble sugar and total N, grain filling ability, the isoenzyme basis of heterosis, and the nucleohistone in hybrid rice.

In all studies, hybrid rice was superior to the conventional variety checks. This paper presents the results of our studies at Hunan Agricultural College, and explains how each of the characters measured contributes to the yield advantage of hybrid rice.

Amylase and α-amylase activity

Hybrid rice has a higher initial growth rate than conventional varieties. This growth advantage is expressed as early as in seed germination. The germination speed of Nan-You 2 and its three parental lines is in the order of hybrid > R line > B line > A line (Li et al 1982). A similar order is seen in the activity of their α-amylases. The activity of a-amylase in hybrids is 20% higher than the mean value in 3 parental lines (Hunan Agricultural College 1977a). During initial germination (0-3 d), hybrid rice showed obvious heterobeltiosis (202%) in amylase activity and 433% in α-amylase activity. It is generally considered that α-amylase and amylase are synthesized after germination begins. Further study has shown that amylase and α-amylase are conserved in the endosperm of dry seed of hybrid rice and its B line, and that their activity is higher in hybrid rice than in its B line (Table 1, Fig. 1,2). The higher α-amylase and amylase contents reserved and their higher activities make it possible to rapidly hydrolyze the starch in the endosperm, providing energy and assimilates for embryo development.

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Managing hybrid rice seed production

Xu Shijue and Li Bihu

Commercial development of hybrid rice depends on the availability of high-quality seed, which can come about only through improved hybrid seed production techniques. Strict isolation of seed production areas, thorough roguing of off-type plants, and rigid operating rules can help to achieve required seed purity of >99.8%. The factors that control hybrid seed yield are discussed: choice of field plots, optimum flowering time, synchronized flowering, growth regulation, row direction, leaf clipping, and supplementary pollination. Hybrid seed production techniques that need further refinement are discussed, among them increasing panicle exsertion and outcrossing rates, and prolonging pollen vitality.

High hybrid rice yields are obtained by exploiting the heterosis in the F_1. Rapid commercial development of hybrid rice depends on the availability of high, good-quality seed. Improving hybrid seed production techniques is the key.

In 1973, when hybrid seed production started in China, yields were very low (less than 0.1 t/ha). Now, techniques for hybrid seed production have been standardized and seed production has increased annually. In 1982, 0.15 million ha was devoted to hybrid seed production with average yields of 0.9 t/ha. By 1985, the area devoted to seed production fell to 0.12 million ha, but yields had increased to more than 1.5 t/ha.

Hunan Province, the birthplace of hybrid rice, is attaining the highest seed production. In 1983, yields were already more than 1.5 t/ha; by 1985, average yield was 2.07 t/ha and maximum yield was 5.4 t/ha. The total area devoted to hybrid seed production has been reduced by 2/3 while yield has doubled (Table 1). The price of hybrid seed has declined 30-40%, very favorable for rapid expansion of hybrid rice cultivation. The area in China planted to hybrid rice covers 25% of the total rice area, but accounts for 32% of the total yield.

Producing hybrid seed involves numerous technical production combinations. In China, hybrid seed production may be undertaken at any season, depending on the combinations used and ecological conditions. The technical principles and specifications are identical but seeding dates, seedling ages, and row ratios of the parents vary. The emphasis is on ensuring quality seed, in quantity.

Ensuring quality

To obtain high-quality F_1 seeds, the following measures are taken: 1) a three-level production system, 2) strict isolation, 3) thorough roguing, and 4) rigid operating rules.

Table 1. Field area and yield of hybrid seed production in Hunan.

Year	Area (ha × 10^3)	Yield		Total yield (t)
		t/ha	Increase over previous year (%)	
1976	49.2	0.31	–	15,252
1977	55.1	0.38	22.5	20,938
1978	61.4	0.49	28.9	30,086
1979	38.0	0.60	22.4	22,800
1980	42.3	0.71	18.3	30,033
1981	32.1	0.80	12.6	25,680
1982	34.8	0.98	22.5	34,104
1983	25.6	1.74	77.5	44,544
1984	17.0	1.83	5.1	31.110
1985	16.4	2.07	13.1	33,948

A three-level production system. The seed company at the province level is responsible for purification of three parental lines and production of foundation seeds. Seed purity should be > 99.8%.

The seed company at the prefecture level multiplies the cytogenic male sterile (CMS) and restorer lines. Seed purity should be 99%.

The seed company at the county level selects 1-3 local sites to produce hybrid seed for commercial cultivation. Seed purity should be at least 98%.

Strict isolation. Seed production areas are isolated by space, time, barriers, and variety. Hills, rivers, and crops other than paddy rice can define an isolation district. The isolation distance required is more than 100 m.

The heading stage of any rice varieties grown within 100 m of the seed production field should be completed 20 d earlier or later than the heading stage of the CMS line.

Only the same variety as the male parent may be planted in the isolation district. Its purity should be more than 99%.

Cloth or other artificial materials often are used to fence the seed production field. The height should be at least 2.5 m.

Thorough roguing. The seed production field from seedling to heading must be free of rogues. Rogues include maintainer plants and semi-sterile plants, which appear in the CMS line rows, and other off-type plants in male and female rows.

Rigid operating rules. To avoid mechanical admixtures that may occur between seed soaking and harvest, quality checks should be established at all levels.

Obtaining high yields

Hybrid seed yields depend on several factors: choice of field plots, optimum flowering time, synchronized flowering, regulating growth, choice of row direction, leaf clipping, and supplementary pollination.

Prime field plots. A seed production plot requires fertile soil with good physical and chemical characteristics, adequate irrigation and drainage, sufficient sunshine, even topography and regular plot shape, and no serious disease or insect problems, especially those forbidden by quarantine regulations.

Optimum flowering time. Conditions favorable for normal flowering are daily temperature of 24-28 °C, relative humidity of 70-80% difference between day and night temperatures of 8-10 °C, and sunny days with breeze.

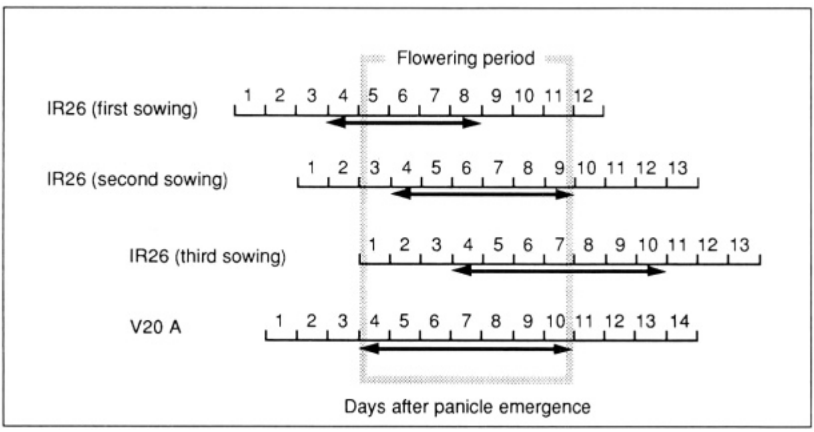
As a rule, in high temperature with low humidity or in low temperature with high humidity, some glumes will not open. This lowers the pollen vitality of the male parent and: the stigma receptivity of the female parent, resulting in low yield. In general, it is better to use cross combinations with shorter growth durations in spring or autumn and cross combinations with longer growth durations in summer or late summer. Flowering should be planned to occur when the high temperature period has ended and the low temperature period has not started.

Synchronized flowering. Synchronizing the flowering of both parents is the key to increased yields. Criteria for synchronizing flowering are shown in Figure 1. Technical measures involve staggering seeding dates of the male and female parents, sowing the male parent three times to extend the time pollen is available, and predicting and adjusting flowering dates.

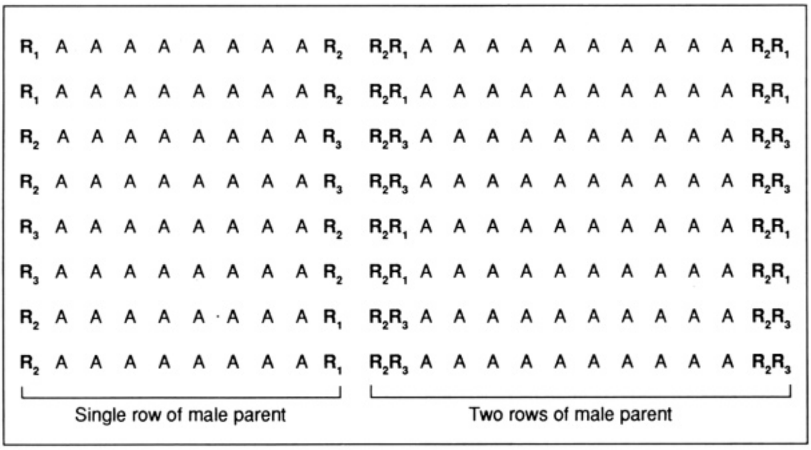
Seeding date is usually determined by leaf age, effective accumulated temperature (EAT), and growth duration. In Shan-You 2, the EAT of its male parent (IR24) from seeding to initial heading is 1133 °C; that of its female parent (Zhen-Shan 97 A), 791 °C. The difference in EAT is 342 °C. When the EAT of IR24 totals 342 °C is the suitable seeding date for Zhen-Shan 97 A.

In general, the period from initial to full heading of a CMS line is 4-6 d longer than for a restorer line. The first sowing of the male parent establishes the dates for second and third sowing. The second sowing is done when the leaf emergence on the first sowing is 1.1; the third sowing when leaf emergence is 2.1. The second sowing is the main parent. Planting ratio is 1:2:1. The two planting patterns used are shown in Figure 2.

Beginning about 30 d before heading, 3 or 4 random samples of the main culm of both parents are taken every 3 d. Young panicle development is compared under magnification. During the first three stages of panicle differentiation, treat the earlier



1. The synchronization of flowering of male and female parents of Wei-You 6.



2. Planting patterns.

developing parent with quick-releasing N fertilizer and spray the later developing parent with potassium dihydrogen phosphate. This adjusts development differences of 4-5 d. During later stages of panicle differentiation, draining water from the field will delay male parent panicle development; higher standing water will speed panicle development.

Regulating growth. The key to regulating growth is to establish a population with large panicles (more than 100 spikelets/panicle), uniform panicles (more than 0.9 million productive panicles per hectare for male parent and more than 3.0 million productive panicles per hectare for female parent), and short flag leaves (about 30 cm for male parent and 25 cm for female parent).

Seedlings with healthy tillers are the basis for increased panicle size (Table 2). For hybrid seed production, the seedlings of both parents should be standardized. For cross combinations with short growth duration, seedlings of the male parent should be 20-30 d old, with 5.5-7 leaves and 2-3 tillers. For cross combinations with longer growth duration, seedlings of the male parent should be 30-35 d old, with 7-8 leaves, and 3-4 tillers. Seedlings of the female parent should be 20–30d old, with 5.5-7 leaves and 2-3 tillers.

Prepare the seedling bed with basal manure. Seed at 150 kg/ha for the female parent and 110-150 kg/ ha for the male parent. Experience has shown that higher row ratios, narrower row spacing, and more seedlings per hill will increase seedlings, panicles, and grain yields (Tables 3-5). In general, tillered seedlings are planted two per hill at a row ratio of 1:10-12 or 2:10-12 spaced 10 × 13.3 cm or 13.3 × 16.7 cm. The male parent should have 450,000 hills and 450,000 basic seedlings per ha. The female parent should have 450,000 hills and 1.8 million basic seedlings per ha. Adequate fertilization is effective in producing short flag leaves (Table 6). In general, a seed production field with moderate fertility should be treated with 200 kg N/ha, 50 kg P/ha, and 150 kg K/ha, 90% applied basally and 10% after panicle differentiation.

Table 2. Economic characters of tillers produced in bed (I) and tillers produced in main field (II).

	Panicle bearing tillers (%)	Panicle length (cm)	Total spikelets/ panicle	Filled spikelets/ panicle	Seed setting rate (%)	Time (d) between main panicle and panicle from tiller
Main panicle		21.4	119.7	37.2	31.1	
I	94.6	20.9	117.4	22.7	19.3	1-5
II	35.6	18.5	69.4	10.1	14.6	8-14

Table 3. Effects of row ratios on panicle structure of female parent and yields.

Row ratio	Productive panicles (× 10^3)	Spikelets/ panicle	Spikelets/ panicle	Seed setting rate (%)	Yield (t/ha)
1:15	2,619	65.9	21.9	33.2	1.6
1:14	2,642	68.6	20.9	30.4	1.6
1:13	2,583	64.8	25.1	38.7	1.9
1:12	2,458	67.9	25.2	37.1	1.8
1:11	2,562	74.5	29.7	39.9	2.3
1:10	2,354	72.4	24.4	33.7	1.8
1:9	2,151	72.0	29.0	40.2	1.8
1:8	2,005	67.0	24.4	36.4	1.4

Note: Combination V20 A/IR26.

The field should be irrigated and drained according to the development of rice plants.

Row direction. Both parents should receive good aeration and equal amounts of sunlight. In addition, row direction nearly perpendicular to prevailing winds at flowering favors cross pollination.

Leaf clipping and GA_3 application. Leaves taller than the panicles are the main obstacle to cross pollination. Clipping leaves 1-2 d before initial heading increases the probability of pollination and the outcrossing rate (Table 7). The blade of the flag leaf is cut back 1/2 to 1/3 from the top. Spraying 75 g GA_3/ ha 2 or 3 times increases panicle exsertion.

Supplementary pollination. On calm days during anthesis, supplementary pollination can be carried out. Panicles of the restorer line are shaken by pulling a long nylon rope (5 mm diam) back and forth every 30 min until no pollen remains on the restorer line. This method is often used on even topography and regularly shaped plots. In hilly, uneven topography with small, irregular plots, a bamboo pole may be used.

Problems and prospects

Techniques of hybrid seed production need further improvement.

Increasing panicle exsertion. Currently, 95% of the CMS lines used in seed production are wild abortive (WA) type. The internodejust below the panicle neck is

Table 4. Effect of plant spacing on panicle structure of female parent and grain yield.

	Plant spacing (cm)	Basic seedlings (× 10^3)	Productive panicles (× 10^3)	Panicle bearing tillers (%)	Spikelets/ panicle (no.)	Filled spikelets/panicle (no.)	Seed setting rate (%)	1,000-grain wt (g)	Yield (t/ha)
	6.7 × 13.3	2,985	3,735	78.0	88.7	43.3	48.8	30.0	3.4
I	6.7 × 16.7	2,385	3,135	79.2	92.6	43.2	46.7	30.2	3.4
	10 × 10	2,655	3,810	82.0	82.6	39.8	48.2	30.1	3.4
	10 × 13.3	3,984	3.420	76.0	76.1	35.8	47.0	29.8	3.4
II	10 × 16.7	3,180	3,465	73.0	78.0	41.4	53.1	29.3	3.9
	13.3 × 13.3	2,985	3,450	85.0	76.8	39.5	51.4	30.7	3.8
	13.3 × 16.7	2,385	3,105	72.2	74.2	31.8	42.8	30.5	3.8

I = 1 plant/hill, II = 2 plants/hill. Combination V20 A/IR26. Row ratio = 1:9.

Table 5. Effect of seedlings planted on panicle structure of female parent and grain yield.

Seedlings/ hill (no.)	Seedlings (× 10^3)	Productive panicles (× 10^3)	Panicle bearing tillers (%)	Spikelets/ panicle (no.)	Filled spikelets/panicle (no.)	Seed setting rate (%)	1,000-grain wt (g)	Yield (t/ha)
1	468	2,520	77.0	99.9	20.1	20.1	30	2.02
2	933	2,985	78.0	67.7	25.6	37.8	30	2.23
3	1,400	3,570	82.0	75.3	28.8	38.3	31	2.66
4	1,866	3,360	85.0	100.2	24.7	24.7	32	2.67
2	933	3,135	89.9	87.2	25.4	29.1	32.3	2.43
4	1,866	3,540	83.5	69.9	26.3	37.6	31.5	2.81
6	2,799	4,290	82.0	75.3	29.0	38.5	32.0	3.08
8	3,732	4,290	83.0	76.8	28.0	36.4	32.0	2.68

Note: Combination V20 A/IR26. Row ratio = 1:9. Plant spacing: 13.3 × 13.3 cm.

Table 6. Effect of fertilizations on flag leaf of female parent.

Treatment[b]	Flag leaf (cm)		Second leaf from top (cm)	
	Length	Width	Length	Width
I	24.1	1.82	34.9	1.5
II	27.9	1.90	35.6	1.5

[a] Fertilizer rate: 240 kg N/ha, 73 kg P/ha, 137 kg K/ha. [b] I = all fertilizers were applied as basal manure, II = 80% of fertilizer applied basally, 20% topdressed.

Table 7. Effect of leaf clipping and GA_3 application on outcrossing rate.

Treatment	Productive panicles/ha (× 10^3)	Panicle bearing tiller (%)	Spikelets/ panicle (no.)	Filled spikelets/panicle (no.)	Seed setting rate (%)
Leaf clipping and GA_3 application	3,187	82.5	68.0	18.5	27.2
Check	2,625	71.4	65.2	11.2	17.2

very short, resulting in a low panicle exsertion. Solving this problem would further increase hybrid seed yields. Regulating growth and applying GA_3 without leaf clipping have made it possible to change the agronomic characters of the parents. For example, flag leaves become shorter and grow horizontally and internodes below the panicle neck become longer. Panicle exsertion percentage is significantly increased, which leads to 5% higher outcrossing and 13% higher seed yield.

Increasing outcrossing rates. A series of new CMS lines with good flowering habits, big stigmas, and high panicle exsertion percentage have been developed. Their outcrossing rate is 30-50% higher than that of the leading CMS line. Meanwhile, plant growth hormones have made it possible to enlarge the angle of the flag leaf of CMS lines by 10.6-66.6 degrees, and the angle of the glume opening by 10 degrees, and to increase the exsertion percentage of stigma by 5.1-43.4%. This means that the outcrossing rate of CMS lines will be significantly increased.

Prolonging pollen vitality. Studies in Hunan recently have shown that under good storage conditions pollen can live for more than 15 d and germination rates can reach 10%.

Notes

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Improving pollination characteristics of japonica rice

H. Namai and H. Kato

To overcome the extremely low seed set percentage of the cytoplasmic male sterile (CMS) seed parent for growing hybrid rice commercially, we have been studying pollination biology of rice. This paper reviews research concerned mainly with necessary conditions for seed setting on CMS seed parents of japonica rice.

Results show that even when only one pollen grain was deposited on a stigma lobe, some spikelets could set seed. Even considering unfavorable pollen viability, 3-4 pollen grains were sufficient for seed setting of CMS seed parents. The flowering duration of spikelet required to deposit 2 or more pollen grains on the stigma lobes of more than 80% of the spikelets in the CMS parent of hybrid rice was 30 min or more.

The most effective floral characteristics for enhancing seed set percentage were frequent stigma exsertion for the CMS seed parent, residual pollen per exserted anther, and many spikelets blooming every day. In japonica rice, effective gene sourcesfor breeding seed and pollen parents of hybrid rice cultivars were the local cultivars, especially contaminated or segregated accessions of temperate japonica and some tropical ones. These gene sources can improve the floral characteristics of seed and pollen parents and enhance their cross-pollination potential.

Finally, we concluded that 1.5 or more airborne pollens per liter and wind velocity of 2-3 m/s were necessary to get more than 50% seed set in CMS seed parents of hybrid rice cultivars.

Wild rice, *Oryza perennis* Moench, is considered an allogamous plant, with 100% outcrossing observed in some populations (Oka and Morishima 1967). On the other hand, cultivated rice, *O. sativa* L., especially improved japonicas, is considered completely autogamous, showing less than 5% outcrossing. However, no conclusions have been made regarding variations in floral characteristics connected with outcrossing for japonicas.

Cytoplasmic male sterility is an essential genetic characteristic for seed parents in hybrid seed production for allogamous and autogamous crops. For successful hybrid seed production, sufficient pollen from the pollen parents must be deposited on the stigma lobes of each spikelet in the seed parents. However, in cytoplasmic or genic male sterile seed parents of rice, extremely low seed set percentage has been reported (Azzini and Rutger 1982, Fujimaki et al 1977, Shinjyo 1975).

In this paper, we report conditions necessary for seed setting in CMS seed parents: 1) the number of pollen grains deposited on stigma lobes of the male sterile seed parent and the necessary period of exposure to airborne pollen, 2) the effective floral characteristics of seed and pollen parents, and 3) environmental factors and cultivation conditions for increasing seed set percentage of CMS seed parents in japonica cultivars.

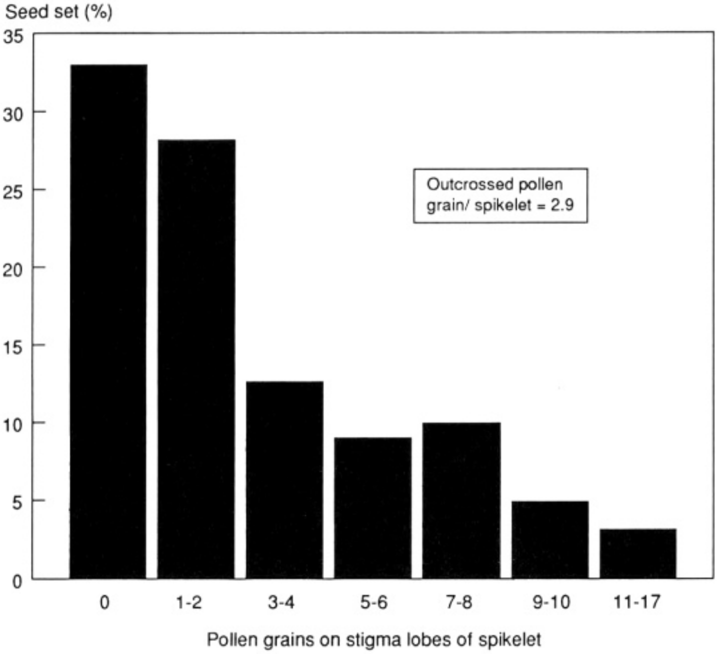
Various factors are related to cross-pollination (Virmani and Edwards 1983). According to their studies and our recent results, the following factors affect natural cross-pollination in CMS seed parents:

1. Flowering behavior of seed and pollen parents
   a. simultaneous initiation and duration of flowering
   b. simultaneous blooming duration of panicles
   c. simultaneous initiation of spikelet opening
2. Floral characteristics of seed parent
   a. large stigma
   b. long style
   c. frequent stigma exsertion
   d. wide angle of spikelet opening
   e. spikelet blooming duration of 30 min or more in seed parent
3. Floral charactenstics of pollen parent
   a. large anther and many pollens per anther
   b. residual pollen number per anther at anther exsertion stage
   c. long filament and frequent anther exsertion
   d. constant spikelet blooming in pollen parent
4. Other morphological characteristics
   a. high tillering capacity
   b. complete panicle exsertion
   c. short and narrow flag leaf
   d. many spikelets per panicle
   e. large length-breadth ratio of seed or spikelet
5. Environmental and cultivation conditions
   a. wind direction and velocity (2-3 or more meters per second)
   b. number of airborne pollen (1.5 or more pollen grains per liter)
   c. short distance from pollen parent to seed parent
   d. seed parent-pollen parent ratio
   e. disparity in plant height (seed parent <pollen parent)

Pollen grains needed for seed setting

To ensure seed setting in the male sterile seed parents, sufficient pollen grains must be deposited on the stigma lobes. Sawada (1978) studied pollen grain deposition at low temperatures (12-15 °C) to examine the mechanisms of cool-summer damage due to floral impotency. He concluded that 10-20 pollen grains were needed to fertilize and develop seed. However, no studies have been made under normal conditions.

Figure 1 shows the frequency distribution of pollen grains deposited on stigma lobes of male sterile plants of a CMS line of Reimei grown in pots and placed in a 0.3-ha blooming field. The number of pollen grains deposited per spikelet was 0-17.



1. Pollen grains deposited on stigma lobes of male sterile plants of a CMS line of Reimei. Sum of data for 4 d in 1984.

Less than 40% of spikelets had 3 or more pollen grains. The mean seed set percentage was 45.3%. Therefore, some spikelets with only 1 or 2 pollen grains deposited on their stigma lobes could develop into seeds. In buckwheat, which, like rice, has 1 ovule/ flower, seed set percentage under favorable conditions was 40% for flowers with only 1 compatible pollen grain/flower and about 70% for flowers with 3-5 compatible pollen grains (Namai and Ohsawa 1986).

Namai and Kato (1987a,b) established simple methods to estimate airborne pollen grains per liter. They found that even when only one pollen grain was deposited, considerable spikelets could set seed. Even with poor pollen viability, 34 pollen grains were sufficient to fertilize and develop seed of CMS seed parents cross-pollinated in the field. Thirty min or more were required to deposit 2 or more pollen grains on the stigma lobes of more than 80% of the spikelets in the CMS seed parent.

Effective floral characteristics for seed parents and pollen parents

Of the floral characteristics needed in seed parents, we especially examined the percentage of spikelets with exserted stigmas. No one has yet proved that this floral trait increases cross-pollination potential of CMS seed parents of hybrid rices.

Table 1 shows the effect of stigma exsertion on seed set percentage of plants of the CMS line Reimei grown in pots and placed in a 0.3-ha blooming field. Seed set percentage of spikelets with exserted stigmas was significantly higher than of spikelets without exserted stigmas. Figure 2 shows the correlation between seed setting percentage in CMS or emasculated plants of 3 japonicas placed in the 0.3-ha blooming ricefield. Kato and Namai (1978a) observed similar correlations.

Parmar et al (1979b) proposed that anther length, which could reflect the amount of pollen per anther, was the most important characteristic for pollen parents of hybrid rice. Joppa et al (l968) investigated the floral characteristics of red spring wheat and durum varieties and concluded that anther exsertion had the largest direct effect on pollen shedding. Virmani and Edwards (1983) predicted that the relative pollen-shedding ability of each cultivar depended on pollen grains per anther, percentage of anther exsertion, and fertile florets per plot.

Counting airborne pollen grains in a short time requires a simple and efficient sampler. To meet this need, Namai and Kato (1987a) devised a rotary sampler (Fig. 3) and developed simple procedures for estimating airborne pollen grains per liter. Research using the sampler showed that anther length did not correlate with percentage or number of residual pollen grains per anther exserted from spikelets (Kato and Namai 1987b). However, residual pollen grains per anther and blooming spikelets per unit area directly effected pollen shedding (Kato and Namai 1987a). Therefore, pollen parents for hybrid rice need numerous residual pollen grains per exserted anther and a large number of spikelets constantly blooming per plant.

Floral characteristics for enhancing cross-pollination of japonicas

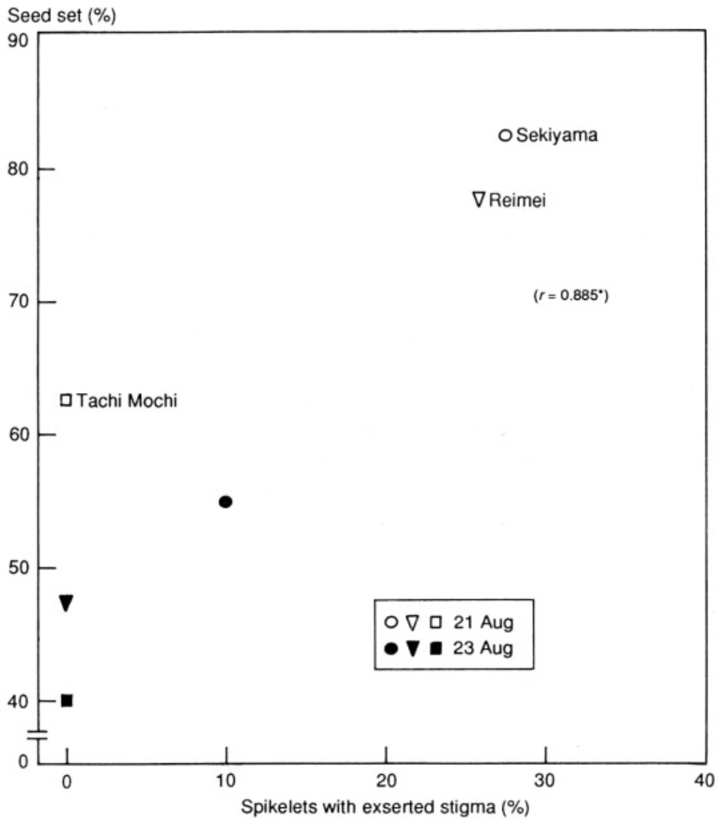
Wild rice and indica rice have many genotypes regarded as sources of effective floral characteristics for seed and pollen parents of hybrid rice (Parmar et al 1979a,b; Virmani and Athwal 1973; Virmani and Edwards 1983). On the other hand, there is no detailed report on japonica rice. Therefore, we have been evaluating efficient floral characteristics for enhancing cross-pollination potential of seed and pollen parents, especially using japonica cultivars preserved by the Ministry of Agriculture, Forestry and Fisheries of Japan.

In the catalog of plant genetic resources of the Ministry, the word *Majiri* is attached to the names of some accessions. Majiri means contamination or mixture. Natural outcrossing is considered a cause of contamination and segregation.

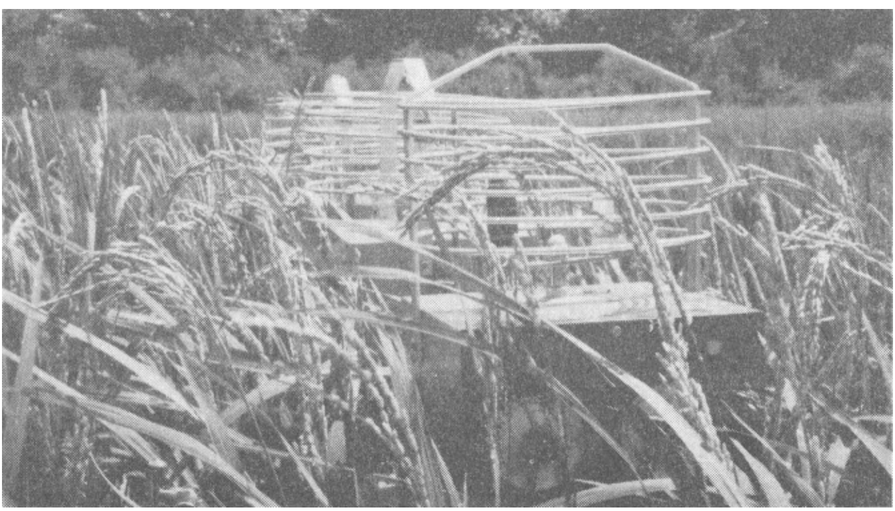
Table 1. Effect of stigma exsertion on seed setting percentage of a japonica CMS line Reimei.

Kind of spikelet	Total	Spikelets		Seed set (%)
		Filled	Unfilled	
With exserted stigma	37	20	17	54.1
Without exserted stigma	218	69	149	31.7

x^2 = 7.02*.



2. Correlation between spikelets with exserted stigmas and seed setting percentage in three cultivars of japonica rice, August 1984.



3. Rotary sampler for airborne pollen.

We found wide variations in floral characteristics of seed and pollen parents of hybrid rice in the Majiri japonica cultivars.

In the seed parents, stigma length ranged from 1.3 to 2.0 mm, and 0-55% of spikelets had exserted stigmas. Some accessions had long stigmas (2.0 mm) and high percentages of spikelets with exserted stigmas (approximately 55%).

In the pollen parents, anther length ranged from 1.7 to 2.8 mm and residual pollen grains per exserted stigma ranged from near 0 to 1,500. Many Majiri accessions had more than 1,000 residual pollen grains, indicating they will certainly be effective gene sources for breeding seed and pollen parents of japonica hybrid rices.

According to Oka (1958) and Oka and Chang (1967), japonicas are classified into temperate and tropical types. Kato and Namai (1987b) found that most tropical japonicas had slender spikelets, which often exserted long stigma lobes and had many residual pollen grains per exserted anther. Some showed exserted stigmas for 80% of spikelets and had more than 1,700 residual pollen grains per anther. Therefore, they will be useful sources for improving floral characteristics of seed and pollen parents of hybrid japonica cultivars.

If we search efficient gene sources on a larger scale, more promising gene sources should appear in tropical and temperate japonicas. Hassan and Siddiq (1984) reported that long anthers were monogenically dominant over short anthers and fully exserted stigma were dominant over partially exserted stigma. Using such gene sources, we should be able to breed for these desirable floral characteristics.

Environmental factors enhancing pollen per liter and seed setting of seed parents

Wind velocity is important in cross-pollination (Whitehead 1983). In wheat, the amount of airborne pollen was related to the natural outcrossing rate (Bitzer and Patterson 1967) and wind direction (De Vries 1974).

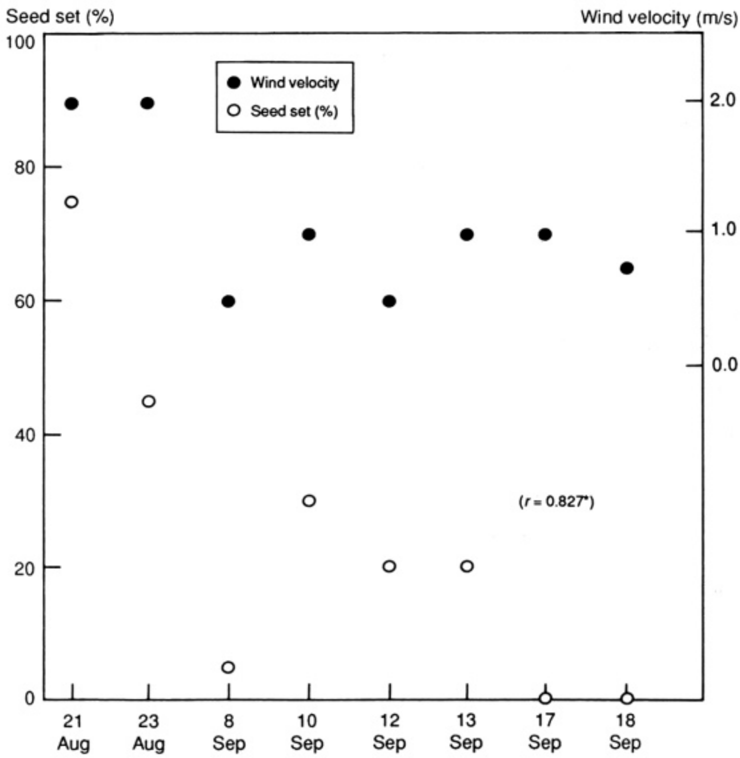
We have researched airborne pollen per liter and its correlations with wind velocity, and outcrossed seed setting percentage of male sterile seed parents of japonica rice.

Table 2 shows the seed setting percentage of the improved cultivar, Reimei, as it relates to outcrossed pollen grains per stigma lobe of a spikelet, airborne pollen grains per liter, and wind velocity. Reimei plants were grown in pots and placed in a 0.3-ha field for 8 d in 1984. Nipponbare, the pollen parent, bloomed in the same period. Reimei had 14.5% of spikelets with exserted stigma lobes.

On 21 and 23 Aug with wind blowing approximately 2.0 m/s, airborne pollen was more than 15 grain/liter, the mean number of outcrossed pollen grains per stigma lobe was approximately 5.0, and seed set percentages were 76.7 on 21 Aug and 47.4% on 23 Aug, greatly exceeding means over the 8 d. On those 2 d, approximately 80 and 50% of stigma lobes were cross-pollinated by 3 or more pollen grains. Figure 4 shows the correlation between wind velocity and seed set percentage and Figure 5 shows the correlation between airborne pollen grains and seed set percentage. Figure 6 shows the correlation between airborne pollen grain per liter and wind velocity. These figures were drawn from the data in Table 2.

Table 2. Relation of floral characteristics and environmental factor to seed setting percentage of CMS. line Reimei.

