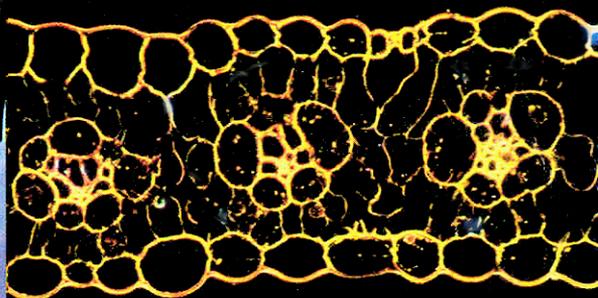
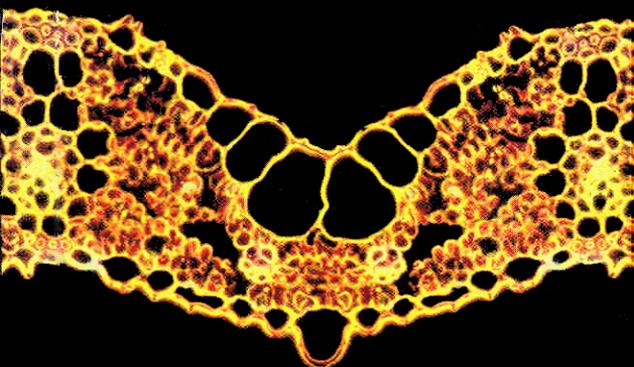


Redesigning Rice Photosynthesis to Increase Yield

Edited by J.E. Sheehy, P.L. Mitchell, and B. Hardy



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*Redesigning Rice Photosynthesis
to Increase Yield*

Cover description:

Left is a photomicrograph of a transverse section of a rice leaf. The bulk of a leaf, the mesophyll (M), is composed of arm-cells with projections of the cell wall into the cell. The larger vascular bundles (dense yellow) have bundle sheath (BS) cells around them. Rice plants appear in middle. Right is a transverse section of a maize leaf. Three vascular bundles appear (dense yellow), surrounded by large BS cells. The M cells are arranged so that almost every M cell is in contact with a BS cell. This is known as Kranz anatomy, characteristic of terrestrial plants with C₄ photosynthesis. (Images left and right by W.C. Taylor and Celia Miller, middle photo by A. Javellana.)

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Redesigning Rice Photosynthesis to Increase Yield

Proceedings of the Workshop on the Quest to Reduce Hunger:
Redesigning Rice Photosynthesis,
held in Los Baños, Philippines, 30 November - 3 December 1999

Edited by

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Foreword

Failure to meet the increasing demand for food from the rising population of Asia will result in misery for hundreds of millions. To be fully confident of avoiding this alarming problem, a 50% increase in Asian rice yields is required by 2050. For 30 years, however, 10 t ha⁻¹ has remained the maximum achievable yield of inbred cultivars of irrigated rice in the tropics, a value long regarded as a yield barrier. Breaking this yield barrier is clearly a formidable challenge. Inevitably, increased rates of resource capture and energy conversion are required to build a faster growing crop. At IRRI, we have explored theoretically yield components and the conversion of solar radiation into the energy in biomass. We concluded that the upper yield limit of rice crops with conventional photosynthetic pathways will take us only halfway to our stated goal of a 50% increase in yield. Improved crop photosynthesis is therefore essential for increased yields.

The principal objective of this workshop was to explore the possibilities for increasing yield in rice, to keep pace with population growth, by redesigning the rice plant's photosynthetic pathway. We wanted to establish at what level we at IRRI should work on this problem, what are the key areas into which we should channel our energies, and, crucially, what partnerships could be constructed to further our aims. We discussed whether the inefficiencies in current photosynthetic systems could be eliminated and if it would be possible to imitate nature and produce a rice plant with the superior photosynthetic capacity of maize. The difficulties in producing such novel plants and the advantages they would offer in terms of yield and use of natural resources were the subject of much discussion. It was concluded that such a plant could revolutionize rice farming, but producing it will not be an easy task.

The biggest single change that could be made to rice would be to make the photosynthetic pathway of rice resemble that of maize, by eliminating photorespiration. In irrigated rice, yield potentials would rise and the increasing demand for rice, driven by the increasing population of Asia, could be met. In drought-prone ecosystems (upland and rainfed lowland rice), yields could be maintained or increased with less water and less fertilizer, especially when coupled with the rising atmospheric concentration of carbon dioxide. The people living at the margins in these ecosystems would see improvements in yield and in yield stability.

We are grateful to the internationally renowned scientists who came to IRRI to participate in the workshop. They all shared their expertise with us and contributed to a fascinating and stimulating debate. The chapters in this book cover various subjects, such as the economic consequences of yield improvements for poverty, the molecular genetics of photosynthesis, and ecophysiological and evolutionary perspectives of photosynthesis in wetlands. IRRI continues to face the challenge of using basic science in the quest to reduce hunger and a workshop such as this one helps us set the agenda.



RONALD P. CANTRELL

Director General

International Rice Research Institute

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Perspectives

The contribution of rice research to poverty alleviation

D. Dawe

Rice is the dominant staple food of Asia, accounting for more than 70% of caloric intake in some countries. Furthermore, Asia is home to approximately 70% of the world's 1.3 billion poor, and the most severe malnutrition in the world occurs in South Asia. These considerations mean that rice research has a key role to play in global poverty alleviation.

Rice research contributes to poverty alleviation through several pathways, and these contributions benefit both producers and consumers. The direct pathway leads to higher productivity and higher profits for farmers. The indirect pathway arises from the lower prices for consumers that are the inevitable result of higher farm productivity for any given level of demand. In the short run, lower prices for consumers reduce poverty because many poor people (the urban poor, the rural landless, and nonrice farmers) are net buyers of rice, and lower prices increase their effective incomes. In the long run, lower prices for consumers reduce the cost to employers of hiring workers (without sacrificing any welfare on the part of those workers). This stimulates job creation in the higher productivity industrial and service sectors of the economy, and eventually draws labor out of agriculture. This structural transformation of the economy is essential for long-term poverty alleviation. In fact, no country has ever become wealthy without removing a significant fraction of its labor force from the agricultural sector.

After the initial success of the Green Revolution, rice yields have stagnated or grown slowly in many countries, and this slow growth retards the process of poverty alleviation. Creation of a C₄ rice plant has the potential to generate substantially higher farm yields and make an important contribution to global poverty alleviation efforts.

Rice is one of the world's most important crops in terms of economic value, and approximately 90% of the world's rice is grown and consumed in Asia in a belt ranging from Pakistan in the west to Japan in the east. Because of the dominance of this region, this chapter will focus on rice production and consumption in Asia.

Rice is very important to Asian consumers, but the magnitude of this importance is astonishing. Rice accounts for more than 70% of total caloric intake for the average citizen in Myanmar and Bangladesh, and it accounts for nearly two-thirds in Vietnam. Even in relatively wealthier countries such as Thailand and Indonesia, it still accounts for nearly 50% of caloric intake (FAO 1999). Furthermore, these figures are for the average citizen, which means that for many citizens (primarily the poor) the share is even higher. Rice is also important to millions

of small Asian farmers who grow the crop on millions of hectares throughout the region, and to the many landless workers who derive income from working on these farms.

Asia is also home to a majority of the world's poor. According to the *Human Development Report* (UNDP 1997), approximately 70% of the world's 1.3 billion poor people live in this part of the world. To some extent, this is because Asia already has such a large population. But even in relative terms, malnutrition appears to affect a substantially larger share of the population in South Asia than in Africa, which are the two poorest regions in the world (Svedberg 1999). Thus, rice research has a key role to play in poverty alleviation at the global scale.

Rice research and poverty alleviation

How does rice research help alleviate poverty? In some ways, this is an easy question to answer. Higher standards of living for rice farmers can only be sustained if farmers are able to produce more rice per unit of input. This higher productivity leads to higher profits from farming and a reduction in poverty. Thus, one way that rice research helps alleviate poverty is by increasing the productivity of farmers. This is the "direct" contribution to poverty alleviation. It is an important one, and it applies primarily to farmers that own land.

If this direct contribution were the only one, then research priorities would be relatively simple to set. The only goal would be to help the poorest farmers directly, and this would mean working with farmers on marginal lands without access to roads and irrigation. But rice research also makes an important indirect contribution to poverty alleviation, and, unfortunately, this contribution is often overlooked. This indirect contribution makes itself felt in both the short term and the long term.

To appreciate the short-term indirect contribution of rice research to poverty alleviation, it is important to understand that not all poor people are farmers who own land, grow rice, and benefit directly from improvements in crop productivity. In fact, several classes of people are very poor but do not reap any direct benefits from rice research. These people are net purchasers of rice, and they reap indirect benefits from rice research in terms of lower prices for the rice they must buy to stay alive. It is true that lower rice prices, *holding all else constant*, adversely affect poor rice farmers who produce a surplus of rice: this is the food price policy dilemma noted by Timmer et al (1983). But agricultural research resolves this dilemma, as will be explained shortly.

Who are the poor people who are net consumers of rice? One increasingly important group is the urban poor. As of 1997, urban dwellers accounted for one-third of the total population in Asia. Although this means that two-thirds of the population still lives in rural areas, urbanization is increasing throughout the developing world, and Asia is no exception. From 1990 to 1997, Asia's rural population grew at a rate of just 0.6% per annum, while the urban population grew more than five times as fast at 3.2% per annum. The urban population increased by 211 million people from 1990 to 1997, whereas the rural population increased by just 93 million. Population projections from the United Nations indicate that the urban population will exceed the rural population in Asia by 2025 (UN 1997). Average levels of income are surely higher in urban areas than in rural areas, but not all urban dwellers are well-to-do. There is little doubt that the number of urban poor is increasing, both in absolute terms and as a share of the total poor (Haddad et al 1999).

Although the share of total poverty that is urban is increasing, it is still probably true that the number of the rural poor exceeds the number of the urban poor. Yet, despite the tremendous importance of rice as a crop in rural Asia, not all rural dwellers are land-owning rice farmers.

Many rural people are landless or near landless and derive most of their income from agricultural labor. In fact, when we think of truly abysmal poverty, we usually think of someone who does not have enough food to eat. Almost by definition, this is not a farmer with sufficient land to grow enough food for his or her family. No doubt many land-owning farmers are poor, but they are not the poorest of the poor.

How numerous are the rural landless or near landless? Data on their numbers are not easy to obtain, but it is clear that they are by no means a small group. In Bangladesh, for example, assuming a paddy rice yield of 2.8 t ha^{-1} (equal to the national average), it would take about 0.2 ha of land to supply an average-size family of 4.7 people with just half of the national average per capita consumption of rice. (This assumes that all of the available land is planted to rice, with nothing left over for vegetables or other crops.) According to an official government survey conducted in 1995-96 (Bangladesh Bureau of Statistics 1998), 48 million Bangladeshis live in households that own less than this amount of land (this is nearly half the rural population). These people have little or no surplus rice to sell, and, if rice prices were lower, they would surely buy more than they currently are able to afford. They are net purchasers of rice, they are very poor, and they would benefit from lower rice prices, especially if those lower prices were induced by higher farm productivity.

In Indonesia, 45% of rural households on Java do not own any land, and another 20% own less than 0.25 ha. In the Philippines, the 1980 census showed that 38% of the rural labor force consisted of landless agricultural laborers (cited in Hayami et al 1990). Many of these work on lands growing maize, sugarcane, coconut, hemp, or tobacco. In Sri Lanka, there are many workers in the estate crops sector (e.g., tea). All of these people and their families would benefit from being able to purchase cheaper rice.

Rural dwellers who own land, but use it to grow nonrice crops, are also poor in many cases. They would benefit from cheaper rice prices. In Indonesia, many farmers plant maize, cassava, and soybeans. In the Philippines, maize and coconut are important crops grown by poor smallholders.

Several studies have compiled data on the position in the income distribution of net buyers and net sellers of rice. Mellor (1978) found that the two poorest deciles of the income distribution in India were net buyers of food, whereas each of the top eight deciles were net sellers of food. Sahn (1988) showed that 83% of rural households in Sri Lanka were net consumers of rice (and, of course, all urban households). In Madagascar, Barrett and Dorosh (1996) found that “the roughly one-third of rice farmers who fall below the poverty line have substantial net purchases of rice, suggesting important negative effects of increases in rice prices on household welfare.” This finding only concerns rice farmers with land, and ignores the rural landless who are even poorer and who are also net purchasers of rice. The authors go on to state that “the poorest rice farmers are quite vulnerable to an increase in the price of their principal crop. . . . Conversely, the largest, wealthiest 10% or so of farmers stand to benefit significantly from rice price increases.” A recent World Bank report on Indonesia states that three out of four Indonesians are net consumers of rice. Clearly, not all of these net rice consumers are poor, but Indonesia is still a poor country, and a substantial number of these net consumers must be poor. Thus, lower rice prices contribute directly to short-term poverty alleviation for these households.

Thus, many poor Asians are net purchasers of rice, and they benefit from the lower rice prices induced indirectly by rice research. Rice constitutes an important part of their daily expenditures, so the contribution of lower prices is not trivial. For example, for the poorest 10% of the urban population in Bangladesh, rice accounts for half of total expenditures. Even for the poorest 60% of the urban population, nearly 40% of expenditures go to rice (Bangladesh

Bureau of Statistics 1998). In Indonesia, rice accounts for 20% of total expenditures for the poorest quarter of the urban population (about 20 million people). For the poorest 5% of the urban population, the share of rice is about 25% (Biro Pusat Statistik 1998).

But poverty alleviation is ultimately a long-term, broad-based process, and higher farm productivity makes at least two important long-term indirect contributions to poverty alleviation. One is through higher wages for farm laborers. As farm yields rise, the value of production increases, which in turn increases the demand for labor. Higher demand for labor leads to higher wages for the poorest of the rural poor, those who work as hired hands on someone else's farm.

The second important long-term indirect contribution is through lower rice prices. To understand this contribution, it is important to view the process of long-term poverty alleviation in the context of the structural transformation of the economy. To raise the incomes of large numbers of poor people in a sustainable manner requires economic growth over a long period of time. This is not always a sufficient condition, but it is certainly necessary. Long-term poverty alleviation also requires the creation of jobs in the relatively higher productivity industrial and service sectors of the economy. This is obviously true if one is trying to reach the urban poor. It is also true for the long-term reduction of rural poverty, because no country in history has managed to grow rich while keeping a large share of its population in agriculture. This is a striking empirical regularity of the development process (Timmer 1988). In other words, long-term poverty alleviation requires a structural transformation of the economy away from agriculture and toward industry and services.

Low rice prices that are the result of higher productivity induced by agricultural research contribute to this structural transformation of the economy. Low rice prices allow wages to be lower from the employer's point of view, without sacrificing any welfare on the part of the workers. In conjunction with other factors, these lower wages stimulate the job creation and growth that are necessary for sustainable poverty alleviation. If a country insists on a high rice price policy instead, workers will legitimately demand higher wages. But these higher wages will discourage investment, both domestic and foreign, and the growth process will be retarded.

Lower rice prices also contribute to a structural transformation of the agricultural sector itself. They allow rice farmers to diversify their cropping patterns by making it cheaper to buy rice from the market. These ex-rice farmers then begin to produce other crops such as fruits and vegetables; this allows consumers to diversify their diets and increase their intake of proteins, vitamins, and minerals.

Yet this does not mean that countries can accelerate the growth process simply by pursuing policies that arbitrarily depress rice (food) prices. This strategy has been tried, and it has not worked. If low farm prices are not accompanied by rising productivity, farmers have little incentive to produce, leading to reduced supplies of food. In conjunction with low prices that encourage high levels of consumption, this results in either black markets (and high prices for those without privileged access to cheap food) or large government fiscal deficits that cannot be sustained.

The only solution to this low price/high price dilemma is agricultural research. Farmers will be encouraged to continue producing even as prices fall only if research leads to higher productivity that compensates for the lower prices. If farmers are able to produce more rice per unit of input, then it is possible for the welfare of everyone to improve. Consumers will gain through lower prices, and the welfare of farmers will also improve as production per unit labor increases. Higher production per unit labor will allow farmers to shift some of their labor into nonfarm work, or will allow family members to migrate to urban areas in search of higher paying jobs. This shedding of labor from agriculture and diversification of income sources are

key components of the structural transformation mentioned above. This process is well under way in Asia, and data from several different countries show that the share of rice in total farm household income is typically less than 50% (Sombilla and Hossain 1999). This also highlights the fact that even many rice farmers ultimately benefit from low rice prices. The rice-growing families of today are progressively diversifying out of rice by growing other crops, moving off the farm, or receiving remittances from family members with jobs in urban areas.

Thus, lower food prices and higher wages that are the result of increased agricultural productivity (as opposed to government decree) contribute to poverty alleviation over the long term. Datt and Ravallion (1998) demonstrate empirically that this process has occurred in India. Using data from 24 household surveys spanning 35 years, they show that “higher farm productivity brought both absolute and relative gains to poor rural households. A large share of the gains was via wages and prices, though these effects took time. The benefits to the poor were not confined to those near the poverty line.”

Timmer (1997) analyzed cross-sectional data from 27 developing countries on income growth, income distribution, and inequality. For countries with relatively equal distributions of income (generally those in the rice-producing countries of East, South, and Southeast Asia), he found that the poorest members of society (those in the bottom quintile of the income distribution) benefited more from agricultural growth than from growth in the industrial and service sectors of the economy. He also found that agricultural growth helps the poor relatively more than the rich in these countries. Agricultural growth is thus both relatively efficient and fair in its effects on poverty alleviation. Since agricultural research is one of the prime determinants of agricultural growth, research clearly has an important role to play in connecting the poor to the growth process.

Long-term trends in rice prices and yields

Since the Green Revolution began in the mid-1960s, rice prices have fallen in real terms (i.e., after adjusting for inflation) and the productivity of rice land has increased dramatically. Both of these developments have made important contributions to poverty alleviation in Asia. From 1950 to 1981 the price of rice on the world market averaged approximately US\$860 t⁻¹ (constant 1998 prices). From 1985 to 1999, however, prices averaged about US\$327 t⁻¹, a decline of 62% (Fig. 1). This is a remarkable decline and it has allowed many rice-deficit nations in Asia (and elsewhere) to turn to the world market for needed supplies at a relatively low cost. Only a small share of world production is traded on the world market, however, so this is not necessarily representative of overall price trends. Long-term data for retail rice prices are not easily available for all countries, but rice prices in domestic markets are generally also lower than they were at the beginning of the Green Revolution. For example, rice prices in Thailand, Indonesia, and the Philippines are about 15–20% lower now than they were in 1968 (Fig. 2). The decline has been greater in India and Bangladesh, where retail prices adjusted for inflation are about 50% lower than they were in 1968 (Fig. 3).

Lower prices are clearly a boon for consumers, but they represent a deterioration in the situation of farmers if all other factors are constant. But other factors have been far from constant. Production per unit land soared in Asia during this period, with paddy rice yields increasing from an average of just 2.1 t ha⁻¹ between 1964 and 1966 to 3.9 t ha⁻¹ from 1996 to 1998, an increase of 89% (Fig. 4). Clearly, not all of the increase in yields is solely attributable to rice research. Increased use of fertilizer and increased investment in irrigation have also made important contributions. Yet rice research allowed these additional inputs and investments to have a large impact, and farmers have benefited tremendously from the resultant gains in

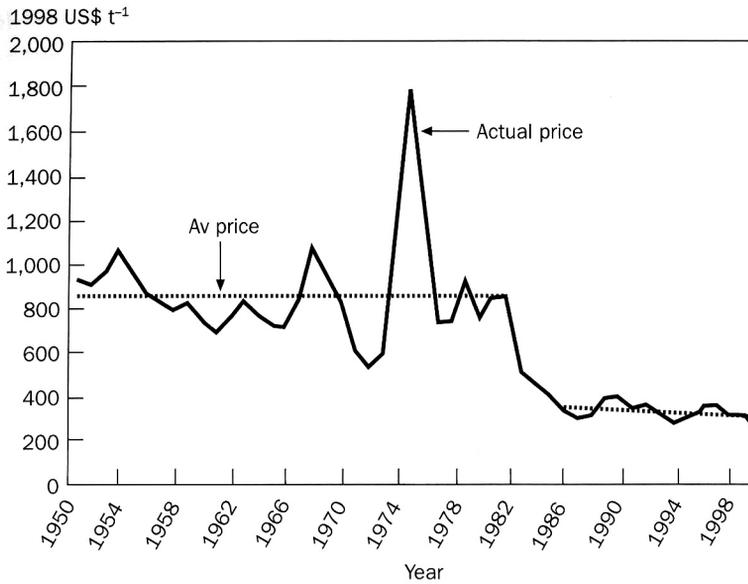


Fig. 1. Real world rice prices, 1950-99. Price quotations are for 100% Grade B rice, FOB Bangkok. Source of raw data: IMF (1999).

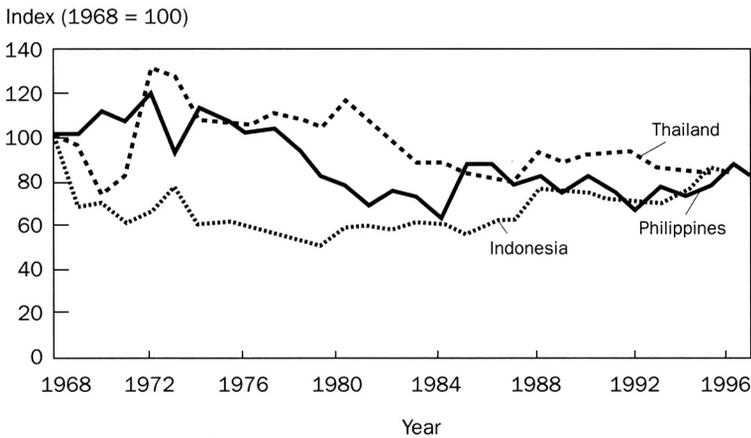


Fig. 2. Trends in real retail rice prices in Thailand, Indonesia, and the Philippines. Sources of raw data: IRR (1995, 2000).

yield. Lower rice prices and higher rice yields have allowed both rice farmers and rice consumers to escape poverty during the past 35 years.

Future trends in rice demand

Will the trend of lower prices and higher yields continue? In the case of prices, the outcome will depend on both supply and demand. In the next 20 years, future demand for rice will

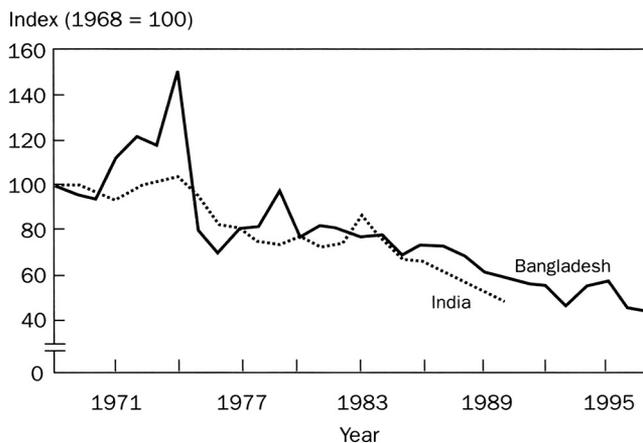


Fig. 3. Trends in real retail prices in India and Bangladesh. Sources of raw data: IRRI (1995, 2000).

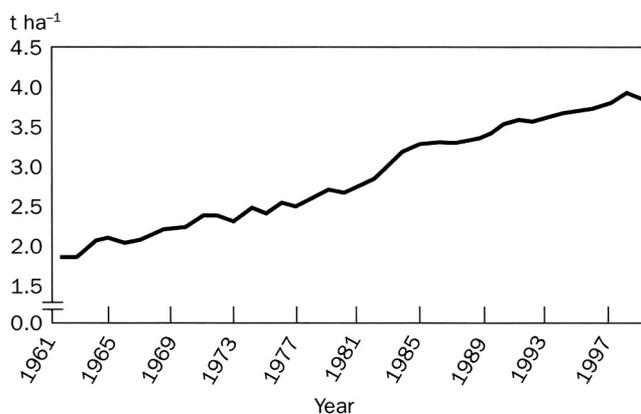


Fig. 4. Paddy rice yields in Asia. Source of raw data: FAO (1999).

depend primarily on population growth. Income growth and urbanization will also affect demand, but in the aggregate these effects are likely to be small. Population growth has been steadily declining in Asia for the past few decades. In the 1960s, population grew at an average annual rate of 2.3%, but this has slowed substantially to just 1.4% from 1990 to 1997 (FAO 1999). Population growth projections from the United Nations estimate that this trend will continue, with average annual population growth in Asia slowing to a rate of 1.08% y^{-1} from 1995 to 2020 (UN 1997). This implies that growth in rice demand in the next 20 years will be much slower than it was in past decades. Thus, estimates made by Rosegrant et al (1995) in the IMPACT model of the International Food Policy Research Institute (IFPRI) are that demand for rice will grow by approximately 1.0% y^{-1} until 2020.

Slower growth in demand does not guarantee lower prices, however, since gains in rice production are becoming more difficult to achieve. Growth in rice area harvested has slowed

to a trickle at just 0.2% per annum from 1984 to 1998, and yield growth is much slower than it was in the past. From 1967 to 1984, rice yields in Asia grew at an average compound rate of 3.5% per annum, while this rate slowed to just 1.2% per annum from 1984 to 1998. (In absolute terms, yield grew at an average annual rate of 67 kg ha⁻¹y⁻¹ in the first period, and just 42 kg ha⁻¹y⁻¹ in the latter period.)

To summarize, although demand growth is slowing down, so is growth in yields, and it is unclear how prices will evolve during the next 20 years. The discussion in an earlier section of this chapter explained why low rice prices contribute to poverty alleviation, so it will be beneficial for prices to be as low as possible in the future, provided that the low prices are due to increased productivity. Thus, the goal of rice research should not be to meet some fixed hypothetical growth rate of future demand, but should be to increase production and yield as much as possible. If the growth of supply exceeds the growth in demand, that will not generate stockpiles of useless grain. Instead, it will push prices lower, and this will hasten the escape from poverty. Without such research, however, there is a real risk of stagnant yields and higher prices, which would throw millions of already poor Asians deeper into poverty. One hopes that it does not take a major food crisis to elicit more funding for agricultural research.

Future constraints to increasing rice production

The most important inputs in rice production are land, labor, water, and fertilizer. Constraints to the future supply of these inputs will affect rice production, and consideration of these constraints gives insights into the type of technologies that are most likely to be helpful in reducing the incidence of poverty.

Land is an increasingly scarce resource in Asia. Urbanization and road construction are placing demands on land use, and it is certain that future gains in rice production will come primarily from increased production per unit land, not from increased area cultivated. If the area cultivated expands significantly, this will lead to environmental problems as production expands to marginal lands that are prone to soil erosion.

Water is another key natural resource that is becoming scarcer as we enter the 21st century. First, demand for water from industries and households is growing rapidly, leaving less water for agriculture than would otherwise be available. Second, the costs of constructing new irrigation systems are increasing rapidly (Rosegrant and Svendsen 1993). The water supplies most suitable for irrigation were naturally developed first, leaving only the sources that are in relatively unfavorable topographic locations. This makes it unlikely that increasing irrigation water supplies will make a large contribution to future increases in rice production.

Although Asia is very densely populated, labor is also becoming scarcer as economic development proceeds and more attractive jobs in urban areas become available. Farmers in many parts of Asia, even in relatively poor countries such as India and Bangladesh, find it increasingly difficult to hire farm workers. Technologies that require large inputs of labor to increase rice production are not likely to be economically attractive to farmers and will not be adopted. For example, the use of deep-placed urea tablets to increase nitrogen-use efficiency was not accepted by many Indonesian farmers because of the additional labor involved in placing the tablets deep into the soil (Cohen 1997).

In contrast to increasing shortages of land, water, and labor, fertilizer is much cheaper now than it was 30 years ago. The world market price of urea (the most common source of nitrogen for rice farmers in Asia) was US\$516 t⁻¹ on average from 1957 to 1961, but declined to US\$174 t⁻¹ on average from 1994 to 1998 (all prices are in constant 1998 dollars; see Fig. 5). Because

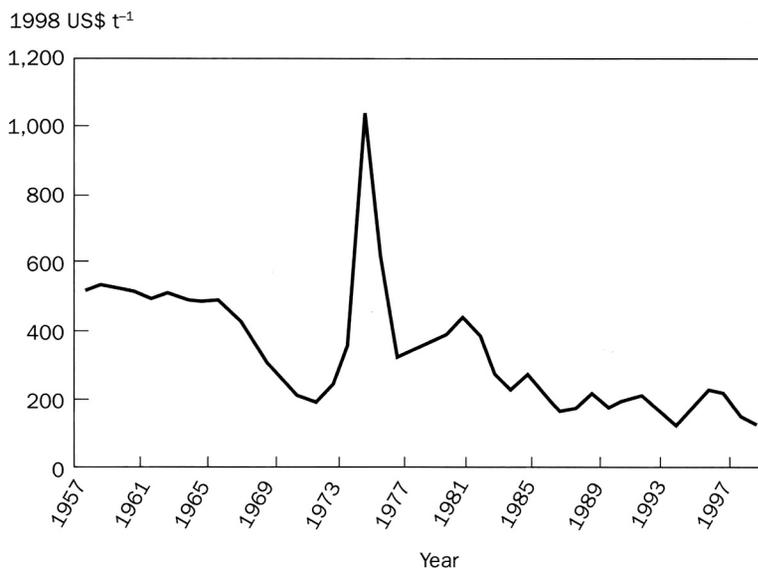


Fig. 5. Real world urea prices, FOB Western Europe, 1957-98. Source of raw data: IMF (1999).

of these low prices, the cost of nitrogen fertilizer is a relatively small share of the total value of production. For example, recent data collected from representative farm enterprises in several key irrigated rice-growing areas showed mean shares of just 3.5% in West Java, Indonesia, 6.7% in the Central Plains of Thailand, and 6.8% in the Mekong Delta of Vietnam (these data are averages of six seasons during the period 1994-98). The share is comparable in typical rainfed systems. It might be supposed that the reason for the low shares of fertilizer costs is heavy government subsidies, but this is not the case (Dawe 2000).

The relatively low cost of nitrogen fertilizer suggests that this is a viable means of increasing future yields. But this will be contingent upon the development of new rice varieties. The Green Revolution began more than 30 years ago, and this has given farmers adequate time to learn the benefits of nitrogen fertilizer. Although farmers' fertilizer management can certainly be improved, they are not grossly underapplying nitrogen given the current varieties available. In other words, increased yields through higher nitrogen applications in farmers' fields will require new varieties developed through more research. If rice can be converted into a C₄ plant, this would open the door for the creation of such new varieties. Such a development would represent a major contribution to poverty alleviation in Asia.

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C₃ versus C₄ photosynthesis in rice: ecophysiological perspectives

R.F. Sage

C₄ photosynthesis confers substantial benefits upon herbaceous plants in tropical environments, most notably in high-light habitats with frequent drought, heat, and salinity stress. In flooded situations, it is less beneficial, for reasons that are not clear. Conditions in wetlands may not enhance CO₂ assimilation rates of C₄ plants to the degree needed to suppress C₃ competitors; alternatively, wetland C₃ plants may be well adapted for marshy environments for reasons unrelated to photosynthetic pathway. If the wetland condition prevents C₄ dominance because C₃ photosynthetic performance is relatively strong in flooded soils, then the development of C₄ rice would probably be of significant benefit in upland situations only, particularly those that experience drought. Alternatively, if C₄ plants are less well adapted to flooded conditions than C₃ plants for reasons unrelated to photosynthetic pathway, then substantial benefits may result from introducing C₄ rice plants into flooded soils. In either case, these considerations must be evaluated in the context of future levels of atmospheric CO₂. Elevated CO₂ will enhance photosynthetic efficiency and yield of C₃ rice plants, perhaps more than might be obtained with C₄ rice. Existing rice varieties may not be adapted to fully exploit the increased productive potential that high CO₂ represents, and thus engineering C₃ rice for a CO₂-enriched environment may be an important way to enhance yield. For example, current varieties of rice plants grown at high CO₂ contain too much Rubisco, and reduction of Rubisco content by antisense technology can further enhance yield and resource-use efficiency. Because the high CO₂ levels favoring C₃ over C₄ photosynthesis may not appear for many decades, however, the C₄ strategy may be the best approach for increasing rice production in the next half-century.

C₄ Photosynthesis is a biochemical and structural syndrome that enhances the concentration of CO₂ in the bundle sheath tissue where ribulose -1.5-bisphosphate carboxylase-oxygenase (Rubisco) is localized. As a consequence, CO₂ is elevated to levels that nearly saturate the carboxylation reaction of Rubisco and the rate of photorespiration is curtailed. This substantially enhances the efficiency of photosynthesis, particularly at higher temperature and in low atmospheric CO₂ conditions that are conducive to high rates of photorespiration (Fig. 1). In C₄ plants, photorespiration is rarely greater than 5% of the rate of photosynthesis; in C₃ plants, it can exceed 30% of the rate of photosynthesis above 30 °C (Fig. 2; Sage and Pearcy 2000). Because of these improvements, C₄ plants have greater maximum productive potential, and greater light-, water-, and nitrogen-use efficiencies of both photosynthesis and growth (Evans 1993, Brown 1999). C₄ photosynthesis also confers numerous indirect benefits in that C₄ biomass

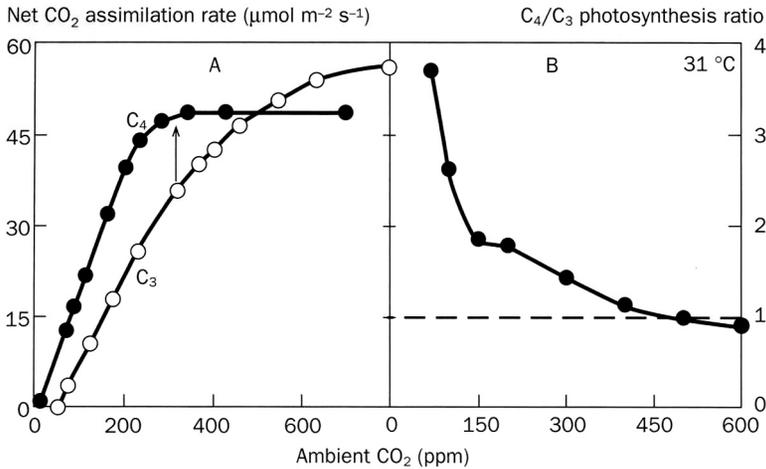


Fig. 1. (A) Responses of net CO₂ assimilation rate to rising ambient CO₂ for a typical C₄ weed (*Amaranthus retroflexus*) and C₃ weed (*Chenopodium album*). The photosynthetic enhancement that might be realized for a C₄ rice plant relative to a C₃ plant is shown by the vertical arrows for current atmospheric conditions. (B) Ratio of C₄ to C₃ photosynthesis corresponding to the curves in panel A. (Adapted from Sage and Pearcy 2000.)

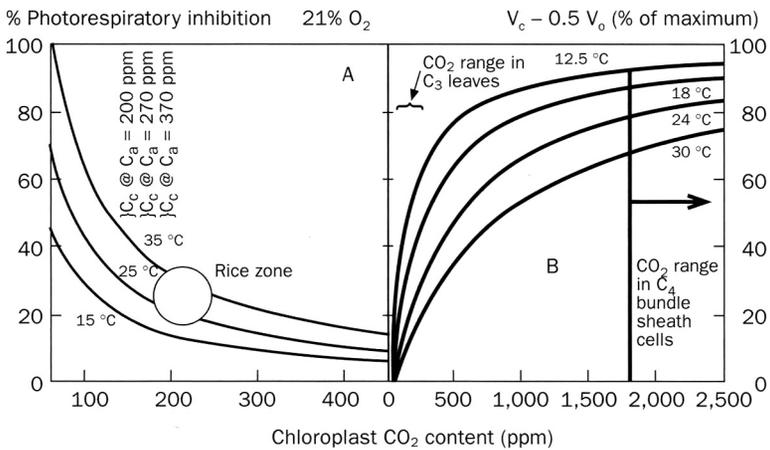


Fig. 2. (A) Modeled percentages of photorespiratory inhibition in C₃ plants as a function of the CO₂ level in the chloroplast (C_c) at 15, 25, and 35 °C. The range of C_c corresponding to atmospheric CO₂ (C_a) levels of the late-Pleistocene (200 ppm), the mid-Holocene (270 ppm), and now (370 ppm) is shown. The "rice zone" indicates the temperature and CO₂ conditions that rice leaves generally experience in the field. (B) The relative substrate saturation curves for the CO₂ response of Rubisco at 12.5 to 30 °C. V_c refers to carboxylation rate; V_o is oxygenation rate. (Modeled according to Sage 1995; adapted from Sage and Pearcy 2000.)

is generally less palatable than C₃ biomass, whereas the reduced requirement for water and nitrogen lowers the need for below-ground biomass allocation relative to C₃ species of similar characteristics. Together, these benefits enable C₄ species to dominate low-latitude grasslands, beaches, deserts, and recently disturbed ground (Table 1).

Table 1. Habitats of the world that can be dominated by C₄ plants. All habitats are low elevation (<2,000 m). Adapted from Sage et al (1999b).

Habitat	Climate zone	Major C ₄ life forms	Notes
Grasslands	Tropical to warm-temperate (includes wet and dry zones)	Grasses, sedges	Temperate dominance depends on summer precipitation.
Savannas (open portions)	Tropical to warm-temperate	Grasses, sedges	Temperate dominance depends on summer precipitation and fire occurrence.
Disturbed ground	Tropical to warm-temperate	Herbaceous weeds	Grasses, sedges, and herbaceous dicots
River and lake margins	Tropics	Grasses and sedges (papyrus)	Generally requires disturbance caused by flooding, animals. or wave action.
Beach dunes and bluffs	Tropics to warm-temperate	Grasses and dicots	Soils here are sandy, salinized, and drought-prone, and are disturbed by wind and waves.
Atolls	Tropics to subtropics	Grasses and dicots	Soils are shallow, salinized, and drought-prone.
Salt marshes	Tropics to cool-temperate	Grasses	Intermediate salinity zones
Saline soils in arid zones	Tropics to temperate	Grasses and dicot shrubs	Soils of Intermediate salinity: many members of the Chenopodiaceae
Arid sand dunes	Tropics to warm-temperate	Grasses and dicot shrubs	

In warm to hot climates, the greater photosynthetic efficiency of C₄ plants enables them to achieve higher radiation-use efficiencies and crop yields than C₃ species (Evans 1993). On average, the efficiency for conversion of photosynthetically active radiation into biomass is more than 40% greater for C₄ crops than for C₃ crops (Long 1999). Among weeds, the C₄ pathway allows for aggressive competition against crops, particularly those of the C₃ pathway. In the tropical lowlands, the most aggressive weeds are C₄, as are most summer weeds in temperate zones (Brown 1999). Because of the higher productive potential of C₄ crops in hot climates and the suppression of C₃ crops by C₄ weeds, the major grain crops that originated in low latitudes use C₄ photosynthesis, with one major exception-rice (Table 2).

From a photosynthetic standpoint, rice is in some ways an enigma. It is tropical in origin and requires relatively high temperature and direct sunlight for successful cultivation. During midday, rice canopies routinely experience temperatures above 30 °C, particularly during periods of full sunlight (O'Toole and Tomar 1982, Huang 1989). In these conditions, photorespiratory inhibition of Rubisco is substantial and the enzyme operates well below CO₂ saturation (Fig. 2). Indeed, the superficial appearance is that rice grows best where the advantages of C₄ over C₃ photosynthesis are substantial, a view supported by high yield suppression that can occur because of infestation by C₄ weeds (Moody 1996). It is not surprising, therefore, that society views the engineering of a C₄ rice plant to be a valuable objective worthy of a substantial research and development effort. However, if C₄ photosynthesis is so robust in tropical settings,

Table 2. The area of Earth occupied by the major grain crops that originated in low-latitude climates (IRRI 1997, Brown 1999). Hectare estimates are for 1992-94.

Crop	Area (millions of ha)
Maize (C ₄)	133
Sorghum (C ₄)	44
Pearl millet (C ₄)	28
Other millets (C ₄)	10
Tef (C ₄)	1.5
Fonio (C ₄)	0.3
Rice (C ₃)	148

then why isn't rice, and all plants like it, already C₄? Are there microclimate and ecological factors associated with tropical wetlands that mitigate the C₄ advantage? To provide a background perspective on these issues, this review will examine the evolutionary history of C₃ and C₄ photosynthesis and provide an ecophysiological assessment of the two photosynthetic pathways in tropical wetlands, with particular emphasis on C₃ rice and its C₄ weeds. I will finish by considering the effect of atmospheric CO₂ enrichment on the future performance of the C₃ and C₄ photosynthetic pathways in wetland habitats.

Origin of the C₄ pathway

C₄ photosynthesis is highly polyphyletic, with at least 30 independent evolutionary origins (Kellogg 1999). In the grass family, C₄ photosynthesis evolved more than six times, whereas, in the dicots, the syndrome probably arose independently in each of the 15 dicot families in which it occurs (Kellogg 1999). The sedges have at least four distinct origins (Soros and Bruhl 2000). Based on molecular phylogenies and estimated genetic divergence rates, C₄ grasses are suggested to have existed between 20 and 30 million years ago (Kellogg 1999). The earliest proven C₄ fossils are 12 million years old, although probable C₄ grass fossils from East Africa are dated at 14.5 million years old (Cerling 1999). There is no evidence for ecological dominance of C₄ plants before 10 million years ago; however, carbon isotope ratios in fossilized plant, animal, and soil remains show that a widespread expansion of C₄-dominated ecosystems occurred between 5 and 10 million years ago (Cerling 1999). By 5 million years ago, C₄-dominated ecosystems were common throughout the tropics, and their expansion has been linked to the evolutionary diversification of major herbivore taxa adapted to grassland biomes (Cerling et al 1997).

The period when C₄ plants first appeared corresponds to a time when atmospheric CO₂ levels were declining and the photorespiratory inhibition of photosynthesis was increasing (Fig. 3). The postulated origin of the C₄ syndrome corresponds to a time when the photorespiratory inhibition of C₃ photosynthesis at 30 °C is modeled to have risen above 20% (Fig. 3). In turn, the subsequent expansion of C₄-dominated biomes 5 to 10 million years ago corresponds to when the potential for photorespiratory inhibition approached that of the present atmosphere. This reduction in atmospheric CO₂ content and the associated increase in photorespiration were probably the principal factors promoting the evolution of the C₄ syndrome (Ehleringer et al 1991, Cerling et al 1997).

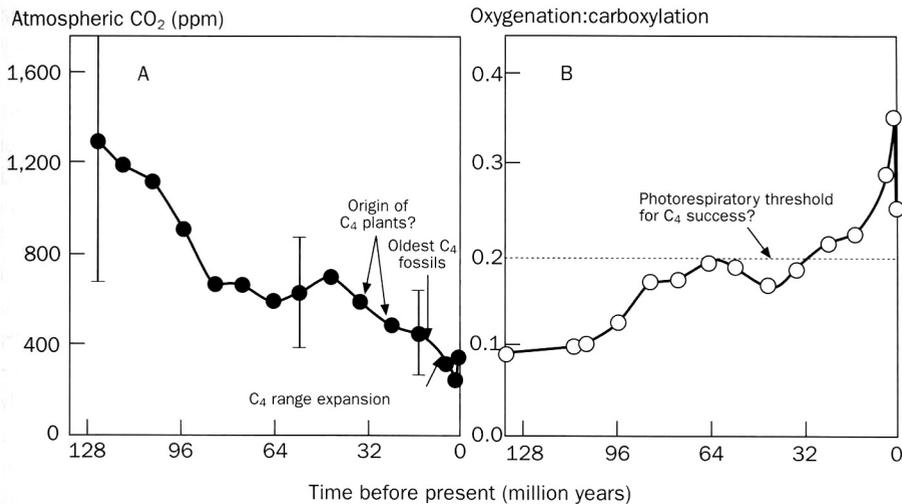


Fig. 3. (A) Modeled atmospheric CO₂ levels over the past 135 million years (Berner 1994) and (B) the corresponding ratio of RuBP oxygenation to carboxylation by Rubisco, assuming a temperature of 30 °C and 210 mbar O₂. (Adapted from Sage 1999.)

Distribution of C₄ photosynthesis in the plant kingdom

Taxonomic and life form distribution

From a floristic survey of photosynthetic pathway in the plant kingdom, it is difficult to conclude that C₄ photosynthesis is the superior syndrome. Of the 280,000 terrestrial plant species, about 7,500 are C₄, or only 3% of the land plant flora (Sage et al 1999a). By contrast, between 20,000 and 30,000 CAM (Crassulacean acid metabolism) species are spread over a greater range of taxonomic groups than C₄ plants (Smith and Winter 1996). As with C₄ plants, most C₃ plants are tropical, and the world's greatest diversity of C₃ species is in the tropical environments where C₄ plants perform well. In absolute terms, the tropical diversity of C₃ plants far exceeds that of C₄. From the standpoint of functional types, the C₄ pathway is largely restricted to graminoid and herbaceous dicot life forms. About 7,000 of the 7,500 or so C₄ species are herbaceous and, of the 500 or so woody species, almost all occur on arid, often saline soils where agriculture is difficult at best. No C₄ trees occur, with the possible exception of an arborescent shrub species from Central Asia and a rare understory tree in Hawaii (Sage et al 1999b).

Economic distribution

Despite its productive superiority, C₄ photosynthesis does not dominate agriculture on a global basis. Of the crops that supply 90% of the world's calories, 80% are C₃ (Harlan 1992, Alberto et al 1996). Major C₃ grain crops include rice, wheat, barley, and rye. All dicot crops are C₃ except for *Amaranthus* spp. and a few very minor species grown as vegetables (Harlan 1992, Brown 1999). Only four C₄ species—maize, sorghum, sugarcane, and pearl millet—are considered major crops, with each being cultivated on more than 18 million ha. Of the remaining C₄ crops of importance (for example, various millets, tef, and fonio), none is cultivated on more than 5 million ha (Brown 1999). In tropical regions wet enough to support rice cultivation, it is usually the dominant grain crop. Where rice cultivation is difficult because of insufficient

water supply, however, the minor C₄ crops in combination with one or more of the major C₄ grains often dominate regional cereal production. In Ethiopia, for example, more than 95% of the cereal cropland below 1,900 m is devoted to sorghum and tef (Pearson 1992).

In contrast to the importance of C₃ crops for production of calories directly consumed by humans, forage production in the tropics and subtropics is heavily dominated by C₄ species, except at high elevation and in wetlands. More than 90% of the forage grasses of tropical regions are C₄, and the major C₃ production on managed rangelands of the lowland tropics is from various legume species or wetland graminoids (Brown 1999, Skerman and Riveros 1990).

The dominance of the C₃ pathway in the major crops used for direct food production is a bit of an enigma, particularly since the atmospheric CO₂ levels of recent geological time have been substantially lower than today. Compared with the current atmospheric level of 370 ppm, the CO₂ content of the atmosphere was approximately 270 ppm between 10,000 and 200 years ago, and about 200 ± 20 ppm between 15,000 and 50,000 years ago (Sage 1995). In these conditions, C₄ plants should have been even more competitive, yet wild progenitors of rice and other C₃ crops not only survived, but were successful enough to attract the attention of protofarmers across the globe and become the major domesticated crops of early agriculture (Table 3). Most leading C₃ grain and legume crops appear to have been domesticated by 7,000 years ago, whereas the major C₄ crops were domesticated at a later time (Harlan 1992). Moreover, rice appears to have been domesticated several times, with each occurring thousands of years before the domestication of the leading C₄ crops (Harlan 1992). While the success of most of these crops can be linked to their temperate habit, rice stands out as having success in a tropical setting where temperature and photoinhibitory potential are high. If C₄ photosynthesis is superior in tropical settings, then what is it about rice that promoted its widespread agricultural success at a time of depleted atmospheric CO₂? Is there something about the wetland habitat that offsets the C₄ advantage?

Ecological distribution of C₄ photosynthesis

Currently, the C₄ pathway is dominant in warm-temperate to tropical habitats that are both warm (greater than 22 °C average growing-season temperature) and well illuminated. The requirement for moderate to high light is instrumental in the failure of C₄ species to exploit forested regions, and may explain in large part why there are virtually no C₄ trees and vines in the world’s forests (Sage et al 1999b). The importance of temperature is reflected in patterns of C₄ abundance along latitude gradients, as is demonstrated from a survey of photosynthetic pathway in the grass floras of the world’s oceanic islands (Fig. 1). On the world’s islands, the grassland flora below 2,000 m is C₃ above 50° latitude, and switches to C₄ dominance in the temperate zones between latitudes of 30° and 40°. In the tropics and subtropics, the degree of C₄ dominance varies between 60% and 100%. The grass floras of small tropical islands and

Table 3. The photosynthetic pathways of major crops under cultivation 6,000 years ago.

Crop	Examples
C ₃	Wheat, barley, rice, oats, beans (all forms), squashes, melons, potatoes, chili peppers, sweet potatoes, yams, cotton
C ₄	<i>Setaria</i> millets, <i>Panicum</i> millets, maize, <i>Amaranthus</i> spp.

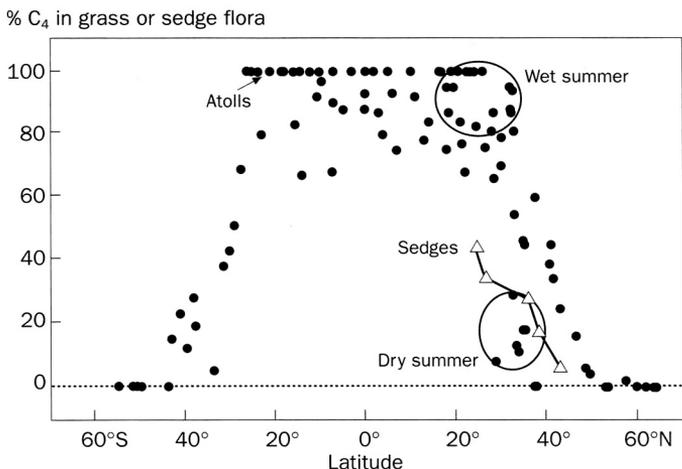


Fig. 4. The relative distribution of C_4 grass species in the grass flora (filled circles) and C_4 sedge species in sedge floras (triangles) of individual marine islands from around the world. (From Sage et al 1999b.) The large ovals surround data from islands of the north-temperate zone that have either wet or dry summers.

atolls are completely C_4 , largely because the soils tend to be shallow, sandy, and drought-prone, and they are often salinized. By contrast, there is a lower frequency of C_4 species on the larger tropical islands where forests and marshes are common. Here, the C_3 contribution to an island's grass flora increases because most forest grasses are C_3 , and C_3 and C_4 grasses tend to be equally represented in the wetland floras of the larger islands. There are similar latitudinal trends in the sedges; however, at a given latitude, there is a lower C_4 contribution to an island's sedge flora (Fig. 4). This largely reflects the wetland habit in which the sedges occur.

The distribution of C_3 and C_4 photosynthesis within a tropical grass flora is well demonstrated by a survey of photosynthetic pathway in the Guianas flora of northern South America (Table 4). A third of the grasses in the flora are C_3 and, of these, half are from forest interiors or margins. Of the C_3 grasses from open habitats, more than half are from wetlands, or about a fourth of the total C_3 grass flora. By contrast, only 11% of the C_4 grass flora is described as favoring wetland habits, but, because there are more C_4 grasses in the flora, the number of C_3 and C_4 wetland grass species is similar. Notably, in high-light, nonflooded locations at low elevation, relatively few C_3 grass species are present (11% of the C_3 grasses, 4% of all grasses). In comparison, 99% of the C_4 grass flora is described as requiring open habitats.

Ecological distributions are also apparent in an overall survey of the grass family. Six subfamilies are currently recognized in the Poaceae (Table 5). Of these, the Chloridoideae and Panicoideae are predominantly C_4 and are mainly from warm-temperate to tropical environments. One largely C_3 subfamily (Arundinoideae) is cosmopolitan, whereas two others (Stipoideae and Pooideae) are exclusively C_3 and are primarily from cool climates of high latitude and altitude. The subfamily Bambusoideae merits special note. This subfamily is largely tropical and comprises most of the shade-adapted forest grasses in the tropics. It also includes the most successful C_3 grasses in open habitats, these being the wetland grasses of the world's tropics. Notably, the tribe Oryzeae falls into this subfamily and is an important contributor to wetland floras of all the world's tropical regions (Vaughan 1994).

Table 4. A summary of the primary habitat distribution of 387 grass species listed in the flora of the Guianas region of tropical South America (wet tropics, 2–7°N latitude). Of the 387 species, 33% are C₃ and 67% are C₄. (Adapted from Judziewicz 1990.)

Habitat	% of C ₃ grasses	% of C ₄ grasses	% of total
Open, disturbed (weedy)	2	19	14
Cultivated ^a	4	13	10
Savanna/grassland	5	52	36
Beaches/sand dunes	0	4	3
Wetlands/marshes	27	11	16
Montane	12	0	4
Forest margins	16	1	6
Forests	34	0	11
Total open habitat	50	99	83
Total closed habitat	50	1	17

^a Cultivated includes row crop, bamboo, and forage grasses.

Table 5. Distribution of photosynthetic pathways in grass subfamilies and tribes. Subfamilies and major grain crops are listed in bold. After Watson and Dallwitz (1998).

Subfamily	Notes								
Stipoideae	5 tribes, 20 genera, all C ₃ ; mainly temperate, often cold, arid regions								
Arundinoideae	9 tribes, 3 with C ₄ genera. Five genera with C ₄ species from about 60 total genera in the subfamily; cosmopolitan								
C ₄ tribes:	<table border="0"> <tr> <td>Aristideae</td> <td>2 C₄ (<i>Aristida</i>, <i>Stipagrostis</i>), 1 C₃ genera</td> </tr> <tr> <td>Danthonieae</td> <td>all C₃ but genus <i>Centropodia</i></td> </tr> <tr> <td>Eriachneae</td> <td>2 C₄ genera (<i>Eriachne</i> and <i>Pheidochloa</i>)</td> </tr> </table>	Aristideae	2 C ₄ (<i>Aristida</i> , <i>Stipagrostis</i>), 1 C ₃ genera	Danthonieae	all C ₃ but genus <i>Centropodia</i>	Eriachneae	2 C ₄ genera (<i>Eriachne</i> and <i>Pheidochloa</i>)		
Aristideae	2 C ₄ (<i>Aristida</i> , <i>Stipagrostis</i>), 1 C ₃ genera								
Danthonieae	all C ₃ but genus <i>Centropodia</i>								
Eriachneae	2 C ₄ genera (<i>Eriachne</i> and <i>Pheidochloa</i>)								
Bambusoideae	15 tribes, 125 genera, all C ₃ ; low latitudes, forests and wetlands								
Main tribes:	<table border="0"> <tr> <td>Bambuseae</td> <td>forest grasses with woody stems, shade-adapted</td> </tr> <tr> <td>Centothecaeae</td> <td>tropical forest shade grasses</td> </tr> <tr> <td>Olyreae</td> <td>semi-woody forest grasses. shade-adapted</td> </tr> <tr> <td>Oryzae</td> <td>marshes, mainly low latitudes (rice)</td> </tr> </table>	Bambuseae	forest grasses with woody stems, shade-adapted	Centothecaeae	tropical forest shade grasses	Olyreae	semi-woody forest grasses. shade-adapted	Oryzae	marshes, mainly low latitudes (rice)
Bambuseae	forest grasses with woody stems, shade-adapted								
Centothecaeae	tropical forest shade grasses								
Olyreae	semi-woody forest grasses. shade-adapted								
Oryzae	marshes, mainly low latitudes (rice)								
Chloridoideae	16 tribes, ~165 genera, all C ₄ except <i>Eragrostis walteri</i> . a revertant to C ₃ ; mainly low latitudes and dry climates								
Main tribes:	Aeluropodeae, Chlorideae, Cynodonteae, Eragrosteae (tef), Jouveae, Lappagineae, Leptureae, Perotideae, Pommereulleae, Spartineae, Sporoboleae, Trageae, Uniioleae								
Panicoideae	6 tribes, C ₃ , C ₃ -C ₄ Intermediate, and C ₄ . Tropical and warm-temperate regions; ~200 C ₄ genera of ~250 total (80% C ₄)								
Tribes:	Andropogoneae (C ₄ : sorghum , sugarcane); Arundinelleae (C ₄ and rare C ₃); Isachneae (C ₃); Maydeae (C ₄ ; maize); Neurachneae (C ₃ , C ₃ -C ₄ intermediate, and C ₄); Paniceae (C ₃ , C ₃ -C ₄ intermediate, C ₄ ; millets , fonios , Echinochloa)								
Pooideae	7 tribes, ~180 genera, all C ₃ ; cool-temperate to polar, tropical mountains								
Tribes:	Triticeae (wheat , barley , rye), Brachypodieae, Bromeae, Aveneae (oats), Poeae, Seslerieae, Meliceae								

Forest and marsh grass ecology in reference to photosynthetic pathway

To understand why rice is successful in flooded situations, it is useful to evaluate the mechanisms contributing to the success of C_3 grasses in tropical environments. This can be done with some certainty for forest grasses, but the situation with marsh grasses is less clear. The experience with forest grasses, however, provides some insight that may help explain the relative success of C_3 marsh grasses at low latitude.

Forests

The failure of C_4 species to dominate the low-light habitats within tropical forests is related to several physiological factors, although none stands out as the leading mechanism. The extra ATP required to operate the C_4 cycle decreases the maximum quantum yield of C_4 plants relative to that of C_3 plants and this could reduce the rate of C_4 relative to C_3 photosynthesis in shaded situations (Pearcy and Ehleringer 1984). Because quantum yield at low light depends on the degree of photorespiratory inhibition in C_3 plants, it declines with increasing temperature and decreasing CO_2 (Fig. 5). By contrast, the quantum yield of C_4 photosynthesis is minimally affected by CO_2 and temperature variation (Fig. 5). At current CO_2 levels, the crossover between C_3 and C_4 quantum yield is near 25 °C, but at higher CO_2 , the crossover occurs at higher temperature. Although tropical climates often experience daytime temperatures well above 25 °C, forest interiors are cooler than surrounding areas and the daytime temperature is usually not much above that required to favor C_4 over C_3 quantum yields (Pearcy and Calkin 1983). Second, relative humidity is greater within forest canopies, allowing for higher stomatal conductance, higher intercellular CO_2 level, and thus higher quantum yield in C_3 species (Bazzaz and Williams 1991, Sage and Pearcy 2000). Third, much of the daily carbon gain (30% to 70%) on the forest floor comes from sunflecks, that is, brief episodes of high light (Pearcy 1990). Although C_4 species are capable of exploiting long-duration sunflecks as well as C_3 species, there is evidence from maize that they are slightly less responsive to short sunflecks of a minute or less than C_3 species (Krall and Pearcy 1993).

Freshwater marshes

Marshes present an interesting case that is intermediate between the terrestrial grasslands that are highly favorable to C_4 species in the tropics and the shade of forests that favors C_3 grasses. On the one hand, tropical marshes experience full illumination, which provides both high light and surface heating that favors C_4 grasses and sedges. Opposed to this are several factors that may mitigate the C_4 advantage. First, high water levels in marshes may enable C_3 species to operate with high stomatal conductances relative to the rate of photosynthesis. Although this would reduce water-use efficiency, high rates of transpirational cooling may result, thereby maintaining leaf temperatures near the thermal optimum of C_3 photosynthesis, and intercellular partial pressures of CO_2 (C_i) may be elevated. Both lower leaf temperature and higher C_i would promote greater efficiency of photosynthesis because photorespiration would be suppressed. Second, leaf canopies within marshes are usually dense, with high leaf area indices and extensive self-shading (Jones 1986). Leaves within marsh canopies would therefore tend to be in a low light and high sunfleck situation similar to that of forest canopies. For C_4 species, the superior performance of the outer canopy may be offset somewhat by light and temperature reduction experienced by leaves within the interior of the canopy. Third, even in tropical regions, daily temperatures favorable to the C_4 habit will only be realized for a portion of the day, typically around the mid- to late-afternoon periods. Only when leaf temperatures rise above 25 to 30 °C

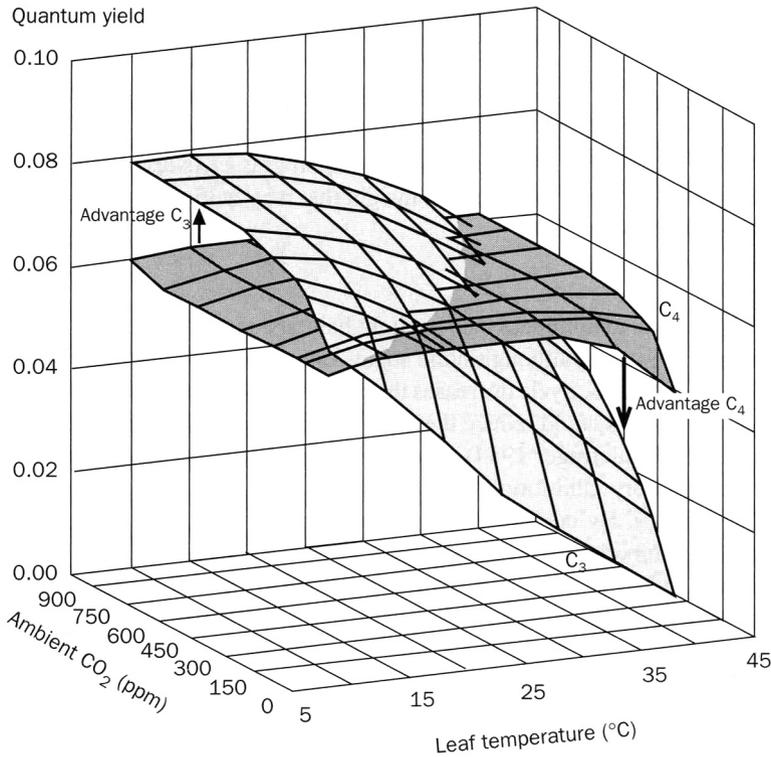


Fig. 5. The theoretical response of the quantum yield of C₃ and C₄ photosynthesis as a function of temperature and ambient CO₂. The response was generated by the Wimovac simulation program (<http://www.life.uiuc.edu/plantbio/wimovac/>).

does the C₄ pathway become clearly favorable (Ehleringer et al 1997). Mornings and evenings are generally favorable to the C₃ habit because of cooler temperature and the lower sun angle that directly illuminates a smaller portion of the canopy. Fourth, in the wet tropics, clouds commonly reduce light levels and surface heating during the afternoon when daytime temperatures would otherwise favor the C₄ pathway. A cool morning, coupled with a cloudy afternoon, could result in daily temperatures that are not generally favorable to C₄ photosynthesis, even though the midday peak temperature may be.

The extent to which conditions in tropical marshes favor C₃ versus C₄ photosynthesis is difficult to evaluate from the existing literature, primarily because there is not a large number of comparative studies. The detailed studies that do exist indicate that C₄ photosynthesis should be the superior pathway in tropical wetlands because it confers superior productive capacity and resource-use efficiency. Some of the highest levels of primary production observed anywhere are reported for C₄ species of tropical floodplains such as *Echinochloa polystachya* and papyrus (*Cyperus papyrus*) (Long 1999). These species form dense, monospecific stands along lake and river margins, and they exhibit over twice the maximum net primary productivity of high-yielding C₃ species such as rice (Long 1999). The high productivity, and dominance of these C₄ plants is attributed to high efficiencies of radiation and nitrogen use, with differences in water-use efficiency (WUE) between C₃ and C₄ wetland species being of little consequence (Jones

1986, Long 1999). Jones (1988) compared the performance of co-occurring C₃ and C₄ species in a papyrus swamp. Although he did not identify a clear mechanism to explain ecological distribution within the swamp, he hypothesized that the higher nitrogen-use efficiency of the C₄ species enabled them to dominate the more nutrient-deficient sites. Unfortunately, Jones' comparison was limited in that it did not account for the thermal environment within the swamp, so that it is difficult to elaborate on the degree of photorespiration that might have been present in the C₃ species. In general, diurnal profiles of leaf and air temperature, canopy light distribution, and associated gas exchange characteristics are needed to fully evaluate the relative performance of the two photosynthetic syndromes in field environments. In light of the limited amount of ecophysiological research on natural wetlands of the tropics, some of the most useful data for comparing the value of photosynthetic pathway in tropical wetlands are found in studies comparing cultivated rice and its C₄ weeds, most notably *Echinochloa crus-galli*.

Ecophysiology of rice and its C₄ weeds

Photosynthetic performance

Photosynthetically, rice is a typical C₃ crop plant. Leaf nitrogen, Rubisco, and chlorophyll contents are similar to those of wheat and other C₃ grasses growing under optimal conditions, and the photosynthetic rates—both the maximum and average—are similar to those of other C₃ grain crops (Evans 1989, Makino et al 1994, 1997). Because rice grows in flooded conditions in humid and warm-to-hot climates, it may use the strategy of maintaining high stomatal conductance and intercellular levels of CO₂ (C_i), thereby gaining some reduction in photorespiration. C₃ species at elevated temperature and high humidity often maintain high stomatal conductances. Chili pepper (*Capsicum annuum*), for example, commonly opens its stomates as temperature rises above 30 °C, increasing its C_i and thermal optimum of photosynthesis (Sage and Sharkey 1987). Under controlled conditions in a laboratory gas exchange system at high humidity, rice can maintain a high ratio of intercellular to ambient partial pressure of CO₂ (C_i/C_a) of 0.8 to 0.9 (Fig. 6). In realistic growth conditions in the field, however, most rice varieties do not appear to operate at such high intercellular CO₂ levels. Values of carbon isotope discrimination (an integrative index of WUE and C_i/C_a) in 190 rice cultivars range between 19.0 and 21.25, which are similar to the range observed in wheat, sunflower, beans, and peanut (Samejima 1985, Dingkuhn et al 1991, Scartazza et al 1998). These discrimination values indicate that, over their lifetime in the field, most rice varieties operate at a C_i/C_a of 0.65 to 0.75, which is typical for C₃ species. The variation present is associated with ecotypic differences in that indica and sinica lines are less water-use-efficient (greater isotopic discrimination and higher C_i at a given C_a) than japonica and javanica types (Samejima 1985, Peng et al 1998). In sum, these studies show that, while rice can operate with wide-open stomates under stable, high-humidity situations, most varieties do not appear to do so in the field to any exceptional degree. The hypothesis that rice maintains unusually high stomatal aperture in wetland settings is not supported in most cases.

The temperature optimum for rice photosynthesis is at the upper range reported for C₃ species, but is not atypical for tropical C₃ plants. Sage and Bayer (Fig. 6) observed a photosynthetic temperature optimum in air of 30 to 35 °C, which is only a few degrees below the thermal optimum of C₄ species such as *Echinochloa crus-galli*. With these thermal responses, it is possible to use field temperatures to evaluate performance of the two species with respect to what would be optimal. O'Toole and Tomar (1982) compared leaf temperature responses for rice and *E. crus-galli* in field environments at IRRI. On a day when the nighttime low was near 23 °C, the daytime air temperature surpassed 30 °C at about 900, peaked near 35 °C at noon,

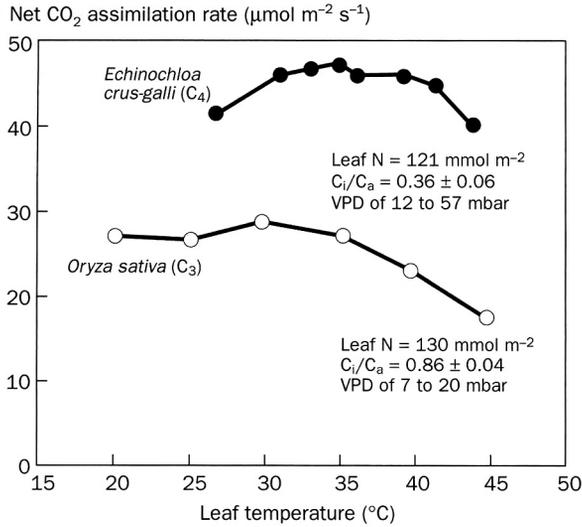


Fig. 6. The temperature response of net CO₂ assimilation in *Echinochloa crus-galli* and rice (*Oryza sativa*). VPD indicates vapor pressure difference between leaf and air. C_i/C_a is the ratio of intercellular to ambient CO₂ partial pressure. (From Sage and Bayer, unpublished results.)

declined slightly with afternoon cloud, yet remained above 30 °C until 1700. During the midday hours when air temperature was above 33 °C (approximately between 1000 and 1600), rice leaf temperature was 3 to 4 °C below air temperature, with a paddy variety operating at a leaf temperature that was 1 to 2 °C below that of an upland variety. Under the same conditions, *E. crus-galli* operated at a leaf temperature that was at most 2 °C below air temperature, but usually within a degree of air temperature. During the peak daytime temperature when conditions were most favorable to the C₄ plant, rice leaf temperature was optimal for photosynthesis (30 to 31 °C) as was the temperature of *E. crus-galli* (33 to 34 °C).

