



Breaking the Yield Barrier

Proceedings of a Workshop
on Rice Yield Potential
in Favorable Environments

Edited by **K.G. Cassman**

IRRI

INTERNATIONAL RICE RESEARCH INSTITUTE

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IRRI, 29 November - 4 December 1993

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1994

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Foreword

World production of rough rice was about 225 million tons when the first modern rice varieties were developed in the early 1960s. Over the next 30 years, world rice production increased by about 300 million tons due to widespread adoption of modern, high-yielding varieties, expansion of irrigated area, increased fertilizer use, and incorporation of pest resistance into the cultivated rice germplasm. Despite this remarkable achievement, the yield potential of modern rice varieties has remained constant since the release of IR8 in 1966.

In the next 30 years, global rice production must be raised by another 300 million tons. With little scope for expanding irrigated area, this challenge must be met by increased yields from the existing riceland. The purpose of this workshop was to delineate the boundaries of our present understanding of yield potential, to explore the frontiers of science in plant biology relevant to yield formation, and to identify the most promising approaches to develop rice varieties with higher yield potential.

IRRI invited scientists who work not only on rice but on other cereal crops as well, in recognition of the unifying nature of processes governing plant growth and yield, and the promise of biotechnology to transfer desired genetic traits from one crop species to another. The starting point for the workshop was a series of papers written by IRRI scientists that summarized past and present research on rice yield potential, or possible avenues to introduce a change. Invited papers were presented by international participants, and these are included as extended abstracts. The IRRI papers are also included, as is a set of Recommendations for future research prepared by the participants.

I am grateful to the overseas participants, and specifically to our colleagues from the University of the Philippines at Los Baños and the Philippine Rice Research Institute who actively participated, and finally to my IRRI colleagues for their contributions. The output of this meeting will help to establish the scientific foundation for our efforts to break through the existing yield potential barrier, a barrier that must be overcome in an environmentally conscious manner to ensure long-term food security in Asia.

Klaus Lampe
Director General

Acknowledgments

The output of this workshop represents the contributions and dedication of many individuals, including the invited participants, IRRI researchers, editors, and secretaries.

The Workshop Organizing Committee, including Gurdev S. Khush, Ben S. Vergara, Tim L. Setter, Martin J. Kropff, Shaobing Peng, and Kenneth G. Cassman (Chair), thank Kate Kirk, Tess Rola, and Reginald MacIntyre for editing the proceedings, and Rafaela Figueroa and Rosario C. Badrina for typing and formatting this document. A special thanks goes to Rafaela Figueroa for her skill in coordinating the workshop itself.

Much of the research reported in Part I is based on recent field studies conducted from 1991 to 1993 that have not previously been reported. The authors of those papers wish to recognize the contributions of: Romeo M. Visperas, Rodolfo C. Aquino, Felipe V. Garcia, and Arnel L. Sanico for research on the "Evolution of the New Plant Ideotype for Increased Yield Potential"; Samuel P. Liboon, Rolando Torres, M. Jacquelin A. Dionora, Helen G. Centeno, and Maridelle A. Dizon for research on the "Quantitative Understanding of Yield Potential"; Rebecca C. Laza, Elizabeth A. Conocono, James A. Egdane, and Samuel P. Liboon for research on "Physiological Considerations and Hybrid Rice"; and Tita V. D. Mew, Arnold S. Parco, Guiquan Zhang, Llevea P. Abenes, and Gerard B. Magpantay for research on "Molecular Approaches".

The Working Group that prepared the Part II section "Research Priorities to Increase Rice Yield Potential" included: Dr. Shigemi Akita, Dr. Araceli Alejar, Dr. P. Stephen Baenziger, Prof. Graham D. Farquhar, Dr. A. Fischer, Dr. Kunio Hamamura, Prof. Peter Horton, Dr. Ganesh M. Kishore, Dr. R. S. Loomis, Dr. John C. O'Toole, Dr. Restituta Robles, and Dr. M. Tollenaar.

Part 1

Raising the Rice Yield Ceiling

Introduction

The four papers in this section of the Proceedings cover past and present efforts to increase rice yield potential, identify gaps in our understanding of the determinants of yield potential, and point to avenues of greatest opportunity to make further research progress. The first paper provides a history and rationale for the new rice plant ideotype as a major research project within the Irrigated Rice Ecosystem Program at IRRI. The second paper applies a quantitative approach to evaluating constraints to increased yield potential, and utilizes a considerable amount of information obtained from research studies done in 1991-93. The third paper covers specific components of our current and planned research on yield potential, and considers several topics of potential importance that are not presently being addressed. The fourth paper examines molecular approaches to increase yield.

IRRI scientists from Plant Physiology, Agroecology, Agronomy, Plant Breeding and Genetics, and Soil Science contributed to these papers. As in most scientific institutes, and indeed in most departments, there is often a diversity of viewpoints on such a general topic as yield potential. We have tried to minimize duplication and contradiction, but we have chosen to include differing perspectives where they occur and deserve consideration.

Our emphasis throughout is tightly focused on yield potential under favorable conditions, when water, nutrients, and pests do not limit plant growth. For rice, these conditions are generally met in irrigated lowland paddies of tropical Asia, especially in the dry season when solar radiation is greatest. The rationale for this focus derives from projections of a 60% increase in global rice demand by the year 2020. At present, 90% of all rice is produced and consumed in Asia, and more than 90% of the projected increase in demand for rice will come from Asia, where population is high and land and water resources are limited.

Although irrigated riceland accounts for about 50% of harvested rice area, irrigated systems produce 75% of total output. However, irrigated land devoted to rice production has declined by 10% in China in the past 20 years, and it is likely that rice area in Asia as a whole will remain stable at best or decline slightly as industrial development accelerates in the region. In 1990, average rice yield on 70 million harvested ha of irrigated riceland in Asia was

4.9 t/ha. Assuming no decrease in irrigated rice area, average yields must increase to about 8.0 t/ha within 30 years to satisfy a conservative projection for 1.6% annual increase in rice demand. A yield of 8.0 t/ha, however, is close to the present yield ceiling of current rice varieties in most tropical and subtropical regions.

Our challenge, therefore, is to generate the knowledge that will allow farmers to produce more rice on less land. Raising the yield potential of rice is a pivotal component of IRRI's strategy to sustain productivity gains for Asian rice farmers and to maintain affordable food prices for rural and urban rice consumers.

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Evolution of the new plant ideotype for increased yield potential

S. Peng, G. S. Khush and K. G. Cassman

Plant type and rice yield potential in retrospect

In pioneering studies, Tsunoda (1959a, b, 1960, 1962) compared yield potential and yield response to nitrogen (N) fertilizer with plant type of different rice genotypes. Varieties with high yield potential and greater responsiveness to applied N had short sturdy stems and leaves that were erect, short, narrow, thick, and dark green. The close association between certain morphological traits and yielding ability in response to N led to the “plant type concept” as a guide for breeding improved varieties (Yoshida 1972). “Crop ideotype” was defined as an idealized plant type with a specific combination of traits deemed favorable for photosynthesis, growth, and grain yield (Donald 1968).

Selection for semidwarf stature in the late 1950s for rice (*Oryza sativa*) and wheat (*Triticum aestivum*) is the most striking example of a successful improvement in plant type. Although selections were guided by short stature, resistance to lodging, and efficient biomass partitioning between grain and straw, breeders were unintentionally selecting for improved canopy architecture, light penetration, and other favorable agronomic characteristics associated with the new plant type (reviewed by Takeda 1984). IR8, the first high-yielding modern rice cultivar, was released by IRRI in 1966. This event marked the start of the “green revolution” in Asia. Compared with traditional cultivars, IR8 was a semidwarf with profuse tillering, stiff culm, erect leaves, photoperiod insensitivity, N responsiveness, and high harvest index (HI) (Chandler 1969). Adoption of high-yielding varieties like IR8 occurred rapidly in South, East and Southeast Asia because farmers obtained a yield advantage of 1 to 2 t/ha over traditional varieties on irrigated land (Chandler 1972). At tropical latitudes in the dry season (DS), when solar radiation is greatest, the yield potential of IR8 is about 10 t/ha. Today, more than 60% of the world’s rice area is planted to semidwarf plant types similar to IR8, and they account for more than 80% of total rice production (Khush 1990).

Subsequent plant breeding efforts were aimed at stabilizing yield and increasing economic value by incorporating disease and insect resistance, better grain quality, and shorter growth duration (Khush and Coffman 1977). One variety, IR36, became the world’s most widely grown rice genotype in the 1970s (IRRI 1982). More recent cultivars, such as IR72, have a yield potential

equivalent to IR8 but have shorter growth duration and improved resistance to a number of important rice diseases and insect pests. When adjusted for earlier maturity, the yield potential of IR72 is 5-10% greater than IR8 on a yield per day basis (see Kropff et al, These Proceedings).

The need for a new plant type

In the 28 years since IR8 was released, rice yield potential has remained constant. This plateau is evident in the yield records from several long-term experiments in which the most recent elite rice genotypes have been substituted continually for older varieties since the experiments began in the 1960s (Flinn et al 1982, Cassman et al 1994). Despite this apparent yield barrier, the quest for higher yield potential continued.

Plant physiologists proposed increased photosynthetic efficiency and greater sink size as possible approaches to increase yield potential (Evans 1972, Yoshida et al 1972). Although genetic variation in single-leaf photosynthetic rates has been reported in rice, increased photosynthetic capacity has not resulted in higher grain yield (Murata 1957, McDonald et al 1974). Moreover, measurements of single-leaf photosynthetic rates in traditional and modern high-yielding varieties did not reveal differences in the historical rice germplasm (Evans et al 1984), and direct selection for higher single-leaf photosynthetic rate sometimes resulted in lower yield (Evans 1990). Genotypic variation in panicle size has been documented as well, but heavy panicle genotypes were generally found in upland rice germplasm that lacked agronomic fitness in irrigated environments (see next section) and did not produce enough biomass and spikelets per unit ground area to give higher yields.

To break through the apparent yield potential barrier, IRRI scientists proposed further modifications of the present high-yielding plant type that would support a significant increase in rice yield potential, particularly for direct-seeded crop establishment. The emphasis on direct seeding was based on projections of increased labor cost and labor shortages as industrial development accelerates in rice-growing countries of Asia. Although the proposed characteristics of the new ideotype came from several different perspectives (Vergara 1988, Janoria 1989, Dingkuhn et al 1991), the major components were essentially the same:

- Low tillering capacity with 3-4 panicles per plant when direct-seeded
- No unproductive tillers
- 200-250 grains per panicle
- 90-100 cm tall
- Sturdy stems
- Vigorous root system
- Multiple disease and insect resistance
- 110-130 days growth duration
- Harvest index of 0.6
- 13-15 t/ha yield potential

This ideotype became the “New Plant Type” highlighted in IRRI’s strategic plan (IRRI 1989a), and the breeding effort to develop this germplasm became a major core research project of the 1990-94 work plan (IRRI 1989b). The goal was to develop a new plant type within 8-10 years with 30-50% higher yield potential than the existing semidwarf varieties in tropical environments during the dry season (Khush 1990).

Rationale for the new plant type

Although the choice of plant attributes for the new plant type was the topic of several publications (Vergara 1988, Janoria 1989, Dingkuhn et al 1991), an in-depth scientific evaluation of the proposed new ideotype does not exist. A major constraint to such an evaluation was the inability in the late 1970s and the 1980s to grow current varieties in the field at yield levels near the yield potential threshold (see Kropff et al, These Proceedings). Thus, plant traits that were thought to increase yield potential beyond 10 t/ha were evaluated at yield levels of 6-8 t/ha.

In the following sections, the literature relevant to the evolution of the new plant type concept is reviewed. The present status of the breeding program for the new plant type is also discussed. In research conducted since 1991, the constraints to expression of full yield potential of current rice varieties were identified, and crop management practices improved, so that the physiological basis of a 10 t/ha yield potential in existing inbred indica varieties could be studied under field conditions. This research is reported in later sections (see also Kropff et al and Setter et al, These Proceedings).

Reduced tillering and large panicles

Increases in the yield potential of other cereals such as maize (*Zea mays*) and sorghum (*Sorghum bicolor*) resulted from increases in sink size. Selection and breeding for large sink size was accompanied by a decrease in tiller number: modern maize and sorghum varieties are unicum whereas primitive maize and sorghum have a large number of tillers and small cobs or heads (Khush 1990). By contrast, modern rice varieties tiller profusely. Each rice hill includes 3-5 plants and produces 30-40 tillers under favorable growth conditions, but only about 20 produce panicles. Unproductive tillers compete for resources with tillers that later produce panicles. Eliminating the unproductive tillers would allow more solar energy and mineral nutrients—particularly N—for growth of productive tillers, but the magnitude of the potential contribution to yield has not been quantified. Furthermore, the dense canopy that results from excess tiller production creates a humid microenvironment favorable for diseases, especially endogenous pathogens like sheath blight (*Rhizoctonia solani*) and stem rot (*Sclerotium oryzae*) that thrive in N-rich canopies (Mew 1991).

Kim and Vergara (1990) studied the tillering behavior of low-tillering (IR25588) and high-tillering (IR58) rice plants in pots. They reported that IR58

had earlier tillering, a faster tillering rate and longer tillering duration than IR25588. Differences in the number of secondary and tertiary tillers accounted for the difference in total tiller number of the two genotypes. A subsequent pot experiment was conducted to study the morpho-anatomical characteristics of the different panicles of the same low and high tillering cultivars (Kim and Vergara 1991). The low-tillering IR25588 had more inner and outer vascular bundles, and greater peduncle diameter and peduncle thickness just below the neck node than the high-tillering IR58. The number of inner and outer vascular bundles was associated with a larger number of rachis-branches, and more spikelets and grain weight per panicle. A subsequent field study conducted in the dry season indicated an 8% grain yield advantage for the low-tillering/ large panicle genotype (7.1 vs 6.6 t/ha) compared with IR58 when both genotypes were grown with high N supply and close spacing (Kim and Vergara 1992).

Reduced tillering is thought to facilitate synchronous flowering and maturity, more uniform panicle size, and efficient use of horizontal space (Janoria 1989). Low-tillering genotypes are also reported to have a larger proportion of high-density grains (Padmaja Rao 1987b). A single semidominant gene controlled the low tillering trait, and this gene had pleiotropic effects on culm length and thickness, and panicle size (Ise 1992). Therefore, the low tillering trait was hypothesized to be associated with larger panicle size, and it became a target trait for IRRI's new plant type.

The number of spikelets per unit land area, or sink size, is the primary determinant of grain yield in cereal crops grown in high-yield environments without stress (Fischer 1983, Takeda 1984). The number of spikelets per unit land area can be increased by increasing the number of panicles, or the number of spikelets per panicle. Modern high-yielding rice varieties have many more panicles than the traditional rice varieties they replaced. There is a limit, however, to how far panicle number can be increased. Additional tillers become unproductive and lead to excessive leaf area index (LAI) and vegetative growth, and a higher percentage of unfilled grains. To achieve increased sink size in the new plant type, reduced panicle number in low-tillering plant types must be compensated by a relatively greater increase in panicle size.

Grain size and high density grain

Yearly variation of grain weight in barley (*Hordeum vulgare*) can be as large as 50% (Thorne 1966), and the variation of wheat grain weight as large as 30% (Asana and Williams 1965). Rice grain weight is considered to be a stable varietal character, with less than 5% coefficient of variation among different years at the same site (Yoshida 1972). However, Venkateswarlu et al (1986b) found 43% variation in the weight of single rice grains within a panicle.

High density grains are those grains that remain submerged in a solution of specific gravity >1.20. Regardless of the growth duration of the varieties studied, high density grains represented 70-85% of filled grain at the top of the panicle (superior spikelet positions) and only 10-50% of inferior spikelets in the

lower portion of the panicle (Padmaja Rao 1987). High-density grains tended to occur on the primary branches of the panicle, while the spikelets of the secondary branches had lower grain weight (Ahn 1986), and the proportion of high density grain was 15% greater on primary tillers than on secondary or tertiary tillers of short-duration cultivars (Padmaja Rao 1987b).

These findings led to the hypothesis that selection for a higher proportion of high-density grains in the new plant type would contribute to higher yield potential, particularly in low-tillering, large-panicle genotypes with thick culms (Vergara 1988). Varietal differences in the number of high-density grains per panicle were found, and this trait appeared to be heritable (Venkateswarlu et al 1986b). Based on these results, it was suggested that rice grain yield could be increased by 30% if all the spikelets of an 8 t/ha crop were high-density grains. High-density grains also gave higher milling recovery and head rice yield (Venkateswarlu et al 1986a).

The hypothesis that selection for high-density grain genotypes would result in greater yield potential assumes sufficient assimilates, or source, to make heavier grains. In more recent work, Iwasaki et al (1992, 1993) found that superior spikelets are the first to accumulate dry matter and N during grain filling, while inferior spikelets do not begin to fill until the dry weight accumulation in superior spikelets is nearly finished. This apical dominance within the panicle was immediately altered upon removal of superior spikelets, which indicates that the delayed filling of inferior spikelets results from source limitation and regulation of assimilate allocation within the panicle. Thus, the high density grain trait may reflect differences in assimilate supply among cultivars that is related to general agronomic fitness. At issue is whether the high grain density trait itself can confer higher yield potential in agronomically fit genotypes.

At present, the new plant type is not directly selected for increased high-density grain, and such an emphasis is premature until the role of source limitation in governing the proportion of high-density grains is clarified.

Canopy and leaf characteristics

Erect leaf angle is a desirable trait for high-yielding varieties. Light is used more efficiently at high LAI in an erect-leaved canopy (Yoshida 1976). Carbon assimilation of a leaf exposed to light on only one side is lower than when the leaf is exposed on both sides if total light intensity is equal for each case, and this difference is greatest when leaves have high N content and greater thickness. Therefore, a plant community with vertically oriented leaves gives better light penetration and higher carbon assimilation per unit of leaf area (Tanaka 1976). Droopy or horizontal leaves increase the relative humidity and decrease the temperature inside the canopy due to reduced light penetration and air movement (Akiyama and Yingchol 1972), and such a microclimate provides a more favorable environment for many diseases and some insect pests of rice (Yoshida 1976).

Although a positive association between leaf thickness and yield potential has not been documented for rice, leaf thickness is positively correlated with leaf photosynthetic rate (Murata 1961). Thick leaves are therefore thought to be a desirable trait (Yoshida 1972), and this trait provides a visual selection criterion for the new plant type.

Crop growth duration

The optimal growth duration for maximum yield of inbred indica varieties in the tropics is thought to be about 120 days from seed to seed (Tanaka et al 1966, Tanaka and Vergara 1967). Akita (1989) found a linear increase in total biomass of recent IRRI varieties as growth duration increased from 95 to 135 days, whereas maximum grain yield of about 8-9 t/ha was constant in varieties of 110-130 days. Varieties of shorter growth duration usually give lower yields at typical hill spacing in transplanted rice due to insufficient vegetative growth for maximum yield levels (Yoshida 1976). Although early maturing varieties of 100-105 days may have greater grain production per day, medium growth duration varieties of 110-120 days can produce higher total yield and still fit into the annual double-crop rice and rice-wheat systems that dominate on 40 million ha of irrigated land in Asia.

Variation in growth duration largely reflects differences in the vegetative growth period (Vergara et al 1969). There is, however, a positive correlation between growth duration and the time from panicle initiation to heading. Thus, an early maturing rice crop has a relatively short period for panicle growth before heading, and spikelet number is positively correlated with crop growth rate during the period from panicle initiation to flowering (see Increased Sink Size section of Kropff et al, These Proceedings). Shorter duration for panicle growth is often accompanied by decreased grain yield (Yoshida 1972). The question remains whether panicle growth and grain filling periods can be extended independent of total growth duration. For both practical considerations of intensive double-crop rice systems and the potential loss of yield from short duration varieties, the new plant type is selected for a growth duration of 110-120 days.

Plant height, stem thickness, biomass production and harvest index

Short stature reduces the susceptibility to lodging and increases the HI (Tsunoda 1962). Shorter culms also require less maintenance respiration and contribute to an improved photosynthesis-respiration balance (Tanaka et al 1966). A decrease in plant height from present levels of current semidwarf varieties would reduce total dry matter unless thicker culms are produced (Vergara and Visperas 1977). Thicker culms could serve to accumulate more assimilates to offset the decrease in total panicle number in the new plant type. It is estimated that stem reserves contribute about 2 t/ha to grain yield (see section Carbohydrate Mobilization in Setter et al, These Proceedings).

Yield is a function of total dry matter and HI. Vergara et al (1966) demonstrated that grain yield was not correlated with total dry matter

although high dry matter production is required for high grain yields. Compared with other C_3 species, rice has a relatively high single-leaf net photosynthetic rate per unit leaf area, with a typical range from 25 to 32 $\mu\text{mol CO}_2/\text{m}^2$ per s (Yoshida 1983). The maximum crop growth rate of rice is around 30–36 g/m^2 per d in the Philippines (Yoshida and Cock 1971). Akita (1989) reported a crop growth rate of 40 g/m^2 per d with a maximum LAI of 20 in a high-N supply outdoor solution-culture system.

Increased biomass production is not difficult to achieve when the rice crop is grown in a high solar radiation environment similar to DS conditions at IRRI, and provided with a luxuriant supply of N (Akita 1989). Without a strong, thick culm and proper partitioning, however, increased biomass production results in lodging, increased disease incidence, and decreased grain yield (Vergara 1988). If lodging and disease problems can be solved, increased biomass production should contribute to increased yield potential in tropical environments.

Harvest index decreases with increased growth duration (Vergara et al 1966). Akita (1989) found HI to decrease from 55% to 35% as growth duration increased from 95 to 135 days. Modern, high yielding indica varieties with a growth duration between 100 and 130 days typically have an HI of 0.50 or more (Vergara and Visperas 1977). Year-round monthly planting experiments with different varieties demonstrate that HI is higher in the DS and lower in the wet season (WS), with a range from 0.44 to 0.58 for improved varieties and 0.12 to 0.48 for traditional varieties (Vergara and Visperas 1977). Clearly, there are limits to how far HI can be further increased in improved varieties. Austin et al (1980) estimated a maximum possible HI of 0.63 in wheat. If the same were true for rice, the present yield potential of 10 t/ha with an HI of 0.53 would increase to 12 t/ha assuming an HI of 0.63 for a new rice plant type (Evans 1990).

Estimates of potential yield for the new plant ideotype

Rice yield potential in tropical lowland environments (such as the IRRI research farm) was estimated to be 9.5 t/ha in the WS and 15.9 t/ha in the DS based on 12.6 and 20.9 MJ/m^2 per d solar radiation, respectively, and ambient CO_2 concentration (Yoshida and Oka 1982). These estimates were based on assuming a grain filling period of 25 days, that all net assimilation in the grain filling period was converted to grain yield, and a 3.5% conversion efficiency of incident solar radiation to carbohydrate (Yoshida 1981). At IRRI, IR24 produced the highest recorded yield of 11.0 t/ha (IRRI 1973), which is much lower than Yoshida's estimated yield potential. Although actual yields achieved by farmers in paddy yield competitions in India were 15.7 t/ha (Freeman et al 1990) and 17.8 t/ha with the cultivar Jaya in Maharashtra State at a research station (Suetsugu 1975), climatic conditions were not reported. Such high yields probably reflect climatic conditions more favorable to yield formation than in the lowland tropics, where high mean daily temperatures increase respiration losses and decrease grain filling duration.

The target yield of 15 t/ha at 14% moisture content (13.3 t/ha oven-dry weight basis with 3% grain moisture content) at IRRI during the DS would require 25 t/ha total aboveground biomass (oven-dry basis), assuming an HI of 0.53. In the high-N supply outdoor solution culture experiment of Akita (1989), maximum biomass of 22 t/ha was achieved with a 120-day breeding line (IR29723-143-3-2-1) in the DS at IRRI. Thus, to achieve the target grain yield potential of 15 t/ha (14% moisture content) in the new plant type, either total biomass production must increase through longer growth duration without a decrease in HI, HI must increase to 0.6 with 22 t/ha total biomass, or photosynthetic efficiency must increase.

Present status of breeding for the new plant type

Breeding work on the new plant type began in 1989, when two thousand entries from the IRRI germplasm bank were evaluated to identify donors for various traits. Most of these entries were *bulus*, also called javanicas, from Indonesia. This germplasm is known for large panicles, low tillering, and sturdy stems. The javanica rices are genetically very close to the japonicas grown in temperate areas. On the basis of allelic constitution at 15 isozyme loci, Glaszmann (1987) showed that javanicas and japonicas belong to the same varietal group. We therefore now refer to javanicas as tropical japonicas.

Crosses between tropical and temperate japonicas are fully fertile and there are no barriers to recombination. On the other hand, crosses between indicas and japonicas have varying levels of sterility and give poor recombinant progenies due to restrictions to recombination (Khush and Aquino 1994). A decision was made to limit the hybridization work for the new plant type to the tropical japonica germplasm to avoid problems of sterility and restrictions to recombination. On the basis of systematic germplasm evaluation, we have identified tropical japonica varieties not only from Indonesia, but also from Malaysia, Philippines, Vietnam, Laos, Thailand, and Myanmar. Some of the donors identified for various traits are listed in Table 1.

Table 1. Donors for various traits being used for developing the new plant type.

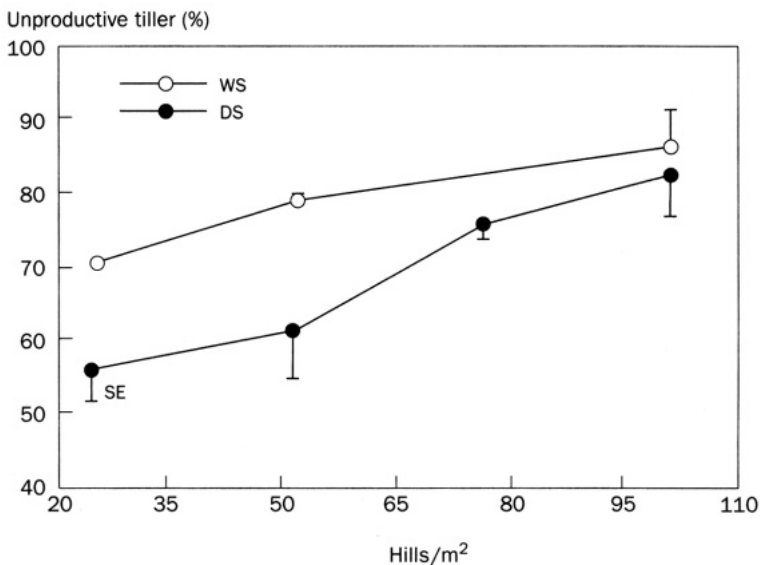
Trait	Donors
Short stature	MD2, Sheng-Nung 89-366
Low tillering	Merim, Gaok, Gendjah Gempol, Gendjah Wangkal
Large panicles	Daringan, Djawa Serang, Ketan Gubat
Thick stems	Sengkeu, Sipapak, Sirah Bareh
Grain quality	Jhum Paddy, WRC4, Azucena, Turpan4
Resistance:	
Bacterial blight	Ketan Lumbu, Laos Gedjah, Tulak Bala
Blast	Moroberekan, Pring, Ketan Aram, Mauni
Tungro	Gundil Kuning, Djawa Serut, Jimbrug, Lembang
Green leafhopper	Pulut Cenrana, Pulut Senteus, Tua Dikin

Hybridization work was undertaken in 1990 DS, and F₁ progenies were grown for the first time in 1990 WS, F₂ progenies in 1991 DS, and pedigree nursery in 1991 WS. Since then, 718 crosses have been made and 37,148 pedigree nursery rows have been grown. We have selected breeding lines which have the targeted traits of the proposed ideotype for high yield potential. These were grown in observational trials for the first time in 1993 WS. Their yield potential will be evaluated in 1994 DS in replicated field plots under various management practices.

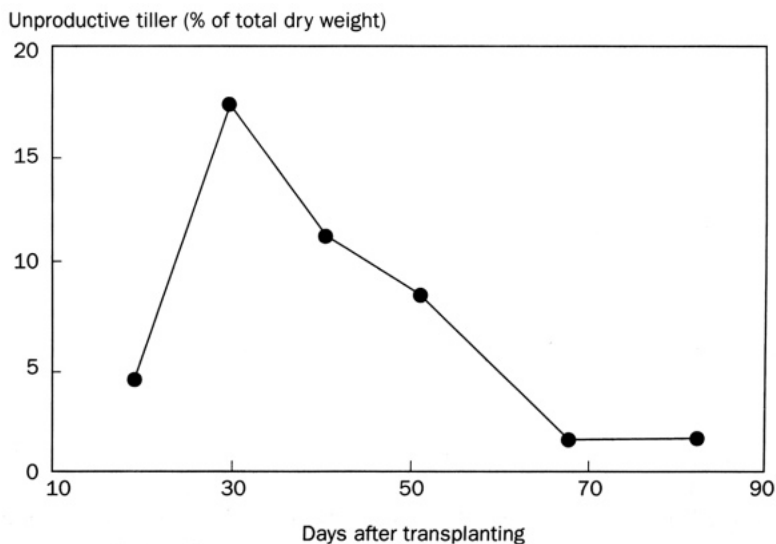
These new plant type breeding lines have semidwarf stature (90-100 cm), sturdy stems, dark green and thick leaves, 8-10 productive tillers when grown at low density in the selection nurseries (versus 20-30 for current indica varieties), no unproductive tillers, and 150-200 grains per panicle. Further improvements are aimed at improving grain quality and incorporation of disease and insect resistance. Most of the tropical japonicas, like their temperate counterparts, have short bold grains. However, preference in the tropics and subtropics is for long slender grains. Donors with long slender grains have been identified within the tropical japonica germplasm (Table 1). Similarly, donors for resistance to blast, bacterial blight, tungro, and green leafhopper have been identified in the tropical japonica germplasm. Although we have not found any donors for resistance to brown planthopper (BPH) in the japonica germplasm, genes for BPH resistance are being transferred from the indicas through backcrossing. It is hoped that improved tropical japonicas with the proposed ideotype, resistant to diseases and insects, and with acceptable grain quality will become available in the next 3-4 years.

Initial evaluation of the new plant type traits: 1991-93

With the recent changes in crop management that allow expression of full yield potential in current indica varieties (see Kropff et al, These Proceedings), the tillering responses of elite semidwarf indica varieties were examined in field experiments with grain yield approaching 10 t/ha in the DS and 6.5 t/ha in the WS. At these yield levels, a large proportion of tillers do not produce panicles: 55% of maximum tiller number in the DS and 70% in the WS were unproductive with a normal hill spacing of 20 × 20 cm and five seedlings per hill (Fig. 1). Increased planting density significantly increased the percentage of unproductive tillers in both seasons. At maximum tillering stage, the biomass of the unproductive tillers was 18% of total dry matter (Fig. 2), and this is probably a conservative estimate because unproductive tillers continuously emerge and disappear so that complete recovery is difficult. But total biomass at maximum tillering stage is only about 2.5 t/ha, so that unproductive tiller biomass represents a small fraction of total primary production in a rice crop yielding 10 t/ha of grain and an HI of 0.53. Although there is a certain degree of remobilization of C and N from unproductive tillers after death to active shoots in wheat (Thorne and Wood 1987), the efficiency of remobilization and the potential contribution to grain yield of rice has not been quantified.

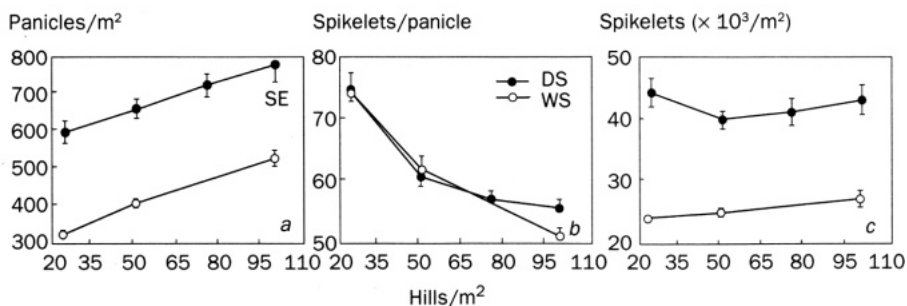


1. Percentage of unproductive tillers of IR72 as a function of hill density in the 1992 DS and WS. Hills with 5 plants each were spaced 20×20 , 14×14 , 11.5×11.5 , and 10×10 cm, respectively (Peng et al, unpubl. data).



2. The biomass of unproductive tillers as percent of total aboveground dry matter of IR72 grown in the 1992 WS (Peng et al, unpubl. data).

The relationship between panicle number/m² and filled spikelets per panicle and plant density was also examined in these recent field studies. Panicle number/m² increased significantly as planting density increased from 25 to 100 hills/m² in both DS and WS (Fig. 3a), but spikelet number per panicle decreased proportionally (Fig. 3b), so that total spikelet number/m² remained



3. Panicles / m² (a), spikelets / panicle (b), and spikelets (× 10³ / m²) (c) as a function of hill density of IR72 grown in the dry (DS) and wet (WS) seasons of 1992. Hills with 5 plants each were spaced at 20 × 20, 14 × 14, 11.5 × 11.5, and 10 × 10 cm, respectively (Peng et al, unpubl. data).

Table 2. Grain yields of IR72 grown at different hill spacing with 5 plants per hill in the dry and wet seasons of 1992 (Peng et al., unpublished data).

Transplanting space (cm)	Hills/m ²	Yield (t/ha)	
		Dry season	Wet season
20 × 20	25	9.6 a ^a	6.0 a
14 × 14	51	8.4 b	5.9 a
11.5 × 11.5	76	8.2 b	
10 × 10	100	8.4 b	6.3 a

^a Comparison within a column by Duncan's Multiple Range Test (P<0.05).

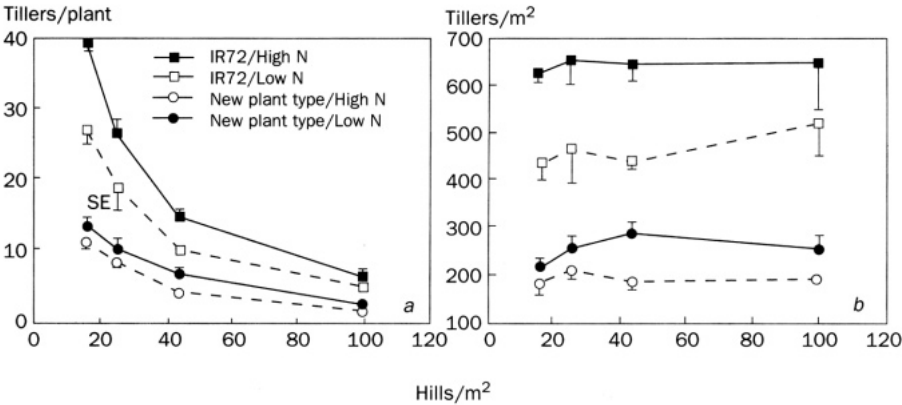
constant (Fig. 3c). Thus, no yield advantage was obtained at higher plant density in either season (Table 2). This dynamic compensation in filled spikelets per panicle as panicle number increases with greater plant density appears to limit potential grain yield gains from higher panicle numbers. In this study, the hill density of 100/m² with five plants per hill represents a typical plant density in direct-seeded rice.

In the WS, however, greater panicle size may confer a yield advantage. The yield and components of yield of a breeding line IR60819-34-2-1 with a relatively larger panicle and lower tillering capacity than IR72 (a high tillering and small panicle type) were compared in the 1992 WS. Highest yields were 5.9 t/ha for IR72 and 6.5 t/ha for IR60819-34-2-1, and the breeding line significantly outyielded IR72 across the different N supply treatments (Table 3). Growth duration was 110 d for IR72 and 112 d for IR60819-34-2-1. The number of spikelets per panicle of IR60819-34-2-1 was 28% greater than for IR72, which contributed to 15% more spikelets/m² for the breeding line. Other components of yield and total biomass were similar in the two genotypes. At issue is whether the yield advantage of the breeding line was related to the more regulated tillering habit compared with IR72, which produced 32% more total tillers and 42% more unproductive tillers. However, the yield advantage of the larger panicle type IR60819-34-2-1 was not obtained in the 1993 DS (unpubl. data). Lack of benefit from reduced tillering habit and larger panicle size in the DS appears to reflect the lower proportion of unproductive tillers (Fig. 1), and

Table 3. Grain yield of IR72 and IR60819-34-2-1 grown with different fertilizer-N treatments, and yield components, total biomass production, and harvest index averaged across N treatments, 1992 WS (Peng et al., unpublished data).

	IR60819-34-2-1	IR72
Grain yield (t/ha)		
0- 0- 0- 0 ^a	4.3 a ^b	4.0 b
0- 0-30-30	6.0 a	5.2 b
0-30-30-30	6.5 a	5.8 b
30-30-30-30	6.3 a	5.9 a
Yield components		
Spikelets/m ² (x10 ³)	30.1 a	25.9 b
Panicles/m ²	312 a	342 a
Spikelets/panicle	97 a	76 b
Filled spikelets (%)	83.2 a	83.3 a
1000-grain wt (g)	21.8 a	22.2 a
Total biomass (t/ha)	11.5 a	10.9 a
Harvest index (%)	49.0 a	45.6 b

^aN management (kg N/ha) at basal, midtillering, panicle initiation, and flowering. ^bComparison within a row by Duncan's Multiple Range Test (P<0.05).



4. Tillers/plant (a) and tillers/m² (b) as a function of hill density of IR72 and new plant type grown under different N supply in the 1993 WS. Each hill had a single plant in treatments with 25 × 25, 20 × 20, 15 × 15, or 10 × 10 cm in hill spacing (Peng et al, unpubl. data).

greater total panicle number (Fig. 3a), of the high-tillering IR72 in the DS, when solar radiation is greater than in the WS.

More recently in the 1993 WS, eight early-generation lines of the new plant type were evaluated for stability in yield components in different plant density and N supply environments. Only data on tillering are available. At low hill density, the high-tillering IR72 had 3 times more tillers per plant than the new low-tillering plant type regardless of N supply (Fig. 4a). For both genotypes, increased plant density had only a small effect on total tiller number, so that the new plant type had 2 to 3 times fewer total tillers regardless of plant density

(Fig. 4b). Thus, the low tillering habit of the new plant type is expressed across a wide range of plant density and N supply environments. Yield comparisons from this WS, however, will be confounded by the lack of disease resistance of the new plant types. Tungro virus, which is endemic at the IRRI research farm, occurred in most of the eight new lines that were evaluated. Transfer of disease and insect resistance into the new plant type genotypes is in progress (Table 1, and earlier section).

In the 1994 DS, we will evaluate the morphological traits and yield potential of new-plant-type lines using both transplanted and direct wet-seeded crop establishment. Higher solar radiation and lower relative humidity reduce disease and insect pressure in the DS. These studies will quantify relationships among yield potential, tillering habit, and yield components. The amount of unproductive tiller biomass and N content will be monitored throughout the crop growth period. The key issue is whether the low-tillering habit of the new plant type confers a yield advantage, and if so, the physiological basis for this increase.