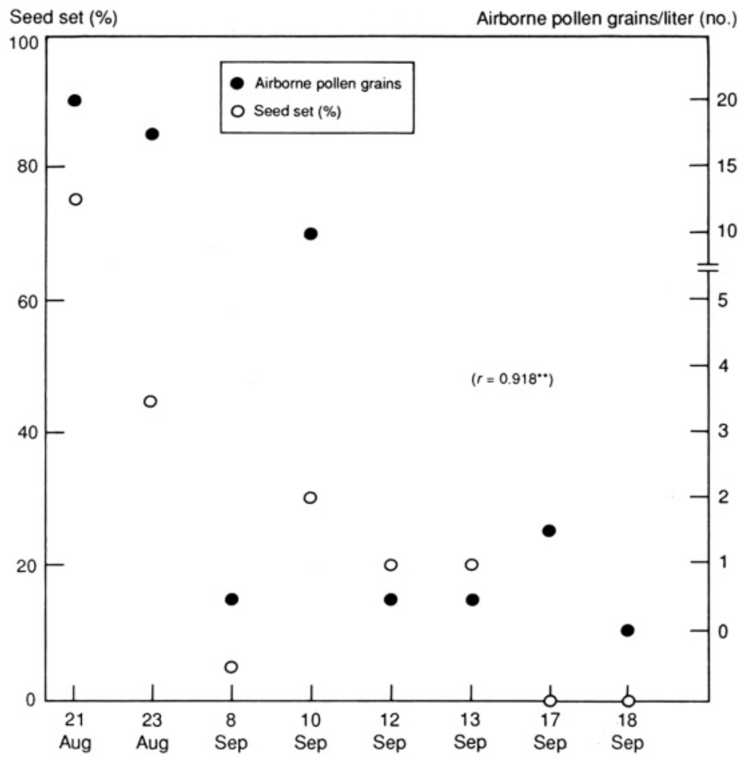
	21 Aug	23 Aug	8 Sep	10 Sep	12 Sep	13 Sep	17 Sep	18 Sep	Total
Spikelets that bloomed	73	19	31	16	61	28	20	7	255
Spikelets that set seeds	56	9	2	5	11	6	0	0	89
Seed setting (%)	76.7	47.4	6.5	31.3	18.0	21.4	0.0	0.0	34.9 (mean)
Mean no. of pollen grains	6.1	4.1	–	–	1.2	0.5	–	–	–
deposited (range)	(1-13)	(0-17)			(0-5)	(0-3)			–
No, of airborne pollen per liter	19.5	16.7	0.3	11.2	0.3	0.3	1.3	0.1	
Wind velocity (m/s)	1.9	2.1	0.6	0.9	0.6	1.1	0.9	0.8	



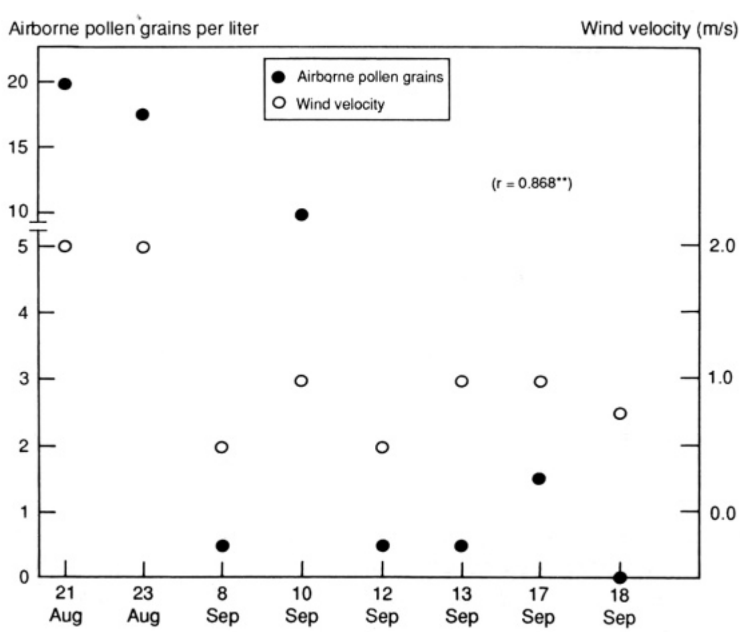
4. Correlation between wind velocity and seed setting percentage in CMS line Reimei, 1984.

As data show, wind velocity enhanced pollen distribution (r = 0.868**) and seed set (r = 0.827*). Airborne pollen per liter was significantly correlated with seed set (r = 0.918**).

Kato and Namai (1987) reported more detailed data for male sterile plants in 4 japonica cultivars. Percentage of spikelets with exserted stigmas ranged from 9.3 to 50.0%. 1.5 or more airborne pollen grains per liter and 2-3 m/s wind velocity were estimated to be necessary to get more than 50% seed set.



5. Correlation between airborne pollen grains and seed setting percentage in CMS line Reimei, 1984.



6. Correlation between wind velocity and airborne pollen grains in CMS line Reimei, 1984.

Further research can clarify relations among the seed set percentage of CMS seed parents, blooming spikelets per unit area, airborne pollen grains, wind direction and velocity, distance from pollen parent to seed parent, and seed and pollen parent row ratio.

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Improving outcrossing rate in rice (*Oryza sativa* L.)

J. TAILLEBOIS AND E. P. GUIMARAES

Floral traits that affect the outcrossing rate in rice are discussed; those that facilitate pollen dispersal and those that facilitate stigma reception of pollen are enumerated. Except for the variability of stigma and anther characteristics, variability for nearly all traits that influence outcrossing is large in *Oryza sativa.* Variability for stigma and anther characteristics is found in allogamous species. Two approaches (backcrosses and pedigree selection, and recurrent selection) used to transfer the long stigmas of wild species to *O. sativa* are described, and results are given.

Success in breeding and growing hybrid rice commercially in China has created great interest among rice breeders throughout the world. However, the natural outcrossing rate in rice cultivars is extremely low and hybrid seed production, using male sterility or gametocides, presents some difficulties. Increasing the potential outcrossing rate would facilitate use of hybrids in other areas of the world.

Floral traits and outcrossing aptitudes of rice species

The *Oryza sativa* species

The inflorescence of *O. sativa* is a terminal panicle of perfect flowers. Each spikelet has a branched stigma, six stamens, and two lodicules. At blooming, the flowers open rapidly. Usually the anthers dehisce just before or immediately after the spikelets open. The flowers remain open from 1 to 3 h and close after anthesis; they never reopen.

Some floral traits, such as stigma size, stigma exsertion, and flower opening duration, affect the rate of outcrossing in rice. *O. sativa* stigmas are small and slightly feathery. Usually the stigma remain inside the glumes during and after anthesis. In some cultivars, some stigmas remain outside the glumes after anthesis and fertilization. This characteristic is more frequent in indicas.

The rate of exserted stigma, which ranges from 0 to 90%, is correlated with stigma length. Parmar et al (1980), Virmani et al (1980), Sarkar and Miah (1983a), and Taillebois (1983) reported stigma lengths of 0.4–1.6 mm. The duration of receptivity is variable and can be longer than 5 d (Virmani and Tan 1982, Yoshida 1981).

Anther length ranges from 0.9 mm to 2.8 mm (Parmar et al 1980, Taillebois 1983, Virmani and Athwal 1973) and correlates highly with number of pollen grains per anther. Oka and Morishima (1967) found 700-2,500 pollen grains/anther. After pollen emission, the duration of fertilizing capacity is rather short, generally less than 5 min.

O. sativa, because its floral structure is not well adapted to cross-pollination, is highly autogamous. In general, natural cross-pollination is less than 1%, depending on environmental conditions and cultivars. When male sterile plants are used, the outcrossing rate is much higher; in China, up to 45% seed set was obtained. Sarkar and Miah (1983b) observed natural outcrossing rates of 0.5-2.5%. Silitonga (1985) reported a maximum seed set of 14%, with a mean of 6%. Outcrossing rates up to 92% were reported for individual plants (Virmani and Tan 1982). At the National Research Center for Rice and Beans (CNPAF), in an indica population of fertile and sterile plants, outcrossing averaged 7.8%.

Male sterile spikelets behave differently from fertile spikelets. At CNPAF, male sterile spikelets had better stigma exsertion and stayed open longer. Parmar et al (1980) reported similar observations and also mentioned a relationship between length and exsertion of stigma and size and function of anthers. In general, anthers with sterile pollen are associated with persistent protruding stigmas.

Hoff and De La Torre (1981) reported a high correlation between seed set and stigma exsertion in male sterile plants. Their results suggest that male sterile plants with 100% exserted stigmas should have about 80% seed set in good pollination conditions.

Allogamous species

Some wild *Oryza* species of the sativa group are partially or completely allogamous. Oka and Morishima (1967) gave a natural outcrossing rate of 20-45% for *O. perennis* (asiatic type). Sakai and Narise (1959) observed an outcrossing rate of 7-50% among different strains of *O. sativa* f. *spontanea. O. longistaminata,* a wild species from Africa, is the most allogamous of all *Oryza* species. Some populations of self-incompatible plants depend on outcrossing for seed multiplication.

The main floral traits of these allogamous species are

- large and feathery stigmas (2.5 mm long for *O. longistaminata)* which remain outside the glumes after anthesis;
- very large anthers (5.5 mm) with more than 7,000 pollen grains/anther in some strains of *O. longistaminata* (Oka and Morishima 1967);
- very good panicle exsertion;
- pollen with high fertility duration, up to 9 min for *O. perennis* (Oka and Morishima 1967); and
- pollen emission lasting up to 9 min after flower opening.

Chinese hybrid seed production techniques

China is the only country in the world where farmers use hybrid rice technology on a large scale. To produce hybrid seeds, male and female parents are planted alternately in specific row ratios; the most common ratios are 1:6 and 1:8. Because the natural rate of outcrossing on male sterile plants is insufficient for economic hybrid seed production, the Chinese promote cross-pollination by

- planting across the wind direction to increase pollen dispersal on female plants;

- clipping the flag leaves of male sterile and restorer lines at booting to facilitate pollen circulation;
- applying 20-30 ppm gibberellin to male sterile or restorer lines at initial heading to promote emergence of the basal part of panicle from the leaf sheath;
- supplementing pollination by rope pulling or rod shaking every 30 min during flowering.

Flower synchronization of male and female plants is basic to obtaining a high seed yield. Two planting dates are used for the restorer line to get good synchronization.

With this technique, outcrossing rates range from 15 to 45%, with a maximum 14% reported (Virmani and Tan 1982). Chinese are now getting hybrid seed yields of more than 1 t/ha.

The technique does require a lot of manpower. In China, 1 kg of hybrid seed costs as much as 6-10 kg of conventionally bred varieties. However, only 16-25% of the seeding rate used for conventional varieties is needed for hybrid rice. In countries where manpower is very expensive or in areas where higher seeding rates are necessary, such as in upland rice, this seed production technique is not economically acceptable. Parents perfectly adapted to cross-pollination are needed if hybrid seeds are to be produced for those situations.

Increasing outcrossing rates

Traits that influence outcrossing

Several traits (stigma and anther size, panicle exsertion, aspect of flag leaf) affect outcrossing. All these characters must facilitate pollen dispersal and stigma reception of the pollen grains.

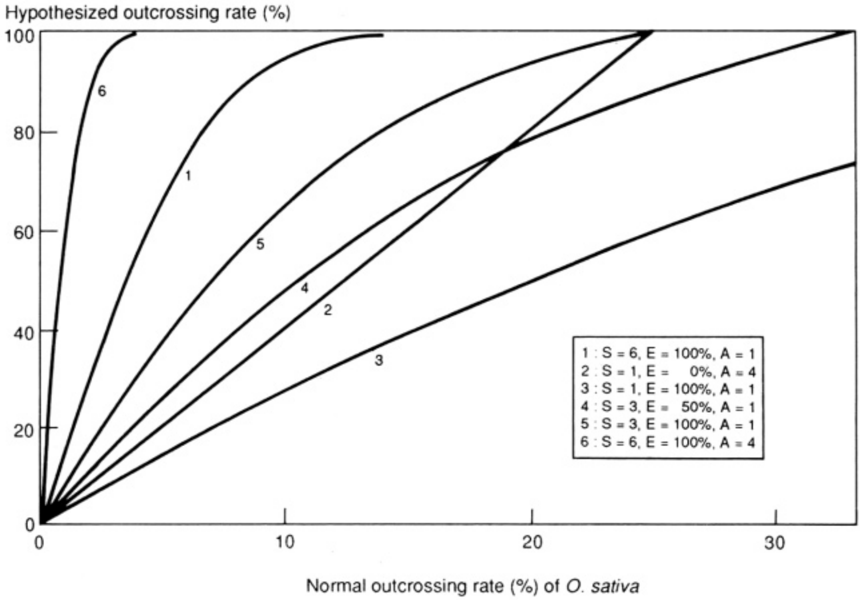
The characteristics that facilitate pollen dispersal are

- many pollen grains per anther;
- long stamen filament and dehiscence of anthers after they are completely outside the glumes;
- pollen with longer capacity of fertilization;
- good male panicle exsertion;
- male parent taller than female parent; and
- small and horizontal flag leaf, which does not interfere with pollen circulation in both parents.

The characteristics that facilitate stigma reception of pollen grains are

- longer and very feathery stigmas, which increase the reception area;
- stigmas that remain outside the glumes after anthesis, increasing the time stigmas are accessible to pollen;
- longer duration of stigma receptivity; and
- good female panicle exsertion and small and horizontal flag leaf, facilitating pollen access to stigma.

Of all the traits influencing outcrossing, stigma characteristics are probably the most efficient in enhancing seed set on male sterile plants. Figure 1 shows hypotheses



1. Theoretical seed set on male sterile plants (based on different hypotheses of stigma area, stigma exsertion, and pollen grains per anther) compared to conventionally bred *O. sativa.* Assumed stigma receptivity period: 3 d. A = pollen grains per anther (1 for *O. sativa,* 4 for *O. longistaminata*); E = stigma exsertion, S = stigma area (1 for *O. sativa,* 6 for *O. longistaminata*).

about the modification of stigma area, stigma exsertion, and number of pollen grains per anther. With a male parent having *O. longistaminata* anthers, a perfect seed set on the male sterile plants is obtainable only when natural outcrossing on normal plants is higher than 25%. With a female parent having *O. longistaminata* stigma, a perfect seed set is obtainable if natural outcrossing on normal plants is higher than 14%.

Selection of plants adapted to outcrossing

The variability in *O. sativa* of nearly all traits that influence outcrossing is very large. There should be no need to resort to wild species to enhance cross-pollination. Using the variability already in *O. sativa* would reduce problems of linkage with undesirable traits and cross-incompatibility and simplify breeding. However, the variability of stigma and anther characteristics in allogamous species is not included in *O. sativa.* As these two traits probably influence outcrossing most, it would be a good alternative to transfer them from wild allogamous species.

The transference of allogamous characteristics from wild species to *O. sativa* was first studied by Virmani and Athwal (l974). The wild species used was *O. sativa* f. *spontanea,* which is cross-compatible with *O. sativa.* They found that shorter anthers and stigmas showed partial dominance. But these wild rice characteristics were linked with genes controlling undesirable wild rice traits. To break the undesirable linkage, a biparental mating approach was proposed.

In Brazil, CNPAF and IRAT are transferring the long stigmas of the wild species *O. longistaminata* to *O. sativa* (indica and japonica types). Two approaches have been used. One is based on successive backcrosses and pedigree selection, the other on recurrent selection. Results so far indicate that

- the *O. longistaminata* stigma is a dominant character;
- the *O. longistaminata* anther is a partially dominant character;
- stigma and large anther are linked characters. No selection pressure was applied for large anthers, although nearly all lines with large stigmas had large anthers. Even so, anthers in the backcross are always smaller than those of *O. longistaminata;*
- perfect *O. longistaminata* stigmas occur in less than 10% of the plants of a backcross. In F_2 generation of a backcross plant with large stigmas, the rate is 5-10%.
- all plants in BC_1 and BC_2 with *O. longistaminata* stigmas are partially or completely male sterile and seed shedding is extremely high, although from BC_3 on, there are no sterility problems and seed shedding decreases.

The use of backcrosses and pedigree selection is expected to result in A and B lines (indica and japonica) with *O. longistaminata* stigmas, by 1987.

Transforming maintainer varieties in A and B lines with *O. longistaminata* stigmas is time-consuming and of low utility, because only a few of the transformed varieties would be used as parents of commercial hybrids. For now, the female parent will be extracted directly from a segregating population where all plants are maintainers with good stigmas. A male sterile gene will be introduced in the population. Such a population will be bred through a process of recurrent selection. To assure a high level of recombination and to maintain selection pressure for outcrossing aptitude, only open-pollinated seeds on male sterile plants will be harvested. After several cycles of selection, the plants should have the *O. longistaminata* stigma character with no undesirable linkages.

The other important favorable traits for outcrossing, such as flag leaf aspect and panicle exsertion, are easily found in *O. sativa.*

Special attention must be given to the “eui” gene described by Rutger and Carnahan (1981). This recessive gene produces nearly twice-as-long uppermost internodes, with no other effects. This character should facilitate windblown dispersal of pollen onto female plants and would make it possible to use another system of hybrid seed production, in which male and female plants could be mixed in transplanting. Because male parents are taller, it is easy to eliminate them after blooming by pulling a herbicide wick across the field at the level of the male panicles.

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Multiple pistillate male sterile rices for hybrid seed production

HAK-SOO SUH

The multiple pistil japonica rice WX154 appeared to be single recessive in the cross between WX154 and a japonica, Suweon 295, but duplicate recessive in the cross between WX154 and an indica, HR1619-6-2-1-2-2. The multiple pistil of the indica cultivar Double Rice was controlled by a single recessive gene in the crosses between Double Rice and indica rice cultivars IR36ms and HR1619-6-2-1-2-2. The florets and grain structure of F_1s of the crosses between the multiple pistillate rice cultivar and the normal pistillate cultivars were normal.

The multiple pistillate lines derived from HR1619-6-2-1-2-2/Double Rice were crossed to the cytoplasmic male sterile (CMS) rice HR1619 A having wild abortive cytoplasm. The sterile F_1 plants were backcrossed to the maintainer multiple pistillate lines. The multiple pistillate cytoplasmic male sterile (MPCMS) lines with semidwarfness could be selected from those backcross progenies. The panicles per hill and florets per panicle of the MPCMS lines did not differ from those of the CMS lines with normal pistils.

The multiple pistillate genetic male sterile (MPGMS) lines could be selected from the F_4 of the cross IR36ms/Double Rice. Fewer panicles per hill and florets per panicle were observed in the MPGMS lines than in the genetic male sterile (GMS) lines with normal pistils.

The average outcrossing of the multiple pistillate male sterile lines in CMS or in GMS rices was statistically not higher than that of the male sterile lines with normal pistils. Larger variation of outcrossing rate was observed in the multiple pistillate male sterile rices than in the normal pistillate male sterile ones. The outcrossed seed setting percentage of the MPCMS lines ranged from 1.1 to 34.8% and that of the MPGMS lines ranged from 0.1 to 40.5%. The outcrossing percentage of the CMS lines ranged from 1.2 to 21.3% while that of the GMS lines ranged from 1.5 to 36.7%.

Seed setting percentage of the artificial cross in the MPCMS lines was significantly higher than in the CMS lines.

The multipistillate mutant in rice was first reported in 1936 by Parthasarathy. He reported that an Indian rice cultivar Plena had multiple pistils controlled by a single recessive gene. Morinaga and Tajiri (1941) found a double seed japonica rice controlled by a single recessive gene. Multiple pistillate (polycaryoptic) mutants having two recessive genes were found by Kasahara (1944) in japonica. Heu and Suh (1976) reported that the polycaryoptic character of the japonica cultivar WX154 was controlled by a single recessive gene and its long empty glume linked it to group IV of

Nagao and Takahashi. Suh et al (1983) reported another multipistillate indica rice, Double Rice. They discovered that the multiple pistils of japonica WX154 and indica Double Rice were governed by different recessive genes.

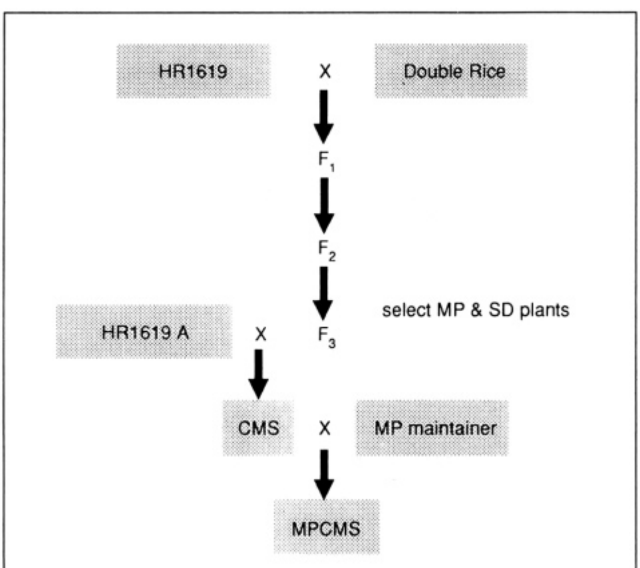
The author attempted to combine the multiple pistil of Double Rice and the cytoplasmic male sterility of V20 A, and to combine the multiple pistil of Double Rice and the genetic male sterility of IR36ms. After selecting multiple pistillate cytoplasmic male sterile (MPCMS) and multiple pistillate genetic male sterile (MPGMS) lines, outcrossing rates were compared with those of male sterile lines having normal pistils to assess the possibility of using the multiple pistillate male sterile rices for hybrid seed production.

Materials and methods

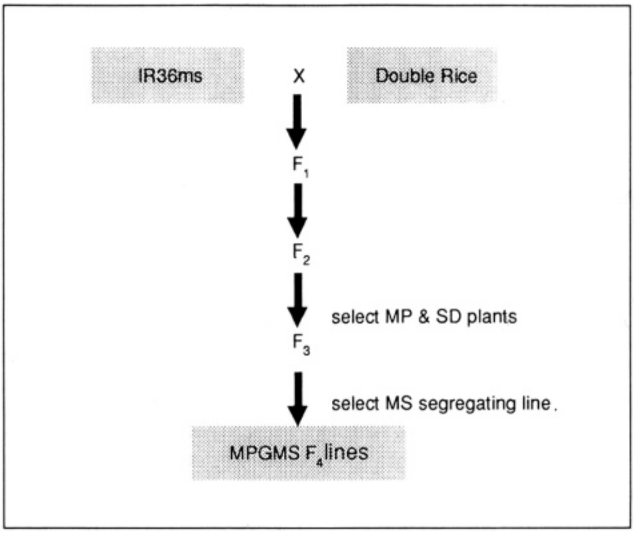
Multiple pistillate japonica WX154 was crossed with japonica Suweon 295 and indica HR1619-6-2-1-2-2. Multiple pistillate indica Double Rice was crossed to indicas IR36ms and HR1619-6-2-1-2-2. The segregation ratios of floral structure were studied in the F_2 generation.

Double Rice was crossed with a Korean semidwarf line HR1619-6-2-1-2-2 and the multiple pistillate lines with semidwarfness were selected from the F_3 of this cross. These multiple pistillate lines were crossed with the CMS line (WA) HR1619 A. After identifying the F_1's sterility, the multiple pistillate lines identified as maintainers were crossed again with the male sterile F_1s and the MPCMS lines were selected from the progenies of those crosses (Fig. 1).

The MPGMS lines with semidwarfness were selected from the F_4 progeny of the cross IR36ms/ Double Rice. The selection process is shown in Figure 2.



1. Selection process for the multiple pistillate cytoplasmic male sterile (MPCMS) lines. MP = multiple pistillate, SD = semidwarf, CMS = cytoplasmic male sterile.



2. Selection process for the multiple pistillate genetic male sterile (MPGMS) lines. MP = multiple pistillate, SD = semidwarf, MS = male sterile.

The MPCMS lines and the CMS lines with normal pistils, and the MPGMS lines and the genetic male sterile (GMS) lines with normal pistils were planted alternately between rows of Suweon 294 at 30- × 15cm spacing. Outcrossed seed set percentage and some agronomic characters were compared between MPCMS and CMS, and MPGMS and GMS lines.

Results and discussion

Inheritance of multiple pistils

All F_1 plants of crosses between the multiple pistillate cultivar and the normal one appeared normal in floret and grain structure. The F_2 plants of the crosses between a multiple pistillate japonica and a normal japonica, WX154/Suweon 295, and between the multiple pistillate indica and the normal indica, IR36ms/Double Rice and HR1619-6-2-1-2-2/Double Rice, segregated at a ratio of 3 normal to 1 multiple pistillate, indicating that the multiple pistils are single recessive. The F_2 of the cross between the multiple pistillate japonica and the normal indica, WX154/HR1619-6-2-1-2-2, segregated at a ratio of 15 normal to 1 multiple pistillate (Table 1). These results are insufficient to explain allelism of the multiple pistils. However, in a previous report (Suh et al 1983) the multiple pistillate cultivars WX154 and Double Rice were identified as having different genes for multiple pistils.

Selecting MPCMS lines

Seven multiple pistillate F_3 plants selected from HR1619-6-2-1-2-21/Double Rice were crossed with CMS HR1619 A. Among the seven crosses, three appeared completely sterile, the rest were incompletely sterile. Thus, the three F_3 lines were proved to be maintainers to HR1619 A. The male sterile F_1s werecrossed again with

Table 1. Segregation of multiple pistil in F_1 and F_2 crosses between multiple pistillate rice cultivars and normal cultivars.

Crosses[a]	F_1	No. of F_2 plants			Ratio	c^2
		Normal	Multiple	Total		
WX154/Suweon 295	Normal	255	68	323	3:1	2.682
WX154/HR1619-6-2-1-2-2	Normal	290	19	309	15:1	0.005
HR1619-6-2-1-2-2/Double Rice	Normal	292	82	374	3:1	1.886
IR36ms/Double Rice	Normal	430	125	555	3:1	1.817

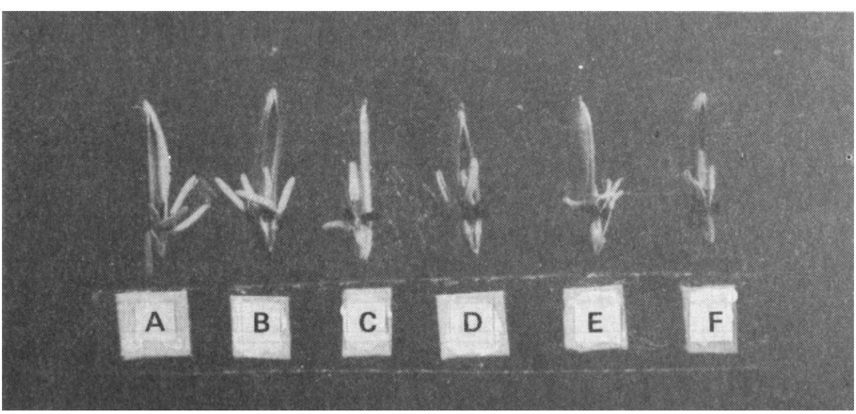
[a]WX154: multiple pistillate japonica; Double Rice: multiple pistillate indica; Suweon 295: normal pistillate japonica; HR1619-6-2-1-2-2; and IR36ms: normal pistillate indica.

the same multiple pistillate lines. From the progeny of these crosses, the MPCMS lines were selected and maintained by backcrossing to the progeny of the selected multiple pistillate maintainers.

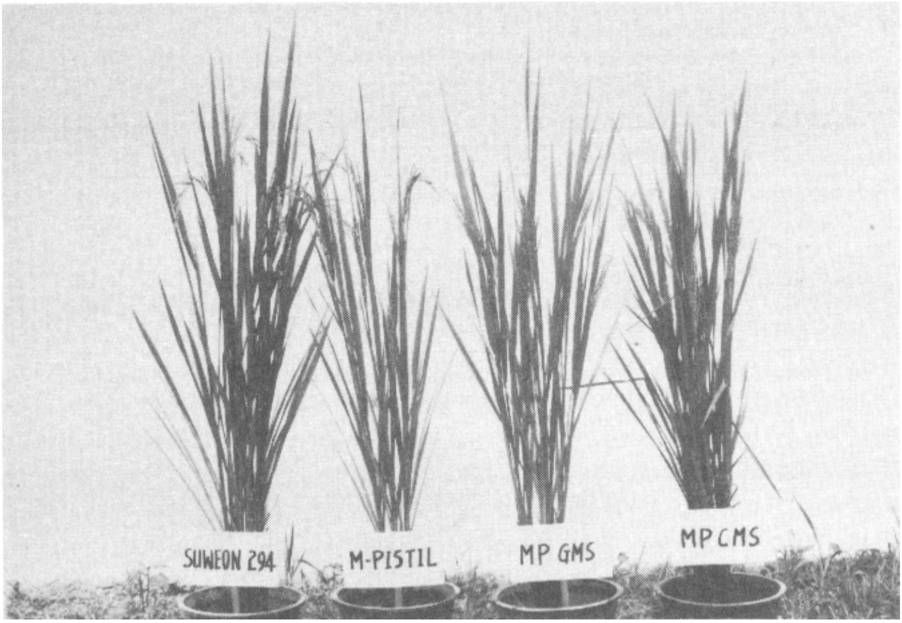
The floret structure and plant type of the MPCMS are shown in Figures 3 and 4. The MPCMS lines with semidwarf plant type and heading dates ranging from 10 to 20 Aug were selected. Panicle per hill and florets per panicle of the MPCMS lines did not differ statistically from those of the CMS lines (Table 2).

Selecting MPGMS lines

The MPGMS lines with semidwarf plant type and heading dates ranging from 10 to 20 Aug were selected from F_4 progenies of IR36ms/ Double Rice. Figure 2 shows the selection process. Figures 3 and 4 show the floret structure and plant type of MPGMS lines. MPGMS lines had statistically fewer panicles per hill and florets per panicle than the GMS lines (Table 2).



3. Floret appearance of the normal (A), multiple pistillate male fertile (B), normal pistillate cytoplasmic male sterile (C), multiple pistillate cytoplasmic male sterile (D), normal pistillate genetic male sterile (E), and multiple pistillate genetic male sterile (F) rices.



4. Plant appearance of the multiple pistillate cytoplasmic male sterile (MPCMS), multiple pistillate genetic male sterile (MPGMS), and multiple pistillate male fertile (M-PISTIL) rices compared with the normal Korean cultivar Suweon 294.

Table 2. Panicles per hill and spikelets per panicle in multiple pistillate male sterile rices and normal pistillate male steriles.

Type of male sterility [a]	Panicles per hill (no.)		Spikelets per panicle (no.)	
	Mean	CV	Mean	CV
MPCMS	9.9	0.41	113	0.27
CMS	10.7	0.36	125	0.26
Significance	ns	–	ns	–
MPGMS	9.4	0.31	167	0.32
GMS	11.9	0.24	198	0.16
Significance	**	–	*	–

[a] MPCMS = multiple pistillate cytoplasmic male sterile, CMS = normal pistillate cytoplasmic male sterile, MPGMS = multiple pistillate genetic male sterile, GMS = normal pistillate genetic male sterile.

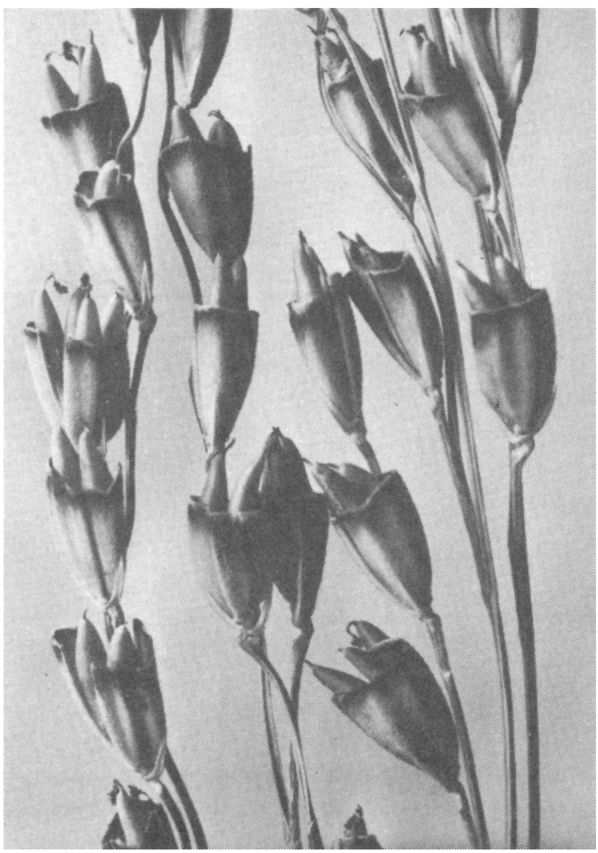
Outcrossing ability of multiple pistillate male sterile lines

The average outcrossing percentages were 13.6% for MPCMS lines, 16.6% for CMS lines, 10.7% for MPGMS lines, and 11.9% for GMS lines. The average outcrossing percentage of multiple pistillate male sterile lines was slightly less than that of normal pistillate male sterile lines, although the differences were not statistically significant.

Table 3. Natural outcrossing ability of multiple pistillate male sterile rices and normal pistillate male steriles.

Types of mate sterility[a]	Seed-setting percentage of natural outcross			Outcrossed seed weight per hill (g/hill)		
	Mean	Range	CV	Mean	Range	CV
MPCMS	13.6	1.1-34.8	1.03	3.0	0.21-11.0	0.97
CMS	16.6	1.2-21.3	0.69	4.1	0.1 2- 9.9	0.66
Significance	ns	–	–	ns	–	–
MPGMS	10.7	0.1-40.5	1.10	3.0	0.02-23.2	1.27
GMS	11.9	1.5-36.7	0.84	5.7	0.72-21.1	0.89
Significance	ns	–	–	ns	–	–

[a]MPCMS = multiple pistillate cytoplasmic male sterile, CMS = normal pistillate cytoplasmic male sterile, MPGMS = multiple pistillate genetic male sterile, GMS = normal pistillate genetic male sterile.



5. Seed set appearance on the multiple pistillate cytoplasmic male sterile rice in artificial cross.

However, MPCMS and MPGMS lines had larger CV values in seed-setting percentage than CMS and GMS lines (Table 3). The multiple pistillate male sterile lines also exhibited higher variation in outcrossed seed weight than the normal pistillate male sterile lines.

Most multiple pistillate lines with cytoplasmic or genetic male sterility appeared to have abnormal florets and a low natural outcrossing rate. However, some MPCMS lines had good floret structure and anthesis character for outcrossing, showing higher natural and artificial outcrossing rates than male sterile lines having normal pistils. A very high outcrossing rate was observed on the selected MPCMS lines in artificial crosses (Fig. 5). Many artificially crossed seeds of selected MPCMS lines were double- or triple-seeded; a few were tetra-seeded. The results mean that most pistils of the selected MPCMS lines are functional. If the multiple pistillate male sterile lines with good floral structure and good anthesis character could be bred true, they could be used to increase hybrid seed production in rice.

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Disease and insect resistance in hybrid rice

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Reports from China show that the incidences of stem borer (SB), white-backed planthopper, leaf roller, bacterial blight (BB), sheath blight, and virus diseases are more frequent on hybrid rice than on conventional varieties. Local outbreaks of diseases such as downy mildew, false smut, and kernel smut occur frequently on hybrid rice. Brown planthopper (BPH) infestation, however, is negligible when IR26, which possesses a dominant gene *Bph-1* for resistance, is used as the R line in the hybrid combination. Although SB occurs often on hybrids, their growth vigor and higher tillering capacity appear to compensate for damage.

Resistance of either the A or R line appears to adequately confer resistance to the hybrid. Several hybrid combinations derive their resistance to BPH and green leafhopper from either parental line. Resistance to insect vectors extends to protection from virus diseases. Inheritance of resistance to BB and blast is often dominant or partially dominant, indicating the potential to breed resistance into hybrid rice.

Although there is no evidence that susceptibility to any rice pest or disease is related to cytoplasmic factors, pest incidence on hybrid rice should be monitored and male sterile cytoplasm evaluated to ensure genetic strength.

Since the first hybrid variety was developed by Chinese scientists more than a decade ago, hybrid rices have shown 20-3% yield increases over conventional varieties (Virmani and Edwards 1983). In China, the cultivation of hybrid rice has increased exponentially from 150,000 ha in 1976 to 33 million ha in 1985 (Yuan 1985).

When hybrid rice was initially developed, the extent of hybrid vigor for yield increase was the major concern. However, as more ricefields were planted, insect and disease problems increased. Compared with conventional varieties, hybrids apparently are more responsive to fertilizers, more vigorous in growth with higher tillering capacity, and more adaptable to different crop environments. These attributes are closely associated with vulnerability to disease epidemics and insect outbreaks.

As more national programs seek to emulate China's success, resistance will have to be a key component in hybrid combinations.