At these temperatures, *E. crus-galli* has clear superiority over rice in terms of absolute photosynthetic rate, quantum yield, and photosynthetic nitrogen-use efficiency (PNUE). Based on comparisons of Ehleringer and Pearcy (1983) for species similar to rice and *E. crus-galli*, the quantum yield of *E. crus-galli* would be 10% to 20% higher than that of rice at their respective photosynthetic thermal optima. This difference would have the most significance for shaded leaves, but because quantum yield also reflects energy costs of photorespiration, it indicates greater inefficiency of the C₃ plants at high light as well. One way that this inefficiency is manifested is as reduced efficiency of nitrogen use. At temperatures close to their respective thermal optima (33 °C for *E. crus-galli* and either 33 or 25 °C for rice, depending on the study), PNUE in *E. crus-galli* was nearly threefold greater than that of rice when both were measured at air levels of CO₂ (Fig. 7). Thus, at a given N level, *E. crus-galli* has a substantially higher photosynthetic rate than rice. The significance of this PNUE difference is not immediately clear because rice fields are not commonly low in nitrogen and plants can exploit higher PNUE in multiple ways. On the one hand, plants with higher PNUE may realize greater carbon gain per unit leaf area; alternatively, they may reduce the N investment in photosynthetic capacity per unit area, and instead invest in greater leaf or root production (Sage and Pearcy 1987a, Long 1999). Where N is abundant, as on fertilized fields, the less N-use-efficient species may

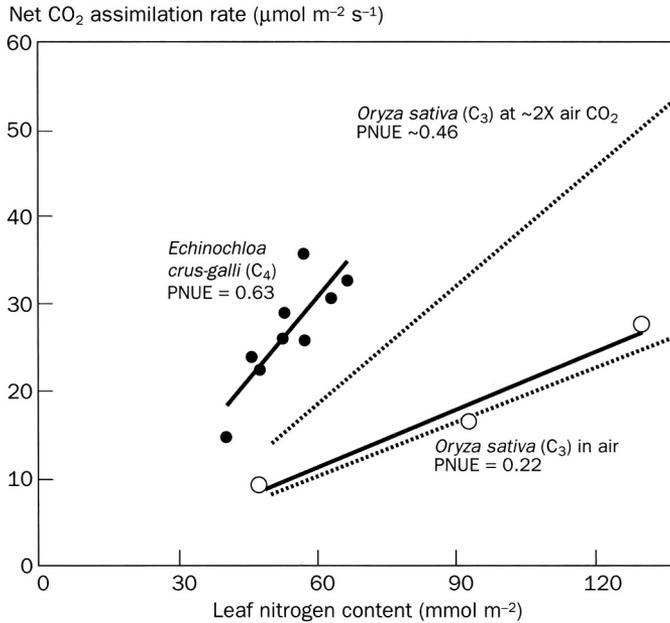


Fig. 7. The nitrogen response of net CO_2 assimilation rate for *Echinochloa crus-galli* and *Oryza sativa* at 33 °C (solid lines and symbols; Sage and Bayer, unpublished results), or for *Oryza sativa* at 25 °C (broken lines; Makino et al 1994). The photosynthetic nitrogen-use efficiency (PNUE) values are calculated as the slope of net CO_2 assimilation rate versus leaf N content.

operate at higher leaf N content, and thus compensate for the differences in PNUE. In plants grown at high soil fertility, C_3 species commonly have 30% to 30% higher leaf N content than C_4 species and often have similar CO_2 assimilation rates (Jones 1986, 1988, Sage and Pearcy 1987b, 2000). These patterns also occur in competitive situations in the field: for example, in temperate grasslands and tropical marshes, high soil N status also allows C_3 plants to outperform C_4 plants that have higher PNUE (Jones 1986, Wedin and Tilman 1996, Sage et al 1999b).

Ironically, the difference in PNUE between the photosynthetic syndromes may increase the agricultural value of the C_3 species such as rice relative to morphologically similar C_4 species because the C_3 leaves may be greater reservoirs of N that can be mobilized to the developing grain (J. Sheehy, personal communication). This raises several interesting issues concerning grain development between C_3 and C_4 species. C_3 species generally have larger seed than C_4 species (maize is the major exception) for reasons that are not clear (Guo and Brown 1997). It may be that the different NUE of the C_3 and C_4 syndromes influenced evolutionary possibilities for seed development—perhaps the smaller C_4 seeds reflect lower N stores in subtending leaves. Alternatively, the evolution of smaller seeds of C_4 plants may reflect a lower N requirement for establishment of C_4 seedlings. In any case, the availability of N for seed fill is an issue that warrants further investigation given that it may be an important factor that determines the agronomic success of C_4 rice plants.

Rice, C_4 weeds, and flooded soils

The general cultivation schemes for rice are the upland habitat where rice is grown in moist but nonflooded soils and three flooded situations involving controlled irrigation, rainfed paddies,

or deepwater flooding (IRRI 1999). An advantage of growing rice in flooded soils is that it checks aggression from C₄ weeds (Vergara 1992, Ampong-Nyarko 1991). For example, Moody (1996) observed for flooded conditions in the Philippines that C₃ weeds are more important and yield loss of rice in plots with no weed control is near 50%: in the absence of flooding, C₄ weeds predominate and yield loss is more than 90% (Table 6). In Malaysian experiments, transplanting of rice seedlings into flooded paddies produced a stand where the weeds were mainly C₃ and aquatic; delaying flooding to allow seeded rice to establish resulted in C₄ weed dominance (Table 6). Similarly, Thai experiments showed that delaying flooding to reduce *E. crus-galli* aggression resulted in a wider range of C₄ weed activity (Table 6).

The experience with C₄ weeds and flooding in rice cultivation is consistent with the patterns generally observed in tropical wetlands. In flooded situations, C₃ sedges, marsh grasses, rice, and various C₃ weeds remain competitive against C₄ weeds, but, as the system shifts to dryland settings, the significance of C₄ competition increases (Hanfei 1992). These patterns demonstrate that flooding in some ways promotes the performance of the C₃ plants, but, as described above, the mechanism is not obvious. It may be that, in upland habitat at low latitude, the occurrence of water deficiency, either seasonally or diurnally, closes stomata enough so that rice often operates at a low C_i in which case photorespiration is very high and the C₄ advantage is great. Carbon isotope ratios from upland rice plants exposed to drought indicate significant stomatal closure and CO₂ reduction (Scartazza et al 1998). Under the high solar loads and temperatures in tropical settings, transitory water stress can develop in rice fields after only a few days of no rain. Because rice is not a drought-tolerant crop, it may be particularly sensitive to episodic soil drying, whereas the competing C₄ vegetation may be better adapted to such events.

Table 6. The major weeds of rice in Asia (after Moody 1996) and their photosynthetic pathway. Rankings are in order of importance. Aq indicates aquatic plants.

<i>In the Philippines</i>	<i>Nonflooded conditions</i>
Flooded conditions (53% yield loss)	(91% yield loss)
<i>Echinochloa crus-galli</i> (C ₄)	<i>Echinochloa colona</i> (C ₄)
<i>Cyperus difformis</i> (C ₃)	<i>Cyperus iria</i> (C ₄)
<i>Eclipta prostrata</i> (C ₃)	<i>Eclipta prostrata</i> (C ₃)
<i>Ammannia baccifera</i> (C ₃)	
<i>Marsilea quadrifolia</i> (C ₃ -Aq)	
<i>In Malaysia</i>	<i>Seeded rice</i>
Transplanted rice seedlings	
<i>Monochoria vaginalis</i> (C ₃ -Aq)	<i>Echinochloa crus-galli</i> (C ₄)
<i>Ludwigia hyssopifolia</i> (C ₃ -Aq)	<i>Leptochloa chinensis</i> (C ₄)
<i>Fimbristylis miliacea</i> (C ₄)	<i>Fimbristylis nifliacea</i> (C ₄)
<i>Cyperus difformis</i> (C ₃)	<i>Marsilea minuta</i> (C ₃ -Aq)
<i>Limnocharis flava</i> (C ₃ -Aq)	<i>Marsilea vagmatum</i> (C ₃ -Aq)
<i>In Thailand</i>	
Delayed flooding to control <i>E. crus-galli</i>	
<i>Leptochloa chinensis</i> (C ₄)	
<i>Cyperus iria</i> (C ₄)	
<i>Fimbristylis miliacea</i> (C ₄)	
<i>Echinochloa colona</i> (C ₄)	
<i>Ischaemum rugosum</i> (C ₄)	

Alternatively, the advantage of C_3 species in flooded situations may not be directly related to photosynthetic pathway, but rather to a specialized set of nonphotosynthetic characteristics that C_3 marsh species such as rice may have. Unlike many of its C_4 weeds, rice is well adapted to flooded soils through a combination of root anatomy, leaf and stem structure, and root physiology (Catling 1992). For example, the surface of rice is modified to form hydrophobic grooves that allow extensive air layers to form along the length of the leaf and stem. This facilitates the transmission of O_2 from the air to the root zone (Raskin and Kende 1985), perhaps to a greater degree than occurs in C_4 weeds. In addition, most C_4 weeds do not establish well in submerged soils (Ampong-Nyarko and Datta 1991). From an evolutionary perspective, it may be that the C_4 weeds originated in upland, nonflooded conditions and only recently spread into marshy habitats. Thus, they may lack many specialized adaptations that older, more established C_3 marsh vegetation may have long ago acquired.

In summary, from the gas exchange perspective, C_4 photosynthesis appears advantageous both in the upland settings where drought can occur and in flooded situations where C_3 marsh grasses are common. Incorporating the C_4 pathway into rice could enhance the capacity for photosynthetic carbon assimilation, but the C_4 pathway may be unable to support greater yield if higher PNUE leads to a reduction in N availability during grain fill. However, these assessments are based on physiological and ecological performance in atmospheric CO_2 levels of the present and recent past, rather than the high levels of CO_2 predicted for later this century.

Atmospheric CO_2 variation and the success of the C_4 habit

The benefits of C_4 photosynthesis are greatest in atmospheres of low CO_2 (Fig. 1). At elevated CO_2 , the net benefits of the C_4 pathway are attenuated and differences in yield of C_3 and C_4 plants can be substantially reduced (Patterson and Flint 1990). In the context of engineering C_4 rice, an important question is whether the anthropogenic increase in atmospheric CO_2 predicted for later this century will enable C_3 rice to also exhibit the high yields forecast for a C_4 rice, and whether it would be more competitive against C_4 weeds. A second consideration is whether C_3 weeds would become serious problems for C_4 rice, thereby offsetting much of the benefit of the engineering effort.

Rising atmospheric CO_2 directly affects plants through enhancements of photosynthesis or reductions in respiration and stomatal conductance (Sage 1995). Increasing photosynthesis has immediate benefits in terms of increased carbon supply within the plant, and this leads to enhanced short-term photosynthesis and growth rates. For C_3 crops and weeds, typical growth and yield enhancements are 20% to 50%, but substantial genotypic variation is present. In rice under optimal growth conditions (well-fertilized, 25 to 29 °C day temperatures), increases in biomass as a result of doubling CO_2 range from 10% to 250%, but are 40% to 60% in most studies (Baker et al 1990, Baker and Allen 1993, Ziska et al 1996). The effect of CO_2 on photosynthesis and growth is temperature-dependent, reflecting the effect of temperature on photorespiration and the CO_2 demand of Rubisco. At high temperature, the short-term stimulation of C_3 photosynthesis can be substantial, leading to suggestions that growth responses at high temperature should also be more pronounced (Long and Drake 1992, Sage et al 1995). It is difficult to conclude this for rice, however, Ziska et al (1996) showed that at 29/21 °C day/night temperature, a doubling of atmospheric CO_2 during growth caused an average biomass enhancement of 70% in 17 rice varieties: by contrast, doubling CO_2 at 37/29 °C caused a growth enhancement of only 23%. Similarly, Baker et al (1993) observed that daytime growth temperature above 28 °C reduced rice grain yield regardless of growth CO_2 . Elevated CO_2 can confer mild heat tolerance in C_3 plants (Sage 1995); despite this, however, numerous

developmental processes are inhibited by heat in a manner that is independent of carbon assimilation rate. In rice, this is a particularly serious problem because temperatures above 33 °C promote spikelet sterility, regardless of CO₂ supply (Ziska and Manalo 1996). High CO₂ can even exacerbate the heat inhibition of grain set in rice because the reduced transpiration associated with CO₂ enrichment promotes higher canopy temperature (Matsui et al 1997). Leaf growth and canopy closure can also be attenuated by temperature above the photosynthetic optimum because of effects on nonphotosynthetic functions, such as wall elasticity and the cell cycle (Ahmed et al 1993, Cowling and Sage 1998). Unless these effects of heat are overcome, introducing C₄ photosynthesis into rice may have little effect. This would be particularly true if the canopy of a C₄ rice crop operated at higher temperature, as it should because latent cooling would be reduced.

The magnitude of the C₄ photosynthetic response to doubling CO₂ is relatively small (0 to 10%; Patterson and Flint 1990, Sage 1994, Ghannoum 1997), and this has led many to predict relatively low responses of C₄ plant growth to rising atmospheric CO₂. There are, however, several important points to consider in evaluating future C₄ performance. First, at higher temperature (>34 °C), the operational C_i of C₄ plants tends to fall below their CO₂ saturation point for photosynthesis, and therefore the photosynthetic enhancement brought about by high CO₂ tends to be larger (Ghannoum 1997, Pittermann and Sage 2000). This has significance for rice systems because paddies generally experience warmer temperature where C₄ photosynthesis might operate below its CO₂ saturation point. If temperatures rise appreciably because of climate warming, then the response of C₄ photosynthesis to rising CO₂ will likely increase. Second, most of the studies of plant responses to CO₂ enrichment have focused on single species growing in isolation from their competitors. In competitive settings, species responses may shift substantially. Recently, using a greenhouse system, the relative performance of rice versus a C₄ weed (*Echinochloa glabrescens*) was evaluated in a 2 × 2 factorial experiment between temperature (27/21 °C and 37/29 °C) and CO₂ (ambient and ambient + 200 ppm) (Alberto et al 1996). In pure stands, rice biomass production was reduced approximately 20% in the warmer treatment relative to the cooler treatment, while the warmer treatment stimulated *E. glabrescens* production by 20%. Increased CO₂ marginally enhanced yield in pure stands of either species (at most 10%), in part because of nutrient limitations. In mixed stands at ambient CO₂, *E. glabrescens* suppressed rice production at both growth temperatures. High CO₂ enabled rice to overcome this suppression and, in some mixtures, rice at high CO₂ was able to moderately inhibit biomass production by the C₄ weed.

Third, the reduction in stomatal conductance with rising CO₂ appears to be of similar magnitude in C₄ and C₃ species, so that substantial enhancements in water-use efficiency are possible for both photosynthetic types (Maroco et al 1999, Sage and Pearcy 2000). In grassland ecosystems that are prone to drought, such as the tall grass prairie of central Kansas (USA), reduced water consumption by a mixed C₃/C₄ sward led to higher plant water potentials and longer growing seasons (Owensby et al 1996). Because drought is generally more severe during the warmer periods of a year, C₄ plants are more likely to experience the benefit of rising CO₂ than C₃ species (Sage and Pearcy 2000). In rice cultivation, the effect of increases in WUE will likely be most significant in upland soils prone to drought. Here, the photosynthetic benefit of engineering the C₄ pathway into rice could be added to the WUE benefit of reduced stomatal conductance caused by both high CO₂ and the introduction of the C₄ pathway. Together, these improvements could have additional benefits in that irrigation requirements for rice could be substantially reduced, and the area where rice can be cultivated could greatly expand. Indeed, with the addition of the C₄ pathway and the advent of high CO₂, rice could conceivably become the principal grain of the dryland tropics.

High CO₂ will also enhance the PNUE of C₃ plants, but these enhancements do not appear to completely offset the greater PNUE of C₄ plants at warmer temperature. Doubling atmospheric CO₂ doubles the PNUE of rice, but it is still approximately 35% below the PNUE of *E. crus-galli* (Fig. 7). Similar results have been observed in other C₃ and C₄ comparisons. In the dicot herbs *Amaranthus retroflexus* (C₄) and *Chenopodium album* (C₃) at 34 °C, the PNUE of *A. retroflexus* was 0.52 at 34 °C and CO₂ saturation; in *C. album*, it was 0.24 at air levels of CO₂ and 0.38 at saturating CO₂ (Sage and Pearcy 1987b, Sage et al 1990). The failure of C₃ species to match the PNUE of C₄ plants at high CO₂ results from several factors. First, at high CO₂, C₃ species still invest substantial quantities of their N supply in Rubisco capacity and photorespiratory enzymes, despite the reduced need for these components. Although acclimation processes may reduce investment in Rubisco capacity, substantial excess capacity remains (typically 15% or more; Sage 1994, Sage et al 1997). Second, at high CO₂, C₃ plants are strongly limited by RuBP (ribulose biphosphate) regeneration capacity, which prevents the plants from exploiting the increased photosynthetic potential that high CO₂ represents (von Caemmerer and Farquhar 1984, Sage et al 1989). In many cases, limitation in RuBP regeneration capacity may reflect sink limitations that feed back through carbohydrate metabolism to reduce photosynthetic capacity at high CO₂ (Moore et al 1999). Such sink limitations are important concerns for agricultural productivity in the future because they may be major constraints that prevent crops from taking advantage of the increased productive potential that high CO₂ represents.

Theoretically, C₃ photosynthesis at two to three times current CO₂ levels should have higher PNUE than C₄ photosynthesis because C₃ plants do not require the N investment in the C₄-cycle reactions. Compared with their counterparts adapted to CO₂-depleted atmospheres, C₃ plants perfectly optimized for high CO₂ should have about half the Rubisco capacity and virtually no investment in photorespiratory enzymes for a given CO₂ assimilation rate (Fig. 8, see also Woodrow 1994). Because Rubisco is a large N pool in C₃ leaves (about 20% to 30% of total leaf nitrogen in rice and other C₃ crops; Evans 1989, Makino and Mae 1999), reducing its content would free up substantial quantities of nitrogen for other important processes. In rice leaves photosynthesizing at a rate of 30 mmol m⁻² s⁻¹ in the current atmosphere, the nitrogen cost of Rubisco is modeled to be approximately 40 mmol N m⁻² (see Fig. 8 for model conditions), which is approximately 30% of the total N in the rice leaf (based on a leaf N level of 150 mmol m⁻² at which photosynthesis is 30 μmol m⁻² s⁻¹; Fig. 7). At double ambient CO₂, approximately 20 mmol m⁻² of N in Rubisco is required for a CO₂ assimilation rate of 30 mmol m⁻² s⁻¹; this is about a 15% reduction in leaf N. Makino et al (1997) used antisense constructs against Rubisco to reduce the Rubisco investment in rice leaves to below 20% of leaf nitrogen. At triple ambient levels of CO₂, the effect of this reduction was a 20% enhancement in the chlorophyll, cytochrome f, and coupling factor, which contributed to a 20% enhancement in the rate of CO₂ fixation and an approximate 25% enhancement in PNUE.

In addition to changing allocation patterns, the quality of specific components could be altered to enhance resource-use efficiency. For example, C₄ plants often have a form of Rubisco that has a 30% to 50% higher turnover capacity (k_{cat}) compared with C₃ plants (Seemann et al 1984). At high CO₂, a high k_{cat} form of Rubisco has a greater turnover rate *in vivo*, and thus greater CO₂ assimilation rate, if RuBP regeneration capacity is nonlimiting. Theoretically, for a CO₂ assimilation rate of 30 mmol m⁻² s⁻¹, an increase in the k_{cat} of Rubisco from 25 to 40 mol mol⁻¹ s⁻¹ would reduce the N cost of Rubisco by a further 8 mmol m⁻² at double the current CO₂ level (Fig. 8).

Similarly, constraints that restrict growth responses to rising CO₂ at the whole-plant level could be removed by selective breeding or molecular engineering. Using an economic analogy

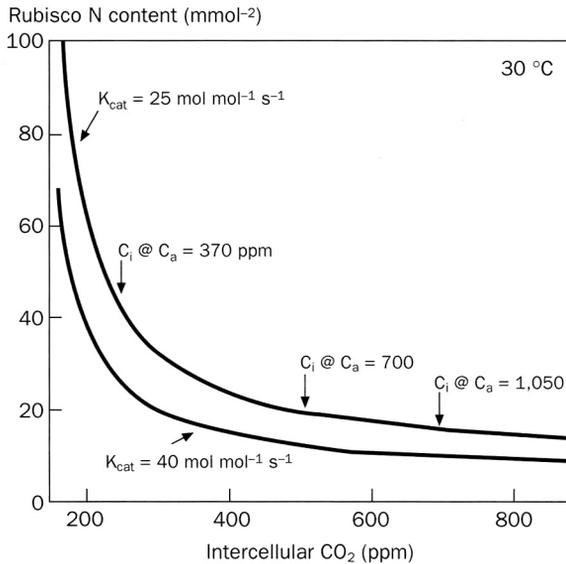


Fig. 8. The theoretical relationship between intercellular CO₂ level and the amount of Rubisco (expressed in terms of its N content) required for a C₃ rice leaf to maintain a gross CO₂ assimilation rate of 30 μmol m⁻² s⁻¹ at 30 °C.

Responses modeled by rearranging the equations for Rubisco-limited assimilation to solve for the V_{cmax} of Rubisco (Farquhar and von Caemmerer 1982). From the predicted V_{cmax}, the amount of Rubisco was calculated using a k_{cat} of either 25 mol CO₂ mol⁻¹ Rubisco s⁻¹ (directly determined for rice at 30 °C by Sage, unpublished) or 40 mol mol⁻¹ s⁻¹. The nitrogen content of Rubisco was estimated by assuming Rubisco is 15% nitrogen (Evans 1983). The value of G was assumed to equal that of spinach (Brooks and Farquhar 1985); the k_c and k_o were 11 μmol and 400 μmol, respectively (after Makino et al 1988, for rice, with adjustment from 25 to 30 °C using the proportional response to temperature determined by Jordan and Ogren 1984 for spinach). The mesophyll transfer conductance used was that of rice (0.50, von Caemmerer and Evans 1991).

commonly employed in ecology, no single process should dominate the control of photosynthesis, growth, or yield in a plant that is optimized for its growth conditions (Bloom et al 1985). Instead, control would be spread over the major processes that contribute to productivity. At the whole-plant level, the optimum would occur when resources within a plant were allocated so that neither sink strength nor source activity would be limiting. Compared with cultivars in today's atmosphere, future cultivars would have reduced investment in photosynthetic enzyme level and greater root mass to acquire limiting soil nutrients. Enhanced root activity appears critical because the availability of mineral resources is one of the greatest limitations to CO₂ responsiveness in C₃ plants. In rice, for example, nitrogen fertilization is required to maintain a positive long-term response to elevated CO₂; without it, little growth enhancement is observed over the growing season (Ziska et al 1996). Increased activity of reproductive sinks could also be realized, but this must also be combined with greater root allocation or fertilizer applications, otherwise mineral elements vital to high grain production might become limiting during grain fill. Finally, C₄ rice leaves and high-CO₂-adapted C₃ rice leaves would likely have a reduced N investment in photosynthetic enzymes, and thus could have less N available for remobilization. If N remobilization becomes a limitation on grain yield, additional storage pools for N may need to be engineered into novel C₄ and C₃ rice varieties.

Conclusions

In summary, although a C₄ rice might confer some benefit, particularly in drought-prone upland settings, an important alternative to C₄ rice would be a C₃ rice variety that is better-designed for a high CO₂ environment. It would probably be easier to engineer a high-CO₂-adapted C₃ rice variety than C₄ rice, as this would involve simple changes in regulatory set points, rather than engineering major changes in leaf anatomy and physiology. Substantial variation already exists in the response of rice to elevated CO₂ (Zisha et al 1996), and many of these traits could be co-opted to engineer superior cultivars for future high CO₂ atmospheres. In addition, the genetics of CO₂ responsiveness is rapidly being elucidated, providing a blueprint for the types of changes that can be exploited to design a rice plant that is optimized for high CO₂. Although the strategy of genetically adapting C₃ rice to high CO₂ is an important alternative, one large uncertainty is when atmospheric CO₂ levels will reach the point at which the high-CO₂-adapted rice is more efficient than C₄ rice. It is commonly stated that atmospheric CO₂ will double within a century, but this estimate is quite uncertain given the problems of predicting human economic and social trends. Currently, CO₂ levels are rising at slightly less than 2 ppm per year. If we assume they will increase over the next 50 years by an average of 3 ppm per year, then the atmospheric CO₂ level will be 520 ppm in 2050. This may not be high enough to allow for C₃ plants to yield more than C₄ plants, even with C₃ plants that are adapted to higher CO₂. Given this, it is reasonable to argue that engineering a C₄ rice may be critical to meeting production goals within the next half-century.

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Photosynthesis, models, structure, and growth

Limits to yield for C₃ and C₄ rice: an agronomist's view

J.E. Sheehy

The conversion of solar energy into grain involves several processes: photosynthesis, respiration, allocation of assimilates to different plant organs, storage, cycling of nitrogen, turnover of short-lived parts, losses of matter owing to biotic and abiotic factors, floret abortion, and sterility. In a growing season of fixed duration, it is inevitable that increased rates of resource capture and retention are required for higher-yielding crops. In this chapter, I assume that mass accumulation is translated into the crop structural and architectural properties required to sustain maximum rates of resource capture. The components of yield and of solar radiation conversion into biomass have been explored using simple theory. I conclude that the upper yield limit of rice crops with conventional photosynthetic pathways will only take us halfway to IRRRI's stated goal of a 50% increase in yield. Future increases in production have to be achieved with less land, less water, less labor, and less pollution. A C₄ rice plant may satisfy all of those requirements and improve yields. Indeed, its greatest impact may be in areas of low rainfall and poor soil conditions. However, the potential yield of such a crop in irrigated systems could be limited by the intrinsically low N content characteristic of C₄ crops.

There must be limits to yield set by the thermodynamics of energy absorption and conversion to biomass. This is not widely appreciated, possibly because it is difficult to determine precisely what those limits are for different ecosystems and crops. Nonetheless, calculating the limits, albeit crudely, is crucial for setting yield targets for the breeding of new, elite germplasm to meet increases in demand for food. Furthermore, future increases in rice production have to be achieved with less land, less water, less labor, and less pollution. As a consequence, defining the agronomic management measures necessary to reach yield limits becomes more complex.

Failure to meet the increasing demand for food from the rising population of Asia will result in misery for hundreds of millions (Mann 1999, Brown 1997, Greenland 1997). To avoid this alarming problem, a 40% to 50% increase in rice yields is required by 2050. However, for 30 years, 10 t ha⁻¹ has remained the maximum yield of inbred cultivars of irrigated rice in the tropics, a value long regarded as a limit to yield or a "yield barrier" (Kropff et al 1994). Breaking the yield barrier is clearly a formidable challenge.

Meeting the increased demand for food will eventually require short-duration cultivars in the tropics capable of yielding close to 15 t ha⁻¹ (14% moisture content). Several factors have to come together if such high yields are to be achieved: (1) crop management has to facilitate resource capture to ensure nonlimiting supplies of water and mineral elements, (2) it is necessary to develop cultivars that make the most effective use of captured resources, including storing

materials for future deployment at critical stages of growth, and (3) plant assets must be protected against pests, diseases, weeds, and damage from adverse environmental conditions.

The primary objectives of the work in this chapter are (1) to determine the principal factors governing the maximum yield limit of rice, (2) to examine yield and radiation-use efficiency, and (3) to quantitatively link leaf and canopy photosynthesis. Secondary objectives are (a) to define the yield limits for rice with redesigned photosynthetic pathways and (b) to consider these issues with respect to sink size and the influence of nitrogen on grain yield.

What governs yield?

The yield equation

A systems analysis approach to the complex issues surrounding yield limits can help in deciding what factors determine yield and their relative importance. It will be used here to derive some simple rules of thumb and provide an overview of the important features of a high-yielding rice system. After that is done, each of the features identified will be analyzed briefly with respect to its contribution to yield. Initially, the mass balance of the whole crop in terms of its inputs and outputs is considered. The inputs to the crop are photosynthate and mineral elements, and the losses from it are respiration and the loss of matter through detachment.

The mass balance of the whole crop (shoots plus roots) at time t can be written as

$$\frac{dW}{dt} = F_{ci}(t) + F_{mi}(t) - F_{co}(t) - F_{do}(t) \quad (1)$$

where $F_{ci}(t)$ is the input of matter associated with instantaneous rate of canopy gross photosynthesis, $F_{mi}(t)$ is the rate of input of mineral elements, $F_{co}(t)$ represents the losses from the system as respiration in the same units as photosynthesis, and $F_{do}(t)$ is the rate of loss of dead matter by detachment. This equation is not always simple to solve, as it is necessary to know how the composition of the plant in terms of carbon and other elements changes with time. Structural, architectural, environmental, pest, and disease problems must be overcome to ensure that the maximum growth rate can be achieved.

The carbon content of rice plants is approximately 40%, rice straw = 38% (Jimenez and Ladha 1993), and rice grain = 39.8% (Ladha, personal communication, IRRI, 2000) so that calculating the mass balance of a plant in terms of carbohydrates gives an acceptable prediction of weight change. To be precise, the fraction of carbon in the dry weight, f_c , needs to be known for each organ and a conversion to dry matter of the form $DW = \text{weight of } CH_2O (0.4/f_c)$ should be used. Using daily time steps, equation (1) can now be written in units of CH_2O as

$$\frac{dW}{dt} = P_g(t) - R_s(t) - R_m(t) - D(t) \quad (2)$$

where P_g is gross photosynthesis (shoot net photosynthesis plus shoot respiration for the daylight hours), R_s is the crop synthetic respiration (shoots plus roots), R_m is the crop maintenance respiration, and D is the daily loss of matter through detachment, assuming that each variable has been measured for a day. If it is assumed that synthetic respiration is equivalent to $0.25P_g$, and maintenance respiration can be written as the product of a maintenance coefficient and crop weight (Thornley 1976), equation (2) can be rewritten as

$$\frac{dW}{dt} = 0.75P_g(t) - m_T W(t) - D(t) \quad (3)$$

where m_T is the maintenance respiration coefficient. The maintenance coefficient, m_T , is assumed to have a Q_{10} of 2 from a reference temperature of 20 °C and may be written as

$$m_T = m_{20} 2^{(T(t) - 20)/10} \quad (4)$$

where m_{20} (g CH₂O g⁻¹DW d⁻¹) is the value of the maintenance respiration coefficient at 20 °C and $T(t)$ is the instantaneous value of temperature (°C).

To make progress, we assume that on average the root weight ratio is 0.15, thus, $W_r = 0.18 W_s$, and that detachment is proportional to weight, using W_r for root weight and W_s for shoot weight. We can then substitute in equation (3) and write

$$1.18 \frac{dW_s}{dt} = 0.75P_g(t) - 1.18 m_T W_s(t) - 1.18 D_s(t) \quad (5)$$

Dividing throughout by 1.18 gives

$$\frac{dW_s}{dt} = 0.64P_g(t) - m_T W_s(t) - D_s(t) \quad (6)$$

For young crops, when D_s is negligible, relative growth rate can be written as

$$\frac{1}{W_s} \frac{dW_s}{dt} = \frac{0.64P_g}{dt} - m_T \quad (7)$$

Akita (1989) explored various approaches to yield limits and one of his treatments included the use of a culture solution system in the field. His maximum reported growth rates were 40 g m⁻²d⁻¹ and the shoot biomass was 1,200 g m⁻². Assuming that D_s was negligible, equation (6) can be used to calculate the canopy gross photosynthetic rate, which I calculated to be 90 g CH₂O m⁻² d⁻¹ during the period of maximum growth. Rates of maintenance and synthetic respiration of the shoots are estimated to have been approximately 22.8 g CH₂O m⁻² d⁻¹ and 22.5 g CH₂O m⁻² d⁻¹, respectively. The total calculated shoot respiration rate of 45.3 g CH₂O m⁻² d⁻¹ is in good agreement with the value reported by Akita (1989) for respiration of more than 40 g DW m⁻² d⁻¹. For the same cultivar grown normally in the field (Akita 1989), the maximum canopy photosynthetic rate can be calculated to have been 60 g CH₂O m⁻² d⁻¹ and shoot respiration to have been 49% of that value.

The common agronomic equation for grain yield, Y , links aboveground biomass to grain yield through the harvest index, H . The equation can be obtained by integrating equation (5) over the growing season from transplanting (tr) to maturity (tm) and multiplying it by H , giving

$$Y = H \int_{tr}^{tm} [0.64P_g(t) - m_T W_s(t) - D_s(t)] dt \quad (8)$$

where H in high-yielding rice is approximately 0.5 and the maximum theoretical value may be close to the value calculated for wheat by Austin et al (1980) of 0.6. It is clear that, to maximize yield, P_g must be maintained at its highest value throughout the growing season, and m_T and D_s must be minimized over that period.

Radiation conversion

Hay and Walker (1989) gave a general equation for crop yield that is based on the concept of solar energy conversion into biomass, which forms Monteith's method of analyzing crop growth (Monteith 1977, Russell et al 1989). The general equation can be developed by integrating over the growing season from transplanting (tr) to maturity (tm) 40 that

$$Y = H\epsilon \int_{tr}^{tm} [Q(t) f(t)] dt \quad (9)$$

where ϵ is the radiation conversion factor (RCF) in g DM MJ⁻¹, t is time, tr is date of transplanting, tm is time of maturity, Q(t) is the photosynthetically active radiation (PAR) incident on the crop at time t (MJ m⁻²), and f(t) is the fraction of radiation intercepted at time t.

The instantaneous value of the radiation conversion factor, ϵ , expressed as g DW MJ⁻¹, can be written as

$$\epsilon = \frac{1}{I_{int}(t)} \frac{dW_s}{dt} \quad (10)$$

Substituting for $\frac{dW_s}{dt}$ from equation (6) gives

$$\epsilon = \frac{0.64P_g(t) - m_T W_s(t) - D_s(t)}{I_{int}} \quad (11)$$

where I_{int} is the daily total of intercepted PAR (MJ m⁻² d⁻¹). When the crop is vegetative, and losses from death and detachment are negligible, $D_s(t) = 0$. The theoretical maximum value of RCF for the aboveground biomass of a C₃ rice crop in the tropics is approximately 2.9 g CH₂O MJ⁻¹ (PAR) (Mitchell et al 1998). The measured quantum yield of an individual rice leaf ranges from approximately 6 to 10 g CH₂O MJ⁻¹ (absorbed PAR) (Yeo et al 1994). It is worth noting in equation (9) that ϵ is outside the integral because it is assumed to remain constant for the growing season. Monteith (1977) and Russell et al (1989) justified this by finding that ϵ is a conservative quantity (at least compared with the fraction of radiation intercepted) unless other factors change during the growing season; notably the availability of water and soil nutrients, especially nitrogen (Sinclair and Muchow 1998).

Mitchell et al (1998) concluded from a literature survey that the RCF of wheat, grown with adequate water and nutrients, was 2.7 g CH₂O MJ⁻¹ (intercepted PAR), a value approximately 25% higher than that observed for rice (Fig. 1). This suggests that, if the gap between wheat and rice could be closed, yields of rice would rise to about 12.5 t ha⁻¹ in the tropics. For the same RCF value, yield in a temperate environment should be greater than in the tropical environment because of the longer crop duration (Table 1).

Canopy photosynthesis

Canopy photosynthesis is an important determinant of yield and RCF. However, measuring canopy photosynthesis accurately in irrigated rice presents a formidable challenge. There are many possible sources of error, the most difficult to eliminate arising from the diffusion of CO₂ into the enclosure from the water-air interface and from the aerenchyma. In dryland crops, a positive pressure in the canopy enclosure can be used to force mass flow through the soil and prevent soil CO₂ from influencing the measurement; without that exclusion, what is measured is not canopy photosynthesis. Here, a more theoretical approach to understanding the factors determining canopy photosynthesis is used. Canopy photosynthesis is influenced by the amount of PAR, the fraction intercepted by the leaves, its distribution in the canopy, and the response

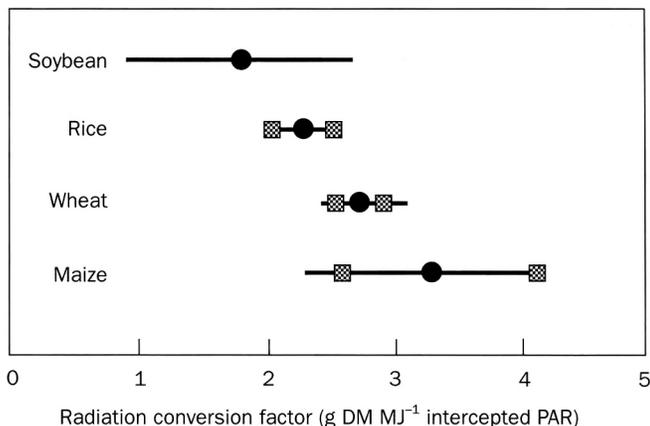


Fig. 1. The radiation conversion factors for soybean, rice, wheat, and maize for the vegetative period. The range is the line, the average is the circle, and the 95% confidence interval is shown by the squares (Mitchell et al 1998).

Table 1. Potential rice yields in tropical and temperate environments.

RCF ^a (gDM MJ ⁻¹)	Yield by environment (t ha ⁻¹)	
	Tropics	Temperate
2.2	10.0	15.0
2.7	12.3	18.4
3.3	15.0	22.5

^aRCF= radiation conversion factor.
Source: Mitchell et al (1998).

of individual leaf photosynthesis to PAR. Interception and distribution of PAR are both influenced by leaf area index (LAI) and the angular arrangement of the leaves with respect to solar elevation. To clarify some of the issues addressed in this chapter concerning canopy photosynthesis, it is convenient to divide the leaf area into three components. Monteith (1965) considered two categories of photosynthetically active leaf area: a sunlit fraction and a once-shaded fraction. Leaves shaded more than once compose a third component that is unimportant photosynthetically, but important as a nitrogen store (Sinclair and Sheehy 1999). The interception and distribution of irradiance in a Monteithian canopy are defined by a parameter S, where S defines the fraction of irradiance that passes through unit LAI without interception. The relationship between the extinction coefficient k (Bouguer-Lambert law extinction coefficient) and S was defined by Sheehy and Johnson (1988) as

$$S = \frac{e^{-k} - \tau}{1 - \tau} \quad (12)$$

where τ is the leaf transmission coefficient for PAR. The sunlit area index, A_0 , is described by the equation

$$A_0 = \frac{1 - S^L}{1 - S} \quad (13)$$

where L is the LAI of live leaves.

The once-shaded area index, A_1 , is given by

$$A_1 = \frac{1 - S^L - (1 - S)LS^{L-1}}{1 - S} \quad (14)$$

The value of S for an erect-leaved canopy is approximately 0.7. For a rice crop at high LAI (LAI = 11.2, a high value recorded for the new plant type) where $S = 0.7$, $A_0 = 3.3$, and $A_1 = 3.0$, the difference between the live LAI and A_0 and A_1 is the area L_N used for storing nitrogen (Sinclair and Sheehy 1999)

$$L_N = L - (A_0 + A_1) \quad (15)$$

and in this example $L_N = 4.9$. The relationship between the photosynthetically active LAI and total LAI is shown in Figure 2: it can be seen that the maximum possible photosynthetically active area index is approximately 6.6. In the experiments of Akita (1989), with cultivar IR29723-143-3-2-1 grown in field-based culture solution, the maximum observed LAI was 17 and I calculated the total photosynthetically active area to have been approximately 6.6, suggesting that L_N was approximately 10.4.

The gross rate of photosynthesis of an individual sunlit leaf, p_{g0} , was described by a simple hyperbolic equation (Monteith 1965) of the form

$$p_{g0} = \frac{\alpha (1 - S)I_0 p_{m0}}{\alpha (1 - S)I_0 + p_{m0}} \quad (16)$$

A similar equation can be used for the shaded leaves:

$$p_{g1} = \frac{\alpha \tau (1 - S)I_0 p_{m1}}{\alpha \tau (1 - S)I_0 + p_{m1}} \quad (17)$$

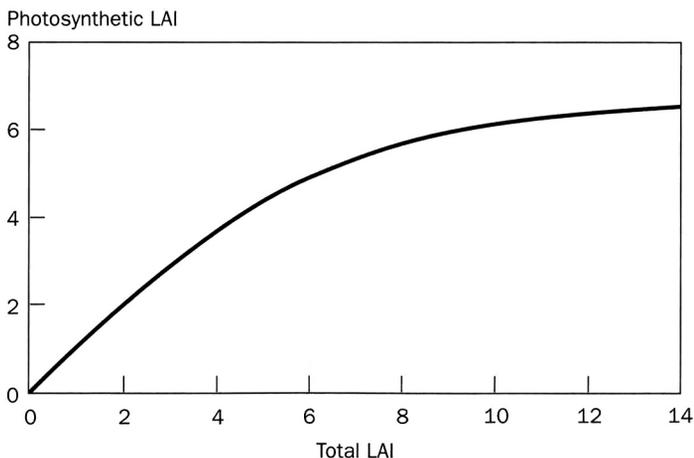


Fig. 2. The relationship between photosynthetically active leaf area index and total leaf area index.

where p_{m0} is the maximum rate of individual leaf photosynthesis for young sunlit leaves, p_{m1} is the maximum rate of individual leaf photosynthesis for older shaded leaves. α is the quantum yield (photochemical efficiency) of an individual leaf, t is approximately 0.1, and I_0 is the instantaneous value of irradiance (PAR). Note that the irradiance on the once-shaded leaves is $t(1-S)I_0$, that is, the PAR passing through one leaf.

The gross photosynthetic rate of the canopy is given by

$$P_g = \int (A_0 p_{g0} + A_1 p_{g1}) dt \quad (18)$$

where the instantaneous values are integrated over the day. The maximum rate of canopy photosynthesis is

$$P_{gmax} = A_0 p_{m0} + A_1 p_{m1}, \text{ as } I \rightarrow \infty \quad (19)$$

The quantum yield of the canopy is given by

$$\frac{dP_g}{dI_0} = \alpha (1-S)(A_0 + \tau A_1), \text{ as } I \rightarrow 0 \quad (20)$$

The maximum quantum yield of a crop intercepting all incident PAR is given by

$$\frac{dP_g}{dI_0} = \alpha (1 + \tau), \text{ as } I \rightarrow 0 \quad (21)$$

This expression has approximately the same numerical value as the more common one given by Sheehy and Johnson (1988).

The rates of individual leaf photosynthesis can be calculated assuming high PAR, 450 W m^{-2} , very high quantum yield ($\alpha = 10.0 \text{ g CH}_2\text{O MJ}^{-1}$), $p_{m0} = 3.0 \text{ g CH}_2\text{O m}^{-2} \text{ h}^{-1}$, and $p_{m1} = 1.5 \text{ g CH}_2\text{O m}^{-2} \text{ h}^{-1}$, that is, once-shaded leaves with half the maximum rates of sunlit leaves. The rate of leaf photosynthesis for sunlit leaves in full PAR, p_{g0} , is $2.5 \text{ g CH}_2\text{O m}^{-2} \text{ h}^{-1}$ and the rate for once-shaded leaves, p_{g1} , is $0.8 \text{ g CH}_2\text{O m}^{-2} \text{ h}^{-1}$.

For the parameter values described above, the rate of gross photosynthesis at 450 W m^{-2} for all the sunlit leaves of the canopy is $8.3 \text{ g CH}_2\text{O m}^{-2} \text{ h}^{-1}$ and the rate for all the once-shaded leaves is $2.3 \text{ g CH}_2\text{O m}^{-2} \text{ h}^{-1}$. The total rate of canopy gross photosynthesis is $10.6 \text{ g CH}_2\text{O m}^{-2} \text{ h}^{-1}$. The shaded leaves contributing 22% of that rate. Dingkuhn et al (1990) reported values of net canopy photosynthesis at high PAR for transplanted rice of approximately $5.0 \text{ g CH}_2\text{O m}^{-2} \text{ h}^{-1}$.

It is interesting to note that the senescence of leaves equivalent to an LAI of 7.9 ($L_N + A_1$) would reduce canopy photosynthesis by only 22%. Furthermore, even if the rate of individual leaf photosynthesis for the once-shaded leaves were as high as for the sunlit leaves, the loss of that LAI would result in only a 27% decline in canopy photosynthesis. This emphasizes the point that nitrogen and carbohydrates can be recycled from approximately 70% of the leaves with canopy photosynthesis declining by only about 25%. The shaded lower leaves of the crop act as a store for nitrogen; the more erect the leaves of the canopy, the larger the fraction of the LAI acting as a nitrogen store (Sinclair and Sheehy 1999).

The nitrogen factor

Ryle and Hesketh (1969) drew attention to the crucial relationship between leaf photosynthesis and leaf nitrogen content. Peng et al (1995) showed that the relationship was linear for rice, suggesting that maximum achievable rates had not been attained. Not surprisingly, high

concentrations of nitrogen in leaves are associated with higher values of the RCF (Sinclair and Horie 1989, Sinclair and Shiraiwa 1993). Work by Horie et al (1997) suggested that the number of spikelets per plant was related to plant nitrogen content (Fig. 3). The rate at which the developing panicle acquires nitrogen exceeds the rate at which the crop acquires it through its roots (Sheehy et al 2000a), emphasizing the requirement for a large “reservoir” in the vegetative tissues. The maximum rate of N accumulation by the crop was $0.38 \text{ g N m}^{-2} \text{ d}^{-1}$ 60 d after transplanting (DAT) and it declined to zero at approximately 105 DAT. The rate of N accumulation by the panicle remained constant at approximately $0.45 \text{ g N m}^{-2} \text{ d}^{-1}$. The essential relationship between high leaf area, erect leaves, and storage of the large amounts of nitrogen associated with high grain yield (Fig. 4) was described by Sinclair and Sheehy (1999). The importance of maintaining at least the minimum concentration of nitrogen (critical N) for maximum metabolic activity, and hence PAR conversion throughout the growth of the crop (Fig. 5), was summarized in a simple model by Greenwood et al (1990). The model predicts that half the total nitrogen accumulated by the crop must be acquired by the time the crop has acquired one quarter of its biomass, between 35 and 50 d following transplanting (Sheehy et al 1998a). Table 2 shows the calculated nitrogen requirements of crops at different grain yields and the amounts of fertilizer required to attain them. Generally, the quantity of N fertilizer applied to rice is much less than 250 kg ha^{-1} (Dobermann, this volume) and so it is not surprising that yields are usually far less than 10 t ha^{-1} . Moreover, in monsoon Asia, crops are often underfertilized to prevent lodging, which remains a significant problem.

In the dry season at IRRI, grain yields of approximately 12 t ha^{-1} and RCFs of approximately 2.6 g DM MJ^{-1} were obtained for crops in which lodging was prevented artificially and large amounts of nitrogen were applied (Sheehy et al 2000b). High application rates of nitrogen may be unrealistic for commercial use, not because the cost of fertilizer is an economic limitation (Dawe 2000), but because environmental considerations are against excessive fertilization. Nonetheless, the combination of nitrogen management and new cultivars possessing superior mechanical strength, high LAI, and low specific leaf area is the way to achieve yields close to the limit of rice with conventional C_3 photosynthetic pathways.

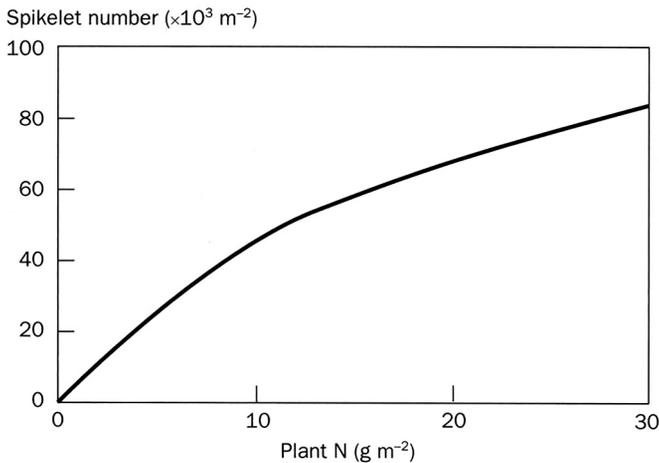


Fig. 3. The relationship between spikelet number and plant nitrogen Content (adapted from Horie et al 1997).

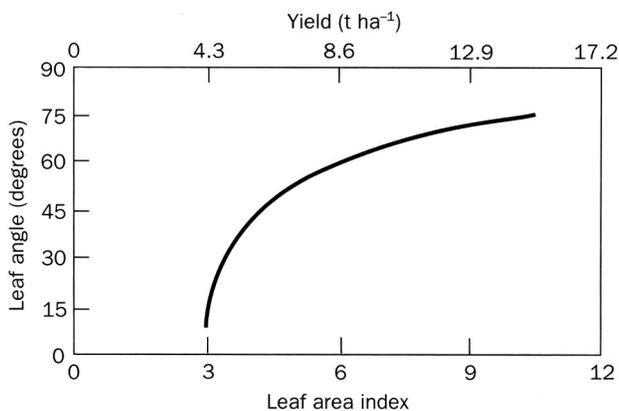


Fig. 4. The relationship among leaf area index, leaf angle, and grain yield (Sinclair and Sheehy 1999).

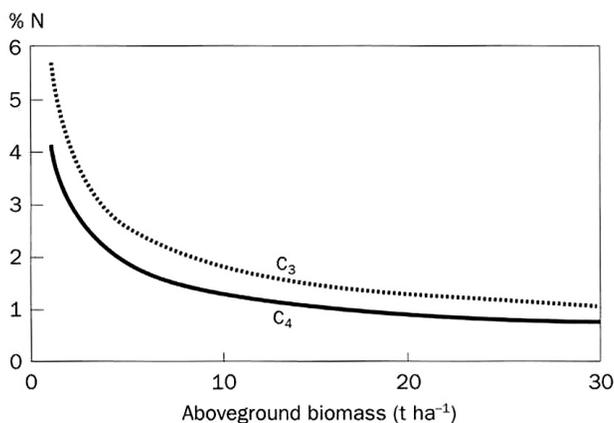


Fig. 5. The relationship between critical N and aboveground biomass for C₃ and C₄ crops (adapted from Greenwood et al 1990).

Table 2. The nitrogen requirements of rice crops at different yields assuming 1.4% N content for grain and 0.8% N content for straw.

Grain yield at 14% moisture content (t ha ⁻¹)	Shoot demand	Assumed intrinsic soil supply	Demand balance	Fertilizer at 50% recovery
		(kg ha ⁻¹)		
10	195	70	125	250
12	234	70	164	328
15	292	70	222	444

Yield and redesigned photosynthesis

On the basis of current evidence, it would appear that achieving a 40% to 50% increase in yield is probably beyond the capacity of rice with its current photosynthetic pathway. Such a large increase in potential yield will certainly require an RCF of 3.3 g DM MJ⁻¹ (the value for maize). As a consequence, achieving yields beyond 12.5 t ha⁻¹ in the next 50 years could require the introduction of the C₄ photosynthetic pathway to rice (Ku et al 1999). Increases in atmospheric CO₂ (Schimel et al 1996) could produce a similar yield increase by about 2050. However, several potential obstacles could prevent crops with very high photosynthetic rates from using the extra photosynthate to increase grain yield without reducing grain quality.

The sink size in rice is proportional to the number of spikelets. At harvest, a large fraction of the spikelets is filled and is known as grain; a smaller fraction remains unfilled or partially filled. Unlike in wheat or barley, grain weight in a rice cultivar is almost constant; the desired range is 23–27 mg. Consequently, yield improvements must result from an increase in spikelet number and that depends on the nitrogen status of the crop (Fig. 3). However, the critical nitrogen content of C₄ crops is less than that of C₃ crops growing under current CO₂ conditions.

Several questions arise if it is assumed that a C₄ rice were to have a critical nitrogen concentration typical of other C₄ crops. Would the number of spikelets per unit ground area be smaller in a C₄ rice crop than in a C₃ rice crop? Would this reduction lead to a limitation of yield potential? Would the C₄ syndrome reduce the protein content of the grain? To answer these questions, it is necessary to make some calculations. A grain yield of 15 t ha⁻¹ (equivalent to 13.3 t ha⁻¹ dry weight) would require 57,826 grains m⁻² each weighing 23 mg. If the filled percentage were 80%, a total of 72,282 spikelets m⁻² would be needed to produce such a yield. The maximum number of juvenile spikelets (glumous flowers at the late differentiation stage of the panicle) observed per square meter in the new plant type in the experiment described above was 116,325. If approximately 65% of these remained after floret abortion, 76% of them would have to be filled to produce the yield required. Therefore, the sink size for a crop with a conventional photosynthetic pathway would be adequate. However, one cannot assume that a rice crop having a C₄ pathway would have the same number of juvenile spikelets because that number depends on plant N status. In an experiment here at IRRI, in which only a moderate amount of N was supplied (120 kg ha⁻¹), the number of juvenile spikelets per square meter was only 54,000.

Horie et al (1997) derived a relationship between spikelet number and the concentration of N at flowering (Fig. 3). The number of spikelets a C₄ rice crop might produce is calculated by assuming that the shoot weight of a C₄ crop at flowering would be 12.4 t ha⁻¹ (a value typical for high-yielding C₃ crops). The Greenwood model suggests that the N content would be approximately 14.5 g N m⁻². The number of spikelets for a C₄ rice crop was calculated to be 60,000 m⁻¹. To obtain a 15 t ha⁻¹ yield, 96% of the spikelets would have to be filled, a remarkably high filling percentage.

The relatively lower intrinsic N content of a C₄ crop may mean that the nitrogen reservoir in its shoots would be inadequate to support the nitrogen demand of the developing grains. To investigate this possibility, the simple model of Greenwood et al (1990) linking critical nitrogen concentrations in the aboveground biomass (%N) and crop dry weight (W) was used. For both C₃ and C₄ crops in the vegetative stage, the model equation is

$$N\% = aW^{-b}, W \geq 1 \text{ t ha}^{-1} \quad (22)$$

Table 3. Crop N contents for two typical grain N content scenarios: (1) high-yielding rice (1.4%) and (2) low-yielding rice (0.9%); straw N is assumed to be constant (0.8%) as is the total crop yield (15 t ha⁻¹).

Panicle	Straw, (kg ha ⁻¹)	Total
186 (1.4% N)	106 (0.8% N)	292
120 (0.9% N)	106 (0.8% N)	226

Greenwood found that for both C₃ and C₄ crops the model accounted for 86% of the variance when $b = 0.5$, $a = 5.7$ (C₃), and $a = 4.1$ (C₄). Using this model, assuming a grain yield of 15 t ha⁻¹, the total nitrogen content in the shoots of a C₃ and a C₄ crop was calculated to be 292 kg ha⁻¹ for the C₃ crop and 211 kg ha⁻¹ for the C₄ crop. Next, the N contents were calculated for two grain N content scenarios: (1) typical of high-yielding rice (1.4%) and (2) typical of low-yielding rice (0.9%) (Table 3). By comparing the N contents in Table 3 with that predicted for a C₄ rice containing the critical nitrogen concentration, it can be seen that such a C₄ rice would contain insufficient N to support a grain yield of 15 t ha⁻¹ at a grain N content as low as 0.9% N. The grain N concentration of a 15 t ha⁻¹ crop would have to be approximately 0.8%, a very low value, assuming that straw N concentration could not be reduced.

A C₃ crop growing under elevated CO₂ conditions with suppressed photorespiration may resemble a C₄ crop in terms of its N content (Nakano et al 1997) and yield. Several experiments have been conducted to investigate such effects. Many such experiments have been carried out in pots or enclosures of limited size and so the results are somewhat difficult to extrapolate to normal field conditions, especially for ceiling-yield scenarios. Ziska et al (1997), using open-top field enclosures, observed increases in grain yield with elevated CO₂, but the control yields were almost 50% lower than the 10 t ha⁻¹ obtained outside the enclosures. Nonetheless, they reported that protein contents in the grain declined significantly with increasing CO₂. Yoshida (1973) elevated CO₂ to approximately 900 ppmv in small open-top field enclosures (0.36 m²) during different growth stages. He reported a yield of 13.3 t ha⁻¹ and a filling percentage of 87.6% when CO₂ was elevated for 30 d before flowering. Baker et al (1992a, 1993), using outdoor sunlit environment chambers, reported no effect of elevated CO₂ on grain yield and in other experiments a significant increase in yield (Baker et al 1990, 1992b, 1997).

If a C₄ rice plant were to be developed and atmospheric CO₂ concentrations continued to rise, so that C₃ and C₄ rice photosynthesized at the same rate, the C₄ rice could use significantly less water in transpiration than the C₃ rice. For example, if CO₂ levels increased to 510 ppm, the stomatal conductance of C₄ rice could fall by more than 50% while maintaining the same rate of photosynthesis per unit leaf area as would occur at current concentrations. The sunlit leaves of a C₄ rice would be unable to shed as much heat as a C₃ rice under such conditions and it can be estimated that close to midday their temperature would rise above that of the C₃ by about 2 °C (Sage, this volume) and their rate of transpiration would be about 50% lower (Sheehy et al 1998b). In a future world of elevated CO₂, the greatest advantage of a C₄ rice may well be its drought tolerance in upland and rainfed ecosystems. The adverse effects on yield of temperature increases that may result from global climate change are documented elsewhere (Matthews et al 1995).

Conclusions

1. Currently, the maximum achievable radiation conversion factor of a C₃ rice is approximately 2.7 g CH₂O MJ⁻¹ (PAR).
2. The ceiling grain yield of a C₃ rice crop (110-d duration) in the tropics at current CO₂ concentrations is predicted to be approximately 12.5 t ha⁻¹.
3. For the same growth duration, grain yields of 15 t ha⁻¹ would require a radiation conversion factor of 3.3 g CH₂O MJ⁻¹ (PAR), the value for maize.
4. Experiments using elevated CO₂ suggest that there will be a yield advantage to be gained from increases in atmospheric CO₂, but the protein content of the rice may be lowered.
5. In canopies of high-yielding crops, approximately 70% of the leaves can become senescent and transfer nitrogen to the grain for a loss of about 25% of the maximum rate of canopy gross photosynthesis.
6. The critical nitrogen content of a C₄ rice crop (or a C₃ rice growing at close to 500 ppmv) may limit sink size (the number of spikelets) and, hence, the yield potential of such a crop.
7. Unless the nitrogen concentration of a C₄ rice were to be greater than the critical nitrogen concentration for C₄ crops as defined by Greenwood et al (1990), its protein content at a yield of 15 t ha⁻¹ could be unacceptably low.
8. If a C₄ rice plant were to be invented, its potential drought tolerance could make it of great value for upland and rainfed rice ecosystems.

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Would C₄ rice produce more biomass than C₃ rice?

J.R. Evans and S. von Caemmerer

Evidence suggests that C₄ plants produce greater amounts of biomass per unit of intercepted photosynthetically active radiation. This is due in large part to two factors. First, C₄ plants have a greater quantum yield than C₃ plants at 30 °C (the C₄ advantage diminishes at lower temperatures and as atmospheric CO₂ partial pressures rise). Second, C₄ plants have greater rates of CO₂ assimilation per unit leaf nitrogen (this benefit diminishes as leaf area index and/or canopy nitrogen content increases). The protein cost of C₄ enzymes per unit chlorophyll is calculated and found to be similar to that of C₃ photosynthesis. However, the rate of CO₂ assimilation per unit nitrogen in C₄ plants is greater than that of C₃ plants because high CO₂ partial pressure in the bundle sheath cells enables Rubisco to operate near its maximum catalytic rate and suppresses photorespiration. Rice leaf anatomy is examined with respect to locating the C₄ metabolism. Chloroplasts in bundle sheath cells represent only a minute fraction of those present in the rice leaf. In addition, whereas mesophyll cells are immediately adjacent to bundle sheath cells in terrestrial C₄ leaves, there are numerous mesophyll cells between adjacent veins in rice, which would diminish the efficiency of the C₄ cycle. To engineer the C₄ pathway into rice is therefore a formidable challenge.

The C₄ photosynthetic pathway has evolved on numerous occasions in diverse plant species. The question is, Would it be useful to genetically engineer the pathway into rice? In this chapter, we wish to examine the evidence that C₄ productivity exceeds that of C₃ species and why this might be so. We will also place the problem of putting C₄ metabolism into rice in the context of its leaf anatomy. The complex details confronting the molecular biologists' task are beyond the scope of this chapter.

CO₂ fixation in C₃ plants is catalyzed by the enzyme Rubisco (ribulose-1,5-bisphosphate carboxylase-oxygenase). Rubisco also catalyzes the reaction with O₂ resulting in photorespiration. With present-day atmospheric CO₂ concentrations, around one-third of the RuBP is consumed by the oxygenase pathway at 30 °C. The C₄ photosynthetic pathway has evolved to virtually eliminate oxygenase activity by Rubisco. This is achieved by bicarbonate reacting with phosphoenolpyruvate, PEP, a carboxylation reaction insensitive to O₂. The C₄ acids formed in the mesophyll cells diffuse into bundle sheath cells where they are decarboxylated. A diffusive resistance restricts CO₂ leakage out of the bundle sheath such that the CO₂ partial pressure in the bundle sheath cells is greatly increased, resulting in the suppression of photorespiration. Whereas C₄ plants from different species share a common carboxylation pathway, there are three major types of decarboxylation pathways, each liberating CO₂ into a different bundle sheath organelle (Hatch 1987).

Radiation-use efficiency

There has been a long and continuing debate in the literature since C_4 photosynthesis was discovered over whether C_4 plants are more productive than C_3 plants. An assemblage of maximum crop growth rates by Evans (1975) led to the suggestion that C_3 and C_4 growth rates were comparable. Monteith (1978) took exception to this and, after giving reasons for excluding half of the values, came up with a different mean based on the four highest crop growth rates of 52 versus 36.5 $\text{g m}^{-2} \text{d}^{-1}$ for C_4 and C_3 plants, respectively. Additional data of final biomass versus length of growing season showed that the C_4 group produced 40% more biomass than the C_3 group on the basis of intercepted solar radiation (Monteith 1978). Subsequently, Snaydon (1991) countered the debate with an analysis of 34 species grown at 88 sites and concluded that C_4 species were not more productive than C_3 species in terms of annual aboveground biomass production. He suggested that previous conclusions about C_4 productivity being greater than that of C_3 were due to “intense selection of data.” Snaydon’s conclusion, however, has been disputed by others, such as Long (1999), who point out that, at low latitudes, there were 10 reports of C_4 plants exceeding 50 t ha^{-1} and only one report for C_3 plants. Long (1999) suggested that the superiority of C_4 plants at low latitudes was consistent with their greater quantum yield (which is dealt with below), but for that to translate into biomass productivity required information on the fraction of CO_2 assimilation consumed in respiration and the allocation to foliage.

One other assemblage of crop growth data by Kiniry et al (1989) should be considered. Sequential harvests of aboveground biomass and estimates of cumulative intercepted photosynthetically active radiation (PAR) were used to calculate the radiation-use efficiency of *Zea mays*, *Sorghum bicolor*, *Triticum aestivum*, and *Oryza sativa*. Table 1 presents mean values for each species. On average, the two C_4 crops had greater radiation-use efficiency than the two C_3 crops. Sorghum and wheat, however, had the same mean values. Kiniry et al (1989) presented two average values for rice, 2.7 and 2.2 g MJ^{-1} , depending on whether one study was included or left out. The literature was again surveyed and assessed by Mitchell et al (1998), who obtained a mean value for rice of 2.2 g MJ^{-1} .

It therefore seems reasonable to proceed on the basis that rice has a lower radiation-use efficiency than the C_4 crops sorghum and maize. In the following sections, we examine two features of C_4 photosynthesis that contribute to this superiority. The first is quantum yield, the amount of CO_2 fixed per quanta absorbed. In a crop canopy, this parameter is very important because light is distributed between the leaves such that the irradiance for any piece of leaf is generally well below light saturation. Consequently, the rate of CO_2 assimilation depends most strongly on the quantum yield. We compare the responses of quantum yield to CO_2 and temperature for C_3 and C_4 leaves. The second feature is that C_4 leaves have a greater rate of CO_2 assimilation per unit of leaf nitrogen than C_3 leaves. We assemble data from the literature to illustrate the allocation of nitrogen between the various proteins necessary for CO_2 assimilation and calculate the CO_2 assimilation rate for a C_3 versus C_4 strategy.

Table 1. Radiation-use efficiency for aboveground biomass production (Kiniry et al 1989), mean \pm S.E. (n).

Crop	E (g MJ^{-1})	e/e_{rice}
Maize	3.5 \pm 0.7 (12)	1.59
Sorghum	2.8 \pm 0.4 (14)	1.27
Wheat	2.8 \pm 0.2 (5)	1.27
Rice	2.2 \pm 0.2 (3)	

Quantum yield

At low irradiances, the rate of CO_2 assimilation increases linearly with increases in irradiance and the quantum yield is the slope of the relationship. When photorespiration is suppressed by either high CO_2 or low O_2 partial pressures, quantum yields are almost identical between diverse C_3 species (Björkman and Demmig 1987). The proportion of RuBP regenerated that is consumed by carboxylation or oxygenation reactions is consistent with the known kinetic properties of Rubisco that have been described in the Farquhar et al (1980) model of C_3 photosynthesis. Carboxylation as a fraction of RuBP regeneration increases as the partial pressure of CO_2 increases, reaching a value of 1 at saturating CO_2 (Fig. 1). The partitioning between carboxylation and oxygenation is governed by the specificity factor of Rubisco, which is related to Γ_* , the CO_2 compensation point in the absence of nonphotorespiratory CO_2 evolution (Farquhar et al 1980). The ratio of carboxylation to oxygenation decreases as temperature increases because Γ_* increases with temperature (Brooks and Farquhar 1985). Thus, for any given CO_2 partial pressure, a smaller proportion of carboxylation reactions occur at 30 compared with 20 °C (Fig. 1). By contrast, the CO_2 -concentrating mechanism of the C_4 pathway suppresses oxygenation reactions and the quantum yields of C_4 leaves are independent of temperature (Ehleringer and Bjorkman 1977, Fig. 1).

For each CO_2 fixed in C_3 photosynthesis, 2 NADPH and 3 ATP molecules are required. To regenerate each NADPH, 2 electrons must be transported from water, via both photosystems, which requires that each photosystem absorb 2 quanta. Thus, 4 quanta are needed per NADPH or 8 quanta for the 2 NADPH per CO_2 fixed, assuming 100% efficiency of both photosystems.

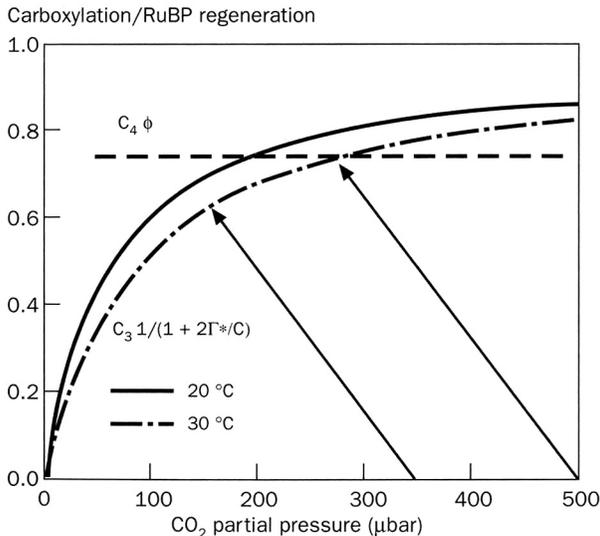


Fig. 1. The dependence on CO_2 partial pressure of the proportion of RuBP regeneration that is consumed by Rubisco carboxylation. The curves are calculated according to the Farquhar et al model (1980) such that the ratio of Rubisco carboxylation to RuBP regeneration is given by $1/(1 + 2\Gamma_*/C)$, where Γ_* is the CO_2 compensation point in the absence of nonphotorespiratory CO_2 release and C is the partial pressure of CO_2 at the sites of carboxylation. The curves are drawn for 20 and 30 °C. Also shown is the quantum yield of a C_4 leaf relative to a C_3 leaf in the absence of photorespiration. The lines with arrows pointing to the 30 °C curve represent the present position for C_3 plants and the point when the quantum yield will equal that of C_4 leaves due to the rising atmospheric CO_2 partial pressure.

This would result in the evolution of 1 O₂ per CO₂ fixed. In the absence of photorespiration, the change in the rate of CO₂ or O₂ exchange for a change in absorbed quanta should be the same. In practice, however, it is generally not possible to measure both CO₂ and O₂ fluxes and the units used to express quantum yields depend on the experimental setup. The calculation for ATP is less certain as the number of protons required to regenerate each ATP could be 3 or 4. Each electron transported via both photosystems will result in 3 protons being deposited in the thylakoid lumen, or 1.5 H⁺ per quanta, assuming the Q cycle operates. Thus, 12 protons will be available from the 8 quanta required for the NADP reduction for each CO₂ fixed, yielding 3 or 4 ATP. Consequently, with 8 quanta, there may be exactly the amount of NADPH and ATP formed that is needed to fix one CO₂, or there may be an extra ATP formed. This would predict that the maximum quantum yield would be 0.125 mol O₂ (mol absorbed quanta)⁻¹. A large survey of C³ plants obtained a highly consistent value of 0.106 mol O₂ (mol absorbed quanta)⁻¹ (Bjorkman and Demmig 1987). The 15% discrepancy may in large part be attributed to the spectral distribution of the light, comprehensively studied by McCree (1972). The wavelength dependence of quantum yield is consistent with the two photosystems having independent pigment antennae that have slightly different absorption spectra that result in photosystem II absorbing about 57.5% of the white light compared with 42.5% by photosystem I (Evans 1987). In practice, quantum yields for CO₂ assimilation are lower because photosystem II does not operate with 100% efficiency (Genty and Harbinson 1996) and there may be electron transport for other processes such as nitrate reduction, or errors in measurement.