References cited

- Ahn J K (1986) Physiological factors affecting grain filling in rice. Ph.D. thesis, Univ. of the Philippines at Los Baños.
- Akita S (1989) Improving yield potential in tropical rice. In: *Progress in Irrigated Rice Research*. Proc. Intl. Rice Res. Conf. 21-25 Sept. 1987, Hangzhou, China.
- Akiyama T, Yingchol P (1972) Studies on response to nitrogen of rice plant as affected by difference in plant type between Thai native and improved varieties. *Proc. Crop Sci. Soc. Japan* 41:126-132.
- Asana R D, Williams R F (1965) The effect of temperature stress on grain development in wheat. *Aust. J. Agr. Res.* 16:1-13.
- Austin R B, Bingham J, Blackwell R D, Evans L T, Ford M A, Morgan C L, Taylor M (1980) Genetic improvements in winter wheat yields since 1990 and associated physiological changes. *J. Agric. Sci. (Cambr.)* 94:675-689.
- Cassman K G, De Datta S K, Olk D C, Alcantara J, Samson M, Descalsota J, Dizon M (1994) Yield decline and the nitrogen economy of long-term experiments on continuous, irrigated rice systems in the tropics. *Advances in Soil Science* (In press).
- Chandler R F Jr. (1969) Plant morphology and stand geometry in relation to nitrogen. Pages 265-285. In: J.D. Eastin, F.A. Haskins, C.Y. Sullivan, C.H.M. van Bavel, ed. *Physiological Aspects of Crop Yield*. ASA, Madison, Wisconsin.
- Chandler R F Jr. (1972) The impact of the improved tropical plant type on rice yields in South and Southeast Asia. Pages 77-85. In: *Rice Breeding*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Dingkuhn M, Penning de Vries F W T, De Datta S K, van Laar H H (1991) Concepts for a new plant type for direct seeded flooded tropical rice. Pages 17-38. In: *Direct Seeded Flooded Rice in the Tropics*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Donald C M (1968) The breeding of crop ideotypes. *Euphytica* 17:385-403.

- Evans L T (1972) Storage capacity as a limitation on grain yield. Pages 499-511. In: *Rice Breeding*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Evans L T (1990) Raising the ceiling to yield: The key role of synergisms between agronomy and plant breeding. Pages 103-107. In: K. Muralidharan and E.A. Siddiq ed. *New Frontiers in Rice Research*. Directorate of Rice Research, Hyderabad, India.
- Evans L T, Visperas R M, Vergara B S (1984) Morphological and physiological changes among rice varieties used in the Philippines over the last seventy years. *Field Crops Res.* 8:105-124.
- Fischer R A (1983) Wheat. Pages 129-154. In: *Potential Productivity of Field Crops Under Different Environments*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Flinn J C, De Datta S K, Labadan E (1982) An analysis of long-term rice yields in a wetland soil. *Field Crops Res.* 5:201-216.
- Freeman W H, Saxena N P, Siddiq E A (1990) Physiological approaches for genetic improvement of yield potential in irrigated rice. Pages 116-124. In: K. Muralidharan and E.A. Siddiq, ed. *New Frontiers in Rice Research*. Directorate of Rice Research, Hyderabad, India.
- Glaszmann J C (1987) Isozymes and classification of Asian rice varieties. *Theor. Appl. Genet.* 74:21-30.
- IRRI-International Rice Research Institute (1973) Annual Report for 1972. Page 266. P.O. Box 933, Manila, Philippines.
- IRRI-International Rice Research Institute (1982) Research highlights for 1981. Page 13. P.O. Box 933, Manila, Philippines.
- IRRI-International Rice Research Institute (1989a) IRRI towards 2000 and beyond. Pages 36-37. P.O. Box 933, Manila, Philippines.
- IRRI-International Rice Research Institute (1989b) Implementing the strategy: Work plan for 1990-1994. Pages 15-17. P.O. Box 933, Manila, Philippines.
- Ise K (1992) Inheritance of a low-tillering plant type in rice. *Intl. Rice Res. Newsl.* 17(4):5-6.
- Iwasaki Y, Mae T, Chikafusa C, Makino A, Ohira K, Ojima K (1993) Glutenin accumulation and changes in the levels of its mRNA in the superior and inferior spikelets of rice ear during ripening. Pages 231-234 In: N.J. Barrow, ed. *Developments in Plant and Soil Science Vol. 54, Plant Nutrition: from Genetic Engineering to Field Practice*. Kluwer Academic Publishers, Dordrecht.
- Iwasaki Y, Mae T, Makino A, Ohira K, Ojima K (1992) Nitrogen accumulation in the inferior spikelet of rice ear during ripening. *Soil Sci. Plant Nutr.* 38(3):517-525.
- Janoria M P (1989) A basic plant ideotype for rice. *Intl. Rice Res. Newsl.* 14(3):12-13.
- Khush G S (1990) Varietal needs for different environments and breeding strategies. Pages 68-75. In: K. Muralidharan and E.A. Siddiq, ed. *New Frontiers in Rice Research*. Directorate of Rice Research, Hyderabad, India.
- Khush G S, Aquino R (1994) Breeding tropical japonica for hybrid rice production. In: Proceedings of the 1992 International Rice Research Conference, Intl Rice Res. Inst. Los Baños, Philippines (in press).
- Khush G S, Coffman W R (1977) Genetic Evaluation and Utilization (GEU), the rice improvement program at the International Rice Research Institute. *Theor. Appl. Genet.* 51:97-110.
- Kim J K, Vergara B S (1990) Tillering behavior of low and high tillering rices. *Korean J. Crop Sci.* 35:512-517.

- Kim J K, Vergara B S (1991) Morpho-anatomical characteristics of different panicles in low and high tillering rices. *Korean J. Crop Sci.* 36:568-575.
- Kim J K, Vergara B S (1992) Grain yield potential of a low-tillering large panicle type in rice. *Korean J. Crop Sci.* 37:361-371.
- McDonald D J, Stansel J W, Gilmore E C (1974) Breeding for high photosynthetic rate in rice. *Indian J. Genet.* 34A:1067-1073.
- Mew T (1991) Disease management in rice. Pages 279-299. In: D. Pimentel (ed) *CRC Handbook of Pest Management in Agriculture*, Second Edition, Vol. III. CRC Press Inc., Boston.
- Murata Y (1957) Photosynthetic characteristics of rice varieties. *Nogyo-gijitsu* 12:460-462.
- Murata Y (1961) Studies on the photosynthesis of rice plants and its cultural significance [in Japanese, English summary]. *Bull. Nat. Inst. Agr. Sci. Jap. Ser. D*, 9:1-169.
- Padmaja Rao S (1987a) Panicle type: few structural considerations for higher yield potential in rice. *Indian J. Plant Physiol.* 30(1):87-90.
- Padmaja Rao S (1987b) High density grain among primary and secondary tillers of short- and long-duration rices. *Intl. Rice Res. Newsl.* 12(4):12.
- Suetsugu I (1975) Records of high rice yields in India. I. Judging method of "All India Crop Competition" and results. *Agric. Technol.* 30:212-215.
- Takeda T (1984) Physiological and ecological characteristics of high yielding varieties of lowland rice. In: *Proc. Intl. Crop Science Symposium*. Oct. 17-20. Fukuoka, Japan.
- Tanaka T (1976) Regulation of plant type and carbon assimilation of rice. *JARQ* 10(4):161-167.
- Tanaka A, Kawano K, Yamaguchi J (1966) Photosynthesis, respiration, and plant type of the tropical rice plant. *Intl. Rice Res. Inst. Tech. Bull.* No. 7.
- Tanaka A, Vergara B S (1967) Growth habit and ripening of rice plants in relation to the environmental conditions in the Far East. *Intl. Rice Comm. Newsl.* (Special Issue):26-42.
- Thorne G N (1966) Physiological aspects of grain yield in cereals. Pages 88-105. In: F.L. Milthorpe and J.D. Ivins, ed. *The Growth of Cereals and Grasses*. Butterworths, London.
- Thorne G N, Wood D W (1987) The fate of carbon in dying tillers of winter wheat. *J. Agric. Sci. (Cambr.)* 108:515-522.
- Tsunoda S (1959a) A developmental analysis of yielding ability in varieties of field crops. I. Leaf area per plant and leaf area ratio. *Jap. J. Breed.* 9:161-168.
- Tsunoda S (1959b) A developmental analysis of yielding ability in varieties of field crops. II. The assimilation-system of plants as affected by the form, direction and arrangement of single leaves. *Jap. J. Breed.* 9:237-244.
- Tsunoda S (1960) A developmental analysis of yielding ability in varieties of field crops. III. The depth of green color and the nitrogen content of leaves. *Jap. J. Breed.* 10:107-111.
- Tsunoda S (1962) A developmental analysis of yielding ability in varieties of field crops. IV. Quantitative and spatial development of the stem-system. *Jap. J. Breed.* 12:49-55.
- Venkateswarlu B, Parao F T, Visperas R M, Vergara B S (1986a) Screening quality grains of rice with a seed blower. *SABRAO J.* 18(1):19-24.
- Venkateswarlu B, Vergara B S, Parao F T, Visperas R M (1986b) Enhanced grain yield potentials in rice by increasing the number of high density grains. *Philipp. J. Crop Sci.* 11:145-152.
- Vergara B S (1988) Raising the yield potential of rice. *Philipp. Tech. J.* 13:3-9.

- Vergara B S, Chang T T, Lilis R (1969) The flowering response of the rice plant to photoperiod. *Intl. Rice Res. Inst. Tech. Bull.* 8.
- Vergara B S, Tanaka A, Lilis R, Puranabhavung S (1966) Relationship between growth duration and grain yield of rice plants. *Soil Sci. Plant Nutr.* 12:31-39.
- Vergara B S, Visperas R M (1977) Harvest index: Criterion for selecting rice plants with high yielding ability. 10 Sept. IRRI Saturday Seminar, Intl. Rice Res. Inst., Los Baños, Philippines.
- Yoshida S (1972) Physiological aspects of grain yield. *Ann. Rev. Plant Physiol.* 23:437-464.
- Yoshida S (1976) Physiological consequences of altering plant type and maturity. In: *Proc. of Intl. Rice Res. Conf.*, Intl. Rice Res. Inst., Los Baños, Philippines.
- Yoshida S (1981) International Rice Research Institute Fundamentals of rice crop science. Intl. Rice Res. Inst., Los Baños, Philippines.
- Yoshida S (1983) Rice. Pages 103-127. In: *Potential Productivity of Field Crops under Different Environments*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Yoshida S, Cock J H (1971) Growth performance of an improved rice variety in the tropics. *Intl. Rice Comm. Newsl.* 20:1-15.
- Yoshida S, Cock J H, Parao F T (1972) Physiological aspects of high yields. Pages 455-469. In: *Rice Breeding*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Yoshida S, Oka I N (1982) Factors influencing rice yield production potential and stability. Pages 51-70. In: *Rice Research Strategies for the Future*. Intl. Rice Res. Inst., Los Baños, Philippines.

Quantitative understanding of yield potential

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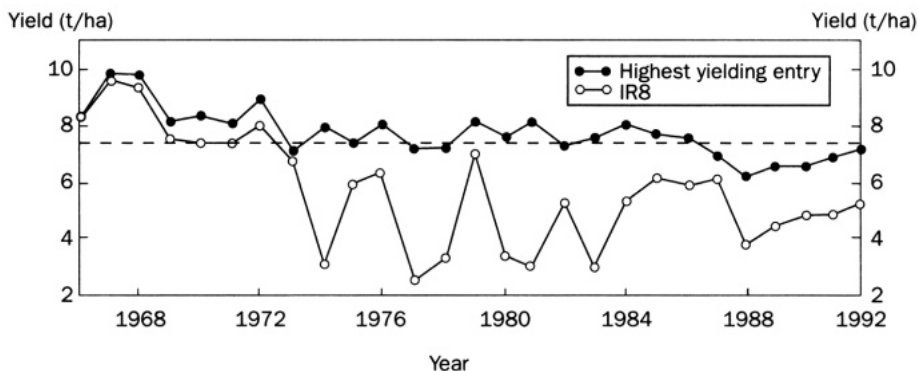
Background of simulation modeling at IRRI

Yield potential is defined as the grain yield obtained when growth is not limited by water, nutrients, or pests. In a specific environment, yield potential is determined by varietal characteristics and climatic variables such as temperature and solar radiation during the growing season. Thus, crop yield potential differs by location, and at the same location it differs by year and season. Maximum rice yields of 10-11 t/ha have been achieved in tropical environments, and yields of 13-15 t/ha are possible in temperate rice-growing environments such as China, Australia and California (Yoshida 1981, Williams 1992). Unfortunately, few data sets of sufficient detail were available before 1985 to provide a quantitative understanding of the determinants of rice yield potential.

Initial rice simulation models developed at IRRI were calibrated using the data sets from field studies conducted in the mid to late 1980s. These models predicted maximum potential yields of 8 t/ha at the IRRI research farm based on dry season (DS) weather conditions (Herrera-Reyes and Penning de Vries 1989, Penning de Vries 1991,1992, U. Singh, pers. commun.). This simulated yield was considerably less than documented yields of 9-10 t/ha that were regularly achieved by the first modern rice varieties (such as IR8 and IR24) at IRRI in the late 1960s and early 1970s (IRRI 1968,1973).

Lower yields in recent years reflect a phenomenon called the “yield decline,” which appears to be a common occurrence in long-term experiments with continuous double-crop rice systems at IRRI (Flinn et al 1982), at other locations in the Philippines (Flinn and De Datta 1984), and in India (Nambiar and Gosh 1984). A similar yield decline has also occurred in the replicated trials with elite breeding lines at IRRI (Fig. 1). These negative yield trends raised the issue of whether a decrease had occurred in the yield potential of more recent IRRI varieties, and whether this putative decrease contributed to the observed yield declines in long-term experiments.

By the late 1980s, rice yields of the best varieties and breeding lines with “best management” typically ranged from 6.5 to 7.5 t/ha in the DS at IRRI. Akita (1989) measured yields of around 10 t/ha in a few experiments in the



1. Grain yield of IR8 and the highest yielding single entry in dry season, replicated yield trials with 300-400 elite lines conducted each year from 1966 to 1992 by the Plant Breeding, Genetics, and Biochemistry Division at IRRI (G.S. Khush, unpubl. data).

mid-1980s, but achievement of such yield levels was inconsistent. Where Akita achieved yields of nearly 10 t/ha, he attributed these higher yield levels to increased N supply and greater crop N uptake than in the other experiments at IRRI. He suggested that the current low yields at IRRI's farm were partly related to a change in the N supply environment which resulted in suboptimal leaf N content, particularly during the grain-filling period. More recent evidence from analysis of yield trends in long-term field experiments at IRRI indicates a decrease in the "effective" N-supplying capacity of soil as the primary cause of declining yield trends, and that yields can be restored to 9-10 t/ha with higher rates of fertilizer-N inputs (Cassman et al 1994). The need for greater N inputs to achieve full yield potential has also been demonstrated in a number of recent field studies at different locations on the IRRI research farm (Kropff et al 1993a,b, Cassman et al 1993).

An improved simulation model for potential rice growth (ORYZA1) was developed using the detailed data sets from these recent field studies. This simulation model can now be used to quantify the yield potential of existing IRRI varieties, and to explore the basis for increasing the yield potential of indica rice grown in tropical environments.

Yield potential of current varieties

Recent experimental results

Early IRRI varieties such as IR8, IR24, IR36, and IR64 become extremely sensitive to insect pests and diseases in areas where intensive rice cropping is practiced. This sensitivity is evident in the yield trends of IR8 in the elite breeding nurseries at IRRI (Fig. 1), and it precludes a direct field comparison of yield potential in the early and more recent IRRI varieties. We therefore evaluated crop growth rate, leaf area development, N accumulation, and components of yield in the best semidwarf indica rice varieties (IR72 and IR58109-113-3-3-2) and a new indica hybrid (IR64616H). These genotypes were grown with different fertilizer-N supply levels at the IRRI research farm in Los Baños in 1991, 1992, and 1993, and at the Philippine Rice Research

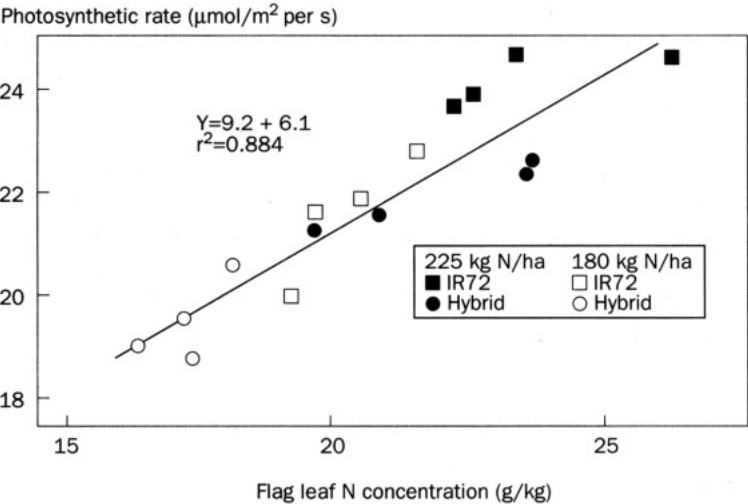
Institute (PhilRice) in Muñoz in 1993. The highest N rates used in these studies were about 50% greater than the recommended rate at both sites, and in some treatments additional N was applied at flowering to maintain leaf N status during grain filling. Prophylactic fungicide applications were made to minimize potential disease problems that become more severe in crops which receive high rates of fertilizer N.

Yields achieved in these experiments were comparable to those reported in the late 1960s and early 1970s for IR8 in field studies (Yoshida 1981), and also in the long-term experiments (Flinn and De Datta 1984, Cassman et al 1994): about 6 t/ha in the wet season (WS) and 9-10 t/ha in the DS (Table 1). These

Table 1. Grain yield (14% moisture content) of IR72 and a tropical indica hybrid IR64616H in recent field experiments conducted in the dry season at the IRRI Research Farm in Los Baños, Laguna, and at the Philippine Rice Research Institute (PhilRice) in Muñoz, Nueva Ecija (Cassman, Kropff, and Peng, unpubl. data).

Fertilizer-N inputs ^a					IRRI				PhilRice	
B	MT	PI	FL	Total	IR72			Hybrid	IR72	Hybrid
					M10 ^b 1992	K3 1992	M10 1993	M10 1992	—1993—	
kg N/ha					t/ha					
0	0	0	0	0	5.4	-	5.1	5.3	5.5	5.6
0	60	60	0	120	-	-	8.6	-	8.9	9.8
0	60	60	45	165	-	9.1	9.8	-	9.6	10.0
60	60	60	45	225	9.5	9.5	10.2	10.7	9.9	10.2

^aB = basal incorporated before transplanting; MT = mid-tillering; PI = panicle initiation stage; FL = flowering stage.
^bM10 and K3 refer to different experiments conducted at different sites on the IRRI farm.



2. Relationship between the rate of net photosynthesis and N concentration in the flag leaf at 16 days after flowering in the inbred indica variety IR72 and an indica tropical hybrid IR64616H in treatments with (225 kg N/ha) and without (180 kg N/ha) an N application at flowering. IRRI, 1992 DS (S. Peng, unpubl. data).

Table 2. Comparison of grain yield (at 14% moisture content) and determinants of yield for IR72 and a tropical indica hybrid IR64616H as influenced by N supply, 1992 DS at IRRI (Cassman, Kropff, and Peng, unpubl. data).

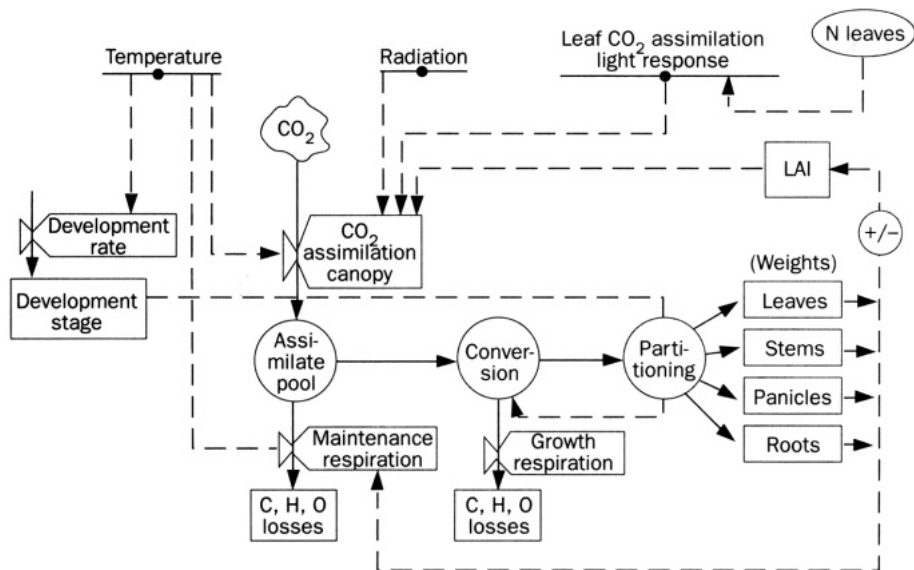
Variety	N Rate	Grain Yield	Total Dry Matter Yield	Harvest Index ^a	Components of Yield			
					Panicles	Spikelets		1000 grain weight
						filled	unfilled	
	kg/ha	t/ha		%		no./m ²		g
IR72	0	5.4	9.5	50	347	21,930	2780	24.0
	180	9.1	16.5	49	553	36,430	5210	26.0
	225 ^b	9.5	17.3	49	562	40,490	4340	24.4
Hybrid	0	5.3	8.8	54	330	22,330	5670	24.5
	180	9.8	17.7	49	557	37,920	11,880	26.3
	225 ^b	10.7	18.5	51	554	41,470	11,980	25.5
AOV								
N Rate		***C	***	*	***	***	***	NS
Variety		***	NS	P=0.06	NS	NS	***	NS
NxVariety		**	NS	NS	NS	NS	NS	NS

^aBased on grain yield corrected to an oven-dry moisture content of 3%. ^bIncludes 45 kg N/ha applied at flowering in addition to 180 kgN/ha applied in split applications before PI. ^c*, **, *** Indicates a significant Fvalue at P<0.05, 0.01, and 0.001, respectively.

results confirmed that the genetic yield potential of recently released IRRI varieties has not changed despite differences in growth duration (IR72 has a growth duration of about 115 days versus 125 days for IR8). The highest yield obtained in these recent experiments was 10.7 t/ha with the hybrid in the 1992 DS. A late-season N application at flowering was required to achieve this yield level for the hybrid, and this response was related to increased leaf N, higher rates of photosynthesis during grain-filling (Fig. 21, and an increase of 2-3 days in grain filling duration. The hybrid yield of 10.7 t/ha was attained despite 22% unfilled spikelets (Table 21. With a 50% reduction in the percentage of unfilled spikelets in the hybrid to 11% (about the same as IR72), the potential yield could reach 12 t/ha, assuming that assimilate supply was sufficient to fill the extra spikelets (see later section and Setter et al, These Proceedings). These results demonstrate the crucial role of adequate N supply throughout the growing season to fully express the yield potential of existing varieties in tropical environments, where high temperatures lead to rapid gaseous losses of applied N from the soil-floodwater system, and where soil N-supplying capacity appears to decrease with continuous rice cropping under submerged soil conditions.

An eco-physiological model of rice growth and yield

The model ORYZA1 was developed and evaluated using the data sets from field experiments conducted in 1991-93 (Kropff et al 1993c). The model includes components from the Wageningen models for potential production: INTERCOM (Kropff and Spitters 1992, Kropff and van Laar 1993), SUCROS (Spitters et al 1989), and the MACROS module LID (Penning de Vries et al



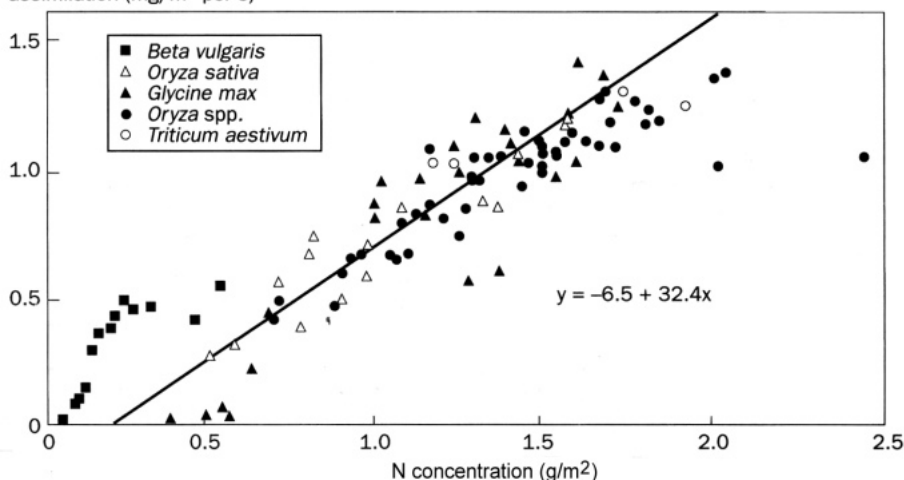
3. A schematic representation of the ORYZA1 model. Boxes are state variables, valves are rate variables, circles are intermediate variables. Solid lines are flows of materials, dotted lines are flows of information (Kropff et al 1993c).

1989). These model frameworks rely on input of detailed eco-physiological information — information that is crucial for theoretical evaluation of plant types for increased yield potential.

The general structure of the ORYZA1 model is presented in Figure 3. Under favorable growth conditions, light and temperature are the main factors determining crop growth rate. From the leaf area index, the vertical distribution of leaf area and light profile within the canopy are calculated. Single leaf photosynthesis is calculated based on leaf N concentration and light intensity, and the photosynthesis profile of the full canopy is obtained from the light and N canopy profiles. The maximum rate of CO₂ assimilation at high radiation levels (the asymptote A_m) depends upon the leaf N concentration. The relationship between A_m and the leaf N concentration is linear and shows little variation across environments and species (Fig. 4, van Keulen and Seligman 1987, Cook and Evans 1983). Thus, the relationship between A_m and leaf N given in Figure 4 is used in the model to estimate single leaf photosynthesis. More recent evidence from direct field measurements on rice at several growth stages indicates a slight deviation from this seminal relationship (see Setter et al, These Proceedings).

In the model, leaf N content is expressed on a leaf area basis and not as a percentage of dry weight because A_m depends on the amount of rubisco per unit leaf area (Makino et al 1984, Makino et al 1985). The vertical distribution of N in the canopy profile is accounted for by a generic exponential distribution derived from the detailed data sets of our recent experiments. Total daily CO_2 assimilation is then obtained by integrating the instantaneous rates of CO_2

Rate of net CO₂
assimilation (mg/m² per s)



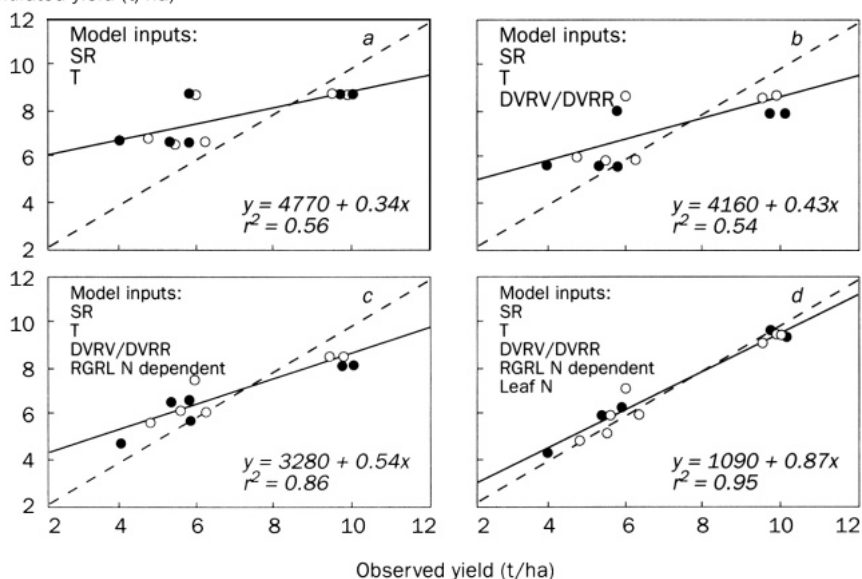
4. The relationship between the maximum rate of CO₂ assimilation of single leaves and the leaf N concentration on a leaf area basis (g/m²) (redrawn after van Keulen and Seligman 1987).

assimilation over the LAI on a daily time-step. Net daily growth rate (kg/ha per d) is obtained after subtraction of respiration requirements and accounting for energy losses in the conversion of carbohydrates into structural dry matter.

The accumulated dry matter is partitioned among the various plant organs. Phenological development rate is tracked as a function of ambient mean daily air temperature. When the canopy is not yet closed, leaf area development is calculated from mean daily temperature. The LAI can be simulated or the measured LAI values for a specific experiment can be used as input to the model. When the canopy closes, the increase in leaf area is obtained from the increase in leaf dry matter accumulation. Calculation of net daily growth rate combines the dry weight increase of leaves, stems, and grain using partitioning coefficients that depend on the stage of phenological development.

Input requirements of the model are: geographic latitude, standard daily weather data, plant density, date of crop emergence or transplanting, and species-specific parameter values that define morphological and physiological characteristics such as development rate constants, partitioning functions, and the stem carbohydrate translocation factor. The most recent version of the model simulates spikelet numbers/m² to allow estimation of sink limitation. Spikelet number is a function of dry matter production between panicle initiation (PI) and flowering (Kobata and Notsu 1991). Temperature effects on spikelet fertility are simulated according to procedures developed by Horie (1992, 1993) for high temperature during flowering and for low temperature from booting (microspore) to flowering stage.

Simulated yield (t/ha)



5. Simulated rice yields in relation to observed yields in 12 treatments (two varieties IR72 (open symbols) and IR358109-113-3-2 (closed symbols); two seasons 1991 WS and 1992 DS; and three N levels in each season) with different model inputs (see Section Model Performance) (Kropff et al 1993b).

Model performance

The model was evaluated with the data sets from the 1991 WS and the 1992 DS experiments with IR72 and IR58109-113-3-2, using the same set of species-specific parameters for all treatments, actual solar radiation (SR), mean daily temperature (T), and the variety-specific coefficients for development rate in the vegetative stage (DVRV) and grain-filling phase (DVRR), the effect of N status on the relative growth rate of the leaf area (RGRL), and the measured specific leaf N (Kropff et al 1993a,b).

The relative contribution of environmental variables, varietal characteristics, and crop N status to the accuracy of model simulations was evaluated by comparing predicted and observed rice yields when different parameter inputs were used to explain yield variation due to N treatment \times variety \times year effects. In the first analysis, all model coefficients and inputs were the same, with the mean of the three variety-specific coefficients and the mean specific leaf N for both varieties, and actual daily SR and T (Fig. 5a). Only 56% of the variation was explained, and the predicted-observed relationship deviated strongly from 1:1. If, in addition to SR and T, variety-specific coefficients for DVRV and the grain-filling phase DVRR were inputs to the model, the regression line came closer to 1:1, but no additional variation was explained (Fig. 5b). Adding the effect of N status on the relative growth rate of the leaf area (RGRL, used in the model for early growth stages) increased the r^2 (86% of total variation), although the deviation from the 1:1 relationship was not

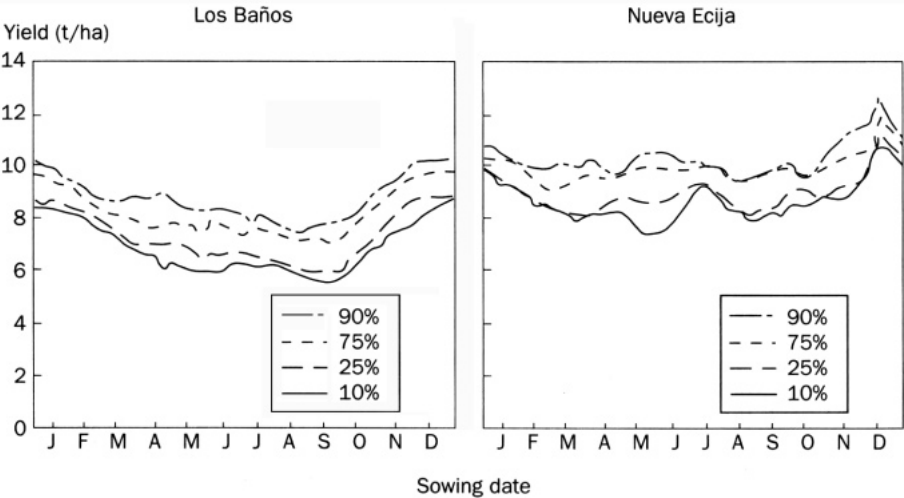
improved (Fig. 5c). Reasonable simulations were obtained only when the actual specific leaf N for each of the 12 treatment-seasons was also included as input (Fig. 5d).

This analysis demonstrates that the large differences in yield due to season, variety, year, and N supply can be explained by the eco-physiological ORYZA1 model with a relatively small number of variables as input. It also emphasizes the importance of N on leaf area development and photosynthesis for explaining yield differences. Further development of the model will focus on the impact of N on leaf area development, and the processes governing tiller development and source-sink relationships.

Rice yield potential in different environments

The yield potential of the same rice variety at higher latitudes (lower average temperatures) is greater than in the tropics with similar solar radiation in both cases. In China, Australia, and California, yields of over 14 t/ha have been reported. This can be explained by the ORYZA1 model based on differences in radiation and temperature. For example, the model predicts that rice yield potential in Los Baños increases by 2 t/ha with a reduction in mean temperature of 3 °C, mainly as a result of a longer growth duration, greater LAI, and a longer grain-filling period. This temperature effect on the length of grain filling is consistent with the data of Yoshida (1981).

Potential yield probabilities can be predicted by the model based on long-term weather records. This is illustrated for the variety IR72 at IRRI, and at PhilRice which is 300 km north of Los Baños (Kropff et al 1993b). Large within-year and year-to-year variation in yield potential is evident, which reflects yearly differences in weather at both sites (Fig. 6). At PhilRice, yield differences



6. Yield probabilities of IR72 as simulated by the model ORYZA1 for Los Baños and Nueva Ecija (Philippines) using long-term weather data and the measured leaf N content in the 1992 DS high N treatment as model input (Kropff et al 1993b).

between the DS and WS are not as distinct as at IRRI, and maximum yield potential is higher than at IRRI. When yield potential was simulated with high or low leaf N status, leaf N status had a greater impact on yield potential in the DS than in the WS at both sites. For example, higher leaf N increased the 75% probability yield from 6.0 to 7.6 t/ha in the WS and from 7.1 to 9.7 t/ha in the DS at IRRI.

With the same parameterization (except for cold tolerance) the model was run with weather data from Yanco, Australia, where yields of over 14 t/ha are achieved (Williams 1992). Yields of 15 t/ha were simulated with the ORYZA1 model. These high yields were mainly because of the lower temperature than in the tropics, which results in longer duration of all growth phases, and particularly grain filling. Longer growth duration of later-maturing varieties in the tropics, however, does not translate into higher yield potential, because grain-filling duration is shorter than in temperate climates due to higher mean temperature, and grain-filling duration of late, medium, and early maturing varieties is the same (see Peng et al, These Proceedings, and later section).

These results demonstrate the importance of the environmental variables of temperature and radiation on rice yield potential, and the capability of an eco-physiological simulation model to explain and predict the influence of these variables on yield development.

A simple framework for estimating yield potential

A simple analysis of the requirements for increased yield potential was presented by Kropff et al (1994) and is summarized here. Plant characteristics and components of yield that determine yield potential were defined by Yoshida (1981).

The driving forces in crop yield formation are well known: both a source of carbohydrates and a sink (spikelets) for these carbohydrates are needed. The source is formed by chlorophyll-containing tissue, mainly in the leaves. Stems, leaf sheaths, and the panicle contribute marginally to canopy photosynthesis. The amount of dry matter stored in the grains (the sink) comes from allocated stem reserves, which are produced in the vegetative phase, and assimilates produced in the grain-filling period. Thus, the source for grain production is determined by three components, 1) amount of stem reserves allocated to the grains, 2) rate of dry matter production in the grain-filling period, and 3) length of the grain-filling period or growth rate duration.

Climate and the N supply environment largely determine these components for irrigated rice (see earlier section). Temperature affects the length of the vegetative and grain-filling periods. Temperature and radiation determine crop growth rate and the amount of stored carbohydrate in stems that becomes available for translocation to grain. The growth rate at a given radiation level depends on crop LAI and leaf N content. Therefore, a simple model for grain yield formation is:

$$Y = S + G \times D$$

in which Y is grain yield, S is the net amount of stem reserves remobilized to grain, G is the average growth rate per day during the grain-filling period, and D is the length of the grain-filling period. Yoshida (1981) estimated that stem reserves provide assimilate sufficient for 2.0-2.5 t/ha of grain. In the tropics, effective (linear) grain-filling duration is about 25 days. Therefore, to achieve 10 t/ha grain yield requires net dry matter accumulation of 8 t/ha (2.0-2.5 t/ha stem reserves) at 14% water content (the standard moisture content for reporting rice grain yield) in 25 d, equivalent to 284 kg/ha per d (oven-dry basis at 3% moisture content) during the effective grain-filling period. This is a reasonable estimate: rice in tropical environments can produce 300-360 kg dry matter/ha per d with high solar radiation, high LAI, and a high leaf N content (Yoshida 1981, Akita 1989). This simple model for yield formation indicates that yields beyond 10 t/ha must result from either increased accumulation and allocation of stem reserves, a prolonged grain-filling period, or an increased growth rate during grain filling.

Increasing stem carbohydrate reserves beyond 2.5 t/ha is not feasible without increasing net assimilation in the vegetative period, which cannot be achieved without providing a luxuriant N supply that leads to lodging and disease problems (see section "Greater Resistance to Lodging and Diseases" in Setter et al, These Proceedings). Moreover, although the new plant type is selected for thicker stems, total culm number will be reduced in the low-tillering new plant ideotype so that increased carbohydrate storage per stem would be offset by fewer stems/m². For a grain yield of 15t/ha at 14% moisture content, 13.3 t dry matter/ha on an oven-dry basis (3% moisture content) is needed. With a sustained growth rate of 284 kg/ha per d during effective grain filling and 2.5 t/ha dry matter contribution from stem reserves, 38 d of effective grain filling is needed to produce the remaining 10.8 t/ha. In essence, these calculations suggest that a genotype with a 15 t/ha yield potential in the tropics must have a grain-filling duration comparable with that of current varieties grown in temperate environments.

To achieve 15 t/ha yields, increased sink size is also required. Both IR72 and the new hybrid attain a grain yield of about 10 t/ha with 40,000 filled grains/m² and a 1000 seed weight of 25 g (Table 2). A yield of 15 t/ha requires 60,000 spikelets/m², or 13% more than the total number of filled and unfilled spikelets produced by the hybrid, and 33% greater than for the inbred variety IR72. The key issue therefore, is whether present genotypes have the capacity to produce sufficient assimilate to support the development and filling of a larger sink. A simple analysis like the one presented in this section cannot address this issue. It is useful, however, to help focus on the basic parameters that must be modified to achieve a major increase in yield potential of rice grown in tropical environments. These fundamental parameters are: increased sink size (i.e., spikelets/m²), a longer period of effective grain filling, and a longer duration of green leaf area and active canopy photosynthesis to match the increase in grain-filling duration.