This paper reviews disease and insect problems associated with hybrid rice, and resistance of hybrids to diseases and insects. Because of China's large-scale, long-term hybrid rice experience, references to insect and disease problems are based primarily on literature published in China.

Insect and disease occurrence on hybrid rice

Crop monoculture and genetic uniformity invite epidemics. Thus it may be a mistake to convert most commercial cultivars to a single source of cytoplasm. How long can a rice hybrid be planted over a wide geographic area before a disease epidemic or insect outbreak halts its cultivation? Reports from China have shown that certain diseases and insects are more prevalent on hybrid rice than on conventional varieties. In Zhejiang Province, in fields planted to late hybrids, bacterial blight, virus diseases, and striped stem borer (SSB) infestations have been more frequent and severe than with conventional varieties (Anonymous 1979). Similar patterns were observed in other provinces (Peng et al 1982, Wang 1981, Wu 1983).

Insects

Stem borers. Rice striped stem borer (*Chilo suppressalis* Walker) is one of Hunan's major insect pests (Tan et al 1983). During the 1970s, the population increased very rapidly and seriously damaged the crop. More importantly, SSB has become a pest, not just of early rice, but of late rice. This parallels spread of hybrid rice with its longer durations. When introduced to an area of early rice, a hybrid extends the crop growth duration to overlap the late rice crop season. In 1979, when the late rice crop was planted after the first crop of hybrid rice, the SSB population was estimated to be 45,900/ha, about 34 times more than in 1974 when hybrid rice was not planted as the first crop.

After hybrid rice was introduced to farmers, the cropping systems changed rapidly. Sowing and transplanting of late rice crop were moved earlier. The seedbed of late rice crop has bridged the second generation of SSB from early rice crop to the late rice crop in the main field.

The density of adult SSB on hybrid rice was 20-35% higher than on conventional varieties, and the egg mass was 107-170% higher. In a survey, more than 50% of SSB-infested plants had 2-10 larvae/plant, a high number rarely observed on conventional indica and never found on japonica grown in the same season in nearby fields (Tan et al 1983).

Others also have reported stem borers (SB) on hybrid rice (Wu 1984, Liu 1979). According to Liu, SB have become a major pest potentially limiting the planting of hybrid rice in many areas.

Despite high insect numbers, hybrids appear to be more tolerant of SB damage than are conventional varieties. Again, Tan and his colleagues (1983) compared Dong-Ting, a late indica rice, with Li-Ming A, a late indica hybrid, on tolerance for SB damage. Whether plants were at heading or ripening stage, the hybrids had higher larvae counts. However, the panicle weight of single tillers of hybrids with SB was 6% less than that of the healthy tiller, but on the conventional, it was 54% of the hybrid. This could be because hybrid rices have a higher tillering capacity and more nodes than conventional varieties. In a survey in Li-Ling county, 83% of the conventional main tillers and 17% of the secondary tillers had deadheart, while on the hybrid 40% of main tillers and 60% of secondary tillers had deadheart.

Planthoppers. Brown planthopper *Nilaparvata lugens* (BPH), whitebacked planthopper *Sogatella furcifera* (WBPH), and green leafhopper *Nephotettix cincticeps* (GLH) are the three major hoppers on hybrid rice. The effect of extended areas of hybrid rice has mixed results on the outbreak of hoppers.

Before Nan-You 6 and Wei-You 6 were planted, BPH outbreaks were recorded in many districts in Hunan in 1979. When the two hybrids were planted, BPH occurrence became negligible (Wu 1985). This is because IR26, the restorer line, possesses dominant gene *Bph-1* for BPH resistance. The resistance was very effective in many farmers' fields.

On the other hand, WBPH has become a major insect pest of hybrids. In the early growth stage, population on hybrids and conventional varieties was not significantly different. In late season, the population was 2 and 11 times higher on 2 hybrids, Shan-You 6 and Shan-You 28, respectively, than on 3 conventional varieties (Cai and Zhong 1980). Proportionally, WBPH was dominant on Shan-You 6, small brown (or gray) planthopper dominated on Shan-You 28, and BPH dominated conventional varieties. Cai and Zhong (1980) found that planthopper populations on hybrids and conventional varieties differed toward the end of the season. Insect incidences were 12% of BPH, 16% of small BPH, and 72% of WBPH on hybrids; on the 3 conventional varieties, BPH incidence was 94%, small BPH 5%, and WBPH 1%.

If resistance is not incorporated in hybrid breeding, WBPH will be a major pest problem. Insecticides are now being used for WBPH control. However, when insect pressure is high, 4 applications at 2-wk interval are needed to keep the insect population below the threshold level in fields where moderate rate of fertilizer is applied. In Jiangsu, in high-fertilizer fields, the insect population would increase to above threshold level toward end of July or early August (Yang and Hu 1984).

Other insect pests. Rice leaffolder *Cnaphalocrocis medinalis* (LF) has also become common on hybrid rice. In Guangdong, infestation was often higher on certain hybrids than on conventional varieties (Wu 1985). In Zhejiang, the adult LF population on hybrids was less than on conventional varieties (Cai and Zhong 1983).

In Jiangsu, planthoppers, LF, SB, and thrips are considered the four major insect pests of hybrid rice (Wang 1982).

Diseases

Two major diseases, bacterial blight (BB) and sheath blight (ShB), threaten the continuous cultivation of hybrid rice in Jiangsu Province, China (Wang 1982). False smut appears increasing in severity in fields for hybrid seed production, and kernel smut is a new problem. In other provinces, blast (Bl) and stem rot have been reported (Cai and Zhong), but the information is not well documented.

Bacterial diseases. BB caused by *Xanthomonas campestris* pv. *oryzae* (Dowson) Dye constitutes the major threat to hybrid rice production in Jiangsu Province. It reduces yields 10-30%. When seedbed is submerged or flooded, kresek incidence is common, sometimes as high as 80% (Chen et al 1980).

The bacterial pathogen can be seed transmitted. Since the cultivation of hybrid rice, hybrid seed is usually produced in Hainan Island in a disease-conducive environment. One month after harvest, the seeds are shipped for commercial planting in Jiangsu. Because of this short time between harvest and planting, the seeds carry a large quantity of inoculum and BB occurs more often (Wang 1983). Furthermore, the rice stubbles from kresek-infected plants and the rhizosphere have carried the inoculum over winter to the following rice crop.

BB is a common disease on hybrid rice in Zhejiang Province (Cai and Zhong 1982).

Fungal diseases. ShB caused by *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris* (Frank) Donk.) is very common in Jiangsu. Annually, the disease affects more than 133,000 ha mostly hybrid, According to Wang (1981), ShB is more severe on hybrids than on other varieties because of the hybrids' luxuriant growth, and a humid microclimate that favors the disease. Hybrid's apparent greater susceptibility to ShB might be partly a response to fertilizer. The incubation period of ShB after inoculation on hybrid rice was 3 d, but 4-7 d on conventional varieties. Furthermore, the lesion number, lesion area, and sclerotia number were all higher on hybrids than on conventional varieties. The effect on grain reduction was 3-4% when the disease was light, 10% when moderate, and 27-29% when severe.

B1 caused by *Pyricularia oryzae* was recently epidemic in the provinces of Guangdong, Fujian, Zhejiang, and Sichuan. The epidemics are closely related to monoculture of a single hybrid over wide geographic regions. In Sichuan, for instance, G group was the predominant race in 1978. Its virulence to the then popular hybrid Shan-You 2 was weak. The area planted to this hybrid increased every year, and in many districts Shan-You 2 was the only rice being cultivated. The B group race which is highly virulent to Shan-You 2 emerged from the *P. oryzae* population. The distribution frequency of the B race was 66.7% in 1983 and 94.1 % in 1984. The resistance of Shan-You 2 was matched, producing the epidemic in 1984. Shan-You 6 in Li-shue district of Zhejiang Province and Shao-Kun district of Guangdong, which was resistant to B1, was overcome by matching races group B and C evolving rapidly in 1982 and 1983 and became susceptible. Similar situations were reported in Hunan and Fujian. To control B1, the current solution is to plant hybrids resistant to races B and C. In Shao-Kun, Guangdong, farmers plant Shan-You 36 and Shan-You 63, resistant to the B races, and the disease is under control.

False smut caused by *Ustilaginoidea virens* (Cke.) Tak. strikes mid and late rice crops. Farmers normally have associated it with a good crop and the disease seldom affected grain yield. However, since the planting of hybrid rice, the disease has gradually increased (Wang 1981). Based on the survey in Jiangsu, panicle infection ranged from 10 to 50% and occasionally 100%. Some panicles had 120 spore balls compared to 1 or 2 in the past.

Kernel smut is caused by *Tilletia barclayana* (Bref.) Sacc. & Syd. The disease was occasionally observed on mid and late rice in Jiangsu. Since the extensive cultivation of hybrid rice, the disease often occurs in seed production fields. According to the survey (Wang 1981), kernel smut primarily infected the maternal plants of the hybrid combination. In 1980 in Kan-yu county, in 174 ha of seed production fields, about 40% of the maternal plants were infected; infection reached 80% in some fields. Maternal plants were usually shorter than paternal plants, suggesting it is easier for fungal spores to land on stigmas of maternal plants. Susceptibility appears related to a hybrid's flowering characteristics. During flowering, if a hybrid variety has a wider opening angle of lemma and the anthesis duration is longer, the exposed stigma becomes more vulnerable to infection. V41A and Zhen-Shan 97A belong to this group.

Downy mildew caused by *Sclerophthora macrospora* (Sacc.) Thirum., Shaw & Naras is not considered a major disease of rice. The disease has been reported in Japan, Italy, Australia, China, and USA (Ou 1985). Usually the disease does little damage. Recently, hybrid rice in Jiangsu suffered more infection than previously known in conventional varieties (Fu 1984). A 16-21% grain yield reduction has been recorded. In hybrid seed production, as high as 40% of seedlings were badly damaged by the disease, prohibiting transplanting (Fu 1984). The disease can be minimized by water management, i.e., avoiding submerging seedlings at 2-leaf stage. No hybrid combinations tested seem resistant to the disease.

Virus and viruslike diseases. Three major viruses or viruslike diseases appear to be more severe on hybrid rice than on conventional varieties (Anonymous 1979). These are yellow dwarf (caused by mycoplasm), dwarf (virus), and yellow stunt (virus). They usually strike hybrid rice 10 d earlier than conventional varieties. Yellow dwarf is more common. Yellow dwarf and dwarf infections begin in seedbeds.

Damage is greater on late crop hybrid rice, because it is sown and transplanted when the regular late rice crop is tillering and there is a heavy migration of vectors. GLH was detected on the hybrid rice immediately after transplanting. Resistance of hybrid rice to GLH was similar to or lower than that of conventional varieties. Because of hybrids' growth vigor and good tillering capacity, even infected plants could reach heading and produce grains.

Insect resistance and virus resistance

The insect density varies on different hybrids (Peng et al 1979). Si-You 2, Si-You 4, Si-You 6, all having V41A as maternal line, were resistant to GLH. CMS lines V20A, Chao-Yang 1 A, Gung-Chao A, Zhen-Shan 97 A, Li-Ming A, Gung-Zai-Zhan A, and Jin-Nan-Te 43A, and the restorer lines IR26, IR24, Gu 154, Zhao-Hui 1,Ze-Ye-Qing 8, Gu 223, and Padi are susceptible to GLH. Therefore their hybrid combinations are also susceptible to GLH.

Reaction of CMS and restorer lines also varied for BPH (Huang and Li 1980, Peng et al 1979). Wei-You 6, Shan-You 6, and Zhen-Shan 97A/ Wen-Hui 1 were resistant to BPH in both greenhouse and field tests (Huang and Li 1980).

Wen-Hui 1, a restorer line, is highly resistant to BPH. Its hybrid combination with Zhen-Shan 97A, the male sterile line, also showed high resistance. Wen-Hui 1 was selected from the cross Ho-Zin-Gu/ Jin-Mei. Among the popular commercial hybrids, Shan-You 6 and Wei-You 7 expressed high resistance while Shan-You 2 was susceptible to BPH.

V41A as CMS line and its hybrids Si-You 2 and Si-You 4 were susceptible to BPH. The restorer IR26 and its hybrids Nan-You 6 and V-You 6 were susceptible to GLH but resistant to BPH. Only Si-You 6, a hybrid of CMS line V41A and restorer IR26, was resistant to both GLH and BPH. It is obvious that the GLH resistance gene of V41A and BPH resistance gene *Bph-1* of IR26 are dominant and also independently segregated. The hybrid has demonstrated the heterosis of both parents (Table 1).

Gu 154 (a restorer line) and its hybrid combinations were susceptible to GLH, but resistant to both dwarf and yellow dwarf. V41A and its hybrids, except Si-You4, are resistant to GLH but susceptible to virus diseases. These results clearly show that resistance to GLH is different from that to viruses (Table 2). However, field surveys also indicate that, as a male sterile line, V41A combinations all possess high field resistance (Peng 1982). Apparently, V41A's field resistance to virus diseases is related to its resistance to GLH, a result of both antibiosis and nonpreference.

Response of hybrids to BPH directly relates to genes for resistance. In those hybrids resistant to BPH, either the male sterile lines or restorer lines are resistant. Table 3 shows such combinations.

The resistance mechanism of the hybrids appears to be determined by the CMS/ restorer lines (Table 4): Most hybrids resistant to BPH and GLH were found due to antibiosis while those resistant to WBPH were determined by tolerance (Wu 1986).

Table 1. Resistance of hybrids to GLH and BPH in relation to resistance of the cytoplasmic male sterile and restorer lines (modified after Peng 1982).

Hybrid	Reaction to		CMS/restorer	Reaction to[a]	
	GLH	BPH		GLH	BPH
Si-You 2	HR	S	V41 A/IR24	HR/S	S/S
Si-You 4	HR	S	V41 A/Gu 154	HR/S	S/S
Si-You 6	HR	MR	V41 A/IR26	HR/S	S/R
Nan-You 6	S	R	Er-Jiu-Nan A/IR26	S/S	S/R
V-You 6	S	R	V20 A/IR26	S/S	S/R

[a]Reaction of CMS/restorer. HR = highly resistant, R = resistant, MR = moderately resistant, and S = susceptible. IR26 possesses *Bph-1* gene from Mudgo for BPH resistance.

Table 2. Relation of vector (green leafhopper) resistance to virus (yellow dwarf and dwarf) reaction of hybrids (modified after Peng 1982).

Hybrid	Disease incidence (%)	Reaction[a] to GLH	CMS/restorer	Reaction[a] of CMS/Restorer to GLH
Si-You 4	2.5	HR	V41 A/Gu 154	R/S
Nan-You 4	2.6	S	Er-Jiu-Nan A/Gu 154	S/S
Shan-You 4	3.9	S	Zhen-Shan 97 A/Gu 154	S/S
V-You 4	6.3	S	V20 A/Gu 154	S/S
V-You 6	31.8	S	V20 A/IR26	S/S

[a]HR = highly resistant, R = resistant, S = susceptible.

Table 3. Relation of vector (green leafhopper) resistance to virus reaction of hybrids (modified after Peng 1982).

Hybrid	Disease incidence (%)	Reactions to GLH	CMS/Restorer	Reactions of CMS/Restorer to GLH
Si-You 4	2.5	HR	V41 A/Gu 154	R/S
Nan-You 4	2.6	S	Er-Jiu-Nan A/Gu 154	S/S
Shan-You 4	3.9	S	Zhen-Shan 97 A/Gu 154	S/S
V-You 4	6.3	S	V20 A/Gu 154	S/S
V-You 6	31.8	S	V20 A/IR26	S/S

[a]HR = highly resistant, R = resistant, S = susceptible.

Disease resistance

Little is known about disease resistance of hybrids. Wang and his colleagues (1985) in Jiangsu tested 33 hybrid combinations and identified no combination showing resistance to BB. However, Shan-You 6, Nan-You 6, and Wei-You 6 were moderately resistant. A new restorer line, No. 2317-18, when combined with CMS lines Zhen-Shan 97A, Yun-Eng-Chao, and Er-Jiu-Nan A, developed hybrids Shan-You 18, Tan-You 18, and Nan-You 18 resistant to both bacterial blight and blast. In Guangshi Province, new restorer lines No. 30-S and Gui 33 had resistance to BB comparable to that of IR26 (Sun et al 1984).

Because most resistance to diseases is controlled by dominant or partially dominant genes, it is possible to select appropriate parents for CMS and restorer lines for disease resistance in hybrids. Because hybrid rice uses the F_1 hybrid vigor, its resistance expression is very closely related to resistance of parents. Studies in Jiangshi and Jiangsu Provinces have shown that inheritance of resistance to BB is either dominant or incompletely dominant (Wang 1982). Further, IR26, IR28, and other IR lines which carry the dominant gene *Xa-4* are widely used as restorer lines. Hybrids involving IR26 or IR28 should be resistant to BB. Other dominant genes are being identified (Khush and Virmani 1985, Mew and Khush 1981), and could be transferred to CMS or restorer lines or both. In resistance to BB, pathogen variation is important. In the tropics, strong interactions between pathogen isolates and rice varieties differing in resistance have been demonstrated (Mew and Vera Cruz 1979, Mew et al 1982, Horino et al 1984). The same phenomenon has been shown in China (Fang et al 1981). For instance, Nan-Geng 15 was susceptible to Yan-Cheng isolate KS-8-4, but resistant to Yang-zhou isolate KS-6-6, while Kinmaze was susceptible to KS-6-6 but resistant to KS-8-4.

Pathogen variation of *Pyricularia oryzae* is well known. In B1, therefore, many genes control resistance to the disease (Kiyosawa 1982). Tetep carries several genes for resistance (Yu et al 1986), and could be used in F_1 hybrids. However, *P. oryzae* is highly variable. As mentioned, the shift in virulence of the pathogen population is also relatively rapid (Wang 1985).

So far, no released hybrid has shown particular resistance to ShB, compared with conventional varieties. Since the inherent level of resistance to ShB is low, and

Table 4. Resistance of selected commercial hybrids to brown planthopper (BPH) and whitebacked planthopper (WBPH) (modified from Wu 1986).

Hybrid	Reaction[a] to		CMS/restorer	Reaction[a] of CMS/restorer to	
	BPH	WBPH		BPH	WBPH
Shan-You 2888	8	9	Zhen-Shan 97 A/No. 2888	S/S	S/—
Qing-You-Zao	9	6	Qing-Si-Ai A/Hong-Mei-Zao	S/S	S/—
Shan-You 23	9	5	Zhen-Shan 97 A/No. 2317	S/S	S/—
Shan-You 36	9	4	Zhen-Shan 97 A/IR36	S/R	S/S
Shan-You 32	8	6	ZhenShan 97 A/IR32	S/R	S/S
Shan-You 64	2	2	ZhenShan 97 A/Ce 64	S/R	S/MR
Wei-You 98	6	2	V20 A/98	MR/–	S/–
Wei-You 64	1	2	V20 A/Ce 64[b]	MR/R	S/MR
Wei-You 35	1	2	V20 A126 Zhai Zao	MR/R	S/S
TN1	9	9	Check	S	S
Mudgo	1	–	Check	R	R
N22	–	2	Check	–	R
Colombo	1	1	Check	R	R

[a]Evaluated by the modified seedling bulk test. 1 = highly resistant, 9 = highly susceptible. R = resistant, MR = moderately resistant, S = susceptible.
[b]IR9761-19-1-64.

assuming such low level of resistance is quantitatively inherited, F_1 hybrids could express the resistance if resistance of CMS and restorer lines is properly developed. In the US., hybrid rice has been more resistant to ShB than have conventional varieties (Calub and Norcio-Locker 1985).

As mentioned, hybrid rice in China seems susceptible to “minor” diseases such as downy mildew, false smut, and kernel smut. Most researchers attribute susceptibility to the hybrid’s plant characters, such as grain size and number related to false smut. On kernel smut, however, the CMS lines appear more susceptible. Whether this relates to any cytoplasmic factor for susceptibility is not known. Among the CMS lines, V41A was most susceptible, followed by Zhen-Shan 97A, then Er-Jiu-Nan A, with disease incidence of 95% (grain infection 70-80%), 62-65% (grain infection 12-13%), and 65% (grain infection 5.2%), respectively. However, disease incidence varied among hybrid combinations—Si-You4 was most susceptible with 73.3% incidence (infected grain: 4.9%) while others had less than 1%.

Potential cytoplasmic susceptibility to rice pests

The southern leaf blight epidemic on hybrid corn in the U.S. in 1969-70 showed the potential danger of basing a crop on a single source of cytoplasm. The T-cytoplasm for hybrid corn production constituted the dominant parameter in the epidemic of leaf blight caused by *Helminthosporium maydis.*

In rice, four cytoplasm male sterility types have been identified (Yuan 1985, Gao 1980). WA (wild abortive) is derived from a wild rice *Oryza sativa* f. *spontanea* L. At present, 200 MS lines have been developed from WA through successive backcrosses and wide testcrosses. CMS lines Zhen-Shan 97A, V20A, and V41A are widely used, dominating hybrids in South China.

A second type of cytoplasmic male sterility is GAW, derived from a late-maturing indica variety originating in Gambia. In 1985, its hybrid combinations were grown in more than 100,000 ha in Sichuan Province. The third male sterile cytoplasm is the CMS-boro or BT type developed in Japan, used in developing japonica hybrids. The fourth male sterile cytoplasm, Qing-Si-Ai A, is anindica type. Unlike the former three which are sporophytic, Qing-Si-Ai A exhibits gametophytic male sterility. Its hybrid combinations dominate in Guangdong Province.

We have no knowledge of how cytoplasmic factor is related to susceptibility of rice pests and diseases. Hooker (1974) indicated that cytoplasmic susceptibility to disease is rare but important when it occurs. To link cytoplasm to the inheritance of pest susceptibility, plant types must be compared that differ in cytoplasms but have identical nuclear genotypes (after Hooker 1974).

Hooker and his associates tested several maize cytoplasms for reaction to diseases (Hooker 1972) after the epidemic of corn leaf blight. Numerous disease tests were made on seedlings in the greenhouse and on plants in the field. No cytoplasm effect showed for most diseases. However, four cytoplasms conditioned susceptibility to *Phyllosticta maydis* and *H. maydis* race T. Leaf blight also predisposed maize plants to root and stalk rots. Hence, secondary effects of plant cytoplasm on other diseases may contribute to crop loss.

Rice hosts 40 fungal, 20 viral, 12 bacterial, 8 nematode, and 2 mycoplasmalike pathogens. Among them, 20 cause crop damage of economic importance in certain rice culture types and climatic and geographical environments. Also, races of pathogens differ in different localities (Ou 1985). In addition, more than 100 species of insects attack rice and 20 are of economic importance (Khush 1984). CMS cytoplasm must be evaluated for pest resistance to ensure the genetic strength of hybrid rice.

Hooker (1974) indicated several experimental tests available to detect cytoplasmic inheritance. One preliminary test is to make reciprocal crosses between two parents that differ in the expression of the resistance. Another is to substitute the genome of one plant into the cytoplasm of another by backcrossing, or to transfer by repeated backcrossing a series of genetically different nuclei from various plants to the common cytoplasm of one plant.

Today, molecular genetics permits direct analysis of the mitochondrial DNA to distinguish CMS from normal cytoplasm (Hanson and Conde 1985).

It is premature to speculate if susceptibility to any rice disease or insect pest is cytoplasmic in inheritance. Rath and Padmaaabhan (1972) found differences between reciprocal crosses in rice to two races of *Pyricularia oryzae,* and interpreted them as cytoplasmic effects. Hooker (1974) pointed out, however, that the variance values for disease reaction, lesion type, and lesion number for each of several crosses and reciprocal crosses showed no consistent pattern for variance size or maternal effects for lesion number, lesion type, or reaction to the two pathogen races of the different crosses.

Because some insect pests or diseases occur more often on hybrids than on regular varieties, testing for cytoplasmic inheritance to susceptibility is essential. Plants with male sterile cytoplasm and normal cytoplasm should be tested for pest resistance. Because races of the pathogens differ in different regions or countries, evaluation of CMS lines for pest resistance needs international coordination.

When the corn hybrid with the T-cytoplasm was found susceptible to isolates of *Helminthosporium maydis* in the Philippines (Villareal and Lantican 1965), no one paid much attention. When an oat cultivar Victoria and derivatives of Victoria carrying the gene for resistance to all races of crown rust were widely planted, it succumbed to *Helminthosporium victoria* arising from wild grasses (NAS 1972).

Summary

Crop monoculture and genetic uniformity invite disease epidemics and insect outbreaks. Reports in China have shown that incidences of SB, WBPH, leaf rollers, BB, ShB, and virus diseases are more frequent on hybrid rice than on conventional varieties.

The reports of normally minor diseases, such as downy mildew, false smut, and kernel smut deserve close attention.

On the other hand, BPH infestation becomes negligible when IR26, a restorer line, is used in the hybrid combinations. IR26 possesses a dominant gene *Bph-1* for BPH resistance.

Although SB occur often on hybrid rice, growth vigor and higher tillering capacity appear to compensate for damage compared to infestation on traditional varieties.

Apparently, resistance of either the CMS or restorer line adequately confers resistance to the hybrid. Several hybrid combinations derived their resistance to BPH and GLH from either parental line. Resistance to insect vectors also extends protection to virus diseases.

With BB and B1, inheritance of resistance very often is either dominant or incompletely dominant, indicating potential to breed resistance into hybrid rice.

So far there is no evidence of cytoplasmic factor to susceptibility of any rice pests and diseases. Even so, care should be taken to monitor pest incidence on hybrid rice and to evaluate CMS cytoplasm for pest resistance to ensure the genetic strength of hybrid rice.

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Grain quality of hybrid rice

G. S. Khush, I. Kumar, and S. S. Virmani

Grains harvested from commercial F_1 rice hybrids represent F_2 seed generation and vary in grain characteristics. We studied the effect of parental differences on grain quality of the derived hybrids.

Higher head rice recovery was obtained mostly from crosses of parents with good head rice recovery. Physical properties like differences between parents in length, breadth, shape, and weight of grain do not pose problems because seeds borne on hybrid plants do not vary from each other. Rather, hybrids with desired physical properties of grain can be produced by selecting the parents of desired grain size. Crosses among parents of different endosperm appearance, on the other hand, produced grains with different types of endosperms in the bulk sample from the hybrid plants. Such variation in grain appearance would affect market acceptability. The use of a waxy or dull endosperm parent with a translucent parent should be avoided.

When parents differed widely in amylose content, the F_2 single grains were clearly classifiable into two, three, or four categories. However, segregation for amylose content did not hurt cooking and eating quality of hybrid grains. Similarly, different classes for gelatinization temperature in low/intermediate, low/low, or intermediate/intermediate crosses did not produce detectable differences in cooked rice samples. With appropriate selection of parents, the hybrids with desired tenderness, cohesiveness, and aroma can be produced to meet consumer preferences.

The success of hybrid rice in China has encouraged breeders all over the world to explore developing hybrids for their countries. Hybrids have shown a yield advantage of about 20% over conventional varieties (Lin and Yuan 1980, Virmani et al 1981). The F_1 hybrids also seem superior in adaptability and various agronomic features. To develop F_1 rice hybrids, various cytoplasmic male sterile lines, and their maintainers and restorers have been identified in China, IRRI, and elsewhere. These parents differ widely from one another in their agronomic characteristics and grain properties. In general, the extent of heterosis for agronomic traits depends on genetic diversity of parents. Although F_1 plants are uniform, seeds borne on them represent F_2 seed generation and are expected to segregate for some grain characteristics. We must, therefore, consider the effect of this segregation on grain quality.

Major determinants of grain quality in hybrid rice are 1) milling and head rice recovery; 2) size, shape, and appearance; and 3) cooking and eating characteristics. Consumer acceptance of grain quality of F_1 rice hybrids will have an important bearing on the adoption of this technology.

Milling and head rice recovery

Unless hybrids have milling quality equal to or higher than either parent, the full advantage of their high yielding ability cannot be realized. Head rice, the proportion of whole milled grains from a unit weight of rough rice, is commercially vital. If a hybrid has a higher broken-grain percentage, its marketability will be reduced. Head rice recovery is a genetic character, although it is affected by environment during grain ripening, postharvest handling, and storage. High head rice recovery is also associated with hardness, absence of chalkiness, and grain size and shape.

Rutger and Bollich (1985) reported low milling recovery as the most significant deficiency of rice hybrids introduced from China and USA. They attributed this deficiency to cytoplasmic male sterile (CMS) lines. In our study of 75 hybrids, heterobeltiosis for head rice recovery ranged from –67% to +49%. Of the 75 hybrids tested, head rice recovery was lower than that of the better parent in 34 crosses and higher than that of the better parent in 41 crosses. Some experimental hybrids with better head rice recovery are listed in Table 1. The highest head rice recovery (65.1%) was obtained from a cross of parents having 57.4 and 5 1.4% head rice recovery. This study and others indicate that hybrids with higher head rice recovery can be obtained if the parents are selected carefully. If either parent has a higher tendency for grain breakage, the F_1 hybrids would normally give lower head rice recovery than the better parent.

Size, shape, and appearance of grains

Grain size, shape, and uniformity

Rice grain is marketed according to its size and shape. Uniformity in physical dimensions such as grain length, breadth, shape, and weight is of prime importance. Grain length is controlled by one gene (Ramiah et al 193l), two genes (Bollich 1957), three genes (Ramiah and Parthasarathy 1933), or polygenes (Chang 1974, Mitra 1962, Nakatat and Jackson 1973, Somrithet al 1979). Similarly, breadth, shape, and

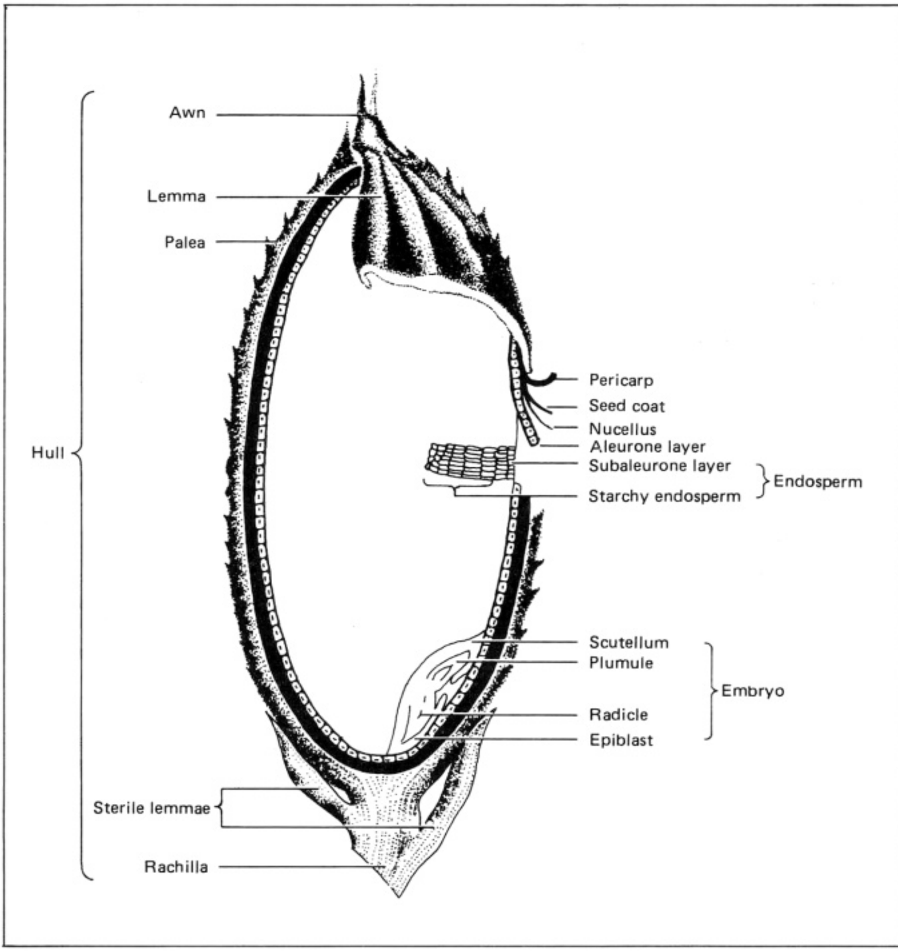
Table 1. Experimental hybrids with high milling and head rice recovery.

Hybrid	Milling yield (%)	Head rice (%)	Heterobeltiosis (for head rice recovery) (%)
IR19647-27/1R36	71	61	6
IR21845-90-3-1/IR21912-9-2	67	57	13
IR21845-90-31/IR1129-749-3-3	68	58	24
IR19746-27-3-3/IR2797-105-2-2	68	64	32
IR19746-27-3-3/IR46	67	62	7
IR19746-27-3-3/IR21526-4-33	70	65	13
IR19746-27-3-3/IR19661-63-1-2-3	68	60	5
MR365/IR36	67	50	49
IR17525-27-1-1/IR19058-107-1	70	60	12
IR19746-27-33/IR183-22-1-2	68	64	4

grain weight have been reported to be polygenic in inheritance (Chang 1974, Lin 1978, Nakatat and Jackson 1973, Ramiah and Parthasarathy 1933, Somrith et al 1979).

Some rice grain tissues are of maternal origin and some result from fertilization and union of genetically diverse gametes (Fig. 1). The lemma and palea of the rice hull are maternal tissues. Seed size and shape are determined by shape and size of hulls. Since genetic segregation for shape and size of hulls of spikelets borne on the F_1 plants does not occur, all F_2 seeds have identical dimensions even though the parents' grain may differ greatly in shape and size.

In general, the length and shape of F_1 grains are between those of the parents. In a study of 75 hybrids, 25% had longer grain, 37% shorter, and 38% almost the same as that of the midparent value. Grain shape trend was similar. Grain borne on F_1



1. The various parts of a rice grain. Some tissues are of maternal origin and some result from the union of diverse gametes.

plants never exceeded the long, slender-grained parent either in grain length or shape. Therefore, to develop medium-grain hybrids, parents having long and short grains may be used, but to produce long-grain hybrids, both parents must have long slender grains.

Table 2. Endosperm characteristics of varieties and breeding lines used for studying endosperm translucency of hybrids.

Parent	Endosperm appearance	Av amylose content (%) mean	Gel temperature
IR29	Opaque	0.0	Low
IR37307-8	Dull	7.4	Low
IR24	Translucent	15.1	Low
IR 3351 -38	Translucent	12.6	Low
BPI 121-407	Translucent	24.9	Low
IR24632-34	Translucent	21.8	Low
IR8	Translucent	27.3	Low

Table 3. Endosperm characteristics of F_1 and F_2 seeds of crosses of lines and varieties with IR29.

Cross	Endosperm appearance [a]	Amylose content (%)	
		F_1 seeds	F_2 seeds
IR29/IR37307-8	W	1.3	4.2
IR37307-8/IR29	W	3.0	3.1
IR29/IR24	W	–	1.5
	H	5.6	8.6
	T	–	13.9
	F_2 bulk	–	9.6
IR24/IR29	W	–	0.4
	H	–	7.8
	T	12.2	14.9
	F_2 bulk	–	9.6
IR29/BPI 121-407	W	–	1.0
	T	22.9	23.5
	F_2 bulk	–	17.6
BPI 121-407/IR29	W	–	0.5
	T	22.4	24.6
	F_2 bulk	–	18.5
IR29/IR8	W	–	1.4
	T	23.9	28.0
	F_2 bulk	–	20.5
IR8/IR29	W	–	0.9
	T	27.5	25.8
	F_2 bulk	–	19.7

[a] W = waxy, H = hazy, T = translucent.

Endosperm translucency

The endosperm is triploid tissue from the union of one male nucleus with two female nuclei (polar nuclei). If the parents differ in endosperm translucency, the F_2 grains show clear-cut segregation (Kumar and Khush 1986).

Based on endosperm opacity, the rice endosperm is classified as waxy or nonwaxy. Waxy rices are devoid of or have only traces of amylose content and are opaque. Nonwaxy rices have varying amylose levels (2.1-32%) and are dull, hazy, or translucent.

We crossed parents having different endosperm appearance (Table 2). The phenotypic appearance of endosperm of seeds borne on hybrid plants varied in crosses involving waxy or dull endosperm parents on one hand, and translucent endosperm parents on the other (Table 3, 4, 5). The F_2 seeds of hybrids between waxy and low-amylose translucent parent, IR24, could be classified into 3 categories: waxy, hazy, and translucent (Fig. 2), in 1:1:2 ratio. In crosses of waxy parent with intermediate- or high-amylosecontent parents, 2 classes with waxy and translucent endosperm were observed in a ratio of 1:3 (Fig. 3, 4). All these classes were phenotypically distinct.