A similar exercise can be carried out for the quantum yield of C₄ photosynthesis. In addition to the quanta required for the C₃ cycle, the equivalent of 2 ATP are needed for each CO₂ fixed by PEP carboxylase. This can be achieved most efficiently via cyclic electron transport around photosystem I, which, with the Q cycle operating, would yield 2 H⁺ per quanta. This is the most efficient mechanism, but other less efficient options exist, such as pseudocyclic electron flow to oxygen, which lead to lower quantum yields (see Furbank et al 1990). Thus, an extra 3 or 4 quanta would be needed in the absence of any leakage of CO₂ from the bundle sheath. The ATP requirement for the C₄ cycle is 2/(1 - f), where f is the proportion of CO₂ fixed by PEP carboxylase that leaks out of the bundle sheath (Caemmerer and Furbank 1999). The value of f at moderate to high irradiance has been calculated to be about 0.2 (Henderson et al 1992, 1994), but it is not known whether this holds true at very low irradiances. If we assume that it does, then the quantum requirement (the reciprocal of quantum yield) for C₄ leaves is expected to be 9.4 for the C₃ cycle (1/0.106) plus 3.75 to 5 for the C₄ cycle, giving a total quantum requirement of 13.15 to 14.4 or a quantum yield of 0.069 to 0.076 mol O₂ (mol absorbed quanta)⁻¹.

Measurements of quantum yield always have an element of uncertainty because of measurement error. Thus, the absolute values between studies are not necessarily comparable. Also, growth conditions may influence the result. The temperature at which the C₄ NAD-ME (malic enzyme) dicot *Atriplex rosea* had a quantum yield equal to that of the C₃ *Encelia californica* was 28 °C (0.053 mol CO₂ [mol absorbed quanta]⁻¹, Ehleringer and Bjorkman 1977). In another study with species from a cool shaded habitat in Hawaii, the C₄ NAD-ME dicot *Euphorbia forbesii* and the C₃ *Claoxylon sandwicense* had equal quantum yields at 22 °C (0.062 mol CO₂ [mol absorbed quanta]⁻¹, Robichaux and Pearcy 1980). It is perhaps significant that, in these two studies, the type of C₄ pathway differed.

In an extensive survey of quantum yields of C₄ species, Ehleringer and Pearcy (1983) measured 16 dicot and 16 monocot species. The nine species of NAD-ME dicots had a mean quantum yield of 0.053 mol CO₂ (mol absorbed quanta)⁻¹ at 30 °C and 330 mbar CO₂, which was the same value determined for 23 C₃ monocot and dicot species. By contrast, the NADP-

ME dicots and all the C_4 monocots, regardless of decarboxylation type, had quantum yields of $0.064 \text{ mol CO}_2 (\text{mol absorbed quanta})^{-1}$. This value is close to that calculated for C_4 leaves above ($0.069\text{--}0.076$). The higher temperature necessary for the C_3 quantum yield to equal that of *A. rosea* may be largely because *A. rosea* is an NAD-ME dicot, the type with the lowest quantum yield. Therefore, at 30°C and $330 \mu\text{bar CO}_2$, the quantum yield of C_4 monocots is 20% greater than that of C_3 plants.

The advantage for C_4 plants increases with temperature but will decrease as atmospheric CO_2 concentrations continue to rise. An increase of $150 \mu\text{bar}$ will be sufficient to eliminate the current difference between the C_3 and C_4 quantum yields (see parallel arrows in Fig. 11. As this is likely to occur around 2050 (Houghton et al 1995), the C_4 quantum yield advantage would appear to have a limited life span.

Nitrogen cost of C_4 versus C_3 photosynthetic pathways

In this section, we will assemble data to illustrate the nitrogen cost of the soluble enzymes directly involved in CO_2 assimilation. In C_3 leaves, Rubisco dominates the profile of leaf protein, typically representing one-third of the soluble protein. The remaining 11 enzymes of the photosynthetic carbon reduction (PCR) cycle account for only another 10% of the soluble protein (Evans and Seemann 1989). The enzymes necessary for the C_4 cycle are also present in C_3 leaves, but at low levels, and account for roughly 1% of the protein allocated to the sum of Rubisco, the other PCR enzymes, and the C_4 enzymes (Fig. 2).

There are few published measurements of the abundance of C_4 enzymes. First, we calculate the amount of protein present in each enzyme, expressed per unit chlorophyll, because this is the only available basis for comparison at present. Then we calculate the rate of CO_2 assimilation achieved for a given amount of nitrogen. Hatch (1987) provides a compilation of the eight

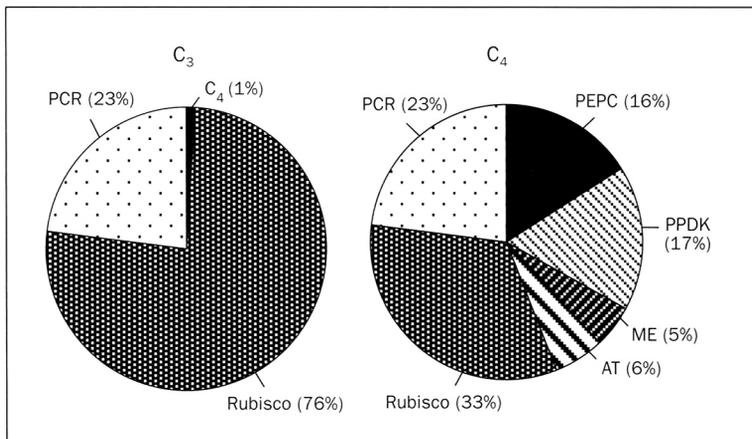


Fig. 2. Diagrammatic representation of the allocation of proteins between the enzymes directly involved with CO_2 assimilation for C_3 and C_4 leaves. The enzymes have been divided into Rubisco, the other photosynthetic carbon reduction (PCR) cycle enzymes, and those for the C_4 cycle (see Table 2 and text for details). For C_3 leaves, PEPC and the other C_4 enzymes account for about 1% of this protein pool. The amount of these proteins per unit chlorophyll is similar for the C_3 and C_4 leaves, $6.1\text{--}6.5 \text{ g protein (g Chl)}^{-1}$, accounting for about 20–25% of leaf N. PEPC = phosphoenolpyruvate carboxylase, AT = aspartate aminotransferase, ME = malic enzyme, PPK = pyruvate, Pi dikinase.

enzymes necessary for the C₄ cycle and their activities expressed per unit of chlorophyll in both C₃ and C₄ plants. To convert these to protein amounts, we have taken the molecular weights and specific activities (corrected to 25 °C where necessary using a Q₁₀ of 2) from Kanai and Edwards (1999). The amount of protein for each enzyme, N₄ (g protein g⁻¹ Chl), is calculated as

$$N_4 = (V_3 \times V_4/V_3 \times T \times MW)/k_{cat}$$

where v₃ is the average rate for each enzyme in a C₃ leaf, v₄/v₃ is the ratio of the C₄ to C₃ rates, T is the C₄ decarboxylation type, MW is the molecular weight of the enzyme, and k_{cat} is the specific activity of each enzyme (Table 2). The protein cost of the C₄ enzymes in C₃ leaves is 0.08 g (g Chl)⁻¹ and ranges from 2.5 to 2.9 g (g Chl)⁻¹ for the three C₄ decarboxylation types. To this must be added the cost of Rubisco and other PCR enzymes. For Rubisco, an average value for C₃ plants is 4.9 g (g Chl)⁻¹ (5.0, *Triticum aestivum*, Evans 1983; 5.4, *Chenopodium album*, Sage et al 1987; 5.0, *Spinacea oleracea*, Terashima and Evans 1988; 4.2, *Nicotiana tabacum*, Evans et al 1994). Sage et al (1987) compared the C₄ dicot *Amaranthus retroflexus* with *C. album*, which had on average only 0.43 times as much Rubisco as the C₃ plant for a given nitrogen content per unit leaf area. As a first approximation, we assume the same cost of other PCR enzymes in both leaf types. When added together, the protein costs (g [g Chl]⁻¹) are remarkably similar for C₃ and C₄ leaves, being 6.48 for C₃, 6.1 for NADP-ME, 6.35 for NAD-ME, and 6.5 for PCK (PEP carboxykinase) types (Table 2). Additional enzymes likely to be less abundant in C₄ compared with C₃ leaves are Rubisco activase, glycine decarboxylase, and carbonic anhydrase in bundle sheath cells, although carbonic anhydrase in mesophyll cells would need to be relocated from the chloroplast to the cytosol.

The values calculated in Table 2 can only be compared with two other determinations in the literature. Sugiyama et al (1984) grew maize under a range of nitrate concentrations and quantified soluble protein, Rubisco, PEPC (phosphoenolpyruvate carboxylase), and PPK

Table 2. C₄ enzymes and their properties and amounts.

Enzyme ^a	MW ^b	k _{cat} ^{b,c}	C ₃ rate ^d	C ₄ /C ₃ ^{d,e}	NADP ^f	NAD ^f	PCK ^f	NAD ^g
PEPC	440	10,400	0.8	16–30	1	1	1	1.02
PPDK	376	2,725	0.8	40–80	1	1	1	1.10
NADP-MDH	86	60,500	0.4	2 ⁷	10 ^h	1	1	0.002
NADP-ME	248	12,700	0.3	25–40	1	0	0	0
NAD-ME	504	38,870	0.3	18–60	0	1	0	0.23
PCK	408	17,700	0.15	40–110	0	0	1	0
Asp AT	84	18,200	1.7	16–30	0.1	1	1	0.24
Ala AT	100	39,500	2.5	12–25	0.1	1	1	0.16
C ₄ enzyme subtotal ⁱ			0.08		2.50	2.75	2.90	2.75
Rubisco ^j			4.9	0.43	2.1	2.1	2.1	
PCR ^{i,k}			1.5	1 ^f	1.5	1.5	1.5	
Total ^l			6.48		6.1	6.35	6.5	

^a PEPC = phosphoenolpyruvate carboxylase, PPDK = pyruvate, Pi dikinase, NADP-MDH = NADP malate dehydrogenase. NADP-ME = NADP malic enzyme, NAD-ME = NAD malic enzyme, PCK = PEP carboxykinase, Asp AT = aspartate aminotransferase, Ala AT = alanine aminotransferase, PCR = photosynthetic carbon reduction. ^b Molecular weight (kDa), Kanai and Edwards (1999). ^c Specific activity, corrected where necessary to 25 °C using a Q₁₀ of 2 (mol [mol enz]⁻¹ min⁻¹). ^d Enzyme activity typical of a C₃ leaf (mmol min⁻¹ g⁻¹ Chl), Hatch (1987). ^e Ratio of activity from a C₄ leaf compared with that of a C₃ leaf. The upper bound is used for the calculation. ^f C₄ decarboxylation type. These columns adjust the activities in the C₄/C₃ column for each C₄ type. ^g Example amount shown for the NAD type, calculated as C₃ rate × C₄/C₃ × NAD × MW/k_{cat} (g protein g⁻¹ Chl). Other types calculated using the NADP or PCK column instead of the NAD one. ^h NADP-ME types have 12-21 times the C₃ rate of NADP-MDH. ⁱ (g protein [g Chl]⁻¹). ^j See text for derivation. ^k PCR cycle enzymes apart from Rubisco, Evans and Seemann (1989). ^l Assumed.

(pyruvate. Pi dikinase). The relative abundances of PEPC and PPDK were similar to each other, confirming the estimate in Table 2. However, each represented about one-fifth of Rubisco content, considerably less than the estimate in Table 2. Sage et al (1987) measured Rubisco and PEPC in *A. retroflexus* and found that PEPC represented just less than half of the protein in Rubisco, in agreement with the estimate in Table 2. It should be remembered that the calculations in Table 2 were based on the maximum value of a given enzyme's activity and in some cases the lower bound value may have been more appropriate for a given species. Although the values calculated in Table 2 are clearly uncertain, they provide the best estimate at present for the nitrogen cost of C_4 photosynthetic enzymes (Fig. 2).

Although the C_3 and C_4 pathways have similar protein costs per unit of chlorophyll, this does not mean that they have similar rates of CO_2 assimilation per unit of nitrogen. A rough estimate can be provided by calculating the Rubisco activity at 25 °C in vivo, V_c , as follows: $V_c = E \cdot k_c \cdot (C - G_*) / (C + K')$, where E is the amount of Rubisco (1 mol sites), k_c is the specific activity (3 and 4 mol CO_2 mol⁻¹ sites s⁻¹ for C_3 and C_4 Rubisco, respectively, Caemmerer and Furbank 1999), C is the intercellular partial pressure of CO_2 ($0.7 \times 350 = 245$ mbar), G_* is the compensation point in the absence of nonphotorespiratory CO_2 evolution (37 mbar, Caemmerer et al 1994), and K' is the Michaelis–Menten constant for CO_2 (730 mbar, Caemmerer et al 1994). For the C_4 leaf, there will be only 0.43 mol Rubisco sites, each operating at 0.9 of V_{max} . The values of V_c for the C_3 and C_4 leaves are therefore $[1 \times 3 \times (245 - 37) / (245 + 730)] = 0.6$ and $(0.43 \times 4 \times 0.9) = 1.5$. Thus, the C_4 leaf has 2.5 times (1.5/0.6) the rate of carboxylation compared with that of the C_3 leaf for a given chlorophyll content per unit leaf area. This is approximately equivalent to 2.5 times the rate for a given nitrogen content per unit leaf area because C_3 and C_4 plants have a similar chlorophyll to nitrogen ratio (e.g., Sage et al 1987) and is consistent with leaf gas exchange measurements (Fig. 3).

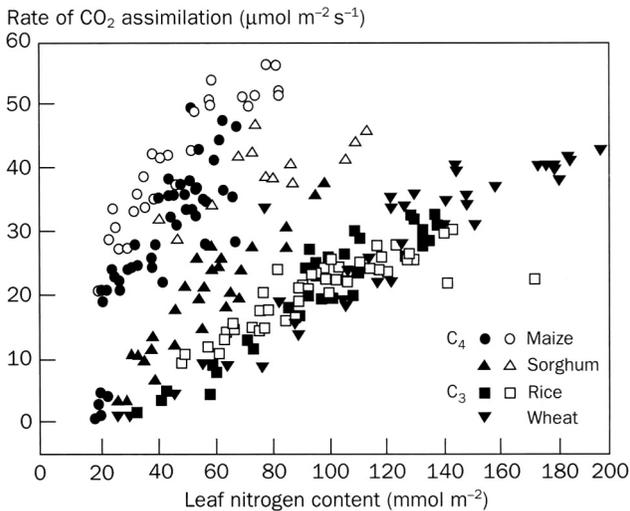


Fig. 3. The relationships between rate of CO_2 assimilation and nitrogen content per unit leaf area. Data for maize (○) from Wong et al (1985), 30 °C, (●) Sinclair and Horie (1989); sorghum (△) from Henderson et al (1994), 29 °C, (◻) Anten et al (1995), 31 °C; rice (◻) Makino et al (1988), 25 °C, (◻) Cook and Evans (1983), 27 °C; wheat (◻) Makino et al (1988), 25 °C; see also Evans (1983).

The superiority that C₄ leaves have over C₃ leaves in terms of their greater rate of CO₂ assimilation under high irradiance for a given nitrogen content per unit leaf area has long been recognized (e.g., Bolton and Brown 1980, Wong et al 1985, Sage and Pearcy 1987). This is illustrated for several monocot species in Figure 3. *Triticum* and *Oryza* share a common relationship, achieving rates of around 16 μmol m⁻² s⁻¹ for nitrogen contents of 80 mmol m⁻². By contrast, *Sorghum* and *Zea* have rates of 30 and 45 μmol m⁻² s⁻¹, respectively, at that same nitrogen content per unit leaf area. As these rates are measured under high irradiance, quantum yield differences are unimportant. Leaf temperatures ranged from 25 to 31 °C (see legend to Fig. 3), being slightly lower for the C₃ species, but this temperature difference is not sufficient to confound the comparison. When comparisons have been made between C₃ and C₄ species at the same temperatures, rates of CO₂ assimilation by the C₄ species are greater than those of the C₃ species at a given leaf nitrogen content (Sage and Pearcy 1987). The superiority of the C₄ species reflects the fact that they do not suffer a one-third loss to photorespiration and their Rubisco operates at near CO₂ saturation. Leaves of C₄ plants often have lower nitrogen contents per unit area than C₃ plants, but, because their rates of CO₂ assimilation are so much greater per unit nitrogen, C₄ leaves generally still have greater rates of CO₂ assimilation per unit leaf area than C₃ leaves.

Photosynthetic performance in a canopy

The apparent superiority of C₄ relative to C₃ plants in terms of radiation-use efficiency was 27% to 60% (Table 1). The difference in quantum yields at 30 °C was about 20%. It is appropriate to use 30 °C for tropical rice, as it is grown with an average maximum temperature of 31.6 °C (Sheehy, personal communication), but clearly where rice is grown in cooler regions, the difference would not be as great (Fig. 1). Because the difference in radiation-use efficiency between C₃ and C₄ plants was more than the difference in quantum yields, additional factors are likely to be involved. The second factor discussed above was the greater photosynthetic rate at high irradiance for a given nitrogen content per unit leaf area. We can compare irradiance response curves for C₃ and C₄ leaves containing 80 mmol N m⁻². The C₄ leaf has a quantum yield and maximum rate of CO₂ assimilation of 0.062 mol CO₂ (mol absorbed quanta)⁻¹ and 30 μmol m⁻² s⁻¹, respectively, whereas the C₃ leaf has values of 0.052 mol CO₂ (mol absorbed quanta)⁻¹ and 16 μmol m⁻² s⁻¹ (Fig. 4). The superiority of the C₄ leaf increases from 20% at low irradiance to 90% at high irradiance. In a crop canopy where the leaf area index (LAI) is high, however, the picture is slightly different. For ease of calculation, we have simply taken the "big leaf" approach to modeling the canopy (e.g., Evans and Farquhar 1991). Although more sophisticated models exist, the conclusions reached would be qualitatively the same. The big leaf model assumes that leaf nitrogen and hence photosynthetic capacity vary through the canopy in proportion to absorbed irradiance. This enables the rate of CO₂ assimilation by the canopy to be calculated in the same way as a single leaf, but with the maximum rate of CO₂ assimilation being simply the sum of the values for each of the leaves. We have based our calculations on two sets of rice canopy data. Anten et al (1995) described a rice canopy that had an LAI of 6 and a mean N content of 80 mmol m⁻². The current agronomic practice for ceiling yields applies much greater amounts of nitrogen fertilizer and typically results in rice canopies with a maximum LAI of 10 and an N content of 100 mmol m⁻² (Sheehy, personal communication). A simulated response of CO₂ assimilation rate to irradiance for a canopy with an LAI of 6, having either C₃ or C₄ leaf characteristics, is shown in Figure 5A. The response curves are much straighter than was the case for single leaves (Fig. 4) and do not approach light saturation. Consequently, the ratio of rates for the C₄ versus the C₃ canopy only increases

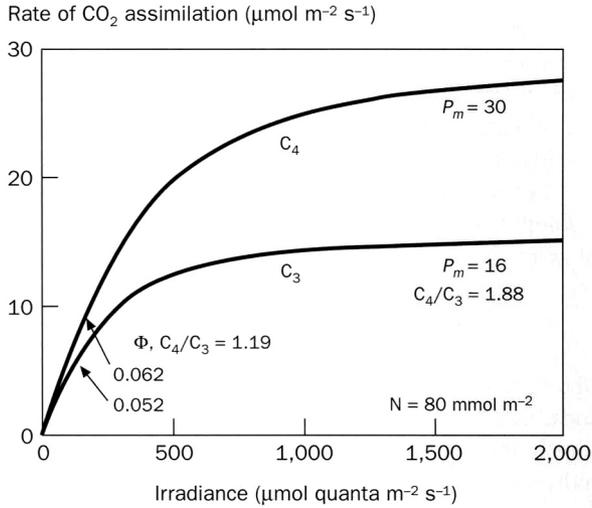


Fig. 4. The relationship between rate of CO₂ assimilation, *A*, and irradiance, *I*, for two leaves containing 80 mmol N m⁻². The values for maximum quantum yield (*f*) were 0.062 and 0.052 mol CO₂ (mol absorbed quanta)⁻¹ for the C₄ and C₃ leaves, respectively, and 30 and 16 mmol m⁻² s⁻¹ for their maximum rates of CO₂ assimilation (*P_m*). The ratios between C₄ and C₃ leaves for *f* and *P_m* were 1.19 and 1.88, respectively. Calculated according to Evans and Farquhar (1991), $A = \{(I + P_m) - [(I + P_m)^2 - 4 Q I P_m^{0.5}]\} / (2 Q)$, with the curvature factor 0 = 0.7.

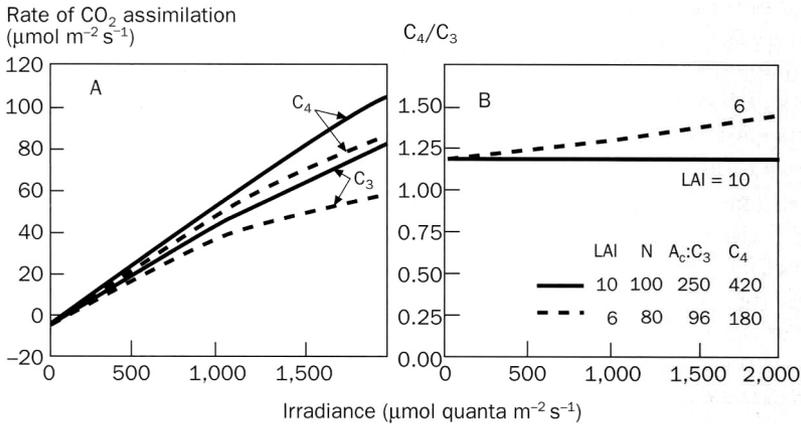


Fig. 5. (A) The relationship between canopy CO₂ assimilation rates and irradiance for a C₄ or C₃ leaf type with a leaf area index (LAI) = 6 and an average nitrogen content per unit leaf area of 80 mmol N m⁻², which results in maximum rates of canopy CO₂ assimilation of 180 and 96 mmol m⁻²s⁻¹ for the C₄ and C₃ leaf types, respectively. At LAI = 10, the average nitrogen contents were 100 mmol N m⁻², which results in maximum rates of canopy CO₂ assimilation of 420 and 250 for the C₄ and C₃ leaf types, respectively. (B) The ratio of the canopy rates of CO₂ assimilation for the C₄/C₃ leaf types for an LAI of 6 or 10.

from 20% to 45% (Fig. 5B) rather than to 90% as with the single leaf (Fig. 4). When the same calculation is made for a canopy with an LAI of 10, the irradiance response curves become even more linear and the ratio of C₄ to C₃ rates now increases only from 20% to 25% (Fig. 5).

From these simple canopy simulations, it is possible to see how radiation-use efficiency of a C₄ relative to a C₃ canopy exceeds that of the ratio of quantum yields and how the advantage declines as LAI and canopy nitrogen increase. Of course, additional factors come into play, such as the proportion of daily carbon gain that is consumed in respiration. For the scenario being proposed here, however, where the rice plant is “simply” engineered to have the C₄ metabolic pathway added, it seems reasonable enough to begin by assuming that the fraction respired is unaltered. Interestingly, one of the factors contributing to the greater radiation-use efficiency of maize compared with sorghum could be the greater rate of CO₂ assimilation at high irradiance for a given nitrogen content per unit leaf area.

Leaf anatomy

Leaves possessing the C₄ pathway stand out because of their Kranz anatomy, recognized by Haberlandt (1914) long before the biochemical significance was known. Around each vascular bundle is a wreath of cells, the bundle sheath, where CO₂ is concentrated and Rubisco is located. Surrounding the bundle sheath are mesophyll cells that first fix the CO₂ into C₄ acids. For the successful operation of C₄ metabolism, it is necessary to differentiate this special anatomy. Given that the C₄ pathway has evolved many times in both monocot and dicot species, the ability of leaves to alter their differentiation to accommodate the C₄ pathway is clearly possible.

Figure 6 shows transverse sections of rice leaves along with *Zea mays* (Fig. 6D) for comparison. The two most important differences between rice and maize are that the interveinal distance is much greater and chloroplasts are almost exclusively located in the mesophyll in rice leaves. The rice leaf contains a regular array of parallel vascular bundles, with major veins occurring every six or so veins (Fig. 6B,E). Each vein has a clearly defined bundle sheath, linked to each epidermis by a fibrous bundle of cells (Fig. 6C). Between each pair of veins on the adaxial epidermis are the massive bulliform cells that enable the lamina to roll. The mesophyll cells are densely packed and highly lobed, making it difficult to discern individual cells. However, there are many mesophyll cells between adjacent veins in rice, but only one or two in maize (Fig. 6D). In Figure 6A (lower half), a lateral vein can be seen extending upward from the minor vein. The limited number of sections examined did not allow us to determine whether a bundle sheath is formed around these lateral veins. Another prominent feature evident in Figure 6F is the aerenchyma in the midvein of the leaf. While this provides a channel for oxygen to diffuse down into the submerged parts of the rice plant, it may also contain higher partial pressures of CO₂ arising from respiration lower in the plant that may be captured in photosynthesis.

Both interveinal distance and the number of mesophyll cells between bundle sheath cells from adjacent veins (lateral cell count) have been found to differ between C₃ and C₄ monocot leaves (Table 3). The overlap in values for interveinal distance between rice and several of the C₄ species suggests that rice leaves may not need to be altered by much. In fact, variation may exist between cultivars, with some possessing closer venation. More of a problem is the lateral cell count being so high. Normally, C₄ acids just have to diffuse into the adjacent cell. With a transformed rice leaf, C₄ acids produced in many of the mesophyll cells would face a tortuous path through numerous cells before reaching a bundle sheath cell where decarboxylation could take place. This is likely to reduce the efficiency of the pathway by requiring a more complex diffusion gradient through the tissue for the C₄ acids moving toward the bundle sheath and the returning flux of pyruvate or alanine.

Rather than trying to reduce the lateral cell count in rice, an alternative strategy may be to attempt to copy the physiology of the submerged monocot *Hydrilla verticillata*. It is possible

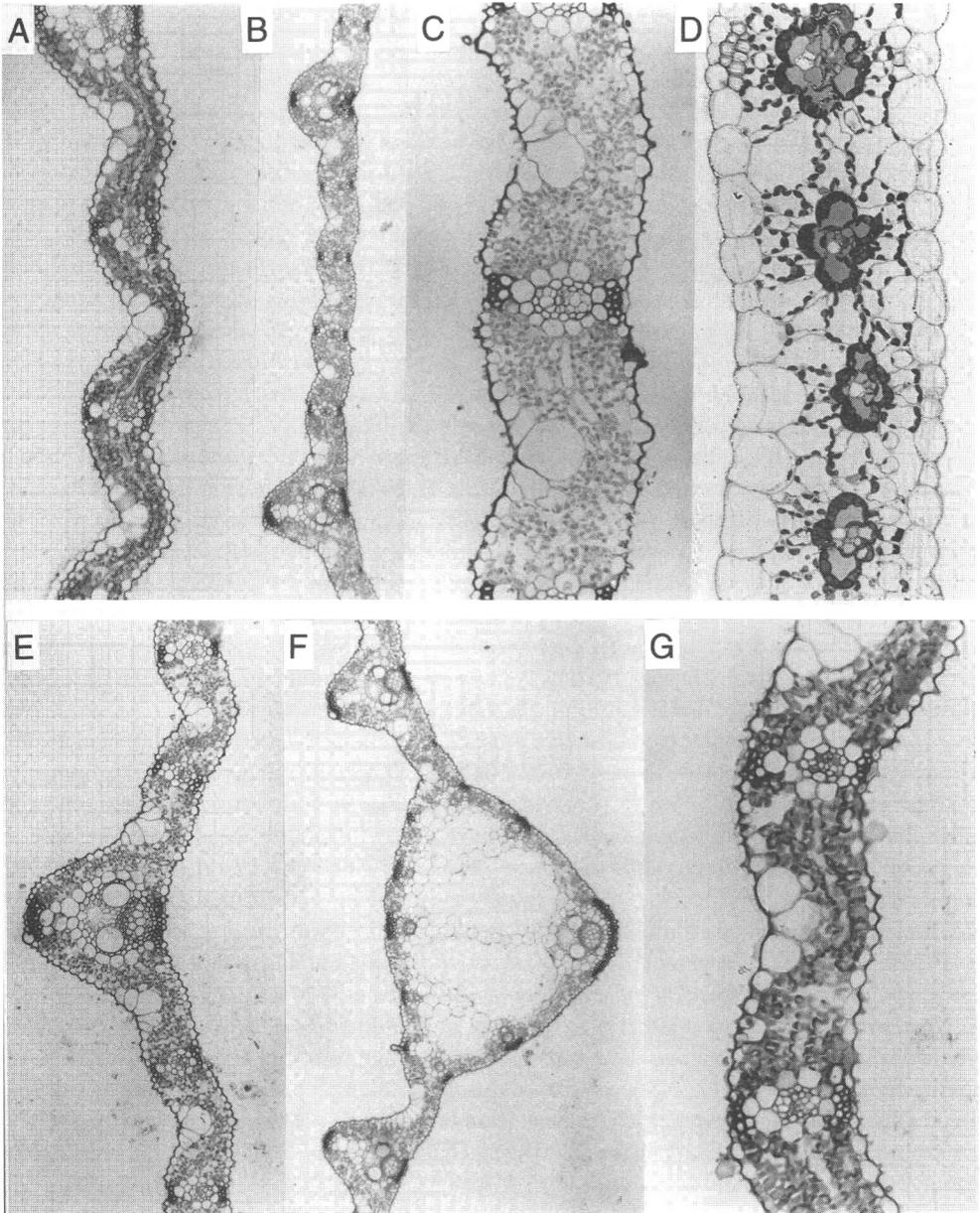


Fig. 6. Light micrographs of transverse sections of *Oryza sativa* and *Zea mays* (D) leaves. Magnifications $\times 100$ B, F; $\times 200$ A, E; $\times 400$ C, D, G. The adaxial surface is to the left.

to induce the C_4 cycle in this plant by culturing it under long photoperiods and high temperatures, which result in limiting dissolved CO_2 concentrations and high O_2 concentrations in the water (Magnin et al 1997). PEP carboxylase, PDK, asp AT, ala AT, and NADP-ME were all induced during a period of acclimation, during which the CO_2 compensation point and O_2 sensitivity of photosynthesis declined. The significant feature of this plant is that the leaf is only two cells

Table 3. Interveinal distance and lateral cell counts for C₃ and C₄ species.

Reference	Interveinal distance (μm)		Lateral cell count	
	C ₃	C ₄	C ₃	C ₄
Morgan and Brown (1979)	120–460	70–180	4.2–13	2–3.2
Bolton and Brown (1980)	145–195 ^a	93–113 ^b		
Dengler et al (1994)	257	115–150		
Hattersley and Watson (1975)			>4	2–4
Parker and Ford (1982)	237, 306 ^c			
This study	157, 209 ^d	100–225 ^e		

^a*Panicum milioides*. ^b*Panicum maximum*. ^c*Triticum monoccocum* and *T. aestivum*. ^d*Oryza sativa*. C₄ species studied by Henderson et al (1992).

thick and the C₄ cycle appears to operate inside every cell. CO₂ in the cytosol is fixed into C₄ acids, which are decarboxylated in the chloroplast by NADP-ME, raising the estimated CO₂ concentration surrounding Rubisco to 400 μM in the C₄-type leaf versus 7 μM for the C₃-type leaf (Reiskind et al 1997). It is not clear what prevents significant leakage of CO₂ from the chloroplast back into the cytosol and hence futile cycling of CO₂. Presumably, carbonic anhydrase activity must be low or absent from the chloroplast but present in the cytosol to reduce bicarbonate leakage out of the chloroplast yet enable PEP carboxylase to function efficiently in the cytosol. The fact that the *Hydrilla* leaf is also submerged imposes a significant diffusion barrier and one that is not available to terrestrial leaves of rice.

In *Hydrilla*, chloroplasts represent only 4% of the cellular volume (Reiskind et al 1997), compared with 16.4% to 19% in mesophyll cells from *Hordeum* (Winter et al 1993), *Spinacea* (Winter et al 1994), and *Solanum* (Leidreiter et al 1995). Because *Hydrilla* is a submerged plant, the chloroplasts in *Hydrilla* cells do not have intercellular airspaces on the other side of their cell walls, which would tend to reduce the rate at which CO₂ could diffuse away. For terrestrial plants, limited access to CO₂ is usually the problem, so their mesophyll chloroplasts are mainly located adjacent to intercellular airspaces. Indeed, in rice (Fig. 6G, Chonan et al 1977) as in wheat (Dunstone and Evans 1974) and other species (Haberlandt 1914), mesophyll cells have numerous lobes that increase their surface area to volume ratio. This increases the surface area adjacent to intercellular airspace available for chloroplasts. However, this characteristic would maximize the chance of CO₂ leakage were the *Hydrilla* C₄ cycle to be engineered into rice leaves. To increase the cytosolic pathlength between the chloroplasts and intercellular airspace to reduce CO₂ leakage, it would be necessary to interfere with the process controlling chloroplast location in the cell. The mechanism for this is not known, but presumably involves the cytoskeleton.

A balancing act

Bearing in mind the possibility of putting C₄ into a single cell, we return to considering the prospect of engineering C₄ with Kranz anatomy. Having functional C₄ metabolism is not just a matter of expressing all of the C₄ enzymes (5 to × depending on the decarboxylation type) in their appropriate cellular and organellar location (3 or 4) at the appropriate level. It may also be necessary to express altered amounts of membrane transporters that enable the rapid diffusion of metabolites within and between cells. Then, to work efficiently, the rates of the C₄ and C₃ cycles have to match one another to either prevent wasteful leakage of CO₂ from the bundle

sheath because of excessive C_4 activity or avoid inadequate C_4 activity, leaving Rubisco starved of CO_2 .

It has been possible to model C_4 photosynthesis to reveal solutions that have many of the observed characteristics of C_4 leaves (Caemmerer and Furbank 1999). Figure 7 presents a diagrammatic representation of the model. Because of the low Michaelis—Menten constant of PEP carboxylase, PEP carboxylation rate increases rapidly with increasing mesophyll CO_2 partial pressures and is likely to operate at nearly its substrate saturated rate. The decarboxylation of the C_4 acids in the bundle sheath increases the CO_2 partial pressure because leakage back to the mesophyll is restricted by a low bundle sheath conductance. The diagrammatic representation in Figure 7 is not drawn to scale because, in reality, bundle sheath CO_2 partial pressures are much greater than that in the mesophyll, to suppress Rubisco oxygenase. The increased partial pressure of CO_2 around Rubisco enables it to react at a greater rate. The interaction among bundle sheath conductance to CO_2 , PEP carboxylation rate, and Rubisco activity determines the partial pressure of CO_2 reached in the bundle sheath and how much leaks back out. For a given PEP carboxylation rate, the greater the bundle sheath conductance, the lower the bundle sheath CO_2 partial pressure and Rubisco carboxylation rate, and the greater the proportion of CO_2 that leaks back out. If the amount of Rubisco is reduced, all else being equal, the Rubisco carboxylation rate declines, so the bundle sheath CO_2 partial pressure and the amount of leakage both increase (Fig. 7).

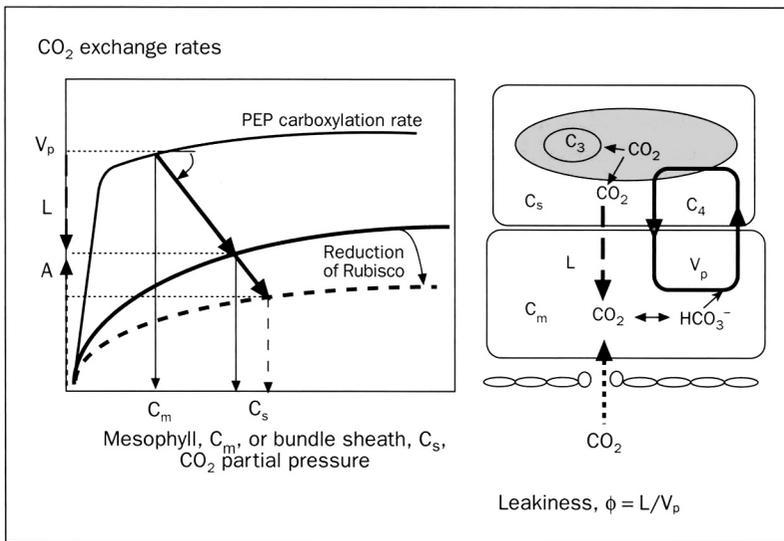


Fig. 7. Diagrammatic representation of the C_4 model. V_p is the phosphoenolpyruvate (PEP) carboxylation rate, L is the rate of leakage of CO_2 from the bundle sheath, A is the rate of CO_2 fixation by Rubisco, g_b is the bundle sheath conductance, C_m and C_s are the CO_2 partial pressures in the mesophyll cytosol and bundle sheath, respectively, and f is leakiness, the proportion of CO_2 fixed by PEP carboxylase that leaks back to the mesophyll. See text for detailed explanation. On the right-hand side, the carbon flows are drawn with respect to cellular location. CO_2 enters through stomata and dissolves in the cytosol of the mesophyll cell. PEP carboxylation draws on this CO_2 and pumps it into the bundle sheath, where it is either fixed in the C_3 cycle or leaks back to the mesophyll, depending on the PEP and Rubisco carboxylation rates and the bundle sheath conductance. If Rubisco activity were to be reduced and PEP carboxylation remained the same, bundle sheath CO_2 partial pressures would rise, which would in turn increase the rate of leakage, which could be seen as an increase in leakiness.

Transformation of *Flaveria bidentis* (an NADP-ME C₄ dicot) with an antisense gene for the RNA of Rubisco produced a revealing phenotype (Caemmerer et al 1997a,b). The amount of Rubisco per unit leaf area was reduced to one-third of that of the wild-type. The response of CO₂ assimilation rate to intercellular CO₂ was unaltered at very low intercellular partial pressures, but reached a CO₂ saturated rate that was 30% less than that of the wild-type. Analysis of carbon isotopic discrimination during photosynthesis enabled the leakiness from the bundle sheath to be calculated. Reduction in the amount of Rubisco led to an increase in the leakiness from 0.24 in the wild-type to 0.37 in the transgenic plants measured under high irradiance, ambient CO₂, and 25 °C. The increase in leakiness (leak rate/PEP carboxylation rate) is consistent with the expectation shown in Figure 7. Previous work with *Sorghum bicolor* (Henderson et al 1992, 1994) revealed that leakiness did not vary over a wide range in irradiance (mean value 0.21), although the rate of CO₂ assimilation did. This revealed that there is normally a considerable degree of regulation between the C₃ and C₄ cycles.

A low conductance to CO₂ diffusion across the bundle sheath is an essential feature of the C₄ pathway. The conductance needs to be low enough to enable CO₂ partial pressures to build up in the bundle sheath, yet not so low as to prevent the escape of oxygen. If oxygen were prevented from diffusing away, the increase in oxygen partial pressure would counter the benefit of concentrating CO₂. In the absence of the C₄ cycle, CO₂ diffuses to Rubisco at very low rates. This was shown by chemical inhibition of PEP carboxylase, which effectively eliminated CO₂ exchange with the normal atmosphere (Jenkins 1989, Brown 1997). The conductance to CO₂ diffusion across the mesophyll bundle sheath interface has been measured for different C₄ species by various techniques. Estimates range from 0.6 to 1.5 mmol m⁻² s⁻¹ on a leaf area basis or 0.5 to 0.9 mmol m⁻² s⁻¹ on an exposed bundle sheath area basis (Jenkins et al 1989, Brown and Byrd 1993). This compares with the internal conductance in C₃ leaves, such as tobacco, of 370 and 25 mmol m⁻² s⁻¹ on the basis of leaf area or exposed chloroplast area, respectively (Evans et al 1994). It has not been possible to measure the conductance across mesophyll cell walls in C₄ plants, but it would appear to be more than twice that of C₃ leaves when expressed per unit mesophyll cell surface exposed to intercellular airspace (Evans and Caemmerer 1996). This could in part reflect that CO₂ only has to reach the cytosol in C₄ mesophyll cells and not diffuse into the chloroplast, or be related to cell wall thickness.

The mechanism behind the 25–50-fold reduction in conductance across the bundle sheath observed in C₄ leaves is not known. Key features likely to be involved are, first, the physical anatomical dimensions of the bundle sheath, which has a limited surface area per unit leaf area—especially compared with mesophyll cells (Evans and Caemmerer 1996). In addition, C₄ leaves generally have no carbonic anhydrase activity in the bundle sheath to minimize facilitated diffusion out of the bundle sheath in the form of bicarbonate (Burnell and Hatch 1988). An attempt to assess the effect of carbonic anhydrase activity in the bundle sheath cells on C₄ function was made with another transformation of *Flaveria bidentis*, in which leaves were produced that had a 2–4-fold increase in carbonic anhydrase activity in the bundle sheath (Caemmerer et al 1997b). Although the leaves had similar Rubisco contents, their rates of CO₂ assimilation were 20% less than that of the wild-type. Measurements of carbon isotope discrimination revealed an increase in discrimination. While this could result from increased leakiness, consistent with expectations, the interpretation is uncertain. This is because assumptions made on the CO₂:bicarbonate equilibrium affect the calculation of leakiness (see Caemmerer et al 1997b for more detail).

Getting the balance right is a formidable challenge at several levels. First, considering light reactions, it is necessary to balance excitation energy between the two photosystems at low irradiance to achieve the maximum quantum yields. The problem is compounded in C₄

leaves because it is necessary to balance energy capture by the mesophyll and bundle sheath cells unless considerable metabolite shuttling takes place. Since the vast bulk of chloroplasts are in the mesophyll cells of rice leaves, it would be necessary to greatly reduce the thylakoid components in the mesophyll and increase them in the bundle sheath. Second, having selected the decarboxylation type and succeeded in getting expression of all the C_4 enzymes at an appropriate level, as well as stopping the expression of Rubisco in the mesophyll and carbonic anhydrase in the bundle sheath, it is necessary to ensure that the C_4 and C_3 cycles operate at appropriate rates with respect to one another. Otherwise, either leakage is excessive or Rubisco is not operating maximally, both of which would result in a photosynthetic system that was no more efficient than the original C_3 starting point or worse.

A much simpler alternative would be to engineer rice expressing a novel Rubisco possessing a lower Γ^* . This would improve both the quantum yield and the rate at high irradiance for a given nitrogen content per unit leaf area (Fig. 8). While there is a correlation between Γ^* and specific activity for Rubisco from green algae and higher plants, this does not appear to hold across more diverse comparisons from red algae (T.J. Andrews, personal communication), which raises the possibility of either discovering or engineering a Rubisco with better kinetic properties. The ratio of the rates of CO_2 assimilation for the two Γ^* types changes, depending on whether the rate of CO_2 assimilation is limited by electron transport rate, as at low irradiance, or Rubisco activity, as at higher irradiances. Another way to look at how a 30% reduction in Γ^* affects quantum yield is that it would be equivalent to a leaf having the 20 °C curve instead of the 30 °C curve in Figure 1. This would result in the quantum yield being close to that of the C_4 leaf at 30 °C. Then, as atmospheric CO_2 concentrations continue to rise, the quantum yield would in fact surpass that of the C_4 leaf. As discussed above in the canopy photosynthesis section, when a canopy has a high LAI, the improvement in quantum yield is more important than that in rate at high irradiance. There would also be an additional benefit to canopy photosynthesis as atmospheric CO_2 concentrations rise. Although the amino acid changes needed to create such a Rubisco are as yet unknown, were they to be discovered, then genetically

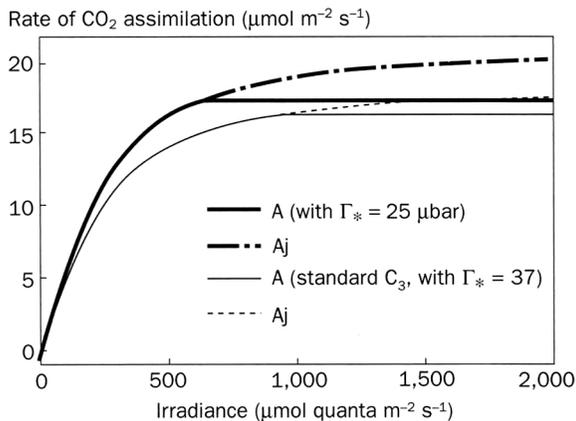


Fig. 8. The relationship between CO_2 assimilation rate and irradiance for two leaves containing Rubisco with different Γ^* values. Parameter values for leaves at 25 °C: V_c 80 $\mu mol CO_2 m^{-2} s^{-1}$, J_m 120 $\mu mol e^{-} m^{-2} s^{-1}$, K_c 404 μbar , K_o 248 mbar, O 200 mbar, C 250 μbar , Θ 0.7, R 1 $\mu mol CO_2 m^{-2} s^{-1}$, and Γ^* 37 or 25 μbar . The irradiance response follows the minimum of the following two equations, which represent RuBP regeneration-limiting and Rubisco-limiting conditions: $J = \left\{ (I + J_m) - \left[(I + J_m)^2 - 4\Theta J_m \right]^{0.5} \right\} / (2\Theta)$ and $A = J(C - \Gamma^*) / (4C + 8\Gamma^*) - R$, or $A = V_c(C - \Gamma^*) / [C + K_c(1 + O/K_o)] - R$.

engineering the novel Rubisco into rice or any other C_3 crop ought to be considerably simpler than the complex task of establishing a functional C_4 metabolic pathway.

Could rice use the extra photosynthate?

The debate about what limits plant growth oscillates between those who argue that carbon gain by photosynthesis sets the potential growth rate and those who argue that it is the sink's ability to grow and use photosynthate that regulates carbon gain. Therefore, if a C_4 rice plant were engineered having a greater potential canopy rate of CO_2 assimilation, would the growth and yield increase? One way to address this question is to look at the situation where rice has been grown under elevated atmospheric CO_2 . While this does not eliminate photorespiration, it provides an analogy for what might be expected for a C_4 -like plant.

Numerous reports mention rice that has been grown under elevated atmospheric CO_2 , but the outcome can be illustrated with a select few. Imai et al (1985) grew rice under 350 and 700 $\mu L L^{-1} CO_2$. They showed that elevated CO_2 led to earlier flowering (another potential benefit of creating C_4 photosynthesis that cannot be covered here) and increased biomass at tillering (94%), ripening (51%), and grain yield (39%). Baker et al (1990), using paddy culture in naturally lit controlled environment chambers with six CO_2 treatments ranging from 160 to 900 $\mu L L^{-1} CO_2$, found that, as atmospheric CO_2 concentration increased, tillering, leaf area, biomass, and number of panicles increased. The grain yield in the 660 versus the 330 $\mu L L^{-1} CO_2$ treatment increased by 32%. Ziska and Teramura (1992) grew two rice cultivars under 360 or 660 $\mu L L^{-1} CO_2$ for 113 d and also found that the increased CO_2 level increased biomass in the two cultivars by 27% and 34%, respectively. Gas exchange measurements revealed that CO_2 assimilation rate increased by 50% in the elevated CO_2 treatment. Although this was offset by a decrease in leaf area per unit leaf dry mass, it demonstrates that, if the rates of CO_2 assimilation per unit leaf area can be increased in rice plants, enhanced growth will follow.

One final note of caution. The harvested grain contains a significant amount of protein and hence nitrogen. When yields are increased, it will be necessary to ensure that the protein content is maintained. This is especially important for rice, which already has the lowest grain protein content among the cereals. If canopy nitrogen contents were not increased as yield increased, a larger fraction of plant nitrogen would need to be remobilized into the grain than at present just to maintain current grain protein content (Feil 1997). Alternatively, canopy nitrogen contents need to rise. This could happen by either increasing nitrogen contents per unit leaf area or increasing LAI. Because C_4 leaves generally have less nitrogen per unit leaf area than C_3 leaves, the former seems unlikely. The prospect of having crops with an LAI much greater than 10 is also daunting, as this would require an even more erect habit than is currently achieved (Sinclair and Sheehy 1999). Given that CO_2 assimilation rate per unit nitrogen is greater in C_4 leaves, this implies that a different balance would be expected for grain yield and grain nitrogen in C_4 versus C_3 crops. However, as they tend not to be grown in the same environments, paired comparisons of nitrogen harvest index between the C_4 cereals (maize, sorghum, and millet) and rice are not made easily.

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Will increased photosynthetic efficiency lead to increased yield in rice?

T.D. Sharkey, M.M. Laporte, and E.L. Kruger

Plant mass is primarily derived from photosynthesis and so it is surprising that final plant mass (yield) and photosynthetic rate of leaves are often not well correlated. The rate of plant respiration and loss of plant matter through detachment also influence yield. The lack of correlation also reflects the fact that photosynthate availability is just one of many signals that affect plant growth and development. Plants grown in elevated carbon dioxide normally have increased yield, which tells us that increasing the availability of photosynthate is likely to increase yield, though perhaps not as much as might be expected. In redesigning photosynthesis for increased yield, we can focus on the inputs, fundamental mechanisms, or outputs. Given the importance of the relationship between photosynthesis and plant growth, this chapter focuses on the outputs of photosynthesis and their immediate use, especially the enzyme sucrose-phosphate synthase (SPS). Plants that are transformed to express more sucrose-phosphate synthase sometimes have higher yields than untransformed plants. Two hypotheses were tested to explain this variability in response: (1) Does expression of the gene in nonphotosynthetic tissue affect yield? (2) Is there an optimum level of SPS that should be sought? It was found that expression in nonphotosynthetic tissue was not important but that there was an optimum level of SPS activity. Too much or too little of this enzyme results in lowered yield and it may be that most plants have a level appropriate to the preindustrial atmospheric carbon dioxide concentration or a level that results in a more conservative strategy than is required for crop plants.

It is an exciting time in biology when the conversion of a C_3 species to a C_4 species can be seriously considered. Genetically engineering single-gene traits into plants is now commonplace and genetically engineering multigenic traits can now be considered. In the case before us, the consideration is whether rice genetically engineered to carry out C_4 photosynthesis would be superior to the current C_3 rice. There are several reasons to consider rice for this change: (1) it normally grows in high temperature, under which photorespiration is a significant problem; (2) it is a grass and therefore may be more amenable to the C_4 syndrome than a dicot. One factor that is weighted against an effort to engineer a C_4 rice is the rising global atmospheric CO_2 concentration, which could eventually favor C_3 over C_4 metabolism. There is a risk that the advantages of C_4 metabolism would be achieved without genetic engineering, simply as a result of the increasing atmospheric CO_2 concentration. This risk depends on the rate of progress in engineering the C_4 syndrome versus the rate of CO_2 increase in the atmosphere. Studies of rice grown at high CO_2 may help us predict the effect of making rice C_4 .

What advantages are expected in a C₄ rice? Plants with C₄ metabolism normally have higher rates of photosynthesis and lower amounts of protein per square meter of leaf. These changes result from metabolism that uses energy to concentrate CO₂. It is generally assumed that increasing photosynthetic rates will increase yield since a large fraction (>80%) of plant dry matter is derived from carbon fixed by photosynthesis. However, higher rates of leaf photosynthesis are not always linked to higher biomass accumulation or grain yield. Evans (1994) describes what he calls the “photosynthetic paradox” that increased yield is often negatively correlated with the leaf area-based photosynthetic rate. This paradox can be explained to some degree by correlations between area-based photosynthetic rate and leaf size or leaf longevity (Evans 1994). What is in fact expected is a correlation between whole-plant photosynthetic rate, excluding root respiration, and the relative growth rate of plants (including the roots). Several reports of correlations between whole-plant photosynthetic rate per gram of plant and relative growth rate have been reported (Farquhar and Sharkey 1994) and Figure 1 shows a recent example.

Students studying ecophysiology at the University of Wisconsin-Madison undertook an experiment. Four plant species, *Zea mays*, *Phaseolus vulgaris*, *Ricinus communis*, and *Helianthus annuus*, were grown in relatively warm (28 °C day) or cool (20 °C day) conditions, in full sun or 50% shade. The relative growth rate (RGR) between 5 and 9 wk after planting was a function of whole-plant photosynthesis with a coefficient of determination (r^2) of 0.92 for plants in the cool condition and 0.86 for plants in the warm condition. The correlation with either area-based photosynthetic rate or mass-based photosynthetic rate was much lower. The lower RGR at high temperature could reflect respiration.

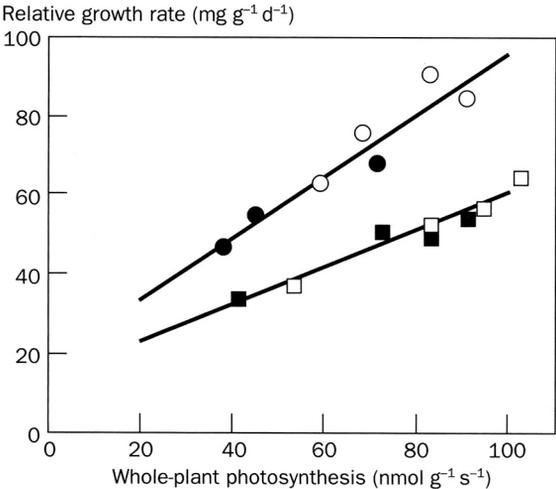


Fig. 1. Relative growth rate as a function of whole-plant photosynthesis rate per gram of plant. Whole-plant photosynthesis was determined as the net photosynthetic rate measured for individual leaves multiplied by the total leaf area for the plant divided by the weight of the plant. Four plant species, *Zea mays*, *Phaseolus vulgaris*, *Ricinus communis*, and *Helianthus annuus*, were grown in relatively warm (28 °C day) or cool (20 °C day) air-conditioned greenhouses in the University of Wisconsin-Madison biotron in full sun or 50% shade. The relative growth rate between 4 and 8 wk was determined from dry weights of harvested plants using both roots and shoots. Circles are data from cool conditions, squares from warm conditions, open symbols from full sun, and filled symbols from 50% shaded conditions.

Plant mass at the end of the experiment depended on light level and was not affected by temperature (Fig. 2), unlike RGR. Part of this paradox can be explained by much faster growth soon after germination, so that the plants in the warm environment got a much quicker start. Similarly, plant growth before 5 wk allowed plants in high light to get established and then a similar subsequent RGR produced a much bigger plant in high light than in low light. The advantage of this early growth was maintained throughout the growth of the plants. Because of these considerations, the data cannot be construed to mean that the growth rate was lower in high temperature, only the relative growth rate evaluated between 5 and 9 wk. Thus, phenological considerations play a large role in determining yield. The allocation of resources to increased leaf area, roots, reproductive structures, etc., has a large effect on the trajectory of whole-plant photosynthetic rate throughout the life cycle of the plant.

A full analysis of the factors that determine allocation to roots, shoots, and fruits is still beyond our capability. However, experiments have been reported that take the first steps toward controlling partitioning of photosynthate by genetic modification. The partitioning of recent photosynthate among sucrose, transitory starch, and amino acids has been called primary partitioning to distinguish it from partitioning of resources among roots, shoots, and fruits (Lunn and Hatch 1995). Substantial variation exists among plants in the ratio of starch to sucrose during primary partitioning. For example, spinach leaves store significant quantities of sucrose in their vacuoles before starch synthesis begins (Gerhardt et al 1987, Servaites et al 1989). The mechanism of control of primary partitioning has been investigated and some of the strong control points are regulation of cytosolic fructose bisphosphatase (FBPase), chloroplastic FBPase, adenosine 5'-diphosphate (ADP)-glucose pyrophosphorylase, and sucrose-phosphate synthase (SPS) (Fig. 3, Herzog et al 1984, Heldt and Stitt 1987, Stitt and Quick 1989, Neuhaus and Stitt 1990, Stitt 1990, 1994). Understanding primary partitioning is a useful step toward understanding how to modify how plants partition additional photosynthate that becomes available regardless of whether increased atmospheric CO₂ or C₄ metabolism causes the increased photosynthate.

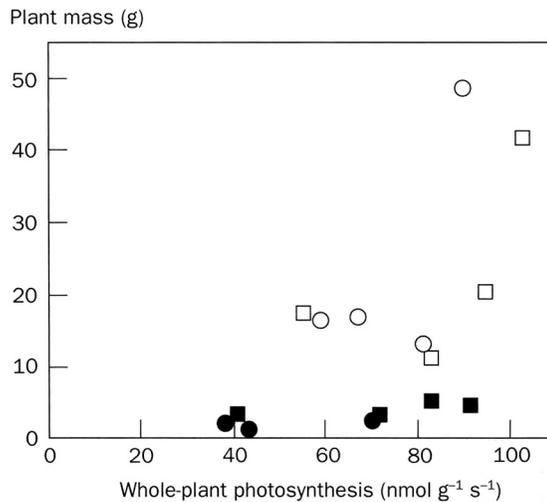


Fig. 2. Total plant mass after 10 wk of growth versus whole-plant photosynthesis rate per gram of plant at 40 d after planting. Conditions and symbols as in Figure 1.

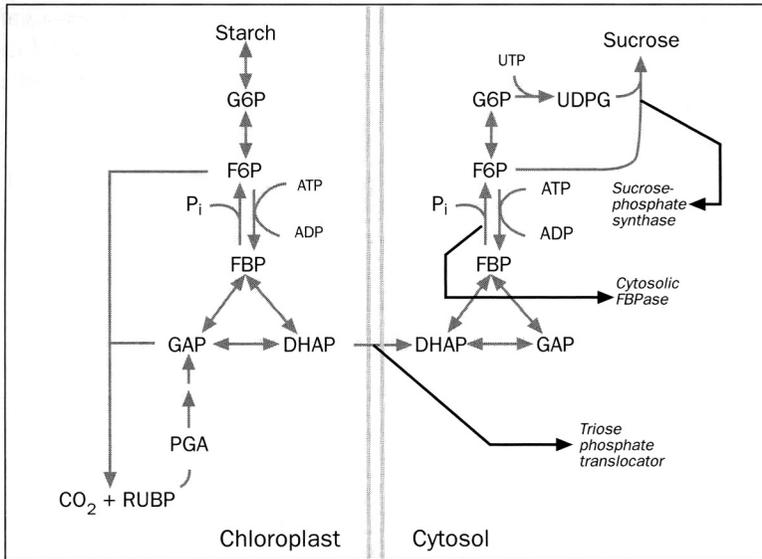


Fig. 3. Carbon export from chloroplasts. ADP = adenosine 5'-diphosphate, ATP = adenosine triphosphate, DHAP = dihydroxyacetone phosphate, FBP = cytosolic fructose bisphosphatase, F6P = fructose 6-phosphate, G6P = glucose 6-phosphate, GAP = glyceraldehyde 3-phosphate, PGA = 3-phosphoglycerate, UDPG = uridine 5-diphosphate glucose.

Using studies on elevated CO₂ to predict yields of C₄ rice

To answer how rice will respond to conversion to C₄ metabolism, we can first assume that the effect of the conversion will be to make photosynthesis more efficient. The overall effect should be similar to growing C₃ rice in elevated CO₂. Rice grown in elevated CO₂ exhibits higher yield than rice grown at normal levels of CO₂ (Rowland-Bamford et al 1991, Nakano et al 1997). This effect is perhaps the most direct demonstration that increased photosynthesis does lead to increased yield (Farquhar and Sharkey 1994). However, the effect of increased CO₂ on yield may depend on changes in development that occur within 2 wk of planting (Jilta et al 1997), analogous to the effects of growth in warm temperature or high light described above (Nakano et al 1997). The effect of increased CO₂ has been modeled based on the partitioning of fixed carbon throughout the plant's life (Boote and Tollenaar 1994).

The increase in yield caused by growth at high CO₂ is not as substantial as might be predicted. Because the capacity for plant growth depends on the amount of leaf area, and leaf area depends on photosynthesis, plant growth can increase exponentially. If elevated CO₂ increases photosynthesis by 60% (Vu et al 1997) and half of that increase is used to stimulate growth, then the relative growth rate should increase by 30%. For rice, the RGR can be 0.10g g⁻¹ d⁻¹ (Makino et al 1997a) so that under elevated CO₂ an RGR of 0.13 g g⁻¹ d⁻¹ should be observed. After 1 d, this increased RGR should stimulate biomass accumulation by 32%, but after 1 wk the increase should be 46% and after 1 mo 154% because of the exponential nature of growth. Rice shoot biomass was increased by 43% by growth in 100 Pa CO₂ relative to 36 Pa (Makino et al 1997a), whereas grain yield was increased by 57% by growth at 70 Pa relative to 36 Pa (Jilta et al 1997) in two recent typical reports.

Thus, the increases in the yield of rice grown in elevated CO₂ are modest relative to the increased photosynthesis rate. In addition, yield increases occur as the CO₂ concentration is

raised to 50 Pa but not beyond (Baker et al 1990, Rowland-Bamford et al 1991). Instantaneous rates of photosynthesis increase with CO₂ increases beyond 50 Pa, but yield often does not, indicating a limit to how far increased photosynthetic rates alone can increase yield. The increase in yield that can be caused by stimulating photosynthetic rate varies among species and with environmental conditions. Nevertheless, from studies on elevated CO₂, it is reasonable to guess that conversion of rice to C₄ metabolism could increase yield up to 50%. However, much of that increase in yield might be expected to occur without engineering C₄ metabolism into rice as a result of increased global CO₂ concentration.

It is most unlikely that conversion to C₄ and increasing CO₂ will produce additive effects for two reasons. First, conversion to C₄ converts to a metabolism nearly saturated by current levels of CO₂. Second, the effect of increased photosynthesis on yield is constrained by the metabolism and physiology of the rest of the plant.

Modifying plant physiology to take advantage of additional photosynthate

Additional genetic modifications are likely to be needed if higher yield increases are to be realized. One of the modifications that has shown promise is increasing the capacity for sucrose synthesis during the day. This shifts carbon partitioning and has allowed substantial increases in yields of tomatoes, even without increased rates of photosynthesis. Hussain et al (1999) have argued that changes in carbon partitioning brought about by changes in SPS activity may be important in adaptation of rice to increased availability, of photosynthate. Similarly, Nakano et al (1997) showed that increasing nitrogen nutrition increases SPS activity. SPS activity increased by 10% to 20% by growth at 66 Pa CO₂, whereas increasing leaf nitrogen content fourfold increased SPS activity by twofold.

Will this increase in SPS activity be enough to handle the increased flux of photosynthate? There is evidence to suggest that it will not. For example, Nakano et al (1997) found that the doubling of SPS activity happened over a range of leaf nitrogen contents that led to a fourfold increase in Rubisco activity. It is likely that genetically engineering increased SPS activity will allow a C₄ rice to take better advantage of the increased availability of photosynthate. Work on this transformation has the advantage that it is likely, to be useful whether the increased photosynthesis comes about as a result of changing rice to C₄ or by increases in atmospheric CO₂ concentration making C₃ metabolism more efficient.

Manipulation of SPS is a first step between manipulation of photosynthesis and manipulation of the physiology of the rest of the plant. It is a way to affect the interface between photosynthesis and the plant.

Transitory starch

Photosynthesis in plants takes place in the chloroplast, believed to have developed as a result of an endosymbiosis (or several endosymbioses). Thus, plants can be viewed as a combination of the autotrophic plastids and the heterotrophic cytosol. Coordination of photosynthesis and heterotrophic processes such as growth is critical to the success of the plant. Photosynthesis allows chloroplasts to provide reduced carbon to the rest of the plant during the day. In addition, chloroplasts store reduced carbon as starch during the day and break it down at night so that chloroplasts supply reduced carbon at night as well. The starch that builds up and breaks down daily is called transitory starch, to distinguish it from starch that accumulates in storage organs such as the rice grain or potato tubers. In C₄ plants, there is the additional complication of starch accumulating in mesophyll cell chloroplasts or bundle sheath cell chloroplasts. In *Zea*

mays, starch accumulates preferentially in mesophyll cells even though carbon fixation occurs in the bundle sheath cells (Lunn and Furbank 1997). How this is controlled and what advantages there are to one location versus the other for transitory starch accumulation are unclear.

Carbon export during the day

Daytime export of reduced carbon from chloroplasts is relatively well understood. Walker and his colleagues showed that the chloroplast produces triose phosphate, which exchanges for phosphate from the surroundings during photosynthesis (Fig. 3. Walker and Herold 1977). Heldt and colleagues discovered the triose phosphate translocator. This antiporter exchanges phosphate, triose phosphates, and 3-phosphoglycerate (PGA) across the inner membrane of the chloroplast envelope. Metabolism in the chloroplast and cytosol establishes a gradient that causes dihydroxyacetone phosphate (DHAP) from the chloroplast to exchange for phosphate from the cytosol. The triose phosphate translocator has been cloned and sequenced and antisense plants have been made to study its role in regulating photosynthesis (Riesmeier et al 1993, Heineke et al 1994, Hausler et al 1998).

Nighttime carbon export

Nighttime export of reduced carbon from the transitory starch reserves is not as well understood as daytime export. The pathway of transitory starch breakdown is unknown. Export of carbon from chloroplasts at night appears to be fundamentally different from daytime export. One common interpretation of observations made with mutants and transgenic plants, for example, plants lacking cytosolic FBPase (Sharkey et al 1992, Zrenner et al 1996), is that carbon is exported as hexose at night. It has been known for some time that chloroplasts can take up glucose (Schafer et al 1977) and also maltose (Herold et al 1981). With either glucose or maltose export, both the triose phosphate translocator and the cytosolic FBPase are bypassed.

Since the cytosolic FBPase is an important regulatory step, it could be that the hexose export pathway is suppressed in normal plants and is only used when the triose phosphate export pathway is blocked. To test this, nuclear magnetic resonance (NMR) was used to examine the incorporation of deuterium from deuterium-enriched water into glucose used to make sucrose at night. The experiments showed that, in tomato and bean, at least 75% and possibly all carbon converted from starch to sucrose at night is never broken down to the level of a triose phosphate (Schleucher et al 1998). Thus, the hexose export pathway is the normal pathway for carbon export from leaves at night.

Plastids in starch-accumulating organs have a hexose phosphate transporter but chloroplasts of leaves do not (Kammerer et al 1998) so hexose-phosphate export appears ruled out. It is unclear, however, whether export at night occurs as free hexose (glucose), maltose, or possibly even a higher maltodextrin (but these are probably ruled out by the low permeability of the chloroplast envelope for maltotriose and higher maltodextrins) (Rost et al 1996). In addition, this hexose pathway must not operate during the day, accounting for the shift to nighttime export in antisense and mutant plants lacking cytosolic FBPase. Daytime operation of this pathway would bypass all of the regulation at cytosolic FBPase.

Altering daytime versus nighttime export of carbon

Substantial variation occurs in partitioning of newly fixed carbon between sucrose and starch synthesis among plants. Some plants, such as barley, accumulate substantial amounts of sucrose and make relatively little transitory starch (Sicher et al 1981). Other plants can make substantial amounts of starch, especially when photosynthetic rates are high as a result of either high light or high CO₂ (e.g., *Phaseolus vulgaris*) (Sharkey et al 1985). Rice appears to make relatively

more sucrose than starch (Nakano et al 1997, Hussain et al 1999). The amount of transitory starch made each day by plants is subject to environmental controls (Chatterton and Silvius 1979) and is often larger in plants that are adapted to life near the equator, where daylengths during the growing season are shorter than in more northern and southern latitudes.

Changes in partitioning of newly fixed carbon between starch and sucrose synthesis are thought to be controlled by cytosolic reactions (Stitt and Quick 1989). In particular, the activity of SPS could be an important determinant of this partitioning, and this would regulate how much carbon is exported during the day and how much at night. Substantial changes in partitioning can occur without affecting the net rate of photosynthesis (Kruckeberg et al 1989), but under some conditions photosynthesis can be limited by the total capacity for starch plus sucrose synthesis (Sharkey 1990), especially when both capacities are reduced through antisense technology (Hattenbach et al 1997).

Partitioning within the leaf can be modified by modifying starch or sucrose synthesis. If starch synthesis is halted, more photosynthate is directed toward sucrose synthesis (Leidreiter et al 1995). If sucrose synthesis is inhibited, more carbon is diverted to starch synthesis (Heineke et al 1994, Zrenner et al 1996). Mutant plants lacking cytosolic FBPase divert all of their carbon fixed in the light to starch (Sharkey et al 1992). These plants and other plants lacking enzymes needed only for the triose phosphate export of carbon (specifically the triose phosphate translocator and cytosolic FBPase) reduce daytime export and increase nighttime export of carbon from photosynthesizing leaves (Sharkey et al 1992, Heineke et al 1994, Zrenner et al 1996).

The limitation on photosynthesis caused by limited capacity for starch and sucrose synthesis is observed as a loss of photosynthetic response to both oxygen and CO₂ (Sharkey 1985) and decarboxylation of Rubisco (Sharkey 1990). Rice grown with high nitrogen nutrition has a high ratio of Rubisco to SPS and exhibits decarboxylation at high CO₂ (Nakano et al 1997), indicating that rice may already be limited by SPS activity to some degree.

Increasing yield by increasing sucrose-phosphate synthase

Sucrose-phosphate synthase was selected for modification to see if the interaction between the chloroplast and the rest of the plant could be modified to increase yield. Worrel et al (1991) reported on tomatoes transformed with a gene for *Zea mays* SPS with a Rubisco small subunit promoter (SSU-SPS plants). In most cases, these plants did not have increased yields (Galtier et al 1993, Laporte et al 1997, Signora et al 1998), though, in one case, substantially increased yields were observed (Micallef et al 1995). In all cases, area-based photosynthetic rates were the same or only marginally increased at elevated CO₂, indicating that the extra SPS modified how the plant handled photosynthate rather than increased the immediate availability of photosynthate.