Physiological traits that govern yield potential: a quantitative perspective

The physiological traits needed to support greater yield potential have been evaluated using simulation models (Penning de Vries 1991, Dingkuhn et al 1991, Kropff et al 1993a, 1994). In this section, the proposed traits that may contribute to increased yield potential are critically evaluated from a quantitative perspective made possible by the results from recent field experiments, and the development of the ORYZA1 simulation model.

Source versus sink limitation

The issue of source and sink limitation to yield potential was addressed in the new plant type by an emphasis on greater spikelets per panicle and a larger flag leaf (Vergara et al 1990). Grain-filling duration was not considered in the new plant ideotype, and thus the source of the increased assimilate for a 50% increase in yield potential is not clear. The effective grain-filling period must increase from the present 25 days to 38 days to produce the required dry matter for a yield of 15 t/ha. There is no evidence, however, that direct selection for larger panicle size will indirectly lead to a longer grain-filling duration.

Yoshida (1981) compared the effect of CO₂ enrichment before or after flowering on rice yield to determine if grain yield was most limited by sink or source. Grain yield increased from 9.0 to 11.6 t/ha when CO₂ enrichment occurred before flowering, due to an increase in spikelets/m². A smaller increase in grain yield, from 9.0 to 10.9 t/ha, occurred when CO₂ was applied after flowering during the grain-filling period. At first glance, these results suggest that both source and sink limited potential yield in the study, but the factors that govern yield are more complex than this first impression indicates. For example, increased CO₂ before flowering could increase flag leaf area, causing a higher growth rate during grain filling in tandem with an increase in spikelet number. To identify cause and effect among the interacting factors that govern yield potential, systems simulation and analysis provides a useful approach to further our understanding of key processes. Such an approach will be required to quantify the relative contribution of source or sink limitation to rice yield potential. At present, only a few data sets are available with sufficient detail for such simulation analyses (see later section).

Contribution of stem reserves

Dingkuhn et al (1991) proposed a plant type with a greater production of stem reserves through an increased partitioning of assimilates from leaves to stems. Using an earlier version of the rice simulation model (MACROS), they changed the dry matter allocation pattern between leaves and stem and predicted that a yield increase of 25% was possible. However, in their model the allocation of dry matter to leaf growth stops at PI, which is not feasible because several leaves, including the flag leaf, must still emerge and expand. The positive effect on yield in their simulation must have resulted from an overestimation of leaf area development in early growth stages by the MACROS model.

In the 1992 DS experiments conducted at IRRI, the decrease in stem (and leaf sheath) dry matter in the period from anthesis to physiological maturity was 2.0 t/ha in the hybrid (IR64616H) at yield levels of 10.7 t/ha, but only 0.5 t/ha in IR72 with a yield of 9.5 t/ha (Kropff et al 1993c). These results suggest that an increase in the net contribution of assimilates produced during the vegetative stage to the grain could enhance the yield of IR72, while the hybrid appears to be functioning near the upper threshold of remobilization efficiency. The smaller net allocation from stem reserves by IR72 reflects a decrease in nonstructural stem carbohydrates during the initial phase of grain filling, and an accumulation of stem reserves in the last days of grain filling (see Setter et al, These Proceedings; Setter, unpubl. data). This apparent "shut-down" in assimilate remobilization from the stem may be due to the smaller sink size of IR72 (Table 2) and a ratooning response triggered by the loss of apical dominance of the panicles towards the end of grain filling.

Dry matter production during grain filling

The maximum growth rate of closed canopies varies little among C₃ crop species (Evans 1990). For several indica hybrid rice genotypes, Akita (1988) reported that the maximum growth rate was not different from the inbred parents. In general, efforts to increase growth rates through increasing the rate of net photosynthesis have met with little success for a number of crop species (Gifford et al 1984).

Several options have been proposed to increase the maximum rate of net assimilation by the rice plant. Modification of physiological processes such as suppression of photorespiration and reduction of maintenance respiration were proposed by Penning de Vries (1991), although these propositions are highly speculative. There is little evidence that photorespiration can be suppressed in C₃ plants, and while there is evidence of genetic variation in maintenance respiration, the magnitude of such differences is small (Gifford et al 1984). Penning de Vries (1991) also proposed to increase the flux of CO₂ from the soil through the root aerenchyma to the leaves, providing an additional source of CO₂ for photosynthesis. This latter proposal is discussed in more detail in Setter et al (These Proceedings). Dingkuhn et al (1991) suggested increasing the vertical N gradient in the canopy so that a greater proportion of leaf N was displayed where light intensity is highest. Preliminary analyses using the ORYZA1 model, however, indicate little gain from this strategy because the extinction coefficients are similar for N and light distribution in the rice canopy.

Based on our observation that the relative panicle position differs among varieties, the effect of the panicle position and effects on light interception by the leaves were evaluated using a detailed model for interplant competition for light capture (Kropff and van Laar 1993; Kropff et al 1994). In the 1991 wet season, the panicle area index (PAI) reached values of 0.6-0.9 m² panicle per m² ground, and the model predicted that panicles located in the top 10 cm of the canopy could reduce canopy photosynthesis by 25% with a typical LAI of 4 in

the WS. By contrast, when panicles are positioned 20 cm below the top of the flag leaves, canopy photosynthesis was reduced by only 10%. Research is in progress to validate whether panicle height can have a significant impact on yield potential under field conditions.

Prolonged grain filling and green leaf area duration

The ORYZA1 model was also used to evaluate the outcome of an increase in grain-filling period (Kropff et al 1994). Panicle dry matter was simulated to increase from 9.3 to 11.7 t/ha with an increase in grain filling duration from 24 to 31 days. A further increase to 14.7 t/ha was predicted with a grain-filling duration of 40 days and a harvest index of 0.6. This is very close to the 38 days calculated with the simple concept of grain filling (see earlier section "A Simple Framework for Estimating Yield Potential"), and illustrates the relatively simple structure of the eco-physiological model. However, in these simulations a substantial amount of green leaf area is specified to remain active for the duration of grain filling, a situation which will not occur in the tropics without manipulation of the leaf senescence process.

For several crop species, increased 'stay green' (i.e., longer green leaf duration during grain filling) has been a major achievement of breeders in the past decades (Evans 1990). The duration of canopy photosynthetic activity can likewise be prolonged by fertilizer-N application later in the growing season, and by improved crop protection against endemic late season diseases—both of which are the basis of increasing yield trends for wheat in northern Europe (Spiertz and Vos 1985). Increased late season N application to wheat also "protects" rubisco from degradation, which delays senescence of the flag leaf and contributes to a yield increase from greater grain weight (Wuest and Cassman 1992, Cassman et al 1992).

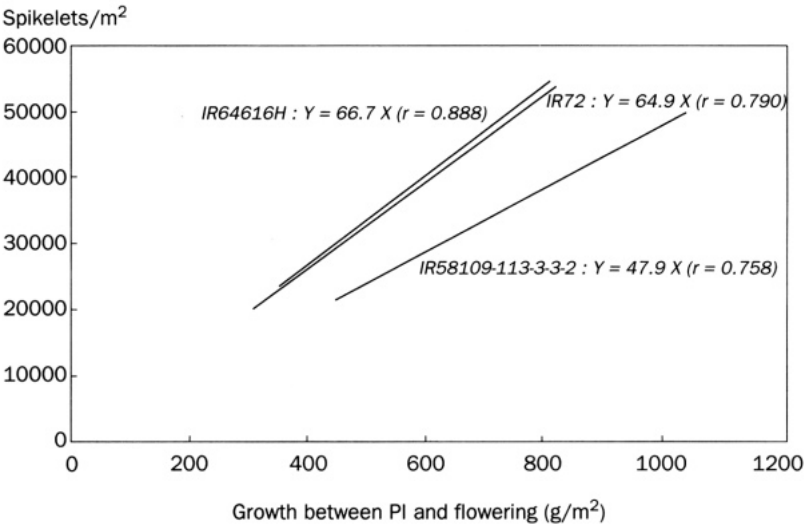
In rice, the relationship between total leaf N and rubisco content appears to be linear during the leaf expansion phase regardless of N supply, so that rubisco typically represents about 30% of total leaf N (Makino et al 1984). During leaf senescence, rubisco is rapidly degraded, with a parallel decrease in net assimilation rate (Makino et al 1985). Higher leaf N content results in greater leaf rubisco and increased net assimilation during the grain-filling period, particularly in the later stages of grain filling. Recent results from field studies at IRRI demonstrate a response to a late-season N application when solar radiation and temperature create high-yield conditions (Table2), and this response is associated with increased rates of net carbon assimilation (Fig. 2). To achieve yields of 13-15 t/ha in the tropics without an increase in the maximum rate of net carbon assimilation of the rice plant (see earlier section on "Dry Matter Production During Grain Filling") will require a considerable delay in leaf senescence and maintenance of leaf N status during an extended grain-filling duration. The mechanisms that govern leaf senescence, however, are poorly understood.

The possibility of a significant extension in grain-filling duration depends on whether genetic variability exists in the length of the grain-filling period,

and whether leaf senescence can be delayed for substantial periods. Senadhira and Li (1989) studied the length of grain filling in 21 rice cultivars at IRRI's experimental farm during the 1987 DS. Grain-filling duration ranged from 16 to 40 days. The rate of grain filling, however, was slow in the long duration varieties, resulting in lower panicle weights. These data are confounded by the agronomic practices used in the study: 1 plant per hill at 30 × 20 cm spacing and low fertilizer N supply. Interpretation of these data may also be confounded because only main culm panicles were monitored. In recent work at IRRI, variation among varieties was found in grain-filling duration of the main-culm panicles, but not for the entire population of panicles in the hill (Dionora and Kropff, unpubl. data). Lack of variation in grain-filling duration of the panicle population may indicate a source limitation. Evidence for a source limitation is also suggested by the grain-filling sequence within a single panicle as discussed by Peng et al (These Proceedings).

Increased sink size

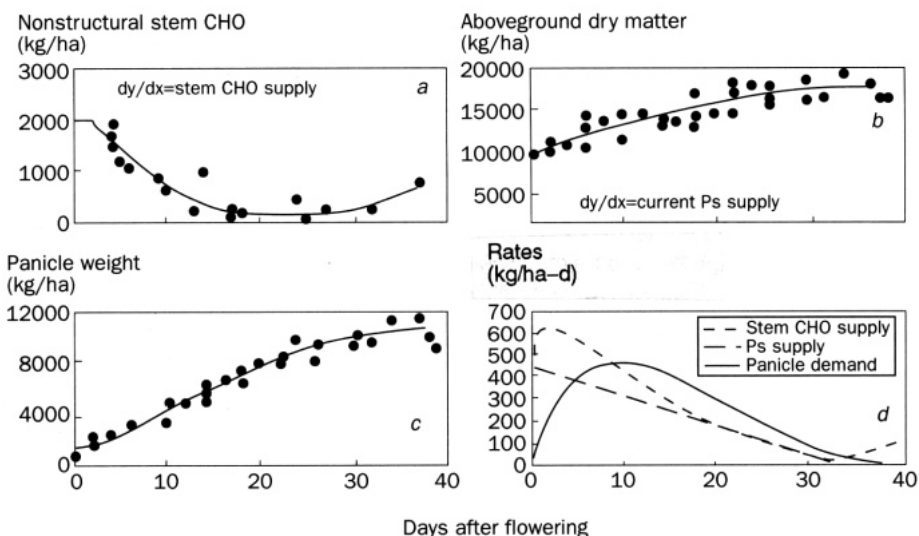
About 60,000 grains/m² would be needed for 15 t/ha yields with a 1000-grain weight of 25 g in the tropics. Analysis of 15 t/ha rice crops in temperate environments or high altitude subtropical environments suggests plasticity in the components of yield to achieve these yield levels. In Yanco, Australia, 15 t/ha yields are obtained with 700 panicles/m², 100 spikelets/panicle, 80% filled spikelets, and 25 mg/grain with the locally bred japonica variety Amaroo (Williams 1992). In Yunnan Province, South China, at 1100 m altitude, 15 t/ha yield levels are achieved by IR72 (an indica variety) with 400 panicles/m², 160



7. Relationship between total spikelet number and dry matter accumulation in the panicle formation period (panicle initiation to flowering) for three varieties in several wet season and dry season field experiments at IRRI. Regression lines are forced through the origin. The mean duration of the panicle formation period was 29, 23, and 27 developmental days for IR64616H, IR72, and IR58109-113-3-3-2, respectively (R.B. Matthews, unpubl. data).

spikelets/panicle, 84% filled spikelets and 30 mg/grain. At IRRI in the lowland tropics, IR72 yields 9.5 t/ha with 560 panicles/m², 80 spikelets/panicle, 90% filled spikelets, and 24 mg/grain. These data demonstrate the plasticity in yield components of modern rice varieties. Moreover, analysis of our recent experiments at IRRI suggests that the crop exhibits strong compensation mechanisms, particularly in panicle number and spikelet number per panicle. As hill density increases, panicle number increases but spikelet number/m² remains constant (see Fig. 3 of Peng et al, These Proceedings).

In the ORYZA1 model, spikelet number is directly related to the crop growth rate in the period from PI to flowering by a variety-specific constant. The relation is derived from data of our recent experiments and holds across a wide range of yields (Fig. 7). A similar relationship was demonstrated by Kobata and Notsu (1991) for japonica varieties. Akita (1989) proposed a higher spikelet formation efficiency per unit of growth to obtain an increase in sink size, but whether sink size limits yield potential remains to be determined. For example, spikelet number of the hybrid in our 1992 DS field experiment clearly did not limit yield because unfilled spikelets accounted for 22% of total spikelet number (Table 2). Likewise, differences in spikelet formation efficiency per unit growth as indicated in Figure 7 were not related to grain yield, but instead reflected differences in growth duration in the period from PI to flowering which was 20 d for the hybrid, 23 d for IR72, and 27 d for IR58109-113-3-3-2.



8. Pattern of assimilate supply from remobilized stem carbohydrate (CHO) reserves (a) and current net photosynthesis (Ps) quantified by the rate of aboveground dry matter accumulation (b), and the demand for assimilate reflected by the dry matter increase of panicles (c). For both assimilate supply and demand, daily rates are plotted in (d) as the derivative of each supply or demand function. Data represent measurements taken in the 1993 DS at IRRI on three varieties that achieved grain yields of 9-10 t/ha (Dionora et al, unpubl. data).

Conclusions from quantitative analysis of yield potential

Based on the reviews presented here and in Peng et al (These Proceedings), it appears that the main constraint to increased yield potential is the maximum, sustainable dry matter accumulation rate of about 300 kg/ha per d, which is governed by the rate of net CO₂ assimilation. Small yield gains may be possible from increased storage and remobilization of stem carbohydrate reserves and a further push to increase the HI. Whether an increase in sink size alone will lead to an increase in yield potential remains an issue, but the existing evidence suggests it will not.

Integrating the recent information on the relationship between dry matter accumulation rate in the panicle formation stage (Fig. 7) and the patterns of assimilate supply and demand during grain filling (Fig. 8) provides a framework to address the issue of source versus sink limitation. Although these data are preliminary, the patterns indicate a critical point at which the daily rate of assimilate demand in the panicle equals the available supply from current photosynthesis and stem reserves. At this point, the available assimilate supply could be evenly distributed among all the existing spikelets that are unfilled — resulting in shrunken seed — or directed to only a portion of the remaining unfilled spikelets. With unfilled spikelet number typically about 15% in our field studies at IRRI at yield levels of 10 t/ha and also in the higher yielding temperate environments with yields of 15 t/ha, it seems that current rice varieties regulate their allocation pattern so that only a portion of the remaining unfilled spikelets continue to fill. Such a regulation of assimilate supply within the panicle is consistent with the findings of Iwasaki et al (1992, 1993). The mechanisms that govern this allocation process are unknown, and the time at which spikelets lose viability for further filling remains to be determined.

Research in progress will further quantify the assimilate supply and demand patterns during grain filling. A key issue is the time at which spikelet infertility is determined, and whether there is any scope to decrease the typical levels of spikelet infertility (i.e., 15% unfilled spikelets) while maintaining high rates of net assimilation during early grain filling and delaying leaf senescence in the later phase of grain filling. Although it should be possible to increase net assimilation in the later phase by an additional N application at 10-14 days after flowering to maintain leaf N status, the benefit will depend upon whether unfilled spikelets are still viable.

References cited

- Akita S (1988) Physiological bases of heterosis in rice. Pages 67-77. In: *Hybrid Rice*. Intl Rice Res. Inst., Los Baños, Philippines.
- Akita S (1989) Improving yield potential in tropical rice. Pages 41-73. In: *Progress in Irrigated Rice Research*. Intl Rice Res. Inst., Los Baños, Philippines.
- Cassman K G, Bryant D C, Fulton A, Jackson L F (1992) Nitrogen supply effects on partitioning of dry matter and nitrogen to grain of irrigated wheat. *Crop Sci.* 32:1251-1258.

- Cassman K G, De Datta S K, Olk D C, Alcantara J M, Samson M I, Descalsota J P, Dizon M A (1994) Yield decline and the nitrogen economy of long-term experiments on continuous, irrigated rice systems in the tropics. *Advances in Soil Science*. (In press)
- Cassman K G, Kropff M J, Gaunt J, Peng S (1993) Nitrogen use efficiency of rice reconsidered: What are the key constraints? *Plant and Soil* 155/156:359-362.
- Cook M G, Evans L T (1983) Nutrient responses of seedlings of wild and cultivated *Oryza* species. *Field Crops Research* 6:205-218.
- Dingkuhn M, Penning de Vries F W T, De Datta S K, van Laar H H (1991) Concept for a new plant type for direct seeded flooded tropical rice. Pages 17-38. In: *Direct seeded flooded rice in the tropics*. Selected papers from the International Rice Research Conference, 27-31 August 1990, Seoul, Korea. Intl Rice Res. Inst., Los Baños, Philippines.
- Evans L T (1990) Assimilation, allocation, explanation, extrapolation. Pages 77-87. In: R. Rabbinge, J. Goudriaan, H. van Keulen, F.W.T. Penning de Vries and H.H. van Laar, ed. *Theoretical Production Ecology: Reflections and Prospects*. Simulation Monographs 34, Pudoc, Wageningen, The Netherlands.
- Flinn J C, De Datta S K (1984) Trends in irrigated-rice yields under intensive cropping at Philippine research stations. *Field Crops Res.* 9:1-15.
- Flinn J C, De Datta S K, Labadan E (1982) An analysis of long-term rice yields in a wetland soil. *Field Crops Res.* 5:201-216.
- Gifford R M, Thorne J H, Hitz W D, Gianquinta R T (1984) Crop productivity and photoassimilate partitioning. *Science* 225:801-808.
- Herrera-Reyes C G, Penning de Vries F W T (1989) Evaluation of a model for simulating the potential production of rice. *Philipp. J. Crop Sci.* 14:21-32.
- Horie T (1992) Yield forecasting. *Agricultural Systems* 40:211-236.
- Horie T (1993) Predicting the effect of climatic variation and elevated CO₂ on rice yield in Japan. *J. Agric. Met.* 48:567-574.
- IRRI (International Rice Research Institute) 1967. Annual Report. IRRI, P.O. Box 933, Manila, Philippines.
- IRRI (International Rice Research Institute) 1973. Annual Report. IRRI, P.O. Box 933, Manila, Philippines.
- Iwasaki Y, Mae T, Chikafusa C, Makino A, Ohira K, Ojima K (1993) Glutenin accumulation and changes in the levels of its mRNA in the superior and inferior spikelets of rice ear during ripening. Pages 231-234 In: N.J. Barrow ed. *Developments in Plant and Soil Science Vol. 54, Plant Nutrition: from Genetic Engineering to Field Practice*. Kluwer Academic Publishers, Dordrecht.
- Iwasaki Y, Mae T, Makino A, Ohira K, Ojima K (1992) Nitrogen accumulation in the inferior spikelet of rice ear during ripening. *Soil Sci. Plant Nutr.* 38(3):517-525.
- van Keulen H, Seligman N G (1987) Simulation of water use, nitrogen and growth of a spring wheat crop. Simulation Monographs, Pudoc, Wageningen, 310 pp.
- Kobata T, Notsu T (1991) Relationships between spikelet number and dry matter production or leaf area before heading in the rice plant. *Bull. Fac. Agric. Shimane Univ.* 25:1-4.
- Kropff M J, Cassman K G, van Laar H H (1994) Quantitative understanding of the irrigated rice ecosystem for increased yield potential. In: *Proceedings of the 1992 International Rice Research Conference*. Intl. Rice Res. Inst., Los Baños, Philippines. (In press)

- Kropff M J, Cassman K G, van Laar H H, Peng S (1993b) Nitrogen and yield potential of irrigated rice. *Plant and Soil* 155/156:391-394.
- Kropff M J, Cassman K G, Penning de Vries F W T, van Laar H H (1993a) Increasing the yield plateau and the impact of global climate change. *J. Agric. Meteorol.* 48:795-798.
- Kropff M J, van Laar H H ed. (1993) Modeling crop-weed interactions, CAB-IRRI, ISBN 0851987451, 269 pp.
- Kropff M J, van Laar H H, ten Berge H F M (1993c) ORYZA1, a basic model for irrigated lowland rice production. Intl. Rice Res. Inst., Los Baños, Philippines.
- Kropff M J, Spitters C J T (1992) An eco-physiological model for interspecific competition, applied to the influence of *Chenopodium album* L. on sugar beet. I. Model description and parameterization. *Weed Res.* 32:437-450.
- Makino A, Mae T, Ohira K (1984) Relation between nitrogen and ribulose-1,5-biphosphate carboxylase in rice leaves from emergence through senescence. *Plant and Cell* 25:429-437.
- Makino A, Mae T, Ohira K (1985) Photosynthesis and ribulose-1,5-biphosphate carboxylase in rice leaves from emergence through senescence. Quantitative analysis by carboxylation/oxygenation and regeneration of ribulose-1,5-biphosphate carboxylase. *Planta* 166:414-420.
- Nambiar K K M, Ghosh A B (1984) Highlights of Research of Long-Term Fertilizer Experiments in India. In: *LTFE Bulletin No.1*. Indian Council of Agric. Research, New Delhi. 97p.
- Penning de Vries F W T (1991) Improving yields: designing and testing VHYVs. In: *Systems Simulation at IRRI*. IRRI Research Paper Series, November 1991, Number 151, 13-19.
- Penning de Vries F W T (1992) Rice production and global climate change. Pages 175-189. In: F.W.T. Penning de Vries, P.S. Teng, and K. Metselaar ed. *Systems Approaches for Agricultural Development*. Kluwer Academic Publishers, Dordrecht/Boston/London and IRRI, Los Baños, Philippines.
- Penning de Vries F W T, Jansen D M, ten Berge H F M, Bakema A (1989) Simulation of ecophysiological processes of growth of several annual crops. Simulation Monographs 29. Pudoc, Wageningen and IRRI, Los Baños. 271 pp.
- Senadhira D, Li G F (1989) Pages 8-9. In: *Variability in rice grain-filling duration*. IRRN 14:1.
- Spiertz J H J, Vos (1985) Grain growth of wheat and its limitation by carbohydrate and nitrogen supply. Pages 129-141 In: W. Day & R.K. Atkin ed. *Wheat growth and modelling*. NATO ASI series. Series A: Life Sciences, vol 86. Plenum Press, New York.
- Spitters C J T, van Keulen H, van Kraalingen G W D (1989) A simple and universal crop growth simulator: SUCROS87. *Simulation Monographs*, Pudoc, Wageningen. pp. 147-181.
- Vergara B S, Venkateswarlu B, Janoria M, Ahn J K, Kim J K, Visperas R M (1990) Rationale for a low-tillering rice plant type with high density grains. *Philipp. J. Crop Sci.* 15:33-40.
- Williams R L (1992) Physiological comparison of Amaro and YRL39. In: H.G. Beecher and B.W. Dunn ed. *Yanco Agricultural Annual Report*. Yanco Agricultural Institute, NSW Australia.
- Wuest S B, Cassman K G (1992) Fertilizer-N use efficiency of irrigated wheat. II. Partitioning efficiency of preplant versus late-season applied N. *Agron. J.* 84:689-694.
- Yoshida S (1981) Fundamentals of rice crop science. Intl. Rice Res. Inst., Los Baños, Philippines. 269 pp.

Physiological considerations and hybrid rice

T. L. Setter, S. Peng, G. J. D. Kirk, S. S. Virmani, M. J. Kropff, and K. G. Cassman

Photosynthesis and leaf nitrogen content in rice

Increased crop production in the last half century is largely attributed to the increased use of N fertilizer (Sinclair and Horie 1989). Leaf N contributes to crop biomass production by affecting leaf CO₂ assimilation rates. Accurate quantification of the relationship between maximum C assimilation rate and leaf N content is a prerequisite for estimating yield potential (see Kropff et al, These Proceedings).

Mitsui and Ishii (1938, 1939) reported that net photosynthetic rate (P_n) was linearly correlated with leaf N concentration per unit dry weight (N_{DW}) between 33 and 64 g N/kg. Similar results were subsequently reported by Tsuno et al (1959) and Takeda (1961). Other studies also indicated a linear relationship between P_n and leaf N content per unit leaf area (N_{LA}) (Tsuno 1971, Yoshida and Coronel 1976, Uchida et al 1982, Cook and Evans 1983a and b). On the other hand, a curvilinear relation between P_n and N_{LA} was reported by Takano and Tsunoda (1971) in a study of 40 rice genotypes from diverse genetic origins.

Van Keulen and Seligman (1987) reported a generic relationship between P_n and N_{LA} based on a data set that included observations from a number of crop species (*Beta vulgaris*, seven *Oryza* spp. including 25 genotypes of *O. sativa*, *Glycine max*, and *Triticum aestivum*). The generic equation for this relationship

$$P_n (\mu\text{mol}/\text{m}^2/\text{s}) = -4.10 + 20.45 N_{LA} (\text{g}/\text{m}^2) \quad (1)$$

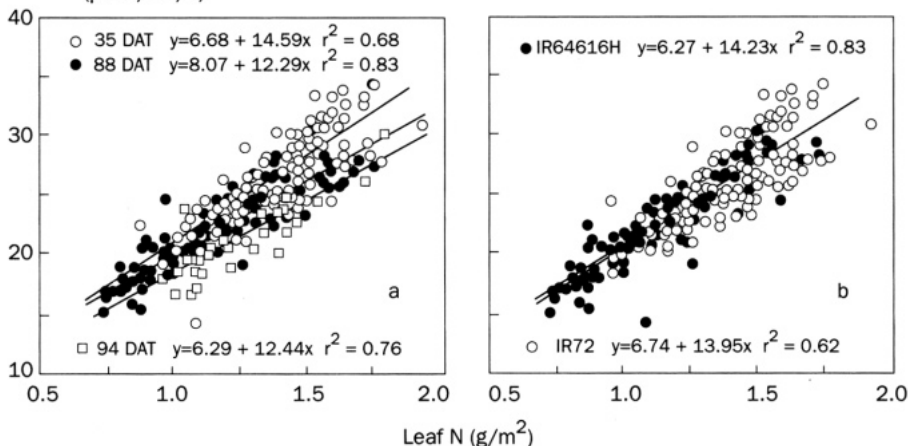
is presently used in the simulation model (ORYZAI) to estimate the rate of C assimilation by a rice crop (Kropff et al 1993). Sinclair and Horie (1989) established a logistic equation based on the reported relationship between P_n and N_{LA} of rice as follows:

$$P_n = 34.1 \left\{ \frac{2}{1 + \exp (-1.4(N_{LA} - 0.3))} - 1 \right\} \quad (2)$$

This equation was based on data from previous studies, and it was used by Sinclair and Horie to examine the effect of leaf N on biomass production and to estimate the radiation use efficiency of rice.

Most of the data in previous studies of the relationship between photosynthesis and leaf N were obtained from rice plants grown in pot or solution-

Net carbon assimilation rate
($\mu\text{mol}/\text{m}^2/\text{s}$)



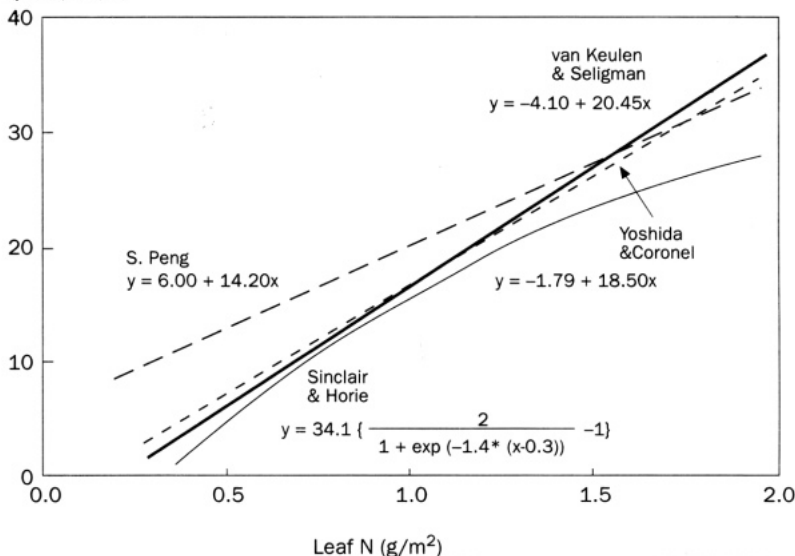
1. Relationship between net carbon assimilation rate and leaf N content per unit area in the 1993 dry season (a) at 35, 88, and 94 days after transplanting and (b) for IR64616H and IR72. The pooled regression for all DAT and both varieties is: $y = 6.00 + 14.20x$, $r^2 = 0.75$ (Peng, unpubl. data).

culture systems under greenhouse conditions. Field conditions, however, are very different from the greenhouse environment, and hence it is important to quantify the relationship between P_n and N_{LA} in field-grown rice plants. In the dry season of 1993, we measured P_n and N_{LA} of IR72 and the IR64164H tropical hybrid in field plots in treatments with a wide range of N fertilizer rates and timing. Gas exchange measurements were conducted on the uppermost fully expanded leaves under full sunshine at different growth stages. A linear relationship between P_n and N_{LA} was found at 35, 88, 94 days after transplanting (DAT) with only slight differences among them (Fig. 1a). The relationship was similar in the two varieties (Fig. 1b), and a pooled regression equation was found to provide a reasonable estimate of net leaf photosynthetic rate in relation to leaf N as follows:

$$P_n = 6.00 + 14.2 N_{LA} \quad (3)$$

Compared with equation (3), equation (2) underestimates P_n across the entire range of leaf N while equation (1) underestimates P_n when N_{LA} is less than $1.5 \text{ g}/\text{m}^2$ (Fig. 2). These discrepancies may reflect the different conditions between greenhouse and field, differences between the rice genotypes used in these studies, and the improvement in the measurement techniques that were employed in the recent field measurements (Peng et al 1991). In addition, equations (1) and (3) are linear; (2) is curvilinear. For a given genotype, most studies indicated a linear relation between P_n and N_{LA} over the range of 0.5 - $2.1 \text{ g N}/\text{m}^2$. The curvilinear pattern of equation (2) from Sinclair and Hone may reflect the use of data from Takano and Tsunoda (1971) in which P_n and N_{LA} were determined for 40 rice genotypes from diverse genetic origins. Such studies often include genotypes that are simply not adapted or lack general agronomic fitness to the growth environment. Thus, the curvilinear relation-

Net carbon assimilation rate
($\mu\text{mol}/\text{m}^2/\text{s}$)



2. Comparison of relationships between net carbon assimilation rate and leaf N content determined by Yoshida and Coronel (1976), van Keulen and Seligman (1987), Sinclair and Horie (1989), and Peng (unpubl. data).

ship of equation (2) may reflect growth limitation by factors other than leaf N in some of the genotypes, which in turn influences the relationship between photosynthesis and leaf N status.

We will continue to quantify the relationship between P_n and N_{LA} under field conditions and validate the new regression equation using the ORYZA1 simulation model by comparing simulated and observed biomass production. The data from our recent studies also confirm the relatively low N_{LA} of rice compared with other cereal crops such as wheat. For example, maximum N_{LA} values of about 1.75 $\text{g N}/\text{m}^2$ (Fig. 1) were obtained in treatment plots that produced a grain yield of nearly 10 t/ha. Makino et al (1988) found a similar maximum N_{LA} in a japonica rice cultivar, but values up to 2.7 $\text{g N}/\text{m}^2$ in a wheat cultivar. At issue is whether it is possible to select rice genotypes with greater N_{LA} to achieve higher rates of canopy photosynthesis at the same LAI during grain filling, and whether such a trait would contribute to higher yield potential.

The broader issue of potential improvement in photosynthetic efficiency of rice also needs to be revisited as measurement techniques are refined and the basic biochemistry and genetics of the photosynthetic components are better understood. Although earlier work suggests no relationship between grain yield and photosynthetic characteristics of traditional or improved rice varieties (Murata 1964, Evans 1972), recent evidence from Japan suggests that there has been a 30% increase in single leaf photosynthesis rates in modern japonica varieties released since 1950 compared with older varieties released from 1882

to 1913 when measurements are made at the mid-grain filling stage, and only in treatments that receive an additional N topdressing at flowering (Ishii 1988). Further work is needed to determine whether genetic variation exists in photosynthetic efficiency on a leaf N basis.

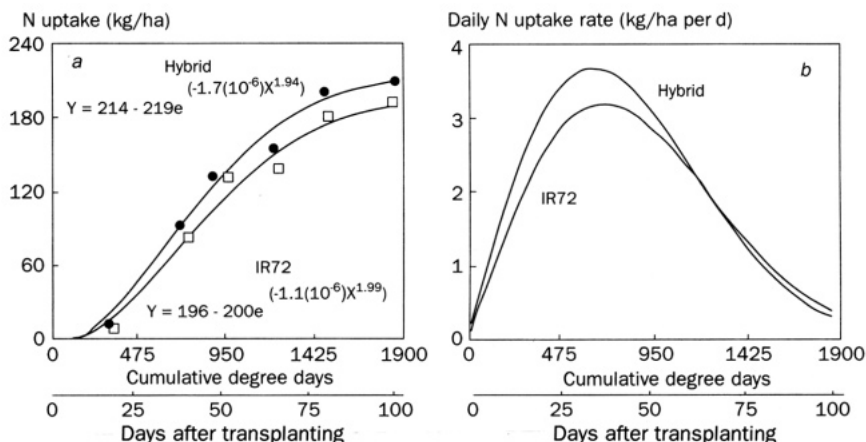
Root system efficiency and higher yield potential

We can question whether the rice root system can supply sufficient nutrients to the rice crop for an increase in yield potential from 10 to 15 t/ha in tropical environments. Clearly the rice root system is adequate in the temperate areas of Australia and China where yields of 15 t/ha are achieved. But the question is complicated by the potential environmental and economic costs of adding the large quantities of fertilizer required to produce higher yields at current levels of efficiency, and the management skills and access to information that would be needed to increase efficiency at higher rates of addition. These issues are particularly important for N inputs because excess N can be leached from the soil profile or lost to the atmosphere as ammonia and nitrous oxide gases, the latter a greenhouse gas of major concern. The relevant questions are therefore: 1) does the rice root system have the capacity to provide adequate nutrient uptake to support higher yield potential in the tropics, where growth duration is much shorter than in temperate environments? 2) can the management of N inputs be improved to maximize capture and utilization by the rice plant? and, 3) can the rice root system itself be modified to increase N acquisition?

Nitrogen requirements and uptake capacity

Recent experiments on yield potential at IRRI's farm demonstrate that it is essential to match the seasonal pattern of the N supply to the N requirements of the crop at each stage of development to achieve full yield potential. Because the effective soil N supply provides only about 50-80 kg N/ha per crop cycle in most double- and triple-crop irrigated rice systems (Cassman et al 1993), the N uptake requirements for high yield levels must be met by additions of fertilizer N at the key growth stages. Increasing yield potential from 10 t/ha to 15 t/ha will have major implications for N management. Nitrogen uptake of 200 kg/ha is required for a rice crop yielding 10 t/ha, and we estimate that 300 kg/ha N uptake will be required for 15 t/ha.

Reducing the grain N content could be an option, but rice grain protein is already low relative to other cereals, and a further reduction would reduce milling quality and protein supply in countries where rice contributes more than 50% of all dietary calories. Because rice soils supply the crop with 50-80 kg N/ha and uptake efficiency from applied N is typically 50% with good management in irrigated rice systems (Cassman et al 1993), fertilizer N inputs of 440-500 kg N/ha will be needed to support a 15 t/ha crop. This requirement could be reduced to 290-330 kg N if uptake efficiency from applied N was increased to 75%. Such quantities of N input are indeed being applied to rice



3. Nitrogen accumulation in aboveground dry matter of the inbred indica variety IR72 and the tropical indica hybrid IR64616H in relation to cumulative degree days (base temperature = 8°C) and converted into days after transplanting (DAT) in the 1992 dry season at IRRI (A) and the daily rate of N uptake (B) based on the relationships shown in A.

Table 1. Effect of fertilizer-N rate and plant density on aboveground dry matter and N accumulation from early tillering 17 days after transplanting (DAT) to 52 DAT (or 11 days after panicle initiation at 41 DAT), and final grain yield of IR72, 1992 dry season at IRRI (Peng et al, unpublished data).

Fertilizer-N addition			Hill density ^a	Plant dry matter ^b				Plant nitrogen ^b				N uptake rate			Grain yield
0	17	41		17	31	41	52	17	31	41	52	17-31	31-41	41-52	
DAT			DAT	DAT	DAT	DAT	DAT	DAT	DAT	DAT	DAT	DAT	DAT	DAT	t/ha
-kg	N/ha-		no./m ²	-kg/ha-				-kg				-kg	N/ha	per d-	
60	60	60	25	300	2130	4250	6810	11	67	97	138	4.0	3.0	3.7	9.6
			50	540	2650	4210	7430	21	74	90	148	3.8	1.6	5.2	8.4
120	60	60	25	430	2970	4830	7520	18	95	112	150	5.5	1.7	3.5	9.3
			50	570	3410	5640	8350	24	107	115	172	5.9	0.8	5.2	7.9

^a Five plants were transplanted in each hill so that total plant density was 125 or 250 plants/m² with hill spacing of 20×20 and 14×14 cm, respectively. The coefficient of variation in treatment means for dry matter averaged 5.6% on all sample dates, and 6.7% for plant nitrogen means.

crops in China where rice yields of 15 t/ha are achieved in Yunnan Province (Gaoqun Yang, Yunnan Acad. Agric. Sci., pers. commun.). In these areas, however, much of the N is supplied from high rates of manure application, an option not feasible in most of the intensive double-crop rice-rice and rice-wheat systems in Asia.