When dull endosperm parent IR37307-8, having very low amylose content, was crossed with low-amylose-content variety IR24, it produced 3 distinct categories of

Table 4. Endosperm characteristics of F_1 and F_2 seeds of crosses of varieties and breeding lines with IR37307-8.

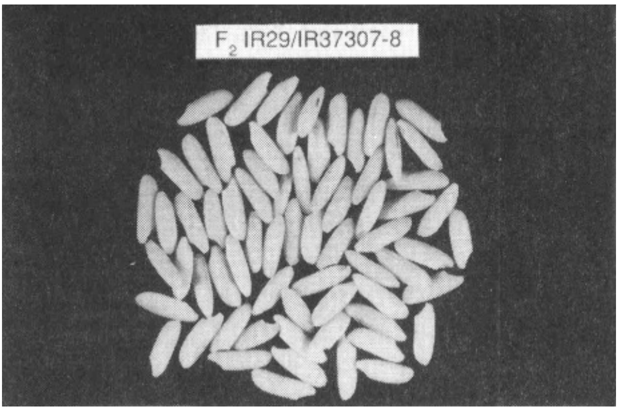
Cross	Endosperm appearance[a]	Amylose content (%)	
		F_1 seeds	F_2 seeds
IR24/IR37307-8	D	–	6.6
	H	–	9.4
	T	12.9	12.8
	F_2 bulk		10.6
IR37307-8/IR24	D	6.9	4.7
	H & T	–	11.6
	F_2 bulk	–	9.5
IR37307-8/BPI 121-407	D	13.9	6.6
	H	–	13.0
	T	–	19.9
	F_2 bulk –		14.8
BPI 121-407/IR37307-8	D	–	5.8
	H	–	11.3
	T	18.6	18.0
	F_2 bulk	–	13.0
IR37307-8/IR8	D	–	6.4
	T	23.5	25.1
	F_2 bulk	–	20.3
IR8/IR37307-8	D	–	8.6
	T	26.9	26.1
	F_2 bulk	–	22.1

[a]D = dull, H = hazy, T = translucent.

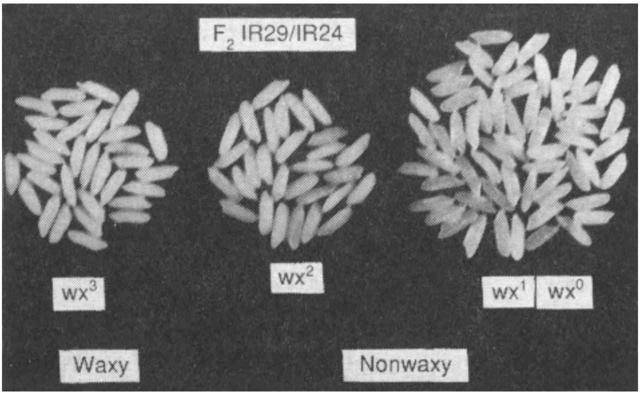
Table 5. Endosperm characteristics of F_1 and F_2 grains of crosses among varieties and breeding lines having translucent grains.

Cross	Endosperm appearance [a]	Amylose content of F_1 seeds (%)	Amylose content of F_2 bulk seeds (%)
IR24/BPI 121-407	T	27.2	22.7
BPI 121-407/IR24	T	24.3	21.3
IR24/IR8	T	28.0	24.6
IR8/IR24	T	25.2	23.8
BPI 121-407/IR8	T	26.4	27.2
IR8/BPI 121-407	T	29.3	25.2
IR3351-38/1R24	T	9.4	14.2
IR2/IR3351-38	T	14.1	14.2
IR24632-34/BPI 121-407	T	22.8	23.9
BPI 121-407/IR24632-34	T	23.6	25.4

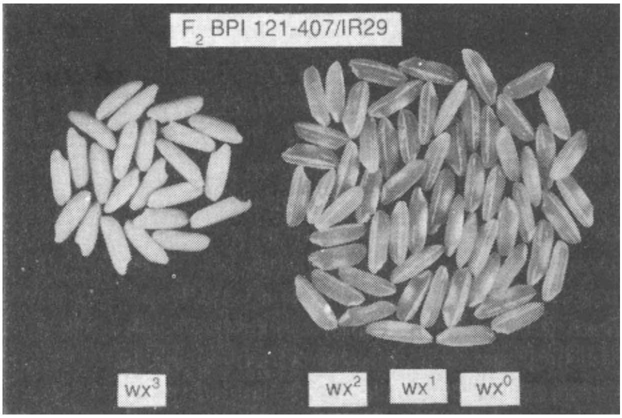
[a] T = translucent.



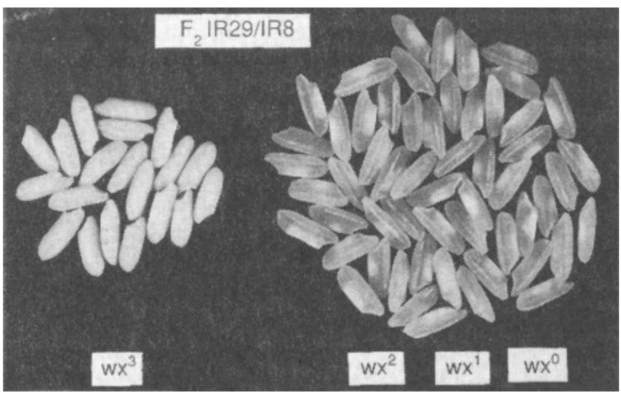
2. Endosperm appearance of F_2 seeds of cross IR29/IR37307-8.



3. Endosperm appearance of F_2 seeds of cross IR29/IR24.



4. Endosperm appearance of F_2 seeds of cross IR29/BPI 121-407.



5. Endosperm appearance of F_2 seeds of cross IR29/IR8.

F_2 seeds having dull, hazy, and translucent endosperm (Fig. 5) in a ratio of 1:1:2. F_2 seeds of the cross between IR37307-8 and BPI 121-407, a translucent variety with intermediate amylose content, similarly differed in amylose content (Fig. 6).

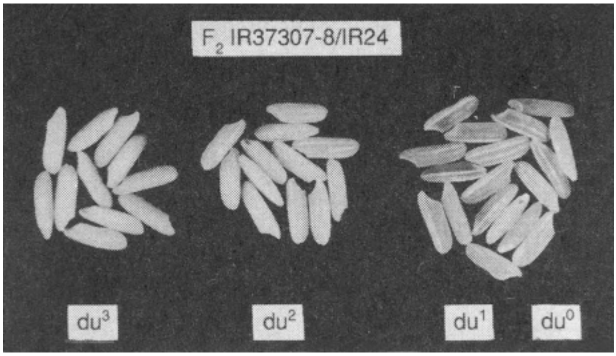
The cross between IR37307-8 and the high-amylose translucent variety, IR8, produced dull and translucent F_2 (Fig. 7) in a ratio of 1:3.

In crosses between dull and waxy parents, no distinct segregation for endosperm appearance was evident (Fig. 8) because of parental similarity. In crosses between translucent endosperm parents, all seeds had translucent endosperm.

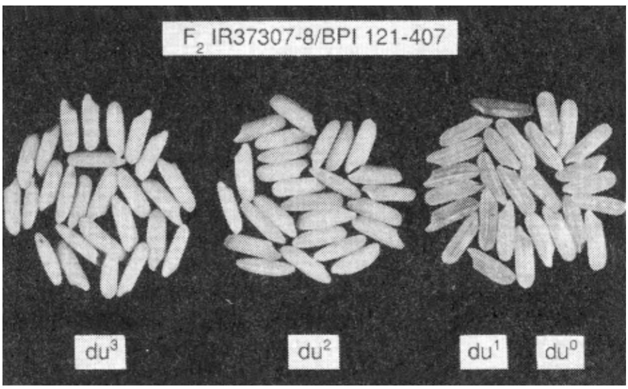
To obtain hybrids with uniform grain appearance, both parents should be of the same endosperm type.

Chalkiness

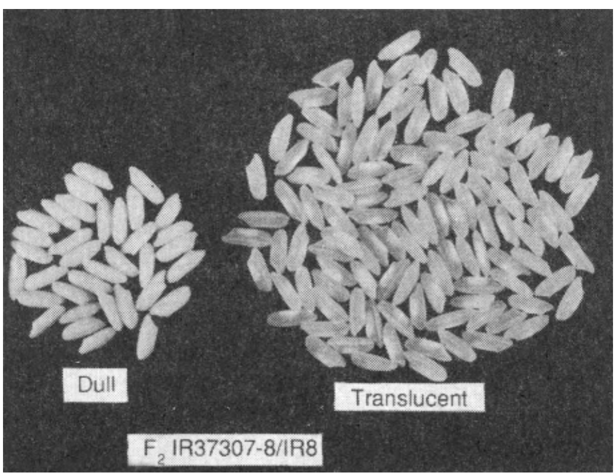
Endosperm appearance also varies by degree of chalkiness—white center, white belly, or white back. White centers and white bellies are reported to be governed by a single recessive gene (USDA 1963). White bellies are also reported to be controlled



6. Endosperm appearance of F_2 seeds of cross IR37307-8/IR24.



7. Endosperm appearance of F_2 seeds of cross IR37307-8/BPI 121-407.



8. Endosperm appearance of F_2 seeds of cross IR37307-8/IR8.

by a dominant gene (Chalam and Venkateswarlu 1965, Nagai 1958) and by polygenes (Nakatat and Jackson 1973, Somoto and Hamamura 1973, Somrith et al 1979). Chalkiness has been reported to be due to loose packing of starch granules (Ando and Ichikawa 1974, Blakenley 1980, Del Rosario et al 1968, Evers and Juliano 1976, Tashiro and Ebata 1975). Chalky grains are more likely to break during milling. Of 75 hybrids involving 18 parents, 44 hybrids had less chalkiness, 15 had more, and 16 had the same as the midparent value. Thirteen hybrids showed better translucence than their better translucent parent.

Grain chalkiness may reduce market acceptability and milling recovery of hybrid grains. However, a low proportion of chalky grains does not improve cooking and eating characteristics, because cooking hides chalkiness. For better grain quality, the parents of hybrid varieties should be free from chalkiness.

Pericarp color

Pericarp is a maternal tissue. Colored pericarp (red or purple) is a dominant trait. If one parent has colored pericarp, all F_2 seeds should have colored pericarp. Because few consumers accept colored pericarp rice, parents with colored pericarp should not be used in developing hybrid rice.

Cooking and eating characteristics

The cooking and eating quality of milled rice is related to starch properties such as amylose content, gelatinization temperature (GT), gel consistency (GC), and aroma.

Amylose content

Amylose content largely determines texture of cooked rice.

We crossed parents of different amylose levels (Table 2) to evaluate the F_2 grains for their means and variations in amylose content and effects on eating quality.

The mean amylose content of F_2 bulk seed samples was between that of the parents in all crosses. In general, the F_2 mean was lower than the F_1 mean, particularly in crosses where gene dosage effects for amylose content were either absent or very small. In crosses where dosage effects were present, the F_2 mean was close to the mean of the parents and F_1s (direct and reciprocal).

The mean amylose contents of phenotypic categories of seeds, obtained in crosses involving IR29 or IR37307-8, are given in Tables 3 and 4.

The mean amylose contents of categories in a cross differed greatly from each other. In crosses involving IR29, the waxy, hazy, and translucent categories obtained in F_2 had amylose content in ascending order. Similarly, in crosses with IR37307-8, significant differences for amylose content were observed in ascending order for dull, hazy, and translucent seeds.

In the crosses involving parents with greater differences in amylose content and lack of gene dosage effects (particularly the ones involving IR8), the F_2 seeds could be classified into 2 categories (waxy/ translucent or dull/ translucent) in a ratio of 1:3. In crosses like IR29/IR24 and IR37307-8/BPI 121-407, where the gene dosage effects for amylose content were present, 4 categories of seeds having 0, 1, 2, or 3 doses of the gene for higher amylose content were observed in F_2 seeds in a 1:1:1:1 ratio. In the crosses IR29/IR37307-8 and TR37307-8/IR24 with little difference in amylose content of parents, a unimodal distribution curve for amylose content indicated lack of distinct categories.

The more parents differed in amylose content, the more F_2 seeds varied. Amylose content ranged from 0 to 11% in F_2 seeds of cross IR29/IR37307-8 and from 0 to 33% in IR29/IR8. Similarly, F_2 seeds of IR37307-8/1R24 ranged 1-18%. In IR37307-8/IR8 and its reciprocal, amylose varied from 2 to 34%. The difference in amylose content between various categories (waxy, very low, low, intermediate, and high) has been found to be governed by one major gene (Kumar and Khush 1986a,b,c; Kumar et al 1987).

Variance was calculated for each phenotypic category. Variance within each category was always lower than that of all F_2 seeds (Table 3,4). Thus, high variance in the bulk sample was primarily due to greater differences in amylose content between categories and not to variation within categories.

The F_2 seeds from the crosses involving translucent endosperm parents, however, had no recognizable classes of endosperm appearance. The variance in bulk F_2 seeds was much lower in crosses involving parents with lesser difference in amylose content than those of widely different parents. For example, in the cross IR24/ BPI 121-407 or its reciprocal, the variance was almost half that of IR24/IR8, although a bimodal distribution was observed in both crosses. Similarly, in F_2 seeds of cross between intermediate- and high-amylose-content parent, the variance was much less and the frequency curve showed a unimodal distribution pattern for amylose content. In crosses involving parents IR24 and IR3351-38 or IR24632-34 and BPI 121-407, a unimodal distribution pattern, and much less variation in F_2 grains was observed for amylose content (Table 5). These parents differ little in amylose content.

Cooking characteristics of bulk F_2 seeds

The bulk F_2 seeds of different crosses and their parents were cooked in an electric rice cooker and served to a taste panel for evaluation (Table 6). Scores for characteristics are given in Table 7.

The gloss of cooked bulk F_2 sample was intermediate to high if one parent was glossy. Similarly, glossiness was greater if the difference in amylose content of glossy parent and nonglossy parent was low. For example, the cooked F_2 grains of crosses IR29/IR37307-8 and IR29/IR24 had more gloss than those of IR29/BPI 121-407 and IR29/IR8. The gloss of F_2 sample thus seems to depend on amylose content of parents.

Tenderness and cohesiveness of cooked rice also varied according to parents' amylose content, and always was intermediate between that of parents. Cooked F_2 grains from hybrids whose parents differed in amylose content were indistinguishable. In hybrids involving high- and low- or intermediate- and low-amylose parents, the cooked F_2 product was not sticky. Possibly low-amylose grains released too little amylopectin in the cooking water to make all grains sticky. Moreover, grains with low amylose content (only 25% of the F_2 bulk) get randomly distributed among the high-amylose grains. The amylopectin released from the low-amylose

Table 6. The eating quality scale employed for the taste-panel evaluation of cooked rice.

Eating quality score	Characteristic				
	Aroma/flavor	Tenderness	Cohesiveness	Whiteness	Gloss
7	Very strong	–	–	–	–
6	Moderately strong	Very tender	Very sticky	White	Very glossy
5	Slightly strong	Moderately tender	Moderately sticky	Creamish white	Moderately glossy
4	Slightly weak	Slightly tender	Slightly sticky	Greyish white	Slightly glossy
3	Moderately weak	Slightly tough	Slightly separated	White with brown streaks	Slightly dull
2	Very weak	Moderately tough	Moderately separated	White with black streaks	Moderately dull
1	None	Very tough	Well separated	Brown (with streaks)	Very dull

Table 7. Taste panel evaluation scores of cooked rice of parents with different amylose content and their hybrids.

Cross (P_1/P_2)	Gloss				Tenderness				Cohesiveness				Whiteness			
		F_2 bulk seeds				F_2 bulk seeds				F_2 bulk seeds				F_2 bulk seeds		
	P_1	P_1/P_2	P_2/P_1	P_2	P_1	P_1/P_2	P_2/P_1	P_2	P_1	P_1/P_2	P_2/P_1	P_2	P_1	P_1/P_2	P_2/P_1	P_2
IR29/IR37307-8	5.9	4.1	5.0	4.3	5.9	5.0	5.1	5.1	6.0	5.1	4.7	4.7	4.4	4.7	4.3	4.4
IR29/IR24	5.9	4.1	3.7	3.6	5.9	4.6	5.1	4.0	6.0	5.1	5.1	3.4	4.4	4.3	4.0	5.3
IR29/BPI 121-407	5.9	2.9	3.9	2.3	5.9	3.0	4.4	2.4	6.0	3.1	3.9	1.7	4.4	4.7	4.7	3.3
IR29/IR8	5.9	3.9	3.7	2.4	5.9	3.9	3.9	2.3	6.0	3.6	3.6	1.6	4.4	4.3	4.4	6.0
IR37307-8/1R24	4.3	3.3	3.1	3.6	5.1	3.4	4.0	4.0	4.7	3.4	3.4	3.4	4.4	4.7	3.7	5.3
IR37307-8/BPI 121-407	4.3	4.3	3.6	2.3	5.1	4.3	4.1	2.4	4.7	3.9	3.6	1.2	4.4	5.9	4.4	3.3
IR37307-8/IR8	4.3	3.9	3.6	2.4	5.1	2.7	4.0	2.3	4.7	2.9	3.4	1.6	4.4	4.9	4.9	6.0
IR24/BPI 121-407	3.6	2.7	2.9	2.3	4.0	2.0	3.4	2.4	3.4	2.1	3.3	1.7	5.3	4.0	3.9	3.3
IR24/IR8	3.6	2.6	2.9	2.4	4.0	2.4	3.3	2.3	3.4	1.9	3.3	1.6	5.3	4.1	4.0	6.0
BPI 121-407/1R8	2.3	3.3	2.6	2.4	2.4	3.1	2.9	2.3	1.7	2.0	2.3	1.6	3.3	4.4	3.0	6.0

grains coats even the high-amylose grains which become softer without becoming sticky. Similar results were also obtained with maintainer lines and restorer lines (Table 8) which had low, intermediate, or high amylose content.

Thus, when parents differ widely in amylose content, the raw F_2 grains are distinctly classifiable into different amylose categories. But when cooked in bulk, the grains do not vary in cooking and eating characteristics. An analysis of the bulk F_2 grain sample of the hybrids between low- and high-amylose parents shows intermediate amylose content (Bollich and Webb 1965, Ghosh and Govindaswamy 1972, McKenzie and Rutger 1983, Seetharaman 1959, Stansel 1966). Intermediate-amylose-content varieties are preferred in many countries (Khush et al 1979). In these countries, hybrids between low- and high-amylose parents will have greater acceptance for grain quality. Several hybrids such as Wei-You 2, Shan-You 2, Si-You 2, and Nan-You 2 derived from high-amylose CMS lines and low-amylose restorer variety IR24 have been produced and grown on a large scale in China. No adverse reports exist about the grain quality of these hybrids. Thus, heterogeneity of individual grains for amylose content does not seem to reduce cooking and eating qualities. Rather, cooked rice with desirable gloss, tenderness, and cohesiveness can be produced by selecting the suitable parents.

Gelatinization temperature

The gelatinization temperature also influences cooking quality (Juliano et al 1964, 1965, 1969). Rices with high GT take longer to cook than do low-GT rices. Bhattacharya and Sowbhagya (197l), on the other hand, observed that water uptake and hence cooking time was strongly influenced by size and shape of rice grain and only marginally by GT. No sensory difference in the texture of cooked rice was observed in the cooked F_2 bulk grain sample of the crosses IR46831 A/IR64 and Iri 356 A/IR64 involving low- and intermediate-GT parents (Table 8) or in PAU269-1/IR64 and PAU269-1 A/IR46 involving high-intermediate and low- or intermediate-GT parents. Because grain size and shape are always uniform in rice hybrids, it appears that when a bulk sample of low- and intermediate-GT grains is cooked, low-GT grains get cooked first and release heat which helps cook intermediate-GT grains and the bulk sample appears uniformly cooked.

Breeding programs select lines with low or intermediate GT. Most elite breeding lines used as maintainers and restorers have low or intermediate GT. Differences in GT, therefore, should not pose any problem in the cooking and eating qualities of hybrid rice.

Gel consistency

The gel consistency of a sample determines the softness of cooked rice. Upon cooling, a sample with high GC gets hard, whereas one with intermediate or low GC remains soft. In study of a cross between high- and low-GC parents, Chang and Li (1981) reported a single major gene controlling this character, with high GC being dominant. In the bulk F_2 sample of hybrids involving high, intermediate-, or low-GC parents, we observed a similar situation for GC as for amylose content and gloss of cooked rice (Table 8). In our study of 75 crosses involving parents with high, soft, and medium GC, we observed both positive and negative heterosis. This

Table 8. Taste panel evaluation score of cooked rice of maintainers, restorers, and their hybrids.

Designation[a]	Characteristic					
	Aroma/flavor	Tenderness	Cohesiveness	Whiteness	Gloss	Head rice recovery (%)
Iri 356 B*	1.1	4.7	3.6	5.0	3.6	58.2
Iri 356 A/IR46	1.6	3.1	2.4	4.7	2.9	58.5
IR46	1.2	2.6	2.9	3.0	2.6	43.7
IR46831 B*	1.1	3.1	2.1	4.9	2.9	43.1
IR46831 A/IR64	2.6	3.6	2.3	6.0	3.6	61.8
IR64	1.3	5.4	4.9	5.0	3.9	60.8
IR54758 B*	4.7	4.7	4.1	3.3	3.0	45.2
IR54758 A/IR64	2.4	4.0	2.3	5.4	3.7	60.8
IR64	1.3	5.4	4.9	5.0	3.9	60.8
IR46831 B*	1.1	3.1	2.1	4.9	2.9	43.1
IR46831 A/IR46	1.6	3.4	2.4	4.6	3.1	57.4
IR46	1.3	2.6	2.9	3.0	2.6	43.7
IR54758 B*	4.7	4.7	4.1	3.3	3.0	45.2
IR354758 A/IR46	4.7	3.7	3.0	4.6	3.1	53.7
IR46	1.3	2.6	2.9	3.0	2.6	45.2
Iri 356 B*	1.1	4.7	3.6	5.0	3.6	58.2
Iri 356 A/IR64	2.0	4.7	2.9	4.9	3.0	65.8
IR64	1.3	5.4	4.9	5.0	3.9	60.8

[a]* = cytoplasmic male sterile lines in these genotypes used to develop the corresponding F_1 hybrids.

suggests that GC of the hybrid depends on the specific combination, but segregation for GC does not interfere with cooking and eating qualities.

Aroma

Aromatic rices are greatly preferred in many parts of the world and get a premium in the market. In crosses between an aromatic male sterile line IR54758 A and nonaromatic varieties such as IR46 and IR64, the bulk grain sample had moderately weak to slightly strong aroma (Table 8). So, if aromatic rices are preferred, at least one parent must be aromatic.

Conclusions

We examined whether the use of genetically divergent parents would pose problems in grain quality by studying bulk grain samples of several F_1 hybrids. We present these conclusions:

- Hybrids will have superior head rice recovery if both parents have high head rice recovery.
- Hybrids of medium grain size and shape can be produced by intercrossing long-, medium-, or short-grained parents. To produce long-grained hybrids, both parents should have long grains.

- For hybrids with better appearance and market acceptability, parents having widely different endosperm appearance should not be crossed.
- Segregation for different starch characteristics in bulk F_2 sample does not pose any problem in the eating and cooking qualities.
- The cooking characteristics of hybrid bulk grains are intermediate between those of parents. Therefore, to produce hybrids with desirable tenderness, cohesiveness, gloss, and aroma, parents should be selected so that the weighted averages of the grains in bulk sample match consumer preferences.

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Notes

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Agronomic management of rice hybrids compared with conventional varieties

Yan Zhende

Agronomic management of hybrid rice differs considerably from that of conventional varieties, primarily because of heterosis. Differences are most pronounced at the seedling and the vegetative growth stages. Fertilizer management, and practices to manipulate yield components, canopy structure, and field population are outlined. Several cropping systems in which hybrids are used as late rice in a double-cropping system are compared.

Commercial hybrid rice production has prompted new research in agronomic management. We studied the differences in major characteristics of hybrid rice and conventional rice and developed a management approach to fully use hybrid rice potential. Heterosis is the major difference between hybrid rice and conventional rice; it varies with growth stage and variety.

Seedling cultivation

Although hybrid rice seed production is only about 20% of conventional rice, planting density is also much lower. Changing from the dense sowing used for conventional rice nurseries to sparse sowing for hybrid rice nurseries is recommended. Sow 75% less seed. Normally, the amount of hybrid rice seed needed is only 15-25 kg/ ha, compared with 110-180 kg/ ha for conventional rice.

Allow 4-5 times more space per hybrid rice seedling in the nursery than for conventional rice. This favors tillering in the nursery and makes it possible to transplant a single hybrid rice plant/hill with 3-4 tillers compared with 3-4 conventional rice plants/hill without tillers.

Because hybrid rice has high tillering capacity, plant density will be less than for conventional rice. In the doublecrop rice area, plant density is about 30-37 hills/ m^2 for hyrid rice and 45-60 hills/ m^2 for conventional rice. In the single-crop rice area, plant density is about 27-33 hills/m^2 for hybrid rice. Plant density varies with soil fertility, and is generally lower in fertile soil.

Managing vegetative growth

The heterosis for yield in hybrid rice comes mainly from vegetative growth. During vegetative growth, hybrid rice accumulates more dry matter, which results in more spikelets per panicle. Conventional rice, which does not have such heterosis, basically depends on accumulation of assimilates after heading.

In early-maturing japonica varieties in North China, about 90% of the grain carbohydrate comes from photosynthetic assimilation after heading; in hybrid rice, 30-40% of the grain carbohydrate comes from assimilation before heading.

During vegetative growth, the leaf area index (LAI) of hybrid rice increases rapidly and the leaf area duration (LAD) is significantly longer than it is in conventional rice. The LAD of Gang-Hua 2 and its parents (IR24/Shan-Dung 1) are shown in Table 1. The rapid increase in LAI of hybrid rice at early and middle growth stages favors the production of more carbohydrate from photosynthesis. The photosynthetic rate of a single leaf does not increase. For example, at 34 d after transplanting (DT), dry weight is 365 g/m^2 for Gang-Hua 2,241 g/m^2 for Shan-Dung 1, and 187 g/m^2 for IR24. Hybrid rice yields can increase with additional management at early and middle stages.

In Jiangsu Province, farmers plant seedlings of hybrid rice on both sides of the ditches of the conventional rice nursery bed after the conventional rice is transplanted. This reduces labor costs for soil preparation and hastens transplanting by 30 d (Fig. 1). Average yields increased from 4.5 to 5.3 t, with a maximum 9.5 t/ha.

Large amounts of N applied on hybrid rice at vegetative growth sometimes results in overtillering. To control tillering, apply fertilizer or dry the soil according to the rates of tillering at 20-25 DT. For example, if tiller increase in Gang-Hua 2 is less than 15/m^2 per d, 10-15 kg N/ha should be supplied. If it is more than 30/m^2 per d, a light dry soil may be adopted. Dry soil decreases nonproductive tillers, regulates metabolism between C and N, and increases dry matter accumulation. Dry soil also helps establish a good canopy for late growth.

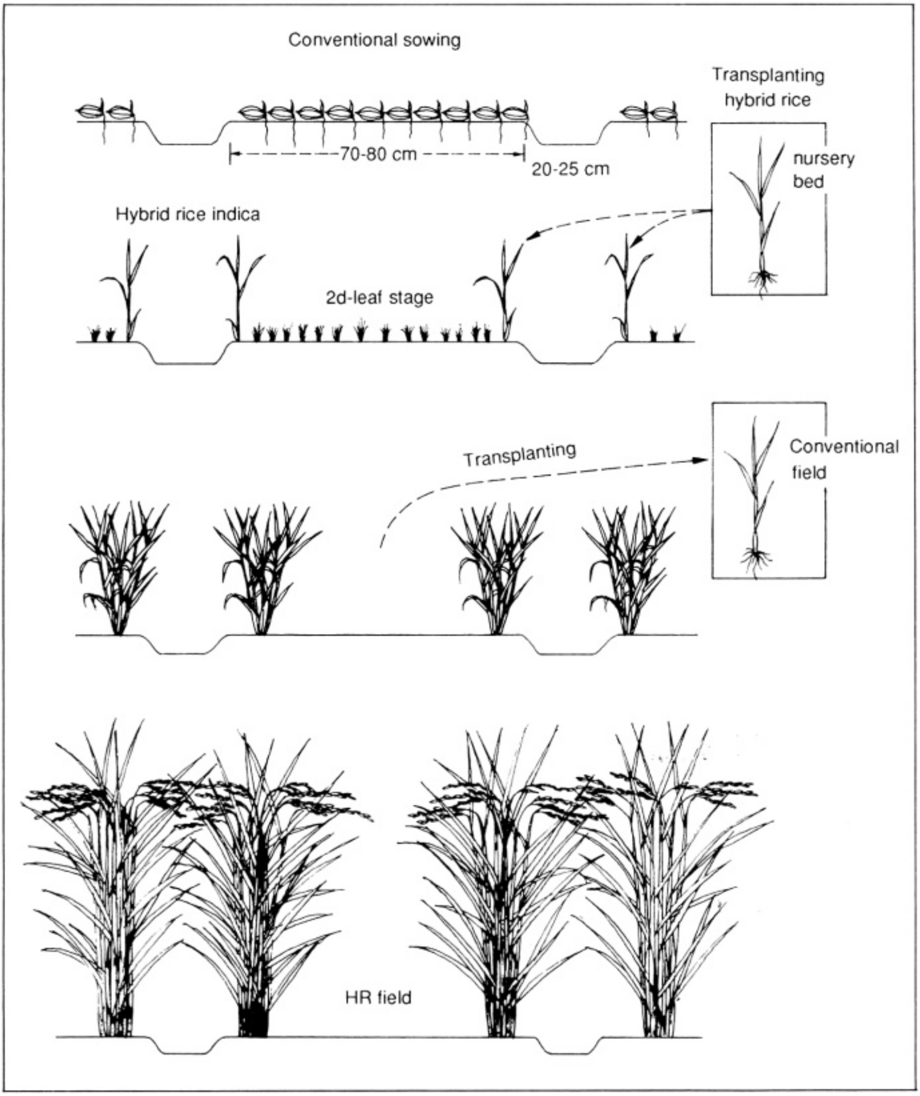
Fertilizer management

The modern short-statured rice varieties are much more N responsive and lodging resistant than conventional varieties. Hybrid rices are more tolerant of fertilizers than conventional rice. In Huai Bei region, Jiangsu Province, traditional tall variety Da-Che-Keng is responsive up to 85 kg N/ ha; short-statured variety Nong-Ken 57 needs 140 kg N/ ha for high yields; hybrid Shan-You 3 needs 180 kg N/ ha. Hybrid Gang-Hua 2 showed the greatest response to N and the highest yield potential (Fig. 2).

Table 1. Leaf area duration (LAD) of hybrid rice Gang-Hua 2 and its parents at indicated days after transplanting (DT).

Variety	LAD (d-m^2/m^2)						
	0-20 DT	22-34 DT	35-49 DT	50-64 DT	65-74 DT	75-90 DT	Total
GangHua 2	13.2	33.3	57.5	80.1	85.1	127.2	396.3
I A24	6.3	17.9	34.4	56.6	72.3	81.6	268.9
Shan-Dung 1	10.1	26.6	53.7		123.3[a]	157.9[b]	371.5

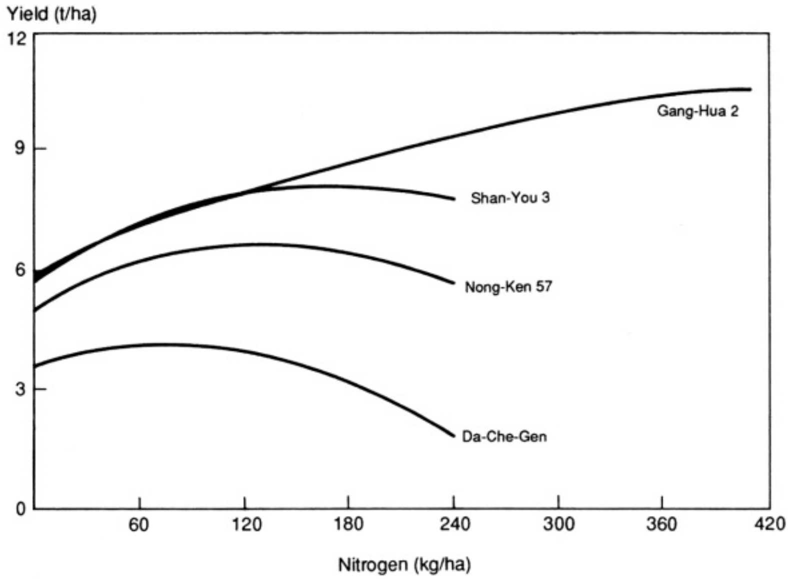
[a]50-74 d, [b]65-90 d.



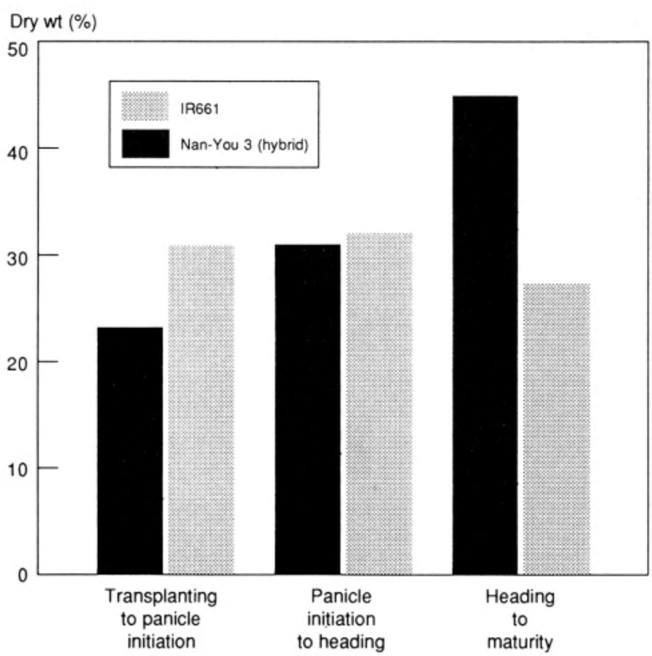
1. Nursery bed of conventional rice; hybrid rice seedlings are on the sides of the ditch.

Dry matter production at different growth stages showed different patterns for hybrid rice and conventional rice. Hybrid rice has more dry matter accumulation in the early and middle growth stages; conventional rice has more in late growth stages. Hybrid rice accumulates more total dry matter than conventional rice (Fig. 3).

N uptake of hybrid rice varies with growth stage. It is 29.1% of the total from recovery to tillering, and 34.3% from tillering to panicle initiation—more than for conventional rice. N uptake of hybrid Nan-You 3 at 40 DT is 71.2%. For hybrid rice, more fertilizer should be applied at early stages.



2. N response of different rice varieties.



3. Dry matter accumulation at different growth stages.

If 160 kg N/ha is used during a cropping season, it is recommended that 80 kg/ ha be applied basally, 50 kg N/ha during tillering, and the remainder after panicle initiation. For conventional rice, more N should be applied during the middle and late stages than during the early stages.

For high hybrid rice yields, organic fertilizers should be applied to maintain soil fertility. In some cases, a ratio of 7:3 or 6:4 organic to inorganic fertilizer is favorable. Hybrid rice absorbs more K and may need additional K fertilizer.