A second series of transformants was made in which the SPS gene was expressed on a cauliflower mosaic virus 35S promoter (35S-SPS plants). These plants did have higher yields than the controls, often by a significant amount (Table 1, Laporte et al 1997, Galtier et al 1995). Sometimes a small reduction in root growth was reported and often there was an increase in harvest index (fruit/plant dry mass, e.g., Table 1). It was unclear what aspect of the 35S promoter allowed the dramatic increase in yield. One possibility is that increased expression of SPS in tissues other than leaves caused the increased yield in 35S-SPS plants relative to SSU-SPS plants. Nguyen-Quoc et al (1999) showed that SSU-SPS plants had more SPS in fruits but not in roots, whereas Laporte et al (1997) showed that 35S-SPS plants had more SPS in roots but not in fruits. Plants transformed with 35S-SPS had about twice the SPS activity in leaves.

whereas SSU-SPS plants had more than four times more SPS activity in leaves than control plants did (Table 2).

Because the SSU-SPS plants did not outyield the control plants. SPS expression in fruits must not be important for yield, though the fruits do have increased sugars (Nguyen-Quoc et al 1999). On the other hand, it was not possible to determine whether increased SPS in the roots caused increased yields. To test this, M.M. Laporte and T.D. Sharkey (unpublished) grafted 35S-SPS transformed shoots onto untransformed roots and vice versa. Plants with transformed shoots had higher yields than plants with untransformed shoots regardless of the rootstock (Fig. 4). Thus, SPS expression in the roots cannot explain the higher yields of the 35S-SPS plants.

The next hypothesis was that there is an optimum SPS expression level. Many plants have a lower than optimum expression, which may be important in the rare year with very unfavorable weather but which limits yield under agronomic conditions. However, if SPS expression is boosted too high, the plants suffer from unbalanced partitioning. To test this hypothesis, Laporte and Sharkey (unpublished) used transformed plants that were backcrossed to untransformed plants twice, then selfed to create a segregating population containing zero, one, or two copies of the 35S-SPS gene or the SSU-SPS gene. From this population, 87 plants were grown to maturity in field conditions. The plants were grouped into normal, medium, and high SPS expression groups based on measurements of SPS activity made at two stages during the season. Plants in the medium category had a significantly increased yield over both the high and low SPS plants regardless of the promoter (Fig. 5). From this it can be concluded that there is an optimum SPS activity and that the optimum is higher than that found in commercial tomato cultivars. The decline in yield in high SPS plants was attributed to reduced partitioning to amino acid synthesis (Fig. 6). In high CO₂ or in a C₄ rice, the requirement for amino acids for photosynthetic proteins may be reduced and so the reduced partitioning to amino acids may be less of a problem.

Table 1. Wild-type and transformed tomato plant yield. Plants were grown in a growth chamber and harvested after 18 wk of growth. The increases in fruit dry mass, whole-plant dry mass, and shoot-root ratio of the 35S plants relative to wild-type plants were significant at the 5% level (means \pm S.E.; n = 5–10).

Yield components	Wild-type	35S-13-2
Fruit dry mass (g)	52 \pm 15	91 \pm 14
Root dry mass (g)	10 \pm 2	10 \pm 1
Whole-plant dry mass (g)	240 \pm 15	305 \pm 20
Harvest index (fruit/plant dry mass)	0.22	0.30
Shoot-root ratio	10 \pm 1	14 \pm 1

Table 2. Sucrose-phosphate synthase (SPS) activity in control plants and plants transformed with 35S- or SSU-promoted SPS.

	Wild-type	35S-13-2	SSU-9-13-13
SPS activity ($\mu\text{mol sucrose m}^{-2}\text{s}^{-1}$)	3.8 \pm 0.9	8.1 \pm 1.4	18.7 \pm 1.8

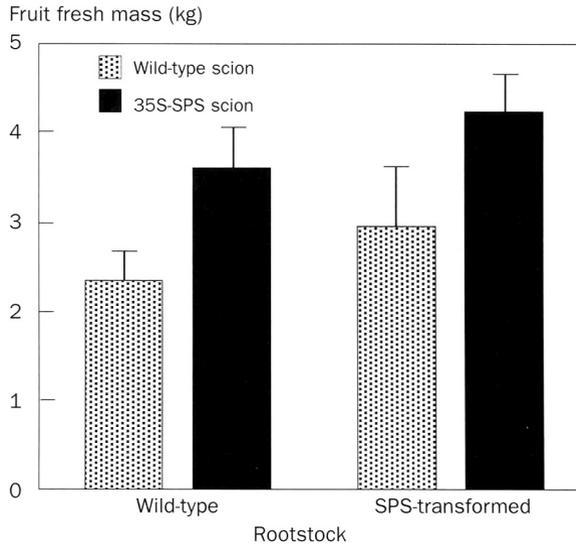


Fig. 4. Effect of sucrose-phosphate synthase (SPS) expression in roots versus shoots on yield. Reciprocal hypocotyl wedge grafts of 13 to 15 wild-type and 35S-SPS transformed plants were made using 6-wk-old plants grown in low light. An equal number of plants was grafted back onto rootstocks of the same genotype. Unpublished data of M.M. Laporte and T.D. Sharkey.

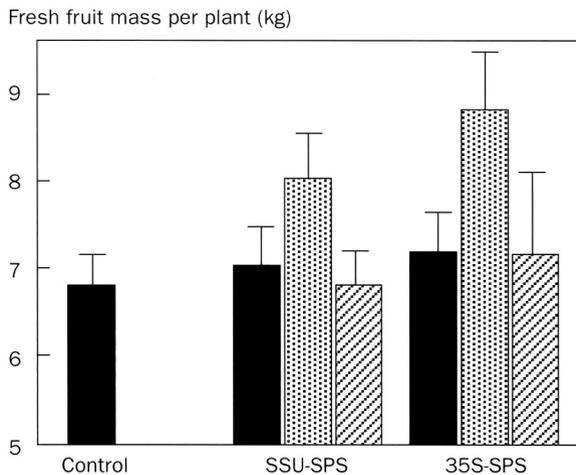


Fig. 5. Effect of promoter and sucrose-phosphate synthase (SPS) activity on yield of tomatoes. Plants ($n = 87$) of segregating populations were grouped into normal (■, av $3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ SPS activity, 1.8 low and 4.6 high), high (▨, av $6.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ SPS activity, 5.0 low and 9.6 high), and very high (▩, av $15.3 \mu\text{mol m}^{-2} \text{s}^{-1}$ SPS activity, 10.1 low and 21.9 high). Data for both promoters include plants derived from two different gene insertion events; no differences between the lines were observed. Unpublished data of M.M. Laporte and T.D. Sharkey.

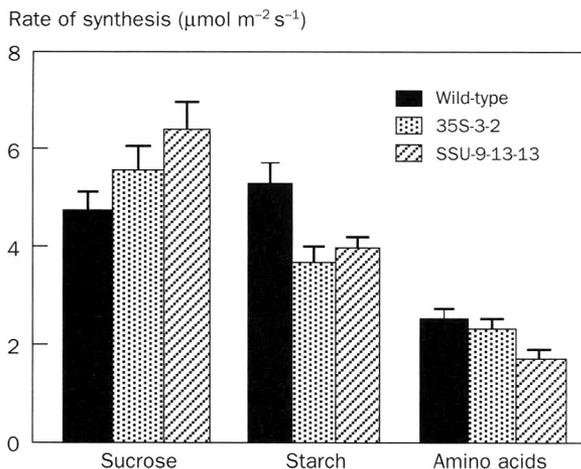


Fig. 6. Partitioning of recent photosynthate determined by feeding $^{14}\text{CO}_2$. The fraction labeled amino acids includes all of the ionic products. Radioactive CO_2 was fed for 10 min at 36 Pa. A 5-min chase followed. Unpublished data of M.M. Laporte and T.D. Sharkey.

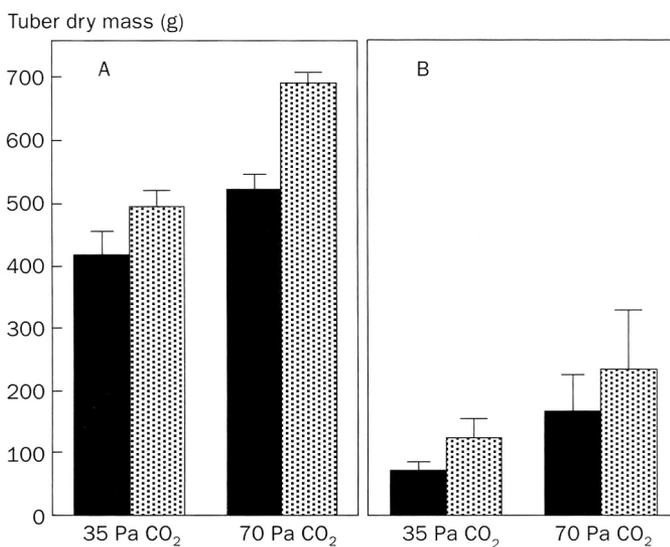


Fig. 7. Tuber dry mass for regenerated control (■) and 3812-24 transgenic (▨) potatoes at 35 and 70 Pa carbon dioxide in (A) high-light growth chambers and (B) open-top chambers in the field. Unpublished data of M.M. Laporte and T.D. Sharkey.

Finally, are the yield enhancements caused by increased photosynthate (e.g., C_4 and its surrogate, high CO_2) and increased SPS additive? In potato, the SSU-SPS transformation increased yield and elevated CO_2 increased yield even further (Fig. 7), indicating that these effects may be additive.

Sucrose-phosphate synthase of rice

Valdez-Alarcón et al (1996) reported that rice has a single gene for SPS, which they cloned and called *sps1*. The promoter had no obvious control regions. The first 700 amino acids had high homology with SPS genes from other plants, but the C terminus was variable. They found no expression of SPS in roots.

The activity of SPS in leaves is correlated with the leaf expansion rate (Seneweera et al 1995). SPS activity of rice does respond to growth in elevated CO₂ (Hussain et al 1999), but the changes in activity are modest. In some cases, the changes in SPS activity caused by elevated CO₂ can be attributed to changes in leaf nitrogen content (Nakano et al 1997). Since both C₄ metabolism and elevated CO₂ reduce the need for nitrogen for Rubisco, plants might be able to partition resources away from Rubisco and toward other enzymes in either the C₄ situation or elevated CO₂. Makino et al (1997b), however, showed that reducing the nitrogen used by Rubisco using antisense technology increased photosynthetic rates at elevated CO₂ by allowing the plant to invest more nitrogen into chlorophyll, cytochrome *f* complex, and the coupling factor. It is reasonable to presume that SPS was also increased in these plants, especially in light of the results of Nakano et al (1997). However, increasing the leaf nitrogen content by fourfold increased Rubisco, chlorophyll, and cytochrome *f* more than SPS, resulting in reduced CO₂ sensitivity at high leaf nitrogen. These results indicate that manipulation of SPS could prove a fruitful way of increasing the yield of rice under conditions of high photosynthate availability, whether that high photosynthate availability is caused by introducing C₄ metabolism, increasing atmospheric CO₂ concentration, or simply improved agronomic practices.

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C₄ rice: What are the lessons from developmental and molecular studies?

W.C. Taylor

The unique features of C₄ leaf anatomy contribute to the photosynthetic and physiological properties of C₄ plants. Our current state of knowledge of the developmental program of C₄ leaves is reviewed. Adding C₄ morphological features to rice leaves will depend on the isolation of genes controlling these traits. Strategies for isolating genes controlling C₄ leaf anatomy, the differentiation of the two photosynthetic cell types, and cell-specific gene expression are discussed. Genes encoding C₄ enzymes are regulated by a diverse set of mechanisms. Although several C₄ genes have been expressed at high levels in transgenic C₃ plants, including rice, at least one C₄ gene is not expressed in C₃ plants.

Morphological features of C₄ leaves

In terrestrial plants, the C₄ pathway of photosynthesis relies on the metabolic cooperation of two photosynthetically active cell types. Mesophyll cells (MC) and bundle sheath cells (BSC) are morphologically distinct and have different biochemical activities. The compartmentalization of the photosynthetic carbon reduction cycle in BSC is a key feature of C₄ plants. The unique features of C₄ leaf anatomy contribute to the photosynthetic and physiological properties of C₄ plants. These features are described for a typical NADP malic enzyme (NADP-ME)-type C₄ plant such as maize. Recent reviews provide more detailed descriptions of C₄ leaf morphology and the extent of variation in different species (Dengler and Nelson 1999, Dengler and Taylor 2000).

- Vein spacing: The most obvious difference between leaves of C₃ and C₄ plants is that the latter are highly vascularized. The maize leaf in Figure 1 shows the typical C₄ pattern of closely spaced veins separated by only two BSC and two MC. In contrast, veins are much more distantly spaced in rice leaves (Fig. 2). In their examination of a wide range of C₄ grasses, Sinha and Kellogg (1996) found that vein spacing was the one consistent characteristic across all evolutionary lineages.
- BSC size: C₄ plants commonly have large BSC, reflecting an increased number of chloroplasts.
- Spatial organization of BSC and MC: Typical C₄ leaves display what is termed “Kranz anatomy,” in which each vein is surrounded by a ring of BSC and in turn by a ring of MC. BSC are tightly packed against the vein, adjacent BSC, and MC such that maximal contact occurs between BSC and MC and only the outer ring of MC is in contact with air.

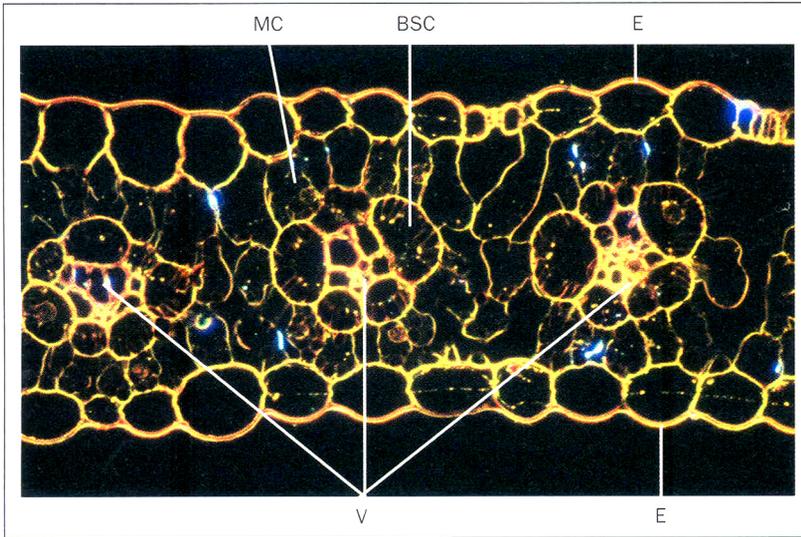


Fig. 1. Cross section of a maize leaf showing typical Kranz anatomy. Tissue was stained with toluidine blue after fixation and sectioning and then photographed under dark field illumination. BSC = bundle sheath cell, E = epidermis, MC = mesophyll cell, V = vein.

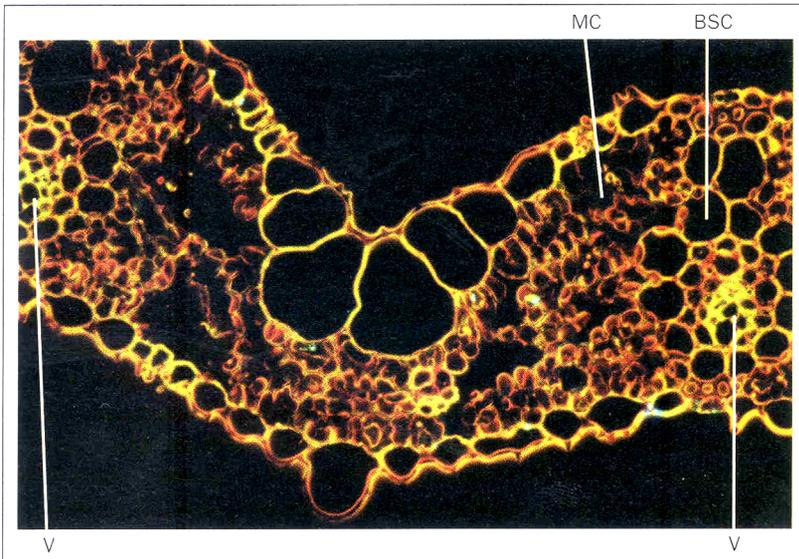


Fig. 2. Cross section of a rice leaf. Tissue was stained with toluidine blue after fixation and sectioning and then photographed under dark field illumination. MC = mesophyll cell, BSC = bundle sheath cell, V = vein.

- MC/BSC interface: Cell walls of BSC are modified in some C_4 species by the addition of a suberin lamella that is thought to impede the leakage of CO_2 out of BSC. The number and size of plasmodesmata connecting BSC and MC are increased over those of C_3 plants to aid in the symplastic diffusion of metabolites (Hatch 1987).

- Differentiation of organelles: BSC have large numbers of chloroplasts. Depending on the C_4 type, BSC chloroplasts may be agranal and may occupy positions adjacent to MC or closer to the vein (Hatch 1987). Maize BSC chloroplasts are organized in a centrifugal position (Fig. 1). The chloroplast position in BSC may reflect different strategies to minimize CO_2 leakage. Mitochondria are the site of C_4 acid decarboxylation in NAD-ME types and the number of mitochondria in BSC is significantly increased. BSC mitochondria also show ultrastructural changes in some C_4 species (Gutierrez et al 1974).

Although there is significant variation between C_4 species in leaf morphology and organelle ultrastructure, the features listed above are believed to play important roles in the compartmentalization of C_4 pathway enzymes, in the intercellular diffusion of pathway intermediates, and in the efficient transfer of photosynthate to the rest of the plant. Therefore, any discussion of adding C_4 characteristics to rice must consider whether changes in leaf morphology are also necessary. This point is probably best illustrated in the genus *Flaveria*, in which some species are C_4 , some are C_3 , and others are intermediates, ranging from C_4 -like to C_3 -like. Among the intermediates, there is a strong correlation among the degree of C_4 photosynthesis, the amount of C_4 enzymes, and the degree of development of Kranz anatomy (Edwards and Ku 1987, Rawsthorne 1992).

Leaf development program of C_4 plants

Development of a C_4 leaf can be viewed as an add-on to the default program followed by C_3 leaves (Dengler and Nelson 1999, Dengler and Taylor 2000). The C_4 program is active at three levels: first, to modify tissue pattern within leaves (spacing of veins); second, to modify cell patterns within tissues (organization of BSC and MC); and, third, to direct differentiation of specialized cells, BSC and MC. In the early stages of leaf development, dermal- and ground-tissue precursors are first established. Provascular strands then appear; those for the major veins occur first and then minor veins develop between the major veins as the leaf elongates and becomes broader (Nelson and Dengler 1997). The C_4 program adds an additional set of veins to give closer spacing of adjacent veins. This is the first obvious modification to basic C_3 developmental programs.

MC develop from the ground meristem located between and around provascular strands and there are no obvious differences between C_3 and C_4 programs at early stages. BSC develop from provascular strands and are distinguishable in C_4 leaves at early stages by their size and chloroplast numbers. More complex patterns of BSC development are found in NAD malic enzyme (NAD-ME)- and phosphoenolpyruvate carboxykinase (PCK)-type C_4 plants (Dengler and Nelson 1999). Differentiation of BSC follows vein development, occurring first around the major veins. Although BSC and MC tend to develop from different cell lineages, cell position plays a major role in the final determination of cell fate. This point was demonstrated by clonal analysis in maize leaves in which cell lineages were marked by mutant sectors (Langdale et al 1989).

The final step in the C_4 program involves the structural and functional differentiation of BSC and MC. This differentiation is the result of cell-specific expression of nuclear and organella genes. In some plants, such as maize, full differentiation is light-dependent. Light is required to promote high gene expression and to suppress expression in the inappropriate cell type (Langdale and Nelson 1991).

Intercellular signaling

A key difference in the C_4 leaf development program is that determination of cell fate takes place at positions different from those in C_3 leaves. Veins, for example, are spaced more closely together. In a recent review of vein pattern formation in plants, Nelson and Dengler (1997) present several models that attempt to explain the regular pattern of veins found in leaves. C_4 leaf development clearly requires a modification of the basic C_3 program regulating vein patterns. At our current stage of relative ignorance, we can only guess that some sort of distant intercellular signaling results in a regular spacing of veins (vein signal, Fig. 3). The timing and spacing of provascular initiation are different in C_4 leaves, but the same or similar intercellular signaling mechanism may be used.

The observation that BSC differentiation follows the position of these cells relative to adjacent developing veins suggests the involvement of intercellular signaling. Langdale and Nelson (1991) found evidence for a signal originating from the vein that promoted differentiation of adjacent cells into C_4 -type BSC. Those cells adjacent to BSC were in turn induced to differentiate into C_4 -type MC after receiving either the same signal at a lower dose or a different signal from BSC (C_4 signal, Fig. 3). MC not adjacent to BSC did not receive a signal and therefore developed via the C_3 default pathway.

What are the prospects for reprogramming rice leaf development?

This question really comes in two parts: What are the prospects for filling our gaps in knowledge about control of leaf development and then what are the prospects for using that knowledge to modify rice? Modification requires first isolating genes that control developmental processes. Because none of these genes that control the key features of C_4 leaves have been isolated, we must therefore ask how they might be found.

Contemporary biology has made use of model genetic systems to identify and isolate genes whose protein product is unknown. Maize and *Arabidopsis thaliana* are often the systems of choice in plants and significant progress has been made in understanding the genetic control of some aspects of development, especially floral morphogenesis. One approach to understanding genetic control of tissue pattern, particularly vein spacing, would be to look for mutants with altered vein patterns. Although mutants are known that affect veins, such as those resulting in loss of midribs, mutants affecting vein patterns have proven difficult to identify.

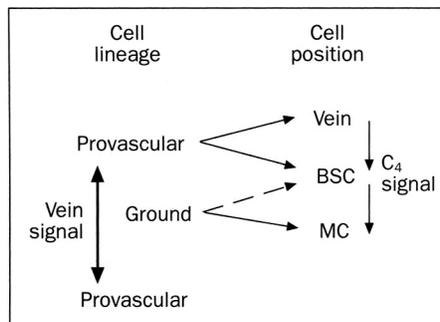


Fig. 3. Mechanisms responsible for the establishment of vein spacing and cell pattern in a C_4 leaf. See text for details. BSC = bundle sheath cell, MC = mesophyll cell.

One aspect of the problem is that there are no good markers to identify provascular strands at their earliest developmental stages when patterns are formed.

Mutations in genes that control the formation of BSC and MC patterns would be very desirable, as would mutants affecting BSC and MC differentiation. It is not obvious, however, what sort of phenotype we should look for. This problem has plagued the efforts to identify “C₄ mutants.” Langdale and colleagues reasoned that mutations affecting BSC and MC differentiation should be photosynthetically impaired. They screened mutagenized maize seedlings for pale green plants that showed altered levels of C₄ enzymes or defective chloroplasts in one cell type. They have identified several mutations specific to BSC and have shown that these *bundle sheath defective* mutants affect chloroplast structure and the accumulation of BSC enzymes (Langdale and Kidner 1994). The *Bsd1* gene has been isolated and shown to encode a transcription factor (Hall et al 1998).

Another approach relies on the major worldwide research activities in structural and functional genomics. Both maize and rice are the subject of major gene sequencing efforts and both are using transposable elements to make large mutant collections so that the phenotype of a knockout mutation in any gene can be determined. Of particular interest would be a comparison of expressed sequence tag (EST) libraries from rice and maize leaves. Each library is a collection of all genes expressed in the leaf. Some of the differences should be due to genes related to the C₄ nature of the maize leaf. A simple comparison, however, may not be adequate to identify the key genes that control C₄ features. It is possible that some important differences between C₃ and C₄ will be due to the timing of gene expression rather than to new genes for the C₄. Vein spacing, for example, could be viewed as an additional set of provascular strands being established after the process would have finished in a C₃ leaf. The comparison between maize and rice will therefore need to rely on microarray technology to compare gene expression programs during all stages of rice and maize leaf development. In this approach, an EST library consisting of perhaps 10,000 clones is put into a microarray on a single glass slide by a robot. RNA from selected stages of leaf development is labeled with a fluorescent dye and then hybridized to the microarray. The extent of fluorescence at each EST gives a quantitative estimate of the expression level of that gene. From the vast amount of expression data generated, it should be possible to identify genes that may be related to C₄ features. The maize transposable element collection can then be screened to look for mutations in these genes and determine their phenotypes. It is fortunate that one of the most widely used model genetic systems in plant research, maize, is a C₄ plant.

Mechanisms controlling C₄ gene expression

Genes coding for C₄ enzymes probably arose by gene duplication followed by the addition of new gene regulatory elements to confer high expression and cell specificity. Given that the nonphotosynthetic isoforms of most C₄ enzymes are found at low levels in several plant tissues, this dramatic change in gene regulation is the major difference between C₄ genes and their relatives. In principle, this evolutionary change could have been accomplished by the addition of a common set of DNA elements controlling BSC or MC specificity coupled with DNA elements for high expression. The expression programs of genes coding for most of the C₄ enzymes have been studied in detail (reviewed in Dengler and Taylor 2000, Sheen 1999). These studies indicate that there is no common mechanism: rather, a diverse set of mechanisms controls the expression of different genes.

There are, however, some common themes in C₄ gene regulation. Several lines of evidence have shown that C₄ genes are up-regulated in chlorenchymous tissue before the differentiation

of BSC and MC, in some cases showing expression in the precursors of both cell types (Langdale et al 1988, Ramsperger et al 1996, Sheen and Bogorad 1987). These observations suggest that part of the enhanced expression of C₄ genes could be due to the acquisition of DNA elements similar to those found on C₃ photosynthetic genes. The C₃ elements could provide the appropriate gene control during leaf development that results in high expression in cells containing chloroplasts.

Dengler and Taylor (2000) presented a hierarchical model to explain C₄ gene regulation. The first level can be considered the C₃ default program. The potential for high gene expression is established in those cells that will become photosynthetically active, including BSC. This level involves positional signaling that distinguishes ground from dermal and provascular tissue, light signals, and signaling between the nucleus and chloroplast. Nuclear genes coding for chloroplast proteins are activated, but not expressed at high levels; chloroplast genes are activated; and chloroplast replication is induced. The fact that some C₄ genes are expressed in a leaf-specific fashion in transgenic C₃ plants provides evidence for this first level of regulation.

The second level of regulation involves BSC and MC differentiation. The same intercellular signaling that promotes cell differentiation also promotes high cell-specific expression of C₄ genes (C₄ signal, Fig. 3). Regulation for some C₄ genes involves both increased expression in one cell type and repression of gene expression in the other.

An additional level of regulation is exerted by the products of photosynthesis (Koch 1996, Jang and Sheen 1997). Nitrogen also affects the expression of C₄ genes and the nitrogen-sensing mechanism has been shown to act through cytokinin (Sakakibara et al 1998).

Several recent reviews provide comprehensive discussions of our current understanding of C₄ gene structure and mechanisms of regulation, including analyses of promoter activities in transgenic C₃ plants (Dengler and Taylor 2000, Ku et al 1996, Sheen 1999). Selected examples will be discussed here.

PEP (phosphoenolpyruvate) carboxylase is encoded by a gene family in maize and *Flaveria* species (Hermans and Westhoff 1990, Ku et al 1996). *Ppc* genes coding for the C₄ isoform have been analyzed in transgenic plants and shown to be transcriptionally regulated by sequences located at the 5' end of the gene (Schaffner and Sheen 1992, Stockhaus et al 1997). The 5' promoter region controls both high-level expression and MC specificity. Yanagisawa and Sheen (1998) have identified a maize transcription factor, Dof1, that activates expression of the C₄ *Ppc* gene but has no effect on *Pdk* or *Cab* (chlorophyll *a/b* apoprotein of photosystem II) promoters.

Matsuoka et al (1994) demonstrated that the maize C₄ *Ppc* gene promoter directs high reporter gene expression in rice. Of particular interest was their observation that this expression was confined to leaf MC. Stockhaus et al (1994) isolated 5' promoter regions from two *PpcA1* genes, one from the C₄ species *Flaveria trinervia*, which encodes the C₄ isoform, and one from the orthologous *PpcA1* gene from the C₃ species *F. pringlei*, which encodes a nonphotosynthetic isoform of PEP carboxylase. The *F. trinervia* promoter directed high leaf-specific expression in transgenic tobacco. This expression was found primarily in palisade mesophyll. Low expression in leaves, stems, and roots was found with the *F. pringlei* promoter. Both research groups concluded that the major change in the evolution of C₄ *Ppc* genes was the acquisition of cis-acting DNA sequences in the 5' promoter region. Furthermore, these sequences are recognized by trans-acting factors in unrelated C₃ plants to give a C₄-like pattern of expression.

The C₄ isoform of pyruvate, orthophosphate dikinase is encoded by a single dual-function gene in maize and *Flaveria* (Glackin and Grula 1990, Rosche and Westhoff 1995, Sheen 1991). These *Pdk* genes are transcribed from two separate promoters. one producing an abundant mRNA specific to MC that encodes the chloroplast-localized C₄ isoform. The other promoter

is located in the large first intron and in roots and stems directs the low accumulation of a shorter mRNA encoding a cytoplasmic isoform of unknown function. The 5' promoter region of the *F. trinervia Pdk* gene directs high MC-specific expression in transformed *F. bidentis* (C_4 species) plants (Rosche et al 1998). Low expression is also seen in stems and roots, suggesting that the chloroplast isoform also has nonphotosynthetic functions. The same region from the maize *Pdk* gene directs high expression in MC protoplasts (Sheen 1991). The *Flaveria* and maize C_4 *Pdk* genes did not evolve through a gene duplication; rather, the existing gene gained new, *cis*-acting regulatory elements in the 5' promoter region that function along with the original promoter elements.

Matsuoka and colleagues (1993) demonstrated that the maize C_4 *Pdk* promoter directed high MC-specific expression in rice. They have also achieved high expression in rice by transferring the entire maize C_4 *Ppc* and *Pdk* genes (Matsuoka et al, this volume).

These experimental results provide encouragement for the possibility of expressing C_4 genes in a C_4 -like pattern in rice. However, a very different story has emerged for the gene encoding the C_4 isoform of NADP-ME. Marshall et al (1996) determined that *Flaveria* species, regardless of photosynthetic type, have two genes coding for chloroplast NADP-ME. The *Mel* gene codes for the C_4 isoform and appears to have arisen by a gene duplication that occurred before speciation within the genus. Experiments in transgenic *F. bidentis* plants showed the regulation of this gene to be complex and different from that of other C_4 genes (Marshall et al 1997, S. Ali and W.C. Taylor, manuscript submitted). Cell specificity is controlled by sequences in the 5' region, either upstream of the transcription initiation site (promoter) or in the 5' untranslated (5' UTR) site of the transcribed part of the gene (Fig. 4). These sequences probably interact with a BSC-specific *trans* factor. Quantitative levels of gene expression are controlled by an interaction of sequences at the N terminus, including the first intron, and at the 3' UTR. This interaction increases transgene expression in C_4 *Flaveria* several hundred-fold. This mechanism controls the accumulation of mRNA, but it is unknown whether it acts through transcriptional or post-transcriptional processes. Surprisingly, none of the transgene constructs showing high BSC-specific expression in *Flaveria* showed any detectable expression in tobacco. We concluded that the interaction between 5' and 3' sequences requires a *trans*-acting factor present only in C_4 *Flaveria*. Evolution of the C_4 *Mel* gene therefore involved acquisition of *cis*-acting DNA elements and at least one *trans*-acting factor. Our data do not provide any clues as to whether the hypothesized *trans* factor controlling quantitative expression is BSC-

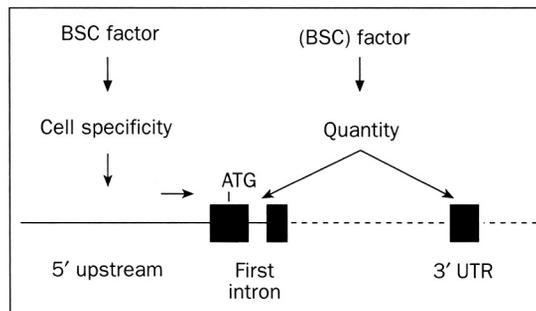


Fig. 4. DNA sequence elements and transacting factors that regulate expression of the *Flaveria* C_4 *Mel* gene. Exons, present in *Mel* mRNA, are indicated by black boxes. ATG = initiation codon, UTR = untranslated region.

specific, or whether it is related to the proposed *trans* factor that controls BSC-specific expression (Fig. 4). Although these results with the *Flaveria Mel* gene do not prove that the orthologous maize gene will be regulated by similar mechanisms, the similarities between maize and *Flaveria Ppc* and *Pdk* genes suggest that might be the case. If so, expression of a *C₄Me* gene in rice may be a challenge.

Expression of genes coding for both subunits of Rubisco is altered in *C₄* plants such that expression is confined to BSC. In maize and in the *C₄* dicot *Amaranthus hypochondriacus*, regulation of *RbcS* genes (coding for the small subunit) involves gene repression in MC and up-regulation in BSC (Langdale et al 1988, Ramsperger et al 1906, Sheen and Bogorad 1987). Positive and negative regulatory DNA elements have been defined (Schattner and Sheen 1991) and sequences at the 3' end of the *RbcS-m3* gene have been shown to be responsible for repressing gene activity in MC (Viret et al 1994). Sheen (1990) found evidence of a post-transcriptional step in repression of MC activity of *RbcS*.

In a detailed study of the patterns of accumulation of mRNAs and proteins during early stages of leaf development in *A. hypochondriacus*, Ramsperger et al (1996) found evidence for translational control of cell-specific accumulation of Rubisco. Translational control is also involved in light regulation of *RbcS* (Berry et al 1997, Wang et al 1993). To explore this level of regulation further, reporter gene constructs have been made to determine which parts of *RbcS* are responsible for translational control. Identification of sequences in the *RbcS* mRNA will then facilitate the study of the mechanism. The 5' and 3' UTRs from an *A. hypochondriacus RbcS* gene have been added to a construct with the cauliflower mosaic virus 35S promoter driving the *gusA* reporter gene and the *nos* 3' terminator (A.C. Corey, J.O. Berry, S. Ali, W.C. Taylor, unpublished). This construct was compared with the original 35S-*gus* in transgenic *C₄ Flaveria*. The addition of the *RbcS* 5' and 3' UTRs increased gene expression about fortyfold. It remains to be determined if the 5' and 3' UTRs will increase gene expression in *C₃* plants. or if they are involved in *C₄*-specific regulation.

When the promoter activity of a maize *RbcS* gene was tested in rice, it was found to direct high expression in MC (Matsuoka et al 1994). The authors concluded that trans-acting factors responsible for repressing MC expression are absent in rice because it is a *C₃* plant.

What are the prospects for *C₄* gene expression in rice?

From the previous discussion, it is clear that it is possible to express some *C₄* genes in rice at high levels. For others, such as *Mel*, expression in a *C₃* plant may prove difficult without extensive reengineering of the gene. One solution may be to use a different promoter, possibly from an *RbcS* gene. It remains to be seen whether *C₄*-like cell-specific expression will be difficult to obtain in rice. The results of Matsuoka et al (1993, 1994) with maize *Pdk* and *Ppc* genes showing apparent MC specificity are encouraging. It must be kept in mind, however, that the BSC of rice are not well developed for photosynthesis, since they possess few, chloroplasts. It may be that all photosynthesis genes will be expressed at low levels in rice BSC, and that attention needs to be focused on enhancing the photosynthetic capabilities of rice BSC.

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Notes

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C₃ and C₄ pathways

Requirements for the CO₂-concentrating mechanism in C₄ plants relative to limitations on carbon assimilation in rice

G.E. Edwards, O. Kirrats, A. Laisk, and T.W. Okita

CO₂-concentrating mechanisms in photosynthetic organisms have in common a requirement for energy (ATP) and investment in proteins (enzymes/transporters). This investment can benefit the plant with a sufficient increase in the supply of CO₂ to Rubisco under CO₂-limited photosynthesis. The effectiveness of the CO₂-concentrating mechanism in a C₄ plant is discussed with respect to overcycling by the C₄ pathway, the energetics of photorespiration, and CO₂ diffusive resistance between Rubisco and phosphoenolpyruvate carboxylase. Comparisons of the minimum calculated quantum requirement for CO₂ fixation at different intercellular levels of CO₂ for a C₄ plant (with variable overcycling and bundle sheath resistance) versus C₃ photosynthesis illustrate when C₄ photosynthesis may be of benefit as well as constraints in engineering C₄ photosynthesis into C₃ plants. Conditions in which photosynthesis is CO₂-limited and conditions where rice may benefit from a CO₂-concentrating mechanism are discussed.

Common features of CO₂-concentrating mechanisms

It is clear that CO₂ limits photosynthesis in plants in certain environments since some species have evolved mechanisms for active accumulation of CO₂. CO₂-concentrating mechanisms have been discovered in a range of organisms from microalgae to higher plants. Although there are differences in the mechanism, they have certain features in common, most notably the ATP-dependent accumulation of CO₂ at the site of ribulose -1,5-bisphosphate carboxylase-oxygenase (Rubisco) (Fig. 1).

In microalgae, including green algae and cyanobacteria, a CO₂-concentrating mechanism is induced when growth occurs under low CO₂ concentrations. Growth under low CO₂ also induces synthesis of carbonic anhydrase (CA, the enzyme that catalyzes the interconversion between CO₂ and bicarbonate) and the appearance of new proteins in the plasma membrane and chloroplast envelope, some of which are believed to be essential components of the CO₂ uptake mechanism (see references in chapter by Leegood, this volume).

In terrestrial plants, two types of CO₂-concentrating mechanisms exist. one in Crassulacean acid metabolism (CAM) and one in C₄ plants. Although they differ in mechanism, these two photosynthetic groups have in common a C₄ pathway of photosynthesis, composed of enzymes that are orders of magnitude more active than those in leaves of C₃ plants. It is this pathway

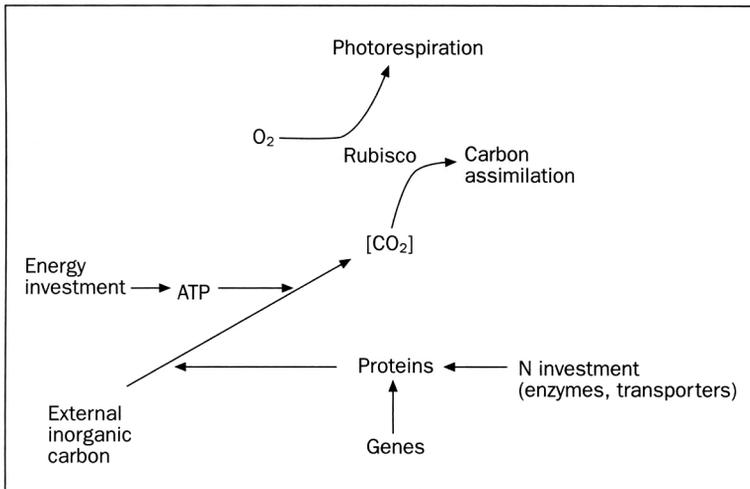


Fig. 1. Illustration of common features of CO₂-concentrating mechanisms in cyanobacteria, microalgae, and higher plants.

that is responsible for the capture of atmospheric CO₂. Some key features of the mechanism of carbon assimilation in C₄ plants are summarized below (see also Sage and Monson 1999 for recent literature).

Terrestrial C₄ plants: subtypes, biochemistry, types of anatomy, and photochemical requirements

Biochemistry and anatomy

Photosynthesis in C₄ plants consists of the coordinated function of two cell types in the leaves, usually designated mesophyll cells (MC) and bundle sheath cells (BSC). In C₄ plants, atmospheric CO₂ enters leaves through stomata and is first accessible to MC, where it is fixed by phosphoenolpyruvate (PEP) carboxylase to form oxaloacetate, and then malate and aspartate. These C₄ dicarboxylic acids are transported to BSC, where they are decarboxylated, and CO₂ is concentrated and refixed by RuBP (ribulose biphosphate) carboxylase and assimilated through the Calvin cycle to form sucrose and starch. Thus, in C₄ plants, there is a spatial separation of CO₂ fixation into malate (occurring in MC) and use of C₄ acids as donors of CO₂ to the C₃ pathway (occurring in BSC). Although anatomical differentiation is apparent in BSC, they are functionally similar in carbon assimilation to MC in C₃ plants, except for the presence of enzymes concerned with decarboxylation of C₄ acids.

In the course of evolution of C₄ plants from C₃ plants, the MC developed a high level of carbonic anhydrase and PEP carboxylase in the cytosol for initial CO₂ fixation in the cytoplasm, and pyruvate, orthophosphate (P₁) dikinase in the chloroplasts for provision of PEP, the HCO₃⁻ acceptor. It is equally important that the synthesis of some key photosynthetic enzymes in carbon metabolism of C₃ photosynthesis is repressed in MC of C₄ plants. These include Rubisco and phosphoribulokinase of the Calvin cycle and enzymes of glycine decarboxylation in MC mitochondria. For reviews of the biochemistry of C₄ photosynthesis, see Kanai and Edwards (1999).

The physiological significance of a separate, but coordinated, function of the two cell types in C_4 photosynthesis is the specialization of MC toward generation of a high concentration of CO_2 in BSC in order to reduce the oxygenase activity of Rubisco and the consequential reduction in photorespiration. C_4 photosynthesis can be visualized as a mechanism to provide Rubisco with near-saturating CO_2 when C_4 plants can afford a high stomatal conductance, or to provide sufficient CO_2 for survival and growth when stomatal conductance is low. Among C_4 plants, three subgroups have been defined based on differences in the decarboxylation step in BSC. These are the NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME), and PEP carboxykinase (PEP-CK) types. Figure 2 outlines the three decarboxylation systems, which is sufficient to visualize the enzymatic steps and the energy requirements. For information on the compartmentation, transport, and regulation of this metabolism in C_4 plants, see the chapter by Leegood (this volume).

As can be seen from the outlines of these cycles, the energy requirement per CO_2 pumped is 2 ATP for malic enzyme cycles and 1 ATP for the PEP-CK cycle. In C_4 plants, which use the PEP-CK located in the cytosol of BSC, the ATP is provided from mitochondrial oxidation of NADH, which is generated from decarboxylation of malate via NAD-ME. The net result is a lower requirement for ATP and higher requirement for reductive power per CO_2 fixed compared with malic enzyme-type C_4 plants. Genetic engineering of rice to put the C_4 form of PEP-CK in the chloroplast (Suzuki et al 1999) may allow decarboxylation to occur using photochemically produced ATP.

Biochemical diversity exists in the development of the C_4 syndrome among higher plants. However, a common feature is that PEP carboxylase is always located in MC and Rubisco and C_4 acid decarboxylases are always located in BSC (e.g., see Sinha and Kellogg 1996, Voznesenskaya et al 1999, Sage and Monson 1999). Structural diversity in the Kranz anatomy is also apparent among monocots and various dicots (Sage and Monson 1999). For example, family Chenopodiaceae has C_4 species with four types of Kranz anatomy: Salsoloid, Suaedoid, Kochioid, and Atriplicoid. In the tribe Salsoleae, family Chenopodiaceae, there is also diversity in the occurrence of C_4 versus C_3 photosynthesis in leaves versus cotyledons. To illustrate this, *Salsola laricina* has Salsoloid-type anatomy with NAD-ME-type photosynthesis in leaves, and Atriplicoid-type anatomy with NAD-ME-type photosynthesis in cotyledons (Pyankov et al 2000). *Haloxylon persicum* has Salsoloid-type anatomy with NADP-ME-type photosynthesis in green shoots and C_3 -type photosynthesis in cotyledons (Pyankov et al 1999, Voznesenskaya et al 1999). This indicates that, within a species, there is genetic control over the types of carbon assimilation occurring in different photosynthetic organs.

Immunolocalization studies demonstrate that PEP carboxylase is located in MC and Rubisco in BSC in both leaves and cotyledons of *Salsola laricina*. Also, the results with cotyledons show that only the MC adjacent to the BSC have PEP carboxylase activity. The distal MC have low levels of Rubisco and lack PEPC activity. In leaves of *S. laricina*, the leaf water storage cells have some Rubisco activity, but lack PEPC activity (Voznesenskaya et al 1999). Thus, the cell-specific expression of these enzymes is strongly regulated and experimental work indicates that the C_4 system is only effective through having the C_4 carboxylation phase in mesophyll cells adjacent to the bundle sheath (see Nelson and Langdale 1992).

Photochemical requirements

The efficiency of use of solar energy for CO_2 assimilation (quantum yield, mol CO_2 fixed or O_2 evolved per mol quanta absorbed by the leaf) depends on light intensity, other environmental factors, and the photosynthetic mechanism of carbon assimilation. Under high light, where the capacity to produce assimilatory power (ATP and NADPH) is high, photosynthesis will more

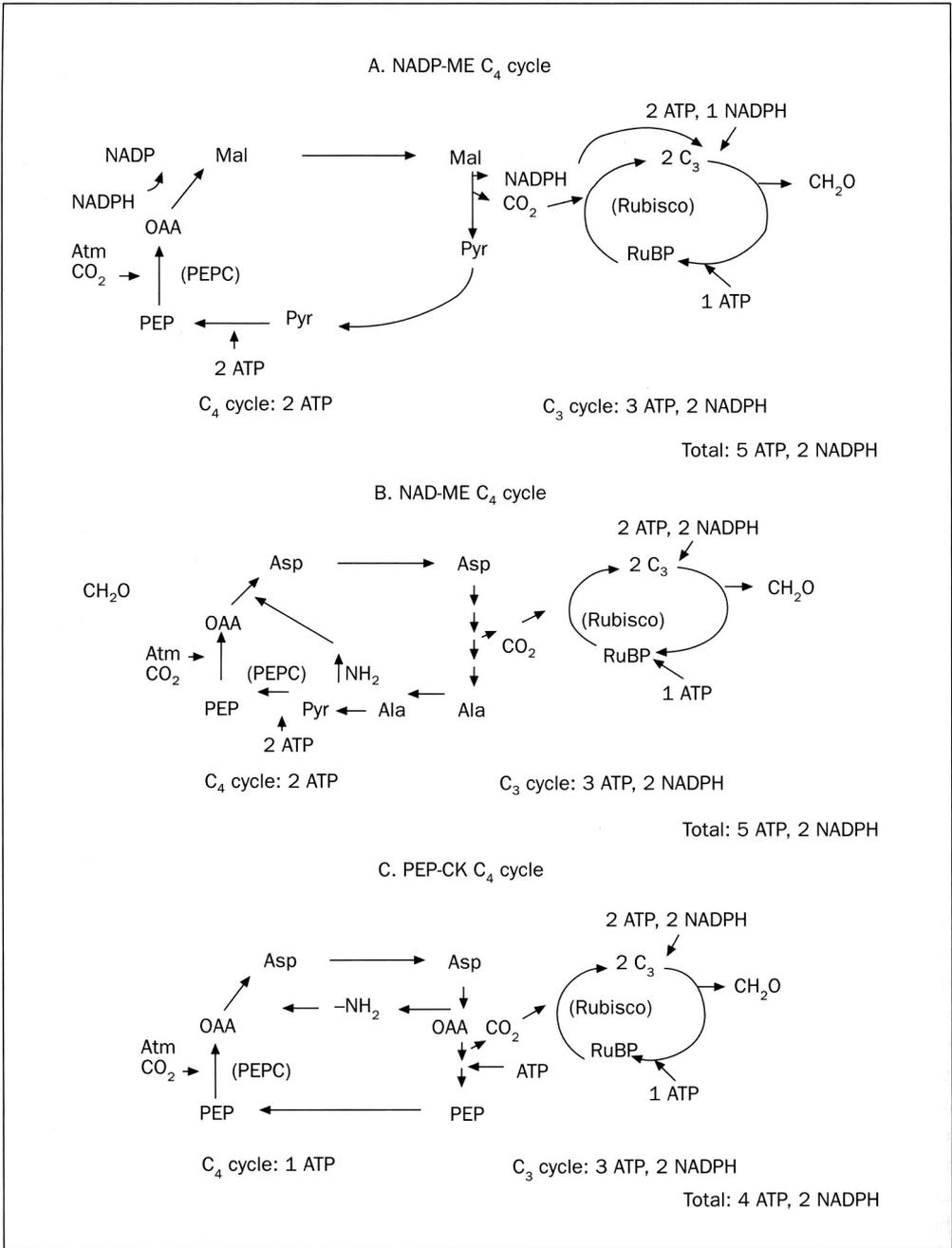


Fig. 2. Key metabolic steps during C₄ photosynthesis through three C₄ cycles and associated energy requirements. In the PEP-CK-type species, the supply of ATP for decarboxylation via PEP-CK is provided through mitochondrial phosphorylation using NADH generated via NAD-malic enzyme. See Kanai and Edwards (1999) as to how this affects the requirements of ATP and NADPH per CO₂ assimilated in these species. (A) NADP-ME C₄ cycle, (B) NAD-ME C₄ cycle, (C) PEP-CK C₄ cycle. Asp = aspartate, Ala = alanine, OAA = oxaloacetate, Pyr = pyruvate, Atm = atmospheric, Mal = malate.

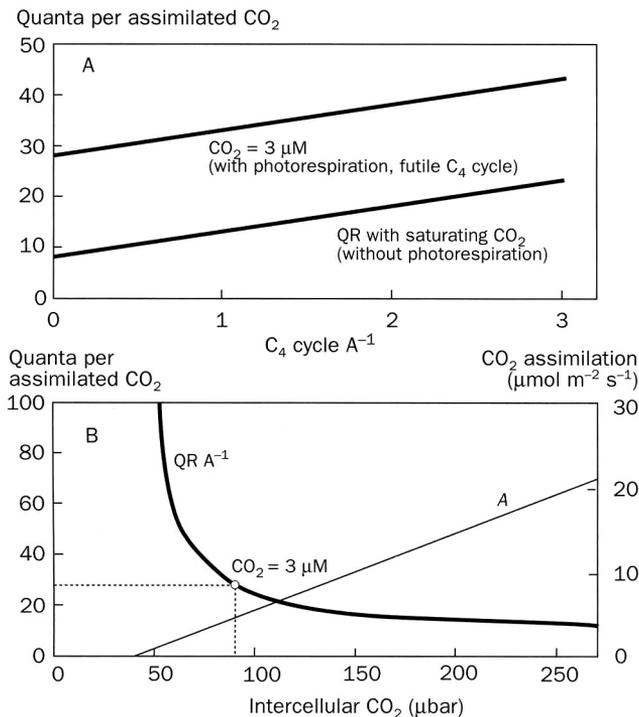


Fig. 4. (A) Minimum quantum requirement (QR) per CO₂ fixed in C₄ plants depending on rate of C₄ cycle (calculated according to pseudocyclic electron flow, Fig. 3), with or without photorespiration. The results with photorespiration were calculated based on 3 μM CO₂ at the site of Rubisco (see panel B for C₃ plant). **(B)** Shows the predicted rates of CO₂ fixation in a C₃ plant with varying levels of CO₂ at the site of Rubisco (equivalent to partial pressures in gas phase), 200 mbar O₂, 25 °C, K_m CO₂ = 11 μM, K_o O₂ = 300 μM, specificity factor q 88, V_{max} = 60 μmol m⁻²s⁻¹. The minimum quantum requirement per CO₂ fixed was calculated considering that 2 NADPH are required per RuBP consumed by Rubisco (see text). A = CO₂ assimilation rate.

by Rubisco where 2 ATP are required per turn of the C₄ cycle through pyruvate, Pi dikinase when decarboxylation occurs via malic enzyme. Results are shown in the absence of photorespiration versus in the presence of photorespiration (with 3 μM CO₂ as in Fig. 4B) when the C₄ cycle was futile, that is, no increase in CO₂ level around Rubisco. If C₃ plants were engineered with PEP-CK as the decarboxylase in the chloroplast and the ATP provided by photophosphorylation, then only 1 ATP would be required per. turn of the C₄ cycle, which would reduce the quantum requirement.

For C₃ plants, the quantum requirement for photochemistry can be calculated as follows (see also Krall and Edwards 1992, Laisk and Loreto 1996). The sum of the velocities of CO₂ and O₂ reacting with RuBP via Rubisco equals $v_c + v_o$. For each CO₂ reacting with RuBP, a minimum of 8 quanta are needed; likewise, for each O₂ reacting with RuBP, the products of photorespiration (3-phosphoglycerate and ammonia) consume ca. 2 equivalents of NADPH, resulting in a requirement of 8 quanta O₂⁻¹ consumed by Rubisco. Since the net rate of CO₂ fixation (A) equals $v_c - 0.5 v_o$, the quantum requirement per CO₂ fixed in C₃ plants equals (i.e., assuming in this illustration that dark-type mitochondrial respiration in the light is negligible)

$$\text{QR/net CO}_2 \text{ fixed} = (8 v_c + 8 v_o)/(v_c - 0.5 v_o)$$

Under current atmospheric conditions at moderate temperatures and without water stress, the v_c/v_o ratio in C_3 plants ranges from about 2 to 3, which, according to the above equation, results in minimum quantum requirements of 13 to 16. Figure 4B illustrates how the minimum quantum requirement based on Rubisco kinetic properties will decrease with increasing CO_2 . The quantum requirement is calculated from the above equation, based on calculated v_c/v_o ratios for a given level of CO_2 at Rubisco under atmospheric levels of O_2 . C_3 plants genetically engineered to supply CO_2 to Rubisco via a C_4 cycle may benefit most from overcycling of the C_4 pathway when the CO_2 supply is most limiting. Obviously, the potential benefit of the C_4 cycle in C_3 plants would depend in part on the degree to which CO_2 is limiting, the degree of overcycling of the C_4 pathway, and the extent to which the supply of CO_2 to Rubisco is increased.

Model of C_4 based on the NADP-ME-type system

For successful incorporation of a CO_2 -concentrating mechanism into a C_3 plant such as rice, it is useful to consider how the C_4 mechanism functions and its important features. While all the enzymatic reactions of the C_4 cycle have been identified and their regulatory properties studied, questions remain about how the system actually functions to concentrate CO_2 around Rubisco. Recently, a model has been developed that includes reactions of the C_4 cycle, the C_3 pathway, and the photorespiratory pathway for an NADP-malic enzyme-type species (Laisk and Edwards, in preparation). The model shows the importance of bundle sheath resistance to effective function of C_4 photosynthesis and the effects of photorespiratory metabolism in bundle sheath and mesophyll cells. To illustrate the effect of variable bundle sheath resistance, Figure 5 shows outputs from the model under high light and $3 \mu M CO_2$ at the site of PEPC in MC (equivalent to about $72 \mu bar$). The BSC of C_4 plants are considered to have a sufficiently high resistance to CO_2 to prevent leakage and futile cycling of the C_4 pathway. Different approaches have been used to evaluate the magnitude of this resistance, resulting in values ranging from ca. 50 to $1,500 m^2 s mol^{-1}$ (ca. 2 to $60 s mm^{-1}$; for conversion to $s mm^{-1}$, the value in $m^2 s mol^{-1}$ is divided by the mole volume of gas at a given temperature and pressure, $V_{mol} = (RT)/P$, which = ca. $25 L mol^{-1}$ at $30^\circ C$) (see summary in He and Edwards 1996). Our recent work with a mutant of *Amaranthus edulis* that has a defective C_4 cycle (courtesy of Dr. P. Lea) indicates that the bundle sheath diffusive resistance in this species is 60 to $180 m^2 s mol^{-1}$ (2.5 to $7.2 s mm^{-1}$) depending on plant age. The output of the model shows that high rates of photosynthesis can be achieved with a bundle sheath resistance of the order that has been suggested based on experimental results. With increasing bundle sheath resistance up to $15 s mm^{-1}$, the CO_2 concentration in the BSC continues to rise. However, a resistance of $2 s mm^{-1}$ causes a sufficient increase of CO_2 in the bundle sheath to restrict v_o (velocity of RuBP oxygenase), and provides relatively high rates of photosynthesis. Below a resistance of $2 s mm^{-1}$, the net rate of CO_2 fixation drops rapidly. At very low resistance ($0.002 s mm^{-1}$), the net rate of CO_2 fixation is only about $6 \mu mol m^{-2} s^{-1}$ and the rate of the C_4 cycle is about 5 times the net rate of CO_2 fixation due to the higher rate of photorespiration and increased leakage of CO_2 from BSC. At such low resistance, the quantum requirement for photosynthesis is high, not only because of photorespiration but also because of the futile C_4 cycle (QR = 34 quanta per assimilated CO_2 at bundle sheath resistance of $0.002 s mm^{-1}$). For comparison to diffusive resistance of BSC, stomatal and aqueous-phase resistances in C_3 plants are on the order of $0.2 s mm^{-1}$ and $0.04 s mm^{-1}$, respectively (see Laisk and Loreto 1996).

Photorespiration is not only repressed in C_4 photosynthesis, it is a part of the CO_2 -concentrating mechanism. Figure 6 illustrates the energy requirements per RuBP consumed as a consequence of photorespiration in C_4 plants (as O_2 reacts with RuBP and the photorespired CO_2 is refixed). It

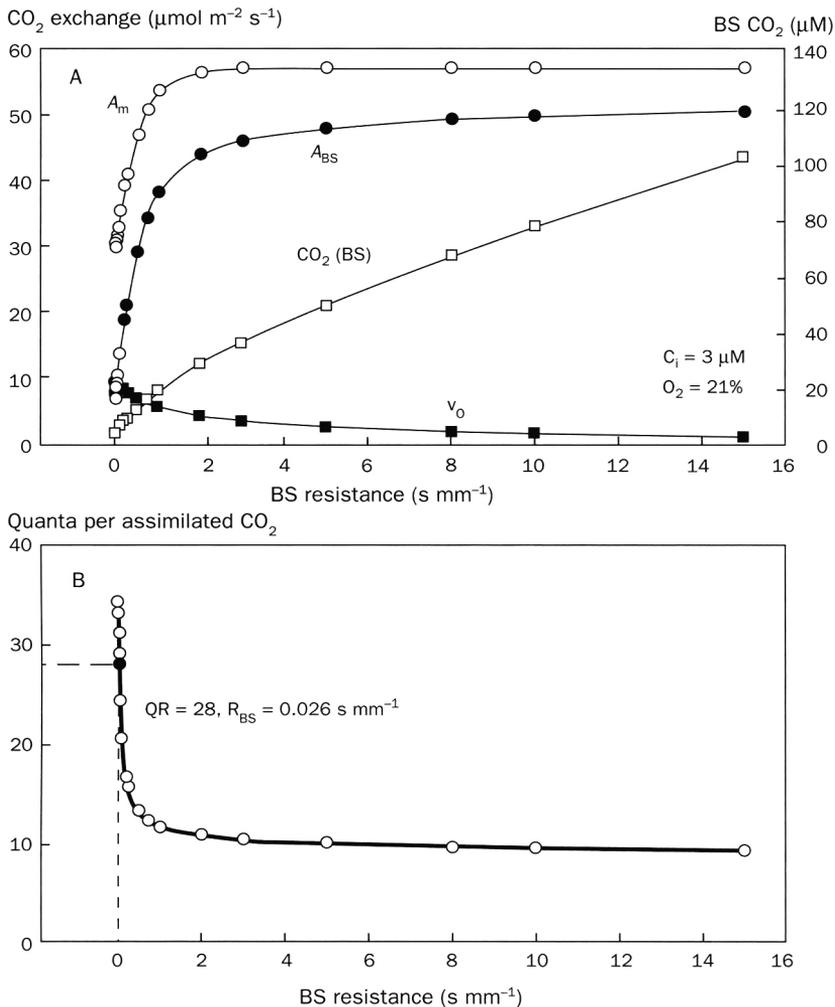


Fig. 5. Effect of bundle sheath (BS) resistance on photosynthesis in C₄ species based on a C₄ model for NADP-ME-type C₄ species. (A) Output for rate of CO₂ fixation in C₄ cycle (A_m), net rate of CO₂ fixation by Rubisco (A_{BS}), CO₂ concentration in bundle sheath cells [CO₂(BS)], and velocity of RuBP oxygenase (v₀) by C₄ model (developed for NADP-ME-type species) at 25 °C, PPFD of 1,600 μmol m⁻² s⁻¹, and 3 mM CO₂ at site of PEPC in mesophyll cells (MC). (B) The minimum quantum requirement (QR) per CO₂ fixed based on linear electron flow in MC (3 ATP per O₂ evolved) and cyclic electron flow in bundle sheath chloroplasts (0.5 versus 1 ATP per quanta with 1 and 2 proton coupling sites), with 2 NADPH per RuBP consumed, and ATP requirements associated with Rubisco activity and C₄ cycle.

shows that, for each RuBP consumed in this way, an additional CO₂ is pumped to the bundle sheath cells by the C₄ cycle (with energy requirements of 5.3 ATP and 2 NADPH per RuBP). This is particularly clear in NADP-ME-type species like sorghum, where the use of NADPH in BSC is obligatorily linked to the C₄ cycle as bundle sheath chloroplasts lack PSII activity.

Evidence for the occurrence of photorespiration *in vivo* in C₄ plants is shown with studies of the C₄ mutant of *Amaranthus edulis*, which lacks PEPC. In the mutant under limiting CO₂, there is an O₂-dependent increase in PSII activity and quanta required per CO₂ fixed (Fig. 7). This O₂-dependent increase in photorespiration will cause CO₂ to be released in bundle sheath

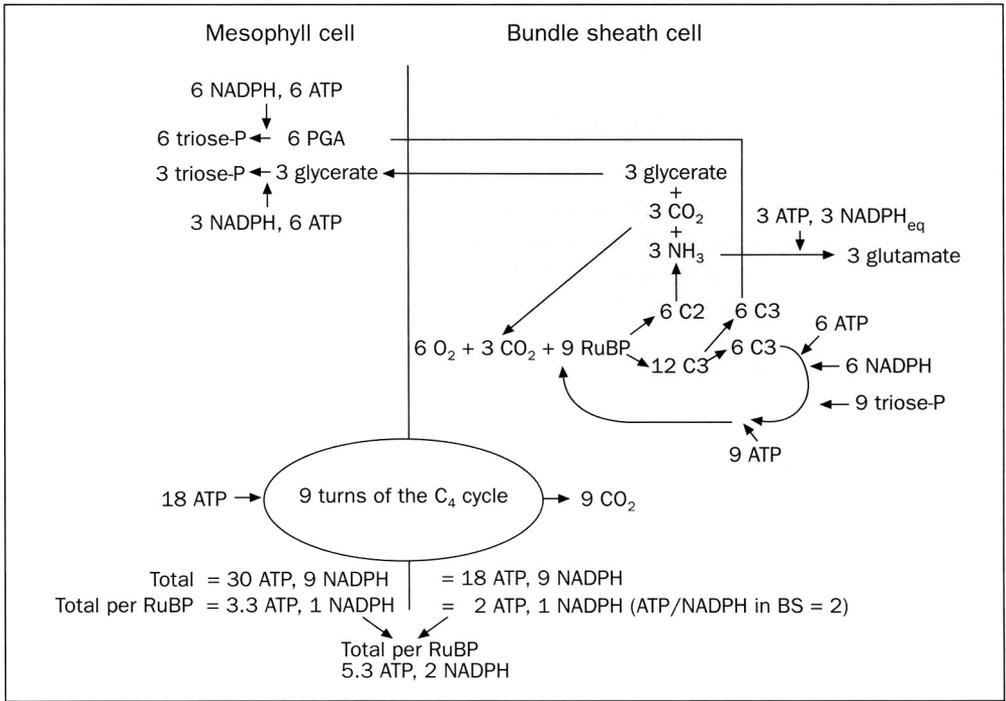


Fig. 6. Proposed consequences of photorespiration on energy requirements for the generation of CO₂ in bundle sheath (BS) cells in C₄ plants. The scheme shows consumption of RuBP as a consequence of photorespiration in C₄ plants. The 6 O₂ and 3 CO₂ react with 9 RuBP; 3 CO₂ are released in bundle sheath cells in the glycolate pathway, resulting in no net change in CO₂. With one turn of the C₄ cycle per RuBP used, 5.3 ATP and 2 NADPH would be consumed in mesophyll and bundle sheath cells.

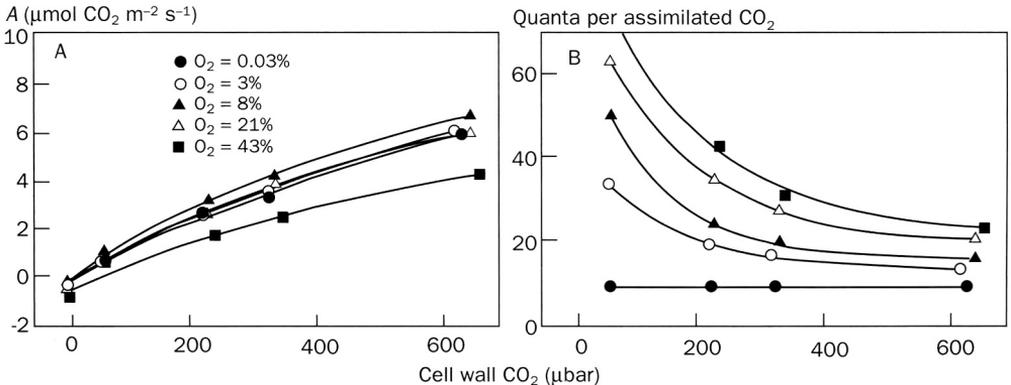


Fig. 7. Evidence for increased photorespiration and generation of CO₂ in bundle sheath cells with increasing O₂ under limiting CO₂ in a C₄ mutant (defective PEP carboxylase) *Amaranthus edulis*. (A) Net rates of CO₂ assimilation with increasing CO₂ at different concentrations of O₂. (B) The minimum quanta use in photochemistry per net CO₂ assimilated was calculated as $8(J_{O_2})/A$ where J_{O_2} is the true rate of O₂ evolution from photosystem II (estimated from analysis of chlorophyll fluorescence), 8 quanta are used for photochemistry in linear electron flow per O₂ evolved, and A is the net rate of CO₂ fixation. The results show that the quanta used in photochemistry per CO₂ fixed increases as CO₂ concentration decreases and O₂ concentration increases. Temperature = 30 °C, photosynthetic photon flux density (PPFD) = 1,800 $\mu\text{mol m}^{-2} \text{ s}^{-1}$.

cells. Since O_2 has little effect on CO_2 fixation, this suggests that the CO_2 generated from photorespiration in the bundle sheath cells is refixed.

Prospects for incorporating the CO_2 -concentrating mechanism into rice

Since the possible introduction of C_4 traits into C_3 plants is a topic of this workshop, we will briefly comment on it here. In general, approaches could include incorporating some features of C_4 photosynthesis into the mesophyll cells of rice (*Oryza sativa*), analogous to *Hydrilla*, the aquatic macrophyte, developing C_3 - C_4 intermediate-type photosynthesis (dependent on mesophyll cells and BSC), or determining the genetic factors required for expression of Kranz-type anatomy.

Currently, key steps of the C_4 pathway are being incorporated into rice (see chapters by Matsuoka et al and Ku et al, this volume). Several considerations affect the possibility of success. A major question is whether the diffusive resistance between atmospheric CO_2 and Rubisco is too low, which could result in a high degree of overcycling with a minimal increase in CO_2 supply. As shown above, in the C_4 model, a minimal resistance is required for the cycle to be effective in concentrating CO_2 . If the strategy is to recapture photorespired CO_2 as in C_4 plants, then a Kranz-type anatomy will be required. Another consideration is whether the chloroplast will be able to generate ATP by cyclic or pseudocyclic photophosphorylation to support the C_4 cycle, in that linear electron flow to NADP only generates sufficient ATP to support the C_3 cycle. One of the key steps for engineering C_4 photosynthesis in rice is expression of the C_4 isoform of PEP carboxylase in the cytosol, where it would fix atmospheric CO_2 . As noted earlier, in C_4 plants, CA in the cytosol of MC is considered to function in generating bicarbonate for PEPC. Thus, a question in genetic engineering of C_4 in rice is whether cytosolic CA will be limiting. To address these types of questions, models for incorporating C_4 photosynthesis into rice that include the enzymatic steps, compartmentation of reactions, and diffusive resistances to CO_2 can be tested to aid genetic engineers in designing rice for improved photosynthesis.

When will photosynthesis in C_3 plants be CO_2 -limited?

In general, photosynthesis in C_3 plants is limited mainly by light harvesting and assimilatory power under low light, and by carboxylation and photorespiration under limiting CO_2 . Under saturating-light and $-CO_2$ conditions, however, photosynthesis can be controlled by processes that convert triose-P into starch and sucrose (Sage 1990, 1994, Stitt 1986, 1996). Thus, the capacity to use triose-P for carbohydrate synthesis can establish an upper limit for the maximum rate of photosynthesis under CO_2 - and light-saturated conditions (Sage 1990, Sharkey et al 1995).

When photosynthesis is CO_2 -limited, then any means of increasing the supply of CO_2 to Rubisco may be beneficial, including genetic modifications, provided there are no offsetting losses (e.g., by increased energy requirements, by decreased Rubisco content, etc.). In C_3 plants, CO_2 limitation increases because of stomatal closure (i.e., with drought, low humidity), an increase in aqueous-phase diffusive resistance (from the plasmamembrane of photosynthetic cells to Rubisco in the chloroplast), or a decrease in solubility of CO_2 (i.e., by increasing temperature, see chapter by Sage, this volume). High light, moderate to high temperature, high stomatal resistance and/or high diffusive resistance, and high Rubisco content would favor CO_2 -limited photosynthesis. Eichelmann and Laisk (1990) showed that liquid-phase diffusional limitations in C_3 leaves may become important above a certain level of Rubisco.