For a 10 t/ha grain yield, a total N uptake of 200 kg/ha must occur in an 80-day period (Fig. 3a) for an average uptake rate of 2.5 kg N/ha per d. Peak demand occurs during midtillering at about 30 days after transplanting (DAT) (Fig. 3b). Because the soil supplies only about 0.6-0.9 kg N/ha per d and

applied fertilizer N is rapidly lost through NH_3 volatilization and nitrification-denitrification from the soil-floodwater system (De Datta et al 1989), the rice plant must acquire most of its N in rapid bursts that follow N topdressings when N uptake rates can exceed 5 kg/ha per d (Table 1). Dry matter accumulation during these periods of rapid N uptake from 41 to 52 DAT averaged 240 and 270 kg/ha per d in treatments with 25 and 50 hills/m², respectively. Such rates are relatively high, and can account for 10 t/ha grain yield with a 25-d linear grain-filling period (see Kropff et al, These Proceedings). Maintaining a N uptake rate of 5 kg/ha per d for 60 d would be adequate to support a 15 t/ha grain yield without an increase in crop duration or grain-filling duration. By contrast, maximum rates of dry matter accumulation are not sufficient for a yield potential goal of 15 t/ha without an increase in grain-filling duration.

In fact, it is often excessive N uptake that is the greater concern, because lush growth stimulated by excess N supply and uptake leads to lodging and increased disease from blast (*Pyricularia oryzae*), sheath blight, and stem rot (see later section on "Greater Resistance to Lodging and Diseases"). Yield losses from excessive N uptake are particularly important in transplanted rice at close hill spacing or in direct-seeded rice, where higher plant density amplifies the effects of excessive N supply.

Despite the transient nature of available N in the soil-floodwater system (De Datta et al 1989), the recovery efficiency of applied N by the rice crop typically ranges from 50 to 60% with good management, even with high N rates to achieve grain yields of 10 t/ha (Cassman et al 1993). Thus, the fertilizer N uptake efficiency by rice is comparable to the efficiency of other cereal crops under well-managed, irrigated conditions (Craswell and Godwin 1984). Maintaining high fertilizer efficiency with increased N inputs sufficient for yields of 13-15 t/ha will require a more information-intensive management strategy with multiple fertilizer-N applications or controlled release fertilizer that matches the available N supply with crop N demand, to avoid periods of N deficiency or excess in the soil-floodwater system. At the foundation of such a strategy is the prediction and in-season estimation of the minimum nitrogen uptake requirement (MNUR) at each stage of development (Cassman et al 1994). To estimate the MNUR throughout the growing season, a user-friendly simulation model will be important for predicting crop N demand in diverse farm environments. A first setup for such a model is being developed. Simple and inexpensive tools such as the chlorophyll meter can also be used to estimate leaf N status as a guide for timing fertilizer N topdressings (Peng et al 1993).

Characteristics and implications of a ventilated root system

It may be possible to modify the rice root system to improve N uptake efficiency. The factors governing nutrient acquisition are: 1) root morphology, 2) root uptake capacity per unit root surface, i.e. rates of transport across cell membranes, 3) rates of transport through the soil to root surfaces by mass flow

and diffusion, and 4) the effect of root-induced changes in the soil on nutrient transport. The dominant form of N taken up by crops growing in fertile aerobic soil is NO_3^- , and NO_3^- is sufficiently mobile in soil that root morphology and uptake characteristics do not limit uptake until soil N levels fall to very low levels (Burns 1980, Drew 1990). However for lowland rice grown in submerged soil, the dominant form of N taken up is NH_4^+ , which is far less mobile, and the situation is entirely different.

There are not many reliable measurements of the nutrient uptake properties of lowland rice roots. It is possible that uptake capacity is compromised by the need for internal root aeration in anaerobic soil because the development of aerenchyma results in an essentially degraded cortex over the bulk of the primary root length, and in spite of morphological adaptations, the O_2 or supply to root cells active in uptake is limited. Calculations with a simple model of nutrient uptake that treats the root as a zero-sink for the nutrient (i.e., the rate of uptake at the root surface is assumed to be so much greater than the rate of transport through the soil that the root uptake characteristics are unimportant) lead to the conclusion that measured root length densities in rice are far greater than necessary to explain measured N uptake rates (Kirk, unpubl. data). There are three possible, not mutually exclusive, explanations for this. One is that uptake capacity is low for the reasons given above. A second is that root length densities are governed by the needs of water uptake, not nutrient uptake. This seems unlikely for water-saturated ricefields, but calculations with available measurements of rice root-surface hydraulic conductivities and typical transpiration rates under tropical conditions indicate that it is a possibility (de Willigen, Wageningen Agric. Univ., pers. commun.). A third explanation is that root-induced changes in the soil that arise as an inevitable consequence of rice root metabolism and nutrient uptake cause an impedance to NH_4^+ diffusion to root surfaces.

Models and experimental studies show that the pH of the rice rhizosphere may be 1-2 pH units lower than that of the soil bulk over a wide range of conditions (Kirk et al 1993). This is due to H^+ generation in the oxidation of ferrous iron by root-released O_2 , and H^+ release from roots to balance excess intake of cations over anions, N being taken up chiefly as NH_4^+ . The acidification would tend to decrease the fraction of exchangeable NH_4^+ in the soil solution, because the NH_4^+ concentration in solution is largely determined by the concentration of anions in solution, and HCO_3^- is the dominant anion in most flooded soils (in aerobic soils it is NO_3^-). HCO_3^- concentrations will decrease as the rhizosphere pH decreases below 6.3, becoming negligible below about pH 5.5, and NH_4^+ mobility will decrease correspondingly. We need more information on these processes and their effects on N uptake.

The extent of genetic variation in uptake efficiency is uncertain (De Datta and Broadbent 1988, Sta Cruz and Wada 1992, Wu et al 1992); it is often difficult to distinguish true differences in efficiency from differences in general agronomic fitness under particular experimental conditions. Because NH_4^+ is relatively immobile in soil, an efficient variety would have long fine roots with

a high NH_4^+ uptake capacity per unit root length and possibly also low O_2 -leakiness. The bulk of the root length in existing varieties does indeed comprise fine laterals (Drenth et al 1991). The O_2 demand of laterals is presumably met by O_2 diffusion through the aerenchyma of the primary roots. This demand is high because, in addition to O_2 consumption in respiration, laterals may leak large quantities of O_2 into the soil because they have high surface area:volume ratios. The O_2 supply may therefore limit the density of laterals borne by a primary root, and consequently limit net root length per unit root mass. We need more information on such relationships to judge how far rice root length density could be manipulated.

Importance of nonstructural carbohydrates during grain filling

The importance of starch and soluble carbohydrates during grain filling to increasing the yield potential of rice assumes source limitations in current genotypes. A source limitation is certainly relevant in the wet season (WS) whereas a sink limitation may exist in the dry season (DS) (see "Conclusion From Quantitative Analysis of Yield Potential" section in Kropff et al, These Proceedings). The supply of carbohydrates during grain filling and limitations to yield involves synthesis, storage and partitioning, and remobilization of carbohydrates.

Carbohydrate Synthesis. The synthesis of carbohydrates during grain filling could be limited due to feedback inhibition from high concentrations of carbohydrates accumulated in leaves prior to flowering, and perhaps also from high concentrations in storage organs. Such limitations could become important as new plant types are developed for higher growth rates and yield under conditions of high radiation and high temperatures in the tropics.

Feedback inhibition of photosynthesis by high carbohydrates was indicated in rice leaves where carbohydrate concentrations were manipulated by different shading levels and cold treatment of the stem at 7°C to limit efflux. When leaf carbohydrates exceeded 6 g/m^2 (approximately 12% dry weight, assuming a specific leaf weight of $50 \text{ g dry weight/m}^2$), there were reductions in photosynthesis of approximately 10% for each additional gram of carbohydrate/ m^2 (or 2% dry weight; calculated from Weng and Chen 1991). It is unknown to what extent feedback inhibition from high carbohydrate concentration in leaves would limit photosynthesis in full sunshine field conditions.

Observations for rice generally indicate that carbohydrate supply does not limit yield in the field. Even though there was a good correlation between the photosynthetic rate of flag leaves of six rice varieties and their crop growth rates, there was little correlation to grain yield, suggesting that carbohydrate supply in these experiments was not limiting (Murata 1965, reviewed by Evans 1972).

Photosynthesis rates respond to the demand for carbohydrates in wheat. During grain filling, ear removal resulted in reduced translocation of ^{14}C assimilated from the flag leaf and a 40% reduction in photosynthetic rate within

3-24 hours (King et al 1967). Unfortunately there were inadequate data to demonstrate a causal relationship between reduced translocation of assimilates and reductions in photosynthesis. There is convincing evidence that carbohydrate synthesis is not limited in highly productive wheat varieties during grain filling since a progressive fall in photosynthesis rate has occurred in the evolution of modern wheats compared with wild relatives, balance sheets of supply and demand demonstrate that adequate assimilates are available, and shading or leaf removal treatments have minimal effects on growth and yield (reviewed by Evans 1972).

Carbohydrate Storage and Partitioning. It could be possible to increase carbohydrates in tissues of most rice genotypes grown in tropical environments based on the variation which occurs in concentrations of carbohydrates prior to grain filling in different genotypes, and for the same genotype grown in different environments. In past experiments, such increases in carbohydrates have often not been translated into greater yields. For example, the increase of 25-50% starch accumulation in Calrose 76 that was thought to be the result of its 18-d longer vegetative growth duration relative to an isogenic line ED7 (Dat and Peterson 1983a), was not translated into greater yield — presumably due to sink limitation from low night temperatures and spikelet sterility for Calrose (yields of 8-9 and 10 t/ha for Calrose and ED7, respectively) (Dat and Peterson 1983b). Similarly in six varieties examined at IRRI, there were almost two-fold differences in starch or soluble carbohydrates at flowering in stems during the DS (Setter et al, unpubl. data), yet these were also not correlated to yield. The lack of relationship between yield and carbohydrate storage in these experiments may be due to differences in yield potential of varieties tested or differences in effective sink size.

Environmental conditions greatly affect carbohydrate concentrations at flowering. The importance of accumulated carbohydrates prior to flowering increases when light intensity is low after heading resulting in low photosynthesis, and N levels are low resulting in higher accumulations of carbohydrates (Wada 1969, Yoshida and Ahn 1968). For rice varieties grown at IRRI, typical values for accumulated shoot carbohydrates range from 10 to 15% (dry weight basis) during the WS, while concentrations of 15-25% occur for varieties grown during the DS (Setter, unpubl. data). In temperate climates such as in rice-growing regions of Australia, values for total shoot carbohydrates are often 30-40% (Williams and Lewin, unpubl. data) presumably due to factors including the greater radiation per day (2.5 vs. 20 MJ/d) and lower night temperatures (15 vs 25°C) than in the tropics.

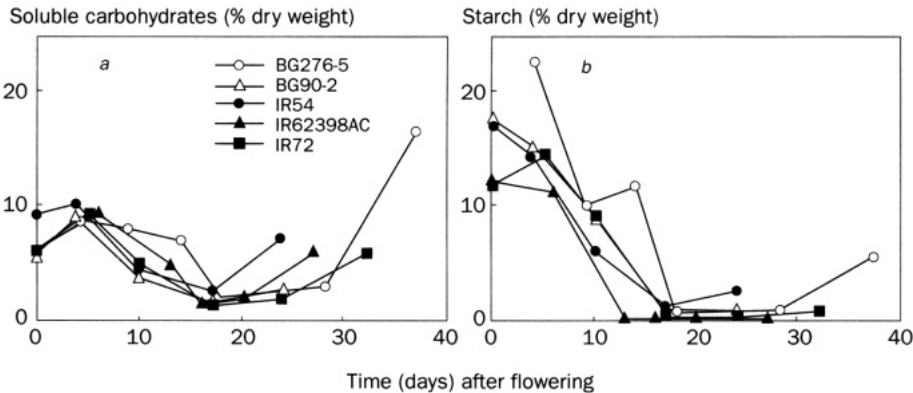
Sheaths serve as a temporary storage site for starch and sugar before heading (Murayama et al 1955, Togari et al 1954). This is consistent with stems containing approximately 15% starch and 10% soluble carbohydrates (dry weight basis) at PI and flowering for many genotypes grown in the DS at IRRI. Leaf blades of rice plants also accumulate starch and soluble carbohydrates, but, concentrations are usually only about 5 and 2%, respectively (Setter, unpubl. data). As the internodes elongate prior to panicle emergence, higher

concentrations of soluble carbohydrates than starch accumulate in the upper internode (approximately 10 and 5%, respectively), while more starch accumulates in the lower internodes (approximately 20% at internode 3) (Peterson 1982).

There is no published information on the accumulation of carbohydrates in rice roots and the extent that this may contribute to grain filling in different genotypes. Efficient future manipulation of these tissues as reserves for carbohydrates depends on their biomass aside from carbohydrate concentrations. For irrigated rice at flowering, the distribution of plant biomass is approximately 45% leaves, 45% sheaths plus culm, and 10% roots (IR58; Akita, unpubl. data).

Carbohydrate Remobilization. The extent of retranslocation of C accumulated before flowering to grain filling seems to vary among rice genotypes and is related to vegetative growth duration (source), reproductive growth duration (sink), and sink size. Murata and Matsushima (1975) observed that carbohydrates only begin to accumulate about 2 weeks before heading in a short-duration variety, they reach a maximum at heading, decline with time, and then may increase slightly prior to maturity. Recent analyses of varieties at IRRI are consistent with these trends (Fig. 4a and b) except that total carbohydrates may be high (25% dry weight) from seedling development until flowering (Setter, unpubl. data).

Varieties are distinguished by three characteristics associated with remobilization of accumulated carbohydrates in stems during grain filling (Fig. 4): the concentration at flowering, the time and rate of decrease after flowering, and increases in carbohydrates near maturity. Decreases in carbohydrate concentrations are probably due to carbohydrate mobilization into panicles, because removal of panicles at flowering prevents decreases, while removal of panicles at approximately 20 days after flowering results in strong increases in stem carbohydrates (Setter, unpubl. data).



4. Soluble Carbohydrates (a) and starch (b) in stems of rice varieties during grain filling. Plants were grown in the dry season at IRRI, Los Baños, Philippines. The SEMs were never more than 1.5 and 2.1% dry weight for (a) and (b) respectively.

It is reasonable to suggest that the differences in the rate of decrease of carbohydrates in storage tissues may be related to the rates of increase in grain filling; indeed, the changes in biomass of storage tissue and panicles have long been used as a basis for calculating carbohydrate mobilization to panicles (e.g. Yoshida 1981). Rates of grain filling are often highly correlated to yield in rice (Jones et al 1979), wheat (Sofield et al 1977) and sometimes maize (see review by Jones et al 1979), but the interpretation why this occurs seems to vary in different experiments. In a recent study at IRRI, this high correlation was largely due to increases in grain number (Kropff et al, unpubl. data). This is reasonable, since varieties with greater grain numbers will probably have greater rates of grain filling.

The frequent occurrence of high concentrations of carbohydrates in stem tissues at maturity (24-37 days after flowering in Fig. 4) seems an obvious source of carbon for increasing yield potential. However, experimental evidence (see above) suggests that for some varieties this may be the result of a limited effective sink size and subsequent reaccumulation of carbohydrates in storage tissues, rather than poor remobilization after flowering. Peterson (1982) concluded that the increases in stem carbohydrates at maturity in some genotypes grown in California were due to an imbalance in source-sink relationships, and that genotypes with considerable residual carbohydrates at maturity have an inadequate (panicle) storage capacity or an inadequate transport system. Alternatively, a limited source at a key time during grain filling may subsequently limit the effective sink, e.g. via abortion of spikelets (see Fig. 8 in Kropff et al, These Proceedings, section "Conclusions From Quantitative Analysis of Yield Potential").

Concentrations of carbohydrates as described above say little about the flux of carbon into and out of different tissues. More accurate measurements are obtained in studies using ^{14}C labeling, although rarely are specific activities evaluated. Specific activities quantify the extent of labeling in soluble and insoluble carbohydrate pool(s) and are therefore necessary to validate interpretation of results. Using ^{14}C -labeling, Cock and Yoshida (1972) observed that 68-77% of the ^{14}C in carbohydrates that had accumulated in one variety at flowering was translocated to the grain by maturity, while about 20% was respired and 12% remained in vegetative parts. The amount of stored ^{14}C -carbohydrate translocated was equal to 26-28% of the grain carbohydrate, i.e., about 2 t/ha at a yield of 7.8 t/ha.

Rice appears to have a considerably greater capacity to utilize accumulated carbohydrates than wheat. When mobilization of accumulated carbohydrates in wheat is estimated from stem dry weight losses less respiration, assimilates mobilized from stems account for only 3-12% of the final grain weight (Rawson and Evans 1971). Similar calculations for rice indicate a contribution of between 10 and 30% (Yoshida 1981, Wei et al 1982). The total export of stem reserves to support grain filling in rice may be underestimated by stem weight loss between flowering and maturity if stem carbohydrate levels increase in the last days of grain filling (Fig. 4). In recent field experiments at IRRI, we

found large varietal differences in carbohydrate export from stems based on the decrease in stem dry weight from flowering to physiological maturity (Kropff et al 1993). Dry weight loss represented 13% in IR72 and 38% in IR58109-113-3-3-2 of stem weight at flowering in treatments that received high rates of N input. Despite these differences, yield of both varieties was 9.5 t/ha.

In summary, some opportunities may exist to increase storage and remobilization of accumulated carbohydrates in rice to increase yield potential given an adequate and effective sink. The physiological mechanisms related to maintenance of effective sinks are an important aspect of this research. Little is known about whether accumulation of nonstructural carbohydrates in the shoot has any importance other than carbon storage for grain filling. For example, maintenance of minimum levels of stem carbohydrates could be important in lodging resistance, whereas it could have adverse effects in relation to increased susceptibility to pests and diseases.

Role of soil-derived inorganic and organic carbon in canopy photosynthesis

It is well known that growth and yield of rice is limited by CO₂ supply. When the ambient CO₂ concentration in air is increased by 2- to 8-fold, increases in dry matter accumulation and yield have been documented for a number of rice varieties under conditions of high light and adequate mineral nutrition. For example, IR8 yields were increased 58-89% at 2400 ppm relative to 300 ppm CO₂ (as cited by Yoshida 1976), cv. Nipponbare yields increased 23-72% at 700 relative to 350 ppm CO₂ (Imai et al 1985), IR30 yields increased 74% at 900 relative to 330 ppm (Baker et al 1990), and cv. Jarrah yields increased by 58% at 700 relative to 350 ppm CO₂ (Seneweera et al, unpubl. data).

It is equally well known that the soil solution in irrigated ricefields is typically in equilibrium with an atmosphere of 300,000 ppm CO₂ about 6 wk after flooding (Ponnamperuma 1976). Yield responses to CO₂ enrichment and high CO₂ in the soil atmosphere raise the question of whether major increases in rice yield could occur by enhancing utilization of inorganic or even organic carbon via root uptake. Indeed, we may ask if rice utilizes these plentiful carbon supplies in soil already, and whether current rates of photosynthesis in rice are often underestimated because available evaluations are based on CO₂ depletion from the air. This means that total CO₂ fixation of rice may be underestimated. There are no suitable data to judge whether the contribution of soil-derived carbon to total carbon assimilation in rice is agronomically significant.

There are three opportunities for utilizing soil-derived carbon, via soil uptake and movement through the root of gaseous CO₂, dissolved CO₂ and bicarbonate, and dissolved organic solutes. The first two possibilities are considered in more detail below, although there is little published information on uptake of organic solutes.

Gaseous CO₂ movement could occur through the aerenchyma, while movement of dissolved carbon could be associated with the uptake of water during growth and development. Utilization of root medium inorganic carbon via gas diffusion through the aerenchyma based on simultaneous field and plant analyses obtained for rice was proposed earlier (Setter et al 1987), while utilization of ethanol from root medium of rice in flooded soils was proposed by Waters et al (1989).

Theoretical considerations - Fick's Law

Fick's first law of diffusion (Nobel 1974) states that the flux (J_j) of species j will depend mainly on the concentration gradient (Δc) and on the distance between the two points (Δx), where D is the diffusion coefficient of the species, i.e. $J_j = -D(\Delta c/\Delta x)$. The concentration gradient of CO₂ is high, being at least 20% vol/vol (calculated from above). Therefore CO₂ will diffuse along a concentration gradient, from high concentrations in the flooded root environment, through the aerenchyma, to low concentrations (equilibrium concentrations <0.005%) in the aerial portions of the shoot. To what extent this predicted root-to-shoot diffusion would function in reassimilation of respired root CO₂ relative to the movement of soil-derived CO₂ is unknown, however this would depend on the concentration gradients and the root membrane permeability to CO₂. A rough estimate can be made about the maximum gain from recycling CO₂ from root respiration. Assuming a shoot/root ratio of 5 and a shoot photosynthesis/root respiration of 3, then the maximum contribution of carbon assimilation from CO₂ via the aerenchyma would be about 7% (1/15) of CO₂ fixation from the air, based on equal gradients of O₂ and CO₂.

Aerenchymous tissue is well known for its importance in supplying meristematic root cells with O₂ for respiration (Armstrong 1979), i.e. for gas diffusion in the opposite direction to the predicted CO₂ diffusion. The concentration gradient for O₂ is similarly about 20% due to O₂ concentrations of 21% in air and equilibrium concentrations of <0.01% in the mitochondria of meristematic cells in the root tip. This similar concentration gradient for O₂ and CO₂ is relevant since O₂ diffusion through aerenchyma of rice roots is responsible for supplying the respiratory requirements for growth in an anoxic root environment (Armstrong 1979).

Carbon assimilation associated with water uptake during plant growth

During growth and development of rice variety IR64 the uptake and transpiration of water amounts to about 1000 kg of water per kg of grain produced (calculated from Yambao and Ingram 1988). The concentration of CO₂ in soil solutions required to produce this yield with this level of water uptake can be calculated based on about 1470 g CO₂ required to produce 1 kg carbohydrate via photosynthesis. Let us assume that (i) CO₂ and HCO₃⁻ is taken up passively by mass flow through roots and completely assimilated in shoots via photosynthesis, (ii) equal concentrations of CO₂ and bicarbonate in the soil solution (as would occur in equilibrium at pH 6.3 and at 30 °C (Umbreit 1964)),

and (iii) all of the bicarbonate can be converted to CO_2 for utilization by photosynthesis in the shoot. This would mean that the concentration of CO_2 in the soil solution would have to be $1470/2(1000) = 0.73$ g/liter, i.e., 17 mol/m³, or be in equilibrium with an atmosphere containing about 620,000 ppm CO_2 (Umbreit 1964). While this is unlikely based on typical CO_2 concentrations observed in flooded soils for rice (Ponnamperuma 1976), contributions of soil-derived CO_2 representing 10-20% of total leaf CO_2 supply are possible.

Contribution of soil-derived CO_2 to carbon assimilation in other plants

In aquatic plant species, there is a remarkable variation in ability to utilize C from the soil via root uptake (Bowes 1987). The aquatic fern *Stylites* obtains C from the soil exclusively by diffusion (Fick's Law) via aerenchyma in the roots; the plants are photosynthetic, have no stomata and a thick cuticle covers the shoots (Keeley et al 1984). Other aquatic species also utilize soil CO_2 via diffusion through aerenchyma, including *Lobelia dortmanna* (Wium-Anderson 1971), *Littorella uniflora* (Sondergaard and Sand-Jensen 1979), *Elodea*, *Egeria*, and *Lagarosiphon* (Brown et al 1974). Although these aquatic plants can utilize soil-derived CO_2 , their growth rates tend to be considerably lower than growth rates of rice under normal field conditions.

There is no good information available on the importance of root uptake of soil inorganic C to growth and yield of rice. Early work involved estimating absorption based on supplying ^{14}C as bicarbonate often at an unknown pH or using unnamed varieties. Additional difficulties in supplying ^{14}C occurred due to lack of information on changes in specific activity of the source with time. In more recent work, it was suggested that CO_2 in the root medium was absorbed by rice roots and transported more via aerenchyma than via xylem flow to the shoot (Higuchi 1982). This was based on observations that there was a five-fold increase in CO_2 concentration of the gaseous medullary cavity and a six-fold increase in the bleeding sap, following a 40-fold increase in exogenously supplied CO_2 in the root medium. Without flux data or isotope studies, such interpretations and the importance to growth are difficult to evaluate.

Other evidence of the importance of gas diffusion rather than xylem flow to root medium carbon movement was obtained later by Higuchi et al (1984). They exposed rice seedling roots to mannitol solutions, which reduced water absorption by 50%. Under these conditions, the movement of ^{14}C from $^{14}\text{CO}_2$ to the shoot was not reduced relative to plants with high water uptake, suggesting that gas diffusion and not xylem flow was involved in uptake and movement of carbon from the root medium. Similar lack of any effect of transpiration (manipulated by light-dark treatments) was observed for movement of methane gas from the roots to shoots of rice (Nouchi et al 1990). Such results for CO_2 should be considered carefully, since much solute transport occurs in the xylem independent of water flow (Marschner 1986) such that solute concentrations could rise in the xylem. This may apply to HCO_3^- and organic acids derived from CO_2 fixed by dark reactions in root tissue.

The movement of gases like CO₂ and methane from roots to shoots appears to occur primarily via the aerenchyma, while the lack of effect of water uptake may be due to the low permeability of the endodermis (surrounding the stele) relative to the rhizodermis. It is unlikely that all sections of the root would be equally responsible for CO₂ uptake, since there is a tendency for decreases in water and solute uptake with greater distance from the tip, presumably due to suberization of the rhizodermis and endodermis (see reviews by Kramer 1983, Marschner 1986). Likewise, O₂ leakage from roots of most wetland species is suggested to decrease at distances >2cm from the tip due to differences in suberization of the rhizodermis (Armstrong 1979).

Although the above work demonstrates that inorganic C can flow from roots to shoots, this has not been adequately quantified in a selection of rice genotypes. Therefore, the impact of carbon supplied from the soil on rice yield remains unknown. This mechanism could be important for plants like rice that grow in a flooded environment rich in inorganic and organic carbon reserves. One way to rapidly screen for such genotypic differences in utilization of root carbon may be via carbon isotope discrimination (Raven et al 1987). In summary, there are three possible sources of carbon in the soil that could supply carbon to the rice plant. Two of these, CO₂ and HCO₃⁻, may contribute to carbon fixation in the shoot. The third organic solutes like ethanol, may also be a substantial contributor to the carbon economy of paddy rice.

Greater resistance to lodging and diseases

The importance of lodging becomes obvious as one imagines how to hold up 15 t of grain almost a meter in the air over an area of 1 ha. Then add another ton for moisture content, 1 or 2 tons more due to rainfall, and then imagine a strong breeze. Such images are relevant to increasing yield potential because lodging is often a constraint to achieving higher yields for current varieties when N supply is high, or when stems are infected by diseases such as sheath blight and stem rot. This section summarizes more extensive reviews on lodging by Chang and Loresto (1985), Chang and Vergara (1972) and Chang (1964, 1967), and a bibliography on Lodging in Rice and Other Cereals by Makinano and Cruz (1982).

The basis for lodging resistance

There are no good estimates of global yield losses of rice due to lodging. Estimates from simulations of lodging by mechanical means can be as high as 80%, while under natural conditions in Japan, serious lodging from storms during grain filling can result in 50% loss of yield. The yield loss due to lodging is usually proportional to the degree of lodging and the number of days before grain ripening. Lodging may result in reductions in grain and straw biomass as well as lower grain quality and greater difficulty in harvesting.

While several different types of lodging can be identified, these can be separated into two main effects: bending or breakage of the shoot and root

upheaval. The buckling of the culm a short distance above ground level soon after heading is the most common type of lodging in the tropics; lodging of tall varieties at IRRI is mostly due to the bending or buckling of basal internodes that are immediately above ground level. Lodging due to upheaval of roots is more common in direct-seeded ricefields, especially those sown by broadcasting. This is presumably due to the poor anchorage of roots relative to transplanted seedlings (Chang and Loresto 1985). Root upheaval will become an important research topic as farmers move to direct broadcast-seeded rice instead of transplanting to reduce labor costs.

Lodging results from the interaction and the balance of three forces: straw strength, environmental factors affecting straw strength in addition to plant characters related to the bending movement, and the impact of external forces such as wind, rain, and falling of neighboring plants. Important environmental factors known to reduce straw strength or enhance lodging susceptibility include excessive N supply, deficiencies of K, Si, and Ca, low light, increased water depth, diseases affecting the leaves, sheaths and culm, and agricultural chemicals affecting plant height such as gibberellin (Chang and Loresto 1985, see also references cited by Makinano and Cruz 1982).

The mechanisms of lodging resistance in varieties are complex and involve several characteristics that have been evaluated by path analysis using progeny from different crosses (Chang and Vergara 1972). These studies identified two major factors—plant height ($r=0.64$) and sheath wrapping ($r=0.43$)—but also minor factors such as basal internode lengths and the cross-sectional area of the culm. The issue for increased lodging resistance is which of these factors is easiest to manipulate (e.g. has the greatest genetic diversity) and has the potential to adequately compensate for the increased load of a 15 t/ha grain crop. The relative importance of each factor depends partly on when lodging occurs. Until internode elongation starts, around PI, the leaf sheaths support the whole plant. Even after the completion of internode elongation, the leaf sheaths contribute to the breaking strength of the shoot by 30-60% (Chang 1964). Therefore the sheath biomass and extent of wrapping will always be an important trait for selection against lodging at all developmental stages. This trait might also be appropriate for aims to increase carbohydrate storage potential as discussed earlier.

Approaches to increase lodging resistance during the reproductive stages include increasing the strength of stems, reducing stem height, and reducing effects of wind through crop management. Increasing stem strength is possible, and genetic studies at IRRI have shown that the above characters associated with lodging resistance are all quantitative and involve multiple genes (Chang and Loresto 1985). Reductions in stem height would not seem feasible in present semidwarf varieties, however it is not the height per se but rather the weight distribution along the axis, which is important, i.e. the height of the center of gravity of the shoot (Seko 1962). Hence, lowering the height of the panicle could have a profound effect on increasing lodging tolerance of rice involving either stem bending or root upheaval.

The impact of lowering the panicle on lodging tolerance has not been considered previously and can be quantified using the rotational inertia equation (e.g. Weber et al 1965). The rotational inertia (I) of an object such as a rice tiller is the product of the weight (m) and the square of the length of the column, i.e., the radius (r), from the base of the stem to the panicle: $I = mr^2$. For variable weights, the rotational inertia is the sum of the components of the individual mass elements along the radius,

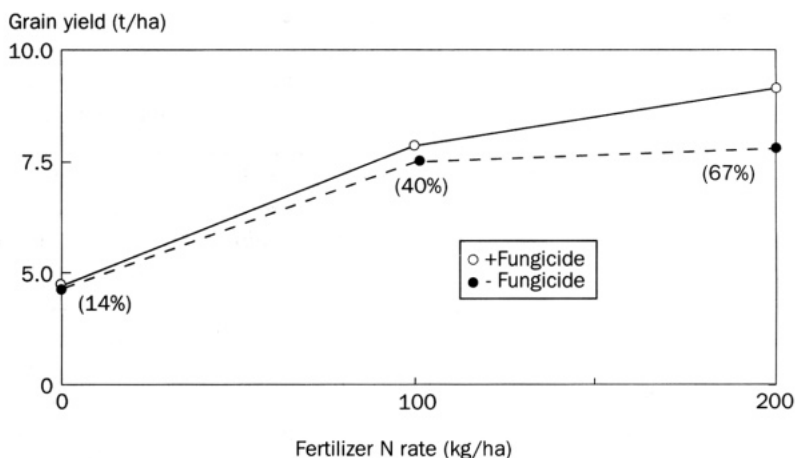
$$I = m_1r_1^2 + m_2r_2^2 + m_3r_3^2 \dots \text{ or } I = \sum m_i r_i^2.$$

This formula is useful for demonstrating the impact of dwarf varieties on the reduction in lodging, however it is equally applicable for evaluating panicle height effects. For simplicity we can assume the dominant mass is the panicle, therefore $I = mr_{\text{pan}}^2$, where r_{pan} is the panicle height. Using a panicle height of 0.8 m and weight of 10 t/ha, the calculated inertia for a typical semidwarf variety would be 6.4. Furthermore, to obtain this same inertia (lodging tendency) at 15 t/ha we would need a panicle height of $(6.4/15)^{1/2}$, or 0.65 m.

Reductions in panicle height to increase lodging resistance would have other positive effects on increasing light interception of leaves during grain filling (see Kropff et al, These Proceedings), although negative effects might include increased susceptibility to flooding or diseases.

Greater resistance to endogenous pathogens

Soil-borne pathogens like sheath blight and stem rot become "endogenous" to intensive rice ecosystems characterized by continuous rice cropping with high levels of nutrient inputs, particularly N (Mew 1991). When disease



5. Grain yield of IR72 in relation to fertilizer-N rate and the intensity of sheath blight infection at flowering stage (% of total tillers infected). Infection intensities are indicated in parentheses under N rate treatments that did not receive fungicide application (- fungicide). Treatments with fungicide application had no disease, and for all data points the standard error is smaller than the symbol. IRRI, 1993 dry season (Cassman et al, unpubl. data).

pressure is high, the achievable yield falls well below the yield potential of the rice variety in question due to lodging and loss of green leaf area during the last half of grain filling (Fig. 5). Although fungicides can be used to eliminate yield loss, farmers must make two to four applications to ensure a disease-free crop where sheath blight has become endemic. We can anticipate that increased N requirements and higher yields will make the problems of endogenous stem diseases and lodging more severe as we raise the yield potential.

Life histories of both sheath blight and stem rot pathogens include a saprophytic stage in soil, plant infection to produce microsclerotia on stems, leaf sheaths, and leaf blades, and recycling of this inoculum with crop residue left in the field. Typically, inoculum levels for these pathogens increase dramatically as rice cropping intensity increases so that the disease becomes endemic. For sheath blight, the most widespread and damaging stem pathogen in the tropics, there are no known sources of resistance in rice germplasm including wild relatives of cultivated rice. It may be argued that success in breaking the present yield threshold of tropical rice will depend not only on improved yield potential per se, but also on increased resistance to sheath blight. Greater understanding of the physiology of the sheath blight infection process is needed, to allow identification of suitable sources of resistance in the plant or animal kingdom for transfer, via molecular approaches, into the new rice plant types of the next century.

Hybrid rice

Tropical indica hybrids

Hybrid rices, exploiting the phenomenon of hybrid vigor, have shown higher yield potential than inbred varieties in various crops including rice. Rice hybrids, yielding on an average 1 t/ha higher than inbreds, helped boost China's rice production by about 10%—more than 200 million tons between 1976 and 1991 (Yuan et al 1992). However, these hybrids were not adapted to the tropics. Research conducted at IRRI (Virmani et al 1982, Ponnuthurai et al 1984, Yuan and Virmani 1988, Young and Virmani 1990, Peng and Virmani 1991, and Virmani et al 1991) has also indicated significant yield superiority of indica rice hybrids over indica rice inbreds. Similar results have also been reported from other tropical rice-growing countries, such as India (Mandal 1982, Nijaguna and Mahadevappa 1983, Singh et al 1984, Prakash and Mahadevappa 1987), Indonesia (Suprihatno 1986), Malaysia (Osman et al 1987), Vietnam (Luat et al 1985), and in recent field experiments at IRRI (see Table 2, Kropff et al, These Proceedings). Increased yield of tropical rice hybrids was obtained due to heterosis in total biomass and spikelet number and to some extent heterosis for 1000 grain weight (Ponnuthurai et al 1984).

Rice hybrids have been commercialized in China by deploying cytoplasmic-genetic male sterility (CMS) and a fertility restoration system. Hundreds of CMS lines have been bred in China for production of hybrid rice seeds. These CMS lines could not be used as such to develop rice hybrids for the tropics because of their susceptibility to major diseases and insects. Therefore new

CMS lines had to be bred at IRRI and national agricultural research systems to develop tropical rice hybrids (Virmani et al 1991). There is no dearth of restorers among elite indica rice cultivars bred for the tropics. With the availability of elite CMS and restorer lines adapted to the tropics, development of tropical rice hybrids has accelerated in recent years.

Recently, thermosensitive genetic male sterility (TGMS) has also been identified in China (Sun et al 1989, Wu et al 1991), Japan (Maruyama et al 1991), and IRRI (Virmani and Voc 1991) for breeding rice hybrids. The TGMS system does not require maintainer lines for multiplication, hence hybrids can be developed using only two lines, the TGMS line and the pollen parent, and the latter does not have to be a restorer. Two-line hybrids are likely to show stronger hybrid vigor because there are fewer restrictions on the choice of parents compared to the CMS system. It should be possible to use the TGMS system to develop rice hybrids in the tropics by utilizing the temperature differences at different altitudes or in different rice-growing seasons. TGMS should make a good alternative to the complex and cumbersome CMS system.

Technology for producing hybrid rice seeds in the tropics has been outlined (Virmani and Sharma 1993). Using this technology yields of 1-2 t/ha of hybrid seed and 1.5-2 t/ha of pollen parent seed have been obtained at IRRI and in some national programs. Seed yields can be increased further by improving the outcrossing potential of the parental lines and fine tuning the technology by the prospective seed growers in the national programs.

Indica × Japonica Hybrids

The magnitude of hybrid vigor (heterosis) depends on the genetic diversity between the two parents of hybrids. The greater the genetic difference in parents, the higher the heterosis. During the past 25 years, genetic diversity among the improved indica rice has narrowed down, due to massive international exchange of germplasm. Indica and japonica germplasm, however, have remained distinct as there has been very little gene flow between these two varietal groups. For example, the new plant type breeding effort is presently only utilizing a tropical japonica germplasm pool while currently used varieties are indicas (see Peng et al, These Proceedings).

Hybrids between indica and japonica parents showed higher heterosis for yield (Yuan et al 1989). Studies conducted at IRRI (IRRI 1990) also indicated that hybrids involving tropical japonicas as one of the parents showed higher total dry matter than other hybrids, although differences were statistically nonsignificant, perhaps due to lower tillering (inherited from tropical japonica parents) and the widely spaced hills (i.e., low plant density) used in this study. Currently, heterosis of indica/tropical japonica and indica/indica hybrids is being studied under normal and closer spacings to determine the potential yield advantage of indica/ tropical japonica hybrids.

Major constraints in utilizing hybrid rice technology for increasing rice production are the need to buy fresh hybrid seeds for every planting season, the high cost of hybrid seeds, and the need to establish seed production infrastructure in developing countries. Farmers would be willing to buy fresh

hybrid seeds at a higher price than inbred rice seeds provided there was a cost:benefit ratio of 1:4 for the more expensive seed. With such a cost:benefit ratio, national programs would also invest to strengthen or establish seed industry in public, private, or cooperative sectors. The constraints can also be overcome if true-breeding hybrids with permanently fixed heterosis are developed by using apomixis, the search for which is in progress in China, IRRI, and elsewhere.