Yield components

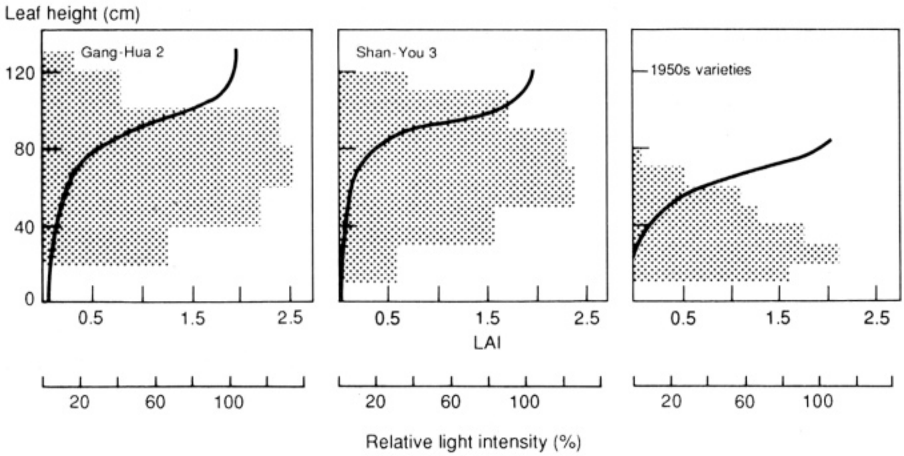
Hybrid rice has bigger panicles and more spikelets per panicle than conventional rice. Gang-Hua 2 (IR24/Shan-Dung 1) had 20% more panicles/ m^2 , 12% more filled spikelets/panicle, 43% more spikelets/m^2, and 34% more filled spikelets than its better parent. But filled spikelet percentage was 6.1% less. Total spikelets and filled spikelets for Gang-Hua 2 can reach 63,700/m^2 and 52,600/m^2; IR24 has only 44,700/m^2 and 39,300/m^2. Gang-Hua 2 yielded about 12.6 t/ha; IR24, about 9.6 t/ha.

Regulating total spikelets is necessary to gain high hybrid rice yields. Optimum spikelet number is 61,400/m^2 for Gang-Hua 2; 50,250/m^2 for Shan-You 3; and 48,720/m^2 for Nan-You 3 in Xuzhou, Jiangsu Province. For conventional rice, the optimum is 25,000-46,000/ m^2.

For high yields in hybrid rice, sink is not the limiting factor that it is in conventional rice. To increase hybrid rice yields, improve nutrient status at late growth, set up a better canopy structure, and regulate the relation between source and sink. To increase conventional rice yields, produce enough panicles and spikelets. Fertilizer management in conventional rice should focus on spikelet initiation; for hybrid rice, it should focus on spikelet filling.

Canopy structure and population adjustment

Yin et al (1959) who studied the population structure of conventional rice observed that the extinction coefficient (K) fluctuates from 0.5 to 0.9 under various treatments and growth stages. The K value of hybrid Nan-You 3 is 0.45; Shan-You 3,0.40; and Gang-Hua 2,0.39. The difference in K between hybrid rice and conventional rice at heading can be explained by the higher LAI and the ratio of upper to middle layer leaf area, as well as better light intensity distribution in hybrid rice (Fig. 4). The increase in upper to middle layer leaf area ratio favors photosynthesis. During panicle initiation, the photosynthetic rate in Gang-Hua 2 is 43.7 mg CO_2/dm^2 per h in the upper leaf layer, 28.6 in the middle layer, and 3.7 in the bottom layer. Because of the difference in canopy structure and interception of light in hybrid rice, populations must be regulated by water, fertilizer, and planting density. The optimum LAI is 7.7 for Nan-You 3, 7.7 for Shan-You 3, and 9.23 for Gang-Hua 2. For conventional Shuang-Chen-Nu it is 7.0; for Xu-Zhou 1,5.2; for Nong-Ken 57, 6.0; and for Nan-Keng 35, 6.63.



4. Leaf layer structures and light intensity distribution in hybrid and nonhybrid rice varieties.

Hybrid rice relies mainly on tillers to obtain the desirable population; conventional rice relies on number of seedlings planted. About 85-90% of productive panicles of hybrid rice come from tillers; in conventional rice, the percentage is 30-40. The maximum number of seedlings and tillers per unit area for conventional rice is 1.3-1.4 times the number of effective panicles, with a 70-80% productive panicle rate. For hybrid rice, the maximum is 1.4-1.5 times, with a 60-70% productive panicle rate.

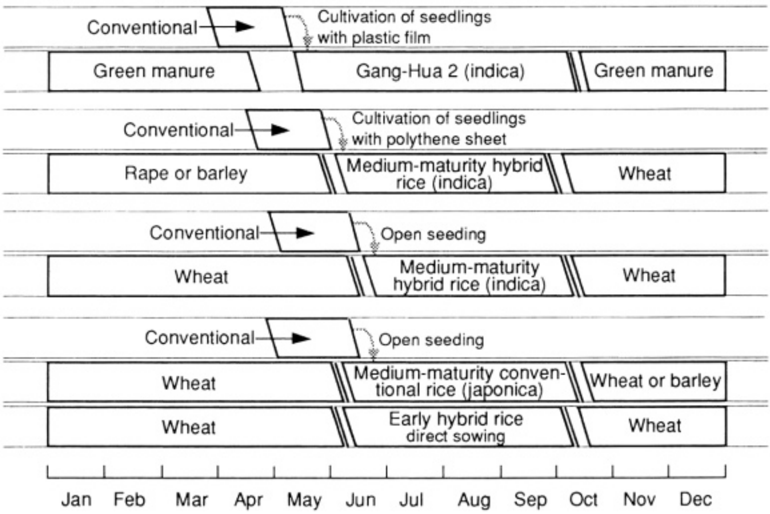
During the middle growth stages, hybrid rice tiller populations that are not high enough to meet the optimum LAI can be increased by applying N 30-35 d before heading. For conventional rice, N must be applied 5-10 d earlier to make nonproductive tillers productive.

Comparing cropping systems

The cropping patterns for hybrid rice depend mainly on growth and development characters. Hybrid rice varieties Nan-You 2, 3, and 6, and Shan-You 2, 3, and 6, usually are used as late rice in the double-cropping region. The cropping pattern is early conventional rice - late hybrid rice. In South China, Shan-You 2, 3, and 6 can be used as the early or late crop. In Nan Ning, Guangxi Province, Shan-You 2 as early rice yields about 8.3 t/ha, and Shan-You 3 as late rice yields about 9.3 t/ha. Recently, late-maturing photoperiod-sensitive indica hybrids such as Shan-You 30 and Wei-You 30, used as late rice in South China, have shown a more stable output.

The Huai Bei area, Jiangsu Province, is a single-crop region in the transitional zone between southern and northern China. The varieties grown in this area include medium-maturity hybrid indica rices and conventional japonica rices, and early-maturing japonica hybrids. Winter crops are wheat, barley, rape, and green manure. The cropping patterns are shown in Figure 5.

Hybrid rice now occupies 70% of the rice area. Yields increased from 4.6 t/ha in 1978 to 6.0 t/ha in 1985.



5. Rice cropping patterns and seedling cultivation technique.

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Nitrogen management for hybrid rice in southern United States

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The performance of tall (Lebonnet), semidwarf (Bellemont and Lemont), and F_1 hybrid (RAX2414) rice cultivars and their N requirements in dry-seeded and water-seeded systems are compared. Agronomic traits measured included maturity, plant height, biomass, grain yield, straw yield, grain-straw ratio (GSR), harvest index (HI), panicles per m^2, filled spikelets per panicle, spikelets per panicle, % sterile spikelets, and 1,000-grain weight. Grain yields were higher for all cultivars in the water-seeded system, with the hybrid yielding significantly more grain than the other cultivars in both the water-seeded and dry-seeded systems. N rates were 34-170 kg N/ha. The GSR and HI of the hybrid and the semidwarf cultivars were higher and more stable than those of the tall cultivar over all N rates. Split N application was about equally efficient as a single preflood or preplant application of total N, probably because the total rate of 102 kg N/ha was near optimum for most cultivars in both cultural systems. RAX2414 appears well adapted to the southern United States.

Rice in southern U.S. is direct seeded in both dry-seeded and water-seeded cultural systems. The dry-seeded system consists of drilling seed into a prepared seedbed which is then flushed with irrigation water or rainfed to germinate the seed and establish a stand. Dry-seeded rice is permanently flooded when the rice is at the fourth-leaf stage (30 d after seeding [DAS]) with a 5-10 cm flood maintained throughout the season (Westcott et al 1986). The water-seeded system consists of dry seedbed preparation followed by flooding and aerial broadcasting of presoaked seed into the flooded field. The floodwater may be drained temporarily in water-seeded rice when the radicle emerges to help seedlings anchor to the soil. A permanent flood is reestablished 2-4 d after draining and a 5-10 cm water depth is maintained until maturity (Westcott et al 1986). The water-seeded system favors early plant growth and hastens maturity (Helms 1984). Input amounts and timing differ between the two systems.

Both systems require special N fertilization practices. In the dry-seeded system, early season N must be sufficient to optimize seedling growth and tillering, but preplant N is subject to loss by denitrification when the permanent flood is established. Timing N application for optimum performance of conventionally developed cultivars in dry-seeded rice requires applying 20-30 kg N/ ha preplant followed by 75% or more of the total N requirement just before permanent flood (Brandon et al 1982). In the water-seeded system, placing ammoniacal N in the dry soil followed by flooding maximizes N efficiency by minimizing NH_3 volatilization and denitrification losses (Mikkelsen et al 1978). In water-seeded rice, 75% or more of the total N requirement should be applied preplant (Brandon and McKenzie 1984).

Improved semidwarf cultivars grown in southern U.S. are more productive than tall cultivars but usually require 30-40 kg more N/ha for optimum yield (Brandon and McKenzie 1984). Semidwarf cultivar Lemont has yielded more than tall Lebonnet in both dry-seeded and water-seeded systems, but seedling establishment has been difficult in the dry-seeded system (McKenzie et al 1983). The shortened mesocotyl associated with semidwarfism in Lemont may reduce seedling vigor in the dry-seeded system (Bollich et al 1985). Preliminary research has indicated that N requirement of an F_1 hybrid rice cultivar may be less and grain yield potential higher than for the semidwarf Lemont (Brandon and McKenzie 1986).

A 1984 study determined the comparative performance of tall, semidwarf, and F_1 hybrid rice cultivars and their N requirements in dry-seeded and water-seeded systems in the southern U.S.

Materials and methods

The experiment was conducted in a Crowley silt loam soil (Typic Albaqualf) in southwestern Louisiana. The cultivars were Lebonnet, a tall, widely grown cultivar; Bellemont, a semidwarf cultivar; Lemont, a semidwarf, widely grown cultivar; and RAX2414, an F_1 hybrid from Ring Around. The tall Lebonnet is a parent of both Bellemont and Lemont. Both semidwarf cultivars possess the semidwarf gene of Taichung Native 1 (Bollich et al 1984).

The dry-seeded system was seeded by drilling and the water-seeded system by broadcasting into the flooded field. Both systems used a seeding rate of 112 kg seed/ha. Total N rates of 0-170 kg/ha in 34 kg N/ha increments were broadcast preflood in the dry-seeded and band-placed preplant in the water-seeded systems. The 102 kg N/ha rate was also split to provide 68 kg N/ha preflood in dry-seeded rice or preplant in water-seeded rice and 34 kg N/ha at panicle initiation or panicle differentiation.

The experimental design was a split-split plot in a randomized complete block with four replications. Cultural systems were the main plots, N treatments the subplots, and cultivars the sub-subplots. Plots were 16.3 m^2. Agronomic traits measured included maturity, plant height, biomass, grain yield, straw yield, grain-straw ratio (GSR), harvest index (HI), panicles per m^2, filled spikelets per panicle, spikelets per panicle, % sterile spikelets, and 1,000-grain weight. The biomass and yield component measurements were taken from a 0.3-m^2 area in each plot.

Results and discussion

Cultural system effects on agronomic traits

Semidwarf Bellemont and Lemont emerged slower than Lebonnet and RAX2414 in the dry-seeded system. Bellemont emerged very slowly in the water-seeded system but Lemont, Lebonnet, and RAX2414 emerged rapidly and equally well. All cultivars developed more rapidly in the water-seeded system, averaging 6 d earlier maturity. The effect of cultural system on biomass yield differed by cultivar. Bellemont and Lemont produced more biomass in the water-seeded system than in the dry-seeded system, however, biomass yields of tall Lebonnet and the F_1 hybrid RAX2414 were about equal in both systems.

Grain yields were greater in the water-seeded (8.6 t/ha) than in the dry-seeded (7.8 t/ha) system primarily because the semidwarfs Bellemont and Lemont performed much better in the water-seeded system than in the dry-seeded system. Grain yields of tall Lebonnet and RAX2414 were only slightly greater in the water-seeded system than in the dry-seeded system. Water-seeding produced significantly greater GSR (0.93 vs 0.79) and HI (0.48 vs 0.44) over all cultivars. Higher grain yields and slightly lower straw yields produced the higher GSR and HI in water-seeded rice.

The water-seeded system produced significantly more panicles per m^2 and higher grain yields for all cultivars. The yield advantage of the water-seeded over the dry-seeded system was much greater for the semidwarf Bellemont (7.9 vs 6.4 t/ha) and Lemont (8.4 vs 7.5 t/ha) than for tall Lebonnet (7.5 vs 7.3 t/ha) and RAX2414 (10.8 vs 10.1 t/ha). The F_1 hybrid RAX2414 yielded significantly more grain than the other cultivars, followed by Lemont, Bellemont, and Lebonnet. The hybrid was well adapted to both systems.

There were significantly more spikelets per panicle in the dry-seeded than in the water-seeded system, however, sterility percentage was significantly greater in the dry-seeded system. The 1,000-grain weight was slightly greater in the dry-seeded system, probably because of fewer panicles per m^2. Correlation regression analysis showed that biomass, straw yield, panicles per m^2, spikelets per panicle, grains per panicle, and 1,000-grain weight were significantly correlated with grain yield. The correlation coefficient between 1,000-grain weight and yield was much higher for the F_1 hybrid (0.56**) than for Bellemont, Lemont, and Lebonnet.

Effect of N rate and timing on agronomic traits

Biomass yield increased quadratically with N up to 136 kg N/ha, and 170 kg N/ha had no additional effect in either system. Grain yield of all cultivars increased quadratically in both cultural systems but the N rate at which maximum yield occurred differed between the systems and among cultivars. All cultivars produced greater yields in the water-seeded system than in the dry-seeded system at low (0-34 kg/ ha) to moderate (68-102 kg/ ha) N rates, but tall Lebonnet lodged at 102 kg N/ha in the water-seeded system and yielded less than in the dry-seeded system.

The tall Lebonnet required only 68 kg N/ha in the water-seeded system while the semidwarf Bellemont and Lemont required 136 kg N/ha for optimum yield. The F_1 hybrid yielded very well at 34-170 kg N/ha, but 102 kg N/ha appeared near optimum. HI and GSR generally increased with the first 34 kg N/ha increment but decreased with additional N increments. The HI and GSR of RAX2414, Bellemont, and Lemont were higher and more stable than those of Lebonnet over all N rates. Spikelets per panicle generally increased with increasing N rates, but increased spikelet sterility with increased N resulted in fewer grains per panicle in some cultivars at high N rates. The 1,000-grain weight increased with the first 34 kg N/ha increment but remained rather stable at higher N rates. The number of panicles per m^2 of all cultivars increased with N up to 136 kg N/ha in both dry-seeded and water-seeded systems.

The effect of split N application on agronomic traits of cultivars depended on cultural system, cultivar, and time of N topdressing. Split N application decreased biomass yield, straw yield, grain yield, and panicles per m^2 compared with a single preplant application of 102 kg N/ha in the water-seeded system when the topdressing was applied at panicle differentiation. The split N application of 68 kg N/ha preflood followed by 34 kg N/ha at panicle initiation in the dry-seeded system increased biomass, straw, and grain yields, and panicles per m^2 over that of a single 102 kg N/ ha rate. The split N treatments generally increased HI and GSR values over that of a single preplant application of 102 kg N/ ha. The equal efficiency of split N applications in relation to a single preflood or preplant application of the total N rate probably occurred because the total rate of 102 kg N/ ha was near optimum for most cultivars in both cultural systems.

Conclusion

The superior performance of the F_1 hybrid RAX2414 over cultural systems and N treatments indicated that this cultivar was well adapted to a wide range of environmental conditions in southern U.S.

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The use of hybrid rice technology: an economic evaluation

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When a new technology is to be popularized in China, it is evaluated mainly on three criteria: 1) Does it increase production? 2) Is it economically efficient? and 3) Are resources available in the adoption area? Social and ecological effects are also considered.

This study on hybrid rice in Jiangsu Province, eastern China, analyzed data from 209 farms according to the above criteria. Hybrid rice yields for individual farmers in 1984 increased 1.0 t/ha over yields of conventional varieties (15% growth) and 0.9 t/ha for society (14% increase). A comparison of hybrids with conventional varieties showed that net returns per hectare rose 37%, returns to labor grew 26%, returns to nonlabor costs increased 12%, and rate of net returns to total cost went up by 30%.

Hybrid seed producers profit much more than field grain growers. The net returns of hybrid seed production per hectare were 3.8 times higher and returns to labor were 2.1 times higher than those for grain producers. The purchase price and sale price of hybrid seeds were 11.3 and 10.7 times those of hybrid grain, respectively. An exorbitant seed price prevents farmers from expanding their hybrid rice hectarage.

Hybrid rice is a key to increasing grain production and enhancing the economic efficiency in China.

In 1984, the area under hybrid rice in Jiangsu Province accounted for 9% of China's hybrid rice area and 33% of the province's rice area. Hybrid rice production in the province was 10% of China's total hybrid rice production and 57% of the provincial hybrid rice production.

Efficiency is the core of any economic activity. How much benefit does hybrid rice production bring to farmers? This is a central question for people guiding and organizing agricultural production.

Under the agreement between the Chinese Academy of Agricultural Sciences and the International Rice Research Institute, a joint study on hybrid rice was conducted in Fujian and Hunan Provinces in 1983 (He Guiting et al 1984). But the 1983 study reported data representing the high-yield hybrid rice-growing regions instead of average-yield regions. We decided to renew the study in six agricultural regions of Jiangsu Province. Growers were randomly selected from different counties. We observed the following criteria in county selection:

1. The county should represent the main characteristics of the different agricultural regions of the province. Jiangsu Province is divided into six geographical and economic regions: Taihu, Hilly, Riverine, Coastal,

Xuhuai, and Lixiahe. Because each region produces large areas of hybrid rice, one county was chosen from each region.

2. The county should be in the median based on the percentage of area planted to hybrid rice. The ratio of hybrid rice area to total rice area in the province was from 10 to 80%. The sample counties were chosen between 50 and 80%.
3. The county should represent the average levels of production and economic situations of the province.

Based on these criteria, Wujin, Taixing, Dafeng, Huaiyin, Jurong, and Jiangdu counties were selected. Thirty farmer households (15 hybrid growers and 15 conventional [indica and japonica] growers) were randomly selected from each county. The total sample of 209 included 90 hybrid growers, 90 conventional growers, and 29 sterile line and hybrid seed producers.

The sample was representative and comparable: 1) the average yields of growers were very close to those of the province as a whole; and 2) the household size of the sample farmers, their education levels, hectarage under rice, and other factors were similar to those of conventional-growing counterparts; and the nonrice and off-farm incomes of both groups were similar (Table 1, 2).

Results

Yield performance of hybrid rice

Calculation methods. It is difficult to measure the increased yield hybrid rice gives over conventional rice. It is necessary to measure the hybrid rice returns to farmers and to society and to distinguish actual yield from nominal. Nominal yield is the output per unit area; actual yield is the nominal yield less the hybrid seed per unit area.

Table 1. Yields (t/ha) of hybrid and conventional rice in the sample and the population, Jiangsu, China, 1984.

		Hybrid	Conventional
Jiangsu	Sample	7.4	6.5
Province	Province	7.6	6.6
Wujin	Sample	8.2	7.4
county	County	8.2	7.6
Jurong	Sample	7.5	6.7
county	County	7.5	6.7
Taixing	Sample	7.6	6.3
county	County	7.6	6.2
Jiangdu	Sample	7.2	6.5
county	County	7.3	6.7
Huaiyin	Sample	7.2	6.5
county	County	7.0	6.3
Dafeng	Sample	6.5	5.7
county	County	6.6	5.7

Table 2. The major characteristics of hybrid and conventional rice growers, Jiangsu, China, 1984.

	Hybrid (n = 90)	Conventional (n = 90)
Size of household (persons)	4.4	4.9
Schooling of labor (years)	5.2	4.8
Average area under rice (ha/household)	0.29	0.33
Ratio of hybrid rice to total (%)	75	36
Ratio of conventional rice to total (%)	25	64
Per capita nonrice income (¥)[a]	439.00	402.00
Per capita off-farm income (¥)[a]	229.00	188.00

[a]2.74 yuan = $1.00.

When a farmer cost-accounts, he figures the net profit after growing rice, i.e., the output of rice per unit area less the seeds per unit area. Hence, the actual yield increase of hybrid rice is the result of comparison. Farmers make their own decisions on distributing grain products—how much grain to sell to the state to fulfill the grain quota, how much to sell in the market, and how much to store for other uses.

At all levels, agricultural production authorities are interested in the total increase of social output. They pay attention to the net contribution of hybrid rice based on the amount of land devoted to it and its seed production compared with the contribution that could have been made by growing conventional rice. When we compute the net output of hybrid rice, we consider its nominal output, the output of hybrid rice seeds and restorer line seeds, the output of sterile line seeds, as well as the losses of conventional rice by using a certain amount of land for hybrid seed production. The above relationship can be expressed in the following formula:

$$Y_{HR} = Y_H - \alpha[(Y_C - S_C) - (Y_R - S_R) - Y_{HS}(1 - K_{HS})] - B[(Y_C - S_C) - (Y_M - S_M) - Y_{MS}(1 - K_{MS})] \quad \text{(Eq. 1)}$$

where

Y_{HR} is net yield of hybrid rice (t/ha),
Y_H is nominal yield of hybrid rice (t/ ha),
Y_C is nominal yield of conventional rice (t/ ha),
S_C is seed used for conventional rice (t/ ha),
Y_R is yield of restorer line of hybrid seed production (t/ha),
S_R is restorer seed used in hybrid seed production (t/ha),
Y_{HS} is yield of hybrid seed (t/ ha),
K_{HS} is proportion of hybrid seed of acceptable standard,
Y_M is yield of maintainer line of male sterile seed multiplication (t/ ha),
S_M is maintainer seed used in male sterile seed multiplication (t/ha),
Y_{MS} is yield of male sterile seed (t/ ha), and
K_{MS} is proportion of male sterile seed of acceptable standard.

The a in equation 1 is the allocation factor of the foregone conventional rice production in the hybrid seed production field and the ß is the allocation factor in the male sterile seed production field. They are expressed as

$$a = \frac{dS_H}{K_{HS} \bullet Y_{HS}}, \text{ and} \qquad \text{(Eq. 2)}$$

$$B = a \frac{dS_{HS}}{K_{MS}\,(Y_{MS} - S_{MS})}, \qquad \text{(Eq. 3)}$$

where

d is the allowance factor for seed,

S_H is hybrid seed used/ ha of hybrid seed production,

S_{HS} is male sterile seed used/ ha of hybrid seed production, and

S_{MS} is male sterile seed used/ ha of male sterile seed multiplication.

Hybrid rice yield increase. Society and farm- level yield increases of hybrid rice (Table 3) were computed using the sample average yield, seed used per hectare, and related data (Table 4). The results show that for farmer households, the actual yield of hybrid rice derived from samples increased 0.7-1.5 t/ha, with average growth of 1.0 t, or a 15% increase. For society, per hectare yield of hybrid rice grew by 0.7-1.4 t, average increase was 0.9 t, or a 14% increase (Table 3). In 1984, the total area under hybrid rice in Jiangsu Province was 820,460 ha. Assuming hybrid rice technology increased output by 0.9 t/ha, the total gain would be 730,000 t, 51% of the province's increased production. Hybrid rice matures 7-10 d earlier than conventional late rice, which is beneficial to subsequent cropping.

Economic returns to hybrid rice production

The absolute economic return simply compares the ratio of outputs to inputs. The relative economic return compares performance of hybrid rice with that of the existing technology. The adoption and popularization of a new technology depends mainly on its relative economic return, or whether the new technology brings greater benefits to farmers.

Table 3. Yield (t/ha) of hybrid rice relative to conventional rice, Jiangsu, China, 1984.

Hybrid	Actual yield [a]				Nominal yield	
	Society		Farmer		Hybrid	Conventional
	Hybrid	Conventional	Hybrid	Conventional		
Sample						
Wujin	8.2	7.3	8.2	7.3	8.2	7.4
Turong	7.5	6.7	7.5	6.7	7.6	6.7
Taixing	7.6	6.2	7.6	6.2	7.6	6.3
Jiangdu	7.2	6.4	7.2	6.4	7.2	6.5
Huaiyin	7.1	6.4	7.1	6.4	7.2	6.5
Dafeng	6.5	5.6	6.5	5.6	6.6	5.7
Provincial total	7.52	6.54	7.59	6.54	7.61	6.64
Province	7.3	6.4	7.4	6.4	7.4	6.5

[a] Conventional varieties include indicas and japonicas.

Table 4. Data used in computing the net yield of hybrid rice in six counties of Jiangsu Province, China, 1984.

Variable[a]	Provincial average	Sample average	County average					
			Wujin	Jurong	Taixing	Dafeng	Huaiyin	Jiangdu
Y_H (t/ha)	7.6	7.4	8.2	7.5	7.6	6.5	7.6	7.2
S_H (kg/ha)	24.0	24.0	16.8	23.6	23.6	33.6	25.3	17.0
Y_C (t/ha)	6.6	6.6	7.4	6.7	6.3	5.7	6.4	6.5
S_C (kg/ha)	95.2	95.2	92.5	75.0	110	129.0	85.0	74.5
Y_R (t/ha)	1.7	1.7	1.5	1.8	1.1	1.5	2.2	2.0
S_R (kg/ha)	9.7	9.7	7.5	12.0	9.0	11.3	9.0	9.7
Y_{HS} (t/ha)	1.6	1.6	1.6	1.5	1.9	1.7	1.5	1.5
S_{HS} (kg/ha)	34.5	34.5	38.3	33.7	33.7	32.3	33.7	36.7
Y_M (t/ha)	1.1	1.1	1.5	1.1	1.1	0.6	1.1[b]	1.2
S_M (kg/ha)	9.7	9.7	7.5	15.7	11.3	7.5	9.7[b]	7.5
Y_{MS} (t/ha)	1.4	1.4	2.0	1.3	1.6	0.9	1.4[b]	1.1
S_{MS} (kg/ha)	27.0	27.0	28.5	36.0	19.5	22.5	27.0	30.1
d	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
k_{HS} (%)	90	90	90	90	90	90	90	90
k_{MS} (%)	60	60	60	60	60	60	60	60

[a]See text for variable definitions. [b]From provincial average.

Absolute economic returns. Table 5 shows the average inputs and outputs of farms sampled. Average yield of hybrid growers was 7.4 t/ha, slightly lower than the provincial average of 7.6 t/ha. The average yield of conventional growers was 6.5 t/ha, also lower than the provincial level of 6.6 t/ha. The yield difference between hybrid and conventional rice was 0.9 t/ha, with a maximum of 1.3 t/ha in Taixing and a minimum of 0.7 t/ha in Huaiyin. Conventional rice used more labor input than hybrid rice, 294.6 workdays/ha compared to 280.7 workdays. The material input of hybrid rice was more than that of conventional rice, 763.00 yuan/ha to 706.00 yuan/ ha. Both conventional rice and hybrid rice used much physical labor, 49 and 46%, respectively.

The absolute economic returns of hybrid rice were measured in terms of land, labor, physical inputs, and capital.

Table 6 shows the absolute economic returns to hybrid rice production. The net returns from growing hybrid rice were 1,270.50 yuan/ ha. The returns to labor were 6.80 yuan/ workday, and returns to material costs were 2.70 yuan. The net returns to total costs were 0.91 yuan.

Relative economic returns. The relative economic returns to hybrid rice can be used to assess whether hybrid rice profitably replaces conventional rice.

As computed on the sample data, the development of hybrid rice gave apparent relative economic gains: hybrid rice yield increased 1 t grain/ ha, output value grew by 18%, net returns per hectare went up 37%, returns to labor increased 26%, and the rate of net returns to total costs increased 30% (Table 7).

As the standard of living rises, people increasingly demand round-grain rice Conventional japonica, if grown properly, gives high yields with good economic

Table 5. The inputs and outputs of hybrid and conventional rice production in sampler from six counties of Jiangsu Province, China, 1984.

County	Conventional					Hybrid				
	Output (t/ha)		Input			Output (t/ha)		Input		
	Grain	Straw	Labor (d/ha)	Nonlabor (yuan[a]/ha)	Indirect (yuan/ha)	Grain	Straw	Labor (d/ha)	Nonlabor (yuan/ha)	Indirect (Yuan/ha)
Wujin	7.4	7.4	304.4	707	88	8.2	8.3	242.1	733	88
Jurong	6.7	6.8	262.5	624	89	7.5	7.5	278.4	747	89
Taixing	6.3	6.2	323.3	633	48	7.6	7.5	293.4	641	48
Jiangdu	6.5	6.5	290.7	630	72	7.3	7.2	279.9	654	72
Huaiyin	6.5	6.5	313.9	570	61	7.2	7.1	301.5	634	61
Dafeng	5.7	5.7	289.8	623	78	6.5	6.5	285.8	698	78
Provincial av	6.5	6.5	294.6	634	73	7.4	7.4	280.7	690	73

[a]2.74 yuan = $1.00.

Table 6. Economic returns[a] to hybrid rice production in six counties of Jiangsu Province, China, 1984.

	Net return (¥[b]/ha)	Return to labor (yuan/workday)[c]	Return to nonlabor cost (yuan/ha)	Benefit-cost ratio (%)
Wujin	1604	8.90	2.95	117
Jurong	1263	6.81	2.51	86
Taixing	1382	7.00	2.96	101
Jiangdu	1256	6.86	2.73	92
Huaiyin	1186	6.20	2.66	85
Dafeng	944	5.60	2.22	66
Sample av	1270	6.60	2.70	91

[a] Output values based on proportional price in Jiangsu Province; the price of hybrid indica was 0.32 yuan/kg. [b]2.74 yuan = $1.00. [c]Labor wage is the average wage of 6 regions, 2.28 yuan/workday.

Table 7. Comparison of economic returns of hybrid rice and conventional indica, 1984.[a]

	Hybrid	Conventional	Increase (%)
Yield[b] (t/ha)	7.4	6.4	16
Output value (yuan[c]/ha)	2673	2266	18
Net returns (yuan/ha)	1271	930	37
Returns to labor (yuan/workday)	6.8	5.4	26
Returns to nonlabor cost (yuan)	2.7	2.4	13
Rate of net returns to total cost (yuan)	0.91	0.70	30.0

[a] Assumes price for hybrid rice at 0.32 yuan/kg; and 0.31 yuan/kg for conventional rice. [b]Nominal yield. [c]2.74 yuan = $1.00.

results (Table 8). Hybrid rice and conventional japonica are both effective in increasing grain production, improving grain quality, and enhancing economic performance.

The average yield of hybrid rice was 7.4 t/ha, 16% higher than that of conventional japonica. People are willing to pay higher prices for japonica, so it raises the economic efficiency. The net returns of growing japonica were 1,437.00 yuan/ ha, 54% higher than growing indica and 22% higher than growing hybrid rice. Hybrid rice and japonica each have advantages and disadvantages. Although hybrid rice gives high yield, the quality is not so good. Research is needed to increase its yield and improve its quality.

If grain is the foundation of the national economy, its economic efficiency is the key to economic development. We should vigorously adopt and develop hybrid rice according to local conditions. To fulfill the state plan of grain production, farmers are encouraged to develop conventional japonicas. A proper combination of hybrids and japonicas can increase total output, improve grain quality, and maximize economic results. Another ideal way is to develop new, highquality hybrid japonicas.

Table 8. Comparison of economic returns of conventional japonica and indica rices, 1984.

	Japonica	indica	Increase (%)
Grain yield (t/ha)	6.7	6.4	5
Gross value (yuan/ha)	2797	2267	23
Net return (yuan)	1437	930	54
Returns to labor (yuan)	7.30	5.40	35

[a]2.74 yuan = $1.00.

Economic results in seed production

Hybrid seed production. In hybrid seed production, a sterile line and a restorer line are used as female and male parents to produce hybrid seeds. Through self-pollination, restorers produce restorer seeds.

Based on the data from 21 hybrid seed producers in 6 counties, the average yield of hybrid seeds was 1.6 t/ha, that of restorer seed was 1.7 t/ha, with 4.5 t/ha of by-products. Output expenses were 373.05 workdays, 1,047.45 yuan of nonlabor costs, and 72.45 yuan of indirect costs.

The hybrid rice production area that 1 ha of hybrid seeds can support is calculated as follows:

$$R_{SHA} = \frac{K_{HS} \cdot Y_{HS}}{dS_H} \qquad \text{(Eq. 4)}$$

where

R_{SHA} is hybrid rice field production area (in hectares) that can be supported/ ha of hybrid seed,
Y_{HS} is hybrid seed yield (kg/ ha),
K_{HS} is proportion of acceptable hybrid seed,
S_H is hybrid seed used/ha of hybrid rice production (kg), and
d is the allowance factor for hybrid seed.

Using the survey data, the R_{SHA} of the samples is:

$$R_{SHA} = \frac{0.9 \cdot 633.13}{1.134.75} = 38.4 \text{ha}$$

That is to say that under 1984 conditions, the output of 1 ha of hybrid seed could support the seed requirement of 38.4 ha of hybrid rice production. Thus, one way to increase the area is to increase the yield of hybrid seed and the proportion of acceptable seed; another way is to save the seed used in field production.

In hybrid seed production, the yield of hybrid seed is lower than that of hybrid rice field production, while the value of the seeds is much higher than that of field grain. According to the sample data, the yields of hybrid seed plus restorer seed were 3.3 t/ ha, 4.1 t/ ha lower than that of hybrid rice. But the output value of hybrid seed production was 6,472.50 yuan/ ha, 3,799.50 yuan/ ha higher than for hybrid rice.

This implies that the value of 1 ha of hybrid seeds is equal to 20.1 t of hybrid rice. After farmers sell their seeds in the market, they can use the money to buy the grain they need or they can trade the hybrid seeds for other grains.

The special techniques of hybrid seed production require substantial manual labor (Table 9). In seed production, it took 373.5 workdays to produce 1 ha of hybrid seeds, 33% higher than for hybrid rice, and 27% higher than for japonica. Among the labor inputs, 43% was physical labor.

The relative economic returns to hybrid seed production can be figured from the uses of land and labor resources.

The yield of hybrid seed was lower than that of hybrid rice, but the price of hybrid seed was 10.7 times that of hybrid rice grain, which made per hectare output value of hybrid seed 1.4 times that of hybrid rice. The increase of output value was much more than the cost increase, which gave a 255% higher net return/ hectare than hybrid rice field production (Table 10). This also made the net returns to labor in hybrid seed production 110% higher than in hybrid rice field production (Table 11).

Hybrid seed production is a sophisticated process requiring skilled farmers. Higher profitability is justifiable. The difference between seed producers and field producers, however, should not be too large. If the seed price is exorbitant, farmers cannot expand hybrid rice hectarage and increase their incomes. The price of seed must be reduced to a level farmers can afford (Table 12). Assuming the purchase

Table 9. The inputs and outputs of hybrid seed production, sample average, 1984.

Item	
Output	
Hybrid seed (t/ha)	1.6
Restorer seed (t/ha)	1.7
Straw (t/ha)	4.5
Input	
Labor (workday/ha)	373
Nonlabor input (yuand/ha)	1047
Indirect input (yuan/ha)	72

[a]2.74 yuan = $1.00.

Table 10. Net returns (yuan/ha)[a] from hybrid seeds and hybrid rice grain production.

	Hybrid seeds	Hybrid rice grain	Increase (%)
Output value[b]	6472	2673	142
Hybrid rice	–	2379	–
Hybrid seeds	5650	–	–
Restorer seeds	643	–	–
Straw	179	294	–
Costs	1970	1402	40
Labor cost	850	640	–
Nonlabor input	1047	690	–
Indirect input	72	72	–
Net returns	4503	1270	254

[a]2.74 yuan = $1.00. [b]Prices used in computation were 3.46 yuan/kg for hybrid seed. 0.38 yuan/kg for restorer seed, and 0.04 yuan/kg for straw.

Table 11. Returns to labor in hybrid seed and grain production, 1984.

Variable	Hybrid seeds	Hybrid rice grain	Increase (%)
Output value (yuan[a] /ha)	6472	2673	142
Nonlabor costs (yuan/ha)	1119	762	47
Net output value (yuan/ha)	5354	1911	180
Workdays/ha	374	280	33
Returns to labor (yuan)	14	7	110

[a] 2.74 yuan = $1.00.