For diffusion of CO₂ in the aqueous phase, whether CA facilitates the supply of CO₂ to Rubisco in C₃ plants continues to be evaluated. It is postulated that three forms of CA may be involved: one in the plasmamembrane, another in the chloroplast stroma, and a third in the thylakoid membranes (Ignatova et al 1998). Diffusion of CO₂ into the chloroplast and its equilibration to bicarbonate by CA provides a larger pool of inorganic carbon to diffuse to sites of CO₂ fixation by Rubisco (see Edwards and Walker 1983). It has also been postulated that the supply of CO₂ to Rubisco in the stroma may be increased by transport of protons (via the proton motive force) and bicarbonate from the stroma to the lumen and their conversion to CO₂ and water by thylakoid CA (Ignatova et al 1998). In considering means to increase the supply of CO₂ to Rubisco in C₃ plants, the functions of these forms of CA need to be elucidated.

CO₂ versus sink limitation of photosynthesis in rice

In our studies with growth-chamber-grown rice, we determined conditions under which photosynthesis is limited by CO₂ supply versus the capacity to use triose-P. Rice (cv. T309) plants were grown with day/night temperatures of 26/24 °C, 10-h photoperiod, and a photosynthetic photon flux density (PPFD) of 700–1,000 μmol m⁻² s⁻¹ (tests indicated that plants were not limited by nitrogen or pot volume, Winder et al 1998). Photosynthesis was measured in flag leaves during heading and grain filling under different environmental conditions. We found that photosynthesis was near saturation under current ambient levels of CO₂ at moderate temperature (25 °C), moderate light intensities, and high humidity (>90%, vapor pressure deficit, VPD, of 2 mbar, Fig. 8A). This was unexpected, since increasing CO₂ above ambient levels is expected to cause a continual rise in photosynthesis by partitioning more of the available RuBP into carboxylase activity and less into oxygenase/photorespiration. This insensitivity of photosynthesis to increasing CO₂ above ambient levels and a concomitant decreased sensitivity to lower O₂ are indications of a limitation on triose-P use.

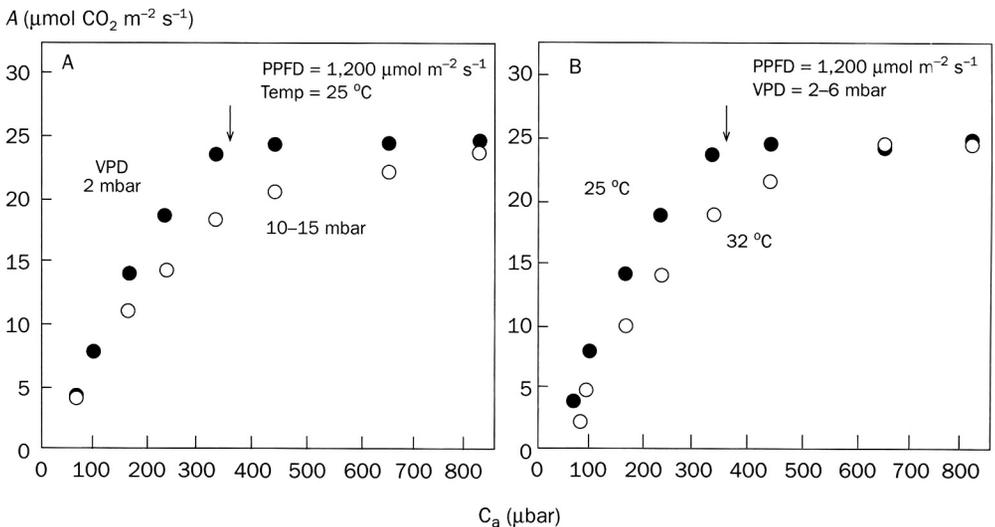


Fig. 8. CO₂-dependent rates of carbon assimilation in rice under low versus high leaf to air vapor pressure differences (A), and under 25 versus 32 °C (B). Rates were measured on the flag leaf of plants during the heading-pollination stage under varying atmospheric levels of CO₂ (C_a) (see also Winder et al 1998). PPFD q photosynthetic photon flux density, VPD = vapor pressure deficit.

However, photosynthesis in rice was clearly not saturated at current ambient levels of CO₂ with high VPD under 25 °C (Fig. 8A). Low humidity (about 60%, VPD of 10–15 mbar) caused CO₂ to be limiting for photosynthesis by decreasing stomatal conductance (He and Edwards 1996, Winder et al 1998, see also studies by Dai et al 1992 with castor bean and other citations therein). This decrease in stomatal conductance with increasing VPD caused a decrease in intercellular levels of CO₂ (He and Edwards 1996). Increasing temperature (under high humidity) resulted in an increase in the level of CO₂ required for saturation of photosynthesis (Fig. 8B) as expected since the solubility of CO₂ decreases and photorespiration increases (see Edwards and Walker 1983).

Studies of the transient changes in photosynthesis upon switching from normal O₂ (200 mbar) to low O₂ (20 mbar) can also be used to test condition where photosynthesis is limited by the capacity to produce triose-P versus the capacity to use triose-P (feedback) (see chapter by Sharkey, this volume). In rice, when photosynthesis is limited by capacity to use triose-P, a transient inhibition of photosynthesis occurs when switching from 20 to 2 mbar O₂, the degree of which is correlated with a terminal, steady-state suppression of low O₂ enhancement of photosynthesis (Sun et al 1999). These symptoms of feedback were very pronounced at 18 °C and current ambient levels of CO₂, whereas at 32 °C no feedback was observed. Feedback was observed with increasing light and CO₂ partial pressures. Rice showed some susceptibility to feedback under moderate temperatures (25 °C) and current atmospheric levels of CO₂.

The above results indicate that, under some conditions (moderate to low temperature, high humidity, and high light), rice productivity may benefit by improvements in the capacity to convert triose-P to carbohydrates (sucrose and starch) in leaves, or increased loading of carbohydrates in the phloem, or increased sink capacity (for further discussion, see Choi et al 1998). In this respect, it is of interest that canopy photosynthesis of field-grown rice plants in Florida was near saturated at atmospheric levels of CO₂ (Rowland-Bamford et al 1991). In some conditions, CO₂ enrichment during grain filling does not lead to an increase in productivity per panicle (Rowland-Bamford et al 1990, Baker et al 1992, Chen and Sung 1994), suggesting a sink limitation and potential to increase yields by genetic modifications in starch biosynthesis in seeds (Choi et al 1998).

Under other conditions (i.e., low humidity, stomatal limitations, high temperatures), rice may benefit by genetic modifications that would increase the supply of atmospheric CO₂ to Rubisco. CO₂ enrichment increased tillering in rice grown in Florida (Rowland-Bamford et al 1990, Baker et al 1992) and the Philippines (Ziska et al 1996). CO₂ enrichment may have its effect by enhancing photosynthesis per leaf area, or by allowing more carbon and nitrogen to be partitioned to tillers by a reduction in the nitrogen investment per leaf area (see Makino and Mae 1999, Winder et al 1998, Ziska et al 1996). The consequences of CO₂ enrichment for rice production may be different than genetic modifications to incorporate an active system to increase the supply of CO₂ to Rubisco, which would require a nitrogen/protein cost (Fig. 1, chapter by Evans and von Caemmerer, this volume). The potential for CO₂-limited versus feedback-limited photosynthesis will vary depending on locality and daily and seasonal changes. Engineering inducible changes in carbon assimilation pathways to maximize carbon gain according to the limitation may be beneficial.

Finally, in considering how photosynthesis in rice responds to varying climatic conditions in the field, it would be advantageous if a measure of carbon fixation could be made on individual leaves without enclosing plants in chambers, which changes the environment. We combined measurements of gross rates of O₂ evolution by chlorophyll fluorescence analysis with a model that predicts stomatal conductance (and inputs of light intensity, humidity, and leaf temperature) to predict rates of CO₂ fixation in rice (He and Edwards 1996). There was good agreement

between the rates of CO₂ fixation predicted by the chlorophyll fluorescence analysis/stomatal conductance model and those measured by infrared gas analysis on leaves of rice under varying VPD, temperature, and light, indicating some potential for this approach to obtaining a noninvasive measure of carbon assimilation.

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Overcoming barriers: CO₂-concentrating mechanisms and C₄ metabolism in relation to transport

R.C. Leegood

The essential features of CO₂-concentrating mechanisms in C₄ plants, algae, and cyanobacteria; in the single cell CO₂-concentrating mechanism found in the aquatic macrophyte *Hydrilla verticillata*; and in the simplest CO₂-salvaging mechanism found in C₃-C₄ intermediates are discussed. Some of the key structural and metabolic aspects that appear to be essential for the operation of C₄ photosynthesis or that are very specialized features in C₄ plants are discussed, including anatomical constraints and the role of plasmodesmata, the regulatory consequences of intercellular metabolite transport, the specialization of metabolite transporters, electron transport pathways, and mitochondrial function, and the mechanisms and functions of the regulation of enzyme activity in C₃ and C₄ plants. Most of these are intimately connected with intra- and intercellular communication. I consider how this communication is achieved at the structural level and how metabolic cross-talk between the two cell types involved in photosynthesis enables integration of function at a metabolic level and draw attention to some of the limitations that may arise if attempts are made to introduce C₄-like characteristics, either at the single-cell level or at the level of the mesophyll and bundle sheath, into C₃ plants such as rice.

Features of CO₂-concentrating mechanisms

Table 1 summarizes the essential features of CO₂-concentrating mechanisms in C₄ plants and in algae and cyanobacteria (adapted from Badger and Spalding 2000), with the addition of the single-cell CO₂-concentrating mechanism found in the submerged aquatic macrophyte *Hydrilla verticillata* (Reiskind et al 1997) and the simplest CO₂-salvaging mechanism found in C₃-C₄, intermediates such as *Moricandia arvensis* (Monson and Rawsthorne 2000). One of the main features that distinguishes the aquatic CO₂-concentrating mechanisms from the aerial CO₂-concentrating mechanism of C₄ photosynthesis is that the latter requires complex intercellular cooperation, whereas the former all possess single-cell CO₂-concentrating mechanisms. These are based, in the algae and cyanobacteria, on an intracellular compartment, the pyrenoid or carboxysome, in which CO₂ can be concentrated in the vicinity of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco). In *Hydrilla*, an inducible C₄-like system operates between the cytosol and chloroplast, but the mechanisms controlling CO₂ leakage from the chloroplast are not known. No other angiosperms appear to have developed a single-cell CO₂-concentrating mechanism, although some bryophytes appear to have retained the ancestral pyrenoid (Smith and Griffiths 1996). Although plants with Crassulacean acid metabolism (CAM) have a CO₂-

Table 1. A comparison of the components of the CO₂-concentrating mechanisms (CCM) present in C₄ and C₃-C₄ intermediate plants, algae, and cyanobacteria. Adapted from Badger and Spalding (2000) and including data on the inducible single-cell C₄ system in the freshwater monocot *Hydrilla verticillata* (Bowes and Salvucci 1983, Reiskind et al 1997) and C₃-C₄ intermediates such as *Moricandia arvensis* (Monson and Rawsthorne 2000)*.

CCM component	Photosynthetic organism			
	C ₄ plants	<i>Moricandia arvensis</i>	<i>Hydrilla verticillata</i>	Algae
1. An active CO ₂ capture system	PEP carboxylase in the cytosol of the specialized mesophyll cells	Nothing in addition to Rubisco	PEP carboxylase in the cytosol of leaf cells	Active CO ₂ and HCO ₃ ⁻ transport on both chloroplast and cell membranes
2. Photosynthetic energy supply	ATP provided by chloroplasts for PEP regeneration	ATP and NADPH requirements of the photorespiratory pathway	ATP provided by chloroplasts for PEP regeneration	Photosynthetically derived ATP and perhaps NADPH energize transport
3. An intermediate pool of captured CO ₂	C ₄ acids—malate and aspartate	Photorespiratory glycine	C ₄ acids—malate and aspartate	HCO ₃ ⁻ in the cytoplasm
4. A mechanism to release CO ₂ from the intermediate CO ₂ pool	Specific C ₄ acid-decarboxylating enzymes in the bundle sheath cells	Glycine decarboxylase located solely in the bundle sheath mitochondria	NADP-malic enzyme in the chloroplasts of leaf cells	Carbonic anhydrase specifically in the carboxysome
5. A compartment to concentrate CO ₂ around Rubisco	The bundle sheath compartment with specialized bundle sheath cells and chloroplasts	The bundle sheath compartment	The chloroplast	The carboxysome compartment in the cytoplasm

Table 1 continued.

CCM component	Photosynthetic organism		
	C ₄ plants	<i>Moricandia arvensis</i>	<i>Hydrilla verticillata</i>
6. A means to reduce CO ₂ leakage from the site of CO ₂ elevation	The bundle sheath structure, including suberized layers, as well as an absence of CA in this compartment	Weakly developed bundle sheath structure	Not known. CA presumably absent from chloroplast stroma
7. Modification of the kinetic properties of Rubisco	An intermediate affinity for CO ₂ , high S _{rel} , and increased V _{max}	None reported	No apparent change in properties between induced and noninduced plants

^a CA = carbonic anhydrase, S_{rel} = relative specificity factor of Rubisco relating the carboxylase to oxygenase kinetics (V_cK_c)/K_cV_o, where V_c and V_o are maximum velocities for carboxylation and oxygenation and K_c and K_o are K_ms for CO₂ and O₂

concentrating mechanism based on a cycle of carboxylation and decarboxylation within a single cell, the mechanism operates over a diurnal cycle (i.e., temporally rather than spatially) and daytime stomatal closure prevents the efflux of CO₂ generated by decarboxylation of C₄ acids, thereby raising the internal concentration of CO₂ around the photosynthetic cells. The primary function of CAM is to conserve water, rather than to increase carbon gain.

Certain C₃-C₄ intermediates (so-called because they have gas-exchange characteristics intermediate between those of C₃ and C₄ plants) have a CO₂-concentrating mechanism based on the recapture of photorespired CO₂ in the bundle sheath, but it does not fulfill all the criteria set out in Table 1 (i.e., there is no active CO₂ capture system). All plants that exhibit C₃-C₄ gas-exchange characteristics (i.e., a CO₂ compensation point intermediate between those of C₃ and C₄ plants) have a weakly developed bundle sheath and lack glycine decarboxylase activity in the mesophyll. The sole location of glycine decarboxylase in the bundle sheath means that the photorespiratory release of CO₂ is confined to the bundle sheath. It is arguable that this specific compartmentation of glycine decarboxylase in the bundle sheath was the first step in the evolution of C₄ photosynthesis, exemplified by *Moricandia arvensis* and *Panicum milioides*, which lack any trace of C₄-like metabolism (Monson and Rawsthorne 2000), whereas other C₃-C₄ intermediates, particularly in the genus *Flaveria*, have C₄ metabolism to varying degrees (Monson et al 1986). In all C₄ plants studied, glycine decarboxylase is also confined to bundle sheath mitochondria (Ohnishi and Kanai 1983), perhaps reflecting the first step in their evolutionary origins.

Some structural and metabolic features of C₄ photosynthesis

C₄ photosynthesis has been defined by Karpilov as cooperative photosynthesis because it involves the cooperation of two cell types, the mesophyll and the chlorenchymatous bundle sheath (it might be noted that a chlorenchymatous bundle sheath is not unique to C₄ plants, but the frequency of chloroplasts is very much lower in the C₃ examples, such as rice and barley). In the following, I discuss some of the key structural and metabolic aspects that appear to be essential for the operation of C₄ photosynthesis or that are very specialized features in C₄ plants. Most of these are concerned with intercellular communication. I consider how this communication is achieved at the structural level and how metabolic cross-talk between the two cell types involved in photosynthesis enables integration of function at a metabolic level.

Anatomical constraints

The necessity for metabolite transport between the mesophyll and bundle sheath sets limits on the amount of mesophyll tissue that can be functionally associated with bundle sheath tissue. Close contact between the two is required, dictating the proximity of mesophyll and bundle sheath cells and, therefore, influencing leaf structure. For this reason, the leaf thickness is limited in C₄ plants and the interveinal distance (i.e., the number of mesophyll cells between adjacent bundle sheaths) is usually smaller than in the leaves of C₃ plants (Hattersley 1992). In a developmental mutant in *Panicum maximum* (*lis1*; Fladung 1994), the interveinal distance is increased from 2 to between 6 and 7 cells (similar to the *Knotted-1* mutant in maize). This results in a lower rate of photosynthesis and an increase in the CO₂ compensation point, partly because it must affect intercellular transport, but also because it influences the development of C₄ metabolism in the mesophyll (Nelson and Langdale 1992). Thus, C₃ plants engineered to express C₄ photosynthesis between the mesophyll and bundle sheath would be compromised in regard to metabolite transport if the interveinal distance were large.

Bundle sheath permeability

An essential part of the C_4 cycle is the transfer of the products of the carboxylation reaction from the mesophyll to the bundle sheath and the return to the mesophyll of the products of the decarboxylation reaction (Fig. 1). Metabolite movement between the bundle sheath and the mesophyll is sustained by diffusion via numerous plasmodesmata, driven by gradients in their concentrations (Leegood 1985, Stitt and Heldt 1985a,b). A striking feature of the leaves of C_4 plants is their high content of metabolites when compared with C_3 plants. For example, contents of triose-P may be 20 times higher in maize than in barley, reflecting the internal metabolite gradients that are established during photosynthesis, and this has important consequences for the regulation of photosynthesis (see below). Hatch and Osmond (1976) estimated that an intercellular gradient of 10 mM of a metabolite such as triose-P or malate would be needed to sustain observed rates of photosynthesis in maize (which has centrifugally arranged bundle sheath chloroplasts and a short diffusion path) and a gradient of 30 mM would be needed in *Amaranthus* (which has centripetally arranged bundle sheath chloroplasts and a longer diffusion path). Comparably large gradients have been observed *in vivo* (Leegood 1985, Stitt and Heldt 1985a,b). However, subsequent direct measurement of diffusion constants in isolated bundle sheath strands for a range of small molecular mass compounds (values of ca. $3 \mu\text{mol min}^{-1} \text{mg}^{-1}$ chlorophyll mM^{-1} gradient) has resulted in a revision of the theoretically required gradient down to 2 mM (Weiner et al 1988). Thus, gradients observed *in vivo* appear to be larger than the theoretical requirement. This might be due, for example, to changes in plasmodesmatal permeability following the isolation of bundle sheath strands.

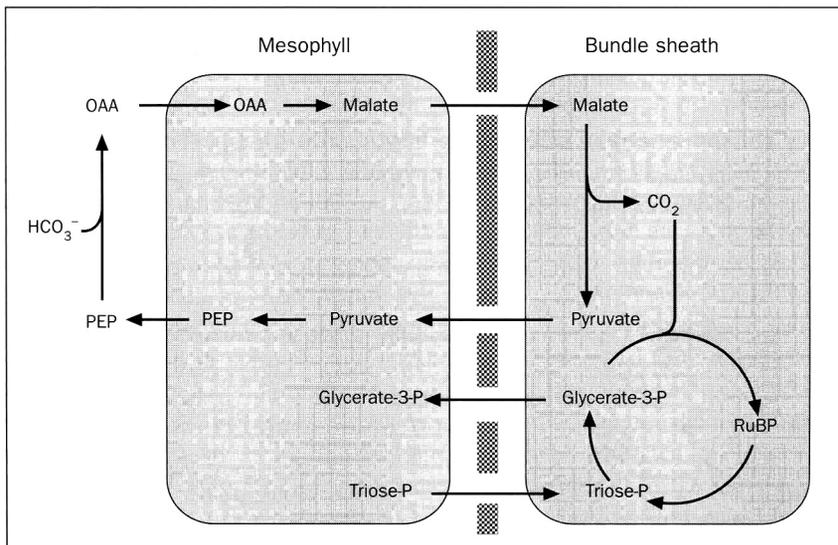


Fig. 1. The simplest form of the C_4 pathway in plants, found in NADP-malic enzyme-type species such as sorghum or sugarcane. The shaded areas represent the mesophyll and bundle sheath chloroplasts. OAA = oxaloacetate, RuBP = ribulose-1,5-bisphosphate.

How far would a plasmodesmatal frequency typical of the C₃ mesophyll–bundle sheath interface limit C₄ photosynthesis? Table 2 shows plasmodesmatal frequencies at this interface in leaves of C₃ and C₄ plants. Although the data are limited, they suggest that the C₃ plasmodesmatal frequency can be a third to a fifth that of the C₄ frequency. As plasmodesmatal frequency appears to be correlated with photosynthetic rate (Botha 1992), suggesting that this is perhaps a major controlling factor, a lower plasmodesmatal frequency could result in proportionately lower fluxes between the mesophyll and bundle sheath in C₃ plants that were engineered to express the C₄ cycle between these cells. Alternatively, proportionately increased metabolite gradients would be required to overcome the diffusive resistance.

How far do the structural modifications that allow metabolite transport also allow leakage of CO₂ out of the bundle sheath? Direct measurements of the inorganic carbon pool show that it is about 10 times the mesophyll CO₂ concentration (Hatch 1999). Detailed modeling of the compartmentation of the inorganic carbon pool in the mesophyll and bundle sheath has shown that the efflux of HCO₃⁻ via plasmodesmata is insignificant compared to the flux of C₄ acids (Furbank and Hatch 1987). It has therefore been suggested that leakage of HCO₃ via the plasmodesmata is not likely to be a serious problem, nor is the leakage of CO₂, because the diffusion coefficients of gases in solution are 10⁴ times less than in air. One factor that may affect leakage of CO₂ from the bundle sheath is the occurrence of a suberized lamella. It is absent in dicotyledonous species, and in grasses is present only in species with either an uneven bundle sheath outline or centrifugally located chloroplasts. In those species with uneven cell outlines, the suberized lamella may be important in restricting CO₂ leakage through the high surface area of the bundle sheath–mesophyll interface (Hattersley 1992). Estimates made by Jenkins et al (1989) suggest that, on average, those species with a suberized lamella do have a lower physical conductance to diffusion of CO₂ than those without it.

All three decarboxylases involved in C₄ photosynthesis release CO₂, which is also the substrate for Rubisco, in contrast to phosphoenolpyruvate (PEP) carboxylase, which uses HCO₃ (Chollet et al 1996). It is therefore crucial that compartments in which inorganic carbon is concentrated around Rubisco, such as the bundle sheath, lack carbonic anhydrase, which would convert the CO₂ to bicarbonate (Table 1, Jenkins et al 1989).

Regulatory consequences of intercellular metabolite transport

Changes in concentration of metabolites are important in coordinating the C₄ cycle with other processes of photosynthetic metabolism in several ways (Walker and Leegood 1999).

Table 2. Plasmodesmatal frequencies at the mesophyll–bundle sheath interface in leaves of various C₃ and C₄ plants.

Plant	Frequency (mm ⁻¹ of interface)	Reference
C₄		
<i>Saccharum hybrid</i>	0.60–0.75	Robinson-Beers and Evert (1991)
<i>Eragrostis plana</i>	1.05	Botha (1992)
<i>Panicum maximum</i>	0.57	Botha (1992)
<i>Themeda triandra</i>	0.35	Botha (1992)
<i>Amaranthus retroflexus</i>	1.09	Fischer and Evert (1982)
C₃		
<i>Coleus blumei</i>	0.12–0.19	Fischer (1986)
<i>Bromus unioloides</i>	0.19	Botha (1992)

Direct coupling. In NADP-malic enzyme (NADP-ME) species, such as maize, the C₄ cycle is coupled to the Benson-Calvin cycle, because NADPH produced by the decarboxylation of malate in bundle sheath chloroplasts is used to reduce glycerate-3-P, an intermediate of the Benson-Calvin cycle.

Interconversion of metabolites of the C₃ and C₄ cycles. Interconversion of glycerate-3-P and PEP, by phosphoglycerate mutase and enolase in the mesophyll cytosol, provides metabolic communication between the C₄ and Benson-Calvin cycles.

Metabolite effectors. Several intermediates of the C₄ and Benson-Calvin cycles act as effectors of enzyme activity. This has important consequences for the regulation of PEP carboxylase and of starch and sucrose synthesis, and perhaps other processes. For example, triose phosphates and hexose phosphates stimulate PEP carboxylase activity, whereas malate inhibits it (Gadal et al 1996).

Consequences for carbohydrate synthesis. In maize, the synthesis of sucrose occurs in the mesophyll cells, although up to a third of the sucrose-phosphate synthase may occur in the bundle sheath of some C₄ plants (Lunn and Furbank 1999), where one of its major functions may be sucrose synthesis following starch degradation at night. The ultimate substrate for sucrose synthesis, triose-P (and hence the amount of fructose-1,6-bisphosphate, FBP), is present in the mesophyll of C₄ plants at far higher concentrations than in C₃ plants. The cytosolic fructose-1,6-bisphosphatase acts as a valve in photosynthetic metabolism in that it not only controls the rate at which carbon is withdrawn for sucrose synthesis but also, via its substrate affinity, sets a threshold for such withdrawal. In maize, the cytosolic fructose-1,6-bisphosphatase has a much higher K_m for FBP (20 mM in maize versus 3 mM in spinach: Stitt and Heldt 1985a).

In leaves from a wide range of C₄ plants grown under normal light conditions, starch is found only in the bundle sheath. However, growth of maize plants in continuous light or at low temperatures leads to starch accumulation in the mesophyll. As with fructose-1,6-bisphosphatase, the properties of ADP-glucose pyrophosphorylase are modified to suit the metabolic environment provided by C₄ photosynthesis. Glycerate-3-P is an activator and P_i an inhibitor, with ratios of glycerate-3-P/P_i for half-maximal activation between 7 and 10 for the bundle sheath enzyme and 9-16 for the mesophyll enzyme, compared with a ratio of less than 1.5 for half-maximal activation of the spinach enzyme (Spilatro and Preiss 1987).

Signal transduction. An increase in glycerate-3-P concentration upon illumination is thought to increase the pH of the mesophyll cytosol, which brings about an increase in the concentration of cytosolic Ca²⁺ by increasing the permeability of Ca²⁺ channels in the tonoplast. An increase in cytosolic Ca²⁺ is proposed to be a component of a signal transduction pathway that leads to the phosphorylation and therefore activation of PEP carboxylase (Giglioli-Guivarch et al 1996).

Reduction of glycerate-3-P in the mesophyll. All C₄ subtypes possess the enzymes for glycerate-3-P reduction in the mesophyll chloroplasts (Hatch and Osmond 1976). This has consequences for the regulation of the C₄ cycle, of carbohydrate synthesis, and of electron transport. In NADP-ME-type species, such as maize and sugarcane, the low photosystem II activity in the bundle sheath means that NADPH can only be generated by NADP-ME, which is sufficient to reduce only 50% of the glycerate-3-P generated in the bundle sheath. The remaining glycerate-3-P is exported to the mesophyll for reduction. However, even in those C₄ subtypes that have photosystem II in the bundle sheath, glycerate-3-P is reduced in the mesophyll. It can thus be inferred that the glycerate-3-P/triose-P shuttle between the bundle sheath and mesophyll is important in C₄ metabolism. Its functions could be

1. Coordination of the Benson-Calvin cycle and C₄ cycle turnover, as discussed above.
2. The shuttle is a means of ensuring H⁺ transport, and hence charge balance, between the

two cell types. The reduction of glycerate-3-P to triose-P in the mesophyll consumes a proton, after which the triose-P is transported to the bundle sheath. The consumption of a proton in this reaction is necessary because a proton is released when CO₂ is hydrated and the resulting HCO₃⁻ is fixed by PEP carboxylase.

3. The shuttle will result in a decrease in the amount of reductant (NADPH) required for reduction of glycerate-3-P in the bundle sheath and hence a decrease in photosynthetic O₂ evolution in the bundle sheath. Photosynthetically generated O₂ may present a problem in all C₄ subtypes because reduced permeability to CO₂ also reduces permeability to O₂, which could lead to a buildup of O₂ in the bundle sheath. Any reduction in the O₂ concentration in the bundle sheath would further favor the carboxylation of ribulose 1, 5-bisphosphate over its oxygenation. In addition, both NAD-ME and PEP carboxykinase (PEPCK)-type species will show enhanced respiratory uptake of O₂ in the bundle sheath.

To what extent would it be necessary to introduce such regulatory mechanisms into genetically engineered CO₂-concentrating mechanisms? In any system, the rate at which CO₂ is delivered to the site of Rubisco has to be closely matched to the rate of its consumption, either by fixation or by leakage. The energy input to the CO₂ pump is wasted if it operates faster than CO₂ can be fixed by Rubisco and the CO₂ pump is clearly ineffective if it works more slowly than CO₂ can be fixed by Rubisco. For C₄ plants, Furbank et al (1990) have considered the energetic implications of slippage between the C₃ and C₄ cycles. It is possible that there would be a degree of regulation by CO₂ concentration alone, since all three decarboxylases involved in C₄ photosynthesis catalyze reactions that are close to equilibrium and the activity of these enzymes will therefore be modulated by CO₂ concentration by simple mass-action. The effectiveness of such a mechanism is open to question.

Specialization of electron transport pathways

The most obvious feature of electron transport in C₄ plants is the very low photosystem II activity in the bundle sheath chloroplasts of NADP-ME plants such as sugarcane, sorghum, and maize. Photosystem I-dependent cyclic electron transport leads to ATP formation (Chapman et al 1980, Leegood 1985). NADPH required for the reductive phase of the Calvin cycle is generated by NADP-ME, which is sufficient to reduce half of the glycerate-3-P, the remainder being exported to the mesophyll. The lack of O₂-evolution in the bundle sheath is presumably an adaptation to reduce the O₂ concentration in the bundle sheath, although export of glycerate-3-P also achieves integrated regulation of metabolism (see above). However, both NAD-ME- and PEPCK-type C₄ plants have normal noncyclic electron flow in the bundle sheath. The second important feature is that the C₄ cycle is essentially an ATP-driven CO₂ pump, requiring 2 ATP per CO₂ transported. This ATP is required by pyruvate. Pi dikinase (PPDK) in the mesophyll and it is notable that C₄ mesophyll chloroplasts readily catalyze cyclic electron transport, presumably to meet this extra ATP demand (Crowther et al 1983), although this might also be met by a Q-cycle mechanism (see von Caemmerer and Furbank 1999 for discussion).

Specialized metabolite transporters

The transport of metabolites between different subcellular compartments is mediated by transport proteins and is an essential component of the C₄ cycle, although relatively little is known about transport into the bundle sheath compared with the mesophyll chloroplasts. The exchange of metabolites occurs at a much greater rate than in C₃ plants and C₄ plants possess translocators

with unique, or considerably altered, kinetic properties (Heldt and Flügge 1992) that can transport key metabolites of the C_4 cycle, such as C_4 acids, pyruvate, oxaloacetate, and PEP. For example, the exchange of PEP, P_i , glycerate-3-P, and triose-P occurs on a common phosphate translocator in the mesophyll chloroplast envelope of C_4 plants. The affinity for PEP of this translocator is very much higher than that of the phosphate translocator in C_3 chloroplasts: the K_i (PEP) (i.e., the concentration of PEP required for 50% inhibition) for P_i transport is 4.7 mM in spinach and 0.086 mM in maize, a 55-fold difference (Gross et al 1990). Chloroplasts also transport pyruvate on a specific carrier in both C_3 and C_4 plants, but again the translocator is much more active and is light-dependent in mesophyll chloroplasts of C_4 plants (Flügge et al 1985).

In C_3 and C_4 plants, the dicarboxylates (oxaloacetate, malate, 2-oxoglutarate, glutamate, and aspartate) undergo counter-exchange across the chloroplast envelope on the dicarboxylate translocator. The K_m for uptake of a particular dicarboxylic acid is similar to the K_i for the inhibition of uptake by other dicarboxylates (Day and Hatch 1981). Oxaloacetate uptake would not occur when oxaloacetate concentrations are several orders of magnitude less than malate concentrations, as occurs in malate-forming C_4 plants such as maize. There is an additional oxaloacetate carrier in maize mesophyll chloroplasts (K_m 45 μ M) that is little affected by malate (K_i 7.5 mM) and this carrier is also present, with a lower capacity, in chloroplasts of the C_3 plant spinach (Hatch et al 1984).

Clearly, limitations imposed by C_3 transporters on the transport of intermediates of the C_4 cycle between the cytosol and the chloroplast could very easily occur, but the extent of these limitations will ultimately depend on the concentrations of all the substrates. It would seem desirable to introduce specific transporters in any program to engineer a CO_2 -concentrating mechanism.

New roles for mitochondria in photosynthesis

It is easy to overlook the fact that, in C_3 plants, mitochondria play a key role in photosynthesis because of their participation in the photorespiratory pathway. In some NADP-ME-type species, such as maize, mitochondria are the site of aspartate transamination, whereas in the simplest C_4 system, such as sugarcane, mitochondria do not appear to be directly involved in photosynthesis (Fig. 1). However, in many other C_4 plants (and in C_3 - C_4 intermediates), there has been enhancement of the role of mitochondria and their frequency in the bundle sheath. Both NAD-ME- and PEPCK-type C_4 plants use NAD-ME to decarboxylate malate in the bundle sheath mitochondria (Carnal et al 1993). Since photosynthetic fluxes vastly exceed respiratory fluxes, this requires some uncoupling of C_4 acid decarboxylation from normal mitochondrial metabolism. In NAD-ME-type species, NAD-ME appears to be freed of respiratory control by engagement of the alternative, cyanide-insensitive pathway of respiration (Agostino et al 1996). In PEPCK-type species, mitochondrial respiration generates the ATP required for the operation of PEPCK (Carnal et al 1993), which is largely due to oxidation of NADH generated in malate oxidation via NAD-ME. As in NAD-ME-type species, there may be some oxidation of NADH via the alternative oxidase (Agostino et al 1996). However, it would appear preferable to decarboxylate via PEPCK, since direct production of PEP and its transport to the mesophyll means that the ATP requirement for PEPCK is circumvented.

Clearly, any genetically modified system would avoid a mitochondrial component. There is no particular reason to suggest that mitochondrial involvement gives NAD-ME or PEPCK plants any particular advantage, although in Australia there appears to be an advantage in having NAD-ME, rather than NADP-ME, in regions of lower rainfall (Henderson et al 1995).

Regulation of enzyme activity in C₃ and C₄ plants

As in the Benson-Calvin cycle, the majority (if not all) of the enzymes involved in the C₄ pathway are subject to control either directly or indirectly by light. Upon illumination, changes in the concentration of many metabolites occur. These changes can modulate enzyme activity in two ways: (1) they may be effectors of an enzyme and alter its activity by directly interacting with it or (2) they may cause a change in the activation state of the enzyme by activating, either directly or indirectly, signal transduction pathways that result in covalent modification of the enzyme by reversible protein phosphorylation, as happens with PEP carboxylase (Giglioli-Guivarc'h et al 1996) and PPDK in the mesophyll and PEPCK in the bundle sheath of some C₄ plants (Walker et al 1997). Light can also activate C₄ enzymes by bringing about a change in conformation as a result of reduction of disulfide groups. This is mediated by a coupling of photosynthetic electron transport to the reduction of thioredoxin, a soluble protein that reduces disulfide groups on proteins. NADP-malate dehydrogenase (NADP-MDH) is regulated in this way, as are five of the Benson-Calvin cycle enzymes (fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, glyceraldehyde-P dehydrogenase, ribulose-5-P kinase, and Rubisco, via Rubisco activase). Illumination can also modulate enzyme activity by bringing about changes in concentrations of ions and pH. For example, upon illumination, the pH of the chloroplast stroma rises from 7 to 8 and the concentration of Mg²⁺ rises from 1-3 mM to 2-6 mM (see Leegood et al 1985); both of these factors bring about an increase in the activity of NADP-ME and probably other enzymes, such as PPDK.

Photosynthetic carbon metabolism in either C₃ or C₄ plants is a remarkable process in that few, if any, other metabolic pathways are capable of such large and rapid flux changes, even increasing rapidly from zero in the case of a darkened leaf. These changes in flux can occur in a matter of a few minutes or seconds under natural conditions, particularly in response to fluctuations in light intensity. As Fell (1997) points out, these large flux changes in a pathway cannot be brought about by changes in the activities of one or two enzymes, and multisite, coordinated modulation is necessary. This is undoubtedly one of the functions of light regulation in photosynthesis, but the function of some phosphorylation mechanisms is unclear. For example, PEP carboxylase takes an hour or more to be fully phosphorylated upon illumination of leaves (Gadal et al 1996). In this case, phosphorylation could simply reinforce metabolite control (phosphorylation of PEP carboxylase changes its sensitivity to metabolite effectors, such as malate and glucose-6-P, Chollet et al 1996).

Thus, in artificially created CO₂-concentrating mechanisms, it would appear necessary to introduce some mechanism that tailors *in vivo* enzyme activity to the flux. This could occur either at the level of metabolite modulation or by covalent modification. In either case, it would be necessary to introduce the enzyme from an appropriate source (i.e., from a plant rather than an unregulated bacterial PEP carboxylase) and to ensure that the appropriate regulatory mechanisms are in place (e.g., kinases).

Conclusions

The above considerations may well induce an air of gloom and despondency. It is apparent that regulation is complex and in many instances appears to be specifically geared to the C₄ syndrome. Are there too many structural and regulatory aspects to manipulate? C₄ photosynthesis has evolved independently many times from C₃ photosynthesis, so this cannot involve too many steps or gross changes in the pattern of regulation and there seem to be several solutions to the same problem within C₄ photosynthesis (exemplified by three biochemical subtypes and their

variants). Evidence shows that the C₄ mechanism is inducible, either in single-cell systems, such as *Hydrilla*, or in its full glory in the amphibious leafless sedge, *Eleocharis vivipara*, which has C₃ biochemical traits under submerged conditions, but develops C₄ biochemical traits, as well as Kranz anatomy, under aerial conditions, a process regulated by abscisic acid (Ueno 1998). An additional observation is that C₄ mutants of *Amaranthus edulis*, lacking either the C₄ isozyme of PEP carboxylase (Dever et al 1995) or NAD-ME activity (Dever et al 1998), and therefore lacking the ability to concentrate CO₂, grow normally (plants are virtually indistinguishable from the wild type) in 0.7% CO₂. This demonstrates that C₃ photosynthesis and carbohydrate synthesis and export can function efficiently in a C₄ structural and regulatory background. How this occurs has not been investigated and it is not necessarily true that the converse applies. However, it gives considerable cause for optimism that the introduction of limited C₄ traits into a C₃ background will function effectively.

It is clear from a consideration of CO₂-concentrating mechanisms in a variety of photosynthetic systems (Table 1) that single-cell CO₂-concentrating mechanisms are effective in algae and cyanobacteria and that a single-cell C₄-like system operates in *Hydrilla verticillata*. A C₄ system lacking Kranz anatomy also appears to operate in aquatic leaves of *Orcuttia* spp., which is a grass that can grow either terrestrially or in seasonal pools (Keeley 1998). Engineering single-cell CO₂-concentrating mechanisms into a leaf is a considerably less complicated task than the additional introduction of the Kranz anatomy found in terrestrial plants. The simplest theoretical system would be PEP carboxylation in the cytosol, followed by oxaloacetate transport into the chloroplast, decarboxylation by PEP carboxykinase in the chloroplast, and then transport of PEP back to the cytosol. This could also be achieved at the expense of 1 ATP per CO₂ transferred. Why no terrestrial plant has developed a single-cell CO₂-concentrating mechanism is not clear—may be that the system is unsuitable for the aerial environment. Thus, it may be necessary to have a compartment in which CO₂ leakage can be minimized.

Recent attempts to engineer the *Hydrilla* -type system into potato suggest that even modest expression of PEP carboxylase and NADP-malic enzyme can influence photorespiratory characteristics. For example, a threefold overexpression of PEP carboxylase from *Corynebacterium glutamicum* in potato led to an increase in the rate of dark respiration and a decrease in the CO₂-compensation point measured in the absence of dark respiration (Hausler et al 1999). Double transformants with an additional three- to fivefold overexpression of *Flaveria pringlei* NADP-ME in the chloroplast showed a temperature-dependent decrease in the electron requirement for CO₂ assimilation, again suggesting the suppression of photorespiration (Lipka et al 1999).

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Notes

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C₄ photosynthesis in rice: some lessons from studies of C₃ photosynthesis in field-grown rice

P. Horton and E.H. Murchie

The sites of limitations on photosynthesis in rice are reviewed for the purpose of developing a strategy to bring about an increase in yield. On the basis of measurements made on irrigated rice under tropical conditions, the sites of real and potential photosynthetic losses are identified. It was found that rice was not optimally adapted for photosynthesis under high irradiance, with light saturation of upper leaves and midmorning depressions of photosynthetic capacity. The limitation of photosynthetic capacity may occur because of the combined effects of developmental programming and incomplete acclimation to high irradiance. Some rice varieties appear to have Rubisco levels well in excess of those required to support measured photosynthetic rates, suggesting its role as a store of leaf N. The important interaction between photosynthetic capacity and the mobilization of Rubisco as a source of leaf N for grain development is discussed. These findings are considered in the context of the introduction of C₄ photosynthesis into rice. In this regard, the implications of the increased ATP/NADPH requirement of C₄ photosynthesis are also assessed in terms of electron transport.

To meet the demand for food from the growing world population, there will have to be significant increases in yield of the major crops grown in developing countries. In rice, for example, an estimated 50% yield increase is needed by 2050. Increasing the maximum yield potential is viewed as an essential part of any strategy, for achieving this increase in yield (Khush and Peng 1996). Since the harvest index for many crops such as rice is approaching a ceiling value, an increase in yield potential will have to involve an increase in crop biomass, that is, there will have to be a higher rate of canopy photosynthesis and an increase in efficiency of radiation conversion (Ying et al 1998, Murchie et al 1999, Horton 2000, Mann 1999a,b). Radiation conversion efficiency can be defined in terms of dry matter produced per unit of intercepted photosynthetically active radiation (PAR) and will be defined here as radiation conversion factor (RCF) (Mitchell et al 1998). An increase in RCF may be achieved by an increase in net canopy photosynthesis per unit PAR intercepted. The absolute amount of PAR intercepted depends on the angular arrangement of the leaves and the leaf area index (LAI). Since LAI is generally already high in rice, increased assimilate production must come from improved photosynthesis at the leaf level.

Photosynthetic rate is determined by the combination of several factors: (1) photosynthetic capacity, essentially the content of the proteins involved in photosynthesis; (2) the delivery of principal inputs—levels of CO₂ and the intensity of sunlight; (3) external factors such as temperature and water availability; (4) removal of carbohydrate products from the leaf for new growth; and (5) regulatory mechanisms that adjust photosynthetic activity to below the maximum capacity.

Each of these factors interacts in different ways according to the climatic conditions and the developmental state of the plant. In terms of crop yield, the decisive factor is not how these behave at the individual leaf level but how the operations of all the leaves are integrated to provide carbon gain to support grain yield. This integration is not just in the cumulative effect of the photosynthetic activity of the leaves. Also important is its formative effect that determines the rate of construction of new photosynthetic area during the vegetative stage of crop growth, and the rate of loss of photosynthetic capacity during reproductive growth associated with leaf senescence and panicle development. Other important factors determining canopy photosynthetic rate are the number of tillers per plant and plant density.

Mathematical models of crop performance can estimate the impact of particular facets of leaf performance on yield. The RCF of rice crops is among the lowest for C_3 species (Mitchell et al 1998). A significant loss arises because of the decrease in radiation conversion resulting from photorespiration. Photorespiration not only results in the loss of fixed carbon but also wastes catalytic turnover of Rubisco and RuBP (ribulose-1,5-bisphosphate) generated from Ru5P (ribulose 5-phosphate) using photosynthetically produced ATP. Since attempts to suppress photorespiration by protein engineering of Rubisco have so far proved unsuccessful, attention has turned to the question of whether introduction of the C_4 pathway into rice represents a feasible strategy for improving crop photosynthesis and yield. In the C_4 pathway, photorespiration is largely suppressed by virtue of a CO_2 -concentrating mechanism in which CO_2 is fixed in organic acids via phosphoenolpyruvate (PEP) carboxylase, the acids later releasing the CO_2 at a higher concentration to be fixed by Rubisco. The exact details of the pathway differ in different C_4 types, but the essential feature is that primary fixation occurs in the mesophyll cells and requires additional ATP input to provide the carboxylation substrate PEP. A second seemingly obligatory feature is that secondary carboxylation via Rubisco occurs in chloroplasts of the bundle sheaths, this being necessary to maintain the CO_2 concentration gradient, and in some types provide a relatively low O_2 environment. As a consequence of the requirement for extra ATP, the theoretical quantum requirement of C_4 photosynthesis is higher than that of C_3 photosynthesis (in high CO_2). Whether the actual quantum yield of C_3 and C_4 photosynthesis differs in the field depends mainly on the external temperature. Other features of C_4 to consider with respect to the rice crop are, first, the improved N-use efficiency of C_4 photosynthesis because of the reduced requirement for Rubisco, and, second, the improved water-use efficiency because of the adequacy of a lower stomatal conductance to optimize the internal CO_2 .

However, photorespiration is not the only factor that may reduce radiation conversion; decreases in the value of RCF may arise from respiration and from canopy Factors (which include light saturation of photosynthesis). It has been concluded that an increase in canopy efficiency has considerable potential for increasing RCF in rice (Mitchell et al 1998) and this would require more leaves operating below light saturation when the quantum yield is highest. Under conditions optimal for plant growth and yield, a limit to RCF occurs due to saturation of photosynthesis because of the ceiling value for the light-saturated rate of photosynthesis (P_{max}) of upper leaves. Developmental status affects P_{max} for a given leaf, and, in rice, P_{max} of fully expanded uppermost leaves declines markedly, well before flowering (Hidema et al 1991, Peng et al 1998). The exact reasons for such declines are not fully understood, but appear to be part of the developmental program of the plant in which photosynthetic rate is tuned to the demands of plant growth and development. Internal and external factors may also determine when (or if) P_{max} is reached in field conditions, and, even under "optimal" conditions, photosynthetic capacity is down-regulated at certain points during the day (Huang et al 1989, Black et al 1995, Murchie et al 1999). Although losses from photorespiratory activity are

relatively easy to estimate, these other effects are much more difficult to quantify. In addition, as we argue below, photosynthesis is closely connected to aspects of nitrogen economy.

Therefore, in terms of estimating the benefits of introducing C₄ photosynthesis into rice, it is necessary to examine all of these aspects of photosynthesis in rice under conditions of high yield where at first sight elimination of photorespiratory activity would appear to be most beneficial.

Losses in operational photosynthesis in the rice canopy

Diurnal depressions in photosynthesis

Midday depression of photosynthesis is a common occurrence in plants. A new plant type (NPT) rice has been developed for high yield using tropical japonica germplasm. In NPT rice, the depression was observed not at midday but during midmorning (Fig. 1, Murchie et al 1999). Because of the erect posture of the upper rice leaves (see below), this result links this phenomenon to attainment of peak irradiance, which occurs around 0900, rather than a diurnal cycle. The speed at which it occurs does not suggest that it arises because of feedback inhibition through buildup of photosynthate (Winder et al 1998)—indeed, measurements of leaf carbohydrates indicate low pool sizes at the time of the onset of inhibition (data not shown). There are many potential sources of lack of balance in the photosynthetic system and, in theory, these could lead to a depression of photosynthesis—in this sense, the depression results from the inability of the photosynthetic reaction to sustain flux at the maximum capacity. Limitations to ATP supply, caused by excessive ATP demand or a restriction on P₁ availability, could arise, although in the laboratory such effects have only been observed with photosynthesis in high CO₂.

A more likely explanation is that the inhibition results from a decrease in stomatal conductance. This could arise because of a developing water deficit despite the availability of water to the root system, which could be triggered by the increase in leaf temperature. A clearer understanding of this phenomenon is urgently required because it results in an estimated loss of 30% daily photosynthesis. It is of interest that the depression in photosynthesis in rice varieties shows genetic variation, and a smaller midday depression was correlated with an increase in yield (Black et al 1995).

The diurnal depression of photosynthesis may be particularly relevant to considering introducing C₄ photosynthesis into rice. Under light-saturating conditions, at leaf temperatures where there is also significant photorespiratory flux, the strains on the electron transport system might be significant. At light saturation and high temperature, rates of ATP synthesis need to be high, requiring a high transthylakoid proton gradient (Δ pH). Under such conditions, there may be three problems: (1) slippage of the protonmotive Q-cycle at high Δ pH, (2) an increased passive permeability of the thylakoid membrane to H⁺, and (3) inhibition of electron transport at high Δ pH. Such effects can be exacerbated by a shortfall in phosphate supply. Moreover, the predicted 5–10% increase in demand for ATP from photorespiratory metabolism, and the consequent requirement for increased H⁺/e ratio, will increase the strain on the electron transport system. Therefore, under these conditions, ATP supply may not match the metabolic demand. A key question could be, What is the extent of flexibility in the electron transport system of a chloroplast? The C₄ pathway exerts a demand for higher ATP/NADPH ratios than C₃ photosynthesis (see Edwards et al, this volume), placing even more strain on the H⁺/e ratio. This important issue will be addressed further below.

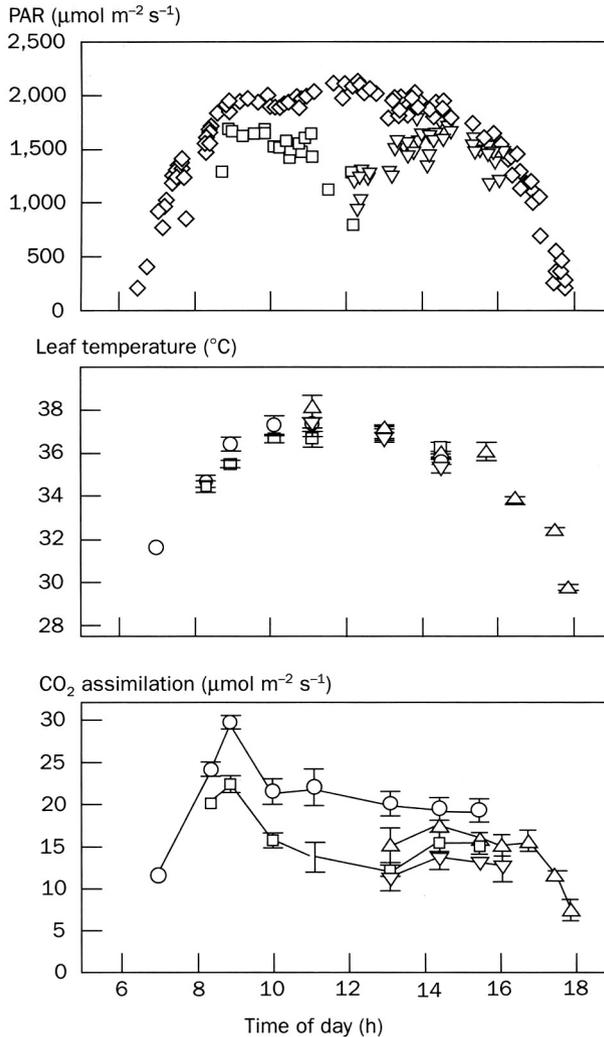


Fig. 1. Daily profiles of photosynthetically active radiation (PAR, $\mu\text{mol m}^{-2} \text{s}^{-1}$), leaf temperature, and photosynthesis for new plant type rice (IR65598-112-2) leaves at 60 d. O = face 1 direct, \square = face 1 *in situ*, \triangle = face 2 direct, ∇ = face 2 *in situ*. Direct and *in situ*, respectively, refer to measurements made with the leaf chamber placed at 90° to sunlight and maintaining the angle as close to its natural, near vertical position as possible. Face 1 and face 2 are the leaf surfaces exposed to full sunlight in the morning and afternoon, respectively. Error bars represent standard errors of the means of at least seven replicates. (Redrawn from Murchie et al 1999.)

Leaf temperature

In the field experiments, leaf temperature, determined during measurement of photosynthesis, reached 35-37 °C for rice under tropical conditions for much of the day (Murchie et al 1999). At this temperature, significant photosynthetic losses arise from increased photorespiration. Differences in P_{max} measured in the field at ambient and saturating CO_2 were large, and were mostly explained by a predicted 40% loss due to photorespiration at this temperature (Leepood and Edwards 1996). Figure 2 shows photosynthesis in air and at 1,000 ppm CO_2 recorded in

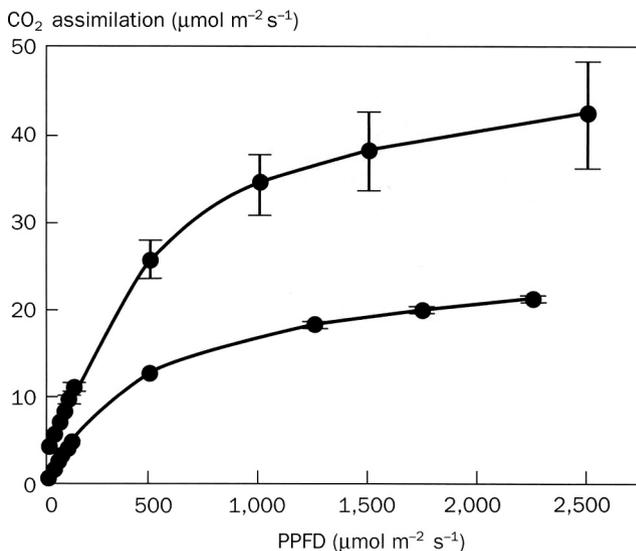


Fig. 2. Light saturation curves of new plant type rice (IR65598-112-2) taken in the field at 350 ppm (lower curve) and 900 ppm (upper curve) CO₂. Irradiance was provided by the LED array of the Li-Cor 6400 IRGA. Measurements were made at around midday 60 d following transplanting. PPFD = photosynthetic photon flux density. (Redrawn from Murchie et al 1999.)

the field with a leaf temperature of 37 °C. Such high leaf temperatures may also affect other aspects of photosynthesis, particularly NADPH/ATP supply (Pastenes and Horton 1996).

Photoinhibition (and down-regulation)

Young leaves, even those uppermost in the canopy, did not suffer chronic photoinhibition at peak irradiance, and any decrease in photochemical efficiency was transient, and reversed as the irradiance declined again (Murchie et al 1999). Examination of kinetics of recovery from potentially photoinhibitory conditions indicated that the dark-adapted F_v/F_m was suppressed over a lag period, but these decreases were relatively small and diminished in line with the change in irradiance.

In older leaves, when a significant proportion adopted a more horizontal orientation, photoinhibition was stronger, and did not recover so readily as in younger erect leaves when the irradiance declined. Again, however, the decline in F_v/F_m did not persist, and recovery was complete overnight. It is hard to argue that, in an irrigated crop, in a tropical environment as found at IRRI, photoinhibition represents a major source of photosynthetic loss.

Equally, however, under these conditions, acclimation to high light reached its maximum in terms of the irradiance-dependent increase in P_{max} and xanthophyll cycle pool size (see Table 1), and could be considered just at the threshold of significant light stress. Therefore, in more extreme conditions, where light intensities are higher and sustained for longer periods, and where water management is perhaps less comprehensive than at IRRI, photoinhibition could become a problem. Certainly, the fact that rice under favorable conditions is so close to light stress indicates that many other crops in more marginal habitats probably suffer considerable photosynthetic losses through photoinhibition.

Table 1. Effect of growth irradiance on photosynthetic characteristics of field-grown rice. New plant type rice (IR65600-42-5-2) plants were grown in the field and exposed to differential shading for 4 wk. The irradiance values are the approximate mean peak irradiances at midday. P_{\max} , Chl content, and xanthophyll cycle contents were determined for upper leaves as described by Murchie et al (1999). Rubisco content was determined following polyacrylamide gel electrophoresis of soluble protein, and quantified by densitometry of the Coomassie stained gel, relative to Rubisco standards. Note the near saturation of P_{\max} at 800, but the large increase in Rubisco at 2,000. Increased photoprotection when acclimation of photosynthesis is saturated at 1,000 is evident from the decrease in Chl and elevation of xanthophyll cycle pool size.

Irradiance ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	P_{\max} ($\mu\text{mol CO}_2 \text{m}^{-2}\text{s}^{-1}$)	Chl content (mg m^{-2})	Rubisco (g m^{-2})	Xanth cycle (% total carotenoid)
200	15.1 \pm 2.4	521 \pm 2	3.20 \pm 0.56	20.3 \pm 0.4
800	21.1 \pm 1.1	485 \pm 2	4.38 \pm 0.55	23.3 \pm 1.0
2,000	24.8 \pm 0.9	401 \pm 2	7.41 \pm 1.02	36.1 \pm 8.0

Demonstration of photosynthetic losses in the field

Visualization of suboptimal radiation conversion may be made by recording photosynthetic activity and irradiance for leaves in the field (Fig. 3). If leaves in a similar position are studied, then irradiance-dependent changes in P_{\max} (see below) or developmental changes (see below) are eliminated. It can be seen that all data fall below an upper limit, which represents the ceiling irradiance response, that is, maximum quantum yield and maximum P_{\max} . The deviations from this line are large, and indicate a high frequency of underperformance of leaf photosynthesis. Such observations have been made previously in natural populations of plants exposed to multiple stresses, and have been attributed to various factors, all of which are discussed above (Cheesman et al 1991). What is remarkable is the extent and frequency of underperformance of photosynthesis under apparently optimal agricultural conditions.

Limitations imposed by photosynthetic capacity

Light saturation of photosynthesis

Photosynthesis of the upper leaves of rice shows a high degree of light saturation, at light levels well below full sunlight, with a consequent decrease in RCF. The extent of light saturation is predicted from the measured irradiance in comparison with a typical irradiance vs photosynthesis curve of NPT rice (Fig. 2). Photosynthesis of field-grown plants saturated at approx. $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$, well below the peak growth light, and consequently field quantum efficiencies drop to low values for large parts of the day. Light saturation can be more easily determined for each leaf from measurement of Chl fluorescence. In the light-limited part of the irradiance curve, the parameter Φ_{PSII} (the quantum efficiency of photosystem II) has a value of 0.75–0.8, close to the maximum quantum yield. Thus, the values of 0.2–0.3 recorded during the peak morning irradiances indicate strong light saturation, and would give rise to a 2.5–4-fold decrease in RCF for those leaves. The light stress imposed under such conditions is also evident—the value of $q\text{P}$, an indication of the oxidation state of photosystem II, falls to values of approximately 0.4 (Fig. 4).

The vertical leaf orientation meant that photosynthetic rates displayed by the upper leaves, which have the largest investment in photosynthetic components, were low at midday due to poor light absorption at high solar angles. In the morning and afternoon, in contrast, the upper leaves were light-saturated. This complexity of the relationship between canopy structure and

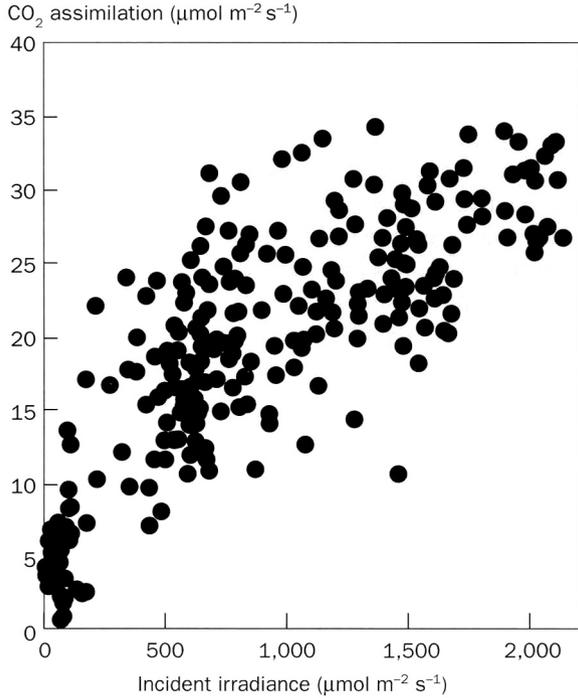


Fig. 3. Net CO₂ assimilation rate plotted as a function of irradiance for new plant type rice leaves in the field. Irradiance and photosynthesis were measured simultaneously on the first leaves, which were at the same stage of development. Points include data obtained at IRRI during the dry season of 1997 and 1998. Each point represents a single measurement.

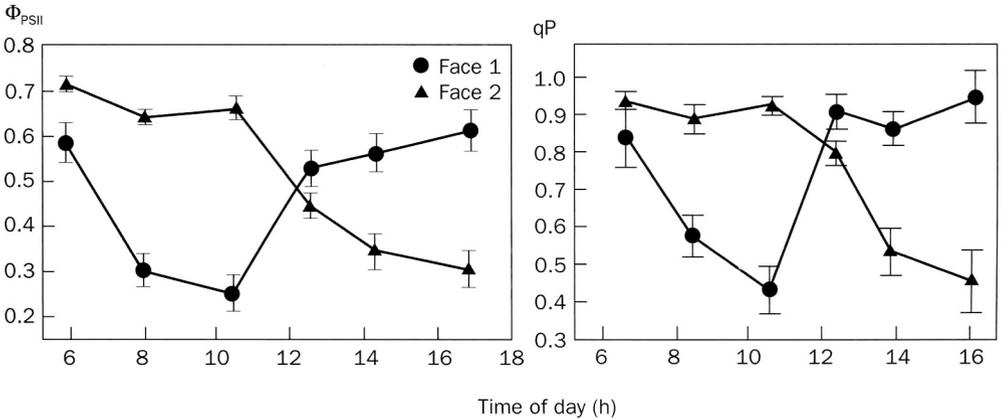


Fig. 4. Daily profiles of photochemical efficiency (Φ_{PSII}) and photochemical quenching (qP) for new plant type rice (IR65598-112-2) at 60 d. Error bars represent standard errors of the means of at least 12 replicates. (Redrawn from Murchie et al 1999.)

solar angle relates to arguments concerning the value of erect leaves in improving efficiency of light use (Sinclair and Sheehy 1999).

The ceiling level for P_{\max}

Most plant species respond to increasing growth irradiance by altering the amounts of photosynthetic proteins, resulting in elevated P_{\max} (Walters and Horton 1994, Murchie and Horton 1998). Such photosynthetic acclimation maintains ambient photosynthesis at a point well below light saturation, maintaining high quantum efficiency (and high RCF). For example, in *Arabidopsis thaliana* grown over a 20-fold range of irradiance, photosystem II efficiency was maintained at greater than 0.75 and qP at 0.9 (Bailey and Horton, unpublished data). However, as growth irradiance increases, P_{\max} may be expected to approach a maximum limit, and an increasing proportion of absorbed photons are in excess. Increasingly, acclimation then becomes not an increase in photosynthetic capacity but an enhancement of photoprotective mechanisms (e.g., Murchie and Horton 1998). Thus, the radiation conversion efficiency will depend on the extent to which the irradiance-dependent increase in P_{\max} is itself saturated.

P_{\max} in rice displayed acclimation to growth irradiance in the field (Table 1), but the acclimation response became saturated well below full sunlight, a 60% decrease in irradiance having only a limited effect on P_{\max} . This explains the high degree of light saturation of photosynthesis during natural conditions, and in fact again demonstrates the poor adaptation of rice photosynthesis to tropical conditions since acclimation to the maximum growth irradiance has not fully occurred. This observation suggests that acclimation is indeed subject to light saturation and that this ceiling is reached under field conditions. Plants fully acclimated to high light intensity would not be expected to show elevated photoprotective responses since they can use a high proportion of the absorbed light for photosynthesis. The clear photoprotective responses to excess light shown by the rice plants grown at high light—a decrease in Chl content and an increase in the content of xanthophyll cycle carotenoids (Table 1, Murchie and Horton 1998)—are therefore consistent with the saturation of photosynthetic acclimation.

As P_{\max} does not increase sufficiently when plants are grown in full sunlight, they suffer effects of absorbing excess light energy—light saturation of photosynthesis resulting in a decline in radiation conversion efficiency, and a potential for photoinhibition. This failure of P_{\max} to acclimate fully to the tropical growth irradiance raises the important question as to what determines this ceiling for the acclimation of P_{\max} . On the one hand, there may be some kind of “physical” limit to P_{\max} that has been reached, for example, perhaps the maximum concentration of Rubisco in the chloroplast has been attained. On the other hand, the acclimation limit could arise because of a developmental limitation to the dynamic range of acclimation that prevents the potential P_{\max} from being attained. Thus, the value of P_{\max} may not represent a true P_{\max} but a value down-regulated by the feedback mechanisms associated with photosynthetic acclimation and development (Fig. 5). In fact, genetic intervention into these mechanisms to improve photosynthetic acclimation to high irradiance could be a most useful strategy to increase P_{\max} . An increased P_{\max} for upper leaves would of course increase RCF since the frequency of leaves displaying high quantum yield of photosynthesis would be increased.

Photosynthesis in lower leaves

The lower leaves, which receive more diffuse light of reduced intensity, contribute significantly to photosynthesis of the rice canopy. In a field experiment carried out at IRRI in 1999, the lower four leaves were removed from NPT rice, leaving just the two upper leaves. Whole-plant photosynthesis was recorded and a 45% reduction was found in midday photosynthetic rate. This observation was made on younger plants prior to panicle formation, and the canopy was fairly open, allowing good light penetration to the lower leaves. In a more mature canopy, the contribution of the photosynthetic activity of lower leaves may be less than 45%.

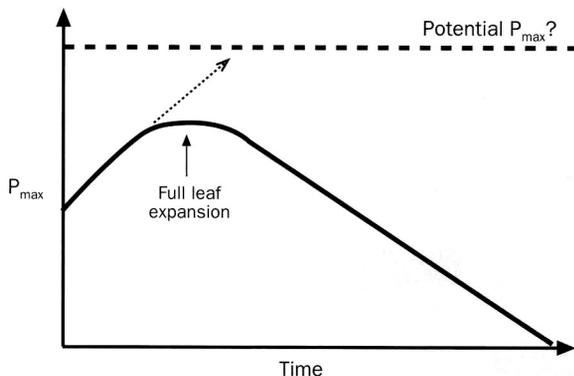


Fig. 5. Schematic representation of the changes in P_{max} of a leaf during development. The key questions are (1) Is the P_{max} at full leaf expansion the maximum theoretical value? (2) Is the decline in P_{max} , which is associated with a decrease in Rubisco content, due to acclimation to low light or to remobilization of N reserves (senescence)? (3) Is the failure to reach the potential P_{max} due to the onset of this decline or to the saturation of the high light acclimation response?

The light environment of the lower leaves is highly heterogeneous with light penetration not only being attenuated by shading but also giving rise to sun-flecks. A key question is whether sun-flecks are fully exploited for carbon gain. This is determined by two features of the photosynthetic apparatus: first, the photosynthetic capacity; second, the kinetic properties of the enzymes whose activity needs to be increased to accommodate the increased flux. The first depends on the extent to which photosynthetic acclimation has tuned the system to the peak light intensity or to the average. In the rice crop, the P_{max} of lower leaves is significantly less than that determined for upper leaves (Fig. 6). Acclimation of photosynthesis undoubtedly contributes to the changes in P_{max} of different leaves in the rice canopy (Table 1). However, P_{max} is lower than that predicted for a light acclimation response alone, and it is suggested that there is also a developmental control over photosynthesis in these leaves. One consequence of the strong decrease in P_{max} of lower leaves is that sun-flecks are expected to readily saturate photosynthesis. Hence, it can be argued that there are further declines in the efficiency of conversion of radiation owing to poor acclimation of lower leaves to irradiance.

Leaf senescence and grain filling

Photosynthesis during grain filling usually accounts for approximately 75% of total C in grain (Yoshida 1981), the remaining 25% coming from stem storage. It is frequently stated that leaf senescence begins during the period of most rapid grain filling, hence the desirability of delaying leaf senescence. Conversely, grain development acts as a dominant sink for photosynthesis and remobilizable resources. If leaf activity were dependent on sink activity, it is conceivable that an increase in leaf photosynthesis would be required during the period of most rapid grain filling, which typically occurs during the 10 d after flowering. We have noted, however, that during this period a significant portion of Chl and N can be lost from leaves without loss of photosynthetic capacity (Murchie et al 1999), which supports the notion of leaf Rubisco as a store of mobilizable N.

We simultaneously measured grain filling and photosynthetic characteristics in NPT and indica varieties during the dry season of 1999. Figure 7 shows relative grain-filling rates and

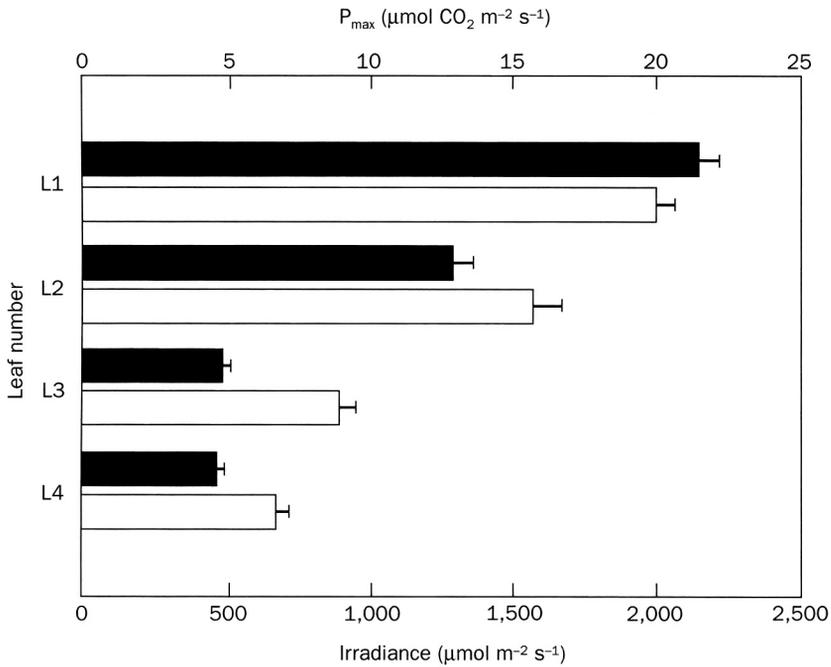


Fig. 6. Light penetration and photosynthetic capacity of a new plant type (IR65598-112-2) rice canopy. Shown are values for P_{max} (black) and irradiance (white) determined for the four leaves of the rice plant. P_{max} values are the maximum for those leaves. Irradiance was recorded at midday at the height of the middle of each leaf.