References cited

- Armstrong W (1979) Aeration in higher plants. *Adv. Bot. Res.* 7:225-332.
- Baker J T, Allen L H, Boote K J (1990) Growth and yield responses of rice to carbon dioxide concentration. *J. Ag. Sci. Camb.* 115:313-320.
- Bowes G (1987) Aquatic plant photosynthesis: strategies that enhance carbon gain. Pages 79-98 in R.M.M. Crawford, ed. *Plant Life in Aquatic and Amphibious Habitats*. British Ecological Society, No. 5. Blackwell Scientific Publ. Oxford.
- Brown J M A, Dromgoole F I, Towsey M W, Browse J (1974) Photosynthesis and photorespiration in aquatic macrophytes. Pages 242-249 in R.L. Bielecki, A.R. Fergusori, and M.M. Cresswell, ed. *Mechanisms of Regulation of Plant Growth*. Bull. 12, The Royal Soc. of New Zealand, Wellington.
- Burns I G (1980) Influence of the spatial distribution of nitrate on the uptake of N by plants: a review and a model for rooting depth. *J. Soil Sci.* 31:155-173.
- Cassman K G, Kropff M J, Gaunt J, Peng S (1993) Nitrogen use efficiency of irrigated rice: what are the key constraints? *Plant and Soil* 155/156:359-362.
- Cassman K G, Kropff M J, Yan Zhende (1994) A conceptual framework for nitrogen management of irrigated rice in high-yield environments. In: *Proceedings of the 1992 International Rice Research Conference*, Intl. Rice Res. Inst., Los Baños, Philippines. (In press)
- Chang T T (1964) Varietal differences in lodging resistance. *Intl. Rice Comm. Newslet.* 13 (4): 1-11.
- Chang T T (1967) Growth characteristics, lodging and grain development. Pages 54-60. *Intl Rice Comm. Newslet. Spec. Issue* (1967 Pacific Sci. Congr. Symp.).
- Chang T T, Loresto G C (1985) Lodging in Rice. Rice Production and Extension. Management Specialist Training Course, September, 1985. IRRI.
- Chang T T, Vergara B S (1972) Ecological and genetic information on adaptability and yielding ability in tropical varieties. Pages 431-453. In: *Rice Breeding*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Cock J H, Yoshida S (1972) Accumulation of ^{14}C -labelled carbohydrate before flowering and the subsequent redistribution and respiration of the rice plant. *Proc. Crop Sci. Soc. Japan* 41:226-234.
- Cook M G, Evans L T (1983a) Nutrient responses of seedlings of wild and cultivated *Oryza* species. *Field Crops Res.* 6:205-218.
- Cook M G, Evans L T (1983b) Some physiological aspects of the domestication and improvement of rice (*Oryza* spp). *Field Crops Res.* 6:219-238.
- Craswell E T, Godwin D C (1984) The efficiency of nitrogen fertilizers applied to cereals in different climates. Pages 1-54 In: P.B. Tinker, and A. Luchli, ed. *Advances in Plant Nutrition*. Praeger Publishers, New York.

- Dat, Tran Van, Peterson M L (1983a) Performance of near-isogenic genotypes of rice differing in growth duration. II. Carbohydrate partitioning during grain filling. *Crop Sci.* 23:243-246.
- Dat, Tran Van, Peterson M L (1983b) Performance of near-isogenic genotypes of rice differing in growth duration. I. Yields and yield components. *Crop Sci.* 23:239-242.
- De Datta S K, Trevitt A C F, Freney J R, Obcemea W N, Real J G, Simpson J R (1989) Measuring nitrogen losses from lowland rice using bulk aerodynamic and nitrogen-15 balance methods. *Soil Sci. Soc. Am. J.* 53: 1275-1281.
- De Datta S K, Broadbent F E (1988) Methodology for evaluating nitrogen use efficiency in rice genotypes. *Agron. J.* 80: 793-798.
- Drenth H, ten Berge H F M, Meijboom F W (1991) Effects of growth medium on porosity and branching of rice roots (*Oryza sativa* L.). Pages 162- 175. In: F.W.T. Penning de Vries, H.H. van Laar and M.J. Kropff. ed. *Simulation and systems analysis for rice production (SARP)*. Pudoc, Wageningen.
- Drew M C (1990) Root function, development, growth and mineral nutrition. Pages 35-57. In: J.M. Lynch. ed. *The Rhizosphere*. Wiley-Interscience, Chichester.
- Evans L T (1972) Storage capacity as a limitation on grain yield. Pages 499-511. In: *Rice Breeding*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Higuchi T (1982) Gaseous CO₂ transport through the aerenchyma and intercellular spaces in relation to uptake of CO₂ by rice roots. *Soil Sci. Plant Nutr.* 28:491-497.
- Higuchi T, Yoda K, Tensho K (1984) Further evidence for gaseous CO₂ transport in relation to root uptake of CO₂ in rice plant. *Soil Sci. Plant Nutr.* 30: 125-136.
- Imai K, Coleman D F, Yanagisawa T (1985) Increase in atmospheric partial pressure of carbon dioxide and growth and yield of rice (*Oryza sativa* L.). *Jap. J. Crop Sci.* 54:413-418.
- International Rice Research Institute (1990) Program Report for 1989. Intl. Rice Res. Inst., Los Baños, Philippines.
- Ishii R (1988) Varietal differences of photosynthesis and grain yield in rice (*Oryza sativa* L.). Korean J. Crop Sci. 33:315-321.
- Jones D B, Peterson M L, Geng S (1979) Association between grain filling rate and duration and yield components in rice. *Crop Sci.* 19:641-644.
- Keeley J E, Osmond C B, Raven J A (1981) *Stylites*, a vascular land plant without stomata absorbs CO₂ via its roots. *Nature* 310:694-695.
- King R W, Wardlaw I F, Evans L T (1967) Effects of assimilate utilisation on photosynthetic rate in wheat. *Planta* 77:261-276.
- Kirk G J D, Begg C B M, Solivas J L (1993) The chemistry of the lowland rice rhizosphere. Pages 87-90 In: N.J. Barrow, ed. *Plant nutrition: from genetic engineering to field practice*. Kluwer Academic Publishers, Dordrecht.
- Kramer P J (1983) Water Relations of Plants. Academic Press. New York. 489 pp.
- Kropff M J, van Laar H H, ten Berg H F M (1993) ORYZA1, a basic model for irrigated lowland rice production. Intl. Rice Res. Inst., Los Baños, Philippines.
- Luat N V, Bong B B, Chandra Mohan J (1985) Evaluation of F₁ hybrids in the Cuu Long Delta, Vietnam. *Int. Rice Res. Newslet.* 10(3):19.
- Makinano S I, Cruz R T (1982) Lodging in rice and other cereals. A selected bibliography. Agron. Dept., Intl. Rice Res. Inst., Los Baños, Philippines. 21 pp.

- Makino A, Mae T, Ohira K (1988) Differences between wheat and rice in the enzymic properties of ribulose-1,5-biphosphate carboxylase/oxygenase and the relationship to photosynthetic gas exchange. *Planta* 174:30-38.
- Mandal B K (1982) Note in the estimates of heterosis for nine quantitative characters in rice. *Indian J. Agric. Sci.* 52:699-700.
- Marschner H (1986) Mineral Nutrition of Higher Plants. Academic Press. New York. 674 pp.
- Maruyama K, Araki H, Kato H (1991) Thermosensitive genetic male sterility induction by irradiation. Pages 227-232. In: *Rice Genetics*. Intl. Rice Res. Inst., Los Baños, Philippines.
- Mew T (1991) Disease management in rice. Pages 279-299. In: D. Pimentel, ed. *CRC Handbook of Pest Management in Agriculture*, Second Edition, Vol. III. CRC Press Inc., Boston.
- Mitsui S, Ishii Y (1938) Effects of supply of three major nutrients on photosynthetic rate of rice seedlings. *J. Sci. Soil Manure (Japan)* 12:287-289.
- Mitsui S, Ishii Y (1939) Effects of nitrogen top-dressing on photosynthetic activity of the rice plant. *J. Sci. Soil Manure (Japan)* 13:309-313.
- Murata Y (1964) Photosynthesis, respiration, and nitrogen response. Pages 385-400. In: *The Mineral Nutrition of the Rice Plant*. The Johns Hopkins Press, Baltimore.
- Murata Y, Matsushima S (1975) Rice. Pages 73-99. In: L.T. Evans, ed. *Crop Physiology, Some Case Histories*. Cambridge University Press.
- Murayama N, Yoshino M, Oshima M, Tsukahara S, Kawarazaki Y (1955) Studies on the accumulation process of carbohydrates associated with growth of rice. *Bull. Natl. Inst. Agric. Sci. (Tokyo)* B4: 123-166.
- Nijaguna G, Mahadevappa M (1983) Heterosis in intervarietal hybrids of rice. *Oryza* 20: 159-161.
- Nobel P S (1974) *Introduction to Biophysical Plant Physiology*. W.H. Freeman and Co. San Francisco. 488 pp.
- Nouchi I, Mariko S, Aoki Y (1990) Mechanism of methane transport from the rhizosphere to the atmosphere through rice plants. *Plant Physiol.* 94: 59-66.
- Osman M, Guok H P, Bin Omar O (1987) Hybrid rice breeding in Malaysia. *Mal. J. Appl. Biol.* 16(1):129-138.
- Peng J Y, Virmani S S (1991) Heterosis in some intervarietal crosses of rice. *Oryza* 28:31-36.
- Peng S, Garcia F, Laza R, Cassman K G (1993) Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. *Agron. J.* 85:987-990.
- Peng S, Krieg D K, Girma F S (1991) Leaf photosynthetic rate is correlated with biomass and grain production in grain sorghum lines. *Photo. Res.* 28:1-7.
- Peterson M (1982) Carbohydrate partitioning during grain filling in rice and its significance in breeding for higher yields. Pages 268-282. In: *Proceedings of the Second National Rice Institute Conference, Egypt, 1982*. CAIRO National Rice Institute, Egypt.
- Ponnamperuma F (1976) Temperature and the chemical kinetics of flooded soils. Pages 249-263. In: *Climate and Rice*. Int. Rice Res. Inst., Los Baños, Philippines.
- Ponnuthurai S, Virmani S S, Vergara B S (1984) Comparative studies on the growth and grain yield of some F₁ rice (*Oryza sativa* L.) hybrids. *Philip. J. Crop Sci.* 9(3):183-193.
- Prakash B G, Mahadevappa M (1987) Evaluation of some experimental rice hybrids for field performance and standard heterosis. *Oryza* 24(1):75-78.

- Raven J A, MacFarlane J J, Griffiths H (1987) The application of carbon isotope discrimination techniques. Pages 79-98 In: Crawford, R.M.M. ed. *Plant life in aquatic and amphibious habitats*. British Ecological Society, No. 5. Blackwell Scientific Publ. Oxford.
- Rawson H M, Evans L T (1971) The contribution of stem reserves to grain development in a range of wheat cultivars of different height. *Aust. J. Ag. Res.* 22:851-863.
- Seko H (1962) Studies on lodging in rice plants. (Japanese, English summary). *B. Kyushu Agric. Exp. Stn.* 7:419-499.
- Setter T L, Kupkanchanakul T, Kupkanchanakul K, Bheksut P, Wiengweera A, Greenway H (1987) Concentrations of CO₂ and O₂ in floodwater and in internodal lacunae of floating rice growing at 1-2 m water depths. *Plant, Cell and Envir.* 10:767-776.
- Sinclair T R, Horie T (1989) Leaf nitrogen, photosynthesis, and crop radiation use efficiency: A review. *Crop Sci.* 29:90-98.
- Singh N B, Singh H G, Singh A K (1984) Hybrid vigor for yield and its components in rice. *Genet. Iber.* 36:53-59.
- Sofield I, Evans L T, Cook M G, Wardlaw I F (1977) Factors influencing the rate and duration of grain filling in wheat. *Aust. J. Plant Physiol.* 4:785-797.
- Sondergaard M, Sand-Jensen K (1979) Carbon uptake by leaves and roots of *Littorella uniflora* (L.) Aschers. *Aquatic Bot.* 6:1-12
- Sta. Cruz P, Wada G (1992) Genetic variation in nitrogen uptake by rice and the effects of management and soil fertility. In: *Proceedings of 1992 International Rice Research Conference*. Int. Rice Res. Inst., Los Baños, Philippines.
- Sun Z X, Xiong Z M, Min S K, Si H M (1989) Identification of the temperature sensitive male sterile rice. *Chinese J. Rice Sci.* 3(2):49-55.
- Suprihatno B (1986) Hybrid rice: Its prospects and problems in Indonesia. *Indonesia Agric. Res. Dev. J.* 8(3-4):51-58.
- Takano Y, Tsunoda S (1971) Curvilinear regression of the leaf photosynthetic rate on leaf nitrogen content among strains of *Oryza* species. *Jpn. J. Breed.* 21:69-76.
- Takeda T (1961) Studies on the photosynthesis and production of dry matter in the community of rice plants. *Jpn. J. Bot.* 17:403-437.
- Togari Y, Okamoto Y, Kumura A (1954) Studies on the production and behavior of carbohydrates in rice plant. I. *Proc. Crop Sci. Soc. Japan* 22:95-97.
- Tsuno Y (1971) Mineral content and photosynthesis of leaves. Pages 82-85. In: Y. Tokari, ed. *Photosynthesis and dry matter production*. Yokendo, Tokyo, Japan.
- Tsuno Y, Inaba N, Shimizu T (1959) Studies on yield-forecast in main crops. V. The effect of light receiving condition and nitrogen content in a plant on the dry matter production of rice plants. *Proc. Crop Sci. Soc. (Japan)* 28:188-190.
- Uchida N, Wada Y, Murata Y (1982) Studies on the changes in the photosynthetic activity of a crop leaf during its development and senescence. II. Effect of nitrogen deficiency on the changes in the senescing leaf of rice. *Jpn. J. Crop Sci.* 51:577-583.
- Umbreit W W (1964) Carbon dioxide and bicarbonate. Pages 18-27. In: W.W. Umbreit, R.H. Burris and J.F. Stauffer, ed. *Manometric Techniques, 4th Edn. Ch. 2*. Burgess Publishers: Minneapolis, Minn.
- van Keulen H, Seligman N G (1987) Simulation of water use, nitrogen and growth of a spring wheat crop. Page 48 In: *Simulation Monographs*, Pudoc, Wageningen.

- Virmani S S, Aquino R C, and Khush G S (1982) Heterosis breeding in rice, *Oryza sativa* L. *Theor. Appl. Genet.* 63:373-380.
- Virmani S S, Sharma H L (1994) Manual for hybrid rice seed production. Intl. Rice Res. Institute, Los Baños, Philippines.
- Virmani S S, Young J B, Moon H P, Kumar I, Flinn J C (1991) Increasing rice yield through exploitation of heterosis. *Int. Rice Res. Inst.*, Los Baños, Philippines.
- Virmani S S, Voc P C (1991) Induction of photo and thermo-sensitive male sterility in indica rice. *Agron. Abst.* 119.
- Wada G (1969) The effects of nitrogenous nutrition on the yield determining processes of the rice plant. *Bull. Natl. Inst. Ag. Sci.* 16:27-167.
- Waters L, Armstrong W, Thompson C J, Setter T L, Adkins S, Gibbs J, Greenway H (1989) Diurnal changes in oxygen transport and ethanol metabolism in roots of submerged and non submerged rice seedlings. *New Phytologist* 11:439-451.
- Weber R L, Manning K V, White M W (1965) College physics. McGraw-Hill Book Co., New York, London, Toronto. Fourth Ed. 710 pp.
- Wei M L, Shen M C, Chen C S, Liu D J (1982) Physiological studies of rice tillers. I. Partition of dry matter, nitrogen and total non structural carbohydrates during grain filling. *Proc. Natl. Sci. Counc. ROC (A)* 6:190-196.
- Weng J H, Chen C Y (1991) Effect of accumulated nonstructural carbohydrates on photosynthesis of rice leaves. *Jpn. J. Crop Sci.* 60: 320-321.
- Wium-Anderson S (1971) Photosynthetic uptake of free CO₂ by the roots of *Lobelia dortmanna*. *Physiol. Plant.* 25: 245-248.
- Wu P, Ladha J K, McCouch S R, Teng S B (1992) Use of molecular markers to evaluate rice genetic variation in associative N₂ fixation, nitrogen uptake and nitrogen use efficiency. In: *Proceedings of 1992 International Rice Research Conference*. Int. Rice Res. Inst., Los Baños, Philippines.
- Wu X J, Yin H Q, Yin H (1991) Preliminary study of the temperature effect on Annon S-1 and W 6154S. *Crop Res. China* 5(2):4-6.
- Yambao E B, Ingram K T (1988) Drought stress index for rice. *Phil. J. Crop Sci.* 13: 105-111.
- Yoshida S (1976) Carbon dioxide and yield of rice. In: *Climate and Rice*. IRRI pp. 211-221.
- Yoshida S (1981) *Fundamentals of Rice Crop Science*. Int. Rice Res. Inst., Los Baños, Philippines.
- Yoshida S, Ahn S B (1968) The accumulation process of carbohydrate content in rice varieties in relation to their response to nitrogen in the tropics. *Soil Sci. Plant Nutr.* 14:153-161.
- Yoshida S, Coronel V (1976) Nitrogen nutrition, leaf resistance, and leaf photosynthetic rate of the rice plant. *Soil Sci. Plant Nutr.* 22(2):207-211.
- Young J B, Virmani S S (1990) Heterosis in rice over environments. *Euphytica* 51:87-93.
- Yuan L P, Virmani S S (1988) Status of hybrid rice research and development. Pages 7-24 In: *Hybrid Rice*. Intl. Rice Res. Inst. Los Baños, Philippines.
- Yuan L P, Virmani S S, Mao C X (1989) Hybrid Rice - Achievements and Future Outlook. Pages 219-235. In: *Progress in Irrigated Rice Research*. Int. Rice Res. Inst., Los Baños, Philippines.
- Yuan L P, Yang Z Y, Yang J B (1992) *Hybrid rice in China*. Paper presented at the Second International Symposium on Hybrid Rice, IRRI, Los Baños, April 21-25, 1992 (In press).

Molecular approaches

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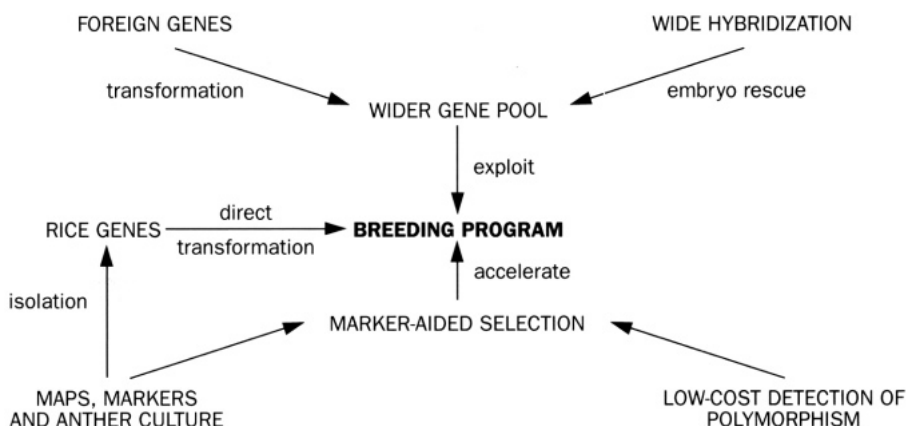
Yield potential shows two of the common attributes of a quantitative trait: it is conditioned by a large number of genes, and its expression is decisively influenced by the environment (Yoshida 1981, Kropff et al 1993, Evans 1993). Until recently, these features rendered quantitative traits difficult to study by molecular techniques, but the advent of a near-saturated genetic map for rice (McCouch et al 1988, Tanksley et al 1992) and of genetic engineering of rice (Shimamoto et al 1989, Christou et al 1991, Shimamoto 1992) has opened up new molecular approaches of considerable power. This paper summarizes IRRI's experience with these techniques and their application to problems other than yield potential, most particularly to the enhancement of host plant resistance to pests and diseases, and then offers some suggestions as to how gene mapping and genetic engineering could help us address yield potential.

Biotechnology at IRRI

IRRI's rice biotechnology program can be summarized in terms of five broad technical approaches that have application in various projects.

- Wide hybridization
- Anther culture
- Mapping of rice nuclear genes and molecular marker-aided selection
- Genetic engineering for the transformation of rice with foreign genes or modified rice genes
- DNA fingerprinting of pests and pathogens.

At present, these five approaches are being exploited in IRRI's program to enhance resistance to biotic and abiotic stresses, and thereby to maximize the attainment of existing yield potential. Wide hybridization and genetic engineering increase the gene pool available to rice breeders. It is now possible through wide hybridization and embryo rescue to access genes from all the wild species of the genus *Oryza*, while genetic engineering opens the door to genes from animals, plants, microorganisms, synthetic genes, and modified genes from rice itself. Gene mapping/marker-aided selection enables IRRI to



1. Biotechnology tools for an enhanced breeding program.

accelerate certain of its breeding programs, especially those that aim to achieve durable stress resistance through the pyramiding of multiple resistance genes (IRRI 1993). Anther culture, through the production of dihaploid lines, accelerates the production of stable breeding lines and mapping populations. DNA fingerprinting permits us to characterize and understand the genetic diversity of populations of pests and pathogens (Leach et al 1992, Levy et al 1991); this understanding has allowed us to formulate new breeding strategies for durable resistance.

The first four of these approaches contribute to the improvement of rice germplasm and are considered in more detail below. The integration of these activities with the breeding program is shown schematically in Fig. 1.

Biotechnology for plant resistance to biotic and abiotic stresses

Through conventional breeding over the last three decades, elite IRRI cultivars have come to possess a wide range of genes for resistance to pests and diseases (Khush 1977, Khush 1989, Bonman et al 1992). These genes were drawn from the primary and secondary gene pools. The primary gene pool consists of the traditional cultivars and landraces of cultivated rice (*Oryza sativa*), whereas the secondary gene pool includes closely related wild species with the AA genome such as *O. nivara*.

Much additional breeding for host plant resistance is required. Resistance frequently breaks down, either because of inadequate screening of lines prior to release, or because of changes in the pest or pathogen populations. IRRI is also placing more effort on breeding for nonirrigated localities and ecosystems, where new problems arise. Finally, some pest and disease problems have simply remained intractable.

Table 1. Application of embryo rescue for the transfer of genes for disease and insect resistance from wild species into rice.

Resistance trait transferred to <i>O. sativa</i> (AA genome)	Donor species (genome)
Brown planthopper	<i>O. officinalis</i> (CC) <i>O. minuta</i> (BBCC) <i>O. latifolia</i> (CCDD) <i>O. australiensis</i> (EE)
Bacterial blight	<i>O. minuta</i> (BBCC) <i>O. latifolia</i> (CCDD) <i>O. australiensis</i> (EE)
Whitebacked planthopper	<i>O. officinalis</i> (CC)
Blast	<i>O. minuta</i> (BBCC)
Yellow stem borer (in progress)	<i>O. brachyantha</i> (FF)

Wide hybridization

IRRI's germplasm collection contains several accessions of distantly related wild *Oryza* species that constitute the tertiary gene pool, and which contain valuable genes for pest and disease resistance. However, the transfer of genes from this gene pool into cultivated rice is normally prevented by the abortion of the hybrid embryos and limited recombination (Khush and Brar 1992). Embryo rescue is a tissue culture technique that enables the hybrids to survive and produce viable plants. Backcrossing to cultivated rice followed by embryo rescue must be repeated several times before fertility and chromosomal stability are restored. The integration of pieces of wild genome into the chromosomes of the elite parent occurs during these cycles (Jena et al 1992, Ishii et al 1994). By this method, several genes have been transferred to cultivated rice from diverse wild species (Jena and Khush 1990, Amante et al 1992, Multani et al 1994). The results of these studies are summarized in Table 1.

Anther culture

New genotypes may also be generated by repeated selfing of a hybrid. At least six cycles of selfing are required to produce stable lines by this method. In areas where only one rice generation is possible per year, anther culture is advantageous because it allows the production of homozygous plants in two generations. Anthers are removed from the floret of the hybrid, sterilized, and placed on a suitable culture medium. A haploid callus develops from the male gametophyte and spontaneously forms dihaploid cells which are homozygous at every locus. Seeds derived from the dihaploid plant are usually viable and give rise to plants that may be evaluated immediately. Early generation selection for recessive traits is one of the major advantages of plants derived from anther culture compared with plants obtained by conventional sexual crossing. Salinity tolerance is one of the traits introduced into elite cultivars at IRRI by this method (Zapata et al 1991; IRRI 1993). However, the efficiency of anther culture is very dependent on genotype (Guiderdoni et al 1992); indica rices are generally much less amenable to anther culture than certain japonica

rices. Anther culture is also important as a source of dihaploid populations for gene mapping.

Molecular markers

In developing improved rice cultivars, IRRI breeders make a large number of crosses to transfer useful genes from donors to elite lines. In the case of genes for resistance to pests and diseases, routine entomology and pathology testing is required to identify progeny containing the desired set of genes. If these genes were tagged with flanking DNA markers, screening for the presence of these genes could be accelerated. Such markers would be especially useful in breeding programs directed against pests and diseases not present at the IRRI farm or only sporadically so, or for genes with overlapping effects (e.g., genes that contribute to a complex and hopefully durable resistance phenotype). The technique most commonly used to identify these marker genes is RFLP analysis (McCouch and Tanksley 1991, Paterson et al 1992). More than 1000 RFLP markers have been placed on the genetic map of rice through co-segregation analysis; the number should soon reach 2000. Traditional morphological markers and isozyme markers are also being placed on the map. Some of the genes for host plant resistance that have been mapped at IRRI and elsewhere are shown in Table 2, along with the DNA marker most closely linked with each gene. Among these are several genes for resistance to the rice blast fungus, and major genes for resistance to bacterial blight and three insects (brown planthopper, whitebacked planthopper, and gall midge) (Ishii et al 1994, McCouch et al 1991a,b, Mohan et al 1994, Ronald et al 1992, Wang et al, unpubl. data, Yoshimura et al 1992, Yu et al 1991).

Table 2. Mapped genes for host plant resistance.

Resistance character	Gene symbol	Chromosomal location	DNA marker
Bacterial blight	<i>Xa-1</i>	4	Npb235
	<i>Xa-3</i>	11	Npb186
	<i>Xa-4</i>	11	Npb181
	<i>xa-5</i>	5	RG207
	<i>xa-8</i>	3	RG944
	<i>xa-13</i>	8	RG136
	<i>Xa-21</i>	11	RG103
Blast	<i>Pi-1</i> (t)	11	Npb181
	<i>Pi-2</i> (t)	6	RG64
	<i>Pi-4</i> (t)	12	RZ397/RG869
	<i>Pi-5</i> (t)	4	RG788
	<i>Pi-zh</i>	8	RG617
	<i>BR14</i>	12	RG869
	<i>BR26</i>	12	RG869
Gall midge	<i>Gm-2</i>	4	RG329
Brown planthopper	<i>Bph</i>	12	RG457
Whitebacked planthopper	<i>Wbh-1</i>	7	RG146

Rice has 12 chromosomes in the haploid set, corresponding to about 430 Mbp of DNA. Current mapping programs are concerned with several abiotic stresses such as salinity, drought and submergence. Mapping results are often immediately applicable to breeding programs (Abenes et al 1993). The inheritance of DNA markers may be monitored in place of phenotypic analyses. Many RFLP markers can be examined simultaneously using the same sample of extracted DNA. Very little tissue (1g) is required for DNA extraction if polymorphisms can be detected by one of the available PCR-based techniques exploiting sequence tagged sites (Williams et al 1991, Tragoonrung et al 1992).

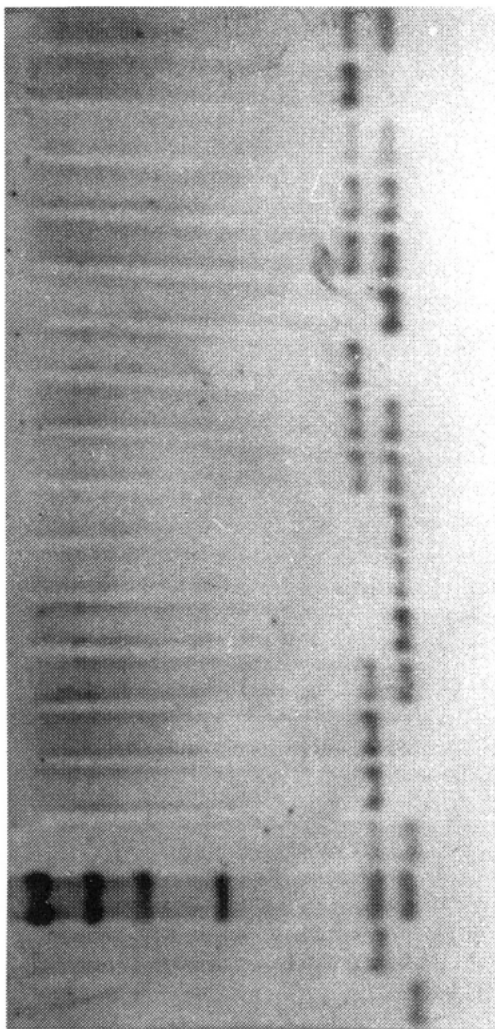
It is possible to tag genes without reference to a map. A technique known as bulked segregant analysis is particularly powerful for tagging a major gene, and may be used with RFLP markers or with so-called RAPD (randomly amplified polymorphic DNA) markers (Michelmore et al 1991). However, it is highly unlikely that the first RFLP or RAPD marker shown to cosegregate with an important gene will be the closest marker. A map provides a logical framework for finding closer markers.

The use of RFLP analysis in mapping the BPH resistance gene introgressed by wide hybridization from *O. australiensis* (Table 1) is illustrated in Figure 2. Monosomic alien addition line analysis indicated that this gene was located on chromosome 12. Only marker RG457 of the 14 polymorphic probes for chromosome 12 detected introgression from *O. australiensis* into *O. sativa* (Ishii et al 1994). Cosegregation between BPH resistance and RG457 in F_2 showed that the gene for BPH resistance is linked to RG457 at a distance of 3.68 cM. Such close linkage is useful for marker-aided selection of BPH resistance. Note that RFLP analysis indicates that the mechanism of alien gene transfer involves multiple genetic recombination events rather than substitution of whole chromosomes or large chromosomal segments (Ishii et al 1994, Jena et al 1992).

IRRI participates in the international efforts to integrate the existing genetic maps of rice in order to provide researchers throughout the world with a single, well-saturated map of the rice genome (Tanksley et al 1992, Kishimoto et al 1993). This effort includes orienting the RFLP maps developed independently at Cornell (USA) and Tsukuba (Japan), locating isozyme and morphological or physiological genetic loci on that map via linkage to existing DNA markers, adding RAPD markers to underpopulated regions of the genome, and developing new kinds of markers aimed at increasing the ease of use and degree of genetic variation detectable by available markers. The IRRI/Cornell and IRRI/Japan Shuttle Programs play a central role in the integration of rice genome data.

Many of the insect pests and viral, bacterial, and fungal pathogens of rice show considerable geographical and temporal diversity. This diversity is usually manifested in terms of differences in virulence and host range. Breeders, entomologists and pathologists respond to this diversity by screening germplasm for new donors of resistance. Application of RFLP and RAPD analysis to the genomes of rice pests and pathogens (Leach et al 1992, Levy et al 1991) is beginning to reveal patterns of relationship or lineages, which are

P₁ P₂ M | | | **F₂** |
 R S R/S S R/S R R R/SR/SR/S S R R/SR/SR/S S R/S



RG457

2. RFLP pattern in the F₂ population of a cross of a BPH-resistant introgression line (P₁) with the recurrent parent (P₂), IRRI 1992. Total DNA was digested with *Eco*RI and probed with RG457 on chromosome 12. Also indicated are the results of BPH resistance tests performed on F₃ progeny of the 16 F₂ plants. R = true breeding for resistance, S = true breeding for susceptibility, R/S = segregating for resistance. The seventh F₂ individual from the marker lane shows the only recombination event between phenotype and genotype for these 16 F₂ individuals.

likely to be important to our eventual understanding of how pests and pathogens evolve, and how new resistant varieties of rice might best be deployed.

Genetic engineering

Genetic engineering permits the introduction into rice of alien genes isolated from organisms that cannot hybridize with rice (Christou et al 1991, Shimamoto 1992). It permits also the reintroduction of rice genes that have been extracted and modified to give altered properties (Shimamoto 1993, Shimada et al 1993). Such gene transfers are beyond the capability of conventional breeding methods. An additional feature of genetic engineering is that it introduces one or two well-characterized genes at a time and therefore does not require the extensive backcrossing needed after conventional sexual hybridization. It does not introduce a large number of undesirable genes along with the gene(s) of interest, or break up polygenic traits laboriously assembled in the recipient cultivar.

IRRI's genetic engineering program involves three phases: 1) developing the capacity to transfer foreign genes into rice, 2) demonstrating that foreign genes function in rice, and 3) fine-tuning the technology to ensure that the foreign genes behave like rice genes and enhance the performance of the plant in the desired manner. At present, IRRI is engaged in phase 1 but is moving rapidly to phase 2, as many foreign genes become available (IRRI 1993).

The genes being used in phase 1 of the genetic engineering program are bacterial genes. The hygromycin phosphotransferase (*hph*) gene from *Escherichia coli* confers resistance to the antibiotic hygromycin B on transformed rice cells (Zheng et al 1991). This gene is used currently as the principal selectable marker gene in rice transformation at IRRI. It is employed either on the same plasmid as the gene of interest (cointegrate mode) or on a separate plasmid mixed with the plasmid containing the gene of interest (co-transformation). Among the selectable marker genes employed with rice in other laboratories (Toki et al 1992, Cao et al 1992, Rathore et al 1993) is the phosphinothricin acetyltransferase (*bar*) gene from *Streptomyces hygroscopicus*, encoding resistance to the herbicide phosphinothricin. In principle, this gene could be utilized to develop herbicide-resistant varieties of value in areas where direct seeding leads to competition with weeds. However, such varieties are unlikely to be released because of the danger that cross-pollination would allow the herbicide resistance gene to escape into local populations of weedy and wild rices and negate the original strategy. IRRI has stated that it will not develop herbicide-resistant varieties, but we may use the *bar* gene as a selectable marker gene for transformation and then eliminate it by classical segregation. A 400 m² transgenic greenhouse is under construction at IRRI to conform with the requirements of the Philippine Biosafety Guidelines. IRRI is also making extensive use of the β -glucuronidase (*gus*) gene from *E. coli* (Jefferson et al 1987), for monitoring transformation efficiency and for studies on promoter function in rice.

IRRI has succeeded in transforming both japonica and indica rice varieties. First successes were achieved in 1991 with protoplast-based transformation, but since February 1993 the pace of success with transformation has accelerated through the use of biolistic transformation. Among the varieties transformed at IRRI by these methods are IR43, IR58, IR57311, Zhong Hua 6 and 10 (from China), Tepi Boro (from Bangladesh), V7 and V9 (from Vietnam), and Anjung Byeo (from Korea).

Just as the wide hybridization program has focused on transferring several genes for pest and disease resistance to cultivated rice, so the first group of useful foreign genes to be put into rice are also intended to enhance pest and disease resistance. These genes include two bacterial genes, the *CryIIA* and *CryIA(b)* endotoxin genes of *Bacillus thuringiensis*, for insect resistance, the soybean trypsin inhibitor also for insect resistance, and two barley genes for resistance to fungal diseases such as sheath blight and blast—the chitinase gene and the ribosome inactivating protein (RIP) gene (IRRI 1993). IRRI also has an important program to enhance submergence tolerance in rice through the manipulation of alcoholic fermentation (IRRI 1993). In addition, a bacterial mannitol 1-phosphate dehydrogenase gene that has provided enhanced salinity tolerance in mature transgenic tobacco through production of linear polyols (Tarcynski et al 1993) will shortly be introduced into rice.

An increasing number of agronomically relevant genes have been introduced into rice to date by transformation, including several genes encoding potentially insecticidal or fungicidal proteins. Fujimoto et al (1993) introduced a *CryIA(b)* δ -endotoxins produced by the bacterium *Bacillus thuringiensis*. Transgenic plants expressing this gene show enhanced resistance to striped stem borer and leaffolder, two of the common lepidopteran pests of rice. The gene used in this case was not the authentic gene from *B. thuringiensis*, but a chemically synthesized gene designed to encode the same amino acid sequence as the authentic gene but with different codons to take account of the differences between rice cells and sporulating bacillus cells in their machinery for protein synthesis. The coat-protein cross-protection approach has proved successful for the rice streak virus, a positive-strand RNA virus (Hayakawa et al 1992). A cDNA from rice dwarf virus genome segment 10 has been moved into rice but its expression level has not been reported (Matsumura et al 1992).

IRRI is involved in testing several transgenic rice plants produced in other laboratories. Yellow stem borer resistance will be studied not only in the above-mentioned plants expressing the synthetic *CryIA(b)* gene, but also in plants expressing various proteinase inhibitors. Tungro virus resistance will be studied in plants expressing the coat protein gene from the DNA-containing rice tungro bacilliform virus (RTBV).

Biotechnology and the basis of yield potential

Tagging quantitative trait loci

The application of marker-aided selection to yield potential is made difficult by the fact that yield is a quantitative trait. It will therefore be necessary to develop larger mapping populations and to use more markers than would be necessary for the mapping of major genes governing pest and disease resistance (Lander and Botstein 1989, Paterson et al 1992). IRRI is accumulating experience with the mapping of quantitative trait loci (QTL) through its current work on QTL for bacterial blight resistance, blast resistance, and drought tolerance (Wang et al, unpublished data). Anther culture will permit the rapid development of dihaploid mapping populations that are highly suitable for detection of QTL. IRRI has already acquired both the molecular and the computational techniques required for QTL analysis, including SAS and Map-Maker QTL software (Lander and Botstein 1989).

Yield and other quantitative traits are often greatly affected by changes in environmental factors ("genotype-environment interactions"). For this reason, gene mapping will be performed in a wide range of environments to identify the environmental sensitivity of individual QTL. Large-scale studies of this type will be facilitated by the recent formation of the Asian Rice Biotechnology Network (ARBN), which will permit scientists in different Asian countries to share mapping populations and thereby jointly characterize major genes and QTL conditioning yield factors. An important benefit of this approach is that it maximizes the payoff derived from the molecular analysis of each mapping population. Marker-based QTL analysis of yield components have been the subject of recent papers on tomato fruit solids content (Paterson et al 1991) and maize (Edwards et al 1992, Stuber et al 1992). Environmental effects on specific QTLs were very evident in the case of tomato (Paterson et al 1991), but not for maize (Stuber et al 1992). The study of Edwards et al (1992) was especially interesting because of their ability to map QTL for 18 different characters related to yield with the one mapping population.

Plant transformation and the determinants of yield potential

Transformation of rice (Shimamoto et al 1989, Cao et al 1992, Christou et al 1991) enables us to introduce single genes that can selectively perturb yield-determining factors. Related studies have already been performed on dicotyledonous plants that are more readily transformed than rice. A key role for sucrose phosphate synthase (SPS) in regulating carbon partitioning between sucrose and starch in leaves was established through the expression in tomato of the SPS gene from maize (Worrell et al 1991). Starch levels and dry matter accumulation were enhanced in potato tubers transformed with the *E. coli* gene encoding ADP-glucose pyrophosphorylase (Stark et al 1992). In both of the above cases, the enzyme encoded by the foreign gene was not regulated in the same way as its endogenous counterpart and alterations in carbohydrate metabolism ensued.

The antisense approach has also been employed: Muller-Rober et al (1992) showed that potato tubers expressing an antisense ADP-glucose pyrophosphorylase, were smaller, more numerous, contained less starch and storage protein and more sugar than controls. Hofgen and Willmitzer (1992) showed that transgenic potato plants expressing an antisense construct of the major tuber protein patatin were grossly depleted in the protein but were otherwise indistinguishable from control plants. A series of papers has appeared in which rubisco, the CO₂-fixing enzyme of photosynthesis, was down-regulated through the antisense approach directed against the expression of the gene encoding the enzyme's small subunit (Quick et al 1992). This genetic alteration affects several aspects of chloroplast metabolism, including photosynthetic rate and nitrogen allocation among chloroplast proteins.