Table 12. The effects of seed price variation on the economic efficiency of hybrid seed and grain production.

Hybrid seed price (yuan[a]/ha)		Net return (yuan/ha)			Net return (yuan/workday)		
Sale price	Purchase price	Hybrid seed	Hybrid rice	Increase (%)	Hybrid seed	Hybrid rice	Increase (%)
3.46	3.64	4506	1270	254	214	102	110
3.00	3.20	3750	1286	192	185	103	80
2.80	3.00	3424	1293	165	172	103	67
2.60	2.80	3097	1300	138	159	103	53
2.40	2.60	2771	1307	112	146	104	41
2.20	2.40	2444	1314	86	132	104	28
2.00	2.20	2117	1320	604	119	104	15

[a] 2.74 yuan = $1.00.

price of seeds is down from 3.46 to between 2.80 and 2.40 yuan per kg, while keeping the sale price at 2.60-2.20 yuan/ kg, the returns to labor of seed production will still be 28-53% higher than those of hybrid rice grain growers. As a result, the farmers will increase their profits about 20.70-30.30 yuan/ ha and net returns to labor 1.40-1.80 yuan/ workday.

In producing hybrid seeds, sterile seed multiplication is needed for female parents. Sterile seed multiplication uses a sterile line as the female parent and a maintainer as the male parent. The hybrid products are sterile seeds. The purpose of multiplication through hybridization and self-pollination of parents is to provide enough female parents for hybrid seed production. Data from 8 sterile seed multiplication growers in 5 counties showed that the average yield of sterile seed was 1.4 t/ha, maintainer seed 1.1 t/ha, and by-products 3.4 t /ha. The average labor input was 397.8 workdays/ha, and the material costs were 1,060.00 yuan/ha (Table 13).

There is a proportional relationship between sterile seed multiplication and hybrid seed production because sterile seed multiplication is used for hybrid seed production. The area of hybrid seed production that 1 ha of sterile seed can serve is calculated as follows:

$$R_{SMSA} = \frac{K_{MS} \cdot Y_{MS}}{dS_{HS}} \qquad \text{(Eq. 5)}$$

Table 13. The inputs and outputs of sterile seed production, sample average, 1984.

Item	Variable
Output	
Sterile line (t/ha)	1.4
Maintainer (t/ha)	1.1
Straw (t/ha)	3.4
Input	
Labor (workday/ha)	398
Nonlabor input (yuan[a] /ha)	988
Indirect input (yuan/ha)	72

[a]2.74 yuan = $1.00.

where

R_{SMSA} is seed production area 1 ha of sterile seeds can serve,
Y_{MS} is yield of sterile seed (kg/ ha),
K_{MS} is proportion of sterile seed of acceptable standard,
S_{HS} is sterile seed used/ ha of hybrid seed production (kg/ ha), and
d is allowance factor of sterile seed.

Substituting the sample data in the formula, we obtain

$$R_{SMSA} = \frac{0.9 \cdot 1369.8}{1.1 \cdot 27.3} = 41.1 \text{ ha.}$$

This result shows that the yield of 1 ha of sterile seeds can support 41.1 ha of hybrid seed production. The supportability of sterile seed multiplication is positively related with the yield of sterile seeds and the proportion of acceptable sterile seeds. It is negatively related with the amount of sterile seed used in hybrid seed production and the allowance factor for sterile seeds. To increase the supportable area of sterile seed multiplication, growers must increase the yield of sterile seeds, improve the quality of sterile seeds, and save the seed used in hybrid seed production.

The output of sterile seed multiplication had the same characteristics as hybrid seed production: the yield of sterile seeds was lower than the yield of hybrid rice, but the value of output was higher. Sterile seed yield was 1.4 t/ha, and restorer seed yield 1.1 t/ha. The total yield was 2.5 t/ha, 4.9 t lower than hybrid rice yield. The output value of the multiplication field was 5,306.00 yuan/ ha, 2,627.00 yuan higher than of production field rice, or a 98% increase. Farmers got almost 2 times higher gross return.

On the input side, sterile seed multiplication used much more labor. Per hectare labor input reached 397.8 workdays. Physical labor accounted for 46% of the total.

The net returns of sterile seed were 4,074.00 yuan/ ha, net returns to labor 12.52 yuan/workday, and net returns to total cost 2.07 yuan (Table 14). Thus, returns to sterile seed multiplication are much higher than those to hybrid rice grain production, and close to those of hybrid seed production.

Ecological and social results of hybrid rice production

Results of surveys show that hybrid rice production fits well in the farming ecosystem. Hybrid rice gives more grain as well as straw. The straw can be used as

Table 14. Economic returns to sterile seed, hybrid seed, and hybrid rice production, 1984.

Variable	Sterile seed	Hybrid seed	Hybrid rice
Net return (¥[a]/ha)	4074	4506	1271
Net return (yuan/yuan output)	2.07	2.28	0.91
Net return (yuan/workday)	12.52	14.30	6.00

[a]2.74 yuan = $1.00.

manure to improve soil texture and fertility. Because hybrid rice increases grain output per hectare, farmers can use more of their land to grow other cash crops, such as oil-bearing crops, cotton, and watermelon. They may engage in poultry and livestock raising. All by-products from the above enterprises, such as oil seedmeals, straw, and barnyard manure, can be used to enrich soil. Two experiments in Huaiyin county in Jiangsu Province showed that using these manures with nitrogen and phosphorus fertilizer keeps soil N balanced and increases soil organic matter.

The social results are also important. Growing 1 ha of hybrid rice could increase grain by 0.8-1.5 t/ha, so more grain could be sold to the state to meet the social demand. When grain output increases, farmers could plant other industrial and oil crops, raise animals, and sell products in the market, which would create better conditions for adjusting the agricultural structure and rural estate structure.

The expansion of hybrid rice production has greatly changed rice cropping patterns. In southern Jiangsu and riverine areas, which were double rice-triple cropping areas before, the dominant cropping pattern has changed to hybrid rice - wheat. These new cropping patterns have better ecological and social results. Under hybrid rice production, the harvesting date of rice moved from late October to early October, so the wheat crop could be sown on 20 Oct instead of mid-November. This has increased wheat yields. For example, average wheat yield in Zhenjin region of Jiangsu Province increased from 1.5 t/ ha to about 3.0 t/ ha.

Although hybrid rice seed production requires highly skilled work, techniques have been standardized, allowing few trained technicians to do this job. Further, hybrid rice has evident hybrid superiority, vigorous growth, and high filled spikelet percentage. Field cultivation and management of hybrid rice are easier than those of conventional rice. Transplanting density is usually 220,000-300,000 plants/ ha, 40-50% less than for conventional rice. This not only increases yield, but saves seed production area, reduces labor input, eases the peak labor demand for harvesting and transplanting in triple-cropped rice, and frees labor from the agricultural sector, providing labor for rural industrial development.

Hybrid rice production promoted the development of new rice cultural techniques. For example, improvements have been made in selecting suitable multiresistant and highly resistant varieties and combining them properly; growing strong seedlings with complete seedling sets; overcoming the seasonal limitation of turnaround time; using tillering superiority and correctly handling the relation between seedling number and spikelets; fully using temperature and light resources;

deciding best sowing and flowering dates; and scientifically managing water and fertility. These efforts also improved the cultural technique of conventional rice.

Hybrid rice has made an important contribution to grain production and enhancement of economic efficiency in China. The major task is to create more favorable conditions to introduce hybrid rice technology where it is feasible.

Problems of further popularizing hybrid rice

Increasing hybrid rice-growing area. In recent years, the percentage of hybrid rice-growing area in Jiangsu Province stabilized at 30%. This percentage is limited by social-economic conditions, turnaround time, and grain quality. With the development of mechanization and the popularization of new, high-quality, resistant varieties and increased demand for rural labor, the percentage of hybrid rice area can be further increased in the province, especially in the northern part.

Effects of prices on economic efficiency of hybrid rice. The economic efficiency of japonicas was higher than that of hybrid rice, partly due to an irrational price system. The current price for japonicas is 0.38 yuan/kg, whereas that for hybrid rice is 0.32 yuan/ kg. This 18% price difference is far beyond the quality difference. If we could properly adjust these prices, the economic efficiency of hybrid rice would improve.

If new seed production techniques reduce the cost, the price of seed will drop. The seed supply and demand will tend to balance, further increasing the economic efficiency of hybrid rice.

Labor efficiency. In hybrid rice production, most field work has been done manually. The high labor input (280.5 workdays/ ha) is inefficient. The cost of labor accounted for 46% of total cost. The grain product per workday was 26.3 kg. Agricultural modernization in China needs to free labor from cropping and transfer it to forestry, animal husbandry, fisheries, and other rural enterprises. Hybrid rice research must improve agricultural mechanization to save labor input.

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Hybrid wheat status and outlook

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The development of superior hybrid wheats *Triticum aestivum* L. has been improved by research in breeding systems based on both cytoplasmic male sterility-fertility restoration and chemical hybridizing agents. Commercial and experimental hybrid wheats produced using both systems currently are being extensively tested to assess yield potential and agronomic and processing characteristics. Technically and commercially, hybrid wheats are a reality. The challenges for hybrid wheat research are to reduce the costs of hybrid seed and to improve hybrid performance.

This paper surveys research on hybrid wheats in the U.S. Topics include pollination control systems, the *T. timopheevi* male sterility-fertility restoration system, chemical hybridizing agents, selection to improve cross-pollination potential of hybrid parents, combining ability and hybrid performance, and research needs.

This paper first surveys briefly current research in hybrid wheat development by commercial seed and chemical companies in the U.S., and describes the status of commercial hybrid wheat seed production. Second, it characterizes the pollination control systems wheat breeders use to develop hybrid wheat seed production. Discussion of parental breeding centers on chemical hybridizing agents (CHAs) rather than cytoplasmic male sterility-fertility restoration systems. Third, the paper describes selection procedures to improve the cross-pollination characteristics of hybrid wheat parents. Fourth, it discusses breeding and testing procedures to improve the combining ability of parents, and discusses the performance of hybrid wheats. Finally, it presents opportunities and constraints to future development of hybrid wheats.

Several recent reviews of hybrid wheat research provide a more comprehensive survey and description (Lucken 1986, McRae 1985, Virmani and Edwards 1983, Wilson 1984, Wilson and Driscoll 1983).

During the 1960s and early 1970s, hybrid wheat research was initiated by state experiment stations in the wheat-growing regions of the U.S. and by several commercial seed companies. At the universities, much of this exploratory research was conducted as MS and Ph D projects supporting varietal breeding programs. As the complexity of parental breeding programs and the expense of commercializing hybrid wheats became evident, state experiment stations reduced their breeding efforts. Except for the program at North Dakota State University (NDSU) and experiments on specific hybrid wheat problems in several states, nearly all hybrid wheat breeding in the U.S. is conducted by commercial seed and chemical companies (Table 1). State yield trials, however, provide useful comparative data on hybrid and varietal yield performance.

The discovery and development of chemical hybridizing agents in the 1970s and 1980s brought additional technical capabilities and additional commercial investments to hybrid wheat research.

Most hybrid wheat breeding involves the hard red winter wheats of the central and southern Great Plains. There also are hybrid breeding programs in the soft red winter wheat region of the Midwest and the hard red spring wheat region of the northern Great Plains.

The concentration of hybrid research on hard red winter wheat matches its hectarage—considerably larger than any other wheat class (26 million ha). Second, large-scale, drill-strip hybrid seed production has been accomplished successfully in the irrigated, high-yielding (and windy) wheat-growing areas of western Kansas and the Texas Panhandle.

Each company has its own strategy for developing hybrid wheats. Some produce varietal seed and hybrids. Others concentrate solely on hybrids; they have concluded that only a product that the farmer has to purchase each year will provide long-term profit. U.S. farmers typically replant their own varietal seed or purchase it from neighbors.

Breeding and seeding production efforts of a few companies center on the *T. timopheevi*-derived cytoplasmic male sterility-fertility restoration system. These companies have 20 yr or more of breeding experiments to improve the fertility restoration capacity of their R lines and to refine seed increase procedures for the cytoplasmic male sterile (CMS) female parents. The complements and restorer and modifier genes used by these programs likely still differ from one another for at least some of the genes.

Other companies have organized their breeding programs to use CHAs. The properties of a CHA can affect how it is used in a breeding program, and favorable response to a CHA becomes a selection criterion for the program's germplasm.

Table 1. A partial list[a] of commercial seed companies breeding hybrid wheat in the United States.

Company	Brand	Wheat class
Cargill, Inc.	Bounty	Hard red winter
HybriTech Seed International, Inc.	Quantum	Hard red winter
Nickerson American Plant Breeders	Agripro	Hard red winter Hard red spring
Pioneer Hi-Bred International, Inc.	Pioneer	Hard red winter Soft red winter Soft red spring
Rohm & Haas Seeds, Inc.	Hybrex hybrid	Hard red winter Soft red winter
Trio Research, Inc.		Hard red winter

[a]Other seed and chemical companies are evaluating cereal hybridizing agents and producing experimental hybrid wheats.

Between 10 and 20 commercial hybrid wheats have been released and marketed, mostly hard red winter wheat hybrids. Hectarage planted to hybrids is less than 1% of total U.S. hectarage. At least three companies produce hybrid seed on a commercial scale, with tens of thousands of hectares planted to hybrids. These companies are developing expertise in managing the logistics of hybrid seed production and in marketing seed to U.S. farmers. Both CMS- and CHA-produced hybrids are marketed.

Hybrid wheat seed is sold in 50-lb (22.7 kg) units. So far, most hybrid seed has been priced at $20/unit or higher. Current prices are in the $19.50 range with discounts for early or bulk purchases.

The profitability of growing hybrids can be evaluated by examining the effect of various seed production procedures, and varying seed costs, cash market price of wheat, seeding rates, base yield levels, and hybrid yield advantages. A recent article by Johnson (1985) discusses the impact of some of these variables on the profitability of growing hybrid wheat. The dominant variable in hybrid seed pricing is the seed set on the female parent in hybrid seed production fields, making this trait the major breeding goal, together with hybrid yield advantage.

Technically and commercially, hybrid wheat in the U.S. is a reality. Both CHA- and CMS-based hybrids represent major technological achievements. The challenges for hybrid wheat now are to further reduce the costs of hybrid seed and to further improve hybrid performance. Economies of scale, technical proficiency in producing hybrid seed, and genetic improvement of parental capacity for cross-pollination should reduce the relative costs. The size of this decrease will largely determine the rate and extent that hybrid wheat becomes planted on U.S. wheat hectarage.

Pollination control systems

At least four types of pollination control are available to develop hybrid wheat parents.

Cytoplasmic male sterility-male fertility restoration

The *T. timopheevi*-derived system is used almost exclusively in hybrid wheat research, but several other male sterility-inducing cytoplasms have been introduced into wheat from its wild relatives, mainly diploid and tetraploid *Aegilops* and *Triticum* species. The requisite nuclear-cytoplasmic interaction of fertility restoration also is known for several of these alien cytoplasm substitutions. Extensive breeding and additional genetic research are required to refine alternative systems for use in commercial hybrid seed production, and, consequently, their main use has been to supplement the *T. timopheevi* system.

Nuclear genic male sterility

A recessive gene for male sterility is located on the short arm of chromosome 4A. Driscoll (1972, 1985) has proposed using this gene for pollination control in a system he terms “XYZ,” denoting the types of lines used to maintain and increase male sterility. Standard wheats are used as male parents. This system depends on differential transmission of an extra chromosome which carries the normal allele of the male sterile gene.

Chemical hybridizing agents

More than 35 chemicals have been patented as potential CHAs. The chemistry includes a diverse group of structural families. Extensive developmental research has been conducted on the CHA efficacy of at least two of these families, the Rohm and Haas CHAs and the Shell CHAs.

Chemical restoration of male fertility in genetic male steriles

Presumably, chemical companies do not yet have screening programs to identify fertility-restoring chemicals. These chemicals would be applied to the genetic male sterile parent to induce pollen fertility during female parent increase. Their use would greatly improve the efficiency and logistics of female increase, usually a limiting step with the genetic systems.

In contemplating the relative advantages and disadvantages of each system, it is easy to overlook the obvious: the sole reason for the breeding manipulations of male fertility in cytoplasmic male sterility-fertility restorer and in nuclear genic systems is to accommodate the seed increase of the male sterile parent. In wheat, the impact of these manipulations on breeding is large although not entirely negative. A CHA eliminates the necessity to increase the male sterile. Breeding efficiency is improved as is the efficiency of female parent increase, but the efficiency of actual hybrid seed production is decreased.

The *T. timopheevi* male sterility-fertility restoration system

The *T. timopheevi* cytoplasm has most characteristics needed for hybrid development: highly stable male sterility, effectiveness with most genotypes, minimal effect on plant vigor and other plant traits, and easy incorporation by backcrossing. Its major drawback has been that restoring normal, complete, male fertility in F_1 hybrids has proven to be extremely difficult.

Genetic control of the fertility restoration trait is multigenic. Monosomic analyses show two or three *Rf* genes which, singly or in even combination, when heterozygous, confer only partial fertility restoration to F_1 hybrids (Maan et al 1984, Maan 1985). Breeding and selection efforts spanning many crossing cycles and crop generations have been required to accumulate modifiers of the *Rf* genes and eliminate inhibitors of *Rf* genes to achieve complete phenotypic expression of male fertility. Genes on nearly all chromosomes affect expression of *Rf* genes, and relationships among homeologs of *T. aestivum's* three genomes also have been found.

Breeding of R lines has involved intense selection for male fertility. In the NDSU program, the primary selection criterion for fertility restoration has been anther extrusion. Early-generation spaced plants and subsequent later generation solid-seeded lines are evaluated and selected on the basis of full and complete anther extrusion to the tip of the wheat spike. Examinations of anther morphology and appearance and of seed set augment the anther extrusion observations. Anther extrusion is the most useful selection criterion because thousands of plants or rows can be quickly evaluated visually.

The evaluations of self-fertility have been coupled with testcross evaluations of fertility restoration. Selected R lines are crossed to one or more male steriles, and the fertility of their F_1 hybrids is evaluated. Many completely self-fertile R lines produce F_1 hybrids which have only partial fertility, again reflecting the complex interactions among the complement of *Rf* genes and their modifiers.

The introgression of *T. timopheevi* cytoplasm and *Rf* genes from *T. timopheevi* and other species apparently has required far more modification of the *T. aestivum* genomes than originally envisioned. Genetic compatibility now has been established to the extent that R lines yield comparably to the best conventional wheats, while carrying the required complement of *Rf* genes and modifiers. Further improvement seems necessary and possible.

Based on knowledge of evolutionary relations among *Triticum* and *Aegilops* species, breeders believe that R lines significantly broaden the germplasm base of cultivated wheats.

The selection for anther extrusion and for genes which enhance anther extrusion also has improved the cross-pollination capacity of R lines. As a group, R lines are superior to conventional varieties as male parents in both CMS and CHA hybrid production systems. Breeders using CHA hybrid breeding systems should systematically evaluate their pollen parent breeding lines for anther extrusion.

Chemical hybridizing agents

The commercial appeal of cereal hybridizing agents is that they permit direct commercial production of F_1 seed using standard wheats as parents. CHAs circumvent several of the breeding manipulations required to develop male and female parents, enabling breeders to focus more on improving hybrid performance.

An effective CHA should

- induce complete or near-complete male sterility in most wheats;
- effectively sterilize wheat over a sufficient range of plant developmental stages to allow time to spray large hectarages or to adjust for bad weather; it therefore likely should be systemic;
- not otherwise damage the plant (phytotoxic effects);
- not affect female fertility;
- have small dosage interactions with environments and varieties;
- be environmentally safe and nontoxic to humans and animals;
- be cost effective and reasonably easy to apply.

Since 1981, we have conducted extensive trials with SD 84811, an experimental CHA from Shell Oil Company (Johnson 1984). SD 84811 is azetidine-3-carboxylic acid, a proline analogue.

We first evaluated rates and times of application on hard red spring wheats in North Dakota. We next assessed genetic variation for response to SD 84811 in terms of male sterility and seed set by evaluating a diverse group of spring wheat cultivars.

For the last 3 yr, we have used SD 84811 to screen for seed setting capacity and favorable response to the chemical, and to produce hybrid seed for hybrid and combining ability evaluations.

SD 84811 fulfills most of the requirements listed above. Treatment with 400-700 kg SD 84811/ha causes complete or near-complete male sterility in most hard red spring wheats in North Dakota. The chemical is highly systemic; the period of effective treatment ranges from when spike primordia are 2-3 cm long to just before heading. This period is 7-10 d in North Dakota and is sufficient for timely spraying. The most favorable seed sets are obtained with later treatment. Except where very late tillers develop, male sterility usually is induced in all heads of the female plant canopy.

The phytotoxic effects of SD 84811 are minimal; maturity, plant height, and general plant growth are not affected or are only slightly affected. Seed development is relatively normal, although some changes on cytoplasmic male steriles—larger kernel size, higher protein, and lower test weight—also are found for the CHA-induced male steriles. These differences vary for genotypes and environments. These interactions have not been fully characterized, and breeders are selecting genotypes that minimize seed set reduction or apparent female sterility.

Dosage, timing, and genotypic responses permit the use of SD 84811 in breeding programs to screen genotypes for cross-pollination potential and to produce experimental hybrids. In other words, entire nurseries or crossing blocks can be treated with a single dosage of SD 84811 over a 10-d period, to obtain reliable hybrid seed production and seed set data. However, once commercial production of a specific hybrid combination is planned, time and dosage experiments probably should be conducted to determine optimal rates and timing for the female genotype. Further knowledge of environmental factors affecting the chemical activity also would help in adjusting application dosages.

The traits of interest in CHA trials are male sterility, female sterility, seed set by cross-pollination, and hybrid seed yield. Except in few instances, easily identified marker genes are not available in wheat to classify as selfed or hybrid the seed produced on treated genotypes in a seed production block. Male sterility usually is determined by bagging heads in treated plots before anthesis or by placing a tent in the plot before anthesis, with seed set of the bagged heads or plot measured at maturity. When hundreds of plots are being treated, the determination of male sterility requires substantial effort during the few days between heading and anthesis, 1-3 d in North Dakota. Bagging induces some male sterility, further complicating this evaluation. As the performance of the CHA becomes known and predictable, less bagging is required.

The measurement of female sterility is much more complicated. Seed set on the female is affected by the cross-pollination potential of the female; the pollination capacity of the male; the nick or coincidence of male and female flowering, environmental effects including day-today variations in wind, soil moisture, air temperature, and humidity; and female response to the CHA. Each variable confounds accurate determination of the two principal variables: genotypic differences for cross-pollination potential among females and genotypic differences in response to the CHA among females. Although these traits appear moderately heritable, repeated observations and trials usually are necessary to identify superior genotypes.

Partial results from a CHA crossing block grown during 1985 are shown in Tables 24 to illustrate the type of data obtained.

Table 2. Hybrid seed yields (tlha) from females treated with SD 84811 at 3 rates in R138 and R182 crossing blocks. North Dakota, 1985 (Johnson 1985, unpubl.).

Variety or line	SD 84811 (g/ha)		
	0	400	600
	R138 pollinator		
Bonanza	3.6	2.9	3.0
Veery 1	4.7	3.3	3.4
Glenlea	4.3	3.6	3.0
R148	3.9	3.0	3.0
Mead	3.4	2.8	2.5
	R182 pollinator		
Bonanza	3.4	3.2	3.2
Veery 1	4.1	2.2	2.2
Glenlea	4.3	3.3	3.3
R148	4.0	2.9	2.8
Mean[a]	3.8	2.6	2.5

[a]Av of 10 females including tho 4 listed in the table.

Table 3. Seed set percentages as % of control from females treated with SD 8481 1 at 3 rates in the R138 and R182 crossing blocks. North Dakota, 1985 (Johnson 1985, unpubl.).

Variety or line	SD 84811 (g/ha)		
	0	400	600
	R138 pollinator		
Bonenze	100	78	83
Veery 1	100	72	74
Glenlea	100	85	71
R148	100	78	73
Mead		70	62
	R182 pollinator		
Bonanza	100	95	93
veery 1	100	54	53
Glenlea	100	77	78
R148	100	71	68
Mean[a]		70	67

[a]Av of 10 femalea include the 4 shown in the table.

Table 4. Selfed seed set percentages (wt of 20 bagged heads, % of control) from females treated with SD 84811 at 3 rates in the R138 and R182 crossing blocks. North Dakota, 1985 (Johnson 1985, unpubl.).

Variety or line	SD 84811 (g/ha)		
	0	400	600
	R138 pollinator		
Bonanza	100	3.2	6.6
Veery 1	100	8.3	0.5
Glenlea	100	42.3[a]	5.0
R148	100	13.1	0.7
	R 182 pollinator		
Bonanza	100	2.4	0.9
Veery 1	100	0.1	0.4
Glenlea	100	7.6	0.6
R148	100	1.5	7.9

[a] Treated late.

Selection to improve the cross-pollination potential of hybrid parents

CHAs permit direct production of hybrid seed using standard varieties. Theoretically, varieties and elite lines, the best products of large, intensive breeding efforts to improve yield, disease resistance, and quality, can be used immediately and directly for hybrid seed production.

Unfortunately, most standard wheats lack the floral traits required (either as male or female parents) to produce high, consistent seed set in hybrid seed production fields. This is true for wheat varieties in all wheat classes grown in the U.S. Consequently, improvement of genetic cross-pollination potential, including the seed-setting capacity of the female and the pollinating capacity of the male parent, is a major goal of hybrid wheat breeding programs.

Anther extrusion can be evaluated visually (we use a scale of 1 = low extrusion to 5 = high extrusion). Any program should evaluate its breeding lines and varieties for this trait when choosing candidates for pollinator parents. Breeders should evaluate anther extrusion on single plants or head rows in early generations, followed by selection in solid-seeded rows and replicated yield trials in later generations.

Contributions of the remaining components of pollinating capacity—pollen quantity, viability, durability, etc.—are determined from crossing block comparisons. Isolation requirements limit the number of pollinators that can be critically evaluated to fewer than 30 pollinators yearly for most breeding programs. This hampers selection based on actual seed set in crossing blocks, a major impediment to improving the cross-pollination potential of wheat.

With CMS systems, the potential selection differential for seed-setting capacity among females also is limited. Females can be evaluated for seed set only in crossing blocks after male sterile conversion by backcrossing. A program can usually convert to male steriles fewer than 50 lines/yr.

CHAs greatly increase the opportunity to improve the seed-setting capacity of females by screening more genotypes. In several experiments, we planted several hundred head rows or single replication plots (0.6 m × 2.4 or 1.5 m) in a crossing block arrangement with the pollinator planted in alternate strips throughout the nursery. The female plots are treated with a single application of SD 84811 when about 15% of the plots have just headed. In a nursery of 400 female plots, the spraying can be done in less than an hour.

Near maturity, when kernel size is maximum, visual estimates of seed set are recorded. Head rows that have high seed set can be marked, and the remaining rows discarded. Remnant seed of selected lines can then be increased for further evaluations. The visual evaluation makes it possible for one person to evaluate several hundred rows per day for seed set—or several thousand head rows. In screening trials consisting of from 208 to 400 genotypes grown at 2 locations over 3 crop seasons, correlations between the visual estimates of seed set and actual plot yields have consistently been around 0.7. Selection intensity can be many times higher than when selecting among CMSs.

However, the female fertility response to the CHA also is a component of seed-setting capacity. The result is a reduction in effective population size for the inherent genetic component of seed-setting capacity. Nevertheless, the selection procedures are entirely appropriate for female lines that will be used as parents in CHA hybrid production, because both components of seed-setting capacity are required.

Results from various crossing block and screening trials have revealed genetic variation for seed-setting capacity in this normally self-pollinated crop. The relative contributions of various floral and plant traits to seed-setting capacity still are not well understood. It should be possible for breeders to make rapid progress in enlarging the seed-setting capacity of their female germplasm using available genetic variation and CHA-aided selection techniques. CHAs also should be useful as breeders attempt to identify genes that enhance cross-pollination in populations derived from crosses with wheat's wild relatives and also in population programs to improve seed-setting capacity.

Combining ability and hybrid performance

Fortunately, the two traits critical to hybrid wheat development can be coupled in their evaluation during breeding. When a line's seed-setting capacity is evaluated by cross-pollination with one or several pollinators, the F_1 seed produced can be planted in a yield trial to evaluate the line's combining ability. Consequently, a breeding program can be organized to comparatively and simultaneously improve cross-pollination capacity and the combining of parental lines.

Such evaluations can be done in many ways during the breeding progression. Decisions about testers and numbers of testers, plot sizes, and the scale of yield trial testing depend on several variables, including project resources, the availability of off-season nurseries, and, of course, breeding philosophy.

Hybrid wheat breeding techniques provide direct comparative progeny evaluations. Typically, hybrid wheat seed is produced in isolated crossing blocks having several females planted in alternate strips with a single pollinator. The same set of females likely will be grown in other crossing blocks—with other elite pollinators. The resulting hybrid matrix, f number of females × m number of males, in yield trials provides data which allow rigorous comparisons of the combining abilities of male and female parents (Table 5).

The reliability of the estimate of a line's combining ability depends, of course, on the number of replications, testing sites, number of females (for male comparisons), and number of males (for female comparisons). For example, when a common set of 17 females is crossed with 2 pollinators, and a 3-replication trial is grown at 2 sites, the comparative combining ability of the females is based on 12 plots (2 males × 3 replications/ site × 2 sites) and the comparative combining ability of the males is based on 102 plots (17 females × 3 replications/site × 2 sites) (Table 6). Thus, males can be selected with more confidence than females. In practice, many more females typically will be evaluated than males because it is easier to use more

Table 5. F_1 yields and general combining abilities (GCA) of 9 males and 4 females grown in yield trials at Fargo and Prosper, North Dakota, 1984.[a]

Male	Female			Mean	GCA
	MST-ND496	MST-Butte	MST-Coteau		
		Yield (t/ha)			
R144	5.8	9.3	8.6	9.0	–.36
R159	9.1	10.1	9.3	9.6	–.07
R162	11.3	12.3	11.3	11.3	.61
R163	11.1	10.8	10.2	11.1	.54
Mean	9.6	10.1	9.6		
GCA	–.07	.22	–.07		

[a] F_1 values shown are for 4 males and 3 females only.

Table 6. Means from analysis of variance of the general combining abilities of 2 males and 17 females from F_1 yield trials at Fargo and Prosper, North Dakota, 1984.[a]

Male or female	N[b]	Yield (t/ha)	% of 5 checks
R139	102	8.5	98
R122	102	9.2	105
Glenlea	12	10.0	115
Chora Sib	12	9.7	111
Wheaton	12	9.6	110
Solar	12	9.2	106
Marshall	12	8.3	96
ND599	12	8.1	93

[a] Results shown are for 6 females only.
[b] N = 102 = 17 females/male X 2 sites X 3 replications/site.
N = 12 = 2 males/female X 2 sites X 3 replications/site.

females/crossing block than to add crossing blocks. However, numbers of males and females can be adjusted in combining ability evaluations to meet breeding goals.

In the normal breeding progression, selected males and females would be included in the next cycle of hybrid matrices and also might be used in subsequent years as testers in early-generation evaluations. This testing and selection process should identify increments of yield in both male and female parents which, cumulatively, should contribute to hybrid advantage.

Although simple in concept and execution, such systematic evaluations of parental combining abilities, facilitated by CHAs, should provide breeders with a powerful means of improving wheat yields.

It is difficult to describe and characterize hybrid wheat yields. Most hybrid yield trials have been conducted by commercial companies and their data are proprietary. Hundreds of hybrids now have been tested by these companies in standard yield trials.

The hybrid × environment interactions appear to be the same size as varietal × environment interactions; extensive yield trial testing at several sites over 2 or 3 yr is required to accurately assess relative varietal and hybrid performance. Hybrids respond differently to environment and vary in stability.

Hybrids produced with parents previously screened for combining ability should perform substantially better than those produced simply using good varieties. This alone makes it difficult to generalize about hybrid performance.

The results from hybrid red spring wheats in Table 7 are from 104 hybrids involving 8 males and 52 females; some parents had been screened previously for combining ability, but most had not.

The most extensive hybrid data are from hard red winter wheat hybrids grown in state yield trials in Kansas, Oklahoma, and Colorado. The results perhaps best indicate current status of hybrid wheat in the U.S. Yields of the highest hybrid and highest variety in each of the last 4 yr in the Kansas State Wheat Performance Trials are summarized in Table 8.

Table 7. Summary of R line combining abilities and hybrid yields from hybrid yield trials at Fargo, Prosper, and Langdon, North Dakota, 1985.

Trial	R line	N	Mean hybrid yield[a]			Highest yield
			(t/ha)	(% of ck)[b]	(% of R line)	(% of ck)
30	R159	117	5.15	114	109	120
30	R163	117	5.21	115	105	125
31	R144	117	4.88	107	99	116
31	R146	117	4.78	105	111	113
32	Coteau	117	4.67	100	102	111
32	R148	116	4.91	106	118	115
33	R150	117	5.01	110	110	111
33	R162	116	5.18	111	111	115

[a]Means of hybrid with 13 females; three replications of each trial at each site. [b]Four checks: Stoa, 107%; R122, 105%; Marshall, 95%; and Len, 93%.

Table 8. Summary of yield results for highest hybrid, highest variety, and Arkan for 4 yr in the Kansas Wheat Performance Trials.

Year/Kind	Yield (t/ha)[a]	Hybrid advantage
1982-83		
Bounty 310	4.59	437 kg (111% of Vona and 118% of trial average)
Vona	4.16	
Arkan	4.14	
1983-84		
Bounty 203	4.80	283 kg (106% of TAM 107 and 118% of trial average)
TAM 107	4.51	
Arkan	4.08	
1984-85		
Bounty 203	4.73	518 kg (112% of Pro Brand 830 and 117% of trial average)
Pro Brand	4.21	
Arkan	4.09	
1985-86		
Bounty 122	3.83	193 kg (105% of Century and 116% of trial average)
Century	3.63	
Arkan	3.89	

[a] Av of 16 sites in 1982-83 and 1984-85 and 14 sites in 1983-84 and 1985-86 (Walter 1983, 1984, 1985, 1986).

Research needs

This short survey of research progress in hybrid wheat development does not include significant areas of research, or describes them only slightly. Similar papers could be presented on field-scale hybrid seed production, the breeding of R lines, developmental research for CHAs, the economics of hybrid wheat, breeding methods, and hybrid performance.

Although hybrid wheat still is grown on relatively few hectares, the research progress in hybrid development is impressive. Here are some opportunities for further progress.

- Rigorously apply and refine available breeding techniques and intensively use known genetic variation to develop superior hybrid wheat parents.
- Introduce further genetic diversity for combining ability and heterotic response into various wheat classes 1) by standard crossing procedures; 2) through population improvement procedures; 3) through cytogenetically manipulated stocks derived from crosses with wheat's wild relatives; and 4) through genes introgressed into wheat or modified by genetic engineering techniques.
- Adapt more of the techniques used to breed maize and other cross-pollinated crops including CHA-aided mass selection and recurrent selection.
- Acquire more knowledge about the genetic variation of floral biology including 1) spike and flower morphology; 2) pollen dispersal, buoyancy, durability, and vigor; 3) stigma accessibility, receptivity, and durability; and 4) development of selection screens for these traits.

- Acquire more knowledge about the genetic, physiological, and biochemical bases of male sterility caused by nuclear genes, by cytoplasmic genes, and by CHAs. The goal is the most efficient pollination control possible for both parental breeding and hybrid seed production.
- Control ergot (*Claviceps purpurea*) and other floral diseases in hybrid seed production fields.
- Seek complementary responses (between parents) for traits that can contribute to hybrid advantage, e.g., disease resistance, milling and baking quality, yield components, and plant height.
- Develop procedures for measuring genetic diversity among potential hybrid parents. Biochemical characterization of genotypes in terms of protein or DNA spectrums could supplement parental performance, physiological responses, and pedigrees and geographical characterizations to describe the genotype.
- Produce and test thousands of experimental hybrids in yield trials to identify superior hybrids and parents with exceptional combining ability.

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Heterosis in rice

Genetic distance and heterosis in japonica rice

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Sixty varieties of japonica rice were tested at Hefei and at Anqing, Anhui Province, in 1979. In 1982, 43 varieties, including A, B, and R lines, were tested in a similar manner. The genetic distances (D^2) of quantitative characters associated with yield between any two varieties were estimated. Heterosis was predicted based on these estimated genetic distances, and 96 crosses of F_1 seeds were planted for checking. Correlations were highly significant between the magnitude of genetic distance values and the strength of heterosis (r = 0.5536** at Hefei and r = 0.5814** at Anqing in 1979; r = 0.7601** at Hefei in 1982).