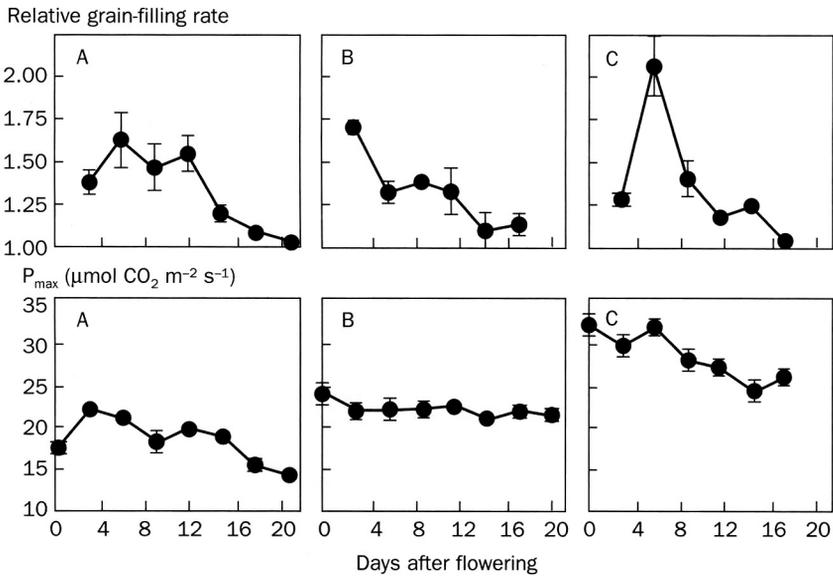


Fig. 7. Photosynthetic capacity (P_{max}) and the relative grain-filling rate of two new plant type lines, IR65600-42-5-2 (A) and IR65600-129-1-1-2 (B), and IR72. The grain-filling rate was determined from the average spikelet dry weight from panicles collected at intervals following flowering.

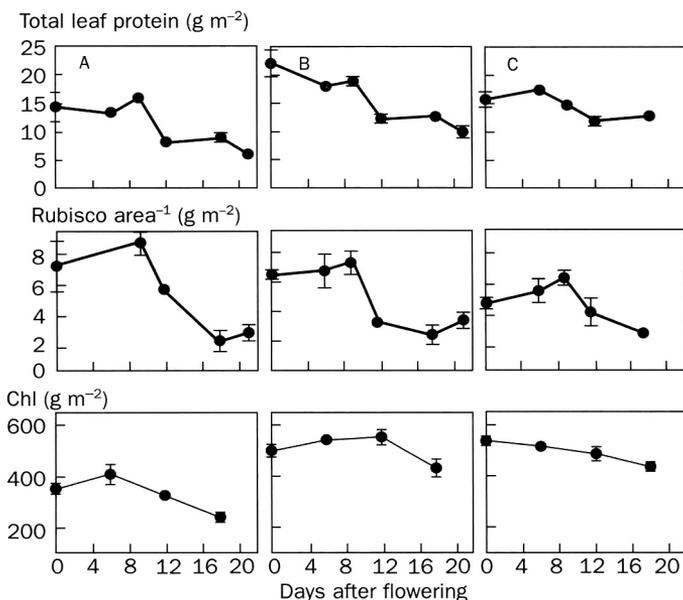


Fig. 8. Total leaf protein, Rubisco protein content, and Chl content of the flag leaf recorded as for the data in Figure 7.

photosynthesis in three varieties. IR72 had the most rapid initial rate of grain filling compared with the NPT lines. P_{max} in IR72 was also higher and, considering the saturation of photosynthesis that occurs in these conditions, we conclude that the most rapid rate of grain filling coincides with the highest rate of photosynthesis. In general, P_{max} did not decline significantly (in some cases not at all) during the period of most rapid grain filling or over the entire grain-filling period in many varieties.

During the grain-filling burst, an increase in Rubisco was observed simultaneously with a decline in total leaf protein. The increase in Rubisco did not coincide with P_{max} (Fig. 8), which may represent an “attempt” by the leaf to increase P_{max} in the face of a large sink demand. Other limitations to photosynthesis may have resulted in this increase in Rubisco content not manifesting in P_{max} . Interestingly, at the same time, a decline in total leaf N was observed.

Following the relaxation of the initial rapid grain-filling rate, a second decline in leaf N and also Rubisco was observed, possibly related to a hormonal trigger from the panicle, which initiates the onset of leaf senescence as defined by a decline in P_{max} as well as N and Chl content of leaves. P_{max} did not decline in line with the classical indicators of leaf senescence, that is, leaf protein and Rubisco (Figs. 7 and 8). In fact, P_{max} was unchanged during a period of significant loss of N from leaves.

These data suggest that P_{max} , leaf N, Rubisco, and Chl can operate independently. Excess leaf N and Rubisco mean that these can be uncoupled from P_{max} during the period of initial N loss. Chl content is dependent upon light intensity during growth (Table 1) as well as leaf N content. It is important to define which of these losses are due to irreversible leaf senescence and which may be part of a reversible acclimation response that is sensitive to sink activity.

We can conclude that there does not appear to be a significant photosynthetic loss from senescence during the grain-filling period. While it is difficult to link grain-filling rate and photosynthesis in a meaningful way, it is clear that because NPT leaves possess the potential

for a higher P_{\max} (as given by the higher leaf N and Rubisco content) they could support a higher grain-filling rate than that shown here.

Leaf nitrogen and Rubisco

An important part of the response to higher irradiance is the increase in content of Rubisco, which is a significant sink for N in C_3 crops such as rice. To improve N economy in a crop, it has been argued that photosynthesis per unit N should be enhanced—i.e., *reducing* the amount of Rubisco but maintaining P_{\max} perhaps by reducing Rubisco oxygenase activity (Mann 1999b). On the other hand, because, over a wide range of N fertilization, photosynthesis correlates positively with leaf N (Peng et al 1995), the suggested way to increase P_{\max} is to *increase* leaf N (and therefore Rubisco content). Both of these views ignore the key role of Rubisco as a reservoir of remobilizable N that is needed to sustain grain yield. Therefore, a strategy that results in lower leaf N may in fact limit crop yield under conditions designed to give high grain yields. It has been argued that the provision of N for grain production is a principal factor in determining canopy architecture (Sinclair and Sheehy 1999). It has been calculated that a 10 t ha^{-1} yield includes the harvest of 140 kg N ha^{-1} , and, since half the grain N must be translocated from leaves, an LAI of 7 is needed simply to store N before transfer to the grain. This LAI is greater than that needed for canopy closure, and the associated high respiratory costs of leaves with low photosynthetic rates is in part offset in a canopy architecture that allows light penetration to lower leaves.

Rubisco content of NPT rice was much higher than in IR72, apparently in excess of that needed to sustain photosynthesis, the higher content in NPT not being reflected in higher P_{\max} (NPT P_{\max} is actually lower than that of IR72), providing further evidence of it being an N store (Fig. 9). The increased content of Rubisco may represent an important strategy for ensuring adequate N reserves in this variety.

Partially shaded plants had a lower Rubisco level even though there was little change in P_{\max} (Table 1)—acclimation of photosynthesis and Rubisco content were not tightly coupled. We suggest that two mechanisms regulate the synthesis of Rubisco protein in rice leaves: one is concerned with maintaining levels that can support the required photosynthetic rate, and the other concerns the role of Rubisco as a store of N that is used in grain formation (Fig. 10). The storage role of Rubisco may be more strongly linked to the relationship between photosynthetic rate and the availability of nutrients (e.g., nitrate or NH_4^+) rather than being mediated by redox sensing, carbohydrate metabolism, or photoreceptors.

The level of Rubisco accumulated per plant (or crop) is determined by the requirement to accumulate sufficient N for grain production. This level of N is determined by N (and hence Rubisco) per unit leaf area multiplied by the LAI. A strategy for improving yield is to optimize the level of Rubisco in the leaf, thus reducing the requirement for excessively high LAI, and decreasing maintenance costs of inefficient highly shaded leaves. Disruption of that part of photosynthetic acclimation that results in a decline in Rubisco content (and P_{\max}) of lower leaves could maintain N storage in these leaves (Fig. 2). Such interventions in the mechanisms that regulate the amount of leaf Rubisco in turn may require a major rethinking of the design of canopy architecture (e.g., more densely packed plants but with fewer leaves per plant). This obviously has to take into account other factors such as susceptibility to lodging, ease of management, incidence of disease, etc.

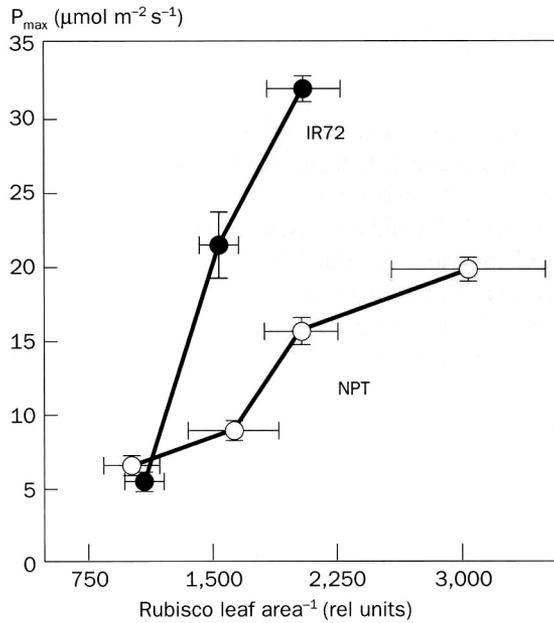


Fig. 9. P_{\max} and Rubisco protein content determined for new plant type and IR72 for four leaves of different age (see Fig. 6 for details).

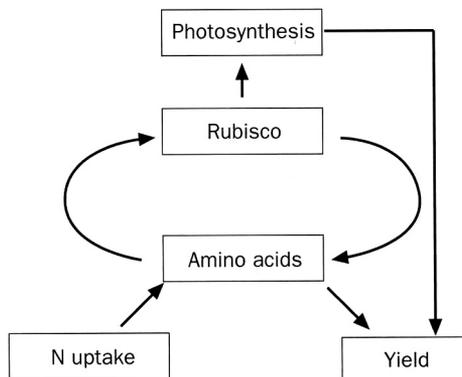


Fig. 10. Importance of optimization of N cycling through Rubisco. For example, if the rate of degradation is too high, premature senescence could restrict C assimilation; an increase in degradation arising from acclimation would lower the total amount of N stored in the lower leaves and possibly limit N availability to the grain. The rate of synthesis should be maximized to enable a large N content per unit leaf area, even though this may be greater than that needed for C assimilation. How these processes are regulated at the leaf level and how they are integrated at the canopy level are the crucial questions.

Conclusions—important questions concerning the introduction of C₄ photosynthesis into rice

Leaf nitrogen

The features of the rice canopy have been developed by plant breeders within the context of C₃ photosynthesis. As discussed above, leaf N and canopy architecture seem closely linked, and a large proportion of leaf N is in Rubisco. Since leaf N is remobilized during grain filling, the main enzyme of C₃ photosynthesis and crop yield are therefore also closely linked. Since the objective of introducing C₄ into rice is to eliminate the consequences of the inherent inefficiency of this enzyme, the effects on grain filling need to be carefully evaluated—any strategy to alter the pathway of carbon assimilation should not lead to a reduction in N available to the developing grain. C₄ plants tend to have reduced leaf N per unit area, presumably because of the reduced need for Rubisco activity. On a whole-plant basis, this is compensated for by a larger leaf area, but this adaptation would still be problematical in terms of crop yield. In the end, yield is expressed per unit land area, not per plant. It is therefore necessary to ensure that the concentration of N per unit land area is maintained after introduction in C₄. Achieving this by an increase in leaf area would not be the answer, since this will result in high respiratory losses and photosynthesis occurring under shaded conditions when C₄ may not be advantageous. Therefore, maintenance of sufficient N per unit leaf area would appear to be necessary even though this may not be required for photosynthesis. It is clear that excess levels of Rubisco can accumulate in rice leaves, particularly in some of the new plant type lines, and knowledge of the control mechanisms operating in these plants should allow such storage N to be maintained even though the requirement for enzyme activity is reduced.

Internal limitations to photosynthesis

The decreases in operational photosynthesis detected in the field suggest internal limitations to photosynthetic activity that may reduce the possible benefits from introduction of C₄ photosynthesis—clearly, much depends on the origin of those limitations. If it were in part a feedback from some product of photosynthesis, this would prevent significant gains from being realized from C₄. On the other hand, if stomatal closure were responsible, then the reduced stomatal conductance required for C₄ fixation would see an increased benefit from C₄ over and above that predicted from simple biochemical considerations.

Light-limiting conditions

An important factor is under what conditions does C₄ photosynthesis give benefit. Clearly, under light saturation at high temperature, significant gains in photosynthesis will result with the provisos described by Edwards et al (this volume). However, the benefit at limiting light is uncertain. Enhanced quantum yield for C₄ has been observed at limiting light, provided temperature exceeds 25 °C as is the norm for tropical rice throughout the day. Important in this consideration, however, is the degree of coupling of the C₄ and C₃ pathways. For example, significant leakage of fixed CO₂ can remove the already marginal quantum yield advantage at low light. Evolution has of course perfected the cellular differentiation and enzyme localization necessary to confer maximum growth advantage from the C₄ pathway. The complexity involved represents an enormous challenge in the genetic manipulation of rice. Without such perfection, introduction of C₄ may lead to a loss of quantum yield and a yield disadvantage under some conditions, such as the tropical wet season when solar radiation is low. Moreover, significant amounts of photosynthesis are carried out at limiting light intensity even under conditions of high solar radiation, and yield losses could be significant under these conditions.

Regulation of P_{\max}

The factors that determine the P_{\max} of a leaf need to be better understood. If the level of P_{\max} were determined developmentally by a signal from a product of photosynthesis, this could negate any benefit of C_4 . It would be critical to know which product was involved. If carbohydrate supply were the key factor, down-regulating P_{\max} when reaching a certain flux, then the extra photosynthetic capacity from C_4 could be removed. However, if a feedback signal from the reduction state of the stroma were involved, P_{\max} would relate to electron transport capacity. In this case, elimination of photorespiration by introduction of C_4 would always lead to higher P_{\max} since it has been estimated from Chl fluorescence data that electron transport rate is the same plus or minus photorespiration (providing there is no limitation to the use of photosynthetic products). In fact, a higher rate of use of NADPH and ATP in C_4 might well lead to a higher sustainable P_{\max} resulting from an induction of an increase in electron transport capacity.

ATP/NADPH requirement and flexibility in electron transport

Elimination of photorespiration by elevation of CO_2 leads to a large increase in photosynthesis in rice. Clearly, there is enough electron transport and Calvin cycle activity to support a much higher rate of carbon assimilation. However, the elimination of photorespiration by, introduction of C_4 is not equivalent to merely elevating the CO_2 level in terms of the requirements for electron transport. The C_4 pathway requires an increase in the ATP requirement relative to NADPH. For C_3 photosynthesis in high CO_2 , this is approximately 1.5, but, in C_4 (of the NADP-ME type), it is estimated to be 2.75. In such C_4 plants, this is achieved by bundle sheath chloroplasts operating only as producers of ATP by cyclic electron transport around PSI. This division of noncyclic and cyclic electron transport between two chloroplast types may be essential to provide this high ratio of ATP/NADPH. Knowledge of thylakoid membrane structure and function now allows us to predict with some certainty the ATP/NADPH production of noncyclic electron transport, since 6 H^+ are translocated for each 2 e transferred from H_2O to NADP, and 4 H^+ are required for each ATP synthesized, that is. the maximum overall stoichiometry is 1.5 ATP/NADPH. To raise this value to that required for C_4 photosynthesis in a single-cell type requires either the operation of cyclic electron transport around PSI or the operation of the Mehler pathway as an alternative pathway to NADP reduction.

The capacity of cyclic electron transport under light-limiting conditions will depend on the partitioning of Chl appropriately between PSII and PSI—the presence of cyclic electron transport requires an excess of PSI over PSII compared with noncyclic transport alone. This partitioning of Chl depends on both the regulation of the content of PSII and PSI reaction centers and the levels of the light-harvesting proteins. This regulation has not been studied in rice, but in *Arcabidopsis thaliana* fairly complex interactions are found: growth in high light results in an increase in PSII reaction centers but a loss of light harvesting. In very low light, the content of PSI increases dramatically. Not only light intensity but also light quality exert control over the levels of these proteins. A comparative study of a range of species showed how the ratio of PSII and PSI is antagonistically controlled by the two components of the shade environment, low irradiance and enrichment in far-red light. The balance between these two forces established how the ratio changed in shade compared with unshaded conditions. There is no information on how these acclimation responses affect the efficiency of cyclic electron transport imposed by the enhanced metabolic demand for ATP. In addition to the acclimation of the thylakoid membrane composition to the requirements of photosystem activation, state transitions provide a mechanism for short-term responses to changes in either the spectral quality of light or the demand for cyclic electron transport. State transitions depend on the reversible phosphorylation of the Lhcb1 and 2 polypeptides of LHCII, and it has been

estimated that the mobile pool of LHCII accounts for about 20% of the antenna of PSII. If the relative antenna size of PSI/PSII were 1:1 in state 1, then in state 2 this ratio could increase to 1.5, provided phospho-LHCII is efficiently coupled to PSI. The latter is substantiated by recent investigation of the state transition in *Arabidopsis* leaves (Horton 1999). In C_4 mesophyll chloroplasts, it was shown that the phosphorylation state of LHCII depended on the metabolic demand for ATP (Horton et al 1990), suggesting that in these chloroplasts at least there is sufficient flexibility in photosystem function to accommodate quite large shifts in ATP/NADPH requirement.

At photosynthetic saturation, electron and proton transport are proceeding close to capacity. ΔpH is high to drive ATP synthesis at high rates. Under such conditions, photosynthetic performance could be impaired by slippage of the Q-cycle, reducing the efficiency of H^+ translocation, and by passive leakage of H^+ through the thylakoid membrane. The higher ATP/NADPH demanded of the C_4 pathway may not be sustainable under these conditions, and may reduce photosynthetic capacity. Conversely, in C_3 chloroplasts, there have been observations consistent with an excessive ΔpH , arising from operation of the Q-cycle and alternative electron transfer pathways, if there are limitations to NADPH turnover. An excessive ΔpH may also arise if the supply of phosphate is in some way limited, as may occur under conditions when triose phosphate supply from the chloroplast exceeds the capacity to synthesize sucrose. An excessive ΔpH can be relieved in isolated chloroplasts by mild uncoupling. In C_4 mesophyll chloroplasts, such inhibition has also been observed when phosphoglycerate is supplied as the only electron acceptor. Interestingly, stimulation of ATP turnover by pyruvate, P_i dikinase activity relieved such inhibition. The higher rate of turnover of ATP in the C_4 pathway could mean that photosynthetic electron transport could proceed at a faster rate.

Grain filling

The decline in photosynthetic capacity observed during grain filling in some rice varieties suggests that an increased rate of photosynthesis from the introduction of C_4 could enhance grain filling. However, as with many of the discussions above, the key questions relate to why photosynthesis is declining. Is the flag leaf photosynthesis so tightly coupled to grain filling that there would be no gain from a higher capacity of photosynthesis? During grain filling, the dominant sink for photosynthate is the developing grain—the level of carbohydrate in the leaf would then serve as a signal that down-regulates photosynthesis to meet the demand of the developing grain. If this were the case, with a C_4 pathway, there would be no gain. On the other hand, however, the C_4 pathway would allow the same rate of photosynthesis with less electron transport capacity and lower Rubisco. Since the supply of N to the grain depends on the degradation of leaf proteins, including thylakoid proteins and Rubisco, C_4 may confer distinct advantages during the grain-filling period, provided that levels of leaf protein are high enough in the first place.

Summary and recommendations

Investigations of photosynthesis in rice under tropical field conditions have revealed several losses in photosynthetic performance related to the responses to principal environmental factors. Also evident from these studies is the importance of the level of Rubisco in terms of the N economy of the plant. A main conclusion of this work is that the rice plant is not optimally adapted for photosynthesis in tropical conditions. Whereas the poor performance of rice at elevated leaf temperatures is recognized as a key factor limiting net photosynthesis under

tropical conditions, poor adaptation to high light levels has not been accurately identified in previous work.

Photosynthesis shows a high degree of light saturation, at light levels well below full sunlight with consequent loss of photosynthetic efficiency, and potential for light-induced stress. The midmorning depression of photosynthesis possibly resulting from stomatal closure means both “lost photosynthesis” and inefficient use of the high N levels needed to establish the photosynthetic capacity. During leaf senescence, a large proportion of chlorophyll and N can be lost from the leaves without loss of photosynthetic capacity. This suggests an overinvestment in photosynthetic machinery, and may again point to poor use of N. Conversely, this apparent overinvestment may indicate an important role for photosynthetic proteins as leaf N stores. The relationship between grain filling and leaf senescence is a complex one, which indicates the interaction between a multiplicity of signals with different “aims.”

While our knowledge of the component reactions of photosynthesis is for the most part well understood, their integration into the whole plant process is not. There is an urgent need for an increased understanding of the underlying processes at the molecular level, and the integration of this knowledge to understand how a plant works. This understanding needs to include the complex interactions among canopy architecture, the growth and development of a leaf, and the biochemistry of the photosynthetic apparatus. Questions concerning the improvement of photosynthesis include: Can these internal limitations be removed? Can the response of the plant to unfavorable external factors be improved? Can the intrinsic capacity of photosynthesis be raised? Can the efficiency of grain filling be improved? Can nitrogen use be optimized? Can rice leaf development be manipulated? Can canopy architecture be further optimized?

Clearly, the introduction of C₄ photosynthesis in rice offers many exciting possibilities to drastically improve photosynthetic efficiency. However, the lack of understanding of the integration of photosynthesis and crop development in rice will hinder the process of accurately predicting the outcome. The above questions need to be answered, not only because the answers will open up possibilities for the genetic improvement of C₃ photosynthesis but also because these answers are needed if the goal of successfully introducing C₄ photosynthesis is to be realized.

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Notes

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Performance of a potential C₄ rice: overview from quantum yield to grain yield

P.L. Mitchell and J.E. Sheehy

The primary determinant of yield in relation to solar radiation and photosynthesis is the radiation conversion factor (RCF, also called radiation-use efficiency): the amount of aboveground dry matter produced from each megajoule of photosynthetically active radiation (PAR) intercepted by the crop. The RCF of rice (2.2 g MJ⁻¹) is lower than that of wheat (2.7 g MJ⁻¹) or maize (3.3 g MJ⁻¹). If genetic engineering could produce rice with C₄ photosynthesis that possessed the RCF of maize, a 50% increase in yield would be conceivable.

Analysis of losses of potential fixed carbon when scaling up from quantum yield to RCF identifies the differences between C₃ and C₄ plants. The scaling-up progresses from photochemistry in the cell (nanoseconds) through leaf and canopy photosynthesis (seconds to hours) to crop growth (weeks to months). Rice and maize differ in several respects. Rice has losses from photorespiration but maize has a lower theoretical quantum yield because of the energy costs of the C₄ pathway. In the hierarchy of scale, there are losses at the cell level from inactive absorption of PAR and these are smaller in maize than in rice. The loss at the transition between leaf and canopy photosynthesis is also smaller in maize. Scaling up from leaf to canopy includes changing from leaf photosynthesis unsaturated for PAR (in which each additional unit of absorbed PAR produces the same additional amount of fixed carbon) to photosynthesis by the canopy with leaves at varying saturation depending on PAR incident on the leaf and on leaf age and photosynthetic capability.

The analysis shows that a C₄ rice with improved RCF, approaching that of maize, must have higher quantum yield and higher rates of leaf photosynthesis. Suppression of photorespiration will increase quantum yield provided that the energy costs of the C₄ pathway, including leakage of carbon dioxide from the bundle sheath, are not much above the minimum observed in C₄ plants. Higher rates of leaf photosynthesis, arising from increased concentration of carbon dioxide around Rubisco, increase canopy photosynthesis and make it less prone to saturation by PAR. Many features of C₄ photosynthesis must be introduced into a C₄ rice, and must operate with high efficiency and coordination, if RCF and yield are to be improved significantly.

Rice production must increase substantially, by 50% by 2050, to meet the demand from the rising population of Asia. Since the cropped area cannot increase, an increase in yield per hectare is required. Some of the increase in rice production will come from improvements in agronomy that raise farm yields but an increase in the potential yield of new cultivars is certainly required.

In 1997, we started to look at the use of intercepted radiation by rice, suspecting that it might shed light on the 10 t ha⁻¹ apparent yield barrier in tropical rice (Kropff et al 1994). We

found that rice in general uses intercepted radiation less effectively than wheat or maize, but some rice cultivars in some conditions made much better use of radiation so that the yield barrier is not a limit set by thermodynamics (Mitchell et al 1998). The comparison with maize naturally prompted the speculation: suppose that a C₄ rice could be produced using genetic engineering. Would it use radiation as well as maize and give correspondingly higher yields? The same thought had occurred to others (Akita 1994, Mann 1999b) and rice transgenic for C₄ enzymes was already being produced (Ku et al 1999). There is now sufficient interest and belief in the idea to warrant a thorough examination of the topic, hence this workshop at IRRI.

The aim of this chapter is to take a broad view of how a C₄ rice could contribute to increasing grain yield. If a rice with leaf photosynthesis using the C₄ pathway could be produced—an ambitious project—what are the implications for a C₄ rice cultivar grown as a crop? Can we be sure that C₄ photosynthesis will produce higher yields? The general experience is that high rates of leaf photosynthesis are only weakly linked with high yields: breeding cultivars with improved leaf photosynthesis has usually proved disappointing (Evans 1993). Our work on how well rice uses intercepted radiation included a scaling-up analysis, trying to connect quantum yield of photosynthesis in the cell to crop growth, across a range of spatial and temporal scales. This provides a framework for comparing rice with maize and predicting how a C₄ rice might perform as a crop.

Radiation conversion factor

The radiation conversion factor (RCF; also called radiation-use efficiency, RUE) links the biomass produced by a crop to the radiation intercepted (Monteith 1977, and see reviews in Russell et al 1989, Mitchell et al 1998, and Sinclair and Muchon 1998). We avoid the word “efficiency,” which means a dimensionless quantity with maximum value of 1, because we wish to work in biomass per unit intercepted radiation (g MJ⁻¹). The RCF is a mathematical conversion factor between two measurable variables; the word “conversion” does not, of course, imply conversion of energy into matter. During vegetative growth and in good growing conditions, the value of RCF varies little for a crop, so it provides a single figure to compare crops and is used in growth models (Russell et al 1989, Prince 1991, Horie et al 1995).

From a survey of the literature, we concluded (Mitchell et al 1998) that general values of RCF for vegetative growth in good conditions were 2.2 g MJ⁻¹ for rice, 2.7 g MJ⁻¹ for wheat, and 3.3 g MJ⁻¹ for maize, all as aboveground dry matter divided by intercepted photosynthetically active radiation (PAR). These values are close to those of Kiniry et al (1989), who used the same units, and are consistent with results tabulated by Prince (1991) and by Sinclair and Muchon (1998) once allowance is made for different units. The RCF of C₄ plants is normally higher than for C₃ plants since C₄ photosynthesis is less easily saturated with PAR.

We used RCF in a simple model to show that for rice 2.2 g MJ⁻¹ was consistent with most recorded yields that were close to potential yield, that is, in optimal growing conditions, free of weeds, pests, and diseases (Mitchell et al 1998). A general equation for crop yield (Hay and Walker 1989) was developed to give

$$Y = H f_{\max} \epsilon_{\max} P \sum_{i=1}^n (Q_{dPARi} P_{fi} P_{ei}) \quad (1)$$

where Y is the yield of grain as dry matter (g m⁻²), so divide by 86 to obtain t ha⁻¹ at 14% moisture content; H is the harvest index (grain as a fraction of the aboveground dry matter);

f_{\max} is the maximum fraction of PAR intercepted; e_{\max} is the maximum value of RCF (g MJ⁻¹; aboveground dry matter, intercepted PAR); P is the length of the n periods into which the growing period is divided (d); Q_{dPARi} is the mean daily total of incident PAR (MJ m⁻²d⁻¹) for the ith period; and p_{fi} and p_{ei} are the proportions of the maximum values of fraction intercepted and RCF, respectively, during the ith period.

The fraction of PAR intercepted by a crop increases as the canopy develops, to a maximum of around 95% with a complete canopy, then declines during the ripening phase as leaves senesce. The value of RCF is nearly constant during vegetative growth and declines during ripening, although only slightly in rice. Shortages of water or nutrients, or low temperatures, reduce the RCF below e_{\max} but these occurrences are least likely in irrigated tropical rice and are minimized by good farming practices. Temporal changes were incorporated in equation 1 by defining maximum values for the fraction intercepted and for RCF as constants that were taken out of the summation term. The values of p_f and p_e can then vary in each of the n periods of growth. The temporal patterns of p_f and p_e were approximate and were developed by trial and error to a state that appeared reasonably close to reality. Monthly periods with P = 30 d provided sufficient resolution in time.

For locations in the tropics with a growth duration of 4 mo. potential yields were around 10 t ha⁻¹. Potential yields of 15 t ha⁻¹ were predicted for temperate regions with a growth duration of 6 mo where only one crop is possible in a year. In the tropics, where the climate and farming system are suitable, two or three crops can be grown in a year and there is no value in increasing growth duration. However, a value of 2.6 g MJ⁻¹ for RCF was required to explain some recent high yields of the elite indica cultivar IR73 grown at IRRI with abundant nitrogen fertilizer, and of Chinese hybrids grown in Yunnan, China (Ying et al 1998).

Given that the model was successful in explaining current potential yields of rice, does it shed light on how higher yields can be achieved? From equation 1, it is clear that yields can be improved by increasing the harvest index, or the fraction of PAR intercepted, or the RCF, or the growth duration (P × n), or the daily total of incident PAR, alone or in any combination. However, most of these variables are nearly fixed, such as growth duration and incident PAR, or difficult to alter much, such as harvest index and fraction of PAR intercepted. The greatest opportunity for improving yields comes from increasing the general value of RCF, that is. E_{\max} the nearly constant value found from sowing or transplanting up to the start of grain filling, for crops in near-optimal conditions (Table 1). Hence, RCF is the primary determinant of yield in relation to PAR and photosynthesis.

Table 1. Potential grain yield (rough rice in t ha⁻¹ at 14% moisture content) calculated when radiation conversion factor (RCF) or harvest index or both are changed from current values for rice. The base yield of 10 t ha⁻¹ is assumed with RCF 2.2 g MJ⁻¹ (aboveground dry matter, intercepted PAR) and harvest index 0.5.

RCF (g MJ ⁻¹)	Harvest index		
	0.47	0.50	0.53
2.2 (rice)	9.4	10.0	10.6
2.5	10.7	11.4	12.0
2.7 (wheat)	11.5	12.3	13.0
3.0	12.8	13.6	14.5
3.3 (maize)	14.1	15.0	15.9

Analysis of losses from quantum yield to RCF

In Mitchell et al (1998), we attempted to connect theoretical quantum yield and RCF through the various losses of potential fixed carbon and the conversions of units. This is a matter of scaling up from the nanosecond photochemistry in the cell, through leaf and canopy photosynthesis (seconds to hours), to the whole crop during vegetative growth (weeks to months). The result is presented in a table that identifies and quantifies the losses of carbon at various stages when read from the top downward. It is convenient to speak of losses of potential fixed carbon, but, for calculations reading down the table, this is quantified as the fraction retained. The table could not be constructed in a top-down sequence, however as measured values were available only at certain points. The procedure is summarized below Table 2 covers quantum yield measured or estimated at various points. Quantum yield, in units of $\text{g CH}_2\text{O MJ}^{-1}$ absorbed PAR, is 138 (that is, 4.6×30) multiplied by quantum yield, in units of $\text{mol CO}_2 \text{ mol}^{-1}$ quanta, which is the reciprocal of quantum requirement (quanta CO_2^{-1}). The 3.6 represents $4.6 \mu\text{mol PAR quanta joule}^{-1}$ in daylight (McCree 1981), and 30 is the molecular weight of CH_2O . The leaf must be defined to be on the linear portion of the photosynthesis versus PAR curve, at low irradiance. Table 3 continues the analysis from measured quantum yield in a leaf to RCF. The temperature sensitivity of photorespiration is set out in Table 4. In these tables, the justifiable precision is two significant figures but more are given to minimize rounding errors in further calculations. The procedure has eight steps.

1. Theoretical quantum yield. On theoretical grounds, the minimum quantum requirement is one molecule of carbon dioxide fixed by 8 quanta in C_3 photosynthesis when the Q-cycle is fully engaged (Rich 1988, Berry and Rumberg 1999) and when one molecule of ATP is synthesized when four hydrogen ions pass through the membrane-bound ATPase (Berry and Rumberg 1996). Two extra ATP are required in C_4 photosynthesis (NADP-ME subtype) to regenerate phosphoenolpyruvate (PEP), and these are probably supplied by cyclic electron flow around photosystem I, again with the Q-cycle, so that each quantum moves two hydrogen ions into the thylakoid lumen; these 4 extra

Table 2. Summary of losses from theoretical to measured quantum yield. The full units are $\text{g CH}_2\text{O MJ}^{-1}$ absorbed PAR, for a leaf when photosynthesis is not saturated for PAR. The measured quantum yields are taken from Ehleringer and Pearcy (1983) using the average for C_3 grasses for rice and wheat, and the average for the NADP-ME (NADP-malic enzyme) subtype of C_4 grasses for maize, all at 30°C leaf temperature and 330 ppmv carbon dioxide.

Quantity Loss or conversion	Value for quantity (g MJ^{-1}) Fraction retained		
	Rice	Wheat	Maize
Theoretical quantum yield for C_3 (8 quanta)	17.25	17.25	17.25
Extra ATP (2) for C_4	-	-	0.667
Theoretical quantum yield for C_4 (12 quanta)	-	-	11.50
Extra ATP (1/2) for leakage	-	-	0.923
Best practical quantum yield for C_4 (13 quanta)	-	-	10.62
Losses at cell level	0.654	0.654	0.845
Quantum yield without photorespiration	11.18	11.18	8.97
Photorespiration at 30°C	0.648	0.648	1.0
Measured quantum yield	7.31	7.31	8.97

Table 3. Summary of losses from measured quantum yield to radiation conversion factor (RCF). For measured quantum yield, the figures are for PAR absorbed (abs.) by unit area of leaf when photosynthesis is not saturated for PAR, as in Table 2. From maximum gross canopy photosynthesis to RCF, the figures are for PAR absorbed or intercepted (int.) by the canopy on unit area of ground with leaves varying in saturation for PAR. Total means aboveground and belowground dry matter (DM), AG means aboveground. The canopy factor is the final calculation to complete the table—see text for details.

Quantity Loss or conversion	Units for quantity Fraction retained	Value for quantity (g MJ ⁻¹)		
		Rice	Wheat	Maize
Measured quantum yield	g CH ₂ O MJ ⁻¹ abs.	7.31	7.31	8.97
Convert from m ² leaf to m ² ground	1.0			
Maximum gross canopy photosynthesis	g CH ₂ O MJ ⁻¹ abs.	7.31	7.31	8.97
Canopy factor at 30 °C	Varies	0.584	0.717	0.715
Gross canopy photosynthesis	g CH ₂ O MJ ⁻¹ abs.	4.27	5.24	6.41
Maintenance respiration	0.85			
Growth respiration	0.75			
Carbohydrate available for growth	g CH ₂ O MJ ⁻¹ abs.	2.72	3.34	4.09
Convert from CH ₂ O to DM	1.0			
RCF (total DM, abs. PAR)	g total DM MJ ⁻¹ abs.	2.72	3.34	4.09
Adjust from absorption to interception	0.95			
RCF (total DM, int. PAR)	g total DM MJ ⁻¹ int.	2.59	3.18	3.88
Allow for allocation to roots	0.85			
RCF (aboveground DM, int. PAR)	g AG DM MJ ⁻¹ int.	2.2	2.7	3.3

Table 4. Summary of losses from quantum yield without photorespiration to gross canopy photosynthesis, taken from Tables 2 and 3 and expanded to show sensitivity to temperature. The full units are g CH₂O MJ⁻¹absorbed PAR but note the critical difference in what absorbed PAR means: for the quantum yields, it is for a leaf when photosynthesis is not saturated by PAR; for canopy photosynthesis, it is for the canopy on unit area of ground with leaves varying in saturation for PAR. The temperature sensitivity of photorespiration is taken from data of Ehleringer and Pearcy (1983) and was applied to their measured quantum yield at 30 °C. The upper half of the table is for C₃ grasses in general, and is thus identical for rice and wheat. The lower half is computed from the bottom of Table 3 upward from radiation conversion factor, so separate values for rice and wheat are given.

Quantity Loss or conversion	Value for quantity (g MJ ⁻¹)				
	Fraction retained				
	Leaf temperature (°C)				
	15	20	25	30	35
Quantum yield without photorespiration				11.18	
Photorespiration(varies)	0.895	0.815	0.735	0.654	0.574
Measured quantum ² yield	10.01	9.11	8.21	7.31	6.42
Convert from m ² leaf to m ² ground (1.0)					
Maximum gross canopy photosynthesis	10.01	9.11	8.21	7.31	6.42
Canopy factor (apparently varies)					
Rice	0.427	0.469	0.520	0.584	0.666
Wheat	0.524	0.576	0.639	0.717	0.817
Gross canopy photosynthesis					
Rice				4.27	
Wheat				5.24	

quanta give a total requirement of 12 quanta for each carbon dioxide. Some leakage of carbon dioxide from the bundle sheath cells back to the mesophyll inevitably occurs but this is difficult to measure. Amid-range estimate of leakiness is 0.2 (von Caemmerer and Furbank 1999), that is, 20% of carbon fixed by phosphoenolpyruvate carboxylase (PEPcase) is not fixed by Rubisco. For each carbon dioxide that leaks, the two ATP that regenerate PEP go to waste. Overall, this adds an extra $\frac{1}{2}$ ATP or 1 quantum as a cost of leakage, per carbon dioxide fixed by Rubisco. so that the best practical C_4 quantum requirement is 13.

2. *Measured quantum yield.* The measured quantum yield of photosynthesis on the basis of absorbed PAR is remarkably uniform across all C_3 plants and within subtypes of C_4 plants (Ehleringer and Pearcy 1983, Long et al 1993). These calculations use data for C_3 grasses and C_4 grasses of the NADP-ME subtype, which contains maize, with quantum yield measured at 30 °C leaf temperature and 330 ppmv carbon dioxide (Ehleringer and Pearcy 1983). We have not adjusted quantum yield to the present atmospheric concentration of carbon dioxide (360–370 ppmv).
3. *Quantum yield without photorespiration.* Ehleringer and Pearcy (1983) measured quantum yield of one C_3 grass in an atmosphere of 2% oxygen in which photorespiration was almost completely suppressed. They computed the quantum yield in 0% oxygen as 0.081 mol CO_2 mol⁻¹ quanta and we have taken this as a general value, converted to 11.18 g CH_2O MJ⁻¹ absorbed PAR. The difference between this and theoretical quantum yield for C_3 is attributed to losses at the cell level. For C_4 plants, these losses occur between the practical best and measured quantum yields. Some radiation is absorbed by structures and nonphotosynthetic pigments in the leaf, so it is not available for photosynthesis. Some of the ATP and NADPH produced in the light reactions of photosynthesis is used in biochemistry not contributing to the fixation of carbon dioxide, especially reduction of nitrate. The difference between quantum yield without photorespiration and measured quantum yield is the loss from photorespiration. The temperature sensitivity of Ehleringer and Pearcy (1983) was used, equivalent to 0.1794 (g CH_2O MJ⁻¹ absorbed PAR) °C⁻¹, for the range 15–40 °C.
4. *Radiation conversion factor:* This is the only other measured quantity (aboveground dry matter, intercepted PAR) at the bottom of Table 3 and specific for a crop. The rest of Table 3 is filled in by calculation.
5. *Radiation conversion factor on other bases.* Working up Table 3, the RCF using total dry matter and absorbed instead of intercepted PAR can be calculated by adding back an allowance for roots (15% of total growth for vegetative cereals) and assuming that 95% of PAR intercepted by a canopy is absorbed.
6. *Carbohydrate and dry matter:* For simplicity, we assumed that dry matter contained 40% carbon, the same fraction as in CH_2O , so that the conversion factor is 1.
7. *Gross canopy photosynthesis.* Continuing up Table 3, we added back respiration, allowing 15% of gross canopy photosynthesis for maintenance respiration, and growth (synthesis) respiration costing 25% of the carbohydrate available.
8. *Scaling up from leaf to canopy photosynthesis.* We now have to connect measured quantum yield with gross canopy photosynthesis. The first step is to use a notional leaf area index (LAI) of 1 m² leaf m⁻² ground to convert photosynthesis between leaf and canopy bases. This gives maximum gross canopy photosynthesis, that is, canopy photosynthesis as if all leaves were never saturated for PAR but operated always on

the linear part of the photosynthesis versus PAR curve. The fraction remaining after scaling up from leaf to canopy photosynthesis is computed as gross canopy photosynthesis divided by maximum gross canopy photosynthesis. We call this the canopy factor. In practice, it will always be less than 1 and so can be interpreted as a canopy efficiency. The canopy factor is dimensionless but carries with it the change of assumptions from a leaf not saturated for PAR to the canopy with leaves at varying saturation (with incident PAR, leafage, and photosynthetic capability. etc.). The canopy factor is apparently temperature-sensitive in C_3 plants (Table 4) because it links measured quantum yield, temperature-sensitive after photorespiratory losses, with gross canopy photosynthesis, invariant with temperature since it arises from RCF and calculations for vegetative growth in general, across a range of temperatures (diurnal, seasonal) in a variety of growing conditions.

This analysis assumes that at each step a constant amount of carbon fixed is associated with one unit of radiation. For quantum yields, this is true at low PAR when leaves are far from saturated. For RCF, it is found experimentally that dry matter produced is linearly related to absorbed or intercepted PAR. Straight lines representing the quantities in Tables 2–3 are shown in Figure 1. The straight line concept is not applicable to canopy photosynthesis of the crop since this is curved with PAR (Fig. 1B). Maximum gross canopy photosynthesis sets an upper limit to the curve. The line representing gross canopy photosynthesis runs from the origin to a point determined by the average PAR absorbed and the average carbohydrate produced. (The line in Figure 1 B is of arbitrary length to show the slope.) The average PAR is for each day and over all days of vegetative growth. The average carbohydrate is over all leaves with their various photosynthetic properties, as determined by the PAR they absorb, throughout vegetative growth. Neither of these nonlinear averages can be measured easily and we have obtained a figure for the slope by calculating up from the bottom of Table 3. The canopy factor is thus the last unknown in the scaling-up analysis. Consequently, it contains all the uncertainties, in particular the complementary temperature sensitivity to photorespiration. an unwanted artifact.

The major losses of potential fixed carbon are at the cell level, from photorespiration. from the canopy factor, and from respiration. The complete sequence in Tables 2–3 can be used to explore the effect of changing the losses. For example, if losses from photorespiration could be halved in rice, then at 30 °C the RCF would increase to 3.78 g MJ⁻¹ (26% increase). Any increase in the canopy factor produces a proportionate increase in RCF, and in theory there is considerable scope for an increase from the value of 0.58 before approaching a limit at 1.0. Breeding crops for reduced respiration has been tried but with no ultimate success (Hay and Walker 1989). In any case, rice does not seem to have a profligate rate of maintenance respiration (Mitchell et al 1998). It is worth noting that the same absolute increase (for example, 0.05) in the fraction retained has a greater effect the higher up the table, since it operates on a larger value of potential fixed carbon in g MJ⁻¹. Consequently, a small reduction in respiration will not produce much improvement in RCF but reductions in losses higher up the table, such as at the cell level and from photorespiration, are more likely to be profitable.

From systems analysis of RCF (Mitchell et al 1998), we estimate that the maximum RCF for rice is 2.9 g MJ⁻¹ (aboveground dry matter, intercepted PAR). This would require, for example, a slight reduction in losses from photorespiration (fraction retained 0.70) and the canopy factor increased to 0.72. Further improvement in RCF will probably require C_4 photosynthesis.

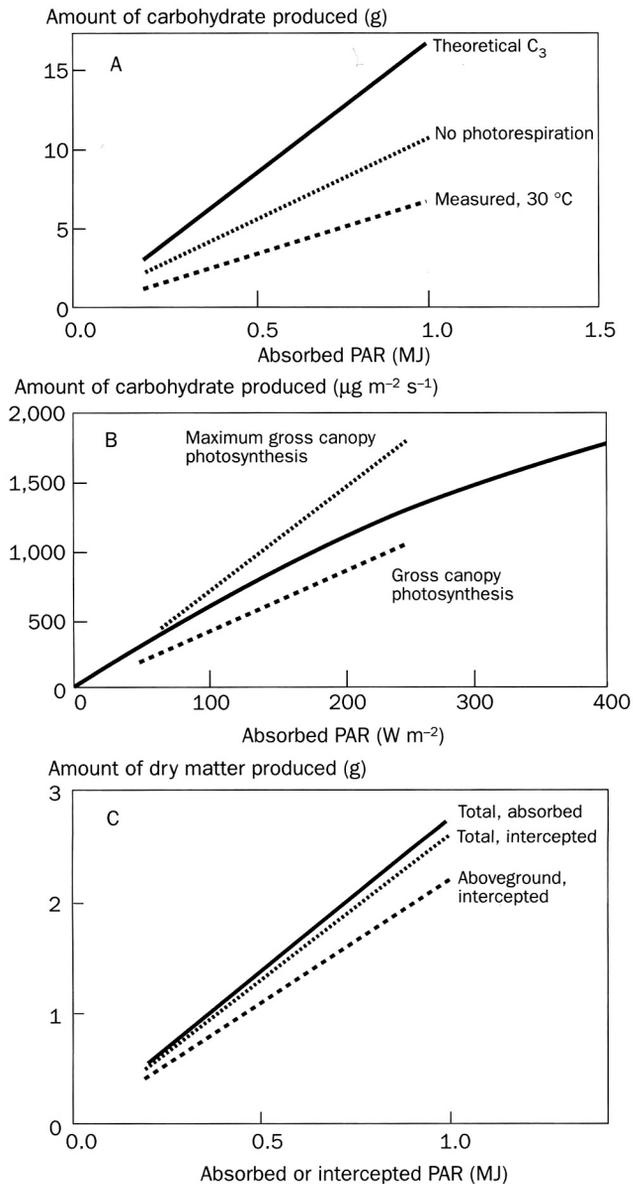


Fig. 1. The quantities in Tables 2 and 3 represented as slopes of lines on graphs of carbohydrate or dry matter versus photosynthetically active radiation (PAR). The difference between slopes of successive lines is the loss of potential fixed carbon. (A) Quantum yields for C₃ grasses (see Table 2 for details). (B) Maximum gross photosynthesis and gross canopy photosynthesis for rice; the difference in slope between the two lines is the canopy factor (see Table 3 and text for details). For comparison, a curve for canopy photosynthesis with parameter values in Table 5 is shown. Note that this curve for a representative rice canopy with LAI of 6 is below the limit set by the line for maximum gross canopy photosynthesis. (C) Radiation conversion factor for rice on different bases, i.e., combinations of total or aboveground dry matter, and intercepted or absorbed PAR. See Table 3 for details.

Comparison of crops

In these approximate calculations, losses from respiration, conversions between PAR absorbed and intercepted and between CH_2O and dry matter, and allocation to roots are assumed to be the same for rice, wheat, and maize. Losses of particular interest here are those at the cell level, from photorespiration, and from the canopy factor, where the crops differ.

In C_3 crops, around a third of potential fixed carbon is lost at the cell level (Table 2) but the loss in maize is less than half this value. Ehleringer and Percy (1983, p. 558) found it “surprising” that the theoretical and measured quantum yields were so close. In terms of quantum requirement, the losses at the cell level account for 4.35 quanta per carbon dioxide fixed in C_3 plants and 2.38 quanta in C_4 plants with leakiness of 0.2 (calculable from Table 2). The reasons for this large difference are not clear.

The losses at the cell level arise from absorption of quanta in the leaf that do not contribute to carbon fixation (Evans 1987, Furbank et al 1990). First, there is absorption by leaf structures that are not completely transparent to PAR. Second, nonphotosynthetic pigments, such as those absorbing ultraviolet radiation in field-grown plants, will absorb a small amount of PAR. Third, the distribution of wavelengths, and hence of quantum energies, in PAR does not exactly match the action spectra of the two photosystems so that some quanta are not used. In addition, the theoretical quantum requirements for ATP and NADPH in the correct proportions have assumed perfect operation of the photosystems but the Q-cycle may not always maintain exact stoichiometry, and some leakage of hydrogen ions across the thylakoid membrane probably occurs. Finally, reduction of nitrate and sulfate can use NADPH generated in the chloroplast.

The quantum requirement for nitrate reduction seems to be small. The ratio of carbon to nitrogen fixed in C_3 plants can be taken as 25:1 (Evans 1987) and in C_4 plants as 35:1 (Kanai and Edwards 1999). Allowing for respiration and composition of dry matter as in Table 3 gives 2.5% N for C_3 and 1.8% N for C_4 , which each correspond to aboveground dry matter of 5.2 t ha^{-1} according to the nitrogen dilution curves of Greenwood et al (1990). Thus, these figures apply to crops during early to middle vegetative growth. The 4 NADPH required to reduce one nitrate (Evans 1987) can be produced by 16 quanta in noncyclic electron flow, along with 6 ATP. If these ATP are not necessary, then presumably the extra hydrogen ions are dissipated by leakage through the thylakoid membrane, or perhaps the Q-cycle does not work at full efficiency so fewer hydrogen ions pass into the thylakoid lumen. But if cyclic electron flow is operating, then the extra 6 ATP can be used instead of ATP from cyclic electron flow, saving 12 quanta and reducing the number of quanta per nitrate to 4. The quantum requirement (per carbon dioxide fixed) is thus $16/25 = 0.64$ for C_3 plants or $4/25 = 0.16$ if cyclic electron flow is operating. For C_4 plants, the figures are 0.46 and 0.11, respectively. Cyclic electron flow is essential for C_4 plants and probably occurs routinely in C_3 plants to provide ATP used in photorespiration so the quantum requirement for nitrate reduction is unlikely to exceed 0.2, a trivial fraction of the 2–4 quanta used in nonphotosynthetic absorption.

As a percentage of measured quantum requirement, the losses at the cell level in C_3 plants decrease from 32% to 20% as temperature increases from 15 to 35 °C. In C_4 plants, these losses range from 15% with leakiness of 0.2 to 22% with no leakage of carbon dioxide from the bundle sheath. Since these losses arise from nonphotosynthetic absorption of quanta, it may be appropriate to deduct them first as a proportion (f_{npa}) of quanta absorbed by the leaf: thus, of 100 quanta in the leaf, only $100(1 - f_{\text{npa}})$ are available for photochemistry leading to fixation of carbon. The value of f_{npa} varies in C_3 plants with temperature and in C_4 plants with leakiness and the ranges overlap slightly. For rice at higher temperatures and maize with low leakage, a general value for f_{npa} of 0.2 will suffice for calculations. For comparison, Loomis

and Amthor (1996) in their calculations for wheat allowed for 10% of radiation being absorbed by nonphotosynthetic structures and pigments, based on measurements of green and albino leaves.

The losses from photorespiration are the same for rice and wheat in Table 2 since they are computed from general data for C₃ photosynthesis. The losses decrease at lower temperatures so that below 20.8 °C the measured quantum yield of C₃ plants exceeds that of C₄ plants. Collatz et al (1998) have modeled this crossover temperature in detail and explored the implications for the comparative ecology of C₃ and C₄ plants.

The canopy factor for maize is a constant 0.71, but for rice and wheat the value is dependent on temperature (Table 4). The higher value for maize is to be expected since leaf photosynthesis of C₄ plants is never fully saturated by PAR. In that sense, the C₄ canopy is more effective since all leaves tend to operate closer to the linear part of the photosynthesis versus PAR curve where each additional unit of absorbed PAR produces the same additional amount of fixed carbon. For rice with an RCF of 2.6 g MJ⁻¹ (aboveground dry matter, intercepted PAR), the canopy factor at 30 °C is 0.69, with all other losses and conversions constant. For the highly fertilized crop of IR72 (Mitchell et al 1998), photosynthesis was probably increased by high concentrations of leaf nitrogen, which is linearly related to the maximum rate of leaf photosynthesis in rice (Peng et al 1995). High leaf nitrogen content is associated with higher RCF (Sinclair and Horie 1989, Sinclair and Shiraiwa 1993).

Owing to the method of calculation, the canopy factors for rice and wheat vary with temperature to complement the temperature sensitivity of photorespiration. The higher canopy factor for wheat arises from its higher RCF. But if the canopy factor were the same for rice and wheat, then greater losses from photorespiration in rice associated with a 10 °C higher temperature would account for the difference in RCF. Note in Table 4 that the canopy factors for rice at 25 °C and wheat at 15 °C are almost identical (0.52) and are close for rice at 35 °C and wheat at 2.5 °C (0.67 and 0.64). Since rice and wheat are both small-grain cereals of similar height and leaf shape and size, with photosynthesis versus PAR curve of the C₃ type, a similar canopy factor is possible. Average leaf temperatures during the day of 15–20 °C for temperate wheat and 25–30 °C for rice in the tropics or warm temperate regions appear plausible for the vegetative phase of growth to which the value of RCF applies. To sum up: photorespiration has the same rate at a given temperature in rice and wheat but rice may have a lower RCF, other things being equal, because its actual losses from photorespiration are higher. The size of the difference arises from rice leaves having daytime temperatures in the vegetative phase about 10 °C higher than wheat leaves.

Radiation conversion factor of a C₄ rice

For comparing rice, maize, and a C₄ rice, Tables 2-3 can be summarized in the equation

$$\text{RCF} = \text{measured quantum yield} \times \text{canopy factor} \times 0.51478 \quad (2)$$

where measured quantum yield (g CH₂O MJ⁻¹ absorbed PAR) is 138 divided by quantum requirement, and 0.51478 represents all other losses and conversions (see Table 3). The RCF of a C₄ rice may be improved by higher quantum yield or higher canopy factor or both. On a graph of measured quantum yield against canopy factor, the RCF can be represented by lines that separate classes of improvement. An increase in RCF up to 10% is small, 10–30% is modest, 30–50% is good, and 50% + is super improvement (Fig. 2). If the measured quantum yield and the canopy factor of maize are taken as maximum values, then super improvement in

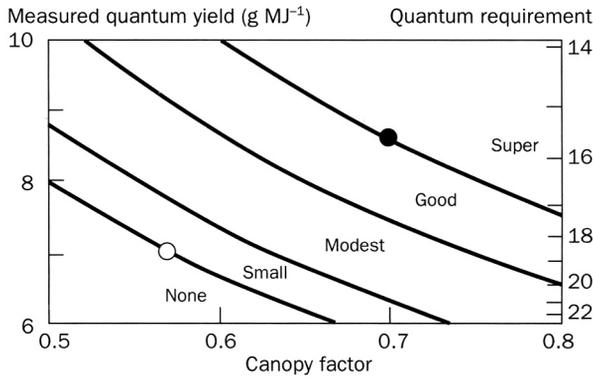


Fig. 2. The radiation conversion factor (RCF) for rice with a range for measured quantum yield and for the canopy factor. The full units for the measured quantum yield are $\text{g CH}_2\text{O MJ}^{-1}$ absorbed PAR. The equivalent quantum requirements are indicated on the y-axis on the right. The curved lines from left to right represent RCF of 2.20, 2.42, 2.86, and 3.30 g MJ^{-1} (aboveground dry matter, intercepted PAR), separating classes of improvement in RCF (none, 0%; small, 10%; modest, 30%; good, 50%; super, >50%). Maize is indicated by a filled circle. Rice at 30°C is indicated by an unfilled circle.

RCF is unattainable, and the region of good improvement is rather small; restricted by horizontal and vertical lines from the maize point on the graph. A measured quantum yield of $8.97 \text{ g CH}_2\text{O MJ}^{-1}$ absorbed PAR may be a maximum but a canopy factor greater than 0.71 is perhaps achievable. A canopy factor of 1.0 represents a canopy with notional LAI of 1 m^2 leaf m^2 ground photosynthesizing as a leaf unsaturated for PAR, integrated over the whole crop growth period.

The losses at the cell level, through photorespiration, and as C_4 leakiness are the variable contributor to quantum yield. In terms of quantum requirement,

$$Q_{\text{leaf}} = \frac{Q_{\text{Calvin}} + Q_{\text{C}_4} + Q_{\text{P/R}} + Q_{\text{leak}}}{1 - f_{\text{npa}}} \quad (3)$$

where Q_{leaf} is the quantum requirement measured on a leaf (absorbed basis); Q_{calvin} is the 8 quanta required for the Calvin cycle, in C_3 and C_4 plants; Q_{C_4} is the 4 quanta required to produce 2 ATP to regenerate PEP in the C_4 pathway; $Q_{\text{P/R}}$ is the quantum cost of photorespiration, 6.52 quanta at 30°C in a C_3 plant; Q_{leak} is the quantum cost of leakage of carbon dioxide from the bundle sheath; and f_{npa} is the fraction of measured leaf quantum requirement used in nonphotosynthetic absorption and nitrate reduction. Here Q_{calvin} is constant and Q_{C_4} is constant in C_3 (0) or C_4 plants (4 quanta). The cost of photorespiration at a given temperature, $Q_{\text{P/R}}(T)$, is the difference between $Q_{\text{leaf}}(T)$ and 12.35 (the quantum requirement of C_3 plants without photorespiration, invariant with temperature). Leakage costs as carbon are leakiness/(1 — leakiness) multiplied by 4 to obtain the quantum cost. Losses at the cell level are computed with f_{npa} , as described above. It can be seen from equation 3 that the C_4 quantum requirement is less than that of C_3 when $Q_{\text{P/R}}$ is small enough to compensate for the nonzero values of Q_{C_4} and Q_{leak} . Conversely, if the C_4 pathway in a C_4 rice were not as effective as in maize, so that $Q_{\text{P/R}}$ was not close to zero and Q_{leak} was substantial, then the quantum requirement could be higher than for C_3 rice.

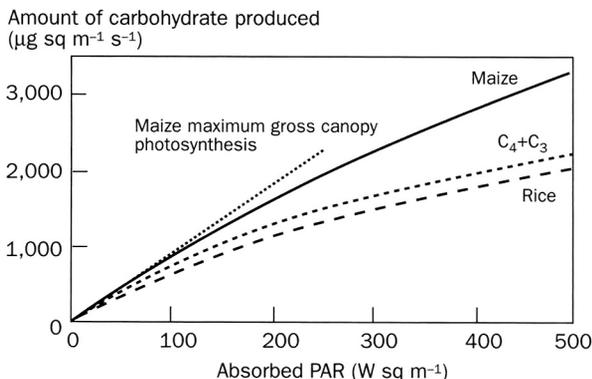


Fig. 3. Comparison of a straight line representing maize maximum gross canopy photosynthesis, an upper limit (from Table 3), and curves for canopy photosynthesis with parameter values in Table 5. The curve C_4+C_3 combines C_4 quantum yield with C_3 maximum rate in the rectangular hyperbola for leaf photosynthesis.

Table 5. Values of parameters of leaf photosynthesis in the canopy photosynthesis model (Sheehy and Johnson 1988) used to construct curves in Figures 1B and 3. The asymptotes were calculated to match maximum measured leaf photosynthesis (Larcher 1995) at $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ incident PAR. The maximum rate of $25 \mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ is found in rice leaves containing 1.34 g N m^{-2} (Peng et al 1995). For rice, the measured quantum yield at 30°C was used. The C_4+C_3 curve is for C_4 quantum yield and C_3 maximum measured leaf photosynthesis. The other parameters in the canopy model were LAI 6, Beer's law extinction coefficient 0.5 (together providing 95% canopy interception of incident PAR), and coefficient of leaf transmittance 0.1. To convert photosynthesis in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$ to $\mu\text{g CH}_2\text{O m}^{-2} \text{s}^{-1}$, multiply by 30. To convert quantum yield in $\text{mol CO}_2 \text{ mol}^{-1}$ quanta absorbed PAR to $\mu\text{g CH}_2\text{O MJ}^{-1}$ absorbed PAR, multiply by 138.

Parameter	Rice	Maize	C_4+C_3
Rate of photosynthesis at $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ incident PAR, equivalent to $1,700 \mu\text{mol m}^{-2} \text{s}^{-1}$ or 370 W m^{-2} absorbed PAR ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$)	25	50	25
Asymptote for photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$)	34.6	91.3	32.3
Initial slope, measured quantum yield ($\text{mol CO}_2 \text{ mol}^{-1}$ quanta absorbed PAR)	0.053	0.065	0.065

The main determinant of the canopy factor is probably the rate of leaf photosynthesis when close to saturation by PAR. This can be examined using a model of canopy photosynthesis (see Appendix). In Figure 3, we compare curves of canopy photosynthesis versus PAR for rice, maize, and C_4+C_3 where C_4 measured quantum yield is combined with a C_3 maximum measured leaf photosynthesis (see Table 5 for parameter values). At first glance, comparing the whole curves, eliminating photorespiration by using the C_4 quantum yield but keeping the C_3 maximum photosynthesis makes only a small improvement on the rice curve. However, although canopy photosynthesis will sometimes be operating at $400\text{--}450 \text{ W m}^{-2}$ absorbed PAR ($2,050\text{--}2,300 \mu\text{mol m}^{-2} \text{s}^{-1}$ incident PAR allowing for 90% absorption by a full canopy), when all times of day and cloudy periods are included the average PAR absorbed will be much lower. For $14\text{--}20 \text{ MJ m}^{-2} \text{d}^{-1}$ of incident solar radiation, the average irradiance of absorbed PAR will be around

150–200 W m⁻² over a 12-h daylength. In this region of the graph, the curves are more evenly spaced vertically so that adding the C₄ quantum yield increases rice canopy photosynthesis by 14% and then adding the C₄ maximum measured leaf photosynthesis contributes a further 26%. These are approximate figures from a crude estimate of average PAR and a simple canopy model but they suggest that increasing both quantum yield and the asymptote of leaf photosynthesis is necessary to swing the line for gross canopy photosynthesis upward (as in Fig. 1B), that is, to increase the rice canopy factor.

The benefit of C₄ photosynthesis is not only the absence of photorespiration and smaller losses at the cell level (in the higher quantum yield) but also the much greater rates of photosynthesis at higher incident PAR. The mechanism concentrating carbon dioxide around Rubisco ensures that photosynthesis is much less easily saturated by PAR. This is a general property of Rubisco and it can be shown in rice. Murchie et al (1999) found that both IR72 and a breeding line of the new plant type doubled their maximum rates of leaf photosynthesis at around 2,000 μmol m⁻² s⁻¹ when the carbon dioxide concentration in the leaf chamber was increased from 350 ppmv to 900 ppmv. The objective of constructing a C₄ rice is to make the plant itself provide such a high carbon dioxide concentration inside the chloroplasts.

Discussion

The scaling-up analysis

The analysis of losses between theoretical quantum yield and RCF in Tables 2–3 contains several assumptions and approximations, stated above or in Mitchell et al (1998). The values used for the losses and conversions affect the value of RCF obtained but these results should be robust in direction or in ranking if not exact quantitatively. In particular, it is hard to conceive of yield improvements in a C₄ rice if RCF decreases.

The starting point for this analysis was the consistent set of carbon dioxide exchange data of Ehleringer and Pearcy (1983): measured quantum yields, photorespiration as the difference between rates in 21% and 2% oxygen, and the temperature dependence of photorespiration. Other published measurements or methods could be used. For example, Long et al (1993) found the average quantum yield without photorespiration for C₃ plants to be 0.093 mol CO₂ mol⁻¹ PAR quanta absorbed by leaf, equivalent to 12.83 g CH₂O MJ⁻¹ absorbed PAR. Substituting in Table 2. this makes the fraction retained after losses at the cell level 0.714. again smaller than for maize. The quantum requirement is 2.75, which represents 13–20% of measured quantum requirement as temperature decreases from 35 to 15 °C. This supports the general value of 0.2 for f_{npa} suggested above, appropriate while we remain ignorant of the exact values for losses at the cell level and why they apparently differ between C₃ and C₄ plants. Kanai and Edwards (1999) calculated the quantum requirement of C₄ photosynthesis from detailed biochemistry. By including leakage from the bundle sheath, a small amount of photorespiration, nitrate assimilation, the energy cost of synthesizing starch, and the efficiency of energy transfer to the chlorophyll reaction centers, they managed to reconcile the calculated and measured quantum yields. The C₄ quantum requirements for a range of leakiness have been calculated by von Caemmerer and Furbank (1999). An alternative approach to quantify photorespiration would be to use Γ^* , the carbon dioxide compensation point without concurrent leaf respiration, that is, the intercellular concentration at which gains by photosynthesis and losses by photorespiration are equal, which is a highly conserved property of Rubisco. The temperature response of Γ^* given by Brooks and Farquhar (1985) is a quadratic equation but the curvature is slight in the range 15–35 °C. These alternative methods and refinements would change the values calculated in Tables 2–4 but are unlikely to alter greatly the conclusions drawn.

Apart from the general value of RCF, measurements specific to rice were not available. Yeo et al (1994) measured quantum yield and photorespiration in rice but no temperature was reported, which is critical given the temperature sensitivity of photorespiration. Differences in rates of photorespiration in rice have been recorded by Hons (1988) and by Kawamitsu et al (1989) and these papers may repay detailed study; we have not used their results here. Reliable values of quantum yield and photorespiration, in particular its response to temperature, are essential information about C₃ rice, the starting point for research to produce a C₄ rice.

Loomis and Amthor (1996) made a similar analysis for wheat. Their range of 3–4 g MJ⁻¹ (total dry matter, absorbed PAR) is consistent with our general and maximum values (Mitchell et al 1998) of 2.7 and 3.1 g MJ⁻¹ (aboveground dry matter, intercepted PAR) whether converted with our factors (Table 3) or theirs to obtain an overall range of 3.2–3.8 g MJ⁻¹ (total dry matter, absorbed PAR). Loomis and Amthor (1996) concluded that in good conditions wheat already attained this maximum RCF so that yield improvements would have to come from greater duration of photosynthesis or higher harvest index. For tropical rice, where losses from photorespiration may be high and it is not desirable to lengthen growth duration, attempts to increase RCF appear worthwhile.

Implications for a C₄ rice

Consideration of the quantum yields in C₄ photosynthesis reveals the much greater involvement of cyclic electron flow, or other mechanisms for generating a hydrogen ion gradient without reducing NADP. If a C₄ rice were constructed by introducing the several enzymes of the C₄ pathway for initial fixation of carbon dioxide and its release close to Rubisco, which is where effort is directed at the moment (Mann 1999b), would extra cyclic electron flow occur automatically, with adjustment of the amounts of photosystems I and II? Would it be necessary to modify this aspect of photosynthesis using genetic engineering as well? In maize, part of the differentiation in function between the mesophyll and bundle sheath is the absence of photosystem II and of carbonic anhydrase from the bundle sheath cells (Leegood et al 1997). This means that no oxygen is produced in those chloroplasts where carbon dioxide is fixed by Rubisco, thus minimizing oxygenase activity. Lack of carbonic anhydrase ensures that carbon dioxide is not rapidly converted to bicarbonate and so remains as the substrate of Rubisco and is not lost by diffusion of bicarbonate down its concentration gradient: bicarbonate concentrations in mesophyll cells are low since bicarbonate is the substrate for PEPcase. Would these features have to be introduced into a C₄ rice?

There appears to be a difference in losses at the cell level between C₃ and C₄ plants; this difference is smaller when expressed as a proportion of measured quantum requirement. If it is not an artifact of our calculations, or the measurements upon which they are based, this C₄ feature will also have to be introduced into a C₄ rice, given the contribution that losses at the cell level make to the high quantum yield of maize (Table 3).

The canopy factor is a proportionality between two quantities calculated from the top downward and the bottom upward in Tables 2 and 3. It boils down into a single figure all the variability in leaf photosynthesis versus PAR curve, and how that depends on leaf age, nitrogen status, and other factors; and the distribution of PAR in the canopy, which varies with time of day, cloudiness, and the growth period of the crop. This is the province of models of canopy photosynthesis. The need for these models, together with reliable data on driving variables and crop properties and parameters, was emphasized by Mitchell et al (1998) and by Murchie et al (1999). Such models could reveal why the canopy factors for rice, maize, and wheat differ and in what respects a C₄ rice canopy must function like a maize canopy to achieve worthwhile

increases in RCF, and hence in grain yield. Canopy scale and architecture differ more between rice and maize than between rice and wheat. For example, dwarfing of maize to increase harvest index was not successful, in contrast to rice and wheat. because the shorter plants produced too much self-shading (Mann 1999a). It is impossible to say at present whether a C₄ rice canopy would function as well as a maize canopy. When a C₄ rice is available, then measurements of canopy photosynthesis can be made but, until then, modeling is the only option.