Several other aspects of plant metabolism affecting processes related to yield have also been manipulated by transformation. Through this powerful approach, we can identify rate-determining and other regulatory steps in the metabolism of source and sink tissues, and in transport between tissues. Two groups have altered starch metabolism in rice itself by introducing antisense constructs directed against the *waxy* gene of rice (Shimamoto 1992, Shimada et al 1993). This gene encodes the starch granule-bound starch synthetase. Reduction in *waxy* gene expression by antisense RNA led to reduced amylose content in rice grains. Comparable results have been seen for tuber starch in potato plants transformed with a similar construct (Visser et al 1991).

Maize transposable elements *Ac* (Murai et al 1991) and *Ds* (Shimamoto et al 1993) have been introduced into rice and are mobile. These developments raise the prospect of using the mutagenic effect of mobile elements to disrupt genes affecting specific phenotypes, including yield. Compared with the approaches mentioned earlier in this section, the use of mobile elements has the advantage of not involving any assumptions as to the nature of the genes affecting yield. Because the sequence of the mobile elements is known, they can be used to isolate the disrupted gene and begin the process of identifying the metabolic role of the gene product. The chief disadvantage of the method is the low probability of disrupting the key genes governing yield.

Key areas that could involve future research on abiotic stresses include:

- enhancing nutrient uptake or modification of root characters,
- carbon assimilation in leaves at low light,
- carbon assimilation from the soil by roots, and
- low or high temperature tolerance of reproductive organs.

Biotechnology options for increasing yield through hybrid rice production

To exploit the yield advantage of hybrid rice fully, it is currently necessary to use female parents displaying cytoplasmic male sterility (CMS) (see Setter et al, These Proceedings). One danger inherent in the use of CMS is that relatively few sources of stable CMS are available, especially in the tropics. The resulting lack of diversity in the cytoplasm of hybrid rices is regarded as a matter for concern, given the well-known susceptibility of maize plants containing the

mitochondrial DNA of the Texas cytoplasm to late blight disease. One option is to transfer novel male-sterile cytoplasms from wild species into cultivated rice by using the wild rice as female parent. This transfer has already been achieved in the case of the CMS character from *O. perennis* (IRRI 1993). Like *O. sativa*, *O. perennis* possesses the AA nuclear genome but it contains a distinct mitochondrial genome.

A second option has been pioneered by Mariani et al (1992), who were the first to develop a novel form of reversible genetic (nuclear) male sterility based on a two-gene system engineered into tobacco and brassica. One gene (encoding a ribonuclease) causes male sterility, whereas the second gene (encoding a ribonuclease inhibitor) blocks the effect of the first gene. Application of this, or an equivalent system, in rice should give the breeder greater flexibility in hybrid rice production, provided that the male fertile and male sterile states are completely stable in the diverse environments in which rice is grown.

References cited

- Abenes M L P, Angeles E R, Khush G S, Huang N (1993) Selection of bacterial blight resistant rice plants in the F₂ generation via their linkage to molecular markers. Rice Genet. Newslett. (in press).
- Amante A D, Sitch L A, Nelson R, Dalmacio R D, Oliva N P, Aswidinoor H, Leung H (1992) Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa*. Theor. Appl. Genet. 84:345-354.
- Bonman J M, Khush G S, Nelson R J (1992) Breeding rice for resistance to pests. Annu. Rev. Phytopathol. 30:507-528.
- Cao J, Duan X, McElroy D, Wu R (1992) Regeneration of herbicide resistant transgenic rice plants following microprojectile-mediated transformation of suspension culture cells. Plant Cell Reports 11:586-591.
- Christou P, Ford T L, Kofron M (1991) Production of transgenic rice (*Oryza sativa* L.) plants from agronomically important indica and japonica varieties via electric discharge particle acceleration of exogenous DNA into immature zygotic embryos. Biotechnology 9:957-962.
- Edwards M D, Helentjaris T, Wright S, Stuber C W (1992) Molecular-marker-facilitated investigations of quantitative trait loci in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. Theor. Appl. Genet. 83:765-774.
- Evans L T (1993) Crop evolution, adaptation, and yield. Cambridge Univ. Press, London.
- Fujimoto H, Itoh K, Yamamoto M, Kyojuka J, Shimamoto K (1993) Insect resistant rice generated by introduction of a modified d-endotoxin gene of *Bacillus thuringiensis*. Biotechnology 11:1151-1155.
- Guiderdoni E, Galinato E, Luistro J, Vergara G (1992) Anther culture of tropical japonica x indica hybrids of rice (*Oryza sativa* L.). Euphytica 62:219-224.
- Hayakawa T, Zh Y, Itoh K, Kimura Y, Izawa T, Shimamoto K, Toriyama S (1992) Genetically engineered rice resistant to rice stripe virus, an insect-transmitted virus. Proc. Natl. Acad. Sci., USA. 89:9865-9869.

- Hofgen R, Willmitzer L (1992) Transgenic potato plants depleted for the major tuber protein patatin via expression of antisense RNA. *Plant Science* 87:45-54.
- IRRI (1993) Cross-ecosystem research - Subprogram I In: Program Report for 1992. International Rice Research Institute, Manila, Philippines, pp. 137-164.
- Ishii T, Brar D S, Multani D S, Khush G S (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa*. *Genome* 37 (in press).
- Jefferson R A, Kavanagh T A, Bevan M W (1987) GUS fusion: β -glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* 6:3901-3907.
- Jena K K, Khush G S (1990) Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice, *O. sativa* L. *Theor. Appl. Genet.* 80:737-745.
- Jena K K, Khush G S, Kochert G (1992) RFLP analysis of rice (*Oryza sativa* L.) introgression lines. *Theor. Appl. Genet.* 84:125-128.
- Kishimoto N, Foolad M R, Shimosaka E, Matsuura S, Saito A (1993) Alignment of molecular and classical linkage maps of rice, *Oryza sativa*. *Plant Cell Reports* 12:457-461.
- Khush G S (1977) Disease and insect resistance in rice. *Adv. Agron.* 29:265-341.
- Khush G S (1989) Multiple disease and insect resistance for increased yield stability in rice. In: Progress in irrigated rice research. International Rice Research Institute, Los Baños, Philippines. pp. 79-92.
- Khush G S, Brar D S (1992) Overcoming barriers in hybridization. Distant hybridization of crop plants. *Theor. Appl. Genet. (monogr.)* 16:47-61.
- Kropff M J, Cassman K G, van Laar H H, Peng S (1993) Nitrogen and yield potential of irrigated rice. pp. 533-536. In N.J. Barrow, ed. Developments in Plant and Soil Science Vol. 54, Plant Nutrition: from Genetic Engineering to Field Practice. Kluwer Academic Publishers, Dordrecht.
- Lander E S, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185-199.
- Leach J E, Rhoads M L, Vera Cruz C M, White F F, Mew T W, Leung H (1992) Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* using a repetitive DNA element. *Appl. Env. Microbiol.* 58:2188-2195.
- Levy M, Romao J, Marchetti M A, Hamer J E (1991) DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. *Plant Cell* 3:95-102.
- Mariani C, Gosele V, DeBeuckeleer M, DeBlock M, Goldberg R B, DeGreef W, Leemans J (1992) A chimaeric ribonuclease-inhibitor gene restores fertility to male sterile plants. *Nature* 357:384-387.
- Matsumura T, Tabayashi N, Uyeda I, Kimura, Shikata E (1992) Production of transgenic rice by introducing rice dwarf virus genome segment 10 cDNA. *J. Fac. Agr. Hokkaido Univ.* 65:343-349.
- McCouch S R, Kochert G, Yu Z H, Wang Z Y, Khush G S, Coffman W R, Tanksley S D (1988) Molecular mapping of rice chromosomes. *Theor. Appl. Genet.* 76:815-829.
- McCouch S R, Abenes M L, Angeles R, Khush G S, Tanksley S D (1991a) Molecular tagging of a recessive gene, *xa-5*, for resistance to bacterial blight of rice. *Rice Genet. Newslett.* 8:143-145.

- McCouch S R, Khush G S, Tanksley S D (1991b) Tagging genes for disease and insect resistance via linkage to RFLP markers. In: Rice Genetics II. International Rice Research Institute, Los Baños, Philippines, pp. 443-449.
- McCouch S R, Tanksley S D (1991) Development and use of restriction fragment length polymorphism in rice breeding and genetics. In: G. S. Khush and G. H. Toenniessen eds. Rice Biotechnology, CAB International, Wallingford, pp. 109-133.
- Michelmore R W, Paran I, Kesseli R V (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA 88:9828-9832.
- Mohan M, Nair S, Bentur J S, Prasada Rao U, Bennett J (1994) RFLP and RAPD mapping of the rice *Gm2* gene that confers resistance to biotype 1 of gall midge (*Orseolia oryzae*). Theor. Appl. Genet. (in press).
- Muller-Rober B, Sonnewald U, Willmitzer L (1992) Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage proteins. EMBO J. 11:1129-1138.
- Multani D S, Jena K K, Brar D S, de los Reyes B G, Angeles E R, Khush G S (1994) Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin. to cultivated rice *O. sativa* L. Theor. Appl. Genet. (in press)
- Murai N, Li Z, Kawagoe Y, Hayashimoto A (1991) Transposition of the maize activator element in transgenic rice plants. Nucleic Acids Res. 19(3):617-622.
- Paterson A D, Damon S, Hewitt J D, Zamir D, Rabinowitch H D, Lincoln S E, Lander E S, Tanksley S D (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127:181-197.
- Paterson A H, Tanksley S D, Sorrells M E (1992) DNA markers in plant improvement. Advances in Agronomy 46:39-90.
- Quick W P, Fichtner K, Schulze E D, Wendler R, Leegood R C, Mooney H, Rodermel S R, Bogorad L, Stitt M (1992) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" *rbcS*. Planta 188:522-531.
- Rathore K S, Chowdhury V K, Hodges T K (1993) Use of *bar* as a selectable marker gene and for the production of herbicide-resistant rice plants from protoplasts. Plant Mol. Biol. 21:871-884.
- Ronald P C, Albano B, Tabien R, Ahenes L, Wu K S, McCouch S, Tanksley S D (1992) Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol. Gen. Genet. 236: 113-120.
- Shimada H, Tada Y, Kawasaki T, Fujimura T (1993) Antisense regulation of the rice *waxy* gene expression using a PCR-amplified fragment of the rice genome reduces the amylose content in grain starch. Theor. Appl. Genet. 86:665-672.
- Shimamoto K (1992) Genetic manipulation of rice: from protoplasts to transgenic plants. Jpn. J. Genet. 67:273-290.
- Shimamoto K, Terada R, Izawa T, Fujimoto H (1989) Fertile transgenic rice plants regenerated from transformed protoplasts. Nature 338:274-276.
- Shimamoto K, Miyazaki C, Hashimoto H, Izawa T, Itoh K, Terada R, Inagaki Y, Iida S (1993) *Trans*-activation and stable integration of the maize transposable element *Ds* cotransfected with the *Ac* transposase gene in transgenic rice plants. Mol. Gen. Genet. 239:354-360.

- Stark D M, Timmerman K P, Barry G F, Preiss J, Kishore G M (1992) Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase. *Science* 258:287-292.
- Stuber C W, Lincoln S E, Wolff D W, Helentjaris T, Lander E S (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* 132:823-839.
- Tanksley S D, Causse M, Fulton T, Ahn N, Wang Z, Wu K, Xiao J, Yu G, Second G, McCouch S (1992) A high density molecular map of the rice genome. *Rice Genet. Newslett.* 9:111-115.
- Tarczynski M C, Jensen R G, Bohnert H J (1993) Stress protection in transgenic tobacco producing a putative osmoprotectant, mannitol. *Science* 259:508-510.
- Toki S, Takamatsu S, Nojiri C, Ooba S, Anzai H, Iwata M, Christensen A H, Quail P H, Uchimiya H (1992) Expression of a maize ubiquitin gene promoter-*bar* chimeric gene in transgenic rice plants. *Plant Physiol.* 100:1503-1507.
- Tragoonrun S, Kanazin V, Hayes P M, Blake T K (1992) Sequence-tagged-site-facilitated PCR for barley genome mapping. *Theor. Appl. Genet.* 84:1002-1008.
- Visser R F G, Somhorst I, Kuipers G J, Ruys N J, Feenstra W J, Jacobsen E (1991) Inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs. *Mol. Gen. Genet.* 225:289-296.
- Williams M N V, Pande N, Nair S, Mohan M, Bennett J (1991) Restriction fragment length polymorphism analysis of polymerase chain reaction products amplified from mapped loci of rice (*Oryza sativa* L.) genomic DNA. *Theor. Appl. Genet.* 82:489-498.
- Worrell A C, Bruneau J M, Summerfelt K, Boersig M, Voelker T A (1991) Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning. *Plant Cell* 3:1121-1130.
- Yoshida S (1981) Fundamentals of rice crop science. International Rice Research Institute, Los Baños, Philippines, pp. 267.
- Yoshimura S, Yoshimura A, Saito A, Kishimoto N, Kawase M, Yano M, Nakagahra M, Ogawa T, Iwata N (1992) RFLP analysis of introgressed chromosomal segments in three near-isogenic lines of rice for bacterial blight resistance. *Japan. J. Genet.* 67:29-37.
- Yu Z H, Mackill D J, Bonman J M, Tanksley S D (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. *Theor. Appl. Genet.* 81:471-476.
- Zapata F J, Alejar M S, Torrizo L B, Novero A U, Singh V, Senadhira D (1991) Field performance of anther culture-derived lines from F₁ crosses of indica rices under saline and non-saline conditions. *Theor. Appl. Genet.* 83:6-11.
- Zheng A, Hayashimoto A, Li Z, Murai N (1991) Hygromycin resistance gene cassettes for vector construction and selection of transformed rice protoplasts. *Plant Physiol.* 97:832-835.

Summary

IRRI scientists began a major effort in 1989 to develop a new concept of a rice plant type that would break through the present yield barrier. Five years on, suitable rice germplasm has been identified, and selections and crosses from that germplasm have been made to generate agronomically fit lines for testing. The selection criteria for the new plant type included large panicles and high spikelet number per panicle, regulated tillering habit, sturdy stems, dark green and erect leaves, and a growth duration of 110-130 days. Elite genotypes that have these traits will be ready for field testing in replicated trials in the 1994 dry season.

The rationale for the new plant type hinged primarily on the need to increase sink size, with some increase in assimilate supply hypothesized to come from greater carbohydrate storage and remobilization from thicker stems, and less waste from unproductive tillers that are over-expressed in current indica varieties. But the contribution of any additional stem reserves in the new plant type will be offset by the reduction in stem number from the reduced tillering habit, and the magnitude of such an increase would not by itself support a large increase in grain yield. Likewise, wasted assimilate in unproductive tillers of present varieties represents a relatively small potential pool of assimilate, because maximum tiller expression occurs early in the growing season, when total biomass is small.

With a goal of increasing rice yield potential by 30-50%, the source of additional assimilate required to support such a yield increase remains a key issue. At the heart of this question is whether the yield potential of present varieties is source or sink limited, because a relatively large portion of total spikelets in current varieties do not fill when yields of 10-11 t/ha are achieved. Similarly, assimilate supply patterns during grain filling do not indicate a surplus to support a substantial increase in sink size.

Opportunities to increase assimilate supply include the extension of the grain-filling period with a concomitant delay in leaf senescence, lower panicle position to increase light interception by the upper leaf canopy, and heterotic vigor from indica hybrids and perhaps from indica x japonica hybrids. These opportunities are presently receiving the major research emphasis at IRRI, and this effort must be expanded to include a broader collaboration with other institutions if we are to be successful, particularly with regard to the mecha-

nisms that govern senescence and N metabolism in the leaf, photo-protection mechanisms that inhibit photosynthesis, and the possibility of manipulating the biochemical components of photosynthesis for greater efficiency.

Selection of appropriate targets for molecular approaches to increase yield potential is also crucial. Based on the uncertainties that arise from our present understanding of yield potential, however, it is difficult to identify the most promising targets for these powerful new tools. On the other hand, molecular approaches are rapidly expanding our knowledge of key processes and mechanisms regulating photosynthetic apparatus and assimilate transport and storage. This knowledge may soon help identify the key targets for molecular manipulation. Certainly the identification of sources of resistance to sheath blight and stem rot diseases, which does not exist in rice germplasm, and transfer to the rice plant, would be a significant contribution from molecular biology to the goal of higher rice yield potential in tropical environments.

In a sense we are fortunate that there remains a relatively large margin between the present rice yield potential of 10 t/ha and average irrigated rice yields of less than 5 t/ha. But this exploitable yield gap will diminish rapidly and disappear early in the next century unless we are successful in breaking through the present yield potential threshold.

Part II

Extended Abstracts of Invited Papers

Optimizing crop-environment relations

R.S. Loomis*

Crop yields are subject to a number of obvious limitations. Foremost are the amount of sunlight received and the capability of plants for photosynthesis. Yield is further limited through partitioning of crop biomass between productive structures necessary for photosynthesis and resource acquisition on the one hand, and economically useful parts on the other. Additional limitations are found in the seasonal patterns and variability of weather, nutrient supply, and farming practice. Where water and nutrients are nonlimiting, the largest yields are generally obtained through rapid attainment of full light interception, so that the crop is source-limited (sink-dominated) for most of its growth cycle.

It appears increasingly unlikely that yield advances can be made through some drastic improvement in leaf photosynthetic capability. A key goal for yield improvement, then, is to improve the fit between phenotype and environment.

Need for optimization

A crop that meshes well with its environment generally provides a greater yield, stability, or quality than one that fits poorly. Poor fit may occur because a crop fails to match the length of the growing season, or because it encounters difficulties with the range or sequence of other factors. Satisfactory meshing distinguishes adapted species and cultivars from those that are not adapted.

Optimization of partitioning is a key feature in the design of ideotypes. It is particularly important to achieve a smooth progression through developmental phases of the crop's life cycle. Although ideotypes can be defined for each environment, we need to keep in mind that environments can also be optimized (e.g., density, nutrient level, or planting date) to suit the phenotype. It will become increasingly important to give greater attention towards fine tuning (i.e., optimization) of the fit between genotype and environment.

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Constraints to optimization

Progress towards ideotypes is subject to a number of constraints. Our knowledge base about adaptive controls of growth and development, for example, is very weak. It also seems that most basic metabolic systems (e.g., photosynthesis, respiration, biosynthesis) represent “efficient evolutions” with little room for further removal of slack in the use of carbon. It remains to be seen whether maintenance activities are also efficient.

The conservation law imposes a fundamental constraint on optimization because carbon, once acquired in reduced form and used by a plant, cannot be used again without energy loss. Respired carbon, for example, is lost completely from the reduced pool, and if a crop’s first use of new assimilate is inappropriate (e.g., too small or too great an investment in leaves, stems or tillers), a loss of seasonal efficiency is inevitable. Selection for specific changes in partitioning (larger/smaller, sooner/later) must affect the rest of the plant through feedback. A key point derived from the conservation law is that yield is a highly polygenic trait. An important corollary is that yield level is the basis from which we establish efficiency of a crop’s use of scarce resources, including human labor.

Because weather and farming practices vary spatially and temporally, optimal meshing is not a straightforward proposition. It must be defined statistically from performance over years and across regions. An additional requirement for high-yielding crops then is that they must vary their patterns of growth and development in ways that make the best use of current circumstances. Such plasticity in carbon partitioning depends in part on feedback control in response to environment, and is one of the more difficult goals in crop improvement.

Quantitative methods for crop ideotype-system designs

Optimal designs for crops and cropping systems must deal on the one hand with great complexity and on the other with the fact that, in agriculture, small differences are important. A 5% change in system performance can be of enormous benefit, yet be very difficult to measure. Coupled with the conservation law, the small-is-important problem points to the need for quantitative tools in ideotype design.

The word “optimum” was coined by biologists in the last century to describe the best conditions for a plant’s growth, but it was quickly adopted and extended by engineers and economists in reference to the best way to control complex systems found in their domains. In Americanese, looking for the optimum became “optimization”. A consequence of looking for the optimal way to run a system is that defects in structure are quickly revealed. That is to say, the same tools can be used in the design of better systems. Those disciplines have now developed formal mathematical approaches including “linear programming” and “optimal control theory” for these purposes.

A few biologists (e.g., Paltridge, Iwasa, Roughgarden, and Cohen) have applied analytical mathematics and techniques of linear programming with some success to several “large” evolutionary questions about plants. Among these are relative advantages of annual vs perennial habit and determinate vs indeterminate development in relation to environment. These mathematical methods can be quickly overwhelmed, however, by nonlinear controls and the fine details of partitioning. Ian Cowan’s (1986) masterful analysis of optimal rooting and stomatal tactics for crops supplied by uncertain rainfall provides insight to the rewards obtained when such detail can be embraced mathematically. His paper bears careful study by serious students of ideotype design.

Quantitative designs for fine tuning of crops such as rice for yield advance will require dynamic models of much greater power than can be found in sets of simple analytical equations (Loomis 1993). Multilevel simulation models, using numerical methods, can embrace much more physiological and morphological detail in explaining yield formation. They thus occupy the middle level between visible crop traits and molecular controls. Such models provide a powerful tool for theoretical evaluation of complex plant traits that influence yield, for example, the optimum distribution of nitrogen among foliage elements or the optimum branching pattern in potato.

Although competent quantitative models are clearly a powerful tool in ideotype analysis, it remains to be seen how much they will contribute. Not many crop models can yet be described as sufficiently “competent.” Given that yield trials will remain the basic tool for yield advance, demonstration by crop models of the importance of even flow in development, for example, suggests new types of screens that look to avoid periods of excessive accumulation of assimilate or down-regulation of photosynthesis.

References cited

- Cowan I (1986) Economics of carbon fixation in higher plants. Pages 133-213 in T.J. Givnish, ed. *On the economy of plant form and function*. Cambridge University Press, Cambridge.
- Loomis R S (1993) Optimization theory and crop improvement. Pages 583-588 in D.R. Buxton et al., ed. *International Crop Science I*. Crop Sci. Soc. Am., Madison, Wisconsin.

Eco-physiological aspects of raising the yield plateau of irrigated rice in the tropics

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Yield of irrigated rice in the tropics has increased little, if at all, since IR8 was released. Yield increase of IR cultivars was attributed to the harvest index (HI) improvement in which two steps were observed (Akita 1988). The first was the introduction of semi-dwarf genes and the second was the utilization of heterosis. Further opportunities to increase yield potential through continued improvement in HI were thought to include reducing hull weight, improving spikelet formation efficiency, and increasing biomass production (Akita 1989). However, increasing nitrogen uptake by the rice crop did not result in higher yields, despite higher growth rates. Thus, heterosis was proposed as the most promising means for increasing biomass production. Recently, IRRI proposed a new irrigated rice plant type that would give up to 30% higher yields (IRRI 1992). This new plant type is characterized by low tillering and many more grains per panicle. The theoretical basis for the proposed semidwarf heavy-panicle type needs to be elucidated for further development of high-yielding cultivars. Raising the yield plateau of irrigated rice in the tropics is discussed from an eco-physiological viewpoint.

Eco-physiological traits for increased yield

IRRI proposed increased photosynthetic efficiency, decreased respiration loss, and increased storage of starch in the grain as the key physiological processes for increased yield potential (IRRI 1993). The first two are closely related to the improvement of biomass production and the last is related to increased HI. However, a further increase in yield potential is dependent on greater N assimilation, but present inbred cultivars cannot utilize increases in plant N efficiently to achieve higher yield potential. Thus, strategies to increase biomass production must be independent of the need for greater N absorption. A further increase in HI is one way to achieve higher yield potential.

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Harvest index

Total biomass production is higher in cultivars with longer growth duration, but is accompanied by a sharp decrease in HI. Thus, there is an opportunity to further increase the HI of long-duration cultivars, whereas there is little scope for cultivars with shorter growth duration in the tropics. Maintenance of higher net energy fixation in the ripening stage (CGR_r) has been the primary approach to achieve higher HI in long-duration cultivars. However, when crop growth rates are increased by increasing plant N supply, there is a strong negative relationship between the vegetative growth rate and CGR_r.

Accumulation of nonstructural carbohydrate and stored nitrogenous compounds is the basis for increased HI in longer duration cultivars, i.e., greater accumulation of these translocatable substances was associated with an increase in net assimilation during the vegetative growth stage. This growth strategy is similar to the yield development of tuber crops in which energy accumulation begins in the vegetative organs and results in a high HI due to a prolonged filling period of the harvested organ.

The positive contribution of heavy panicle type to higher yield potential was supported by the experiences of Korean and Chinese high-yielding semi-dwarf rice cultivars (Akita et al, unpubl. data), and by wheat cultivars (Mizuochi 1988). Heavy panicle cultivars such as IR29723-123-1-1, IR9761-19-1, and most F₁ hybrids also tended to show higher yield than the high panicle number type cultivars in field experiments conducted at IRRI (Akita 1988), although the mechanism responsible for higher yield of the heavy panicle type is not known. In more recent studies, heavy panicle type cultivars were found to have a higher accumulation rate of pre-anthesis nonstructural carbohydrate (Akita 1993a). However, further morphological and/or physiological approaches are required to clarify the basis for higher yield of these cultivars.

A prolonged period of flowering and ripening is also a favorable trait for higher yield potential from an energetic point of view. Such traits, however, may reduce grain quality and increase instability of yield.

Biomass production

Biomass production increases with growth duration of rice cultivars, and also increases with greater N assimilation in a hyperbolic manner. Increased N absorption, however, is accompanied by the inevitable enhancement of respiratory losses in the later growth stages. Thus, increased biomass production in the vegetative stage from greater N uptake was not reflected in the final biomass due to a considerable increase in respiratory losses during the ripening stage, resulting in lower CGR_r (Akita 1988). The greater respiratory loss in later growth stages due to higher N absorption suggests the importance of a strategy to increase energy fixation without the need for greater N assimilation. This strategy will require manipulation of physiological traits related to photosynthesis and respiration for improved biomass production and higher CGR.

The efficiency of the energy harvesting system (quantum yield) shows little variation among the crop species, and therefore the efficiency of the dark reaction process of photosynthesis has been the primary target of manipulation. In general, the rate of photosynthesis per unit land area is closely related to the amount of rubisco, but is not closely related to rubisco activity (Osaki et al 1993). The gene responsible for higher rubisco activity has been identified, although it is not yet known whether incorporation of this gene into rice will improve the overall efficiency of photosynthesis.

Recently, incorporation of C_4 characteristics into a C_3 plant species to increase net energy fixation by rice has been proposed. If successful, this approach would not only result in a greater CGR, but also would contribute to maintaining sustainability in rice cultivation. The conversion from C_3 to C_4 photosynthetic mode in *Eleocharis vivipara* (Ueno et al. 1988) was closely related to leaf dimorphism, which is controlled by a process involving ABA (Golibar and Feldman 1989).

Maintenance respiration has been considered the major energy-consuming process for manipulation. However, the nature of maintenance respiration has not been clearly defined. Energy used in maintenance respiration, such as for maintaining ionic balance, cannot be manipulated easily without adverse effects on plant growth. One component of maintenance respiration which might be manipulated without adverse effect is the energy consumption for remobilizing protein. A reduction in the energy required for remobilizing proteins during leaf senescence would help maintain net assimilation and root activity during later growth stages. Higher root activity would enable the continuous uptake of nutrients during grain filling. Although delayed senescence of the root system may not be crucial to achieve present yield potential levels, it may become a limiting factor for CGR to achieve higher yield potential. Further study is needed to determine whether a reduction in unnecessary dark respiration is feasible, such as an alternative pathway (Akita 1993b).

Yield stability and quality

When national yield trends are considered, yield stabilization is an important factor. In most Asian countries, national average yields have continued to increase since semidwarf cultivars were released in the mid 1960s. This increase has occurred despite little, if any, change in the net assimilation rate of cultivars released since IR8. One of the factors responsible for this continuous increase is the greater resistance to insect pests and diseases that has been incorporated into more recent cultivars. Improvement of pest resistance to and tolerance for various stresses such as lodging should continue to provide opportunities for yield increases at the farm level.

The concept of a new plant type with increased yield potential must also consider improved grain quality. The market value of existing high-yielding cultivars, including F_1 hybrids, is low, although grain quality of recently

developed, high-yielding cultivars has been improved. The strong negative correlation between yield and grain quality or eating quality of high-yielding cultivars needs to be broken.

Future directions

The possibility of further increases in HI was emphasized for cultivars with longer growth duration. Improved energy fixation capacity per unit of acquired N was also highlighted, and may be possible by incorporating C_4 photosynthetic characteristics into rice, not only for increased biomass production but also for maintaining sustainability in rice cultivation. Elucidation of the regulation of dimorphism in the incorporation of C_4 traits and the genetic control of various limiting steps of energy fixation including dark respiration, will be required to succeed in this effort.

Marker-aided selection will greatly facilitate genetic transformation to improve the fundamental processes of yield formation described in IRRI's medium-term plan (IRRI 1993). However, the time horizon for advances using biotechnology tools is long-term because yield-determining processes involve many genes. Consequently, conventional approaches will remain as the primary means for increasing rice yield potential in the short term. Among conventional approaches, improving agronomic practices to allow new rice cultivars to express their full yield potential will be an important strategy, as will the continued development of cultivars with durable pest resistance and tolerance to various stresses. In fact, average rice yield in countries where the dominant rice cultivars have a relatively low yield potential is often higher than in countries growing cultivars with higher yield potential.

References cited

- Akita S (1988) Energetics for rice yield improvement-Accomplishments and future outlook-IRRI Thursday Seminar Paper, October 27, 1988. International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines.
- Akita S (1989) Improving yield potential in tropical rice. Pages 41-73 *in* Progress in Irrigated Rice Research. International Rice Research Institute, Los Baños, Philippines.
- Akita S (1993a) Nonstructural carbohydrate accumulation and crop yield. Pages 11-22 *in* Crop Production Technology and Strategy for the Future, Korean Soc. Crop Sci., Korea.
- Akita S (1993b) Respiration: Variation and potential for manipulation. Pages 799-805 *in* D. R. Buxton et al. (ed.) International Crop Science I. Crop Sci. Soc. America, Madison WI, USA.
- Golibar T E, Feldman L J (1989) Osmotic stress, endogenous abscisic acid and the control of leaf morphology in *Hippris vulgaris* L. Plant Cell Environ. 12:163-171.
- IRRI-International Rice Research Institute (1992). Irrigated rice ecosystems. IRRI Program Report for 1991:5-24. IRRI, P.O. Box 933, 1099 Manila, Philippines.
- IRRI-International Rice Research Institute (1993). Rice research in a time of change—IRRI's medium term plan for 1994-1998. IRRI, P.O. Box 933, 1099 Manila, Philippines.

- Mizuochi T (1988) Prospects for development of high-yielding techniques of winter wheat in Hokkaido. *Nogyo Gijyutsu* 43:337-342 (In Japanese).
- Osaki M, Morikawa K, Yoshida M, Shinano T, Iyoda M, Tadano T (1993) Productivity of high-yielding crops. III. Accumulation of ribulose-1,5-biphosphate carboxylase/oxygenase and chlorophyll in relation to the productivity among high yielding crops. *Soil Sci. Plant Nutr.*, in press.
- Ueno O, Samejima M, Muto S, Miyachi S (1988) Photosynthetic characteristics of an amphibious plant, *Eleocharis vivipara*: Expression of C4 and C3 modes in contrasting environments. *Proc. Natl. Acad. Sci.* 85:6733-6737.

Key issues in wheat yield potential

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At a 1981 symposium at IRRI, I set down a model for the determination of yield potential in wheat which still suits our purposes. Genetic progress in yield potential until then arose entirely through increased kernels/m² (KNO), so the model focuses on KNO determination:

$$\text{KNO} = \text{SDW}_A * (\text{KNO}/\text{SDW}_A), \text{ and}$$

$$\text{SDW}_A = D_s * \text{CGR} * F_s$$

where SDW_A is spike dry weight at anthesis (g/m²), D_s is the duration of the spike growth period (about 500 day degrees preceding anthesis), CGR is the crop growth rate during the period, and F_s is the average partitioning of net assimilate gain to spikes. Until the mid 1970s, all genetic progress at CIMMYT was associated with an increase in F_s , in turn largely associated with reduced stature.

A brief overview of general developments in wheat crop physiology and improvement since then confirms the role of KNO increase and assimilate supply to growing spikes in yield determination in general, and yield progress in particular. The many studies on photosynthetic aspects of wheat including CGR, RUE (radiation use efficiency), P_{\max} (light saturated leaf photosynthetic activity), Δ (carbon 13 discrimination) have not, with two exceptions mentioned below, revealed any association with yield potential. Kernel weight control has been studied intensively, but lack of progress in this yield component is no better understood. Breeding methodologies have become more efficient however, through better biometry, computerization and mechanization, and the key role of agronomic innovation in creating opportunities for breeders is now well recognized.

Progress with spring wheats at CIMMYT has continued since the mid 1970s. The impact of the resultant germplasm remains huge (around 40 million ha in the developing world are currently planted to CIMMYT-derived wheats). Several studies have investigated this progress and the most recent (Rees et al. 1993) is quite enlightening. Increased yield potential of Mexican

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semidwarf bread wheats averaged 0.83%/year between 1962 and 1988. KNO continued to rise, whereas kernel weight, plant height, and flowering date changed little. Yield progress in eight key varieties, studied at the physiological level in the 1992-93 season, was associated with increased flag leaf P_{\max} and greater stomatal conductance, both pre-anthesis and post-anthesis. Moreover, KNO and grain yield were correlated with CGR determined just before anthesis. These results, which have been confirmed at a second hotter location with other genotypes (Delgado et al. 1993), suggest possible indirect selection criteria for even higher yields, including leaf traits related to P_{\max} . They also encourage our efforts to increase P_{\max} through crossing to diploid wheat ancestors known to have higher P_{\max} . Finally, the results explain the positive relationship between yield potential and canopy temperature depression commonly observed at CIMMYT. This suggests that the infrared thermometer could be a useful tool in yield selection.

Other avenues for further yield potential increase in wheat need to be explored. The duration of spike growth (D_s) could be increased, but little is known about this stage. Partitioning of assimilate to the growing spikes (F_s) could probably be increased further via reductions in flag leaf size and further reductions in stem length, because current varieties are probably 10% taller than optimum for maximum yield potential. Kernel number per unit of spike dry weight warrants detailed investigation. Given that it seems possible to prolong crop photosynthetic activity beyond the date of physiological maturity in the spikes in current varieties, ways of extending grain-filling duration should be sought, thereby filling more kernels more slowly, or achieving larger kernels for the same number of kernels. Finally, any increase in yield potential must be accompanied by better lodging resistance and improved adaptation to intensive agronomic management.

Summary

The situation described for determining yield potential in spring wheat has interesting parallels and contrasts with that for irrigated tropical rice as described in Part I of this volume. Actual and record grain yields, and P_{\max} values, do not differ greatly, whereas the critical role of solar radiation before anthesis in determining the potential number of grains is similar, as is the clear need for greater lodging resistance. In contrast, the high level of stem carbohydrate reserves accumulated before flowering is unique to rice, and investment in inflorescence dry weight relative to total biomass at anthesis is smaller than in wheat. The existence of unfilled spikelets in rice could be likened to failures of grain set in wheat, but the relative loss of potential grains appears greater in rice, and the determination of this yield component seems to extend further into the grain-filling period. Another curious difference is the presumed movement of stem reserves into growing grains early in grain filling in rice and much later in the case of wheat. But the smaller final kernel weight of rice combined with synchrony of flowering means a shorter grain-filling

interval and much higher rates of grain weight accumulation (kg/ha per day) - this may necessitate the larger investment in pre-anthesis stem carbohydrate reserves.

References cited

- Delgado M I, Reynolds M P, Larque-Saavedra A. and Nava T (1993) Genetic diversity for photosynthesis in wheat (*Triticum aestivum* L.) under heat stressed field environments and its relation to productivity. (Submitted to Plant Physiology).
- Rees D, Sayre K D, Acevedo E, Nava Sanchez T, Lu, Z, Zeiger E, and Limon A (1993) Canopy temperatures of wheat: relationship of yield and potential as a technique for early generation selection. Wheat Special Report No. 10. CIMMYT, Mexico, D.F.

Use of carbon and oxygen isotope composition and mineral ash content in breeding for improved rice production under favorable, irrigated conditions

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Condon et al (1987) reported positive correlations between carbon isotope discrimination, Δ , and both grain yield and dry matter production in field-grown wheat genotypes when water did not limit growth. They suggested that large Δ could be used to select for increased yield in wheat under optimal conditions. Preliminary data show a positive relationship between yield and Δ among irrigated breeding lines of rice at Yanco, Australia (A.G. Condon and L. Lewin, unpubl. data), indicating, perhaps, an avenue for the International Rice Research Institute to screen for improved yields of irrigated rice growing under favorable conditions in Asia.

Theory for association between carbon isotope discrimination, Δ , and the ratio of intercellular to atmospheric partial pressure of CO_2

Discrimination is basically negatively related to the product of two factors, the CO_2 assimilation rate and the CO_2 diffusion (mainly stomatal) resistance (Farquhar et al 1982). Large values of Δ are caused by either low rates of photosynthesis or small values of stomatal resistance (or both). Put another way, discrimination during photosynthesis is positively related to the ratio of intercellular and ambient CO_2 concentration.

The details of the theory of carbon isotope discrimination have been reviewed recently (Farquhar et al 1989a). Briefly, the primary carboxylating enzyme, rubisco, discriminates against (reacts more slowly with) CO_2 containing the natural, stable isotope, ^{13}C . Thus there is slightly less ^{13}C in plant matter, as a proportion of total carbon, than in atmospheric CO_2 . Discrimination, Δ , is defined as the $^{13}\text{C}:^{12}\text{C}$ molar ratio in atmospheric CO_2 divided by that in the

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plant product, and expressed as a deviation from unity (Farquhar and Richards 1984). It is thus a small, positive quantity and is commonly expressed in parts per thousand (‰).

In a leaf with wide open stomata, the buildup of concentration of $^{13}\text{CO}_2$ in the space around rubisco is avoided because back-diffusion to the atmosphere is rapid. In principle, then, such leaves should show a discrimination near to that of rubisco itself, which is about $30 \times 10^{-3} = 30\text{‰}$ (Roeske and O'Leary 1984; Guy et al 1987), when expressed with respect to gaseous CO_2 (Farquhar et al 1989a). In leaves with nearly closed stomata such back-diffusion is more difficult, and the rubisco is "forced" to make more use of $^{13}\text{CO}_2$. That is, discrimination is small. In fact, diffusion through the stomatal pore and air spaces is also slightly slower for $^{13}\text{CO}_2$, and this process shows a discrimination of 4.4‰. So, during photosynthesis, as stomata open and the ratio of intercellular to atmospheric partial pressure of CO_2 , p_i/p_a , increases from zero towards unity, Δ increases from about 4 to about 30‰. Equations describing these and other effects in more detail were developed by Farquhar et al (1982) and reviewed by Farquhar et al (1989a).