There were also highly significant correlations between sites and years for the genetic distances of the same varieties (r = 0.4645** between sites, r = 0.6123** between years). It appears that genetic distance can be used in selecting parents for wide hybrid combinations. The predicted superior cross combinations provide guidelines for breeding japonica rice hybrids; some have been identified and adopted. The coincidence of achieved combinations with those predicted was 80%.

Major factors affecting D^2 values are discussed. Eigenvalues and corresponding eigenvectors were calculated from genetic correlation matrices by using two groups of varieties. Results show that yield factors (characters of panicle and grain) and growth duration play a major role in complex indicators. In addition, correlations between some factors of the two groups frequently interact with each other. Therefore, ecotopic differences between varieties play an important role in D^2 values and yield.

Contributions of some quantitative characters to genetic diversity in rice

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Before any breeding program is begun, a knowledge of the genetic diversity of parents is essential for obtaining high heterosis in F_1s, creating broad-spectrum variability in segregating generations, and for categorizing varieties. Ten main quantitative characters of 29 indica cultivars were

measured in 1984 by cluster analysis and discriminant analysis. A randomized complete block design with three replications was used.

Through Jacobi's transformation, characteristic roots and vectors were calculated from the matrix of genetic correlation coefficients. Four principal components of each variety were calculated and used to estimate Mahalanobi's D^2. The 29 varieties were divided into 6 groups at the level of $D^2 = 4.5$ following the method described by Tocher.

The results of cluster analysis were subjected to discriminant analysis. The discriminant functions were established as

$$F1y = 0.965X_1+1.581X_5+4.251X_7+7.392X_9+1.811X_{10}-452.699$$
$$F2y = 0.971X_1+1.308X_5+3.544X_7+7.096X_9+1.684X_{10}-363.705$$
$$F3y = 0.442X_1+1.612X_5+3.687X_7+1.759X_9+2.174X_{10}-421.419$$
$$F4y = 0.179X_1+1.487X_5+3.541X_7+9.814X_9+1.314X_{10}-355.945$$
$$F5y = 0.952X_1+1.744X_5+4.285X_7+6.906X_9+1.613X_{10}-447.775$$
$$F6y = 3.477X_1+1.350X_5+5.014X_7+0.530X_9+1.391X_{10}-587.538$$

where X_1 = plant height, X_2 = average number of filled spikelets per panicle, X_7 = percentage of filled spikelets, X_9 = 1,000-grain weight, and X_{10} = heading date.

An equation to estimate a character's contribution to genetic diversity is proposed: the degree to which the function of the character plays a role in varietal taxonomy, whether ecotopic or genetic. If we let Qi represent the i^{th} character's contribution, then

$$Qi = \frac{(Ci\ maximum - Ci\ minimum}{2}$$

where Ci is the coefficient of i^{th} variable in the discriminant function.

Qi can be used as an index for varietal taxonomy and as an index for selecting parents for cross breeding and heterosis.

Genetic effects of cytoplasm on hybrid rice

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The influence of 8 male sterile (A line) cytoplasms (WA, Liuye, Shenqi, Gambiaca, Hongye, BT, Dian 1, and Dian 3) on 12 agronomic characters of hybrid rice was studied, with the following results:

- All had negative effects on plant height, neck length, panicles per plant, percentage of productive tillers, filled spikelets per panicle, filled spikelet percentage, 1,000-grain weight, and grain weight per plant. With different combinations, heading time was apparently delayed. Cytoplasmic effects were similar to zymographed vitality of the peroxidase isozymes. We conclude that the phenotypic effects of sterile cytoplasm on hybrid rice constitute an inheritance phenomenon closely related to sterility.

- The decrease in the yield of hybrid rice derived from the A line is due to the degradation of all yield components. Path analysis showed that the percentage of productive tillers had the greatest direct negative effect on grain weight per plant, followed by total spikelets per panicle and filled spikelets per panicle, and filled spikelet percentage and 1,0000-grain weight.
- Most aF_1 combinations showed positive vigor in grain weight per plant. The means of heterosis indices were in the order: aF_1 of sporophytic sterile type in indica rice (1.39) > aF_1 of gametophytic sterile type in japonica rice (1.19) > aF_1 of gametophytic sterile type in indica rice (1.08). Yet in $aF_1 - bF_1$, the sterile cytoplasmic effects of the heterosis indices are the reverse, –0.31, –0.19, and –0.11. These results show that the negative effect of sterile cytoplasms on heterosis is only relative. If the combinations have high combining and restoring ability, the negative effects are not strong enough to change the direction and degree of heterosis.
- The genetic effects of sterile cytoplasms vary with cytoplasmic sources and combinations. Therefore, it is necessary to evaluate objectively the heterotic influence and direction of A lines with different cytoplasmic sources. We suggest introducing the concept *cytoplasmic effect* or *cytoplasmic heterosis* into heterosis evaluation, and expressing it as a percentage according to the equation

$$\text{Cytoplasmic effect } \% = \frac{aF_1\text{-}bF_1}{bF_1} \times 100\ bF_1$$

where aF_1 is. the sterile line/restorer line F_1 and bF_1 is the maintainer line of the same type/restorer line F_1.

Comparison of heterosis effects of different cytoplasm types in rice

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Cytoplasm affects agronomic characters in many crops. This study attempts to draw inferences from the effects of cytoplasm on rice progeny. Several A lines developed by nucleus substitution, with identical nuclei but different cytoplasm, were hybridized with some R lines. The F populations were then planted to observe the phenotypic differences in agronomic characters and physiobiochemical properties.

Cytoplasmic effects were manifested in various ways such as the number of green leaves at harvest, 1,000-grain weight, days to heading, net photosynthetic rate, and peroxidase zymograms. This indicates the potential for broad cytoplasmic effects on the phenotypes of many characters. Nucleus-cytoplasm interactions were exhibited in panicle length, filled spikelet percentage, and exudation pressure, among others. These results proved that the materials used belong to the nucleus-cytoplasm interaction type.

Hence rice heterosis should result from better combinations between the nuclei of R, B, and A lines. Divergences of different cytoplasms affect heterosis. The R line is likely to affect cytoplasm somewhat; the B line likely affects its direction of expression. To obtain high heterosis, we still must consider renewing R lines and improving B lines. On the other hand, exploiting new sources of sterile cytoplasm may be important in hybrid rice breeding.

Combining ability of some agronomic characters in hybrid rice

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Of the many reports on combining ability in rice, few are devoted to hybrid rice. To determine guidelines for hybrid rice breeding, the combining ability for 12 characters in japonica hybrids was studied in a 7 × 5 diallel including 7 A lines with different cytoplasm and 5 R lines. Most characters were affected by the general combining ability (GCA) effects of A and R lines and the specific combining ability (SCA) effects of their combinations. The number of panicles per plant, however, was mainly affected by GCA effects of A lines, and grain yield and biomass per plant were unaffected by GCA effects of R lines. The GCA effects of A and R lines played a more important role than their SCA effects, and for the GCA effects the A lines were more important than the R lines. For that reason, greater attention must be devoted to developing A lines, and the diversity of R lines must also be considered in the breeding of japonica hybrids. The parents were evaluated according to their comprehensive expression of combining abilities of the main economic characters. The A lines of (BT) Liu-Qian-Xin and (D) Nangeng 34 A and the R lines of Ninhui 3-2 and 77302-1 were recommended as the better parents. The better combinations of (BT) Liu-Qian-Xin A/77302-1, (D) Nangeng 34 A/Ninhui 3-2, (BT) Liu-Qian-Xin A/Ninhui 3-2, and (D) Nangeng 34 A/77302-1 were also identified. These results, which coincided well with current hybrid breeding practices, verified the significance of combining ability in heterosis breeding.

Esterase isozyme and its application in hybrid rice breeding

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Esterase isozymes in the embryos of dry seeds of 164 combinations of A, B, and R lines and their F_1 hybrids were analyzed with polyacrylamide gel electrophoresis. Six bands were identified and named 1 A, 3A, 4A, 5A, 6A, and 7A. Among them, 1 A, 4A, and 7A bands were universally present in the materials analyzed. The occurrence of 3A, 5A. and 6A bands varied by variety. When A lines with the 6A band are crossed with R lines with 3A or 5A, hybrids with complementary combinations of 3A, 6A (or 5A, 6A) might

be obtained. Hybrids with 3A and 6A combinations usually showed apparent heterosis expressed as vigorous vegetative growth in the field. Hybrids with 5A and 6A combinations yielded well.

Zymographs of commercial indica hybrid varieties indicated 14 of them have 5A, 6A complementary combinations. Because these hybrids always have 5A and 6A esterase band patterns, an enzymologic assay of their F_1 seed can be used as a criterion of their purity. Electrophoresis has been improved and simplified; 200 samples can be analyzed in 8 h by 1 person, and the zymogram can be obtained from a single embryo.

Isoesterase in F_1 hybrids of 56 japonicas was studied. The japonica hybrid with band 5A was superior in yield, but still 16.7% lowerthan indica hybrids with 5A, 6A complementary bands. We did not, however, identify any hybrid japonica with 5A, 6A complementary bands. One reason is that of 513 japonicas tested, almost none exhibited the 6A band.

After an extensive survey of germplasm resources, one A line of indica type with the 6A band has finally been obtained, Ai-Jiu-Zao 6 A. Also, Prof. Tsui Jilin and colleagues at Jiangsu Academy of Agricultural Sciences developed a japonica variety No. 02428 with a 5A band by crossing mutants screened for high photosynthetic efficiency. It is highly compatible with indica varieties in hybridization. Most of the F_1 hybrids showed heterosis in higher filled spikelet percentage, comparable to that of normal commercial varieties. Its 5A band could be easily transmitted to its progeny to create an esterase 5A, 6A complementary combination characteristic of japonicas. High-yielding japonica hybrids might be produced by choosing appropriate parents for interspecific hybridization.

Preliminary analysis of heterosis in japonica rice

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More research on heterosis in indica rices has been done than on japonicas. We have investigated heterosis in japonica rice since 1978. Heterosis of principal economic characters in japonicas was marked. The levels and expressions of heterosis were analyzed by performance tests on hybrid progeny.

- The hybrid combinations showed significant heterosis for plant height and 1,000-grain weight. Most combinations showed heterobeltiosis, and many showed heterosis in productive tillers per plant, spikelets per panicle, and filled spikelets per panicle. Heterosis for yield was significant. Hybrid combinations were generally of medium duration, and appeared to be earlier than late parents. Filled spikelet percentage was generally low, varied widely, and differed markedly between combinations.
- Principal economic characters had significant standard heterosis, especially grain weight per plant, filled spikelets per panicle, filled spikelet percentage, and 1,000-grain weight. Principal economic characters in seven hybrid combinations, Xi 14 A/C57-80, Chang-

Bai 6 A/[B8/Jing-Ying 177)/S131], Chang-Bai 6 A/C57-80, Suong-Gian A/C57-80, B_2A/C57-80, Jing-Ying 127 A/C57-80, and Chang-Bai 6/[(B8/Jing-Ying 177)/R_1^{25-4}] were much better than those of the check variety Ji-Gen 60, and the hybrids yielded higher.

- Plant height, filled spikelets per panicle, productive tillers per plant, and filled spikelet percentage in hybrid combinations significantly positively correlated with the mean parental value; correlation coefficients and regression coefficients were all significant. The variation range of hybrid japonicas could be estimated by the mean parental value.

This experiment provides a basis for selecting new hybrid combinations with early maturity, high yield, resistances to diseases and stress, and other superior characters suitable for cultivation in Jilin Province.

Male sterility and fertility restoration

Classification of male sterile cytoplasms in rice by their nuclear-cytoplasm interactions

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The relation between restorers and maintainers, and the transfer of the male sterile heterocytoplasm to homokaryon were studied using the cytoplasm of 34 A lines. From 1974 to 1983, the cytoplasm of A lines collected and bred included 19 *Oryza sativa* f. *spontanea* wild rices along with wild-cultivated crosses, 4 *O. sativa* cultivars along with cultivated-wild crosses, 9 indicas, and 2 japonicas. The crosses were indica/indica, japonica/ japonica, indica/japonica, and japonica/indica. Pollen fertility and anther features, influence on major agronomic characters, and differentiation of restorers and maintainers were determined.

Male sterile pollens such as wild abortive (WA), Gambiaca (Gam), etc. abort at the uninucleate stage. Pollen grains are irregularly shaped, unstainable with I-KI solution, and their sterility is stable. Anthers are arrow shaped and milky-white, and when soaked in water their features differ from those of their maintainer lines. Pollens of male sterile lines of Hong-Lien (HL), Tian-Ji-Du (TD), and Jing-Chuan-Nuo (JN) cytoplasm abort at the binucleate stage. Anther shape is similar to that of the maintainer, but anthers generally do not rupture, except for a few porous ruptures, and pollen grains are not free-spreading. Pollen grains of A lines of Chinsurah Boro II (BT) cytoplasm abort at the trinucleate stage. Pollen grains are round, nonuniform, and are only slightly stainable with I-KI

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solution. Anthers are similar to the B line. Partially normal pollen forms and spreads under optimum temperature, but selfing rate is low, no higher than 11.8%.

The influence of different cytoplasm was measured by dividing male steriles into two groups. Group 1 consisted of Chao-Yang 1 A, which exhibits five kinds of heterocytoplasm, whose B line is sporophytic male sterile, and whose pollen aborts early. Group 2 was Fu-You 1 A, which exhibits four kinds of cytoplasm, whose B line isgametophytic male sterile, and whose pollen aborts late.

Plant height and internode length were affected by sterility, but not by cytoplasm. The A line exhibits more dwarfism than its B line. Dwarfism of the sporophytic male sterile is more obvious than in the gametophytic male sterile. Dwarfism in sporophytics is caused mainly by curtailment in the growth of 1st and 2d internodes, but of only the first internode in gametophytics. Panicles per plant is mainly affected by sterility; the A line has more panicles than the B line. Sterility and cytoplasm affect days from seeding to heading and head node length. Growth duration of the A line exceeds that of the B line. And the A line is more likely to exhibit partly exserted panicles than the B line. The effect of sporophytic male sterile on duration from seeding to heading and partly exserted panicles is more pronounced than that of the gametophytic male sterile.

The cross of the A line of heterocytoplasm-monokaryon and homocytoplasm-heterokaryon showed that cytoplasm controls the restorer-maintainer type, and that the nucleus controls the degree of restoration. Five differential varieties having sterile cytoplasm can be used to test cross for the 34 kinds of male sterile cytoplasm: Chao-Yang 1, IR8608-65-2, IR2055-462-2, IR2865-1-5, and BJ1. Seven types of restorers-maintainers have been identified: 1) Dong-Pu wild rice (DW) restored only by IR2055-462-2,2) HL restored only by Chao-Yang 1,3) WA restored by IR2055-462-2 and IR8608-65-2,4) Long-awn wild rice (LW) restored by IR2055-462-2 and IR2865-1-5, 5) Indonesian paddy (IS) maintained by Chao-Yang 1 and BJ1,6) Gam maintained only by Chao-Yang 1, and 7) BT maintained only by BJ1.

Based on these results, we propose the following five-step taxonomic key to male sterile cytoplasm:

1. inheritance of fertility divided into sporophytic male sterile and gametophytic male sterile,
2. pollen abortion stage divided into a) uninucleate (typical abortion), b) binucleate (round abortion), and c) trinucleate (stained abortion),
3. restorer-maintainer type divided into DW, WA, etc.,
4. manner of nucleus substitution divided into wild-cultivated, cultivated-wild, etc., and
5. varietal differentiation based on cytoplasmic source.

The differentiation of the five varieties of male sterile cytoplasm is one based on observation. Further genetic and biochemical studies are required.

A new cytoplasmic source of male sterility in rice

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Indica hybrid rice is grown commercially on more than 6.6 million ha in China. Because the cytoplasm of these hybrids is basically derived from WA, there is the potential for disease due to the uniformity of the cytoplasm. We screened wild varieties for a new cytoplasmic source of male sterility in rice and identified Jiangxi wild rice. Using its cytoplasm, we have bred the A line You-Zhan A and developed three new parental lines. The heterosis of the combinations is being evaluated.

Jiangxi *Oryza sativa* f. *spontanea,* which grows at 28°4'-10'N latitude, is the northernmost wild rice community in the world. It can withstand low temperatures during seedling and flowering, and it winters naturally at about –7 °C. It shows a higher resistance to yellow dwarf, bacterial blight, blast, and leaffolder in the field and when inoculated. It has excellent grain quality and is high in protein and various amino acids. Its tiller regeneration ability is good and the ratoon crop matures early. Its chromosome number is 2n=24; meiosis in pollen mother cells is normal without obvious chromosomal aberrations. Genome and band pattern analysis showed that only centromere bands (C/C) and short whole band and long centromere band (W/C) are found in this wild rice, but no whole bands (W/W), similar to japonica rices but unlike Guangdong and Guangxi *Oryza sativa* f. *spontanea* and indica. Compatibility is high and fertility is normal when it is crossed with indica, japonica, and Guangdong wild rice. It exhibits restorer ability when crossed with A lines of WA cytoplasm.

Some male sterile plants segregated in the F_2 of a You-Zhan A/indica cross were back crossed to a B line to develop the A line You-Zhan A (B_5F_1). You-Zhan A has extremely fine grain quality and is resistant to bacterial blight. Its restorer and maintainer relation differs from that in WA cytoplasm. You-Zhan A is a new cytoplasm source that can be used for rice heterosis.

Breeding male sterile lines by crossing indica varieties

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Sixteen A lines were derived from crossing indica varieties to produce an A line of nucleus-cytoplasm interaction type for three-line breeding. This A line consisting of the cytoplasm and the nucleus of indica (hsien) type is called Hsien-Hsien type A line (HHTSL), a nucleus-cytoplasm genotype with sterility formed in a series of crosses. HHTSL often displays properties of different pollen abortion types of the same nucleus and cytoplasm background, easily forms a complete set of three lines, and shows excellent sterility restoration. Studies of several groups of A lines with the same nucleus but different cytoplasm gave these results.

1. The pollen abortive manner of indica-type cytoplasm is different from wild abortive (WA) types and its outcrossing habit is significantly better than the WA check.
2. There are distinct differences in zymograms and enzyme activities of isoenzymes of cytochrome oxidase, esterase, and peroxidase between indica-type and WA cytoplasm, and of various indica-type cytoplasm.
3. The negative effect on yield heterosis in indica-type cytoplasm, even though it occurs, is lower than in WA, and indica-type hybrids have more filled spikelets and yield more per plant than WA hybrids.
4. HHTSL with typical pollen abortion is of the sporophytic abortive genotype.

For F_2, F_3, and B_1 incomplete crosses with two sets of 6 × 5 and 4 × 3, there are different male abortive genotypes in different crossing combinations. The sterility of most is controlled by recessive sterile genes, but in some it is controlled by dominant sterile genes. All HHTSLs developed have been used in complete sets of three lines for rice production.

Breeding maintainer lines of a wild abortive cytoplasmic male sterile line

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Many indica varieties used in hybrid breeding carry minor restorer genes. Their fertility increases slightly with successive generations so they cannot be bred into new A lines. Four models for breeding B lines to incorporate into A lines were designed: B/B, B/restorer varieties with minor gene(s), and B/R and B/hybrid rice (F_1). New maintainers that possess desired characters such as complete male sterility, required flowering habit, blast resistance, good grain quality, etc. were developed and have been used to backcross with the earlier A line. Moreover, the new B lines have more genetic diversity than the earlier B lines.

Fertility and sterility are controlled by two pairs of genes, and restoring ability varies between strong and weak genes. When an A line was crossed with the F_2 (B line/strong R line), the percentage of normal pollen in the F_1 was 40.9% of the A line, and only 15.6% with the F_2 (B line/weak R line). The restoring ability of weak restorer genes is only 38% of that of strong restorer genes.

Geographical distribution of fertility restoring genes for cytoplasmic male sterility in sinica rice

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Almost all restorer genes for cytoplasmic male sterile (CMS) japonica rice were derived from indica rices. Therefore, japonica rice restorers must be developed from indica and japonica hybrids, which is difficult, time-consuming, and often results in undesirable characters. The existence and geographical distribution of restorer genes for CMS lines of japonica rice with indica cytoplasm of Chinsurah Boro II (BT), E-Shan-Da-bai-gu (D), wild abortive (WA), and Lead Rice (L) types were determined from among japonica varieties collected from Yunan Province, the Tai Lake Valley, and some foreign countries. Among 595 combinations of F_1 hybrids pollinated with varieties from Yunan, 40 showed a fertility restoring rate of 50% or more, and 3 reached 70-80%. Of 1,146 combinations pollinated with Tai Lake varieties, 35 had a fertility restoring rate of 50% or more, and 3 reached 70-80%. For 717 combinations pollinated with foreign varieties, 36 had a fertility restoring rate of 50% or more, and 15 reached 70-80%. Restoring degree was affected by the cytoplasmic and genetic background of CMS lines as well as by restoring ability of the R lines. The utilization of restorers and some related topics are also discussed.

Parental sources of commercial combinations of hybrid rice in China

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Of the dozens of cytoplasmic male sterile (CMS) lines and several hundred restorer lines, only a few have excellent general and specific combining ability. The parental sources of some old and new hybrid rice combinations widely planted in China were analyzed. Parental sources included in the study were the Nan-You system (Nan-You 2, 3, 6); the Wei-You system (Wei-You 6, 30, 35, 49, 64); the Shan-You system (Shan-You 2, 6, 63 and Shan-You Gui 8, 30, 33, and 34); the Xie-You system; the Shi-You system; and the Il-32-You system. All have one or more high-yielding parental sources either in the CMS line or in the R line.

Lineage analysis of maintainer sources showed that six major indica CMS lines, Er-Jiu-Nan 1 A, V20 A, Zhen-Shan 97A, V41 A, Xie-Qing-Zao A, and 11-32 A have the common parental sources Zhen-Zhu-Zao and Ai-Zai-Zhan 4, although they differ in sterile cytoplasmic sources.

All R lines used in hybrid rice production in China were obtained by screening testcrosses and by cross breeding. Several outstanding IRRI

breeding lines such as IR24, IR661, IR26, IR30, and IR9761-19-1 are directly used as R lines through testcrosses. Nearly all new cross bred R lines recently used in production contain restorer genes from IRRI improved lines. For example, 26 Zhai-Zao, the R line of early-maturing F_1 hybrid Wei-You 35, contains the parental source IR26 and IR661, and Ming-Hui 63, the male parent of F_1 hybrid Shan-You 63 is derived from IR30. Among some new R lines, No. 6161-8 is an IR661/IR2061 cross, 3624-33 is an IR36/IR24 cross, and 3024-1 is an IR30/IR24 cross. A series of japonica restorer genes may come from the same ancestral parents, Peta or Cina.

Evaluation of selective male gametocidal activity of chemical sterilants in rice

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The selective male gametocidal (SMG) activity of two chemical emasculators on three indica cultivars was assessed in the field. Percent selfing seed set (Y_1) was calculated from bagged panicles, and percent hand emasculated seed set (Y) was calculated from unbagged panicles. Percent outcrossing seed set (Y_2) was calculated from the equation

$$Y_2 = Y - Y_1.$$

At the induction of 95% male sterility, percent outcrossing seed set defined as the selective index (S) of male gametocides (MG), or S_{MG}, was calculated from two orthogonal polynomial simulation curves of dosage-selfing seed set and dosage-outcrossing seed set. S_{MG} values were affected by treatment stage, application time, surfactant rate, and field environment. S_{MG} is an acceptable method for evaluating chemical emasculators.

Breeding procedures for hybrid rice

Use of photoperiod-sensitive genic male sterility in rice breeding

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Hubei photoperiod-sensitive, genic, male sterile (PSMS) rice showed complete male sterility under long photoperiods but fertility under short photoperiods. Preliminary studies indicated that the PSMS character is controlled by a recessive key allele.

A new procedure for hybrid seed production based on the PSMS character (two-line system) is believed to be the simplest procedure for hybrid seed production in autogamous crops. New PSMS lines with various nuclear and cytoplasmic backgrounds can be easily developed. Selecting parental pairs is not limited by the relation of the B and R lines.

The joint selection system using recurrent selection programs can produce PSMS lines and R lines simultaneously. A modified simple recurrent selection scheme is suggested as the basic scheme in a breeding program. Other recurrent selection schemes, including a reciprocal recurrent selection scheme, also can be used.

Breeding and use of a cytosterile line

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More than 97% of the indica cytoplasmic male sterile (CMS) lines used in commercial production belong to the wild abortive (WA) system. We studied the cytoplasm of dwarf wild rice originating in Jiangxi Province. This wild rice has elongating stems with high tillering ability. A CMS plant found in 1979 was designated as dwarf abortive (DA).

We used conventional varieties such as Zhu-Jun, Xie-Zhen 1, etc. to transfer the sterility. But these varieties have poor insect and disease resistance and poor flowering hybrid combinations. Jun-Xie/Wen-Xuan-Qing//Qing-Tang-Zao 5 has double-exserted stigmas, good flowering, healthy growth, and high resistance. We selected three elite plants with well-exserted stigmas. Nuclear genes of the elite lines were introduced into the genetic background of the DA wild rice to develop a BC_4F_1 early-maturing indica Xie-Qing-Zao A line.

The A line has stable sterility, good grain quality, high resistance, and good flowering. In general, effective R lines for the WA system are also effective restorer lines for Xie-Qing-Zao A.

Xie-Qing-Zao A has good combining abilities when crossed with R lines Ce 64-7, IR26, Ming-Hui 63, Fei 11, Ce 49, R29, and Fei 1. The hybrid of Xie-You 64 has wider adaptability. In 3-yr provincial and national tests, yields for 6 groups averaged 6.2-7.7 t/ha.

Hunan Hybrid Rice Research Center developed Xie-You 11 and Xie-You 49 by crossing Xie-Qing-Zao A with R lines Fei 11 and Ce 49. Grain quality met the standards of the National Grain Quality Laboratory of the United States Department of Agriculture.

Development of japonica type restorers from indica/japonica crosses

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The development of japonica hybrids has been slowed by the difficulty in breeding restorers. We found that crossing indica and japonica, then repeatedly crossing the F_1, is a practical way to develop restorers. This method uses the indica nucleus as a bridge between indica and japonica. Japonica type restorer C57 possesses high combining ability and vigorous heterosis. It improved plant type and leaf and promoted partitioning between sink and source for high-yielding japonica hybrids.

Japonica hybrids Li-You 57 and Xiu-You 57 were developed using japonica type restorer C57. They exhibit hybrid heterosis derived from their indica and japonica parents. Semidwarf plant type from A and R lines played a more important role than specific combining ability effects. The A lines were more important for general combining ability effects than were R lines. Sterile lines (BT) Liu-Qian-Xin A and (D) Nan-Geng 34 A and R lines Nin-Hui 3-2 and No. 77302-1 are recommended as the best parents for three lines. (BT) Liu-Qian-Xin A/Nin-Hui 3-2 and (D) Nan-Geng 34 A/No. 77302-1 were identified as good combinations.

Using anther culture to develop parental lines for hybrid rice breeding

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Anthers at the uninucleate pollen stage of V20 A (Yebai A line), V20 B (B line), and IR26 (R line) were cultured in vitro. Pollen grains of the A line gave rise to pollen plants. However, green plant induction was low compared with that of the R and B lines. Pollen plant induction of the F_1 hybrids of the crosses V20 A/IR26 and V20 B/IR26 was higher than those of the R and B lines.

Pollen lines derived from the A line segregated, perhaps because of heterozygous sterility in V20 A. This heterozygosity postponed pollen abortion in V20 A longer than in the original Yebai A line. When its homozygosity was recovered by anther culture, some segregants resumed the original sterility characteristics.

Pollen plants derived from culturing anthers of V20 B and IR26 mostly resembled the Band R lines. Some mutants occurred, which might be due to somaclonal variation occurring in anther culture or to residual heterozygosity in the experimental materials.

Through anther culture, homozygosity can be achieved quickly and sterility can be identified in the first generation.

Disease/insect resistance and grain quality

Disease and insect pests in hybrid rice in China

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Diseases and insect pests in hybrid rice are similar to those in ordinary rice—blast, sheath blight, bacterial blight, stem borer (mainly the Asian rice borer), brown planthopper, and leaffolder. Sheath blight has been common in hybrid rice. With higher fertilizer rates, foliage is luxuriant, favoring disease. Blast occurs mainly in the mountain regions of western and southern Hunan Province. Bacterial blight and bacterial leaf streak *Xanthomonas oryzicola* Fang et al also occur in some parts of Hunan Province. The susceptibility period for bacterial blight is longer in hybrid rice than in ordinary rice. Symptoms are not only withered leaves at booting and heading, but kresek type symptoms in seedlings and at tillering.

During grain filling, hybrid rice management practices such as leaf cutting and rope pulling caused wounds, increasing hybrid rice susceptibility to infection and disease.

Yellow stunt virus spread by the rice leafhopper *Nephotettix cincticeps* (Uhler) was heavy on late hybrid rice from the 1970s to the early 1980s in northern and central Hunan Province because highly susceptible combinations were planted. Several flower-infection diseases occur more in hybrid rice; false smut caused by *Ustilaginoidea virens* (Cooke) Takahashi, and rice kernel smut caused by *Tilletia horrida* Takahashi, because the extended angle of the glume is wider in hybrid rice than in conventional rice. The hybrid flowering phase also is longer and the rate of nakedstigma is higher.

Downy mildew caused by *Sclerophthora macrospora* (Saccardo) and sheath rot caused by *Acrocylindrium oryzae* Sawada have also been common.

Populations of stem borers have varied. The Asian rice borer, formerly the dominant species, has been replaced by the yellow rice borer. Damage by rice pink borer *Sesamia inferens* Walker has risen to some extent.

Brown planthopper damage has been less in hybrid rice than in ordinary rice because of the compensation ability of hybrid rice.

Control of diseases and insect pests in hybrid rice has focused on planting resistant varieties and adopting integrated pest management.

Some cross combinations with better resistance, Wei-You 6, Xian-You 1, Wei-You 64, and Wei-You 35, have been identified. Wei-You 64 has a combined resistance to yellow stunt, blast, bacterial blight, brown planthopper, and leafhopper. Currently, it is the best heterosis combination. Since 1983, yellow stunt has been controlled.

Using backcrossing to improve disease resistance of cytosterile Zhen-Shan 97

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To improve Zhen-Shan 97 A resistance to blast caused by *Pyricularia oryzae* and bacterial blight caused by *Xanthomonas campestris* pv. *oryzae,* the inheritance of restoration and resistance in three nonrecurrent varieties (IR29, IR2061-464-2-4-5, and Ge-Ma-Li) was analyzed. Zhen-Shan 97 (male sterile line or maintainer) was the recurrent parent. Male sterile and maintainer lines of improved Zhen-Shan 97 were developed through backcrossing and selfing. In a 1985 field test, the improved hybrid line (No. 8501 A)/IR26 was as resistant to blast and blight as the improved sterile line. Its heterosis compared to that of Shan-You 6.

Analyses of male sterile restoration showed that fertile restoration of the cytosterile line of IR29 is governed by one dominant gene. IR2061 and Ge-Ma-Li each have one partial restoring gene. The Ge-Ma-Li gene also is affected by another gene.

Blast resistance in IR2061 and Ge-Ma-Li is controlled by two independent dominant genes, but the expression of resistance in IR29 is more complicated. The resistant genes are nonallelic.

Blight resistance in IR29 and IR2061 is controlled by the same dominant gene; Ge-Ma-Li has one nonallelic dominant gene affected by one recessive suppressor gene from Zhen-Shan 97.

The original sterile line was used as the recurrent parent at the beginning of backcrossing (or A^n/Rf) when a nonrecurrent parent with a major restoring gene, such as IR29, was used to improve the resistance of the male sterile line. Then the maintainer was used as the female parent for backcrossing (B/(A^n/Rf). However, when a variety with weak restoration ability was used as the nonrecurrent parent, it was better to use the original maintainer as the female parent for backcrossing (B^n/Rf).

Improved grain quality and yielding ability of hybrid rice

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Seven characters—yield potential, protein content, amylose content, gel consistency, head rice percentage, net photosynthetic efficiency, and chlorophyll content—were examined in 14 hybrid rice combinations and their parents. Correlations between hybrid value (F_1)and high parent value (P_1), hybrid value and low parent value (P_2). and hybrid value and the mean value of both parents (MP) also were made.

The hybrid value of two characters, net photosynthetic efficiency, and gel consistency correlated significantly with P_1, P_2, and MP. As the average value of net photosynthetic efficiency rose, F_1 yield increased and amylose content decreased, resulting in higher gel consistency and softer grain quality. To develop new combinations of fine grain quality and high-yield ability, parents should have high net photosynthetic efficiency and high gel consistency.

Outcrossing mechanisms and hybrid seed production

Genetic mechanisms to enhance hybrid rice seed production

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Hybrid rice development *per* se is pursued in the United States by the private sector. Public sector research has examined genetic mechanisms that affect the ability to produce hybrid seed, including recessive tall plant type, cytoplasmic male sterility (CMS), and apomixis (asexual seed production).

A recessive gene for elongated uppermost internode (*eui*) effectively produces a recessive tall plant type by almost doubling the length of the uppermost internode. This gene might be a useful fourth element to complement the other three genetic elements—CMS, maintainers, and restorers—generally used in hybrid seed production. The *eui* gene would be incorporated in pollen fertility restoring parents for hybrid seed production in which a semidwarf F_1 generation is desired. The tall paternal plant type would be desirable for windblown pollen dispersal onto semidwarf female plants; the resulting hybrids would be semidwarf, unlike the usual tall hybrids resulting from semidwarf/tall crosses.

Successful induction of CMS in locally adapted varieties could provide a major short-cut in developing hybrid rice. Seeds of variety M201 were treated with streptomycin or mitomycin in 1983. Male sterility was successfully induced, but it was genetic male sterility, not the desired cytoplasmic type. The experiment was repeated in 1985 with three other varieties. Sterile plants were isolated in all three varieties, and tests are under way to determine if the sterility is cytoplasmic or genetic.

Apomixis can theoretically be used to produce true-breeding F_1 hybrids with permanently fixed heterosis. Discovery and application of apomixis would allow farmers to exploit the increased yield of hybrids. Germplasm collections of cultivated rice and wild rice species are being screened for indications of apomixis.

Techniques to get high yield in hybrid rice seed production in China

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Hybrid seed production research in Sichuan in the past 9 yr has emphasized improving the environmental conditions of both parents. The seed yield obtained from 140,000 ha of seed production fields reached 2.0 t/ha in 1985. The highest yield was 4.1 t/ha on 30 ha.

It is best to produce hybrid seed in Sichuan in the summer. Male parent flowering has been synchronized by seeding on the same date each year. The leaf number, speed of leaf emergence, and effective accumulated temperature of both male and female parents were investigated to determine the sowing date for the female parent by predicting the heading of the male parent. Synchronization of flowering permits the male parent to be seeded three times, thereby providing maximum pollen during the female parent flowering.

To increase the productive tillers of both parents, using a constant row ratio (1:10-1 2 or 2:12-16),2 seedlings were planted per hill instead of 1, and the transplanting rate was increased from 2.4 million/ha to 3 million/ha. To increase productive tillers in the male parent, 2 rows were transplanted instead of a single row, and seedling number was increased to 750,000/ha; in this way the productive tillers of the male parent reached 1.5 million/ha, and the glume ratio of female to male parents was about 1:0.8.

Gibberellin was sprayed (3 or 4 times instead of once or twice) in a larger amount (150 g/ha instead of 15-30 g/ha) and at a higher concentration (40-60 ppm rather than 10-20 ppm) at early heading (rather than when 10% of the panicles had emerged). This technique improved the seed set of the female parent by up to 65%. The average yield of hybrid seed from 1,333 ha was more than 3.0 t/ha.

Impurity prevention and stock renewal of parental lines of hybrid rice

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Degeneration of three-line hybrid rice is caused not only by inadequate separation of breeding seeds from production seeds, and mixing them during seed production, but by varietal variations. Simple, economical, rapid, and effective countermeasures are described for hybrid seed production of both indica and japonica rice.