Other implications of C₄ photosynthesis at the crop scale

In general, C₄ crops have lower concentrations of nitrogen than C₃ crops (Greenwood et al 1990) and C₄ plants have higher rates of photosynthesis relative to leaf nitrogen (Schmitt and Edwards 1981, Field and Mooney 1986). Insofar as this means a smaller demand for nitrogen, or a larger biomass constructed with the same amount, then a C₄ rice could be advantageous. However, Sinclair and Sheehy (1999) have argued eloquently that erect leaf canopies with their higher LAI improved rice yields not by increasing photosynthesis but by providing greater amounts of leaf in which nitrogen can accumulate. The nitrogen is transferred to the grain as the leaves senesce during the later stages of grain filling. If a C₄ rice canopy makes better use of nitrogen in photosynthesis, then the canopy should hold less nitrogen per unit ground area. Together with changes in the compounds in which nitrogen is held—less Rubisco, greater amounts of enzymes associated with C₄ photosynthesis—this altered nitrogen holding may have profound effects on the nitrogen economy of rice during grain filling. The protein content of rice is considered to be low compared with other cereals so that higher yields must maintain it at least. It would be pointless to construct a C₄ rice only to find that its yield was limited by its inability to provide enough nitrogen for filling grains. Modeling is required to simulate these changes and allow an assessment of their importance, and a start has been made by Sheehy (this volume).

Although modern rice cultivars have shorter and stronger stems than their predecessors, crops continue to lodge when wind and rain forces exceed the capacities of the stem and roots to withstand them. If new cultivars producing 25–50% greater yields are seriously contemplated, then exactly how that increased weight is to be supported deserves attention (Setter et al 1994, Cassman 1994, p. 135). Recent findings for wheat (Berry et al 1998) may be transferable in part, allowing for the altered mechanics of the drooping rice panicle compared with the vertical ear of wheat. If panicle weight remains the same, more stems per hectare are needed. If stem number per hectare is constant, individual stems must be stronger. Either possibility, and the intermediates, has implications for the harvest index. The main analysis of this chapter on increasing RCF to improve yields assumes that harvest index will be maintained but note in Table 1 the effect of changing harvest index as well. A small decrease in harvest index may be necessary to improve resistance to lodging. Changes in harvest index also involve nitrogen since the nitrogen concentration is higher in grain than in straw (Sinclair 1998).

Conclusions

Increasing RCF offers the best chance of substantial improvement in rice yields. The scaling-up analysis identified differences between crops in the losses at the cell level, in photorespiration, and in the canopy factor (a measure of canopy performance), with rice apparently worse than wheat and much worse than maize. Improvements in all three areas are required if the RCF of rice is to be increased to that of maize (see Fig. 2) with a consequent improvement in yield.

Current attempts to produce a C₄ rice have concentrated on the enzymes of initial carbon fixation and decarboxylation. These could eliminate photorespiration and increase the maximum rates of leaf photosynthesis, and hence the canopy factor (see Fig. 3), provided that adjustments also occur, or can be introduced, in types of electron flow and the proportions of the two photosystems to produce the required ratio of ATP to NADPH. If the lack of both photosystem II and carbonic anhydrase in the bundle sheath cells of maize makes significant contributions to the higher rate of leaf photosynthesis, then these changes would also need to be introduced into a C₄ rice. These topics deserve intensive research to ensure that a C₄ rice will be successful in increasing yields. Increased yields have implications for the nitrogen economy and for lodging, which also require attention if the benefits of C₄ photosynthesis are not to be outweighed by other features of crop physiology.

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Notes

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Appendix

Curves of canopy photosynthesis versus PAR can be constructed with the model of Sheehy and Johnson (1988). Leaf photosynthesis is assumed to follow a rectangular hyperbola with parameters for the initial slope, given by quantum yield, and the asymptote. This curve does not always fit measurements closely because it is too slow to approach the asymptote (Causton and Dale 1990). Here the asymptotes were calculated to produce maximum measured leaf photosynthesis at $2,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ incident PAR (Table 5). When combined with Beer's law for the attenuation of PAR down the canopy and integrated over LAI, gross canopy photosynthesis is given by

$$P_{\text{canopy}} = \frac{p_{\text{max}}}{k} \ln \left[\frac{\alpha k I + p_{\text{max}} (1 - m)}{\alpha k I \exp(-k L) + p_{\text{max}} (1 - m)} \right] \quad (\text{A1})$$

where P_{canopy} is the rate of canopy photosynthesis ($\mu\text{mol CO}_2 \text{m}^{-2} \text{ground s}^{-1}$); P_{max} is the maximum rate of leaf photosynthesis ($\mu\text{mol CO}_2 \text{m}^{-2} \text{leaves}^{-1}$) at the asymptote; α is the leaf quantum yield ($\text{mol CO}_2 \text{mol}^{-1} \text{PAR quanta}$); k is Beer's law extinction coefficient ($\text{m}^{-2} \text{ground m}^{-2} \text{leaf}$); L is LAI ($\text{m}^2 \text{leaf m}^{-2} \text{ground}$); I is the PAR received by the canopy ($\mu\text{mol m}^{-2} \text{ground s}^{-1}$); and m is the fraction of PAR transmitted by a leaf (dimensionless).

Here a single leaf photosynthesis curve is applied to all leaves, which in reality have a variety of curves depending on nitrogen content, age, etc. The value of this model is its analytical integration to provide one equation, also a rectangular hyperbola, predicting canopy photosynthesis as a function of PAR. Its initial slope (canopy quantum yield) is largely determined by the leaf quantum yield for canopies with nearly complete interception. Its asymptote is given by $p_{\text{max}} L$, that is, all leaves saturated for PAR, which is not approached unless L is small or I is unrealistically large. Canopy photosynthesis is rarely close to saturation. in accordance with observations.

**Genes, physiology,
and function**

How to express some C₄ photosynthesis genes at high levels in rice

M. Matsuoka, H. Fukayama, H. Tsuchida, M. Nomura, S. Agarie, M.S.B. Ku, and M. Miyao

To investigate the difference between Pdk genes that encode pyruvate orthophosphate dikinase (PPDK), a Pdk gene homologous to the maize C₄-type Pdk gene was isolated from a C₃ plant, rice, and compared with the maize gene. The primary structures of the genes are essentially the same, except that the rice gene has two additional introns. A transient expression assay of Pdk promoters using maize mesophyll protoplasts showed that the mode of expression of the maize and rice genes differs only in the expression activity of the promoter for the chloroplast-type PPDK: the maize gene was expressed fourfold higher than the rice gene. It was also found that a chimeric gene containing the maize Pdk promoter and a reporter gene led to high expression of the reporter gene in transgenic rice.

Based on the above observations, the intact genes from maize encoding enzymes for C₄ photosynthesis were introduced into rice to increase the activity of the C₄ enzymes. As expected, the introduction of the maize gene led to high expression of C₄ enzymes in transgenic rice. The activities of phosphoenolpyruvate carboxylase (PEPC) and PPDK increased up to 110- and 40-fold more, respectively, than those of nontransgenic rice. High expression of C₄ enzymes did not result solely from the high expression activity of the maize gene, since the introduction of a maize PPDK cDNA fused to the maize *Pdk* promoter or rice *Cab* promoter did not lead to high expression of PPDK. In some transgenic rice plants carrying the intact maize gene, the level of PPDK protein amounted to 35% of total leaf-soluble protein. The high expression of each C₄ enzyme altered metabolism slightly but did not seem to increase the photosynthetic efficiency of transgenic rice leaves.

Land plants can be divided into three major photosynthetic types—C₃, C₄, and Crassulacean acid metabolism (CAM) plants—based on differences in their mechanism of CO₂ assimilation. In C₃ plants, fixed CO₂ appears in a C₃ compound, phosphoglycerate, through carboxylation of ribulose biphosphate (RuBP) catalyzed by RuBP carboxylase–oxygenase (Rubisco). This reaction is inhibited up to 50% by atmospheric O₂ that competes with CO₂ at the active site of Rubisco, and O₂ fixed in this way wastes energy via the process of photorespiration. Taxonomical and phylogenetic studies suggest that CAM and C₄ plants were derived from C₃ plants (Moore 1982). The C₄ plants have succeeded in eliminating photorespiration by splitting photosynthetic reactions between two morphologically distinct cell types, the bundle sheath and mesophyll cells (Furbank and Taylor 1995). In a C₄ plant, maize, CO₂ is initially fixed by phosphoenolpyruvate carboxylase (PEPC) in the mesophyll cell to form a C₄ compound, oxaloacetate (OAA), which is subsequently converted to malate. Malate diffuses to the bundle sheath cell and is decarboxylated by NADP-dependent malic enzyme. The CO₂ released from this decarboxylation reaction is refixed in the Calvin cycle by Rubisco, as in C₃ plants. To

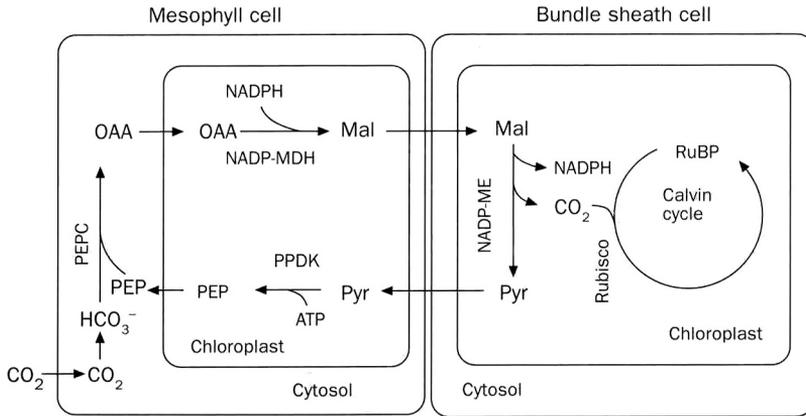


Fig. 1. The C₄ pathway of photosynthesis in maize. The major function of the C₄ pathway is to concentrate CO₂ in the inner bundle sheath cells where Rubisco is located and thus suppress its oxygenase activity and the associated photorespiration. OAA = oxaloacetate, NADP-MDH = NADP-malate dehydrogenase, Mal = malate, PEP = phosphoenolpyruvate, Pyr = pyruvate, RuBP = ribulose-1.5-bisphosphate.

complete the cycle, pyruvate produced by the malate decarboxylation diffuses to the mesophyll chloroplasts and is phosphorylated to phosphoenolpyruvate (PEP) via pyruvate, orthophosphate dikinase (PPDK) (Fig. 1).

The introduction of C₄ traits into C₃ plants to improve their photosynthetic efficiency has been an important target in plant biotechnology. For this purpose, it is necessary to establish techniques to express C₄ enzymes at high levels in C₃ plants. We report here the mechanism of evolution of C₄ photosynthesis genes, and the strategy for high expression of C₄ genes in C₃ plants based on characteristics of the C₄ genes.

Evolution of the C₄ type of *Pdk* gene

The genes involved in C₄ photosynthesis have been found in a variety of organisms, not only in C₄ (Hatch and Slack 1968) and CAM (Kluge and Osmond 1971) plants but also in C₃ plants (Imaizumi et al 1997). However, the activities of C₄ enzymes such as PPDK are much lower in C₃ plants than in C₄ plants. Therefore, the capability of expressing the C₄-type gene for PPDK, *Pdk*, at high levels is thought to be one of the primary events in establishment of the ability to perform C₄ photosynthesis. How did an ancestral gene in C₃ plants gain a C₄-specific mode of expression during the evolution of C₄ plants? Glackin and Grula (1990) and Sheen (1991) found that the C₄-type *Pdk* gene in maize is transcribed from two different initiation sites under the control of two different promoters: the first promoter produces the larger transcript encoding the C₄-type enzyme with the chloroplast transit peptide and the second promoter produces the smaller transcript encoding the C₃-type enzyme that is localized in the cytoplasm. The larger transcript is started from exon 1 by the first promoter, whereas the smaller transcript is started from exon 2 by the second promoter (Fig. 2). To investigate whether this unique mode of expression is also found in the *Pdk* gene in C₃ plants, we isolated and characterized a *Pdk* gene homologous to the maize C₄-type gene from a C₃ plant, rice.

The comparative study revealed that the deduced amino acid sequence of rice PPDK is 88% homologous to the maize C₄-type PPDK in the mature peptide region (Imaizumi et al

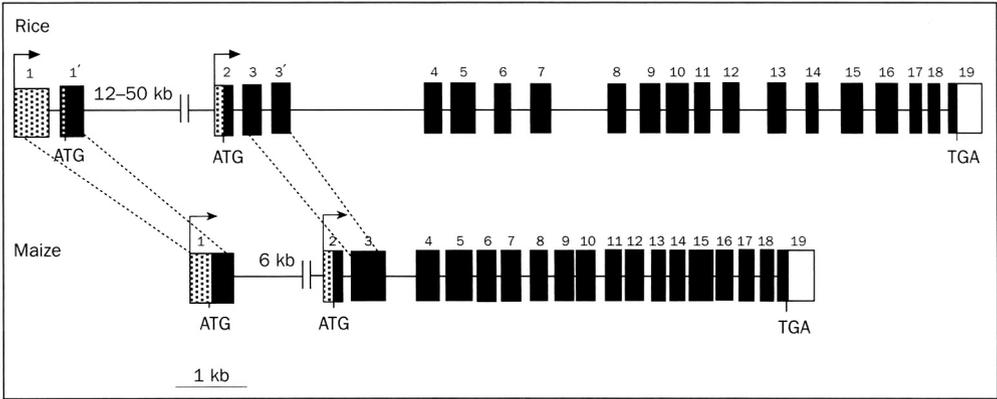


Fig. 2. A comparison of the exon-intron structure of the genes for pyruvate, orthophosphate dikinase (PPDK). In both rice and maize genes, transcription starts at the two sites indicated by bent arrows. The common coding regions of the large and small transcripts are represented by filled boxes and the 5'- and 3'-noncoding regions are represented by open boxes. Dotted boxes in rice exon 2 and maize exon 1 represent the region that encodes the transit peptide, and dotted boxes in rice exon 3 and maize exon 2 represent the coding region unique to the small transcripts. The positions of the initiation and termination codons are indicated by ATG and TGA, respectively. The broken lines linking the first and second diagrams indicate the presence of two extra introns in the rice gene.

1997). The C_4 -like *Pdk* gene in rice is essentially the same as in the maize gene, except that the rice gene has two extra introns (Fig. 2). These observations indicate that the maize C_4 -type *Pdk* gene and the rice C_4 -like *Pdk* gene are similar in terms of the primary structure.

We also examined the transcription activity of the promoters of the rice and maize *Pdk* genes *in vivo*. For each gene, we constructed two chimeric genes with a reporter gene, β -glucuronidase (GUS), under the control of either the first promoter (for the large transcript encoding the chloroplast-type PPDK) or the second promoter (for the small transcript encoding the cytoplasm-type one) (Fig. 3A). These chimeric genes were introduced into maize mesophyll protoplasts by electroporation, and the transient expression of the GUS activity was examined (Fig. 3B). The highest activity was found in protoplasts transfected with the rice cytoplasm-type promoter::GUS construct, indicating the presence of the strong promoter around the 3' end portion of intron 3 in the rice *Pdk* gene. In contrast to the cytoplasm-type promoter, the activity of the rice chloroplast-type promoter was about fourfold lower than that of the maize chloroplast-type promoter. This result is in good agreement with the low expression of the chloroplast-type transcript in rice green leaves compared with that in maize green leaves. The expression of the rice chloroplast-type promoter::GUS is regulated by light and metabolites in the same manner as in the case of the maize chloroplast-type promoter (Ku et al, unpublished data). These findings strongly suggest that both promoters have similar qualitative characters in terms of their regulation by light and metabolites. Taken together, it is demonstrated that the rice C_4 -like *Pdk* is similar to the maize C_4 -type *Pdk*, not only in terms of the primary structure but also in terms of the regulation of expression, except that the strength of the maize promoter for the chloroplast type is much higher than that of rice.

To account for the high expression of the maize chloroplast-type promoter compared with the rice promoter, we have proposed two models (Fig. 4). In the first model, the *Pdk* genes in rice and maize are different in cis-acting elements of their promoter regions. If this is the case, it is not necessary to assume that the machinery regulating expression is totally different in rice

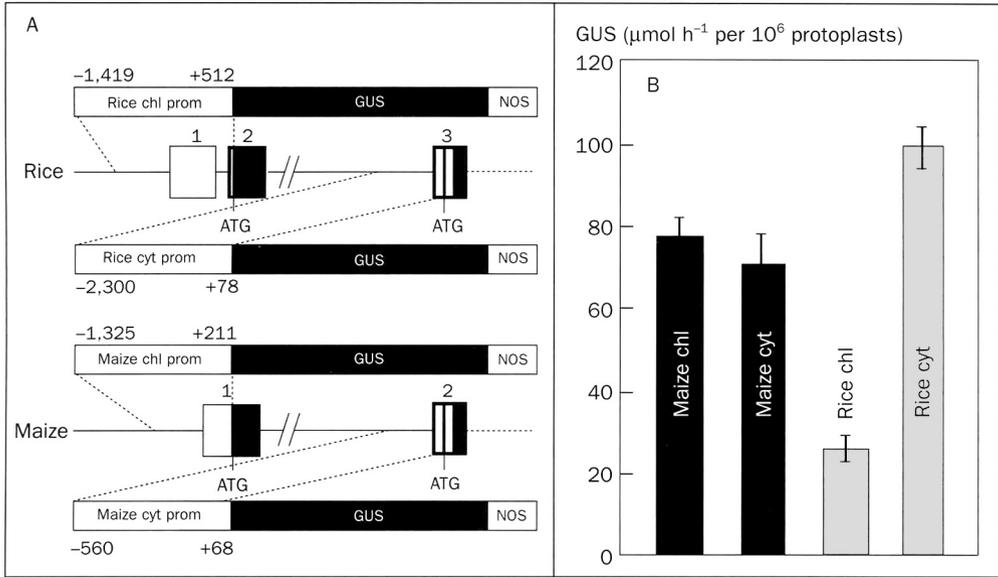


Fig. 3. The activities of the maize and rice promoters of genes for pyruvate, orthophosphate dikinase (PPDK) in maize mesophyll protoplasts. (A) Structures of the chimeric genes. The promoter sequences for the chloroplast-type transcripts (chl) or for the cytoplasmic-type transcripts (cyt) of rice and maize *Pdks* were fused to the GUS coding region. (B) The activities of the maize and rice promoters were assayed as the expression of GUS by the transient expression in isolated maize mesophyll protoplasts. The numbers -1,419, +512, etc., represent nucleotide position relative to the initiation site of transcriptions.

and maize (Fig. 4A). By contrast, in the second model, it is assumed that there are no differences in the cis-acting elements between the C_3 - and C_4 -*Pdk* genes. Rather, differences in *trans*-acting elements would account for the differential expression of the C_4 -type gene in C_3 versus C_4 plants (Fig. 4B). To examine the validity of these models, we introduced into rice the chimeric gene GUS under the control of the first promoter from the maize gene. The introduced gene was expressed at high levels almost exclusively in mesophyll cells in leaves, with no or very low activity in other cells (Matsuoka et al 1993). The expression of the transgene was also induced by light in the same manner as in maize. The high expression of C_4 -specific genes is not limited to the *Pdk* gene since another C_4 photosynthetic gene *PEPC*, *Ppc*, was also expressed in mesophyll cells in light-grown tissue (Matsuoka et al 1994). These results indicate that rice contains *trans*-acting elements that are required not only for expression of the C_4 *Pdk* gene but also for the C_4 *Ppc* gene; this therefore suggests that rice contains most of the *trans*-acting elements required for the expression of C_4 -specific photosynthetic genes. However, this notion must be examined for many other C_4 photosynthetic genes.

High expression of maize C_4 -specific genes in rice

Some attempts have been made in the past to increase the activities of C_4 photosynthesis enzymes in C_3 plants through recombinant DNA techniques (Hudspeth et al 1992, Kogami et al 1994, Gehlen et al 1996). However, the activities of the C_4 enzymes in transgenic C_3 plants were low, and consequently no significant impact on the physiology of photosynthesis was observed. The introduced genes used in these studies mainly consisted of cDNA clones encoding the C_4

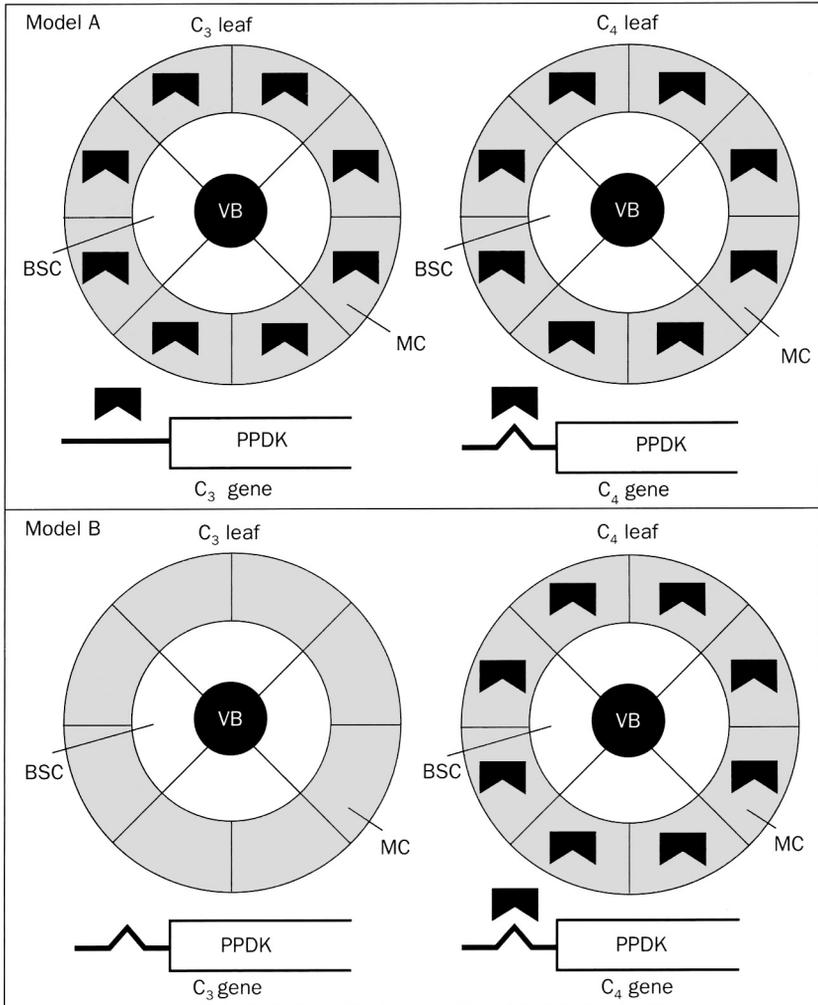


Fig. 4. Hypothetical models that account for the cell-specific expression of C_4 genes. Model A predicts that C_3 - and C_4 -specific genes differ in *cis*-acting elements in their promoter regions. Model B assumes no differences between *cis*-acting elements in C_3 and C_4 genes but predicts that trans-factors are different from each other. VB = vascular bundle, MC = mesophyll cell, BSC = bundle sheath cell, PPDK = pyruvate, orthophosphate dikinase.

enzymes and regulatory sequences, such as the 35S promoter from cauliflower mosaic virus or *Cab* promoters of target C_3 plants. As mentioned previously, we have demonstrated that the promoters in the maize *Ppc* and *Pdk* genes can drive high expression of a reporter gene in rice in a manner similar to that in maize (Matsuoka et al 1994). The finding that rice possesses the necessary regulatory factors for high expression of the C_4 -specific genes led us to examine the possibility that intact maize C_4 -specific genes, containing all exons and introns and their own promoter and terminator sequences, can produce high amounts of C_4 enzymes in mesophyll cells of C_3 plants.

Following this idea, we introduced the intact gene for the C_4 PEPC enzyme of maize into rice using *Agrobacterium tumefaciens* to mediate gene transfer. More than 100 primary

transformants were obtained from antibiotic resistant cells. The transgenic plants showed a wide range of PEPC activity. The majority (85%) had activity 2- to 30-fold higher than that of nontransformed plants, with the remaining 15% showing 30- to 110-fold higher activity, or 1- to 3-fold the maize PEPC activity on the protein basis (Fig. 5). Electrophoretic analysis of total leaf protein extracts revealed the presence of a novel 110-kDa polypeptide, corresponding to that of maize PEPC, in transgenic rice plants. Furthermore, the amount of the polypeptide, as judged from band intensity, correlated well with the enzyme activity. Immunoblotting analysis with the antibody for maize PEPC confirmed that the novel 110-kDa polypeptide was the product of the maize *C₄*-type *Ppc* gene. These results indicate that the elevated PEPC activities in transgenic rice plants were due to the expression of the maize enzyme. To our knowledge, such high expression of a transgene in plants has not been reported previously. Indeed, earlier attempts to increase PEPC activity in transgenic *C₃* plants reported only a 0.5- to 3-fold increase in activity.

The same strategy was also successful in expressing *C₄*-specific PPDK in rice and activity in green leaves increased up to 40-fold more than that of nontransformed rice with the introduction of the intact PPDK gene from maize. In some transformants, the level of the PPDK protein was extraordinarily high: it amounted to 35% of total leaf-soluble protein and was comparable with that of the large subunit of Rubisco, the most abundant protein in leaves of *C₃* plants (Fig. 6). This amount of protein was much higher than that of PEPC in transgenic rice plants carrying the maize PEPC gene. Such high expression was not ascribable solely to the transcriptional activity of the maize PPDK gene, since expression of the corresponding cDNA under the control of either the promoter of the maize *C₄*-specific PPDK gene or the rice *Cab* promoter increased the activity less than fivefold. Therefore, it is possible that the presence of exons and introns or the terminator sequence or both in intact genes acts to confer the high expression. By N-terminal amino acid sequencing, we found that the maize PPDK protein expressed in rice leaves was located exclusively inside the chloroplast, as observed in maize. These results demonstrate that the introduction of intact genes for *C₄*-specific enzymes is a very effective technique for producing transgenic *C₃* plants with high amounts of *C₄*-specific enzymes.

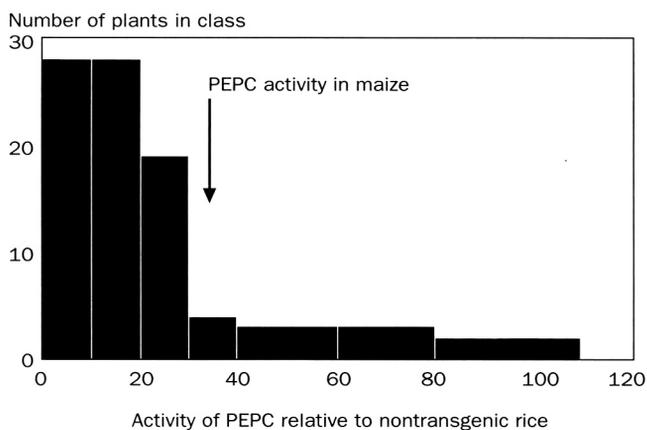


Fig. 5. Frequency distribution of the activities of phosphoenolpyruvate carboxylase (PEPC) in 87 transgenic rice plants. The activity of PEPC is relative to the activity in nontransformed rice plants. Note that PEPC activity of maize leaves is about 35 times higher than that of nontransformed rice.

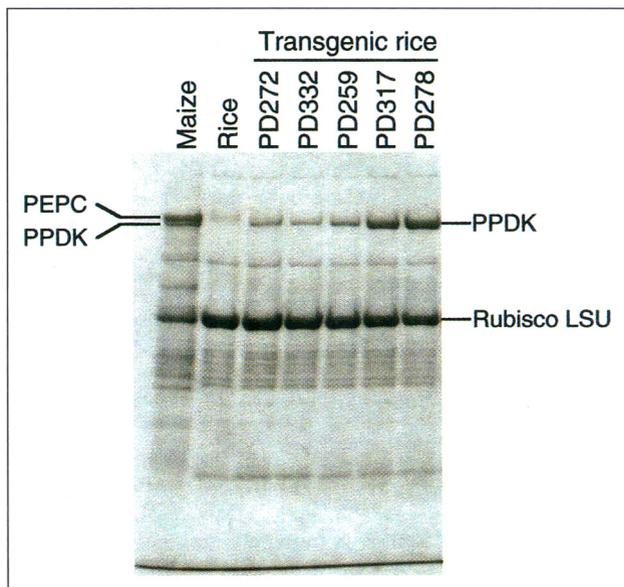


Fig. 6. Polypeptide profiles of leaf-soluble protein in maize, rice, and homozygous lines of transgenic rice carrying the intact maize *Pdk* gene. Protein was stained with Coomassie brilliant blue R-250. LSU = the large subunit of Rubisco, PEPC = phosphoenolpyruvate carboxylase, PPDK = pyruvate, orthophosphate dikinase.

In C_4 plants, the activity of chloroplastic PPDK is strictly regulated by light conditions via the action of the specific regulatory protein (PDRP; Burnell and Hatch 1985). To examine whether a similar regulation mechanism is operative for the maize PPDK protein in transgenic rice plants, the effects of illumination on PPDK activity were investigated. PPDK activity was much higher under illumination than in darkness in all plants examined. However, differences existed in the extent of light activation between maize and rice plants. The light/dark activity ratios were 20 in maize, 6 in nontransformed rice, and 6 to 9 in transgenic rice. If it is assumed that PPDK was fully activated under illumination in maize, only 10% to 30% of PPDK was activated in transgenic rice. These results indicate that the endogenous rice PDRP can regulate the activity of maize PPDK, although the capacity of PDRP to activate PPDK was limited, possibly due to a small amount of PDRP relative to PPDK in transgenic rice.

To examine the effects of high expression of maize PEPC on photosynthetic characteristics, the oxygen sensitivity of photosynthetic CO_2 assimilation of transgenic rice plants was investigated by measuring CO_2 assimilation rates under 21% and 2% oxygen (Fig. 7). Oxygen inhibition of photosynthetic CO_2 assimilation was negatively correlated to the activity of PEPC in transgenic rice plants. This reduced oxygen inhibition was explained by direct CO_2 fixation by the maize PEPC in transgenic rice (Ku et al 1999). However, photosynthetic CO_2 assimilation of transgenic rice decreased with increasing PEPC activity at both oxygen concentrations. In addition, the slope of the regression line was steeper in 2% oxygen than in 21% oxygen. Thus, the observed reduction of oxygen inhibition might result from decreased CO_2 assimilation in 2% oxygen in transgenic rice. Since the photosynthetic rate in 2% oxygen can be limited by P_i regeneration capacity, high expression of PEPC might affect P_i regeneration capacity in transgenic rice.

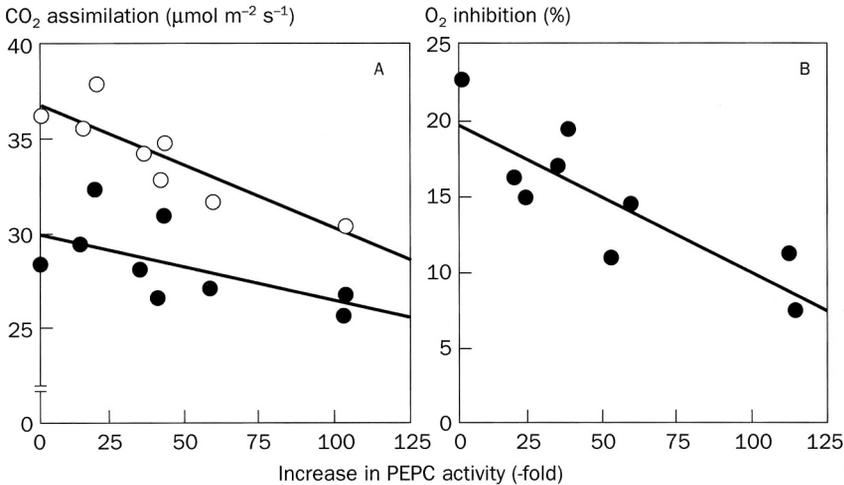


Fig. 7. Photosynthetic CO₂ assimilation rates (A) and oxygen inhibition of photosynthesis (B) as a function of phosphoenol/pyruvate carboxylase (PEPC) activity in transgenic rice carrying the intact maize PEPC gene. Photosynthetic rates were measured under 21% (●) and 2% (○) oxygen. The lines are the regressions of assimilation or inhibition on increase in PEPC activity.

Conclusions

Our strategy for high expression of PEPC and PPDK in rice has succeeded and has made it possible to change the carbon metabolism of rice. Some studies on physiological changes in transgenic rice plants have been described by our collaborators in another chapter in this book (Ku et al, this volume).

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Notes

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Influence of carbon–nitrogen balance on productivity of C₃ plants and effect of high expression of phosphoenolpyruvate carboxylase in transgenic rice

M. Osaki and T. Shinano

A root-shoot interaction model is proposed to explain the high productivity of high-yielding varieties of several crops, based on high-yielding trials. In the high-yielding varieties, nitrogen is always actively absorbed during the vigorous sink organ-filling stage (maturation or the ripening stage of growth). Thus, photosynthetic rate (dry matter increase) and root activity (nutrient absorption) remained constant during maturation in high-yielding varieties because a high photosynthetic rate maintains a high root activity by supplying a sufficient amount of photosynthate to the roots, a phenomenon referred to as root-shoot interaction for high productivity. On the other hand, in the varieties with standard yield, hereafter referred to as standard or old or low-yielding varieties, the photosynthetic rate decreased, followed by a decrease in root activity because of the reduced carbohydrate supply; nitrogen incorporated into leaves and stems before maturation was retranslocated during maturation.

Two carbon-nitrogen (C–N) interaction models are developed. One is $DM_t = DM_o \exp(CNI \times N_t)$ for cereals and the other is $DM_t = DM_o + CNI' \times N_t$ for legumes, where DM_t is the dry weight of a plant at a given time, N_t is the amount of N accumulated in the plant at a given time, DM_o is the initial dry weight, and CNI and CNI' are the C–N indices. Moreover, the productivity per unit amount of N accumulated in legumes is quite low compared with that in cereals during the vegetative growth stage. This is caused by the low growth efficiencies [accumulated dry matter/(accumulated dry matter + respiration)] of whole plants regardless of nitrogen concentration, indicating that the concept of growth and maintenance respiration is not valid.

The fate of photosynthesized $^{14}CO_2$ was quite different between rice and soybean. In soybean, a large amount of photosynthesized $^{14}CO_2$ is respired in the light compared with that in the dark, but in rice the amount of ^{14}C retained in the leaves is similar regardless of light conditions. This high respiratory loss of current photosynthate in soybean in the light can be explained partly by the high rate of photorespiration in the leaves. A large portion of photosynthetically fixed $^{14}CO_2$ in soybean in the light was distributed into organic acids, amino acids, and protein compared with that in rice, where metabolism of newly fixed carbon is mainly regulated by the activity of sucrose-phosphate synthase (SPS) and especially phosphoenolpyruvate carboxylase (PEPC). Thus, it is assumed that the carbon-nitrogen balance of the whole plant is regulated by (1) whether current photosynthate distributes into the tricarboxylic acid cycle or sucrose metabolism in the light, which is regulated by PEPC or SPS, respectively, and (2) whether photorespiratory activity is high or not. This information will help to improve crop productivity through regulation of carbon-nitrogen metabolism.

The distribution of $^{14}\text{CO}_2$ to chemical compounds was studied in transgenic rice plants that showed high expression of the maize PEPC gene. The C/N ratio decreased in transgenic plants compared with controls because of high ^{14}C distribution to organic acids. As the transgenic plant could exude much organic acid from the roots, this plant showed aluminum tolerance for high aluminum in solution.

The old concept of plant productivity had been mainly centered on the carbon balance, with emphasis on plant type in relation to respiratory balance. Jensen (1932) pointed out the importance of leaf area and leaf angle in relation to dry matter production, and then Monsi and Saeki (1953) applied the concept of Jensen for a model of the light regime in relation to plant population geometry. Tsunoda (1959a,b) suggested that plant architecture must be considered in breeding for high-productivity crops. Donald (1968) outlined the ideotype for high yield in the case of wheat as follows: (1) a short, strong stem, (2) few, small, erect leaves, (3) a large erect ear, and (4) a single culm. After the demonstration of the presence of optimum leaf area index (LAI) by Watson (1952), the physiological basis for ideotypes for high yield was considered as follows. The photosynthetic rate increases more or less proportionally to the increase in LAI of a population until a certain LAI value is reached, and the photosynthetic rate may decrease because of mutual shading of leaves when the LAI value exceeds this value. Meanwhile, the respiration rate increases almost proportionally to the increase in LAI because the respiration rate is not affected by mutual shading. For these reasons, dry matter production reaches a maximum value at the optimum LAI (Takeda 1961, Tanaka 1972). According to this concept of productivity, in crops with an unfavorable plant type, lower leaves, culms, and roots respire a large amount of substrate, which is assimilated in the upper leaves. Tanaka and Osaki (1983), however, reported that the ^{14}C retention fraction under field conditions, that is, the fraction of ^{14}C retained in the plant after ^{14}C assimilation to the total amount of ^{14}C assimilated by the plant, increased with growth in rice, spring wheat, maize, potato, and sugar beet. The pattern of the carbon balance of current photosynthate was opposite to that predicted based on the plant-type theory. The ^{14}C retention ratio of tropical rice was also not affected by plant type (Cock and Yoshida 1973) or artificial mutual shading (Osaki et al 1995a). Accordingly, most of the current photosynthate is translocated to new growing organs and is used for respiration at the level of the assimilating leaf or new growing organs, suggesting that, in old organs, materials previously constructed were decomposed and translocated to growing organs or used for respiration in the old organs themselves (Osaki and Tanaka 1979, Osaki et al 1995a, Shinano et al 1996). Consequently, it is now thought that the effect of plant type on high productivity cannot be explained by the respiratory loss of photosynthate from organs at a lower position, which casts doubt on the concept of optimum LAI.

The interpretation of recent experiments (Osaki et al 1991b,c, 1995b) is that nitrogen must continue to be absorbed by the roots during maturation in order to supply the shoots and maintain high photosynthesis, which in turn supplies the roots with carbohydrate. Therefore, the growth or activity of roots (nitrogen absorption) and shoots (carbon assimilation) is assumed to be mutually regulated by each other, which will be discussed later in the root–shoot interaction model for high productivity.

In other experiments for the carbon–nitrogen balance in whole plants during growth, the balance was considerably different between cereal crops and legume crops (Osaki et al 1993c, 1996). As described later, the difference in the carbon–nitrogen balance in whole plants between both crops was ascribed to the difference in the mechanisms of current photosynthate distribution into the tricarboxylic acid (TCA) cycle and amino acids or into sucrose in leaves in the light.

In this section, new concepts on crop productivity are proposed, namely, the hypothesis of carbon–nitrogen balance including root–shoot interactions and interaction of carbon–nitrogen metabolism.

Regulation of root–shoot interactions

High-yielding trials

The productivity of field crops, especially cereal crops, has increased significantly through the improvement of the plant type (semidwarf and upright leaf) by breeding. Nevertheless, according to the above discussion, it is difficult to explain the high productivity of new varieties based only on the carbon (photosynthesis–respiration) balance theory.

Using high-yielding varieties and slow-release fertilizer, a very high yield was attained for various field crops in Sapporo (43°03'N), Japan (Osaki et al 1991c, 1992c, 1995b: Table 1). These high-yielding varieties (HYVs) were characterized by the fact that the total amount of dry matter (DMt) and the amount of nitrogen absorbed (Nt) in a whole plant increased linearly with growth until harvest (Osaki et al 1991b,c), indicating that photosynthetic rate and root activity remained high during maturation. This type is designated as nitrogen absorption type. In contrast, in standard-yielding varieties (SYVs), nitrogen absorption ceased at the maximum shoot growth stage, and at the same time Nt in the nonreproductive organs started to decrease, followed by cessation of the increase in dry matter. This type is designated as nitrogen efficiency type.

For HYVs, the amount of nitrogen absorbed in plants reached a value of 30 to 40 g N m⁻², half of which was absorbed within only 30 d during maturation. According to these results, it is impossible to obtain more than 10 t ha⁻¹ yield under the current methods of cultivation when nitrogen absorption by SYVs is assumed to amount to only 10 to 20 g N m². Consequently, since in the SYVs harvested parts grow by using nitrogen stored or incorporated in leaves and stems before maturation, root activity during maturation does not play a major role. Thus, the basic strategy to achieve high yield should be to transfer the nitrogen efficiency type to the nitrogen absorption type in breeding programs. The issue to address is why the root activity of HYVs remained high during maturation. In this regard, the carbohydrate distribution system is analyzed in the next section.

Photosynthate distribution into roots

Since one unit of internode organs, namely, leaf, axillary organ, and root, can reproduce the whole plant, it is defined as a phytomer or phyton (Evans and Grover 1940, Kawata et al 1963) (Fig. 1). However, since the leaf-upper adventitious root and the branch-lower adventitious root in the phytomer belong to a different phyllotaxy, it is difficult to explain the photosynthate distribution within a phyton or between phytoms. Therefore, we propose a node unit hypothesis.

Table 1. Yields (g m⁻²) from high-yielding trials in Sapporo, Japan.

Yield	Rice	Wheat	Maize	Soybean	Potato	Sugar beet	Sunflower
Biological yield ^a	1,510	2,389	2,240	1,150	2,127	2,400	2,933
Economic yield ^b	750	1,325	1,140	530	1,706	1,470	393

^a= aboveground dry matter (including economic yield). ^b= absolute dry matter and grain yield for cereal crops. Sources: Osaki et al (1991c, 1992c, 1995b).

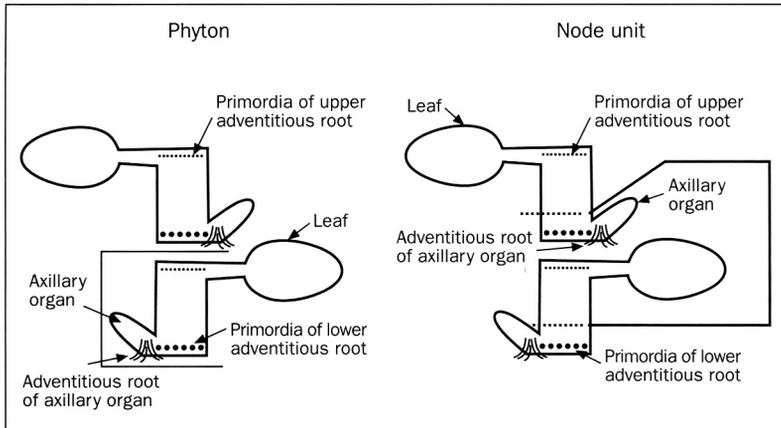


Fig. 1. Diagrams of the phyton and node unit.

with leaf, axillary organ, upper adventitious root, and lower adventitious root in the same phyllotaxy (Fig. 1). According to the node unit hypothesis, the ^{14}C -photosynthate distribution system operates as follows. The interrelationship among organs is strong and in the order of (1) within the node unit > (2) among the node units in the same or adjacent phyllotaxy > (3) in the main root or apical organs adjacent to node units (unpublished data).

In monocotyledonous plants (rice, wheat, and maize) before the floret initiation stage, the node unit is belowground, each normally with lateral roots that it supplies with photosynthate (Fig. 2). After the floret initiation stage, however, as the internode elongates, each node unit in the elongated stem has no roots and the roots depend on the activity of the lower leaves, which decreases with mutual shading and senescence. Therefore, in monocotyledonous plants, it is important to maintain root activity after the floret initiation stage. In dicotyledonous plants, since each node unit normally has no roots, except for the potato plant (germinated from the mother tuber), the system of photosynthate distribution into roots is different from that of monocotyledonous plants. In legumes, the lower node units mainly supply photosynthate to roots (Fig. 2), whereas, in dicotyledonous root crops, all the node units supply photosynthate to the belowground organs.

Thus, cereals and legume crops form strong sinks in aerial parts, and the root–shoot interaction becomes weak with growth. Therefore, plant type or canopy structure plays an important role in the maintenance of a high root activity because the lower leaves mainly supply photosynthate to roots, especially in cereal crops (Tanaha 1961; unpublished data by Osaki et al). The high performance of the semidwarf plant type with upright leaf can be better explained by this phenomenon than by the respiratory loss theory: upright leaves allow more light to the lower leaves that are supplying the roots with photosynthate. On the other hand, root crops can supply carbohydrates to roots because roots are adjacent to the sink organs. In spite of this advantage of root crops, if the sink activity becomes stronger, the root activity decreases because the carbohydrate supply to roots is reduced by the sink demand for carbohydrates. Osaki et al (1991a) reported that the distribution of ^{14}C assimilated in each leaf abruptly decreased in roots due to competition for carbohydrates with sink organs when tuber swelling started.

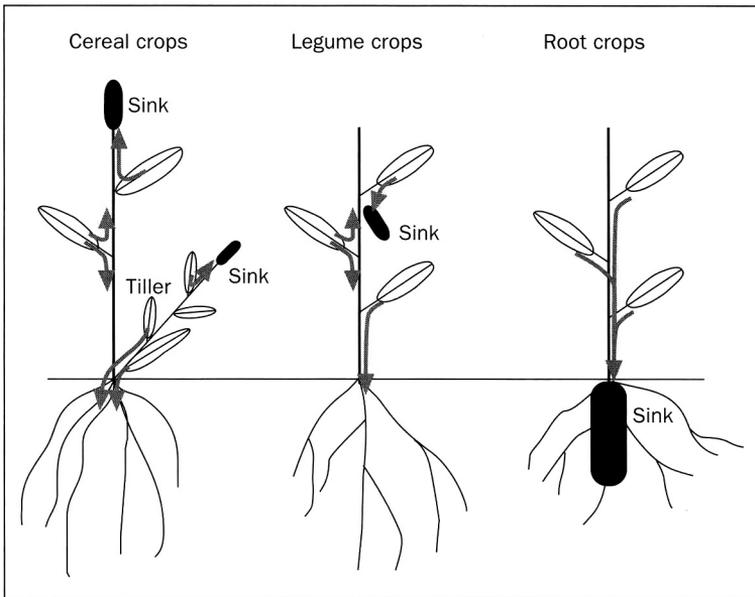


Fig. 2. Scheme of photosynthate distribution into roots based on Tanaka (1961) for rice, Tanaka and Fujita (1979) for field bean, Osaki et al (1991a) for potato, and unpublished data. In cereal crops, because tillers develop in the lower node unit and tillers have roots, photosynthates are actively supplied to roots from the lower node unit before the reproductive stage. After the reproductive stage, because the upper node unit has no roots, a small amount of photosynthates is supplied to the roots. In legume crops, node units have no roots and the connection between node units and roots is weak. In root crops where roots are adjacent to the sink, photosynthates are supplied to the roots from all the node units.

Specific absorption rate of nitrogen by roots (SARN)

To estimate the role of the roots in nitrogen absorption, the specific absorption rate of nitrogen per unit dry weight of root (SARN, $\text{g N g}^{-1} \text{DM d}^{-1}$) is defined (Osaki et al 1995b) as the nitrogen absorption rate (AN, $\text{g N m}^{-2} \text{d}^{-1}$) divided by root weight (RW, g m^{-2}). The SARN of field crops during maturation was higher in HYVs than in SYVs (Osaki et al 1995b). The root weight was not affected by nitrogen absorption or dry matter production, especially during maturation (Osaki et al 1995b), which indicates that root weight itself is not an index of high root activity. Although root respiration is thought to be an indicator of nitrogen absorption activity in roots (de Visser et al 1986, van der Werf et al 1993), root respiration was less affected by SARN (unpublished data). On the contrary, as the nitrogen content in roots was considerably affected by SARN (unpublished data), nitrogen content of roots is a better indicator of root activity in terms of nitrogen absorption than root respiration.

Conclusions

The root-shoot interaction hypothesis for high productivity of plants (not only root crops) alleviates the shortcoming of theories of plant type in relation to the carbon balance. Others have also emphasized the importance of root-shoot interactions (e.g., Hunt 1975, Richards 1976, Jesko 1992). Our trials of HYVs revealed the importance of root-shoot interactions during maturation, particularly when high yield was attained. For the SYVs, the importance of root-shoot interactions was masked since large amounts of nutrients for harvested parts depend

mainly on nutrient retranslocation from shoots where they were stored before maturation. In our high-yielding trials, since the SARN value was not different between the HYVs and SYVs before maturation, studies should focus on root activity at the later growth stage, which is strongly related to the system of carbohydrate supply to roots.

Adjustment of carbon-nitrogen balance in the whole plant

Carbon-nitrogen in the whole plant throughout life

The relationship between the amount of dry matter accumulated (DM_t) and the amount of nitrogen absorbed (N_t) in a whole plant at the successive growth stages was as follows (Osaki et al 1992c):

$$\text{Cereals } DM_t = DM_0 \exp(\text{CNI} \times N_t)$$

$$\text{Legumes } DM_t = DM_0 + \text{CNI}' \times N_t$$

Here, DM_t is the dry weight of a plant at time t , DM_0 is the initial dry weight, CNI and CNI' are the carbon-nitrogen index for cereals and legumes, respectively, and N_t is the amount of N accumulated in the plant at time t .

The CNI of cereal crops varied according to the nitrogen application rate: CNI was high at low nitrogen application rates and vice versa, whereas CNI' of legume crops was quite stable regardless of nitrogen application rates (Osaki et al 1992c) (Fig. 3). This difference in the DM_t - N_t relationship between legumes and cereals cannot be ascribed to the fact that a large amount of energy was lost during nitrogen fixation through respiration in legumes. When the relationship of DM_t and N_t was examined in isogenic lines of soybean (A62-1, nodulated, and A62-2, nonnodulated), the effect of nodulation for nitrogen fixation on the coefficient CNI' was negligible (Osaki et al 1992c), indicating that the respiratory loss of carbohydrates for nitrogen fixation was not large enough to change the relationship in soybean. Thus, as a result of the variation in CNI and CNI', it is assumed that the variation in the DM_t/N_t ratio is large in cereals and negligible in legumes; this was clear from the frequency distribution of the shoot DM_t/N_t ratio in tropical pasture crops (Fig. 3).

It is interesting to determine how the carbon-nitrogen balance changes in a high CO_2 environment because of the increase in the CO_2 concentration in the atmosphere. The effect of CO_2 enrichment on the carbon-nitrogen balance in the whole plant was studied in wheat (spring wheat) and soybean (A62-1 and A62-2) with a combination of two nitrogen application rates (0 and 30 g N m^{-2}) and two temperature treatments (30/20 and 26/16 °C day/night) (Nakamura et al 1997b). Carbon-nitrogen interaction in wheat was strongly affected by the rhizosphere environment (nitrogen nutrition), but not by the atmosphere environment (CO_2 enrichment and temperature), whereas that in soybean was less affected by environments above or below ground. Thus, the root environment and probably root activity are strongly related to the carbon-nitrogen balance in whole plants.

Among the cereal crops, nitrogen-use efficiency in C_4 plants is presumably high because the photosynthetic rate per unit amount of nitrogen in leaf is higher in C_4 plants than in C_3 plants (Sage and Pearcy 1987). However, in the field experiments, nitrogen-use efficiency of whole plants at harvest was only slightly higher in C_4 plants than in C_3 plants when a small amount of nitrogen was absorbed. When a large amount of N was absorbed, however, the values of nitrogen-use efficiency at harvest were similar between C_3 and C_4 plants if nitrogen-use efficiency was compared at the same amount of nitrogen absorbed, which was less affected by weather conditions (Osaki et al 1992c).

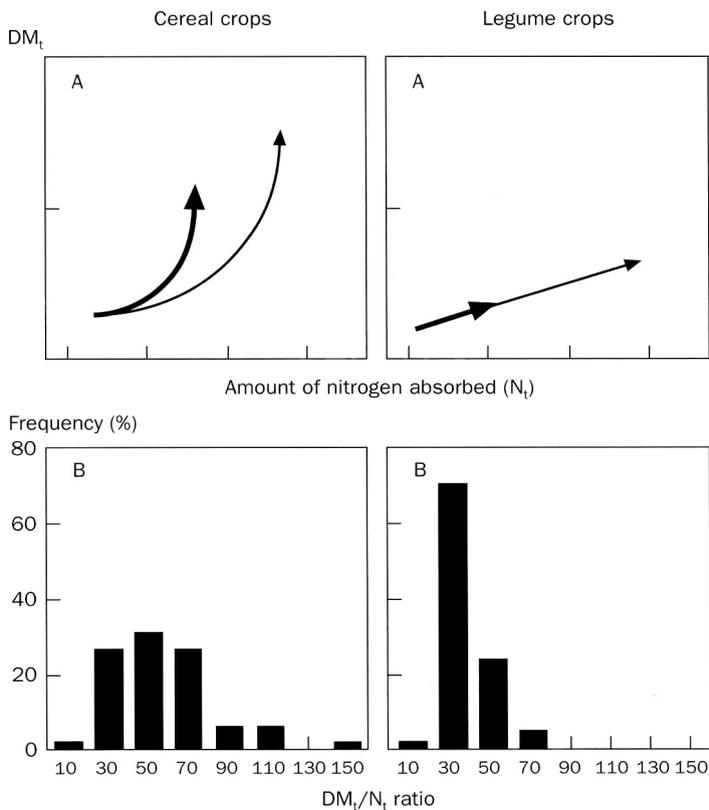


Fig. 3. (A) Sketch of the relationship between the amount of dry matter accumulated (DM_t) and the amount of nitrogen absorbed (N_t) by a whole plant at successive growth stages. See text for equations for the curves. (B) Frequency distributions of the ratio of DM_t/N_t in shoots (vegetative growth stage) of cereals (C_3 plants) and legumes grown in the tropics, calculated from the data of Göhl (1981). The class interval for DM_t/N_t is 20 g g^{-1} and the center of the class is shown; $n = 156$ for cereals and $n = 135$ for legumes.

Thus, even if the photosynthetic rate per unit amount of nitrogen in leaf varies among cereal crops, the difference in the carbon-nitrogen balance of the whole plant becomes negligible among cereal crops because of various metabolic adjustments. It is thus concluded that a large difference exists in metabolic systems between cereals and legume crops.

Growth efficiency

The reason for the large difference in dry matter production per unit amount of nitrogen absorbed between cereals and legume crops is ascribed to the differences in the (1) photosynthetic rate per unit amount of nitrogen and (2) respiratory rate per photosynthetic rate. However, for (1), the difference between cereals and legume crops was not appreciable (unpublished data). Therefore, the respiration-photosynthesis balance in the leaf was examined based on the growth efficiency ($GE = RGR/(RGR + R)$), where RGR is the relative growth rate on a carbon basis and R is the respiratory rate on a carbon basis. To determine the effect of the protein content on GE, both plants (without nodulation in soybean) were grown hydroponically with three nitrogen application rates. It was found that GE was affected more by ontogeny than by the nitrogen

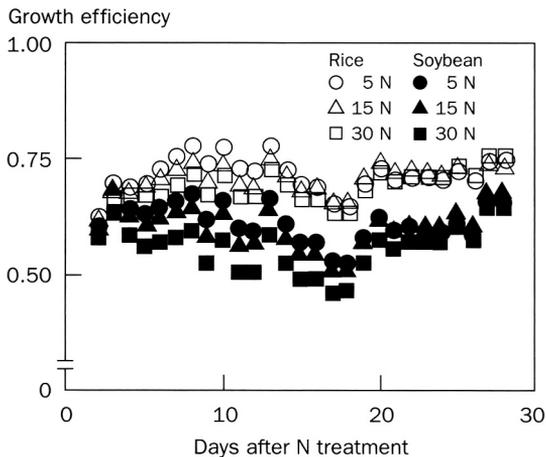


Fig. 4. Growth efficiency of rice and soybean in water culture with three nitrogen treatments.

application rate (Fig. 4; Shinano et al 1995). The effect of other chemical components was also similar in rice and soybean at the vegetative growth stage. If the metabolic processes of growth and maintenance respiration are similar, the GE of both crops should be similar when the protein content in plants is similar—25% in rice and 28% in soybean. However, the GE of rice was 10% to 20% higher than that of soybean even when the protein content was similar, indicating that growth respiration is not a major factor. Since the temperature effect on GE was negligible (Shinano et al 1995), we suggest that maintenance respiration is not an important factor either.

Consequently, based on the data for the carbon–nitrogen or dry matter to nitrogen ratio and GE, it can be seen that the mechanism of regulation of the carbon–nitrogen balance is different between cereals and legumes, and this could not be explained by the concept of growth and maintenance respiration.

Current photosynthate distribution into various compounds in the light

As the GE of shoots in soybean was lower and the respiratory rate in leaves was higher than in rice (Shinano et al 1995), we assume that the balance of the carbon–nitrogen metabolism in the leaf is considerably different between rice and soybean. To examine this difference in biochemical pathways, photosynthesized ^{14}C was traced. A large amount of $^{14}\text{CO}_2$ was released rapidly from the leaf of soybean in both the light and the dark compared with rice, which was less affected by the nitrogen application rate (Shinano et al 1994). Thus, since the respiratory rate was higher in soybean not only in the dark but also in the light, it is necessary to determine how current photosynthate is distributed into each chemical compound, and how photorespiration contributes to the CO_2 release rate and carbon distribution into each chemical compound.

In rice and soybean, $^{14}\text{CO}_2$ was assimilated during 10 min by a leaf under 21 kPa O_2 (21% O_2 treatment) or 2 kPa O_2 (2% O_2 treatment) at the vegetative growth stage (Nakamura et al 1997a). The fractions of ^{14}C in respired CO_2 and crude chemical components (sugars, polysaccharides, organic acids, amino acids, and proteins) in the light were measured (Fig. 5). Since the ^{14}C -distribution mechanism into carbon compounds and nitrogen compounds is of particular interest here, the carbon metabolism pool (C-pool) is defined as sugars and

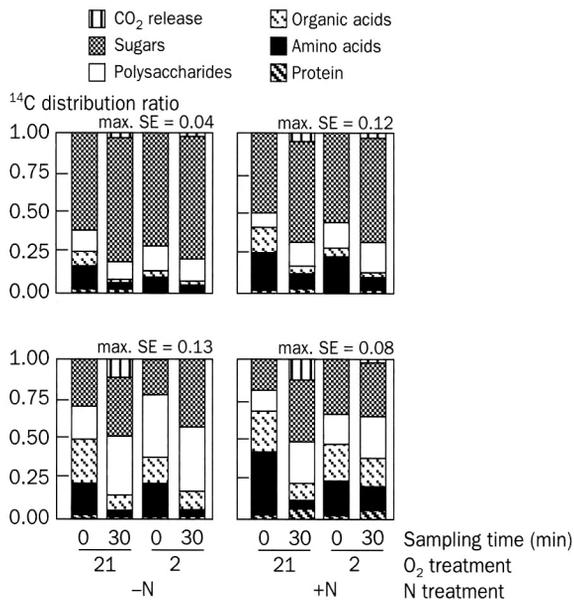


Fig. 5. Current photosynthate distribution for respiration, and into sugars, polysaccharides, organic acids, amino acids, and protein in a leaf of rice (A) and soybean (B) at two nitrogen application rates and two O₂ concentrations in the light. For the ¹⁴C distribution into the N-pool, the amount of ¹⁴C supplied from photorespiration, the phosphoenolpyruvate carboxylase (PEPC) system, or other pathways is not known. Modified from a figure in Nakamura et al (199713). SE = standard error.

polysaccharides, and the nitrogen metabolism pool (N-pool) as the organic acids, amino acids, and proteins. The fraction of ¹⁴C in the N-pool after the 10 min of ¹⁴C assimilation was higher in soybean than in rice, regardless of treatments and growth stages, but decreased markedly in both rice and soybean 30 min later. A larger amount of photosynthesized ¹⁴C was distributed into the N-pool than into the C-pool, especially in soybean in the light. During the transfer, the ¹⁴C compounds in the N-pool were actively respired.

In the 2% O₂ treatment, ¹⁴C distribution into the N-pool decreased in both crops regardless of N treatment, indicating that photorespiration plays an important role in the supply of the previously photosynthesized carbon compounds into the N-pool. In the 2% O₂ treatment, ¹⁴C distribution into the N-pool was higher in soybean than in rice, indicating that triose-P transported from the chloroplast was preferentially distributed into the N-pool. The fractions of ¹⁴C released and in organic acids and amino acids were reduced in 24 oxygen, more so in soybean than in rice (Fig. 5). Nevertheless, the fraction of ¹⁴C in organic and amino acids remained larger in soybean than in rice. Overall, we concluded that photorespiration is closely linked to TCA and amino acid metabolism and that photorespiration affects these pathways more in soybean than in rice.

At the onset of ¹⁴C assimilation, the ¹⁴C amino acid pool was mainly composed of serine and glycine, whose fraction was higher in soybean than in rice because the aspartic acid fraction was higher in rice than in soybean (Nakamura et al 1997a). On the other hand, as the distribution to serine and glycine was also active under the 2% O₂ treatment in both crops, it remains to be determined whether serine and glycine are produced only by photorespiration. When ¹⁴C sucrose or ¹⁴C amino acids were introduced to the leaf tip in rice, these compounds were actively

metabolized into various compounds, with a large amount of glycine and serine in the free amino acid pool (Osaki et al 1992a,b). Thus, glycine and serine were not formed through the photorespiratory pathway alone. Eickenbusch et al (1975) reported that glycolate was produced in the chloroplast from fructose-6-phosphate, which was not affected by O₂ concentration, and not only from RuBP through photorespiration. We assume that photorespiration is also significant for the synthesis (or carbon flow) of other amino acids since, under the 2% O₂ treatment, the distribution of ¹⁴C to aspartic acid decreased in both crops.

In summary, photosynthetically fixed carbon in the light is distributed into the carbon skeleton of sugars and polysaccharides (C-pool), and into the carbon skeleton of organic acids and amino acids (N-pool) (Fig. 6). Champigny and Foyer (1992) suggested that the triose-P distribution into sucrose and organic acids (including amino acids) was regulated by the activities of sucrose-phosphate synthase (SPS) and phospho*enol*pyruvate carboxylase (PEPC), respectively. The activities of PEPC and SPS may be related to this distribution in carbon–nitrogen metabolism because the ratio of PEPC activity to SPS activity was higher in soybean than in rice (Nakamura et al 1997a). Carbon in the N-pool in soybean is remetabolized to the C-pool via various pathways along with the release of a large amount of ¹⁴C. Also, photorespiration contributes more significantly to the photosynthesized carbon distribution to the N-pool in soybean than in rice. In addition, since the system of photosynthesized carbon distribution to the C-pool and N-pool was less affected by nitrogen status (Fig. 5), it was basically regulated by the inherent characteristics of the crop. This system of carbon distribution that was regulated by the growth stages in rice (or photoperiod: unpublished), unlike in soybean, is responsible for the difference in the carbon–nitrogen balance between cereals and legume crops (Osaki et al 1992c). Shinano et al (1994) showed that, since ¹⁴CO₂ released in the light or dark was still more active in soybean than in rice after ¹⁴CO₂ assimilation, carbon compounds (mainly starch in soybean) stored in the C-pool in soybean tended to be used for dark respiratory

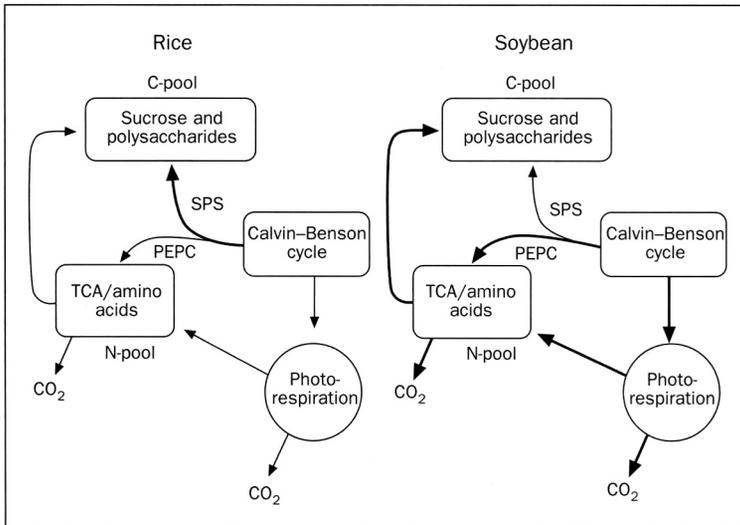


Fig. 6. Scheme of current photosynthate distribution into sugars and polysaccharides (C-pool) and organic acids, amino acids, and protein (N-pool) in rice and soybean in the light. In the dark, substrates in the C-pool are mainly used for dark respiration, especially in soybean.

substrates. In conclusion, the difference in the mechanisms of distribution of current photosynthesized carbon into the C-pool and N-pool between cereals and legume crops results in differences in the carbon–nitrogen balance (Osaki et al 1992c) and growth efficiency between cereals and legume crops (Shinano et al 1995).

¹⁴CO₂ distribution to chemical compounds in the PEPC transgenic rice plant

In this chapter, we assume that PEPC has an important role in the current photosynthate distribution to organic acids and amino acids under light conditions. Ku et al (1999) introduced the intact gene of maize PEPC into the C₃ rice crop, and PEPC activity in leaves of some transgenic rice plants was two- to threefold higher than that in maize. PEPC transgenic rice plants, however, still show C₃ characteristics. As PEPC activity of these transgenic rice plants was high in the cytosol of the leaf and slight in the leaf sheath and stem, it is a good model to elucidate whether PEPC regulates current photosynthate distribution to organic acids.

¹⁴C distribution to organic acids at 0 min after 5 min ¹⁴CO₂ assimilation was almost two times higher in the transgenic rice plant than in the control rice plant (Table 2). However, ¹⁴C distribution to amino acids and proteins in leaves did not increase (Table 2), and, on the contrary, free amino acid concentration in leaves decreased about 20% in the transgenic plant compared with the control plant because of the high requirement of amino acids for construction of PEPC protein, which corresponds to 12% of the total leaf soluble protein (Ku et al 1999). We expected that current photosynthate distribution to amino acids would increase in PEPC transgenic rice plants (as in soybean); however, that system of current photosynthate distribution to amino acids must cooperate with another metabolic pathway, not only PEPC. Nitrogen concentration (mg g⁻¹ dry matter) of the whole plant at the vegetative stage was higher in transgenic rice (15.4) than in the control (11.1), indicating that as a large amount of current photosynthate was distributed to organic acids, current photosynthate was respired more in the transgenic rice plant than in the control. Thus, high PEPC activity in the cytosol will cause a decrease in C/N ratio through carbon loss by respiration.

In the transgenic rice plant, as the PEPC gene is expressed mainly in the leaves, organic acids are also mainly constructed in the leaves. Organic acids were contained in phloem (Jeschke et al 1986) and organic acids translocate from leaves to roots (Ben-Zioni et al 1971, Kirkby and Knight 1977). Therefore, it is expected that the transgenic plant could exude much organic acid from its roots. In tropical soils, low pH and low available P content are serious problems for plant growth (Foth and Ellis 1997). As aluminum is solubilized in low pH soils, it is generally recognized as the major constraint to crop production in highly weathered acid soils in the tropics (Foy et al 1978, Marschner 1995). Moreover, it is assumed that Al³⁺ induces P deficiency in low pH soils because Al³⁺ makes a complex with P, resulting in a decrease in P availability to plants (Foth and Ellis 1997). It was reported that the P concentration in culture solution was decreased by the presence of Al as aluminum phosphate and plant growth, root elongation, and

Table 2. ¹⁴C distribution to each fraction at 0 min after 5 min ¹⁴CO₂ assimilation in PEPC transgenic rice plant.

Variety	Protein	Amino acid	Organic acid	Phosphate ester	Sugar	Polysaccharide	SE
Control	0.002	0.199	0.139	0.228	0.411	0.021	(±0.01)
Transgenic	0.003	0.212	0.228	0.200	0.331	0.026	(±0.04)

^aSE =standard error estimated from all fractions.

amount of phosphate absorbed in rape were inhibited in 150 μM Al in culture solution (Luo et al 1999). Thus, Al has functions in toxicity and P deficiency. It was suggested, however, that citric acid exudation from roots might contribute to the detoxification of Al and to the increase in phosphate availability in the rhizosphere in rape (Luo et al 1999). The exudation of citric acid by white lupine into the rhizosphere could increase P availability by solubilizing P from insoluble iron phosphates and aluminum phosphates in low pH soil (Gardner et al 1982a,b, 1983). In pigeonpea, secretion of piscidic, malonic, and oxalic acid appears to be the mechanism to release P from iron and aluminum phosphates (Ae et al 1990, Otani et al 1996). The secretion of organic acids by roots is not only the plant mechanism for P absorption but also the plant mechanism for Al tolerance. Malic acid secreted from wheat roots gave an Al resistance mechanism (Ryan et al 1993) and citric acid secreted from *Cassia tora* L. roots in response to Al gave Al tolerance (Ma et al 1997). Recently, it was reported that the secretion of oxalic acid from taro roots was stimulated by excess Al, not by P deficiency (Ma and Miyasaka 1998).

After preculture, PEPC transgenic rice seedlings were transferred to hydroponic solution including complete nutrients treated with 0, 5, 10, and 30 ppm Al using $\text{Al}_2(\text{SO}_4)_3$. The Al and P concentrations in the solution medium had been adjusted by adding an adequate amount of Al and P until the Al-P equilibrium state was reached at $\text{pH } 4.0 \pm 0.1$. Relative growth of the control rice plant decreased with an increase in Al application; however, that of the transgenic rice plant increased with an increase in Al application (Table 3). It is generally recognized that Al exerts toxic effects on plant growth and root activity, although many reports indicated a beneficial effect of Al on plant growth (Haridasan 1988, Huang and Bachelard 1993, Konishi et al 1985, Mullette 1975). Organic acid (mainly oxalic acid) exudation from roots of the control plant remained regardless of Al application; however, that of the transgenic plant increased with an increase in Al application (Table 4), similar to the change in relative growth

Table 3. Effect of aluminum on relative growth in transgenic and control rice plants.