In practice, although there are environmental and genetic effects on Δ , its value in C_3 species growing under favorable conditions is typically in the range 18-22‰, corresponding to variations in p_i/p_a of 0.16, or of 60 nbar in p_i (at 1993 levels of CO_2).

Correlations between yield and Δ in monocots

As well as the measurements on yield in relation to Δ across genotypes in wheat and rice noted above, there have been others on monocots. Work on barley has recently been reviewed by Acevedo (1993). He, too, showed a positive relationship, but concluded that an association between earliness of flowering and discrimination confounds any simple interpretation of the yield results in terms of gas exchange parameters. That is, there was a strong negative correlation among the genotypes examined between Δ and days to ear emergence (Crauford et al 1991). With sorghum, Hubick et al (1990) found a negative relationship between yield and discrimination. However, in species like sorghum with the C_4 pathway of photosynthetic carbon assimilation, the initial reaction with bicarbonate catalyzed by PEP carboxylase changes the characteristics of carbon isotope discrimination (Farquhar 1983) and the dependence on p_i/p_a becomes negative (Henderson et al 1992). Thus the negative correlation observed by Hubick et al (1990) between yield and Δ in this C_4 crop is actually equivalent to the positive relationship seen between yield and p_i/p_a other, C_3 species like wheat and rice. Weak, positive relationships have also been reported for crested wheatgrass (*Agropyron desertorum*) by Johnson et al (1993).

We are restricting ourselves here to monocots as the situation with dicots seems more varied. A positive relation between yield and Δ was observed for *Phaseolus vulgaris* lines (White et al 1990), but among peanut (groundnut)

genotypes, the relationship between biomass and **D** was negative (Hubick et al 1988; Wright et al 1988). Across peanut genotypes the source of variation in discrimination is photosynthetic capacity and the low discrimination plants have thicker leaves. However, there is a positive association between discrimination and harvest index so that the relationship between grain yield and **D** is indistinct. Research in this area has been reviewed by Wright et al (1993). In cowpea, there is a similar, but weak, positive association between harvest index and **D**, and a negative one between days to flowering and **D** which confounds direct interpretation of the relationship between yield and **D** (Hall et al 1993).

Of course, it may be that as more data become available, the picture for dicots may become clearer - or the monocots story may become less so. We know of no *a priori* reason why monocots should behave differently from dicots in this context.

Why do monocots with greater carbon isotope discrimination often yield more under well-watered conditions than those with lesser discrimination?

One obvious answer might be that stomatal resistance is the underlying source of variation so that the greater $[CO_2]$ at the sites of carboxylation in the large **D** plants increases their photosynthesis rate (although not in proportion to the increase in conductance). Certainly CO_2 enrichment enhances growth and yield of rice. Rice plants are not adapted to the certainty of irrigation. Their stomatal resistance functions to conserve water and will penalize carbon assimilation somewhat. We suggest that it would be useful to reduce stomatal resistance in the irrigated rice ideotype.

Farquhar et al (1989b) suggested that it may be possible to screen for variation in stomatal resistance using the $^{18}O/^{16}O$ ratio of leaf dry matter. Reduced resistance causes greater transpiration rate, cools the leaf, and diminishes the enrichment of $^{18}O/^{16}O$ in leaf water (Farquhar et al 1993; Flanagan et al 1994).

However, the differences in growth between genotypes of wheat seem somewhat greater than can be explained solely on the basis of a 40 μ bar difference in p_i . Condon et al (1987) suggested that faster early development of thinner leaves, resulting in lower photosynthetic capacity per unit leaf area, but greater light interception per unit ground area, may contribute to the positive relation they observed in wheat. More formally, it could be that, depending on how well the light penetrates the canopy, reducing the photosynthetic capacity per unit leaf area, allowing more leaf area per unit ground area, is advantageous, even allowing for the C, N and other costs of leaf construction. This would probably be most important at lower light intensities per unit leaf area, whether associated with regions of lower intensity per ground area, or with greater leaf area indices. The question remains, however, why in such leaves stomatal resistance does not scale up in proportion.

Similarly, why don't the fastest growing peanut genotypes (and sunflower genotypes, Virgona et al 1990) choose to achieve greatest rates of photosynthesis and growth by "spending" more water with a reduced stomatal resistance and greater intercellular $[CO_2]$, rather than via greater photosynthetic capacities, as they do. The answer presumably is related to the relative "costs" of water and nitrogen, etc., in the environments in which they evolved. Field et al (1983) have discussed the nature of this trade-off.

Another, related, suggestion to explain the positive relationship between yield and **D** was that processes controlling allocation may be somehow associated with processes affecting **D**, with large **D** plants having a greater leaf area ratio. This relationship has been observed (phenotypically but not genetically) across a range of soil strengths (Masle and Farquhar 1988). There is some evidence to support the idea of genetic association (Virgona et al 1990) but on a dicot, sunflower, and possibly confounded by differences in plant mass, and in turn caused by underlying differences in photosynthetic capacity (Virgona and Farquhar, unpubl. data). Masle (1992) saw no relationship between leaf area ratio and discrimination among wheat genotypes grown without intra-plant competition in well-spaced pots. The situation may be different in a densely planted crop and careful measurements in situ are needed.

The positive relationship between yield and discrimination in wheat has been confirmed for wetter sites and seasons in eastern Australia and is in contrast to the negative relationship found in drier sites and seasons (Condon and Richards 1993). The latter reflects the negative relationship between transpiration efficiency and carbon isotope discrimination (Farquhar and Richards 1984) which is observed in many species and reasonably well understood (see Farquhar et al 1989a, and case studies in Ehleringer et al 1993). In this context it is worth noting that the rice cultivar Azucena, which had the lowest **D** of the cultivars screened by Dingkuhn et al (1991), performs well in rainfed upland environments (M.A. Arrauadeau, IRRI pers. commun.).

Ash content (g/g) and carbon isotope discrimination

Masle et al (1992) showed that the ash content of dry matter (g/g) correlated positively, across genotypes of particular species, with **D** in C_3 tissues and negatively across genotypes in C_4 sorghum - in other words positively with intercellular $[CO_2]$ in all cases. Large values of ash content in high **D** genotypes of C_3 species means less organic matter as a proportion of total dry matter, and explains the negative relationship between carbon content and **D** among rice genotypes observed by Dingkuhn et al (1991). This is obviously interesting from the point of view of finding a simple and cheap screen for high intercellular $[CO_2]$.

It is also most interesting from a physiological point of view. Firstly, it is not simply a consequence of passive nutrient uptake, proportional to transpiration rate. When the partial pressure of either water vapor or CO_2 was changed during growth, ash content was hardly changed, even though the

transpiration ratio (transpiration divided by biomass accumulation) changed in the expected manner (Masle et al 1992). Neither can the genetic correlation, in rice at least, be explained on the basis of large Δ plants having greater water content - the relation persists when minerals are expressed on a fresh weight basis among an F_2 population (J. Masle, A. Yeo and G.D. Farquhar, unpubl. data).

Direct versus indirect associations between yield and carbon isotope discrimination

It is important to distinguish effects on yield that are positively correlated with discrimination due to the direct effects of variation in the intercellular $[CO_2]$, from those correlations that reflect co-linear associations with other traits. Such associations probably include those between discrimination and early maturity (several species), or greater harvest index (peanut and cowpea), and are perhaps caused by the relevant genes being reasonably close on a chromosome to those influencing intercellular $[CO_2]$. While these associations may be useful for screening, they do not reveal the character of interest directly, and confound the important effects on plant performance of the product of assimilation rate and stomatal resistance.

On the other hand, it seems likely that such an important compromise as the value of intercellular $[CO_2]$ in a well-adapted plant will not be made in isolation, and there could be adaptive advantage, in the wild, in having a gene promoting early maturity being near, and therefore frequently segregating with, one causing high intercellular $[CO_2]$. For cultivated rice this may still be important because the yield per day is often greater for short-duration varieties, and may lead to even more crops per year than at present (Evans 1993).

Although there are obviously many genes involved in photosynthetic capacity and stomatal resistance, there may be fewer key ones involved in their coordination (which in turn determines carbon isotope discrimination). Mapping in the rice genome of such loci and of those affecting associated traits would be useful. Martin et al (1989) and Masle et al (1993) reported progress in this area with restriction fragment length polymorphisms in tomato and *Arabidopsis thaliana*, respectively.

Recommendations

The relations between yield and Δ (positive in Australia), and those between yield, discrimination and ash content should be tested among genotypes of rice in a favorable Asian environment.

If patterns are confirmed, the underlying nature of the variation should be determined. One useful way of unravelling the factors may be to measure the $^{18}O/^{16}O$ ratio of dried leaves as a surrogate for stomatal resistance. Although conservation of soil water by stomata is an important feature in natural environments, contemporary values of stomatal resistance in modern rice

varieties may place an unnecessary limitation on CO₂ assimilation in an irrigated environment.

Identifying the physiological factors causing differences in growth rate among rice genotypes seems fundamental to success in germplasm development for greater yield potential.

References cited

- Acevedo E (1993) Potential of carbon isotope discrimination as a selection criterion in barley breeding. Pages 399-417 *in* Stable isotopes and plant carbon-water relations. J.R. Ehleringer, A.E. Hall, and G.D. Farquhar, ed. Academic Press, San Diego, USA.
- Condon A G, Richards R A (1993) Exploiting genetic variation in transpiration efficiency in wheat: an agronomic view. Pages 435-450 *in* Stable isotopes and plant-carbon water relations. J.R. Ehleringer, A.E. Hall, and G.D. Farquhar, ed. Academic Press, San Diego, USA.
- Condon A G, Richards R A, Farquhar G D (1987) Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. *Crop Sci.* 27:996-1001.
- Crauford P Q, Austin R B, Acevedo E, Hall MA (1991) Carbon isotope discrimination and grain yield in barley. *Field Crops Res.* 27:301-313.
- Dingkuhn M, Farquhar G D, De Datta S K, O'Toole J C (1991) Discrimination of ¹³C among upland rices having different water use efficiencies. *Aust. J. Agric. Res.* 42:1123-1131.
- Ehleringer J, Hall A E, Farquhar G D ed. (1993) Stable isotopes and plant carbon-water relations. 555 pp. Academic Press, San Diego, USA.
- Evans L T (1993) Crop evolution, adaptation and yield. 500 pp. Cambridge University Press, Great Britain.
- Farquhar G D (1983) On the nature of carbon isotope discrimination in C₄ species. *Aust. J. Plant Physiol.* 72:245-250.
- Farquhar G D, Ehleringer J R, Hubick K T (1989a) Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40:503-537.
- Farquhar G D, Hubick K T, Condon A G, Richards R A (1989b) Carbon isotope fractionation and plant water-use efficiency. Pages 21-40 *in* Stable isotopes in ecological research. P.W. Rundel, J.R. Ehleringer, K.A. Nagy, ed. Springer Verlag, New York.
- Farquhar G D, Lloyd J, Taylor J A, Flanagan L B, Syvertsen J P, Hubick K T, Wong S C, Ehleringer J R (1993) Vegetation effects on the isotopic composition of oxygen in atmospheric CO₂. *Nature* 363:439-443.
- Farquhar G D, O'Leary M H, Berry J A (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.* 9:121-137.
- Farquhar G D, Richards R A (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust. J Plant Physiol.* 11:539-552.
- Flanagan L B, Phillips S L, Ehleringer J R, Lloyd J, Farquhar G D (1994) Effect of changes in leaf water oxygen isotopic composition on discrimination against C¹⁸O¹⁶O during photosynthetic gas exchange. *Aust. J. Plant Physiol.* (in press).
- Field C B, Merino C J, Mooney H A (1983) Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. *Oecologia* 60:384-389.

- Guy R D, Fogel M L, Berry J A, Hoering T C (1987) Isotope fractionation during oxygen production and consumption by plants. *Prog. Photosynth. Res.* 3:597-600.
- Hall A E, Ismail A M, Menendez C M (1993) Implications for plant breeding of genotypic and drought-induced differences in water-use efficiency, carbon isotope discrimination, and gas exchange. Pages 349-369 *in* Stable isotopes and plant carbon-water relations. J.R. Ehleringer, A.E. Hall, and G.D. Farquhar, ed. Academic Press, San Diego, USA.
- Henderson S A, von Caemmerer S, Farquhar G D (1992) Short term measurements of carbon isotope discrimination in several C_4 species. *Aust. J. Plant Physiol.* 19:263-285.
- Hubick K T, Shorter R, Farquhar G D (1988) Heritability and genotype x environment interactions of carbon isotope discrimination and transpiration efficiency in peanut. *Aust. J. Plant Physiol.* 15:799-813.
- Hubick K T, Hammer G L, Farquhar G D, Wade L J, von Caemmerer S, Henderson S A (1990) Carbon isotope discrimination varies genetically in C_4 species. *Plant Physiol.* 91:534-537.
- Johnson D A, Asay K H, Read J J (1993) Genotypic and environmental variation for carbon isotope discrimination in crested wheatgrass, a perennial forage grass. Pages 269-280 *in* Stable isotopes and plant carbon-water relations. J.R. Ehleringer, A.E. Hall, and G.D. Farquhar, ed. Academic Press, San Diego, USA.
- Martin B, Nienhuis J, King G, Schafer A (1989) Restriction fragment length polymorphisms associated with water use efficiency in tomato. *Science* 243:1725-1728.
- Masle J (1992) Genetic variation in the effects of root impedance on growth and transpiration rates of wheat and barley. *Aust. J. Plant Physiol.* 19:109-125.
- Masle J, Farquhar G D (1988) Effects of soil strength in the relation of water-use efficiency and growth to carbon isotope discrimination in wheat seedlings. *Plant Physiol.* 86:32-38.
- Masle J, Shin J S, Farquhar G D (1993) Analysis of restriction fragment length polymorphisms associated with variation of carbon isotope discrimination among ecotypes of *Arabidopsis thaliana*. Pages 371-386 *in* Stable isotopes and plant carbon-water relations. J.R. Ehleringer, A.E. Hall, and G.D. Farquhar, ed. Academic Press, San Diego, USA.
- Masle J, Wong S C, Farquhar G D (1992) Transpiration ratio and plant mineral content are related among genotypes of a range of species. *Aust. J. Plant Physiol.* 19:709-721.
- Roeske M, O'Leary M H (1984) Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulose biphosphate. *Biochem.* 23:6275-6284.
- Virgona J M, Hubick K T, Rawson H M, Farquhar G D, Downes R W (1990) Genotypic variation in transpiration efficiency and carbon allocation during early growth in sunflower. *Aust. J. Plant Physiol.* 17:207-214.
- White J W, Castillo J A, Ehleringer J R (1990) Associations between productivity, root growth and carbon isotope discrimination in *Phaseolus vulgaris* under water deficit. *Aust. J. Plant Physiol.* 17:189-198.
- Wright G C, Hubick K T, Farquhar G D (1988) Discrimination in carbon isotopes of leaves correlates with water-use efficiency of field grown peanut cultivars. *Aust. J. Plant Physiol.* 15:815-825.
- Wright G C, Hubick K T, Farquhar G D, Rao R C N (1993) Genetic and environmental variation in transpiration efficiency and its correlation with carbon isotope discrimination and specific leaf area in peanut. Pages 247-267 *in* Stable isotopes and plant carbon-water relations. J.R. Ehleringer, A.E. Hall, and G.D. Farquhar, ed. Academic Press, San Diego, USA.

Yield potential of maize: impact of stress tolerance

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Understanding the processes underlying yield formation should facilitate the application of new technologies to crop breeding. Basic processes such as photosynthesis and N assimilation are important to crop growth, but it is the integration of single processes over space and time that determines whether modifications of these processes will result in yield improvement. The physiology of whole plants and crop canopies was developed in rice before any other field crop (Matsushima 1967). The constraints to biological productivity of field crops may be similar, and this summary will consider yield potential and potential yield improvement in maize.

Yield potential

Yield potential is a benchmark that can be used as an indicator of yield improvement possible through optimizing agronomic practices and breeding. An estimate of yield potential can be the highest yield ever recorded for a given crop at a certain location, or the theoretical value of maximum biophysical and biochemical efficiencies of the processes involved in the conversion of intercepted solar irradiance into plant dry matter, integrated over the life cycle of the crop. For instance, estimates of maximum crop growth rate and yield in maize are 550 kg/ha per d and 20 t/ha, respectively, based on theoretical maximum potential photosynthetic efficiency, leaf area development, and absorption of incident solar irradiance, and these estimates are similar to maximum rates reported in the literature (Tollenaar 1983). Maximum crop growth rates and grain yields of maize are two to three times higher than those recorded under apparent optimal conditions in commercial maize production, suggesting considerable scope for improvement.

Any estimation of yield potential, however, should not be taken at face value, whether it is based on practical considerations or on apparently sophisticated algorithms in a computer simulation model. In addition, although most biochemical and physiological processes can be potentially changed in this age of molecular biology, extrapolation of these modifications to crop

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performance is frequently highly unrealistic unless they are placed in the multi-dimensional context of yield formation, with rates of reaction ranging from picoseconds for photochemical reactions to months for crop growth rates.

Genetic yield improvement

Grain yield improvement in maize has been considerable over the past six decades, ranging from a four-fold increase between the 1930s and the 1990s in the US, to a five-fold increase between 1950 and 1990 in France (Tollenaar et al 1993). This yield improvement is associated with genetic gain and changes in cultural management and climate. Between 57 and 79% of the yield improvement in the US has been attributed to genetic gain (Russell 1986, Duvick 1992), whereas the genetic gain in Ontario, Canada, has been larger than the overall maize grain yield improvement (Tollenaar 1989). Past yield improvements cannot necessarily be extrapolated to the future, but an analysis of genetic gain during the past does clarify processes and crop characteristics that are important to yield improvement.

A number of factors traditionally emphasized by agronomists, physiologists, and breeders appear to have been of only marginal importance in the yield improvement of maize during the past six decades.

Harvest index

There has been no systematic change in maize harvest index, HI (i.e. grain as a proportion of total above-ground dry matter accumulation) during the past six decades. HI is influenced by management practices, in particular by plant density, and a proper analysis should account for changes in the response of genotypes of different eras to management factors. For instance, new hybrids have a higher HI at high plant densities than old hybrids because of their greater stress tolerance; when compared at their respective optimum plant density for grain yield, differences are negligible.

Potential leaf photosynthesis

Since the partitioning of dry matter has not changed substantially, increased grain yields should be attributable to increased canopy photosynthesis. However, studies conducted by us with Ontario hybrids (Dwyer and Tollenaar 1989) and by Crosbie (1982) with US hybrids have shown no significant differences between new and old hybrids in potential leaf photosynthesis.

Canopy architecture

An increased average leaf angle in the canopy results in a more even distribution of absorbed irradiance and, frequently, in higher photosynthesis per unit irradiance absorbed. Yield improvement in the US Corn Belt has been associated with increased leaf angle (Duvick 1992), but the distribution of absorbed irradiance does not appear to have changed in Ontario hybrids

(Tollenaar and Aguilera 1992). Potential yield improvement due to increased leaf angle in maize is in the order of 20-30%.

Pest resistance

The contribution of pest resistance to yield improvement of many crops is substantial. For example, Ledent and Stoy (1988) showed that virtually all yield improvement in Swedish wheat cultivars representing seven decades of breeding could be attributed to disease and lodging resistance. In contrast, increased resistance is only a minor factor in maize yield improvement, and may be partly attributable to the better physiological “health” of the plants (Tollenaar et al 1993).

Hybrid vigor

Yield improvement in maize has been associated with hybrid development. Yields in the US were basically unchanged from the mid 19th century until the 1930s, and accelerated greatly after the introduction of commercial double-cross hybrids. The subsequent replacement of double-cross hybrids by single-cross hybrids in the 1960s is associated with a second acceleration in maize yield (Tollenaar et al 1993). The yield advantage of hybrids vs cultivars is approximately 15%, but increases beyond yields of the initial double- and single-cross hybrids cannot be attributed to hybrid vigor per se.

Increased “stay green” has been recognized for several decades by maize breeders as an important selection criterion. Studies with US and Ontario hybrids have shown large differences in “stay green” between old and new hybrids (Duvick 1992, Tollenaar and Aguilera 1992). Differences in rates of dry matter accumulation between old and new hybrids were apparent before the onset of rapid leaf senescence, although accelerated senescence was associated with large differences in rate of dry matter accumulation between old and new hybrids (Tollenaar and Aguilera 1992). We have postulated that “stay green” is the result of enhanced stress tolerance.

Stress tolerance

The substantial yield improvement in Ontario maize hybrids between the 1950s and the 1980s appears to be attributable predominantly to increased stress tolerance. We define stress tolerance as the capacity of the plant to cope with biotic and abiotic stresses, in general, analogous to horizontal resistance as defined in disease resistance. Although most of the evidence is still circumstantial, we have observed that recent maize hybrids are more tolerant of a range of different kinds of mild stresses.

Nitrogen

The new hybrid has shown more tolerance for low N than the old hybrid in field and indoor studies. In the field study, two N levels were combined in a factorial design with three weed densities (no weeds, medium, and high weed

pressure). Results in 1991 showed highly significant effects of N and weed pressure on the yield difference between the new (Pioneer 3902) and the old (Pride 5) hybrids. The yield difference was relatively small at high N and no weeds (37%), high N and high weed pressure (47%), and low N and no weeds (52%), but always increased when N stress and weed-pressure occurred together. At low N and high weed pressure the yield difference between the new and old hybrid was 126% (Tollenaar et al 1994). In the indoor study, rate of dry matter accumulation during early phases of development was higher for the old than for the new hybrid at high N, but the opposite was true at low N. Rate of photosynthesis per unit leaf N was significantly higher for the new hybrid at all N levels (McCullough et al 1994).

Soil moisture

The old hybrid also appears to be more affected by soil-moisture stress than the new hybrid. We have observed that under conditions of moisture stress, stomatal conductance and leaf photosynthesis are depressed about 2 h earlier in the old hybrid than in the new (Dwyer et al 1992). Detailed studies on the impact of moisture stress on the hybrids are currently being conducted in our laboratory.

Plant density

Numerous studies have been reported on the effect of plant density on the differences between old and new hybrids (Russell 1986, DuVick 1992, Tollenaar 1989). Plant density is a composite stress, possibly increasing competition for soil moisture and nutrients, and increasing mutual shading. Relative differences in yield are greater at higher plant densities. In our studies, yield differences were lowest at a plant density of 4 plants/m², but increased again when plant density was reduced even more. We have attributed the latter phenomenon to the increased exposure of single-spaced plants to wind and high incident irradiation (photoinhibition), i.e., low plant-density stress (Tollenaar 1992). A similar response has not been reported for US hybrids, possibly because of the larger stature of these hybrids.

Temperature

Potential leaf photosynthetic rate during the first 2 wk after silking appeared to be similar for eight hybrids representing three decades of yield improvement in Ontario (Dwyer and Tollenaar 1989). However, leaf photosynthetic rate of older hybrids declines more than that of new hybrids after a relatively cool night (10°C). In contrast, the oldest and newest hybrid did not differ in their photosynthetic response to low temperature during early phases of development in a growth cabinet study (Tollenaar et al 1991).

Herbicides

Various herbicides affect old and new hybrids differently. We noticed differences in response to the post-emergence herbicide bromoxynil (a photosystem

II inhibitor) for the first time when we scored the hybrids for phytotoxicity after applying this herbicide in our field tests. The response was short-lived and the differences among hybrids was small but significant. We investigated the effect of bromoxynil on seedlings, using chlorophyll fluorescence as the parameter that indicates the impact on leaf photosynthesis. Results indicated that we could separate the hybrids in our test according to decade of release based on the bromoxynil response of the seedlings. Bromoxynil response was correlated ($r=0.91$) with the yield of hybrids in four location/year field tests (Tollenaar and Mihajlovic 1991). The mode of action of bromoxynil tolerance in the hybrids is not known, but results suggest that oxyradical scavenging is involved. We are currently investigating whether modern high-yielding maize hybrids vary in their bromoxynil response, and whether apparent stress-tolerant hybrids (i.e., hybrids that perform relatively well under poor conditions) differ from apparent stress-susceptible hybrids in their bromoxynil response.

Low stress environments

If yield improvement in maize is largely attributable to increased stress tolerance, then yield differences between old and new hybrids should be low or non-existent under low-stress conditions. When the old and the new hybrid were grown hydroponically (i.e., nutrients and water supplied regularly to an inert rooting medium) in the field, yield differences between the hybrids were not significant. These results are particularly interesting in light of results of a growth room study that was conducted more than a decade ago. In that study, Tollenaar and Migus (1984) reported that grain yield and crop growth rates of maize grown under controlled-environment conditions were more than 50% higher than those of field-grown plants, although daily incident photosynthetic active radiation was only approximately half of that received under field conditions. At that time, we were unable to explain the three-fold higher efficiency of indoor-grown plants. The phenomenon of high yields under controlled-environment conditions is not uncommon, although it has rarely been published in scientific literature. The yield level under controlled-environment conditions may be indicative of the potential genetic gain due to stress tolerance.

Conclusions

Further analysis of stress tolerance, and inclusion of a wider range of hybrids from different backgrounds, is required to reach more definite conclusions regarding the role of stress tolerance in the genetic improvement of maize. So far, the small but consistent response to a number of different stresses and the lack of differences in other physiological traits provide the best evidence of the role of stress tolerance in genetic improvement of maize.

There are various reasons why this analysis of yield potential of maize may have little relevance to rice yield. Rice and maize yield formation differ in

many respects. In addition, yield potential provides a good framework for the analysis of yield formation, but this analysis harbors the danger of oversimplification. Finally, even if the factors involved in past yield improvement are identified, extrapolation of past trends to the future may be questionable.

The analysis of yield potential and genetic yield improvement in maize does reveal, however, a number of important general aspects of crop yield improvement. First, current yields are not approaching a theoretical ceiling, and rate of yield improvement could continue at current rates or could even accelerate. The rate of yield improvement in maize has been accelerating since the introduction of hybrids, which resulted in greater commercial incentives for seed companies and allocation of financial resources to yield improvement research in maize. Second, increasing crop yield is not necessarily associated with increasing inputs of N, etc. Selection pressure in maize in North America has not been explicitly on low-input conditions; the apparent increased stress tolerance in maize may be a result of the selection for yield stability in elite material through extensive evaluation in multi-year, multi-location tests. Thirdly, past yield improvements have resulted predominantly from selection for yield per se. Because grain yield is the integration of processes at all levels of organization across the life cycle of the crops, continued success towards higher yield potential will require greater collaboration among plant breeders, plant physiologists, and agronomists.

References cited

- Crosbie T M (1982) Changes in physiological traits associated with long-term breeding efforts to improve grain yield of maize. Pages 206-233 in H.D. Loden and D. Wilkinson, ed. Proc. 37th Annu. Corn and Sorghum Industry Res. Conf., Chicago, IL, Am. Seed Trade Assoc., Washington, D.C.
- Duvick D N (1992) Genetic contributions to advances in yield of U.S. maize. *Maydica* 37:69-79.
- Dwyer L M, Stewart D W, Tollenaar M (1992) Analysis of maize leaf photosynthesis under drought stress. *Can. J. Plant Sci.* 72:477-481.
- Dwyer L M, Tollenaar M (1989) Genetic improvement in photosynthetic response of hybrid maize cultivars, 1959 to 1988. *Can. J. Plant Sci.* 69:81-91.
- Ledent J R, Stoy V (1988) Yield of winter wheat. A comparison of genotypes from 1910 to 1976. *Cereal Res. Commun.* 16:151-156.
- Matsushima S (1967) Crop science in rice: Theory of yield determination and its application. Fuji Publ. Co. Ltd., Tokyo, Japan.
- McCullough D E, Aguilera A, Tollenaar M (1994) N uptake, N partitioning, and N-use efficiency of an old and new maize hybrid. *Can. J. Plant Sci.* 74 (submitted for publication).
- Russell W A (1986) Contribution of breeding to maize improvement in the United States, 1920s - 1980s. *Iowa State J. Res.* 61:5-34.
- Tollenaar M (1983) Potential vegetative productivity in Canada. *Can. J. Plant Sci.* 63:1-10.
- Tollenaar M (1989) Genetic improvement in grain yield of commercial maize hybrid grown in Ontario from 1959 to 1988. *Crop Sci.* 29:1365-1371.

- Tollenaar M (1992) Is low plant density a stress in maize? *Maydica* 37:305-311.
- Tollenaar M, Aguilera A (1992) Radiation use efficiency of an old and new maize hybrid. *Agron. J.* 84:536-541.
- Tollenaar M, McCullough D E, Dwyer L M (1993) Physiological basis of the genetic improvement of corn. Pages 183-236 in G. A. Slafer, ed. *Genetic improvements in field crops: current status and development*. Marcel Dekker Inc., N.Y.
- Tollenaar M, Migus W (1984) Dry matter accumulation of maize grown hydroponically under controlled-environment and field conditions. *Can. J. Plant Sci.* 64:475-485.
- Tollenaar M, Mihajlovic M (1991) Bromoxynil tolerance during the seedling phase is associated with genetic yield improvement in maize. *Can. J. Plant Sci.* 71:1021-1027.
- Tollenaar M, Mihajlovic M, Aguilera A (1991) Temperature response of dry matter accumulation, leaf photosynthesis and chlorophyll fluorescence in an old and new maize hybrid during early development. *Can. J. Plant Sci.* 71:353-359.
- Tollenaar M, Nissanka S P, Aguilera A, Weise S F, Swanton C J (1994) Impact of maize hybrid and soil N on weed interference in maize. *Agron. J.* 86 (submitted for publication).

Limitations to crop yield by photosynthesis

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It is now understood that raising the yield potential of rice without an increase in growth duration will require an increase in the net photosynthetic rate. This can be achieved either by removing internal and external limitations to the achievement of existing photosynthetic potential or by increasing intrinsic photosynthetic efficiency and capacity. However, improvement in photosynthesis has not been used as a selection parameter in breeding programs in the past. There are a number of reasons for this, both practical and theoretical. However, studies on the role of photosynthetic processes in limiting plant growth have undergone a radical change in emphasis in recent years. Whilst the focus in the past has been upon whether intrinsic photosynthetic processes could be improved (e.g. RuBP carboxylase vs oxygenase), recent progress in basic research makes it possible to identify several other aspects of photosynthesis that are equally important. With the development of new genetic methods, it is now possible to conceive of redesigning crop plants on the basis of this new knowledge.

It is likely that photosynthesis rarely achieves its potential capacity in the field. For example, one field study of mangrove showed that the curve describing the irradiance response of photosynthesis represents only the ceiling below which instantaneous measurements of photosynthetic rate fall in an apparently random manner (Cheesman et al 1991). Regaining this lost photosynthesis represents an important objective and provides a good opportunity for crop yield increase. Achieving this objective would be complementary to the goal of increasing photosynthesis via an increase in photosynthetic capacity (i.e. the light-saturated rate of photosynthesis per unit leaf area). There are many reasons for the under-achievement in photosynthesis, but at least in part it results from environmentally and metabolically induced decreases in photosynthetic quantum yield and capacity.

Whenever the rate of light absorption exceeds the capacity for electron transport (this occurs at all light intensities above the 'quantum yield' region

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of the irradiance curve) the efficiency of light utilization is down-regulated to offset the tendency for saturation (Weis and Berry 1989). An 'active' decrease in quantum efficiency, which is rapidly reversible upon darkening, is linked to the more sustained light-stress-related down-regulation of photon yield, which in turn is often associated with actual damage to the photosynthetic reaction centers (Horton and Ruban 1992). The recovery times of the latter processes mean that significant losses of photosynthesis occur, particularly under fluctuating climatic conditions. There is direct evidence from the field that there can be a decrease in crop photosynthesis (Stirling et al 1991). Such responses could be significant even under apparently favorable conditions for plant growth.

The response to high light intensity may then be an important determinant of carbon gain. It is to be emphasized also that light can be especially damaging, even at low intensity, if there are other restrictions on photosynthetic rate such as drought or temperature extremes, which may occur during the dry season. The short-term response to high light intensity tends to offset longer-term photoinhibitory effects, and hence is a photoprotective process. In a study of a range of species, it is now clear that the capacity of the former (and by inference the extent of the latter) is variable between species (Johnson et al 1993). Species genetically adapted to survive in extreme environments have a capacity for short-term photoprotection that is five times greater than all crop plants so far investigated, including pea, barley, spinach, maize, tomato, tobacco, and potato (Ruban et al 1993). Up to now there has been little investigation of these events in rice.

The main reversible photoprotective mechanism is the induction of energy dissipation—conditions of excess irradiation are 'sensed' by the chloroplast thylakoid membranes, resulting in a down-regulation of energy-capture efficiency because of the increases in rate of non-radiative energy dissipation (Horton and Ruban 1992). The dissipation of energy as heat can be readily detected by the consequent decrease in fluorescence emission from chlorophyll, the so-called non-photochemical quenching (qN). With improvements in methodology, it is now possible to rapidly determine qN in whole leaves (Horton and Bowyer 1990, Krause and Weis 1992). There has been considerable progress recently in determining the molecular mechanism of qN (Horton and Ruban 1992, Ruban et al 1993), allowing some specific suggestions to be made for the genetic improvement in stress resistance for a species such as rice.

It has to be considered, however, that this form of photoprotection is integrated with other processes in the whole plant to give its overall growth strategy. This will have many facets. For example, it is known that there is a variable degree of plasticity within a species to control energy dissipation capacity through a long-term adaptation to exposure to a high-intensity light environment (Ruban et al 1993, Demmig-Adams and Adams 1992). Also, other mechanisms exist for relieving energy buildup—charge recombination in PSII, electron cycling, and transfer to O₂ or other alternative acceptors (Mehler reactions, photorespiration, oxaloacetate reduction). Furthermore, the response

to light is linked to (determined by or limited by) the presence of other environmental factors—low and high temperature, drought, heat, salt, nutrient supply. The actual inhibitory effect of excess light will also be determined by rates of turnover and replacement of damaged proteins and by the activity of enzymes (superoxide dismutase, catalase, etc.) involved in preventing oxidative stress. Finally, the effectiveness of light interception, determined by leaf architecture, will also influence the need for photoprotective mechanisms.

Whilst the common questions that have been asked about these processes in relation to plant growth concern resistance to environmental stresses, an alternative question is whether the protective mechanisms, and other regulatory processes, are optimized for maximum biomass production and yield under favorable agricultural conditions. It may be that attention should be given to removal of 'over regulation'. Detailed studies of photosynthesis in the rice canopy are therefore needed to determine the direction of future research and the strategy for genetic improvement.

Internal metabolic factors may also limit the achievement of potential photosynthetic capacity. Firstly, feedback control over photosynthesis can occur on sink limitation. Feedback inhibition of photosynthesis occurs when the capacity for sucrose synthesis is less than chloroplast photosynthetic capacity. This can arise because of an intrinsic limitation to the cytoplasmic pathway of sucrose synthesis (exposed for example as O_2 -insensitive photosynthesis [Pammenter et al 1992]), or because of an accumulation of sucrose that leads to a regulated decrease in pathway capacity. The latter will arise under sink-limiting conditions. It is important to point out that the effect is ultimately on the chloroplast reactions and this has to be mediated by, for example, P_i supply to the thylakoid ATP synthase with the resultant effect on ΔpH and electron transport rate. Again it is necessary to consider if such effects may be important in holding back potential yield.

In fact, recent work on transgenic tomato has shown that it is possible to bring about an increase in biomass by expression of a maize gene for sucrose phosphate synthase (Galtier N, Alred R, Quick W P, and Foyer C H, unpublished data). In the transgenic plants, elevated leaf sucrose levels can be sustained without the normal feedback regulation, providing there is an increase in the sucrose gradient from source to sink; increases of about 5-10% in photosynthetic rate have been measured. Further opportunities may be offered by tissue-specific expression of the enzymes of carbohydrate transport and metabolism that could remove both source and sink limitations and allow simulation of grain filling; this may have to be linked to alteration of carbohydrate metabolism in the grain. However, it is vital that careful analyses of photosynthesis during plant development are made, since not only are small increases in photosynthesis significant when integrated over a growing period, but at present we do not yet understand the complex network of controls involved.

A parameter of great interest in this regard is leaf longevity, since changes in source/sink interactions are associated with leaf senescence. Increasing

source strength by manipulating photosynthesis may only result in an accelerated leaf senescence, as a result of a kind of whole plant "homeostasis". Moreover, it is well known that leaf senescence occurs during the grain-filling period of rice and that it may be the source of a major limitation to yield potential. However, again there are already opportunities for delaying leaf senescence, for example, by the suppression of ethylene synthesis. In tomato plants transformed with antisense to the gene for the ACCO enzyme, significant delays in chlorophyll loss have been observed (Picton et al 1993), and it has been shown that full photosynthetic activity remains in these leaves (John I, Drake R, Farrell A, Cooper W, Lee P J, Horton P and Grierson D., unpubl. data), but studies are needed to see if this transformation leads to increased biomass in the field.

Summary

There are now opportunities to increase the efficiency with which potential photosynthetic capacity can be used for plant growth and crop yield. This need not necessarily involve increasing photosynthetic capacity per se, but removing internal metabolic limitations to carbohydrate metabolism, increasing the functional lifetime of the leaf, and forming a photosynthetic membrane that has an optimized response to environmental conditions. Because of the complex interactions involved in optimizing whole plant performance, and the likely occurrence of pleiotropic effects of most genetic interventions, research has to proceed in a step-wise manner in which the effects of each manipulation are carefully analyzed, since it is unlikely that a single change will give the desired phenotype. With the development of rapid non-invasive methods to measure the key aspects of photosynthesis, such an approach becomes feasible.

References cited

- Cheesman J M, Clough B F, Carter D R, Lovelock C E, Eong O J, Sim R G (1991) The analysis of photosynthetic performance in leaves under field conditions: a case study using *Bruguiera* mangroves. *Photosynth. Res.* 29:11-22.
- Demmig-Adams B, Adams W W (1992) Photoprotection and other responses of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:599-626.
- Horton P, Bowyer J (1990) Chlorophyll fluorescence transients. Pages 259-295 in *Methods in plant biochemistry*. J.L. Harwood, J. Bowyer, ed. Academic Press.
- Horton P, Ruban A V (1992) Regulation of photosystem II. *Photosynth. Res.* 34:375-385.
- Johnson G N, Young A J, Scholes J D, Horton P (1993) The dissipation of excess excitation energy in British plant species. *Plant Cell Environ.* 16:673-679.
- Krause G H, Weis E (1992) Chlorophyll fluorescence and photosynthesis: the basics. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:313-349.
- Pammenter N W, Loreta F, Sharkey T D (1992) End product feedback effects on photosynthetic electron transport. *Photosynth. Res.* 35:5-14.

- Picton S, Barton S L, Bouzayen M, Hamilton A J, Grierson D (1993) Altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene-forming enzyme transgene. *Plant J.* 3:469-481.
- Ruban A V, Young A J, Horton P (1993) Induction of nonphotochemical energy dissipation and absorbance changes in leaves. Evidence for changes in the state of light harvesting system of photosystem II in vivo. *Plant Physiol.* 102:741-750.
- Stirling C M, Nie G Y, Agulera L, Nugaweda A, Long S P, Baker N R (1991) Photosynthetic productivity of an immature maize crop changes in quantum yield, CO₂ assimilation, conversion efficiency and thylakoid proteins. *Plant Cell Environ.* 14:947-954.
- Weis E, Berry J (1989) Quantum efficiency of PSII in relation to energy dependent quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta.* 894:198-208.