Conditions and techniques for achieving high yields of pure seed as well as the phenomena and causes of purity and impurity in three-line hybrid rice were studied. Badly mixed production fields were examined first to trace seed production, propagation, and the purities of the three

lines themselves. Hybrid rice impurities were caused not only by careless isolation in breeding and seed production fields and by mechanical mixing in seed production, but by variations in varietal characters of the A, B, and R lines themselves. Variation of the A line normally follows that of the B line.

Mechanical mixing of seed is common, but easy to eliminate. Artificial mixing can be avoided by a set breeding base, basic equipment, and simple operating rules. Biological mixing, caused by pollen from plants in adjoining fields mixing with that of plants in breeding and seed production fields, can be solved by strict isolation of centralized breeding and production fields.

This research shows that eliminating biological mixing, brought about by variations of A, B, and R lines and by selfing in the filial generations, is not sufficient in itself. Strict isolation and impurity prevention are also required.

Two techniques, one for producing seed stock of three lines and the other for renewal over time, have been established. The seed production system relies on economical equipment, simple procedures, and a short production period. The purification technique lends itself to purifying seed production after serious mixing or to purifying recently produced parental lines. By alternate use of the two techniques, we achieve stable crosses over time with high combining ability.

Using isozymes to determine purity of hybrid rice seed

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The esterase isozymes present in rice embryos were analyzed by disc polyacrylamide gel electrophoresis to test the seed purity of 146 samples of hybrid rice(including Shan-You 3, Shan-You 2, Shan-You 6, Wei-You 6, and Si-You 6). Seed purity assessed through isoesterase analysis was the same as with small plot tests and field investigations at the 95% level of confidence.

The esterase electrophoresis method was improved and simplified so that 200 samples can be analyzed in 8 h by 1 person, and the zymogram can be clearly shown from the embryo of 1 seed.

Suitability of hybrid rice seed production techniques in Indonesia

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Hybrid rice seed production techniques developed in China for both cytoplasmic male sterile (CMS) seed multiplication and hybrid seed production were found suitable for Indonesian conditions. Standard

Chinese practices were followed, except gibberellin application (because of its cost).

Seed yield of MR365A/MR365 B, a promising CMS line from IRRI, during the 1984-85 wet season at Sukamandi ranged from 1.2 to 1.8 t/ha–higher than in the 1983-84 wet season. The crops were planted using a male-female ratio of 2:3 to 2:8 and a plant spacing of 20 × 20 cm. The highest seed yield was obtained with the ratio of 2:5. Leaf clipping did not significantly affect total filled grain/20 panicles and total seed yield/150 hills, although it indeed produced significantly higher percentage seed set, The nonsignificant effect on seed yield was due mainly to fewer spikelets/20 panicles.

On V41 A, clipping leaves gave significantly higher percentage seed set but no significant difference in spikelets per panicle.

In the 1985 dry season, we multiplied V41 A and MR365 A in 0.5-ha fields using a male-female ratio of 2:4. The harvest was 334 kg seed of V41 A, 570 kg V41 B, 383 kg MR365 A, and 364 kg MR365 B—comparable to yields in China. Seed set was 38.4% for V41 A and 39.3% for MR365 A.

In the hybrid seed production plot at Sukamandi in the 1983-84 wet season using MR365A as the CMS, and IR54 and Sadang as restorers, we obtained seed sets of 13.7-19.4%—low compared with that of CMS multiplication, probably due to lack of synchronization of flowering. Furthermore, all the CMS lines are susceptible to major pests and diseases prevalent in Indonesia.

Cultural management of hybrid rice

Nutrient requirement and fertilizer management in hybrid rice

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The relation between fertilizer levels and late-maturing hybrid rice yields was studied in the greenhouse and in the field. Uptake, distribution, and translocation of NPK within vegetative and reproductive stages were studied. Nutrients were characterized by kind of nutrient and amount absorbed at various growth stages. The ratio of NPK uptake, the effect of time and rate of fertilizer application on yield, and recovery rate of fertilizer were monitored.

Hybrid rice yields were 0.9 t/ha more than those of conventional varieties at the same level of fertilizer application. Yields of hybrid rice were highest at rates of 180-225 kg N/ha, 11-22 kg P/ha, and 60-120 kg K/ha. At higher rates, yields declined. For hybrid rice yielding more than 7.5 t/ha, the NPK ratio required was 1:0.52:1.47. The amounts of nutrients absorbed were 208 kg N/ha, 24 kg P/ha, and 190 kg K/ha.

Nitrogen uptake was 3.2% more than in conventional rice, P uptake was 7.8% more, and K uptake was 12.7% higher. More than half the total nutrients were absorbed in the middle growth stages. With increased N and K fertilizer application, *luxury absorption* of N and K was more critical in hybrid rice than in conventional varieties. Yields of late-maturing hybrid rice declined with increasing N ratio; they remained unchanged with increasing P and K ratios. The effect of N on hybrid rice yields is related to P and K applied. Late-maturing hybrid rice absorbed 19% of total N and 8.7% of total K after full heading. Topdressing a small part of N fertilizer in the late growth stage, while maintaining a steady supply of NPK during early growth, increased yields and N fertilizer recovery of late-maturing hybrids. This practice may increase the yield potential of late-maturing hybrid rice by preventing premature senescence.

Optimum plant population of hybrid rice in single cropping

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The plant population, number of tillers, and leaf area index (LAI) required for the optimum number of panicles to give maximum filled spikelets per panicle for high yields of hybrid rice were determined in experiments conducted from 1977 to 1979. The optimum panicle number to achieve the highest filled spikelet percentage was 289 panicles/m^2. The optimum number of spikelets per panicle was 179. The effect of plant population on panicle number, grain weight, and filled spikelet percentage is described.

Panicle number per unit area is a function of leaf area. The leaf area of a single tiller of hybrid rice was 240-250 cm^2 at heading, about 50 cm^2 more than that of IR24, indicating that the capacity of IR24 for producing effective tillers is less than that of hybrid rices. Shan-You 2 yields highest at maximum LAI of 7.6 at heading. For Shan-You, Nan-You, etc., 3 million panicles/ha is the theoretical optimum.

Filled spikelet percentage and grain weight are functions of net photosynthetic rate and dry matter accumulation. There is significant positive correlation between dry weight of stems and leaf sheath per spikelet and the percentage of filled spikelets (r = 0.712**, DF= 16). If LAI is 6.3-7.3 at heading, net photosynthetic rate remains higher during the 20 d after heading and dry matter accumulation is 3.8-5.7 t/ha.

Cultural manipulation of plant type in hybrid rice

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The effects of different cultural practices on organogenesis and plant type in hybrid rice are described. Generally, direct-seeded and young-seedling transplanted hybrid rice produce a loose, radiating plant type with more and shorter tillers; transplanted older seedlings with developed tillers develop into a bee-waisted plant type with discontinuous tillering; and densely planted seedlings without tillers form tall plants with tillers forming high on the main stem due to slow tillering in the field. The relation between cultural plant type and agronomic characters is also described. Through proper selection of seedling density and seedling age in the nursery, the cultural plant type of hybrid rice can be manipulated. Seedlings of Nan-You 3, at a planting density of 225 plants/m^2 and transplanted at the 10-leaf stage, developed 2-6 vigorous tillers, resulting in a plant type that was compact, erect, and strongly adaptable to the environment.

The large-seedling cultural method has two main features: 1) accurate control of seedling density and seedling age, and 2) proper regulation of fertilizer input and irrigation throughout crop growth. An empirical formula for calculating proper seedling density to achieve the maximum number of effective tillers for different cultural types is given.

$$\text{Seedlings/ha} = \frac{\text{Optimum no. of panicles}}{A + Br_1 + (N - n - C)r_2A}$$

where A = no. of large tillers with 3 or more leaves/seedling, B = no. of tillers with 2 or fewer leaves/seedling, r_1 = transplanting survival percentage of small tillers with 2 or fewer leaves, N = leaf no. of the main stem, n = no. of nodes extended, C = leaf age of seedling transplanted, and r_2 = percentage of emerged effective tillers. Three kinds of seedling integration for stable high yields based on the cultural method described are discussed.

Minimum tillage and direct seeding in hybrid rice

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New techniques for hybrid rice cultivation, minimum tillage, double-cropping, and broadcast seeding are described. Early-maturing hybrid rice varieties V49 and V35 were the experimental materials. Since the new techniques were adopted in 1983, hybrid rice yields have averaged 15 t/ha over 2 crops, 10-20% higher than yields of hybrid rice grown with conventional cultivation. Significant increases in productivity and profit

result from savings in labor, seeds, and land no longer devoted to seedling beds.

The techniques improve soil physical conditions through better aeration; increase bacteria in the soil and NPK availability; and facilitate the activities of hydrogen peroxidase and urease. They also positively affect agronomic characters necessary for high yield: optimum plant population, well-developed root system, lower tillering nodes, healthy plants, higher photosynthetic efficiency, uniform heading, more productive panicles, and good leaf color.

Ratooning in hybrid rice

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Results of a cooperative research program on ratooning hybrid rices to prolong use of ricefields and to increase rice yield are described. Plot experiments and field production demonstrations were conducted in southeast Sichuan Province, China, from 1981 to 1985. Some medium-maturing hybrid varieties that yielded high in the main crop and had high ratooning ability were identified. They markedly increased grain yield. Number of productive panicles per unit area and percentage of fertility are the chief factors affecting ratoon grain yield.

Best results are obtained when growth of the 2d, 3d, and 4th nodes (top to bottom) of the main crop stems sprout and grow well after main crop harvest. Main crop should be sown and harvested early to ensure safe heading of the ratoon crop before autumn low temperatures. Optimum timing in southeast Sichuan is sowing in early March, harvest before 15 Aug, and ratoon crop heading in early September. Applying fertilizer at 35-70 kg N/ha 17 d after main crop heading promoted tiller regeneration and increased the number of panicles per unit area. Increasing cutting height of the main crop to preserve the 2d node on the stubble promoted tiller regeneration and early maturity of the crop.

When the cultural practices described were applied on a production demonstration area of 1,346 ha, average main crop yields were 7.9 t/ha, equal to those of popular single cropping hybrids; ratoon crop yields averaged 1.8 t/ha, 22% that of the main crop.

Physiological and genetic research in hybrid rice

Physiological and biochemical characters of hybrid rice in Dongting Lake region, China

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In two yield trials of V64 type combinations, hybrids outyielded conventional varieties by 15-28%. Hybrids have these advantages:

- Good photosynthetic characters. Leaf area index (LAI) in hybrids was 17-34% higher than that of conventional varieties at heading, and photosynthetic potential 21-26% higher from tillering to heading. In the Dongting Lake region,' solar radiation is 84.9 kcal/cm^2 from April to November. Hybrid rice can absorb more solar energy because of larger LAI. The photosynthetic intensity was 8-15% higher for hybrids than for conventional varieties at early growth and 6-27% higher at middle growth stages.
- Stronger root systems at early and middle growth stages. Hybrid rootability was 21-34% higher than for the conventional check at the seedling stage. Exudative intensity was 3-34% bigger at middle growth stages. This indicates that hybrids have wider root distribution and more ability to absorb water and fertilizer.
- Lower respiration intensity. Average dry matter accumulation in the hybrids was 46-99 kg/ha per d more than in the check.
- Shorter growth duration. Because of their shorter growth duration, hybrids mature before the cool fall temperatures, which limited production of earlier hybrids in the Dongting Lake region.
- Virus disease resistance. Disease percentage in V64 infected by green leafhopper (1.5% transmission rate) was 11-12%.

However, larger sink size and insufficient source in the new hybrids resulted in more unfilled panicles. Percentages of unfilled grain were 23% in V35, 29% in V64, 23% in V98, and 15% in the conventional check. Source was the major factor affecting yield. Crop management in late growth stages should promote root system development. Higher photosynthetic activity maintained in functional leaves would correct the imbalance between sink and source.

Seasonal variation in dry matter production of hybrid rice in Changsha, China

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Seeding dates for three hybrid rice combinations, Wei-You 35 (early maturity), Wei-You 6 (late maturity), and Shan-You 6 (late maturity), were studied in 1982 and 1983. Seeding began 1 Apr and continued at 10-d intervals for 10-12 plantings.

The net assimilation rate of the hybrids gradually declined as plants developed. It was higher in Wei-You 35 than Wei-You 6 at all stages in 1983.

Optimum seeding times were identified by dry matter production, grain yield, productivity, and rate of solar energy use.

The caloric value of dry matter was higher in Wei-You 6 than in Wei-You 35. Net assimilation rate and solar energy use rate of Wei-You 6 were lower than those of Wei-You 35.

The highest potential grain yield for late rice was in the second optimum seeding time.

The two optimum seeding times under the inland-monsoon humid climate in Changsha are early April and mid-June for late-maturing combinations, and early April and early July for early-maturing combinations. Late summer to early autumn, with high temperatures and low humidity, might not be safe for hybrid rice.

Response of hybrid rice combinations to photoperiod and temperature

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We tested photoperiod sensitivity and thermosensitivity and basic vegetative time of 104 hybrid combinations and their restorer and male sterile lines. The hybrids came from WA, Gambiaca, ET, Tien, Liu WA, Nanxin, and Hong-Lien types. Heading acceleration with a shorter photoperiod ranged from –3.0 to 60%. Heading acceleration with high temperature ranged from 10 to 45%. About 80% of the combinations had shorter vegetative time than the parental mean. The hybrid combinations tested were classified into 1 1 thermo-photosensitive types, 60% in the midrange.

The F_1s exhibited three types: 1) male parent type, 2) female parent type, and 3) the midtype. They also showed tendencies toward the parent with the greater value or the parent with the smaller value on the three characters measured. No significant differences were found between male sterile lines with different cytoplasm sources and the combinations produced from them. The relative heterosis of the F_1 hybrids indicated that about 60% of the combinations exhibited transgressive inheritance in photoperiod sensitivity and thermosensitivity, and incomplete dominance in vegetative time and heading date.

Physiological characteristics of hybrid rice roots

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During field experiments in 1983 and 1984 we evaluated morphological, physiological, and biochemical functions of root systems of hybrid combinations at transplanting, tillering, panicle differentiation, booting, heading, and milk stages to improve late modern varieties (MV). The hybrids used were Shan-You 6 (Zhen-Shan 97 A/IR26) and Wei-You 6 (Wei 20 A/IR26); the late rices were Zhao-Jin-Fong 5, Zhe-Gen 6, and Nong-Hu 3-2.

The hybrids had larger and deeper root systems than the conventional varieties. The root system density of hybrids was twice that of MVs. The superficial roots of hybrids were more developed and vital, about 4-5 times heavier than those of the MVs.

The hybrids were stronger in aerobic respiration and energy metabolism than MVs. Dehydrogenase and cytochrome oxidase activity and the root system oxidizing power were much higher in all development stages. Adenotriphosphate of root systems was also much greater. The oxidizing power of root systems at heading correlated positively with grain yield (r = 0.932*). More interesting is the closer relationship between the oxidizing power of the superficial root and grain yield (0.954*). Dehydrogenase activities correlated significantly with grain yield at transplanting (0.956**) and at heading (0.946**). ATP content correlated significantly with yield at transplanting (0.958**) and at heading (0.897*).

Relations between physiological heterosis of root and shoot systems of hybrid rice

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Hybrid rice Shan-You 6 as a late crop outperformed its three parents and conventional cultivars in root growth, N uptake, ability to synthesize amino acids, and root activity.

Growth vigor of the root system occurred at seed germination. Heterosis in seed germination is mainly a function of high respiratory rate and diastase activity. a-amylase in the endosperm of the dry seed is the important physiological basis of high diastase activity at early germination, resulting in seedling growth vigor.

The hybrid displayed its N uptake vigor in absorption rate per plant. Nutrient uptake and dry matter production are closely related.

Free amino acids separated from the bleeding sap of the hybrid included 20 protein amino acids and 11 nonprotein amino acids. The high number of free amino acids shows its distinct heterosis. There are quantitative differences between varieties, but qualitative differences in

free amino acids in the exuding sap of intact and clipped plants suggest a nutrition feedback from the aboveground parts to the underground parts.

Root activity is low at tillering, increasing gradually to panicle initiation, reaching maximum at booting, then dropping.

Mid- and late-growth stage of hybrid root activity could be depressed by N deficiency and promoted by N fertilizer application. Improving aeration at late growth stages gave effects similar to those of N fertilization. Improved aeration may maintain the chlorophyll function of rice leaves better than N application.

Early diagnostic indices of heterosis might be diastase activity of seed 1-3 d after soaking and percentage root surface that is actively absorbing at seedling stage. Root activity from booting to milk stage, reduction in root activity, and aging leaf indices might forecast yield.

Using radioactive tracers to predict grain yield of hybrid rice[1]

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Radioactive-labeled ^{32}P, ^{35}S, and ^{14}C photosynthates were used to study translocation and distribution in leaf, sheath, culm, seedling, and panicle at seedling and milk stages of F_1 hybrids; percent translocation of ^{32}P, ^{35}S, and ^{14}C photosynthates from leaf to other organs; and the relation between the distribution of ^{35}S and ^{14}C in the panicle and grain yield.

Yield of the F_1 hybrid can be predicted by

- Distribution of ^{32}P in tillers. F_1 hybrids have high tiller capacity, more tillers, more absorbed ^{32}P, and more ^{32}P translocated to the tillers. The correlation coefficient between tiller number and percent ^{32}P distributed in tillers was r = 0.660*. The regression equation is $y = 0.03x + 2.86$.
- Translocation of ^{14}C photosynthates from leaf to other organs. F_1 hybrid plants Shan-You 2, Shan-You 3, Shan-You 6, Shan-You-Gui 33, Shan-You 36, Shan-You 63, Shan-You 64, Shan-You 2888, Shan-You-Zhi-Long, Zhen-You 63, Zhen-You 64, Ging-You-Zao, Ging-You-Zhi, and Ging-You-Jan 2 have shown higher ^{14}C photosynthates translocated percentage from leaf and ^{14}C total (cpm) in panicle than the restorer line and the check variety.
- Distribution of ^{14}C photosynthates in panicle at the milk stage. Correlation coefficient between yield and percent ^{14}C photosynthates in the panicle at milk stage was r = 0.698**. The regression analysis is $y = 0.04x + 8.05$. Percent ^{14}C photosynthates distributed in the panicles of hybrids was higher than in the check Shuang-Cui 36.
- Input accumulation percentage. Input accumulation percentage (IAP) of ^{14}C photosynthates is a predictor of heterosis. IAP is calculated as percentage ^{14}C photosynthate translocated from leaf × percentage ^{14}C photosynthate distributed to panicle/100.

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Mitochondrial DNA modifications associated with cytoplasmic male sterility

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Restriction analysis of isolated mitochondrial DNA (mtDNA) showed specific modifications in the male sterile cytoplasm of several plants. In addition to the main mtDNA, mitochondria from plant species have been shown to contain low-molecular-weight DNA. Here we report the isolation and preliminary characterization of mtDNA in fertile and male sterile lines of rice. This line was developed from a wild rice *Oryza sativa* f. *spontanea* L. cytoplasmic source designated as WA and the nuclear genotype of Chinese variety Zhen-Shan 97.

Mitochondrial DNA was prepared from a fertile and a sterile cytoplasm of indica WA type. Analysis of isolated mtDNA by agarose gel electrophoresis revealed discrete bands of low molecular weight, reminiscent of mitochondrial plasmidlike molecules identified in other species in the fertile cytoplasm. An additional band of low molecular weight was found in the sterile cytoplasm. We characterized these low molecular weights by S1 nuclease treatment. The mitochondria of fertile cytoplasm Zhen-Shan 97 B and male sterile Zhen-Shan 97 A contain three small, supercoiled DNA molecules. Male sterile cytoplasm is distinguished by the presence of one additional supercoiled DNA. The linear forms measured 1.0, 1.35, and 1.35 kb for the common molecules and 2.1 kb for the plasmid specifically found in the sterile line. Electron microscopy analysis, cloning, and sequencing of the different plasmids will give us definitive characterization.

We also analyzed the high-molecular-weight mitochondrial DNA of both rice cytoplasms after restriction with the endonucleases EcoRI, BamHI, SaLI, and Pstl. While most restriction fragments were common to both cytoplasms, several bands were specifically modified in the male sterile line.

Molecular cloning and sequencing of small-circular DNAs in male sterile cytoplasm of rice

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We used Boro-type cytoplasm to study cytoplasmic male sterility. We found two low-molecular-weight DNAs in the mitochondrial DNA fraction of the callus produced from seeds of a line (msRfRf) of Taichung 65 to which male sterile cytoplasm and a fertility-restoring gene from Chinsurah Boro II were introduced.

Under electron microscope examination, these DNAs, designated as B-1 and B-2, werecircular. B-1 DNA was 0.475 μm long (about 2000 base pairs), and B-2 was 0.375 μm long (about 1600 base pairs).

Neither DNA was able to be digested by specific restriction enzymes, such as EcoRI, Hind III, BamHI, and PstI. However, DNAs were digested into a few fragments with Hae III. We cloned these fragments into the PUC13-SmaI site. Using *E. coli* JM107 as a host, many transformations were obtained. Using Southern hybridization, we ascertained that fragments derived from B-1 and B-2 DNAs were inserted into each plasmid. The maintainer of male sterile cytoplasm did not carry B-1 and B-2 DNAs. Both DNAs replicated autonomously. According to the nucleotide sequences for these fragments, they were highly adenine-thymine rich and contained short open reading frames.

Plasmidlike DNA in mitochondria of WA type rice

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We investigated mitochondrial DNA isolated from rice of the BT and wild abortive (WA) types. In the BT type, the plasmidlike DNAs were found in mitochondria of both normal and male sterile cytoplasm.

Mitochondrial DNA of WA type rice was analyzed by agarose gel electrophoresis. Plasmidlike DNAs of 1.2 and 1.5 kb were found in a WA cytoplasmic male sterile (CMS) line (V20 A), its maintainer line (V20 B), and an F_1 hybrid (VU6). but not in the restorer line (IR26).

After mtDNA was treated by RNase, the main chain mtDNA and plasmidlike DNA were still present, as shown by agarose gel electrophoresis. The two low-molecular-weight bands were probably DNA fragments.

Both the nucleus and the cytoplasm of male sterile lines, maintainer lines, and F_1 hybrids contain factors that may affect the expression of normal fertility, but the nucleus and the cytoplasm of restorer lines are normal. This suggests that mitochondrial plasmidlike DNA may be associated with the expression of male sterility.

Amino acid changes in the development of rice hybrids and parent[1]

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We determined the content of 16 amino acids in the seeds, leaves, and anthers of 4 hybrid rices (Wei-You 6, Wei-You 64, Shan-You 6, and Shan-You 8) by automatic amino acid analyzer. In seeds, the content of six essential amino acids (lysine, valine, leucine, isoleucine, tryptophan, and phenylalanine) of the four hybrids is greatly affected by the female parent. This is significant in selecting and breeding for new hybrids with high essential amino acid contents. The amino acid contents of leaves of Wei-You 6, Shan-You 6, and Shan-You 8 are higher at the vegetative stage than at the seedling and tillering stages, indicating heterosis. By contrast, the higher amino acid content of Wei-You 64 occurs during reproductive growth, not during vegetative growth.

Differences between Wei-You 64 and the other three hybrids show that hybrids have different developmental models. The increase in amino acid content of the steriles in leaves during the differentiation of young panicles shows that male abortion of the steriles occurs not only in the anthers, but in the amino acid metabolism of leaves, and that the steriles display abortion during the differentiation of young panicles. In anthers, the asparagine content in the steriles is apparently high, and the contents of alanine, valine, phenylalanine, glutamine, leucine, lysine, asparagine, and arginine in the restorer and the sterile show changes opposite to those in the four hybrid combinations.

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Nucleoli and sat-chromosomes of japonica hybrids

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In previous studies on sat-chromosomes of rice, we found that indicas have two pairs of sat-chromosomes and japonicas have one pair. In this study, we investigated pollen mother cell meiosis in four japonica hybrid rices and their three parental lines. At pachytene, chromosomes and one or two nucleoli can be observed easily and clearly. Each nucleolus sticks on a bivalent. There are two nucleoli per cell, identical in size, which often develop into one large nucleolus and one small one. At diakinesis, these two nucleoli merge into one nucleolus, which sticks on two bivalents. The japonica restorer lines having indica consanguinity usually have two pairs of sat-chromosomes identical to those of indica, or two pairs of sat-chromosomes at a higher ratio. An increase in nucleoli and sat-chromosomes indicates an increase in rRNA, to promote protein synthesis. Therefore, in indica/japonica hybridization, selecting japonica restorer lines having two pairs of sat-chromosomes or having two pairs of sat-chromosomes at a higher ratio, and having a restorer gene, might be advantageous for raising the fertility and heterosis of japonica hybrids.

Prospects for hybrid rice production through protoplast fusion

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Application of novel genetic manipulation techniques such as somatic hybridization to rice improvement has been restricted by a lack of plant regeneration from rice protoplasts. Somatic hybridization through rice protoplast fusion promises rapid transfer of traits such as cytoplasmic male sterility, novel cytoplasmic recombinations, and transfer of disease resistance and salinity tolerance across sexual hybridization barriers.

Efficient plant regeneration from protoplast-derived callus, and efficient protoplast fusion, culture, and selection methods are essential before such experiments can be undertaken in rice.

Procedures are described for rapid efficient plant regeneration from cell suspension-derived japonica rice protoplasts. These include subjecting freshly isolated protoplasts to heat shock, culture in agarose-solidified medium, and direct colony transfer to a medium favoring regeneration through somatic embryogenesis.

The regenerated plants that arise from well-developed typical embryoids look normal, and set seed at high frequency.

Rice protoplasts have been fused chemically and electrically using two cell suspension protoplast partners or a combination of cell suspension and leaf protoplasts.

Selection schemes are outlined for the recovery of hybrid rice calli and plants including the use of fluorescence-activated cell sorting.

The finding that normal green plants can be efficiently regenerated from rice protoplasts will enable experiments on production of hybrid rice through protoplast fusion.

Hybrid rice worldwide

Hybrid rice breeding in Japan

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The hybrid rice development project of the Ministry of Agriculture, Forestry, and Fisheries of Japan started in 1983, with hybrid rice breeding at one central and five regional agricultural research institutes. This report mainly discusses hybrid rice breeding at Hokuriku National Agricultural Experiment Station.

Cytoplasmic male sterile (CMS) lines are developed by transferring the sterile cytoplasms originating from Chinsurah Boro II and Lead Rice to Japanese leading varieties and breeding lines. The fertility-restoring genes for these sterile cytoplasms are introduced from exotic germplasms and a group of local Japanese varieties. The CMS lines completed nuclear exchange and selected restorer lines were combined. Yield tests were conducted in 1984 and 1985. The hybrid combination yields ranged from 75 to 125% that of the check variety. Three combinations, judged promising by evaluation of their agronomic characters, were named: Hokurikuko 1, Shuko 4770, and Shuko 4771. The seed set of CMS lines in the seed production fields was examined with 15 combinations. Seed setting percentage ranged from 12.4 to 55.9%. Variation was due to differences in synchronization of flowering time between the parents.

The hybrid rice program in India

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Average productivity of rice in India is 1.4 t/ha, 0.6 t/ha in rainfed uplands, 1.3 t/ha in lowlands, and 1.7 t/ha in irrigated lands. The recent success in exploiting the heterosis exhibited in the F_1s through the development of hybrid rice, primarily in China, has encouraged Indian rice breeders to explore the potential of hybrids to increase yields.

The National Programme on Hybrid Rice Breeding was initiated 6 yr ago in collaboration with the International Rice Research Institute. Centers involved are Agricultural Research Station, Aduthurai; Tamil

Nadu Agricultural University, Tamil Nadu; Central Rice Research Institute, Cuttack; Punjab Agricultural University, Kapurthala, Punjab; Indian Agricultural Research Institute, New Delhi; Agricultural Research Station, Mithapur, Bihar; Agricultural Research Station, Maruteru, Andhra Pradesh; and University of Agricultural Sciences, Bangalore, Karnataka.

Work under way includes

- searching for new sources of CMS lines,
- identifying appropriate maintainers and restorers,
- studying heterosis and combining ability,
- studying histology and histochemistry of CMS lines and their maintainers,
- using chemical gametocides to increase the extent of outcrossing in CMS lines,
- determining the genetics and nature of inheritance of fertility restoration and other floral modifications suitable for outcrossing,
- studying the physiological and biochemical basis of heterosis,
- using tissue culture techniques in hybrid rice,
- evaluating yield performance of F_1 hybrid combinations, and
- developing seed production technology to produce CMS lines and F_1 hybrids.

Prospects for hybrid rice in Indonesia

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Yield trials compared hybrid rice varieties with the conventional varieties in Indonesia. Hybrids were derived mostly from Chinese CMS lines, from International Rice Research Institute lines, and from Indonesian restorers. Highest yielders were hybrids, although yield differences between the best performing hybrid and best check were not always significant. Standard heterosis of the best hybrids ranged from 2.6 to 69.8% for productivity per hectare and from 3.0 to 91.2% for productivity per day. Most hybrids matured earlier than the best performing check.

These results indicate that hybrid rice can perform better than conventional varieties and could help maintain self-sufficiency in rice production.

Problems are CMS multiplication and hybrid seed production. The CMS lines derived from China are susceptible to pests and diseases prevalent in Indonesia. Hybrid rices developed using Chinese CMS lines have poor grain quality, whereas that of their restorers is good.

Hybrid rice breeding in Malaysia

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In a 1984 study to evaluate heterosis, 14 of 18 hybrids involving our advanced breeding lines yielded more than check variety Muda, up to 5.7 t/ha compared with 4.5 t/ha, giving the highest standard heterosis of 26.6%. In 2 yield trials, experimental F_1 hybrids from China and IRRI showed standard heterosisfrom −37% to 27.1 %. Using Chinese CMS lines V20 A and Zhen-Shan 97 A in test crosses with 68 advanced lines, only 7.4% were identified as maintainers (MR5, MR83, MR98, Y626, and Y629) and 14.7% as restorers (Setanjung, MR72, MR82, MR87, Y635, Y833, Y837,Y839, AYT43, and YKK52). The CMS system is currently being transferred into MR83, MR98, Y626, and Y629 to develop CMS lines that can adapt to local environments. Fourteen more have been identified as maintainers from 50 testcrosses. To test the extent of cross-pollination, we attempted to multiply male sterile seeds of IR54752 A using techniques developed in China and at IRRI.

Performance of hybrid rices in Texas, USA

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F_1 hybrid and check varieties were included in replicated yield trials at six sites in Texas in 1983 and 1985, and five sites in 1984. The hybrids are proprietary varieties of Ring-Around Products, Inc., and information on the parentage was not available. In 1983 and 1984, five hybrids were common to all tests, but in 1985 they were replaced by three new hybrids. Conventional Chinese varieties Nan-Jing 11, Kwang-Chang-Ai, and Gui-Chao; U.S. varieties LA110, Brazos, Lemont, and Rico 1; and two experimental selections from Beaumont (RU7803097 and RU8103029) were included as checks at all sites in one or more years. The tests were drill-seeded at 80 kg/ha and standard cultural practices were followed. Milling yields and yield components were determined only at the Beaumont site.

Average yields of hybrids were 8.6 t/ha in 1983, 11.0 t/ha in 1984, and 8.6 t/ha in 1985. Those of the checks were 8.0 t/ha in 1983, 8.8 t/ha in 1984, and 7.4 t/ha in 1985, for a hybrid yield advantage of 7.4% in 1983, 25.0% in 1984, and 15.7% in 1985. Over the 3 yr of testing, the highest hybrid yield was 13.8 t/ha compared with 12.5 t/ha for Kwang-Chang-Ai, 12.4 t/ha for Nan-Jing 11, 13.4 t/ha for LA110, and 11.4 t/ha for Rico 1, all in 1984. Gui-Chao, a check only in 1985, produced the highest yield (11.7 t/ha) of any entry and the highest average across all sites, 9.3 t/ha compared with 8.7 t/ha for the best hybrid. Without data on the parents' performance, the yield increase due to hybrid vigor could not be determined. The hybrid varieties and the conventional varieties from

China were all highly productive under Texas environmental conditions and cultural practices.

The major defect in hybrid varieties in these tests is low milling yield. Because the whole-grain milling yield is the principal determinant of the price the producer receives for his rice in the U.S., superior milling is a top breeding objective. In 1983,1984, and 1985, average whole-grain milling yield was 4.1, 3.6, and 3.0 t/ha for the hybrids and 4.7, 5.6, and 4.2 t/ha for the checks, or check advantages of 13% in 1983, 55% in 1984, and 40% in 1985.

The only yield component that differentiated hybrids from checks was individual grain weight. Hybrids averaged 33.8 mg/grain and checks 27.7 mg/grain, a 22% increase for the hybrids. All statistically significant correlations between characters studied were low to moderate except the strong negative correlation (–0.829**) between grain weight and percent whole-grain milling yield.

Hybrid rice research in South Korea

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Hybrid rice research in Korea started in the early 1970s, but was not active until 1982, when some wild abortive (WA)-type cytoplasmic male sterile (CMS) systems were introduced. A Korea-IRRI collaborative research project on hybrid rice was formally established in 1984.

The experimental hybrids have yielded about 20% more than commercial cultivars. The most widely adaptable F_1 hybrid combination V20/Milyang 46 yielded 11.5 t/ha in 1984 and 10.0 t/ha in 1985 at Honam Crop Experiment Station. However, Korean consumers do not prefer its high chalkiness and high-amylose grain.

Several Tongil-type cultivars, Suweon 290, Suweon 296, Suweon 311, Iri 356, Milyang 55, and some breeding lines have been identified as maintainers. Most Tongil-type cultivars showed good fertility restoration for the WA-type CMS system, but no japonica rices were identified as restorers.

Two CMS lines, IR54756 A and IR54757A, developed by transferring the WA CMS system into Korean Tongil-type varieties, are promising. These and some newly developing CMS lines with Korean commercial cultivars are being tested for stability and combining ability.

In experimental hybrid seed production, outcrossing ratios ranged from 2.2 to 30.0%. Hybrid seed yields of 0.97-1.17 t/ha have been obtained in an experimental seed production plot. Hybrid rice appears to be a good prospect for improving grain quality and disease and insect resistance.

The hybrid rice breeding program in Korea will focus on developing CMS for grain quality and cold tolerance, selecting fertility restorers for the developing CMS, accumulating desirable traits into the CMS system, and establishing an adequate hybrid seed production system.

Agronomic performance of hybrid rice in northern Mexico

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Fifteen hybrid rices introduced from IRRl in 1984 were compared with a local improved rice variety in a randomized complete block design with three replications. Plot size was five 5-m-long rows with 25 cm spacing. The seeding rate was 30 kg/ha in an alkali soil (8.3 pH).

Irrigation was intermittent from seeding to harvest, except for static flooding from panicle initiation to complete flowering. For weed control, 3 liters oxadiazon/ha was sprayed preemergence. Urea was applied twice, 100 kg N/ha 35 d after seeding and 50 kg N/ha at panicle initiation.

Thirteen characters were evaluated using the standard evaluation system for rice.

The hybrid MR365 A/IR36 at 3.7 t/ha had the highest yield. Five statistical groups were identified by Duncan's multiple range test. The first included MR365 A/IR36 (3.7 t/ha), MR365 A/IR54 (3.6 t/ha), Culiacan 82 A(3.6t/ha), IR46831 A/IR54 (3.5t/ha), V20 A/IR13420-6-3-3-1 (3.0 t/ha), Zhen-Shan 97 A/IR2307-247-2-2-3 (2.7t/ha), V20 A/IR9761-19-1 (2.4 t/ha), and lR74-7B2-6-3 A/IR50 (2.4 t/ha).

The second group was composed of the hybrids lR365 A/Milyang 54 (2.0t/ha) and V20 A/IR13419-113-1 (1.9 t/ha). The third group included hybrids MR365 A/IR21015-180-3-3 (1.5 t/ha) and MR365 A/IR13524-21-2-3-3-1 (1.3 t/ha). In the 2 other groups, yields were 0.9-1.2 t/ha.

Sterility among the hybrids ranged from 6.7 to 80.4%; the local check variety had no sterility.

The hybrids matured earlier (104-115 d) than the local variety(135 d). Highest daily productivity was 34.4-26.5 kg/d; the local check yielded 26.3 kg/d. Lowest daily productivity was 8.9 kg/d.

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Appendices

Appendix 1. Rice varieties

Appendix 2. CMS types.

Appendix 3. Wheat varieties.

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