Starch biosynthesis in plants: identification of ADP glucose pyrophosphorylase as a rate-limiting step

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Starch biosynthesis plays a pivotal role in plant metabolism, both as a transient storage metabolite of leaf tissue and as an important energy and carbon reserve for sink organs such as seed, root, tuber, and fruit. It has been suggested that starch is a critical determinant of the sink strength of developing sink organs. This has been attributed primarily to the lowering of the intracellular osmotic pressure of the sink cells, resulting in more efficient transport of sugars (hexoses, sucrose, etc.) across the plasma membrane, and to promotion of sugar unloading from the vascular cells of the sink tissue. However, unequivocal data relating starch biosynthesis with sink strength is lacking at present. Our laboratory has been engaged in probing the rate-determining steps of starch biosynthesis in plant cells with the ultimate objective of establishing the relationship between starch and sink strength.

At least three critical enzymatic steps are involved in starch biosynthesis in plants. These include ADP glucose pyrophosphorylase (ADPGPP)-catalyzed formation of ADP glucose (ADPG) from glucose 1-phosphate and ATP, starch synthase-catalyzed transfer of the glucosyl moiety of ADPG to a glucan primer to extend the chain length of the α 1,4 glucan, and the branching enzyme catalyzed-formation of branched glucans or amylopectin.

Of the three reactions described above, we reasoned that ADPGPP is a critical step in starch biosynthesis since it is the first committed step of the pathway and subject to regulation by allosteric effectors such as 3-phosphoglyceric acid (3-PGA) and inorganic phosphate (Pi). However, in vitro measurements had indicated that the specific activity of the starch synthase was the lowest of the three enzymes. Mutants of *Arabidopsis thaliana* deficient in ADPGPP (5% of the specific activity of the wild type), produced nearly 60% as much starch as the wild type. Similar observations had been made with maize and pea mutants deficient in ADPGPP. These results suggested that ADPGPP was not the rate-determining step in starch biosynthesis.

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In order to verify the role of ADPGPP in regulating starch biosynthesis, we used a mutant ADPGPP gene from the bacterium *Escherichia coli*. This bacterium produces glycogen via the same pathway as described above for starch formation in plants. Mutants of *E. coli*, which overproduce glycogen, have been described. One of the mutants, *E. coli* 618, contains a mutant ADPGPP gene, designated *glgC16*. This mutant has a *gly* to *asp* change at position 355. *E. coli* ADPGPP differs from plant ADPGPP in that the former enzyme is a homotetramer and regulated by 5'-AMP and fructose 1,6-bisphosphate, whereas the latter enzymes are heterotetramers regulated by 3-PGA and Pi. The *E. coli* enzyme encoded by *glgC16* gene is substantially less susceptible to regulation by the allosteric effectors. The *glgC16* gene was modified at its 5' end, by addition of the nucleotide sequence encoding the chloroplast transit peptide region of the small subunit of RuBP carboxylase. This chimeric gene encoded a fusion protein that contained the chloroplast-targeting sequence at its N-terminus. In vitro measurements, using radiolabeled chimeric protein and lettuce chloroplast preparations, confirmed that this protein was imported and processed to a mature protein that had ADPGPP activity. We used this chimeric gene for plant transformation.

Initial experiments using the CaMV 35S promoter/Chimeric gene/Nos poly A signal confirmed that this gene was able to increase starch production in tobacco and tomato cells. Similar results were obtained with tomato shoots expressing the chimeric gene. Plants expressing this gene construct, however, did not thrive in the soil. We reasoned that this could be due to the deprivation of sucrose from the source leaf, caused by its conversion to starch. In order to circumvent this problem, we targeted the expression of the chimeric gene to sink tissues. We used potato as our model system and patatin as the promoter to drive the expression of the chimeric gene.

Potato plants expressing the chimeric gene produce tubers with higher dry matter and starch content, under both growth chamber and field conditions. These plants have growth and development profiles similar to the nontransgenic controls. The nature of the starch produced by the tubers containing the chimeric gene is indistinguishable from the control tubers.

Although the starch and dry matter content of tubers expressing the chimeric gene is substantially elevated, there is no direct correlation between the extent of expression of the chimeric gene and starch accumulation. We reason that this is due to the fact that other steps in the pathway become rate-limiting under these conditions; whether these steps are unique to the starch biosynthetic pathway or provide substrates for the starch pathway is unknown at present.

Our work has illustrated that ADPGPP is indeed a critical enzyme in regulating starch biosynthesis in plant tissues. Even in storage organs that have high levels of ADPGPP, its activity is still limiting. This limitation appears to be primarily at the level of allosteric regulation of the enzyme, at least in sink tissues. It should be possible to affect starch production in storage tissues positively by regulated expression of the gene encoding this enzyme.

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Augmenting current efforts to improve crop yield by breeding

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Plant breeding has often been described as a numbers game in which the goal is to have sufficient numbers to identify the rare line that has potential to become a cultivar. Also, plant breeding has been described as being as much an art as it is a science. Although both descriptions are correct in successful breeding programs, numbers alone do not ensure success, nor will the well-trained breeder's "eye" (the art of selection). The momentum of carrying large numbers of lines forward every year can be so overwhelming that plant breeders may have little time to critique their breeding programs and make them more efficient. Plant breeders must retain their curiosity about the plant, its environment, and their procedures. Relying too heavily on "art" will create breeding programs that cannot be described or duplicated adequately by others.

In considering the key issues that affect crop yield potential from a plant breeding perspective, it must be remembered that plant breeding programs have three phases, 1) introduction of genetic variation, 2) segregation and selection of useful variants, and 3) extensive evaluation of elite lines. It should also be recognized that plant breeding has been extremely successful. Changing plant breeding methods will be evolutionary and not revolutionary. To augment traditional plant breeding efforts, the science related to these three phases needs to be enhanced.

Introduction of genetic variation

Most plant breeders have easy access to the domesticated germplasm pool. The critical need is to have the domesticated germplasm pool adequately understood and the documentation and seed readily accessible to all. With the major germplasm collections at the international crop improvement centers and in national collections, much of the work of collecting and maintaining the cultivated materials has been accomplished. Documenting and describing the

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materials is progressing, but much remains to be done. These efforts should be increased (Chang 1992).

In the future, increased use of the wild relatives of crop plants through the elegant use of modern cytogenetics (Sharma and Gill 1983) and use of the potential genetic resources of the biosphere through genetic engineering in plant transformation (Dale et al 1993), will be needed as well. In wheat (*Triticum aestivum* L.), the domesticated gene pool has provided relatively few genetic resources to reduce the devastating effects of some diseases and their vectors, such as barley yellow dwarf virus (Banks et al 1993) and wheat streak mosaic virus (Martin et al 1976, Whelan and Hart 1988). The same is true for physical stresses (e.g. soil toxicity — see Dvorak et al 1992, Schachtman et al 1992). In both diseases and physical stresses, genes from wild relatives are becoming more useful through the breakage of linkage blocks and the use of molecular (RFLPs, RAPDs, isozymes, or antibodies; e.g. Ma et al 1993) or histological markers (in situ hybridization to identify chromosome arms or segments) to track the genes or chromosome fragments (Rayburn and Gill 1985). Genetic engineering is developing new genetic mechanisms and gene pools to alleviate insect, disease, herbicide, and physical stresses, to improve food processing and quality, and to produce specialized chemicals (Dale et al 1993, Tarczyński et al 1993). Though genetic engineering has had relatively little economic impact to date, it is hard to overestimate its long-term potential if for no other reason than that it provides a means to test biochemical, developmental, physiological, and genetic processes in vitro (Dale et al 1993).

Segregation and selection of useful variants

It is in this area that the "art" of plant breeding is most effective. However, the imprecision of any selection method ultimately reduces heritability, thus genetic gain. Hence improvements in the ability to select within the segregating generations should make selection more precise. Increasing selection precision can be achieved by improving selection assays. Currently the greatest interest in selection theory and practice is in using some type of molecular markers as diagnostics or to tag genes of interest. Antibodies are commonly used to identify the presence of diseases that are difficult to diagnose by symptomatology (e.g. Lister et al 1985). Strain or race differences can be identified by first amplifying pathogen DNA (using the polymerase chain reaction), cutting the amplified DNA using restriction enzymes, and separating the DNA fragments on electrophoretic gels (Robertson et al 1991, Henson and French 1993). Knowing the disease race or strain is critical for understanding the genetic basis of resistance and of pathogenicity. Diagnostic uses of molecular markers are becoming standard for understanding the plant phenotype and making selections.

Though our view may be the result of the paucity of our imaginations, we expect that marker-based selection technology will probably be used in very specialized problems, such as 1) traits with phenotypes difficult to assay

properly due to imprecise assays or large environmental effects (e.g. sprouting, Anderson et al 1993), 2) pyramiding genes where the individual genes have the same phenotype and are indistinguishable from the phenotype of the desired plant having multiple resistance genes, 3) potentially in backcrossing to reduce the donor parent genotype and recover the current parent genotype as quickly as possible, 4) tagging alien introgressions and engineered genes (may be easier than evaluating the plant phenotype), and 5) prescreening populations where the obvious poor types have already been removed (Baenziger and Peterson 1992). Where there are simple tests to remove obvious deleterious genes or gene clusters, marker tests may be used in lieu of the more complex phenotypic assays. For example, the wheat-rye (*Secale cereale*) translocation 1B/1R is known to adversely affect hard wheat (bread) quality (Dhaliwal and MacRitchie 1990). This translocation can be rapidly identified using antibodies to a rye seed storage protein (Graybosch et al 1993) which is less time-consuming than doing a predictive micro quality test (the mixograph).

In crops where there are predominant gene pools and intraspecific markers (e.g. the heterotic gene pools in maize, *Zea mays* L.), it may be more useful to use molecular markers. Most introgression and improvement programs will work within relatively narrow germplasm pools and intraspecific markers will attempt to bring new genes into the predominant germplasm pool. The goal in these programs is to add relatively few new genes while maintaining the existing gene pool, or to develop new genetic combinations from the existing gene pool. However, in many crops (e.g. oats (*Avena sativa*) Rodgers et al 1983, soybean (*Glycine max*) Delannay et al 1983, and wheat, Cox et al 1986) the use of common ancestors is decreasing and the genetic diversity is stable or increasing. As the gene pool broadens, many more genes with unknown marker relationships, unknown effects, and unknown epistatic effects, are added to the gene pool. To tag and understand the new genes, more populations will need to be classified phenotypically and with markers.

In addition, many scientists are recognizing that the power of marker-based selection requires very good phenotypic measurements. This means that many of the initial studies using early generation materials were inadequate due to the expected loss of precision by using measurements from lines that could not be replicated or repeated. Similarly, many of the studies were inadequate because they used statistical models that did not account for genotype \times environment interactions and small sample sizes (leading to over parameterizing the data set). These constraints can be removed by using later generation materials, particularly doubled haploid if the doubled haploid process does not induce chromosome rearrangements (Youssef et al 1989), multilocation, replicated field trials, and by increasing the sample size. However, removing these constraints is costly and only very few populations will merit this effort. With the current and foreseeable time constraints and cost of marker analysis, visual selection coupled with natural or artificial selection must remain the primary analytical tool for selection. Simply, the numbers game in successful plant breeding programs is too large for the

routine use of these powerful genetic analysis techniques (Baenziger and Peterson 1992). However, the study of quantitative trait loci through the use of molecular markers and cytogenetic stocks will be very important in developing understanding of the crop genome (Berke et al 1992a,b, Yen and Baenziger 1992).

Evaluation of elite lines

There are two evaluation problems that are difficult for plant breeders: choosing evaluation sites that adequately represent the targeted environments (Brown et al 1983), and producing high quality data from those environments. There is little useful information on how to choose evaluation environments, though there are some indications that using the indigenous biodiversity to identify ecological zones can be a starting point (Peterson 1992). However, within the ecological zones, the breeder must evaluate experimental lines using the "normal" cropping practices, which can be quite diverse. If one assumes that the target environments will include more marginal production environments because the better production areas are already using optimal production practices and cultivars (hence further improvements are more difficult), then the importance of producing high quality data increases. Our experience is that it is very difficult to identify uniformly good land for evaluation trials, but it is impossible to identify uniformly poor land to represent marginal lands. Improving the quality of the data and the ability to interpret the data will require statistical analyses greatly different from the classical blocking designs that are commonly used in evaluation trials (Stroup and Mulitze 1991, Lin et al 1993, Es and Es 1993, Stroup et al, 1993 in press). Similarly, models for understanding how cultivars interact with the environment will probably need to change from the more classical response models (reviewed by Lin et al 1986, Eberhart and Russell 1966) to models that include risk (Eskridge 1990, 1991). In marginal lands, the risk of crop failure may be higher, and cultivars that provide consistent yields under adverse conditions, while taking advantage of better conditions, should be selected so that the marginal farmer will not suffer crop failures that could be disastrous. In breeding for marginal lands, selection may need to be for both stability and yield (Khang 1993).

Conclusions

The domesticated gene pool is generally accessible, but needs better and more accessible documentation. Future efforts on introgressing genes from wild relatives or incorporating completely novel genes via genetic engineering will need to be increased.

For the foreseeable future, effective plant breeding programs will use large populations that can be most efficiently screened visually. Molecular markers will not be used except in specialized populations or for specialized selection

procedures due to their cost, relatively low number of lines that can be evaluated, or the poor merit of most of the lines within a population.

Site selection to optimize predictive value and data analysis to optimize quality of data will remain the two most critical problems facing plant breeders in this area. New statistical approaches are being developed and need to be implemented.

References cited

- Anderson J A, Sorrells M E, Tanksley S D (1993) Detection of QTLs affecting preharvest sprouting resistance in wheat by RFLPs. *Crop Sci.* 33:453-459.
- Baenziger P S, Peterson C J (1992) Genetic variation in self-pollinated species. *In* H. T. Stalker and J. P. Murphy, ed. *Plant Breeding in the 1990s*. CAB International, Wallingford, Oxon, U.K.
- Banks P M, Larkin P J, Bariana H S, Lagudah E S, Appels R, Waterhouse P M, Brettel R I S (1993) Introgression of barley yellow dwarf virus (BYDV) resistance from *Thinopyrum intermedium* to wheat. *Genome* (in press)
- Berke T G, Baenziger P S, Morris R (1992a) Location of wheat quantitative trait loci affecting agronomic performance of seven traits using reciprocal chromosome substitutions. *Crop Sci.* 32:621-627.
- Berke T G, Baenziger P S, Morris R (1992b) Location of wheat quantitative trait loci affecting stability of six traits using reciprocal chromosome substitution. *Crop Sci.* 32:628-633.
- Brown K D, Sorrells M E, Coffman W R (1983) A method of classification and evaluation of testing environments. *Crop Sci.* 23:889-893.
- Chang T T (1992) Availability of plant germplasm for use in crop improvement. *In* H. T. Stalker and J. P. Murphy, ed. *Plant Breeding in the 1990s*. CAB International, Wallingford, Oxon, U.K.
- Cox T S, Murphy J P, Rodgers D M (1986) Changes in genetic diversity in the red winter wheat regions of the United States. *Proc. Natl. Acad. Sci.* 83:5583-5586.
- Dale P J, Irwin J A, Scheffler J A (1993) The experimental and commercial release of transgenic crop plants. *Plant Breeding* 111:1-22.
- Delannay X, Rodgers D M, Palmer R G (1983) Relative genetic contribution among ancestral lines to North American soybean cultivars. *Crop Sci.* 23:944-949.
- Dhaliwal A S, MacRitchie J (1990) Contributions of protein fractions to dough handling properties of wheat-rye translocation cultivars. *J. Cereal Sci.* 12:113-122.
- Dvorak J, Epstein E, Galvez A, Gulick P, Omielan J A (1992) Genetic basis of plant tolerance of soil toxicity. *In* H. T. Stalker and J. P. Murphy, ed. *Plant Breeding in the 1990s*. CAB International, Wallingford, Oxon, U. K.
- Eberhart S A, Russell W A (1966) Stability parameters for comparing varieties. *Crop Sci.* 6:36-40.
- Graybosch R A, Seo Y M, Peterson C J (1993) Detection of wheat-rye chromosomal translocation using an antiseccalin monoclonal antibody. *Cereal Chem.* 70:458-463.
- Henson J M, French R (1993) The polymerase chain reaction and plant disease diagnosis. *Annu. Rev. Phytopathol.* 31:81-109.

- Es H M van, Es E L van (1993) Spatial nature of randomization and its effect on the outcome of field experiments. *Agron. J.* 85:420-428.
- Esikridge K M (1990) Selection of stable cultivars using a safety-first rule. *Crop Sci.* 30:369-374.
- Esikridge K M (1991) Screening cultivars for yield stability to limit the probability of disaster. *Maydica* 36:275-282.
- Khang M S (1993) Simultaneous selection for yield and stability in crop performance trials: consequences for growers. *Agron. J.* 85:754-757.
- Lin C S, Binns M R, Lefkovitch L P (1986) Stability analysis: where do we stand. *Crop. Sci.* 26:894-900.
- Lin C S, Binns M R, Voldeng H D, Guillemette R (1993) Performance of randomized block designs in field experiments. *Agron. J.* 85:168-171.
- Lister R M, Clement D, Skaria M (1985) Stability of ELISA activity of barley yellow dwarf virus in leaf samples and extracts. *Plant Disease* 69:854-857.
- Ma Z Q, Gill B S, Sorrells M E, Tanksley S D (1993) RFLP markers linked to two Hessian fly-resistance genes in wheat (*Triticum aestivum* L.) from *Triticum tauschii* (Coss.) Schmal. *Theor. Appl. Genet.* 85:750-754.
- Martin T J, Harvey T L, Livers R W (1976) Resistance to wheat streak mosaic virus and its vector, *Aceria tulipae*. *Phytopathology* 66:346-349.
- Peterson C J (1992) Similarities among test sites based on cultivar performance in the hard red winter wheat region. *Cop Sci.* 32:907-912.
- Rayburn A L, Gill B S (1985) Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. *J. Hered.* 76:78-81.
- Robertson N L, French R, Gray S M (1991) Use of group-specific primers at the polymerase chain reaction for the detection and identification of luteoviruses. *J. Gen. Virol.* 72:1473-1477.
- Rodgers D M, Murphy J P, Frey K J (1983) Impact of plant breeding on the grain yield and genetic diversity of spring oats *Avena sativa*. *Crop Sci.* 23:737-740.
- Schachtman D P, Lagudah E S, Munns R (1992) The expression of salt tolerance from *Triticum tauschii* in hexaploid wheat. *Theor. Appl. Genet.* 84:714-719.
- Sharma H C, Gill B S (1983) Current status of wide hybridization in wheat. *Euphytica* 32:17-31.
- Stroup W W, Baenziger P S, Mulitze D K (1994) A comparison for removing spatial variation from wheat yield trials. *Crop Sci.* (in press).
- Stroup W W, Mulitze D K (1991) Nearest neighbor adjusted best linear unbiased prediction. *Amer. Stat.* 45:194-200.
- Tarczynski M C, Jensen R G, Bohmer H J (1993) Stress protection of transgenic tobacco by production of the osmolyte mannitol. *Science* 259:508-510.
- Whelan E D P, Hart G E (1988) A spontaneous translocation that transfers wheat curl mite resistance from decaploid *Agropyron elongatum* to common wheat. *Genome* 30:289-292.
- Yen Y, Baenziger P S (1992) A better way to construct recombinant chromosome lines and their controls. *Genome* 35:827-830.
- Youssef S S, Morris R, Baenziger P S, Papa C M (1989) Cytogenetic studies of progenies from crosses between 'Centurk' wheat and its doubled haploids derived from anther culture. *Genome* 32:622-628.

Maintaining rice yields in Japan

K. Hamamura*

Japanese agronomists and farmers have put primary emphasis on grain filling and grain ripening in the later growth stages to secure high yields. This emphasis reflects the availability and use of inexpensive fertilizer inputs to produce a sufficient number of panicles and spikelets for high yield levels. Extension and crop monitoring activities have also contributed to maintaining high yield levels.

More recently, micronutrient deficiencies appear to be contributing to declining yield trends in some parts of Asia. Monitoring systems may be necessary to cope with this decline.

Other possible opportunities for research by the Japan International Research Center for Agricultural Sciences (JIRCAS, formerly TARC) may exist in such areas as phosphorus nutrition and replacement of urea by ammonium sulphate in alkaline soils.

Factors contributing to high yields

Factors that contributed to yield increases and maintenance of high yield levels in Japan during 1950-67 include:

- 1) Incentives for rice farming;
- 2) Expansion of high-yielding areas to the north with the support of protected seedling nurseries;
- 3) Land development that enabled precise irrigation control;
- 4) Mechanization of tillage, planting, and harvesting;
- 5) High-yielding varieties;
- 6) Split application of fertilizers;
- 7) Plant protection utilizing agricultural chemicals;
- 8) Techniques to control yield components at key growth stages; and
- 9) Extension work and monitoring system for pests.

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After 1967, when over-production of rice became a financial burden, emphasis was placed on achieving higher grain quality, crop diversification, and reduction of production costs.

Rice research at JIRCAS

Even before TARC was formally established in 1970, several cooperative research projects were underway in Malaysia, Sri Lanka, Thailand and IRRI. Major fields of research were:

Malaysia (1967-present)

Major projects emphasized double cropping, plant protection and direct seeding with subprojects on breeding, land preparation, irrigation, mechanization, entomology, weed science, farm management, and extension methods.

Sri Lanka (1967-69 and 1978-90)

Projects focused on breeding and cultivation techniques.

Thailand (1969-91)

Research emphasis has been given to cultivation, multiple cropping in low-land rice-based systems, breeding, and postharvest technology.

China (1981-present)

Projects included breeding, rice blast, and advanced technology breeding methods.

Brazil (1982-85 and 1992-present)

Research focused on upland soils and weed control.

IRRI (1968-72, 1975-80, 1982-89)

Research projects included breeding for cold tolerance, insect resistance, high temperature tolerance, and bacterial leaf blight resistance.

CIAT (1992-present)

Research focused on tolerance of acid lateritic soil conditions.

TARC was reorganized into JIRCAS on 1 October 1993 to respond to increasing requests from developing countries. In reality, the limiting factor is human resources.

Future research opportunities

Several subjects of importance include:

Micronutrient status

In IARI, India (Nambiar and Ghosh 1984), a long-term experiment on fertilizer

application has been underway since 1971. The major conclusions are that micronutrient deficiencies such as Fe and Zn become a serious limiting factor after 4-5 years in plots receiving 50% of the recommended NPK input rate, and after 12-15 years in plots receiving 150% of the recommended NPK rate.

Substitution of urea by ammonium sulphate

Even in alkaline soils in India and China, urea is commonly used because it is cheaper and has a higher N concentration. In alkaline soils, ammonium sulphate may be more beneficial to improve nutritional conditions. If by-product sulphur, which comes from generating stations using coal as fuel, is utilized in the form of fertilizer, it is beneficial in avoiding acid rainfall.

Deep placement of ammonium sulphate

Miyoshi et al. (1990) proposed placing soil balls incorporated with ammonium sulphate deep into the soil. The method appears to increase the efficiency of N utilization by rice plants.

Phosphorus absorption mechanism

A peculiar capacity of pigeon pea to absorb iron-bound form of phosphorus by excreting phytic acid was discovered by Japanese and ICRISAT researchers (Ae et al 1990). A similar mechanism could be incorporated into upland rice.

Future outlook

It is also noteworthy that more and more young researchers are acquiring sophisticated techniques in molecular biology. With these new tools, mechanisms of pest resistance, stress tolerance and the process of starch synthesis may be better understood and this information can contribute to breeding for higher yield potential.

References cited

- Nambiar K K M, Ghosh A B (1984) Highlights of research of long-term fertilizer experiments in India. In LTFE Bulletin No. 1. Indian Agricultural Research Institute, New Delhi. 97 p.
- Miyoshi H, Siddique S B, Mazid M A, Chowdhury A A (1990) Studies on the efficiency of dressed nitrogen by transplanted aman rice. JICA (Japan International Cooperative Agency)-BRRI (Bangladesh Rice Research Institute) Publication No. 1.
- Ae N, Arihara J, Okada K, Yoshihara T, Johansen C (1990) Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. Science 248:477-480.

Research priorities to increase rice yield potential

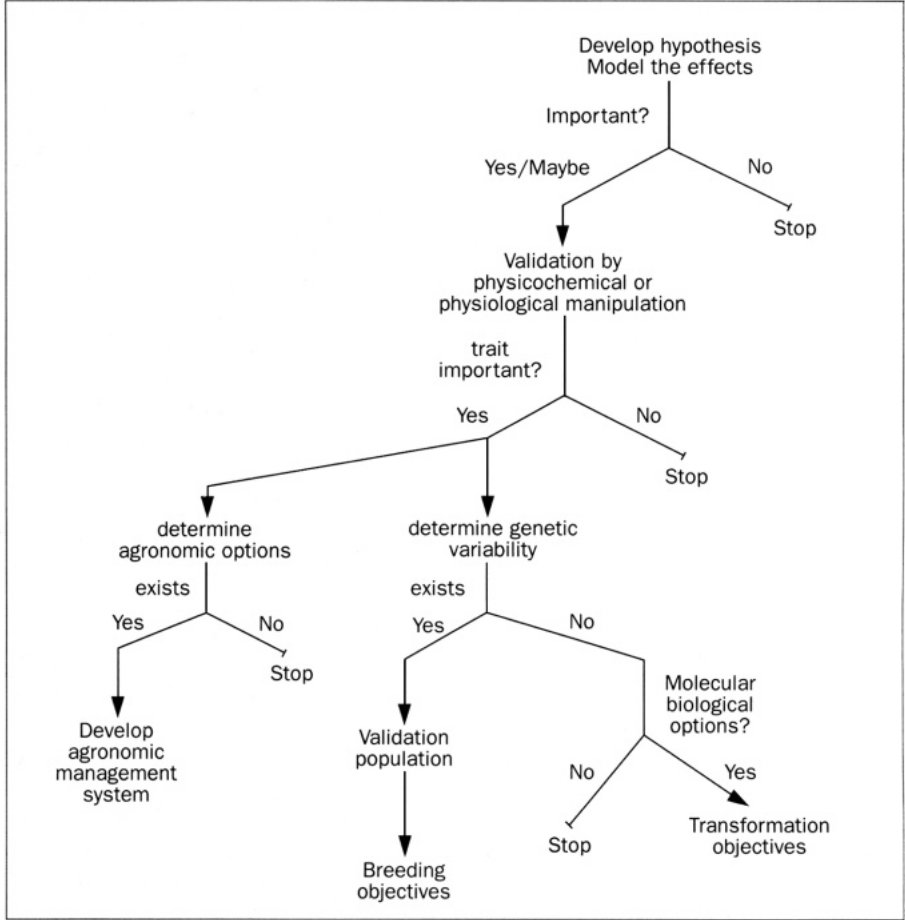
Achieving potential yield in any crop is simple in theory. Carbon and N acquisition should be maximized, and C-partitioning among centers of growth over the course of the season should be optimized. These processes are strongly influenced and controlled by weather and water and nutrient relations.

Protein is required in the basic structure and metabolic elements of every plant cell. The major components of photosynthetic systems, and thus leaves, are composed of protein. The large N requirement imposes significant logistical burdens on plants for uptake and assimilation and for the necessary pH balance. Mobilization of N from senescing tissues is particularly important in the overall N economy. All of these problems are exacerbated as growth and yield are pushed to higher levels.

To achieve potential yield where water and nutrients are not limiting, a crop should reach complete light interception quickly through an appropriate canopy architecture, and remain that way as long as possible. Equally important, it should quickly become source-limited (sink-dominated). When individual plants are appropriately spaced, most crops can attain such conditions. It is easiest to achieve with root and tuber crops, which have strong, indeterminate vegetative sinks. It is generally more difficult with crops where the desired yield accrues after a phasic change to reproductive growth.

Achieving high yield depends on efficient coordination and regulation of assimilation, growth, and senescence. Transition from one phase of development to another, and indeed, from day to night and with changes in weather, should proceed smoothly in time, with accommodation for imbalances between sources and sinks and minimum over- or under-adjustment. Photosynthesis, for example, can be suppressed rather quickly in the absence of sinks. Excessive production of leaves, tillers, roots, or other organs must be avoided. Carbon committed to such structures that is not recovered through senescence and remobilization, as well as that consumed in associated respiration, is lost from the train of yield accumulation. Close coordination and regulation through feedback and other means are clearly prerequisites to high yields and efficient use of resources; at the same time they must be sufficiently plastic to meet expected variations in weather and cropping systems.

Our preceding comments stress the dynamic nature of production processes. Formulating ideotypes for high yield must include a firm understanding of adaptive controls. This suggests that the ideotype must be based not just upon morphological features, as has been the popular understanding of an ideotype, but must also consider questions of physiological and biochemical processes. Breeding for a very high-yielding rice ideotype becomes not only a unique task in the history of modern plant breeding, but also an extremely complex and difficult one. A number of recent developments give some grounds for optimism. These include advances in the use of genetic transformation to explore and validate limiting processes, as well as possibly reducing the constraints they impose on yield. This clearly influenced the thinking of the working group as they advanced a number of objectives for research. The



1. The decision tree could be used to aid evaluation of the traits of the high-yielding ideotype. As transformation becomes easier and cheaper, it may become simpler to use transgenic technology in the place of the physicochemical manipulations. This would obviously change the structure of the decision tree.

various roles of molecular and other tools are outlined in a general way in Figure 1.

Research objectives

Increasing the rate of biomass production

The approach is to begin with exploratory physiological experiments to discover the processes that can be manipulated to increase the rate of net primary production by the rice crop, particularly during the reproductive growth phase. These studies must be conducted in an environment that allows full expression of maximum growth rate, and they are needed to set priorities and optimum methods for genetic manipulation and breeding. Interaction with modelling will be an essential ingredient in the development of this agenda. Six targets for physiological and molecular investigations are considered below for the initial stages of this effort.

- Increased specific leaf N (SLN, g/m) as a mechanism for increasing P_{max} (rate of photosynthesis in saturating light). Finding genotypes with thicker leaves without a corresponding decrease in leaf N content is one option. The difficulty with present indica rice varieties is that increased N supply leads to increased leaf area without an increase in SLN. Over-expression of RuBP carboxylase is an alternative approach.
- Increased P_{max} per unit SLN. Consider greater stomatal conductance, overcoming possible limitations in sucrose metabolism and export from leaf, and other possible avenues of limitation related to sink influence on P_{max} and SLN.
- Raising operational leaf photosynthesis towards the theoretical maximum at each stratum in the canopy. Examine whether photoinhibition is a factor in lowering quantum efficiency. Explore the importance of abiotic stress effects on photosynthesis under favorable conditions—is there afternoon water stress on clear days? Are there other nutrient element deficiencies besides N? Explore the photoprotective mechanisms that may give tolerance for abiotic stress (e.g. oxygen radical scavenging, energy dissipation). Are photoprotective mechanisms and other regulatory processes optimized in humid tropical environments where rapid fluctuation in light intensity is common?
- Canopy-related issues. Full light interception at all times after panicle formation, optimum leaf angle, leaf size, and N deployment (i.e. smaller leaves with higher SLN), and increased photosynthetic capacity of the panicle.
- Reduce respiratory losses. Explore CN-insensitive respiration as a loss of C by application of specific inhibitors. Explore the potential to reduce maintenance respiration.
- Keeping the photosynthetic system functioning longer by extending the supply, uptake, and conservation of N in upper leaves. Test this by

delaying senescence in experiments that employ leaf infusion techniques (e.g., supply of sucrose and glutamine, growth regulators). Develop a rapid screen for exploring existing genetic variation in the rate of leaf senescence during the reproductive growth phase that is independent of the effect of sink size.

Expected output. Identification of germplasm with greater rates of dry matter accumulation in canopies with full light interception.

New research methodologies. An efficient and rapid system of transformation, regeneration, and evaluation capabilities. Carbon and O isotope discrimination and ash content as potential rapid screens, improved methods for measurement of leaf photosynthesis, canopy temperature, chlorophyll fluorescence and other optical properties of photosynthetic surfaces.

Expected time frame. Explorative physiological experiments: 3 years. Molecular biological transformations: 10 years. Emphasis on manipulation of priority targets identified in the explorative phase, stressing priority traits and biochemical processes for which variation within the rice germplasm base is small. In such cases, improvement via molecular transformation would be required.

Increasing sink size

The extent to which sink size limits yield potential must be better understood, and the capability to predict sink size limitation must be further developed. Sink size is defined here as the product of the number of panicles per unit area and number of spikelets per panicle. Compensation precludes increasing any yield component independently of the others, so specification of plant number, panicle number, and panicle size cannot be done independently.

The extent to which sink size is limiting must be quantified by altering source and sink in a range of potential yield environments, and utilizing and refining simulation models to allow prediction of sink limitation. Transgenic plants with modified sink capacity would also provide useful tools. Ways of increasing sink size include:

- A higher rate of biomass accumulation during the period prior to heading, when inflorescence size is determined;
 - Increased partitioning of the available assimilate supply (including stem carbohydrate reserves) to the developing panicle prior to heading;
 - Reducing wasteful tillering;
 - Increasing grain-filling duration by (a) a lower rate of filling of individual grains and/or (b) asynchronous onset of filling within and between panicles.
- Increased sink size, however, will not contribute to increased yield potential unless there is sufficient net assimilation to fill a larger sink. Thus, this objective is tightly linked to increasing the rate of biomass production, and was given the highest priority.

Expected output. Rice genotypes capable of producing sufficient total spikelet number for a 15 t/ha rice crop in the tropics.

Expected time frame. 10 years.

Decreasing susceptibility to lodging

- Develop mechanistic models for the two different types of lodging.
- Seek genetic options for improving the critical components of stem diameter, stem lignification, root depth and angle, and to decouple N level and root growth.
- Optimize cultural practices and management options such as water management, disease control, and the use of plant growth regulators.

Expected output. Genotypes with increased lignification and/or increased stem thickness, and improved root lodging resistance created or identified.

Expected time frame. 5 years if existing genetic variation can be used, 10 years if molecular solutions are required.

New research methodologies. Rapid screening methods to quantify resistance to lodging.

Using genetics, both classical and molecular, to validate the components of the IRRI-specified ideotypes as well as other traits identified in pursuit of objectives

Ideotype breeding may well give higher yields, but IRRI, as a global rice research center, must identify which traits were important in achieving this success, and the physiological basis for such increases.

- Ensure sufficient independent variation in individual ideo-traits to facilitate the validation of their contribution to increased yield potential.
- Plan development of populations and yield testing to achieve the above.
- Give special attention to identifying the most appropriate environments for testing genetic gain in yield potential.
- Determine if the ideotype for a variety is different from the ideotype of the hybrid parent, and whether there are unique ideo-traits required for hybrid parents.
- Ensure that the target for heterosis is at least 30% above the best pure lines, with careful quantification and identification of heterosis based on actual yield increases in the appropriate environment.
- Explore methodologies for more efficient hybrid seed production, both for commercial and experimental use. For example, in RNase or flavone biosynthesis, restoration will occur via RNase inhibitors or external flavone application; chemical hybridizing agents; CMS, TGMS, use of tester theory for identifying parents.
- Select and identify lines with increased stability of yield potential towards achieving the goal of greater yield potential.

Expected output. Germplasm (pure lines and hybrids) with greater yield potential. Validation of an integrated physiological and genetic approach for research to increase yield potential.

Expected time frame. Five years for the first three objectives, 15 years for the next three, and 15 years for the last objective.

New research methodologies. Modern tools to rapidly assay large numbers of lines, and reproducible yield potential assessment in an appropriate set of environments.

Comments. Note that IRRI's new plant type is designed for direct-seeded rice in an environment in which hybrids will play a major role. However, inbred improvement is just as important in a situation in which hybrids dominate as in one using only inbreds. Transformation should be integrated into the conventional breeding program.

Optimizing crop management

Full expression of a crop's potential for high yield depends on management. Management is continually evolving, and this evolution creates opportunities for breeding. Control of time and method of sowing, space relations within the stand, supplies of nutrients and water, and control of weeds are among the factors that can be manipulated. Advancing yield potential should be coupled closely with improving systems, practices, and tools for management. In the following recommendations, attention is called not only to the need for making it easier to implement new practices, but also to accommodate the increasing scarcity of labor.

- Efficiency in the use of nutrients, especially N inputs, must be improved. As yield potential increases, this may require different formulations, placement, or timing from present applications.
- Efforts should move quickly towards a means of spacing plants precisely. Interference among neighboring plants in direct-seeded stands and in transplanted nurseries as well as after transplanting is quite different, and may call for rather different traits in tillering and root growth.
- Attention should be given to the development of simple "tools" to aid in rapid diagnosis of the status of soils and crops. Methods for quick assessment of crop N status, for example, are especially important.
- Efficient use of water is also needed. This entails not only control of water application to the field, but also water control within the field that depends on careful land leveling during tillage and puddling operations.
- Minimizing the potential for undesirable externalities from intensified rice production in support of higher yield potential is also an important objective. Attention should be given to possible effects on ground and surface water resources, and emission of nitrous oxide and methane gases in relation to crop management practices required to achieve high yields.

Expected time frame. Agronomic research must backstop and provide feedback to both the breeding and physiology research on yield potential. This support must be a continuous activity in IRRI's research project on yield potential.

New research and methodologies. The use of hydroponic growth systems in the field is recommended to quantify existing and future yield potential without complications from imperfect plant nutrition, or from negative effects of anaerobiosis and soil physical properties on crop performance.

Conclusions

One common feature here is the role anticipated for molecular investigations and genetic transformation. The workshop participants were swayed in this direction particularly by the recent studies of components of photosynthesis and in sucrose and starch metabolism. The work will, however, require an efficient transformation system in rice, and even so will be resource intensive, accounting for a large investment in scientist-years. The working group was guided in this by Monsanto's recent transformation of Russet Burbank potatoes with the ADP pyrophosphorylase gene (affecting starch synthesis) that was tailored to function only in the tuber. Attaining the goal of increased dry weight content via greater starch synthesis capacity took between 15 and 20 scientist-years to achieve. These sorts of costs are small, however, relative to the potential benefits from progress in rice.

Another interesting aspect of the working group discussion was the reference in several places to the possibility of significant abiotic stresses in what is supposed to be a situation where no factor is limiting. Experience with maize in particular, but also with other crops, supports this view. Growing crops outside inevitably exposes plants to temporal fluctuations in their environment; these fluctuations could be stressful in ways we would not have imagined some years ago. Recent experience confirms that better stress resistance at the higher end of the yield scale can also extend to the lower end of the scale.

Communication and collaboration between disciplines (breeding, agronomy, physiology, biotechnology, etc.) is vital in what is a multidisciplinary task on a very large scale. Coordination will also be essential both within IRRI and between IRRI and partner organizations. The working group did not deal with the question of partner organizations. There will be many possible candidates, but access to realistic field conditions for tropical rice will restrict the list. IRRI seems to be well positioned with the resources and long-term commitment to rice improvement that are required to lead and coordinate the task outlined.

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