Variety	Al treatment (ppm)	Relative growth		
		Shoot	Root	Total
Control	0	1.00	1.00	1.00
	10	0.88	0.89	0.88
	30	0.87	0.80	0.85
Transgenic	0	1.00	1.00	1.00
	10	1.25	2.75	1.30
	30	1.32	3.67	1.40

^aRelative growth = dry weight (Al_x) - dry weight (zero day)/dry weight (Al) - dry weight (zero day), where x = ppm Al.

Table 4. Effect of aluminum on oxalic acid exudation from roots in transgenic and control rice plants.

Variety	Al treatment (ppm)	Oxalic acid exudation rate (nmol g ⁻¹ root fresh weight)
Control	0	5.17 \pm 0.12
	10	6.63 \pm 1.66
	30	5.23 \pm 0.68
Transgenic	0	6.09 \pm 0.58
	10	8.38 \pm 0.76
	30	12.56 \pm 2.17

of the transgenic plants. Thus, it was elucidated that PEPC transgenic plants contribute to Al tolerance through the exudation from roots of organic acids, which are translocated from leaves.

Conclusions

It used to be thought that the carbon–nitrogen balance of the whole plant was regulated by the end-products of each organ. For example, in cereal crops, protein metabolism is active at the vegetative stage because leaf growth is dominant, while cellulose and lignin metabolism is active at the reproductive stage because stem growth is dominant, and then starch metabolism is active at the maturation stage because starch is stored in the grain. If the carbon–nitrogen balance of the whole plant depends on the metabolism of end-products in each organ, it is not appropriate to study its regulation in the whole plant. Alternatively, it is certainly possible that the carbon–nitrogen balance is determined through the current photosynthate distribution into sucrose (C-pool) and organic acids and amino acids (N-pool) before carbohydrates translocate from leaves (Shinano et al 1994, Nakamura et al 1997a).

Since the current photosynthate in legume crops is constantly supplied to the N-pool, shoot growth tends to remain vigorous throughout plant development. In cereal crops, the current photosynthate is actively supplied to the N-pool at the vegetative growth stage, then to the C-pool after the reproductive stage, and its changes are markedly affected by growth stage or photoperiod, and less by nitrogen status (unpublished data). Although it is easy to breed lines with vigorous vegetative growth, it is difficult to repress vigor during the transition from vegetative growth to reproductive growth (K. Goto, personal communication). Although the physiological significance of vigor in plants is still unclear, it is closely related to carbon–nitrogen metabolism since vigor is an indicator of active nitrogen metabolism at the vegetative growth stage. Thus, the regulation of carbon–nitrogen metabolism is a major target for the regulation of plant growth.

The distribution of current photosynthate in the leaf in the light is regulated by photorespiration (Nakamura et al 1997b) and by the enzymes SPS and PEPC (Champigny and Foyer 1992, Nakamura et al 1997b: Fig. 6). The first step of photorespiration is catalyzed by ribulose- 1.5-bisphosphate carboxylases-oxygenase (Rubisco). However, Rubisco also catalyzes CO₂ assimilation, suggesting that it is difficult to change Rubisco activity or Rubisco expression. On the other hand, since glutamine synthetase II (GSII) activity in the chloroplast is strongly associated with photorespiration (Kozaki and Takeba 1996), the regulation of GSII activity is an important target for the carbon–nitrogen balance through photorespiration. In rice, as the PEPC activity/SPS activity ratio decreased with growth following the decrease in photosynthate distribution into the N-pool (unpublished data), the regulation of SPS and PEPC activities will also become an important target for the regulation of the carbon–nitrogen balance. As a result of the PEPC transgenic rice plant, it was confirmed that PEPC in the cytosol can regulate the distribution of current photosynthate to organic acids. When Al was applied to the PEPC transgenic rice plant, these organic acids translocated from leaves to roots, were exuded from roots, and then contributed to detoxifying Al in culture. However, since current photosynthate distribution to amino acids was not increased in the PEPC transgenic plant, a high amount of current photosynthate distribution to amino acids, as in soybean, will be regulated not only by PEPC but also by other metabolic pathways.

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Photosynthetic performance of transgenic rice plants overexpressing maize C₄ photosynthesis enzymes

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Transgenic rice plants overexpressing maize C₄-specific phosphoenolpyruvate carboxylase (PEPC) exhibit a higher photosynthetic rate (up to 30%) and a more reduced O₂ inhibition of photosynthesis than untransformed plants. There is a small increase in the amount of atmospheric CO₂ being directly fixed by PEPC. Similarly, transgenic rice plants overexpressing the maize chloroplastic pyruvate, orthophosphate dikinase (PPDK), also have higher photosynthetic rates (up to 35%) than untransformed plants. This increased photosynthetic capacity is at least in part due to an enhanced stomatal conductance and a higher internal CO₂ concentration. Using conventional hybridization, we have integrated maize PEPC and PPDK genes into the same transgenic rice plants. In the segregating population, the photosynthetic rates of plants with high levels of both maize enzymes are up to 35% higher than those of untransformed plants.

Under full-sunlight conditions, the photosynthetic capacity of field-grown PEPC transgenic rice plants is twice as high as that of untransformed plants. PEPC transgenic plants consistently have a higher photosynthetic quantum yield by photosystem II and a higher capacity to dissipate excess energy photochemically and nonphotochemically. Preliminary data from field tests show that the grain yield is about 10–30% higher in PEPC and 30–35% higher in PPDK transgenic rice plants relative to untransformed plants. Taken together, these results suggest that introduction of C₄ photosynthesis enzymes into rice has a good potential for enhancing the crop's photosynthetic capacity and yield.

Due to the CO₂-concentrating mechanism in the C₄ pathway of photosynthesis, C₄ plants have many desirable agronomic traits, such as high photosynthetic capacity and high mineral-use efficiency, especially under high light, high temperature, and drought conditions (Hatch 1987, Ku et al 1996). On the other hand, plants that assimilate atmospheric CO₂ via the C₃ pathway, including many agronomically important species such as rice, suffer from O₂ inhibition of photosynthesis and the associated photorespiration, and thus exhibit a lower photosynthetic efficiency under these conditions. Conventional hybridization has been employed to transfer C₄ traits to C₃ plants (see Brown and Bouton 1993): however, epistatic interaction between the alleles suppresses the expression of C₄ traits in the progeny, and genes for Kranz leaf anatomy and biochemistry of C₄ photosynthesis are not closely linked. Most importantly, no closely related C₃ and C₄ crops can be hybridized. Several attempts have been made in the past to transfer the genes involved in C₄ photosynthesis into C₃ plants (Hudspeth et al 1991, Kogami et al 1994, Gehlen et al 1996, Gallardo et al 1995, Ishimaru et al 1998) in an effort to tune up

their photosynthetic metabolism. However, limited physiological consequences were observed in these transgenic plants, which may be due to the low levels of expression of these genes.

Using an *Agrobacterium*-based transformation system, we have independently introduced three key C_4 photosynthesis genes from maize into rice with high levels of expression (Agaric et al 1998, Ku et al 1999). These are phosphoenolpyruvate carboxylase (PEPC), pyruvate, orthophosphate dikinase (PPDK), and NADP-malic enzyme (NADP-ME). The major objective of our research is to introduce enzymes involved in C_4 photosynthesis into C_3 plants and test their effects on photosynthesis and plant productivity. By introducing some of the key enzymes of C_4 photosynthesis into C_3 plants with proper intercellular compartmentation, a limited C_4 acid metabolism may be installed for fixing atmospheric CO_2 directly via this pathway and partially concentrating CO_2 in the chloroplast. In this regard, a similar mechanism has been found in the primitive aquatic angiosperm *Hydrilla verticillata* (Magnin et al 1997). The photosynthetic mechanism in *H. verticillata* is considered a primitive form of C_4 photosynthesis. When it is grown under low CO_2 conditions, it shifts from C_3 to C_4 photosynthesis and assimilates atmospheric CO_2 via the C_4 pathway without Kranz leaf anatomy (Bowes and Salvucci 1989). Inorganic carbon is first assimilated into the C_4 acid malate in the cytoplasm via PEPC. Subsequently, malate serves as a donor of CO_2 to Rubisco in the chloroplast by the decarboxylating enzyme NADP-ME. This primitive-type C_4 photosynthesis is sufficient to concentrate CO_2 in the chloroplast and overcome photorespiration (Reiskind et al 1997). It is possible that this archetypal version of C_4 photosynthesis, which does not depend on Kranz compartmentation, can be engineered to function in terrestrial C_3 plants.

Enhanced expression of enzymes of C_4 photosynthesis in C_3 plants may increase carbon and nitrogen metabolism in certain tissues of C_3 plants. All enzymes involved in C_4 photosynthesis are found in leaves of C_3 plants. Although they are low in activity in leaves of C_3 plants, some of them are found at high levels in reproductive tissues. For example, the cytosolic isoform of PPDK occurs at high levels in seeds of both the C_3 plant wheat (Aoyagi and Bassham 1984a,b, Aoyagi and Chua 1988, Blanke and Lenz 1989) and the C_4 plant maize (Imaizumi et al 1997). Thus, PPDK may play an important role in linking carbon and nitrogen metabolism or supply of energy (e.g., release of ATP from PEP catalyzed by PPDK) in reproductive tissues, and enhanced expression of the enzyme may boost seed development and grain productivity.

Enzymes involved in C_4 photosynthesis, although low in C_3 plants, may also play important roles in plant defense responses to biotic and abiotic stress. Metabolic alterations in response to stress allow plants to adapt to adverse conditions. For example, an increase in NADP-ME by wounding, low oxygen, low temperature, salinity, and ultraviolet light has been reported in C_3 plants such as rice (Fushimi et al 1994) and bean (Walter et al 1994, Schaaf et al 1995, Pinto et al 1999) and the C_4 plant maize (Drincovich et al 1998). It is postulated that the reductant (NADPH) released from decarboxylation of malate by NADP-ME may be required for the increased synthesis of secondary metabolites for defense purposes. Furthermore, increased expression of PPDK in C_3 chloroplast may enhance synthesis of aromatic amino acids, such as phenylalanine via the shikimic pathway (Hermann 1995), which serve as the substrate for secondary metabolites (e.g., phenylpropanoids), which are part of the biosynthesis involved in plant defense mechanisms (Douglas 1996). The biosynthesis of phenylpropanoids requires the efficient flow of carbon into phenylalanine biosynthesis. Thus, increased expression of some C_4 photosynthesis enzymes in C_3 plants could confer enhanced tolerance under stress conditions.

In this chapter, we report on the photosynthetic traits of transgenic rice plants that express maize C_4 -specific PEPC and PPDK independently. In addition, the photosynthetic performance of hybrid transgenic plants overexpressing both maize PEPC and PPDK was also measured.

This represents a first step in engineering the biochemical components of C_4 photosynthesis in rice.

Transgenic rice plants overexpressing maize PEPC

In previous studies (Agarie et al 1998, Ku et al 1999), we reported that the primary transgenic rice plants harboring the maize PEPC gene have high levels of expression (up to 12% of total leaf soluble protein) and the enzyme remains active. Immunolabeling studies indicate that the enzyme is localized in the cytosol of mesophyll cells (data not shown). Genetic studies show that the maize gene is stably inherited in a Mendelian manner, with the gene being inserted at one or two loci. Furthermore, the photosynthetic rates, measured under ambient conditions, of these transgenic plants are comparable with or higher than those of untransformed plants (Ku et al 1999). In addition, O_2 inhibition of photosynthesis decreases progressively, with an increasing level of PEPC activity among the transgenic plants. Our preliminary labeling experiments with $^{14}CO_2$ in leaves shows only a small increase (4%) in atmospheric CO_2 being directly fixed by PEPC. The supply of PEP, the substrate for PEPC, may be limited in C_3 leaf. Thus, the biochemical and physiological bases of these alterations in photosynthetic traits remain unclear.

Using the segregating populations from four primary transgenic lines that exhibit high levels of maize PEPC, we have shown that the photosynthetic rates (on a leaf area basis) of flag leaves in most PEPC transgenic rice plants are either similar to or up to 30% higher than those of untransformed plants (average $17.5 \text{ mmol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$, Fig. 1A). The photosynthetic rate begins to decrease as the level of expression reaches very high values, as one would expect. Indeed, transgenic plants with extremely high levels of PEPC have lower chlorophyll contents. Analysis of the relationship between photosynthetic rate and stomatal conductance among these plants shows a good positive correlation between the two parameters (Fig. 1B). Furthermore, stomatal conductance is highly correlated with intercellular CO_2 concentration (Fig. 1C). The intercellular CO_2 concentration in some transgenic plants is as high as 275 mL L^{-1} versus 335 mL L^{-1} in untransformed plants. Thus, part of the higher photosynthetic capacity of the transgenic plants may be due to the ability of the plants to maintain a higher internal CO_2 in the leaf because of increased stomatal opening. The immediate benefit of a higher intercellular CO_2 for the plants is elevated net carbon fixation due to suppression of Rubisco oxygenase and photorespiration. We also observed a consistent upward shift in optimal temperature for photosynthesis by the transgenic plants from 26 to 28–32 °C (data not shown), presumably because of reduced photorespiration. Interestingly, the improvement in wheat grain yield in the past 30 years by CIMMYT (27%) is mainly due to an increased stomatal conductance in the new cultivars (63%), which results in a 23% increase in maximum photosynthetic rate (23%) and a canopy temperature depression of 0.6 °C (Fischer et al 1998). Furthermore, ^{13}C discrimination is also positively correlated with yield progress.

Consistent with the suggestion that PEPC transgenic rice plants have a higher stomatal conductance than untransformed wild-type plants, the $\delta^{13}C$ values for the transgenic plants are 1.5–2.5‰ more negative than that of untransformed plants (–27.5‰) and the value becomes more negative with increasing PEPC activity among the transgenic plants (Fig. 2). It has been shown that an increased stomatal conductance allows more CO_2 to diffuse into the leaf and thus more ^{13}C is discriminated during photosynthesis (Winter et al 1982). However, the possibility that the lower ^{13}C content in the leaves of transgenic plants could be due to refixation of photorespiratory CO_2 by PEPC and then Rubisco again cannot be ruled out. The interesting question here is how transgenic plants manage to maintain a higher stomatal conductance. The

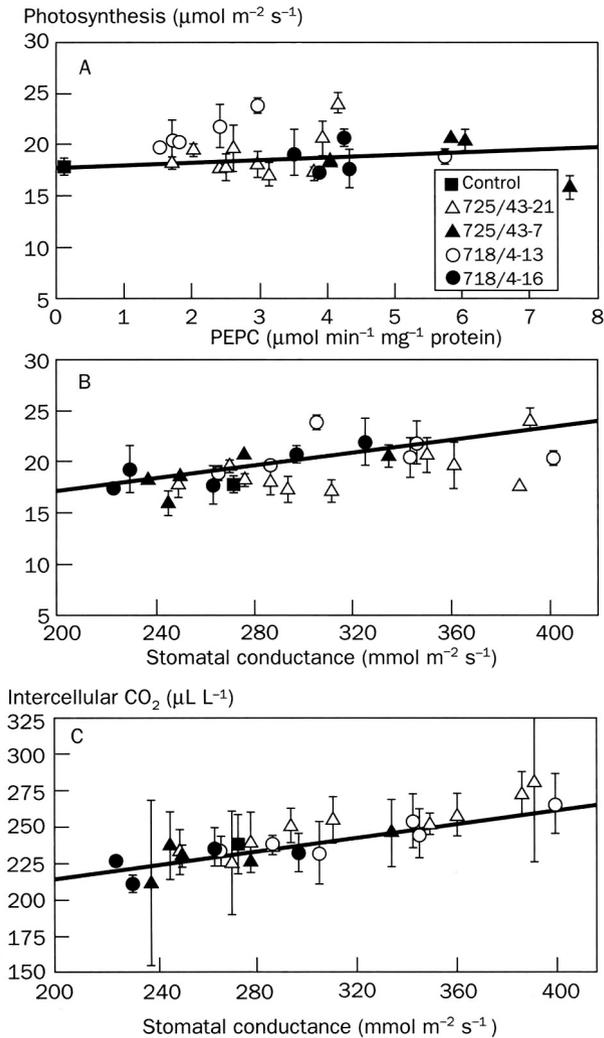


Fig. 1. (A) Photosynthetic rate as a function of PEPC activity, **(B)** relationship between photosynthetic rate and stomatal conductance, and **(C)** relationship between stomatal conductance and intercellular CO₂ concentration in untransformed control (Kitaake) and PEPC transgenic rice plants. Transgenic plants generated from segregating populations of four independent T₁ plants were used for analysis. Photosynthesis was measured in newly matured flag leaves at 26 °C, 1,200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ and 360 $\mu\text{L L}^{-1}$ CO₂. Unpublished data of D. Cho, U. Ranade, and M.S.B. Ku.

mechanism underlying this phenomenon is not quite clear. It is conceivable, however, that an increased expression of PEPC in the guard cells would allow more fixation of atmospheric CO₂ into organic acids such as malate, which are stored in the vacuole. Consequently, inorganic solutes such as potassium move from subsidiary or epidermal cells into guard cells for balance of charge. The accumulation of ions in the vacuole lowers the water potential of the guard cell, thereby stimulating the osmotic uptake of water and increased turgor for opening of stomates.

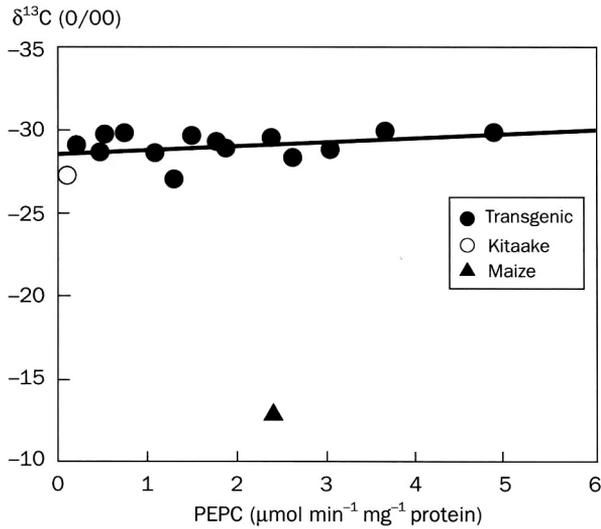


Fig. 2. Leaf $\delta^{13}\text{C}$ value as a function of PEPC activity for maize, untransformed control (Kitaake), and PEPC transgenic rice plants. Newly mature flag leaves from primary transgenic (T_0) plants were used for measurement of ^{13}C content. Unpublished data of J. Ehleringer and M.S.B. Ku.

PEPC transgenic rice plants grown in the field also exhibit a better photosynthetic performance than untransformed plants. The photosynthetic rate of untransformed plants is saturated by half full sunlight, whereas that of PEPC transgenic plants does not show saturation until full sunlight is reached. In one experiment, the light-saturated photosynthetic rates in PEPC transgenic plants ranged from 21.0 to 27.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$, whereas the rates in untransformed plants were around 17.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$. These results suggest that the leaves of PEPC transgenic plants are capable of using full sunlight to maximize carbon gain. On the other hand, photosynthesis by untransformed plants is inhibited by high light (photoinhibition). Consistent with this observation, the intrinsic quantum yield of photosystem II (PSII) as measured by F_v/F_m is less inhibited by full-sunlight treatment alone or by a combination of methyl viologen and full-sunlight treatment in PEPC transgenic plants relative to wild-type plants (Fig. 3). Methyl viologen accepts electrons from PSI and generates oxy-radicals. Under these high-light and photooxidative conditions, PEPC transgenic plants are capable of dissipating excess light energy through photochemical and nonphotochemical means more effectively than untransformed plants, as demonstrated by the measurements of qP (photochemical quenching) and qN (nonphotochemical quenching), respectively. Taken together, these results indicate that PEPC transgenic plants are less susceptible to photoinhibition or photooxidation, which may contribute to the increased photosynthetic capacity. The basis for the superior ability of PEPC transgenic plants to dissipate excess light energy is not known at present.

It is also quite possible that overexpression of the maize C_4 PEPC in the transgenic rice plants (up to 12% in primary transgenic plants, Ku et al 1999) may influence the activities of other photosynthetic enzymes or enzyme kinetics. This needs to be evaluated in relation to the photosynthetic performance of these transgenic plants. In this experiment, the chlorophyll contents were similar between untransformed plants and the transgenic rice plants used. On a chlorophyll basis, Rubisco activities in untransformed and transgenic plants are similar (340

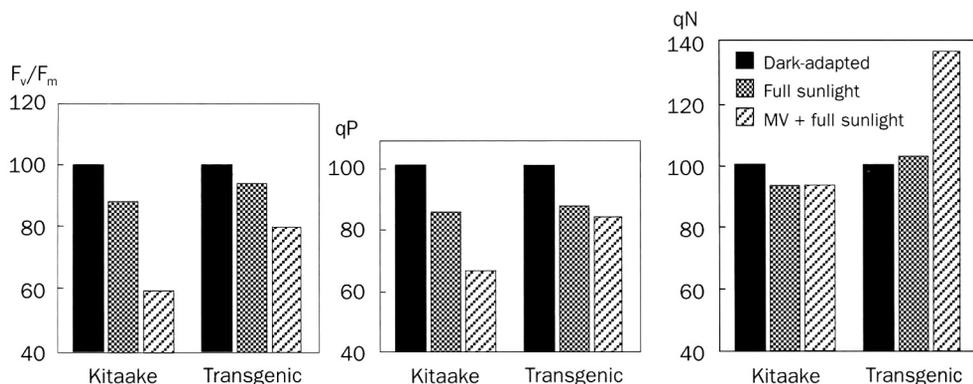


Fig. 3. Intrinsic quantum yield of PSII (F_v/F_m), photochemical quenching (qP), and nonphotochemical quenching (qN) for dark-adapted leaves, leaves illuminated for 3 h under full sunlight, or leaves treated with 1.5 mM methyl viologen (MV) and then illuminated for 3 h under full sunlight in untransformed control (Kitaake) and PEPC transgenic plants. Plants were grown in pots and maintained outdoors during May–August in Nanjiang, China (1999), and newly mature flag leaves and the leaves below were used for measurements. Unpublished data of X. Li, D-M. Jiao, and M.S.B. Ku.

Table 1. Activities of PEP carboxylase (PEPC), carbonic anhydrase (CA), and Rubisco, and kinetics of Rubisco in PEPC transgenic and untransformed (Kitaake) rice plants. Plants were grown in pots and maintained outdoors during May–August (Nanjiang, China, 1999); newly mature flag leaves and the leaves below were used for enzyme extraction after illumination at $1,400 \mu\text{mol photon m}^{-2}\text{s}^{-1}$ for 4–6 h. Enzymes were assayed at 30°C and the data for enzyme activity were means \pm standard deviation from 3–6 replicates of measurements. Unpublished data of X. Li, D-M. Jiao, and M.S.B. Ku.

Enzyme	Kitaake	Transgenic
PEPC ($\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$)	165 \pm 9	1,265 \pm 66
CA ($\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$)	204 \pm 11	577 \pm 28
Rubisco ($\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$)	340 \pm 24	367 \pm 21
Rubisco $K_m(\text{CO}_2)$ (mM)	11.95	11.53
Rubisco V_{max} ($\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$)	2.38	4.77

vs 367 $\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$) (Table 1). Also, the $K_m(\text{CO}_2)$ of Rubisco is the same between the two plants (12.0 vs 11.5 mM). However, the V_{max} of Rubisco on a protein basis is twice as high in PEPC transgenic plants as in untransformed plants (4.77 vs 1.38 $\mu\text{mol mg}^{-1} \text{protein min}^{-1}$). In addition, carbonic anhydrase (CA) is almost three times higher in the transgenic plants (577 vs 204 $\mu\text{mol mg}^{-1} \text{Chl h}^{-1}$). These results suggest that enhanced expression of the maize PEPC in rice may have altered the expression or activation state of other photosynthetic enzymes in the leaves. Similarly, transgenic tobacco overexpressing a gene that encodes chloroplast-localized Cu/Zn superoxide dismutase (3-fold) also exhibits a 3–4-fold increase in ascorbate peroxidase because of increased transcription (Gupta et al 1993). This phenomenon warrants further investigation. In any case, increased CA activity may enhance the fixation of atmospheric CO_2 via PEPC as HCO_3^- is the active CO_2 species for PEPC (Hutch 1987). The higher V_{max} of Rubisco in the leaves of transgenic plants may compensate for its lower amount (as a percentage) because of overexpression of the maize PEPC (Ku et al 1999) and thus allow the plants to maintain a high photosynthetic capacity.

Transgenic rice plants overexpressing maize PPDK

The primary transgenic rice plants harboring the maize chloroplast PPDK gene exhibit a wide range of enzyme activity, up to 10-fold higher than that in untransformed plants (Agarie et al 1998). Studies with isolated chloroplasts from leaves of PPDK transgenic plants indicate that the majority of PPDK in the mesophyll cell is localized in the chloroplast. The photosynthetic performance of PPDK transgenic plants was evaluated using segregating populations from four primary transgenic lines that exhibit high levels of PPDK. As expected, the amount of PPDK varies in segregating populations (Fig. 1). Most of the PPDK transgenic plants exhibit a higher photosynthetic rate (up to 35%) than the wild-type plants, and the higher photosynthetic rates are associated with increased stomatal conductance (Fig. 5) and higher intercellular CO₂ concentration (data not shown), similar to those found in the PEPC transgenic plants. Thus, as with PEPC transgenic plants, PPDK transgenic rice plants may also be able to maintain a

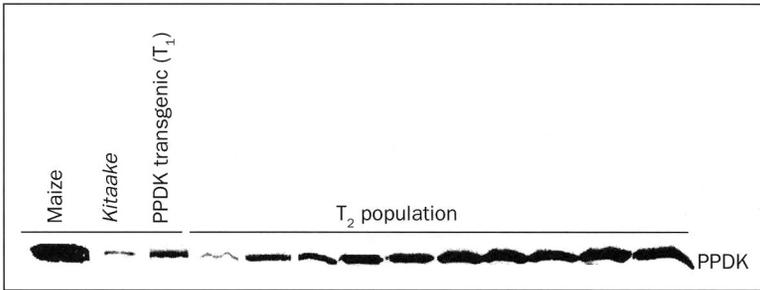


Fig. 4. Immunoblot of PPDK in leaves of maize, untransformed control (Kitaake), and a primary (T₁) PPDK transgenic rice plant and its segregating population. Unpublished data of T.-P. Hsu and M.S.B. Ku.

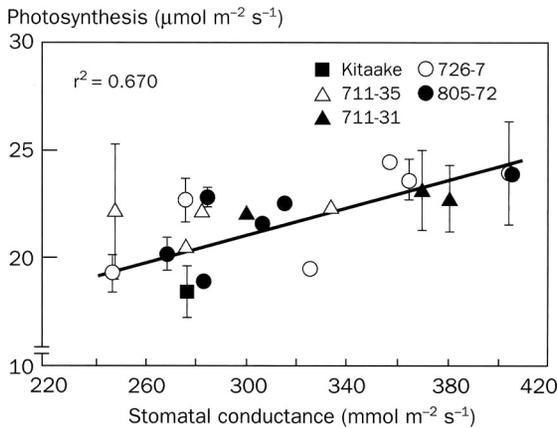


Fig. 5. Relationship between photosynthetic rate and stomatal conductance among untransformed control plant (Kitaake) and segregating populations from four primary (T₀) PPDK transgenic rice plants. Measurement conditions were the same as described in Fig. 1. Unpublished data of D. Cho and M.S.B. Ku.

higher internal CO₂ level due to increased stomatal conductance. Increased expression of PPDK in the guard cells may function to supply PEP, the substrate for PEPC, for synthesis of organic acids. How the elevated PPDK may affect carbon and nitrogen metabolism in leaves of transgenic rice plants awaits further investigation.

The effects of elevated expression of maize PPDK on carbon metabolism in transgenic potatoes (C₃) have been reported recently (Ishimaru et al 1998). PPDK activities in leaves of transgenic potatoes are up to 5-fold higher than those of untransformed control plants. Analysis of metabolites shows that PPDK activity in leaves is negatively correlated with pyruvate content and positively correlated with malate content. It is suggested that elevated PPDK activity in the leaf may lead to a partial function of C₄-type carbon metabolism. However, the altered carbon metabolism does not have any significant effect on other photosynthetic characteristics.

Transgenic rice plants overexpressing both maize PEPC and PPDK

Since PEPC catalyzes the initial fixation of atmospheric CO₂ in the C₄ pathway and PPDK catalyzes the conversion of pyruvate to PEP, overexpression of both enzymes simultaneously may enhance the fixation of atmospheric CO₂ via PEPC. Using conventional hybridization, we have integrated both maize PEPC and PPDK genes into the same plants from two independent homozygous transgenic rice plants that express high levels of the maize enzymes. The amounts of the two enzymes in the F₁ hybrids are about half of those in the parents (Fig. 6). The photosynthetic performance of the transgenic plants expressing varying amounts of the two maize enzymes was first evaluated in the segregating population from one of the F₁ hybrids. As expected, the segregating population exhibits different combinations for the amounts of the two enzymes, with some combinations having only the same amount as the wild-type plants (without the maize gene inserted) and others having twice the amount of the parental transgenic plants (homozygous with respect to the inserted maize gene) (Fig. 7). The activities of each enzyme are well correlated with the amounts of the protein. Although the overall photosynthetic rates in this experiment are somewhat lower than those obtained earlier due to cultivation in smaller pots, the photosynthetic trend for the activities of the two maize enzymes is clear (Fig. 8). Hybrid plants expressing high levels of both PEPC and PPDK tend to have a higher photosynthetic rate (up to 35%) than untransformed plants, again because of higher stomatal conductance and higher intercellular CO₂ (data not shown). It is quite possible that overexpression of both enzymes further enhances the capability of the plants to synthesize organic acids in the guard cells and consequently the conductance of CO₂ into the leaf. With two of the key enzymes of the C₄ pathway introduced into the same plants, experiments are under way to see if the leaves of these plants are capable of fixing more atmospheric CO₂ directly via this route.

Future directions

In summary, our physiological results demonstrate that introduction of maize PEPC and PPDK into rice has the potential to enhance its photosynthetic capacity by increasing stomatal conductance for CO₂ diffusion. PEPC transgenic rice plants are also more tolerant of photoinhibition and photooxidation under field conditions. This trait is important for rice productivity as early senescence of leaves, due to photoinhibition and photooxidation, often occurs in the field, which reduces grain yield. The performance of these transgenic plants under other stress conditions such as water deficit, high and low temperatures, and mineral deficiency needs to be evaluated in the future. The higher stomatal conductance exhibited by

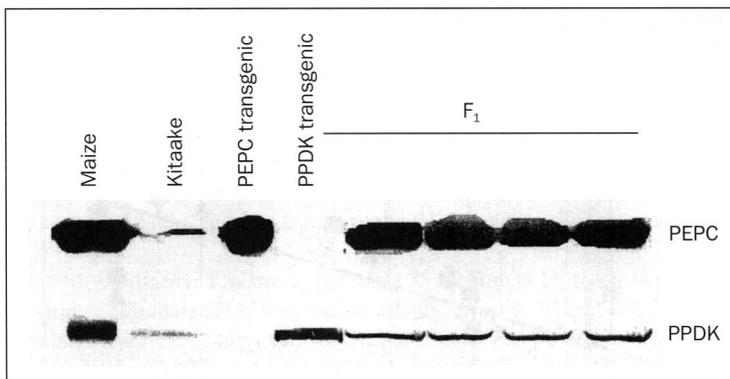


Fig. 6. Immunoblot of PEPC and PPDK in leaves of maize, untransformed control (Kitaake), PEPC, and PPDK transgenic rice plants, and four F_1 hybrids between PEPC and PPDK transgenic plants. Unpublished data of T-P. Hsu, D. Cho, and M.S.B. Ku.

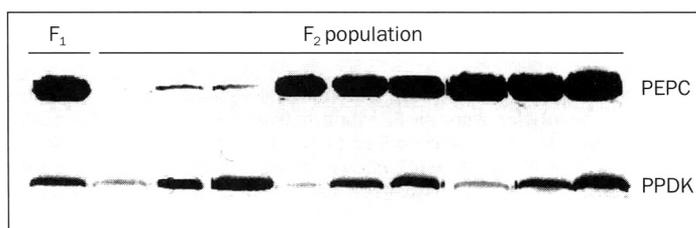


Fig. 7. Immunoblot of PEPC and PPDK in leaves of an F_1 hybrid between PEPC and PPDK transgenic rice plants and the F_2 population. Unpublished data of T-P. Hsu, D. Cho, and M.S.B. Ku.

the transgenic rice plants implies that more water may be needed. However, this may not be a serious problem for paddy rice. A preliminary small-scale test in the field shows that the grain yield is about 10–30% higher in PEPC and 30–35% higher in PPDK transgenic rice plants relative to untransformed plants, in spite of a lower fertility (5–10%). The increases in grain yield are mainly associated with an increased number of panicles per plant (15–30%). These results suggest that these transgenic plants can perform well in variable environments. More field tests, especially on a large scale, will be required to confirm this. Also, whether this trait will be stably inherited in the following generations needs to be evaluated too.

Transgenic rice plants overexpressing PEPC, PPDK, or both may not be capable of fixing a large amount of atmospheric CO_2 directly as in C_4 plants due to a limited supply of substrates or further metabolism of products. However, with the introduction of another key enzyme of the C_4 pathway, NADP-ME in the chloroplast, a limited CO_2 -concentrating mechanism may be achieved. Enhanced expression of other biochemical components of the C_4 pathway, such as CA, NADP-malate dehydrogenase, adenylate kinase, and transporters specific for C_4 metabolites, may allow the cycle to function more effectively. In this regard, the increased activities of CA and Rubisco in PEPC transgenic rice plants are worth noting; some related enzymes in the pathway may be induced or enhanced. This raises an interesting question on metabolic adaptation, and more studies on expression of related genes in the transgenics will

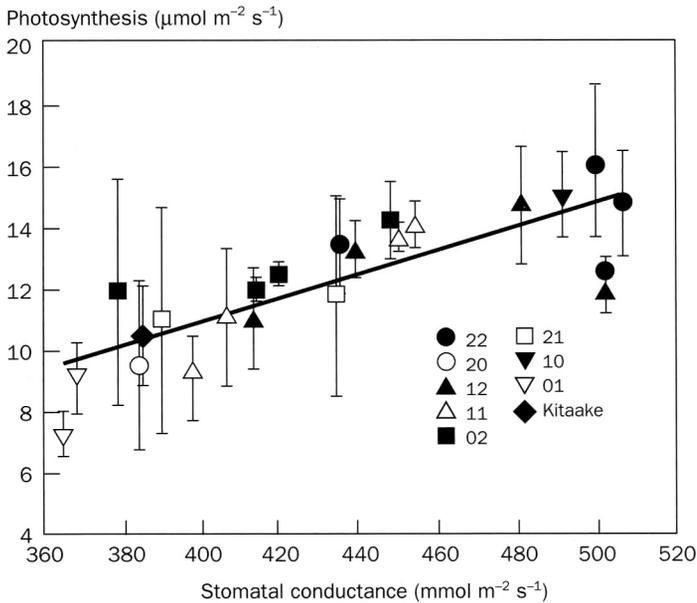


Fig. 8. Relationship between photosynthetic rate and stomatal conductance among untransformed control plant (Kitaake) and the F₂ population from an F₁ hybrid between PEPC and PPK transgenic rice plants. Measurement conditions were the same as described in Fig. 1. Unpublished data of D. Cho and M.S.B. Ku. Designation of plants: “22” represents plants with homozygous maize PEPC and PPK genes and “21” represents plants with homozygous maize PEPC and heterozygous maize PPK genes, while “20” represents plants with homozygous maize PEPC and no maize PPK gene, and so on.

be needed to address this issue. As discussed earlier, a primitive C₄-type photosynthesis has been reported to function in the aquatic angiosperm *H. verticillata* without Kranz anatomy (Magnin et al 1997). Perhaps a similar system can be engineered in terrestrial C₃ plants to concentrate CO₂ in the leaf and reduce carbon loss from photorespiration. A more efficient CO₂-concentrating mechanism by the C₄ pathway would require the concomitant installation of Kranz leaf anatomy. At present, little is known about the biochemical processes or genes regulating the differentiation of Kranz leaf anatomy in C₄ plants.

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Rice: practical issues

Strategies for increasing the yield potential of rice

G.S.Khush

Rice is the most important food crop in the world. Major advances have occurred in rice production as a result of the wide-scale adoption of improved rice varieties. However, demand for rice in low-income countries continues to increase because of increases in the population of rice consumers and improvements in living standards. It is estimated that we will have to produce 50% more rice by 2050. To meet this challenge, we need rice varieties with higher yield potential. Several approaches are being employed for developing rice varieties with increased yield potential, such as population improvement, ideotype breeding, heterosis breeding, wide hybridization, genetic engineering, and molecular breeding.

Major advances have been made in increasing rice production worldwide as a result of the large-scale adoption of modern high-yielding varieties and improved cultural practices. World rice production increased from 257 million tons in 1966 to 570 million tons in 1997. The present world population of 6 billion is likely to reach 8 billion by 2050. More than 70% of this increase in population will occur in Asia, where rice is the staple food. It is estimated that we will have to produce 50% more rice to satisfy the growing demand for food. This increased demand will have to be met from less land, with less water, less labor, and less chemicals. Thus, the challenge for rice improvement is to develop varieties with higher yield potential, more durable resistance to diseases and insects, and higher levels of tolerance for abiotic stresses.

Several approaches for increasing the yield potential of rice are being used, such as population improvement, ideotype breeding, heterosis breeding, wide hybridization, genetic engineering, and molecular breeding.

Population improvement

This is the time-tested strategy for selecting crop cultivars with higher yield potential. It has two phases. The first phase involves the creation of variability; in the second phase, desirable individuals are selected. Selection criteria involve growth duration, lodging resistance, disease and insect resistance, quality considerations, and adaptation to a specific environment. Selected genotypes are evaluated for yield and superior ones are released as varieties. It has been estimated that, on average, about a 1% increase has occurred per year in the yield potential of rice over a 30-y period since the development of the first improved variety, IR8 (Peng et al 1999). Similarly, the yield potential of wheat developed at the International Center for Maize and Wheat

Improvement (CIMMYT) increased at the rate of 0.83% y^{-1} over the past 30 years (Fischer 1994). The yields of most crops for which investment in research is sufficient have been continuously improved and there is no reason why further increases cannot be attained.

Ideotype breeding

Ideotype breeding aimed at modifying the plant architecture is a time-tested strategy to achieve increases in yield potential. Thus, selection for short-statured cereals such as wheat, rice, and sorghum resulted in a doubling of yield potential. Yield potential is determined by the total dry matter or biomass and the harvest index (HI). Tall and traditional rice varieties had an HI of around 0.3 and total biomass of about 12 t ha⁻¹. Thus, their maximum yield was about 4 t. This biomass could not be increased by applications of nitrogenous fertilizers as the plants grew excessively, lodged badly, and yield decreased instead of increasing. To increase the yield potential of tropical rice, it was necessary to improve the harvest index and increase nitrogen responsiveness by increasing lodging resistance. This was accomplished by reducing the plant height by incorporating a recessive gene, *Sd-1*, for short stature.

The first short-statured variety, IR8, developed at IRRI, also had a combination of other desirable traits such as profuse tillering, dark green and erect leaves for good canopy architecture, and sturdy stems. It responded to nitrogenous fertilizer much better and had a higher biomass (about 18 t) and improved HI of 0.45. Its yield potential was 8–9 t ha⁻¹ (Chandler 1969).

To further increase the yield potential of rice, a new plant type was conceptualized in 1988 (IRRI 1989). Modern semidwarf rice varieties produce a large number of unproductive tillers and excessive leaf area, which cause mutual shading and reduce canopy photosynthesis and sink size, especially when they are grown under direct-seeded conditions. To increase the yield potential of these semidwarf rice varieties, IRRI scientists proposed further modifications of plant architecture, with the following characteristics: low tillering capacity (3–4 tillers when direct-seeded); no unproductive tillers; 200–250 grains per panicle; very sturdy stems; dark green, thick, and erect leaves; and a vigorous and deep root system.

This proposed ideotype became the “new plant type” highlighted in IRRI’s strategic plan (IRRI 1989) and the breeding effort to develop this germplasm became a major research project of IRRI. The goal is to increase the yield potential of rice to 12 t ha⁻¹, 3% higher than that of modern high-yielding semidwarf varieties. Donors for developing the new plant type were identified and breeding work began in 1989 (Khush 1993, 1995). Numerous breeding lines with the desired ideotype have been developed and are being evaluated in replicated yield trials. Several of the new plant type lines have outyielded IR72, which is one of the highest-yielding semidwarf modern varieties. Breeding efforts now focus on improving grain quality and incorporating genes for disease and insect resistance.

Heterosis breeding

Yield improvement in maize has been associated with hybrid development. Yields of maize in the United States were basically unchanged from the mid-19th century until 1930; they accelerated sharply after the introduction of commercial double-cross hybrids. The subsequent replacement of double-cross hybrids by single-cross hybrids in 1960 is associated with the second acceleration in maize yields. The average yield advantage of hybrids versus inbred cultivars is approximately 15% (Tollenaar 1994).

Rice hybrids with a yield advantage of about 10–15% over the best inbred varieties were introduced in China in the mid-1970s and are now planted on about 45% of the rice land in that

country. Rice hybrids adapted to the tropics have now been bred at IRRI and these show a similar yield advantage. Almost all the hybrid rice grown in China and India and hybrids developed at IRRI are between indica varieties. The magnitude of heterosis depends on the genetic diversity between the parents. The greater the genetic difference, the higher the heterosis. During the past 30 years, the genetic differences among the improved indica rice varieties have narrowed because of the massive international exchange of germplasm (Khush and Aquino 1994). Indica and japonica germplasm has, however, remained distinct as little gene flow has occurred between these two varietal groups. As expected, hybrids between indica and japonica varieties show higher heterosis for yield than indica/indica hybrids (Yuan et al 1989). IRRI's new plant type program is based on tropical japonica germplasm. These improved tropical japonicas, when used for producing hybrids with modern semidwarf indica varieties, have a higher heterosis.

The increased yield of tropical rice hybrids is due to increased biomass, higher spikelet number, and, to some extent, higher grain weight. The major constraints to the large-scale adoption of hybrid varieties in developing countries is the cost of hybrid seed, which farmers must buy for each crop of rice. To overcome this constraint, several institutes have active research programs to develop apomictic hybrids that will breed true and allow farmers to use their crop harvest for planting the next crop (Khush et al 1994).

Wide hybridization

Crop gene pools are widened through hybridization of crop cultivars with wild species, weedy races, and intrasubspecific crosses. Such gene pools are exploited for improving many traits, including yield. Lawrence and Frey (1976) reported that a quarter of the lines from BC₂-BC₄ segregants from *Avena sativa* × *A. sterilis* matings were significantly higher in grain yield than the cultivated recurrent parent. Nine lines from this study, when tested over years and sites, had agronomic traits similar to those of the recurrent parent and 10–29% higher grain yield. The higher yield potential of these interspecific derivatives was attributed to higher vegetative growth rates or early seedling vigor.

Tongil rice varieties developed in Korea from indica-japonica crosses have a 20% yield advantage over the best japonica parents (Ahn 1995). Xiao et al (1996) reported that some backcross derivatives from a cross between an *Oryza rufipogon* accession from Malaysia and cultivated rice outyielded the recurrent parent by as much as 18%. They identified two quantitative trait loci (QTLs) from wild species with a major contribution to yield increase. These QTLs are now being transferred to several modern semidwarf varieties.

Genetic engineering

Because protocols for transformation of rice are now well established (Shimamoto et al 1989, Christou et al 1991), it is possible to introduce single alien genes that can selectively modify yield-determining processes. 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, roots, tubers, and fruits. Thus, starch is the critical determinant of the sink strength of developing sink organs as well as the source. Several enzymatic steps are involved in starch biosynthesis in plants. ADP glucose pyrophosphorylase (ADPGPP) is a critical enzyme in regulating starch biosynthesis in plant tissues. Even in storage organs with 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 (Kishore 1994). Starch levels and dry matter accumulation were enhanced in potato tubers of plants transformed with the *glgC¹⁶* gene from *Escherichia coli* encoding ADPGPP (Stark et al 1992). The transformed potato plants had tubers with higher dry matter and starch content, under both growth chamber and field conditions. The plants were similar in growth and development to the nontransgenic controls. The nature of starch produced by the tubers containing the *glgC¹⁶* gene was similar to that of the controls. This gene has been introduced into rice and transgenic plants are being evaluated.

In several crop species, incorporation of the “stay-green” trait or slower leaf senescence has been a major achievement of breeders (Evans 1993). In some genotypes with slower senescence (stay green), Rubisco degradation is slower, which results in longer duration of canopy photosynthesis and higher yields. The onset of senescence is controlled by a complement of external and internal factors. Plant hormones such as ethylene and abscisic acid promote senescence, whereas cytokinins are senescence antagonists. Therefore, overproduction of cytokinins can delay senescence. The *ipt* gene from *Agrobacterium tumefaciens* encoding an isopentenyl transferase (Akiyoshi et al 1984) was fused with the senescence-specific promoter SAG12 (Gan and Amasino 1995) and introduced into tobacco plants. Leaf and floral senescence in the transgenic plants was markedly delayed, biomass and seed yield increased, but other aspects of plant growth and development were normal. This approach appears to have great potential for improving crop yields by slowing senescence and Rubisco degradation and thus increasing canopy photosynthesis. The *ipt* gene has been introduced into rice and transgenic plants are being evaluated.

Molecular breeding

Crop yields are polygenically inherited and are strongly influenced by environment. Therefore, determination of genotypic values from phenotypic expression is not precise and selection strategies must take into account low heritabilities. Breeders generally select for yield on the basis of plant type in early segregating generations and on the basis of yield trials when uniform breeding lines become available. Up to now, it has not been possible to select for individual QTLs having positive effects on yield in segregating populations. Recent developments in the construction of saturated molecular maps of several crops based on restriction fragment length polymorphism (RFLP), random amplified polymorphic DNAs (RAPDs), and microsatellites have made it possible to map individual QTLs for yield. Molecular genetic maps consisting of numerous markers covering the entire genome of rice have been prepared. Through linkage analysis of large segregating populations, polymorphic for molecular markers and QTLs for yield, location of the QTLs for yield can be determined. Selection for yield can then be done via closely linked molecular markers. In a cross between parents differing in QTLs for yield, individuals having positive QTL alleles for yield from both parents can be selected. Tanksley et al (1989) and Stuber (1989, 1992) have elaborated the theory behind the molecular basis of selection for QTLs. A project to enhance the yield potential of rice through molecular marker-aided selection is under way at IRRI.

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Single-leaf and canopy photosynthesis of rice

S.Peng

Rice single-leaf photosynthesis was studied intensively in the 1960s and '70s. However, these studies did not contribute significantly to rice crop improvement because of poor correlation between single-leaf photosynthetic rate and grain yield. Canopy photosynthesis has received more attention since then. There is little doubt that canopy net photosynthesis rate correlates with biomass production. Yield enhancement by conventional breeding has mainly resulted from improvement in plant type, which has increased canopy net photosynthesis, especially during the grain-filling period. It is argued that further improvement in canopy net photosynthesis by fine-tuning plant type is difficult because most high-yielding cultivars are close to the optimum canopy architecture. This suggests that increasing single-leaf photosynthesis could be the only way to substantially enhance rice yield potential. With a better understanding of limiting processes in photosynthesis, advances in measurement methodology, and the advent of biotechnology, which enables the modification of content or activity of individual enzymes, the possibility of enhancing biomass production by improving single-leaf photosynthesis should be reexamined. In this chapter, I review the characteristics of rice photosynthesis at the single-leaf and canopy levels by summarizing the external and internal factors that control single-leaf and canopy photosynthetic rates. I also discuss the scenario of increasing rice yield potential by improving single-leaf photosynthesis.

Economic yield is the product of aboveground biomass and the harvest index (HI). For cereal crops, genetic gain in yield potential usually arises from improved HI through modified canopy architecture (Austin et al 1980). Current high-yielding indica rice varieties have a yield potential of 10 t ha^{-1} with HI of 0.5 under tropical irrigated conditions. Because it is difficult to increase HI for many cereals (Austin et al 1980), a further increase in yield potential will be attained mainly by increasing biomass production. This conclusion is indirectly supported by the fact that a yield of 15.7 t ha^{-1} was achieved with HI of 0.47 in the subtropical environment of Yunnan, China (Ying et al 1998). At least 90% of the biomass of higher plants is derived from CO_2 assimilated through photosynthesis (Zelitch 1982), so, the importance of photosynthesis for improving biomass is indisputable. It has been well established that canopy photosynthesis is closely correlated to biomass production and grain yield. Canopy net photosynthetic rate (CP_n) is a function of leaf area index (LAI), canopy structure, and single-leaf net photosynthetic rate (P_n). Whether increasing P_n can improve yield potential or not is controversial because P_n is inconsistently related to biological and economic yields. Crop physiologists have tried to select for high P_n in several crop species, but no cultivar has been released from these selection

programs (Nelson 1988). Direct selection for P_n sometimes resulted in lower yield (Evans 1990). In spite of these problems, the hypothesis that higher P_n is necessary for increased yields is still valid (Elmore 1980). Gupta and Olugbemi (1988) believed that a further enhancement in crop productivity would depend on an increase in P_n . In this chapter, I will review the characteristics of rice photosynthesis at the single-leaf and canopy levels and discuss the possibilities for increasing rice yield potential by improving photosynthesis.

Single-leaf photosynthesis

Rice is classified as a C_3 plant despite isolated claims of C_4 characteristics (Hegde and Joshi 1974, Imaizumi et al 1990). The value of P_n measured under optimal conditions has become increasingly higher in recent years as the accuracy of measuring instruments has been improved (Matsuo et al 1995). Maximum P_n under saturated light ranges from 15 to 32 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Yoshida 1981) and 40 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was measured recently in rice leaves at IRRI. The value of P_n depends on leaf morphology and physiology and environmental factors.

Environmental factors

Photosynthetically active radiation. The values of photosynthetically active radiation (PAR) at the compensation point ($P_n = 0$) and saturation point (P_n is maximum) of rice leaves were 8 to 20 and 880 to 1,170 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, under 25°C and ambient CO_2 concentration (Murata 1961). The saturation point increases with increased CO_2 concentration (Kuroda 1971). Photosynthetic efficiency, defined as the ratio of P_n to PAR, decreases as PAR increases. Ishii et al (1977) reported that the slope of the P_n versus PAR curve was greater before the compensation point than after. This phenomenon is called the Kok effect. The Kok effect is not observed in C_4 plants, so it is assumed that the Kok effect is associated with photorespiration, which may begin near the compensation point.

There is no difference in P_n between the two sides of a leaf when they receive the same PAR (Tanaka 1972). At a given PAR, P_n is highest when the radiation is evenly projected to both sides, intermediate when unevenly projected to both sides, and lowest when the light is projected to only one side. The benefit of two-side exposure becomes greater with thick or N-rich leaf blades. Leaves with two-side exposure never showed saturation up to PAR of 1,221 $\mu\text{mol m}^{-2} \text{s}^{-1}$, whereas leaves with one-side exposure showed saturation at 916 to 1,069 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Tanaka 1972).

When the PAR rises, P_n increases up to a certain limit beyond which further higher PAR results in a decrease in light-harvesting efficiency and photosynthetic capacity, and a loss of chlorophyll (Horton and Ruban 1992). That is because, when the rate of absorption exceeds the capacity for electron transport, the efficiency of light collection is “down-regulated” to prevent overreduction of photosystem II. Although this down-regulation offsets longer-term photoinhibitory effects, significant losses of photosynthesis occur during these processes. Under more severe conditions (such as extremely high PAR, temperature extremes, and water deficit), down-regulation can be very long-lived, and even overloaded, resulting in photodamage (Horton, personal communication).

CO_2 concentration. The CO_2 concentration affects P_n directly since it is the substrate for the dark reaction of photosynthesis. CO_2 concentration also affects P_n indirectly by influencing stomatal aperture and CO_2 diffusion. Stomatal resistance increased as CO_2 increased above 300 ppm (Akita 1980). In rice, the CO_2 compensation point for P_n remained at a constant value of 55 ppm at PAR higher than 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$, significantly higher than for C_4 species. The CO_2 compensation point increased to 103 ppm as PAR decreased from 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to the

compensation point (Ishii et al 1977). The CO₂ saturation point for P_n in rice was about 2,000 ppm, also higher than for C₄ species (Akita 1980). As PAR increases, the CO₂ saturation point increases. Photorespiration was highest at a CO₂ concentration of 300 to 500 ppm, and it decreased as the CO₂ concentration increased beyond that. Photorespiration in the rice plant disappeared when CO₂ concentration reached 1,500 to 2,000 ppm (Matsuo et al 1995). Dark respiration is less sensitive to changes in CO₂ concentration.

Temperature. The optimum temperature for P_n in indica rice is 25 to 35 °C and 20 to 33 °C in japonica rice under saturating PAR. Although the optimum temperature for P_n in rice is lower than for C₄ plants, it is higher than for other C₃ species (Vong and Murata 1977). Under low PAR, effects of temperature on P_n are small because P_n is primarily determined by photochemical reactions that are not sensitive to temperature (Q₁₀ = 1). The Q₁₀ of dark reaction is 2 to 4 (Yoshida et al 1970). The temperature effect differs between gross photosynthesis (P_g) and P_n. Within a temperature range of 15 to 35 °C, P_g increases as the temperature rises. However, P_n remains relatively constant over the range of 18 to 33 °C because of the temperature reaction of photorespiration and dark respiration (Murata 1961). The rate of photorespiration rises as temperature increases because of a decrease in the solubility of CO₂ and an increase in the O₂/CO₂ solubility ratio (Edwards and Walker 1983). Temperature also affects P_n indirectly through changing humidity. A rapid decline in humidity because of a temperature rise causes increased stomatal resistance and decreased P_n (Ishihara and Saito 1987).

Humidity. Horie (1979) found that P_n was highest under relative humidity of 60% to 80%, and it decreased as relative humidity dropped below 60% because of increased stomatal resistance. A decrease in humidity causes a decrease in leaf water content due to intense transpiration and this results in a decrease in P_n (Ishihara et al 1971b). The PAR saturation point of P_n increased from 1,200 to 1,800 μmol m⁻² s⁻¹ in high humidity (Ishihara and Saito 1987).

Morphological traits

Stomatal density. Stomata are found in the leaf blade, leaf sheath, rachis, rachis branch, and the lemma and palea of the spikelet. A small number of poorly developed and uncompleted stomata were found in the plume of the spikelet (Maeda 1972). The stomata of rice plants are smaller than in other plant species, but rice has a relatively high stomatal density. The stomatal density of rice is greater than that of other cereal crops such as maize, wheat, and barley (Chen et al 1990, Teare et al 1971), ranging from 150 to 650 mm⁻², depending on leaf positions on the stem, cultivar, and growing conditions (Matsuo et al 1995). Stomatal density increases with leaf position (Table 1). The stomatal density of the indica type and indica/japonica hybrids is higher than that of the japonica type (Maruyama and Tajima 1986). The distribution of the stomata on the adaxial and abaxial sides depends on leaf position and genotype.

Table 1. Stomatal density of different leaf positions in rice and wheat.

Leaf position	Rice (no. mm ⁻²)	Leaf position	Wheat (no. mm ⁻²)
9th	330 ^a	-4th	51 ^b
10th	363	-3rd	53
11th	399	-2nd	58
12th	448	Flag leaf	63

^aMeasured on abaxial side (Ishihara et al. unpublished). ^bAverage of two sides (Teare et al 1971).

Stomatal aperture. The stomatal aperture of rice is much smaller than in other species, with a maximum aperture of 1.5 μm . The average stomatal aperture of several other species is as high as 6 μm (Meidner and Mansfield 1968). Ishihara et al (1971a) observed a variation in rice stomatal aperture that ranged from 0.5 to 1.2 μm . Stomatal aperture and diffusion conductance in leaves increase with higher leaf N concentration. Stomatal aperture decreases under high solar radiation, high temperature, and low air humidity as a result of the lowered leaf water potential caused by increased transpiration (Iahihara and Saito 1983). A close relationship between stomatal aperture and stomatal conductance was observed in rice leaves with different N concentrations.

Specific leaf area and leaf shape. Specific leaf area (SLA, $\text{m}^2 \text{ leaf g}^{-1} \text{ dry matter}$) is an indication of leaf thickness. Thick leaves (low SLA) usually have a high chlorophyll content and a high content of photosynthetic enzymes per unit leaf area. Leaf thickness is positively correlated with P_n (Murata 1961). A thick leaf has less tendency to expand horizontally and a greater tendency to be erect. After the early vegetative stage, thick leaves are thought to be desirable for improving P_n . Tsunoda and Kishitani (1976) claimed that, at a given leaf area, a narrow leaf can increase P_n by as much as 30% because of reduced boundary layer resistance on the leaf surface.

Physiological factors

Leaf nitrogen concentration. A linear relationship between light-saturated P_n and leaf N concentration per unit leaf area (N_a) was reported in rice when N_a ranged from 0.5 to 2.1 g m^{-2} (Yoshida and Coronel 1976, Makino et al 1988). This relationship holds whether differences in N_a are due to environment or genotype (Tsunoda 1972). On the other hand, a curvilinear relationship between light-saturated P_n and N_a was observed by Takano and Tsunoda (1971) and Cook and Evans (1983): a linear relationship when N_a was below 1.6 g N m^{-2} , but P_n leveled off above 1.6 g N m^{-2} . This curvilinear relationship might have resulted from growth limitations in some of the primitive genotypes caused by factors other than leaf N. Wheat usually contains a higher amount of N per unit leaf area than rice, which may explain the difference in radiation-use efficiency between wheat and rice (see Sheehy, this volume).

Peng et al (1995) determined the relationship between light-saturated P_n and N_a in field-grown rice plants (Fig. 1). Compared with the relationship determined under greenhouse/growth chamber conditions in previous studies, a higher P_n was observed at low N_a (less than 1.0 g N m^{-2}) under field conditions. When the field-derived relationship between light-saturated P_n and N_a was substituted for the greenhouse/growth chamber-derived equation in the rice growth simulation model (ORYZAI), the estimation of dry matter production was improved for rice grown in tropical irrigated environments.

Rubisco. About 50% of total soluble protein and 25% of total N are associated with Rubisco (ribulose-1.5-bisphosphate carboxylase–oxygenase) protein in rice leaves (Makino et al 1984). During leaf senescence, specific carboxylase activity of Rubisco did not change and a decline in activity was caused by the reduction in the amount of Rubisco protein (Makino et al 1983). Rice has a $K_m(\text{CO}_2)$ value and ratio of Rubisco to total soluble protein similar to those of other C_3 plants, but its V_{max} may be much lower (Makino et al 1985). The V_{max} of rice Rubisco is 45% lower than that of wheat. Among species of *Oryza*, the differences in enzymatic properties of Rubisco are small (Makino et al 1987). Within *O. sativa*, the differences in $K_m(\text{CO}_2)$, V_{max} , and the ratio of Rubisco to total soluble protein are small among indica, temperate japonica, and intermediate types (Makino et al 1987). The tropical japonica type has a slightly higher $K_m(\text{CO}_2)$ and V_{max} than other subspecies. The differences are also small among the cultivars within

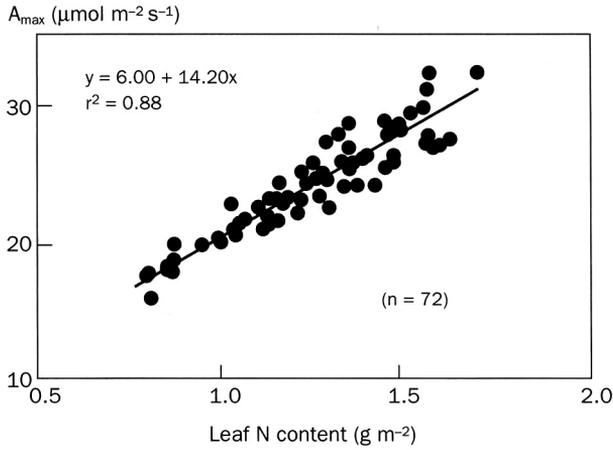


Fig. 1. Relationship between light-saturated single-leaf photosynthetic rate (A_{max}) and leaf N content on a leaf-area basis measured under field conditions (redrawn from Peng et al 1995).

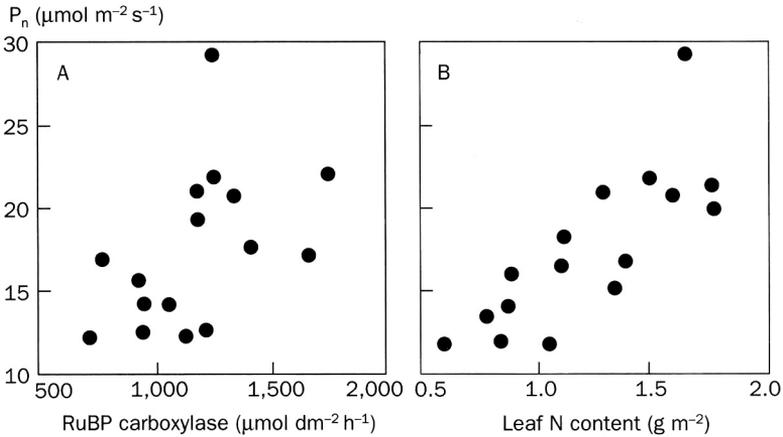


Fig. 2. Relationship between single-leaf net photosynthetic rate (P_n) and RuBP carboxylase activities (A) and between P_n and leaf N content (B) of four rice varieties grown under four nitrogen levels (redrawn from Paulsen 1972).

a subspecies. These results suggest that it is difficult to improve the enzymatic characteristics of Rubisco by crossbreeding since the genetic variation in enzymatic properties is small even when the comparison was expanded to the other species of *Oryza*.

It remains unknown why N_a correlates better with P_n than Rubisco content per unit leaf area (Fig. 2). It was speculated that high N_a reflects small cell size, high stomatal density per unit leaf area, or high surface/volume ratio of cells, which may provide a more direct relation with P_n , (Tsunoda 1972). It could also be because leaf N can be measured more accurately than Rubisco content.

Chlorophyll. Chlorophyll content seldom limits P_n under sufficient PAR (Rabinowitch 1956) because more chlorophyll is contained in an ordinary leaf than necessary. Under low PAR, however, chlorophyll content may limit P_n since the rate of light reaction may limit the overall process of photosynthesis (Murata 1965). Paulsen (1972) reported that P_n and chlorophyll content were closely correlated in six rice cultivars under low PAR but not under high PAR. In rice, chlorophyll content is closely correlated with N concentration, so an apparent close relationship exists between chlorophyll content and P_n (Fig. 3). However, if the variation in chlorophyll content is caused by different genotypes or by other nutrients such as phosphorus or potassium, chlorophyll content is not correlated with P_n (Yoshida et al 1970). During leaf senescence, chlorophyll remains relatively more stable than Rubisco (Makino et al 1983).

Stomatal conductance. High stomatal density compensates for the small aperture so that the stomatal conductance of a rice leaf is comparable to or even greater than that of other species. It is not easy to compare published values of conductance because measurements were made in different conditions and using different gas exchange measurement systems. In addition, different boundary layer conductance may be used to calculate stomatal conductance in various studies.

The 20% to 30% decline in P_n in the afternoon despite sufficient PAR is accompanied by a reduction in stomatal conductance (Ishihara and Saito 1987). Murata (1961) observed midday depression in P_n of a rice leaf. The main cause of midday P_n depression in the rice leaf is also attributed to stomata closure and a reduction in the CO_2 supply to mesophyll cells. O'Toole and Tomar (1982) have shown that rice leaves can suffer water deficits even when the plants are permanently flooded.

Sink limitation. Coordination between source and sink is said to affect P_n . The rate of removal of photosynthetic products from the site controls P_n (Tanaka 1977). King et al (1967) reported that removal of panicles during ripening decreased P_n . When sink capacity is smaller than the source, photosynthetic products accumulate in the leaves and in conductive tissues. Tanaka and Fujita (1971) stated that the accumulation promotes respiration and retards photosynthesis.

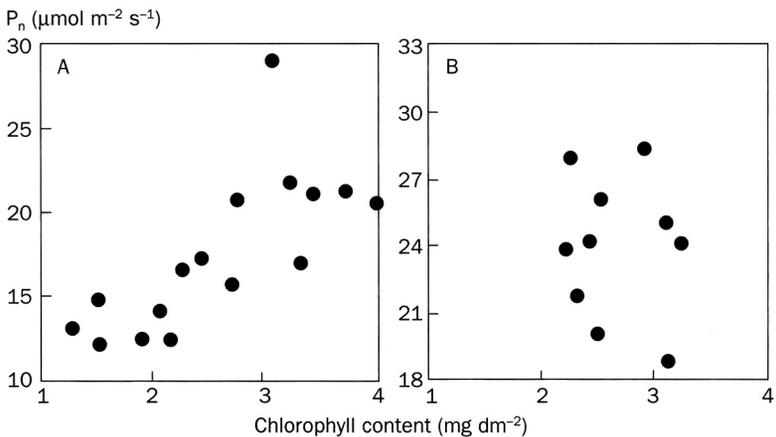


Fig. 3. Relationship between single-leaf net photosynthetic rate (P_n) and chlorophyll content of four rice varieties grown under four nitrogen levels (A) and ten rice genotypes (B) (redrawn from Paulsen 1972).

Photorespiration. About 30% to 40% of the fixed carbon is consumed by photorespiration. This is determined by the increase in apparent photosynthesis rate when the leaf is placed in a low O₂ condition of about 2%. Under this condition, photorespiration is completely inhibited, whereas respiration is not affected. No significant difference in photorespiration rate was observed among cultivars or across the species of *Oryza* (Akita et al 1975).

Respiration. Net photosynthesis is gross photosynthesis minus concurrent respiration. The respiration rate is about 10% of P_n in the range of optimum temperature for net photosynthesis. It is generally believed that the rate of respiration is the same in the light as in the dark (Matsuo et al 1995). Respiratory rate among leaves does not vary as much as photosynthetic rate (Tanaka et al 1966).

Genetic variation in P_n

The cultivated species *Oryza sativa* has a higher P_n than wild species of *Oryza* (Cook and Evans 1983). Among the subspecies of *O. sativa*, the order in P_n is generally indica > temperate japonica > tropical japonica. Murata (1961) compared P_n of 30 varieties and found a 20% variation in P_n among the varieties at the maximum tillering stage and even greater differences at the heading stage. Large differences in P_n occur, but much of the variation is due to the low P_n in poorly adapted varieties. Many adapted varieties have P_n near the maximum (Paulsen 1972). Results on heterosis in P_n are inconsistent, probably because of different hybrid combinations tested and variation in leaf N concentration. The higher P_n of a genotype is usually associated with a higher leaf N concentration, thicker leaf, or narrower leaf.

Canopy photosynthesis

Canopy Photosynthesis is difficult to measure because of carbon dioxide fluxes in the aerenchyma of the plant and from the soil and water. The highest canopy net photosynthetic rate (CP_n) is generally observed at 1000-1100 (Dingkuhn et al 1990b). Maximum CP_n reaches 45 to 50 μmol m⁻² s⁻¹ on a clear day for a high-yielding rice cultivar grown under favorable conditions (Dingkuhn et al 1990b). On a seasonal basis, CP_n reaches a maximum between panicle initiation and the booting stage when LAI is the highest (Tanaka 1972). CP_n is affected by environmental factors such as PAR, temperature, and CO₂ concentration. Plant internal factors such as LAI, canopy structure, P_n, and crop respiration also control CP_n. Among these plant internal factors, LAI is the most important determinant of light interception and therefore of CP_n, with canopy structure being of secondary importance.

Environmental factors

Photosynthetically active radiation. Canopy photosynthesis is saturated at full sunlight when LAI is less than 1. No saturation was observed at higher LAI for the improved cultivars with short and erect leaves (Dingkuhn et al 1990b). Saturation is found in the canopy with long and drooped leaves, regardless of LAI (Tanaka 1972). In addition to LAI and plant type, light incident angle, foliage N concentration, and leaf water content influence the value of light saturation point (Tanaka 1972). At a given PAR, CP_n increases with an increase in the fraction of diffuse PAR (Tanaka 1972). The light compensation point increases with LAI as more leaves are shaded and there is more respiring biomass (Dingkuhn et al 1990b).

Temperature. Short-term temperature changes have a minimal effect on CP_n. These short-term temperature effects are mainly on Photorespiration and respiration, Long-term temperature changes affect CP_n through a direct effect on P_n and indirect effects on other physiological processes such as leaf area development rate, nutrient uptake rate, and growth duration (Matsuo et al 1995).

CO₂ concentration. The responses of photosynthesis to CO₂ concentration are similar at the single-leaf and canopy levels. Atmospheric CO₂ content above the rice canopy showed a 15% diurnal fluctuation, decreasing during the day and increasing during the night. This magnitude of fluctuation may cause a 10% to 20% change in CP_n (Yamada 1963). Higuchi (1982) reported that rice root systems absorbed CO₂ from the soil-floodwater system; the CO₂ diffused upward through the aerenchyma and assimilated in photosynthesizing tissues.

Plant internal factors

Leaf area index. A maximum LAI of 20 in rice was reported by Shi and Akita (1988) but large edge effects on small plots are suspected. More recent general values of LAI are 10–12. Peak LAI is generally observed around the heading stage. At heading, flag leaves contribute 19% of LAI, second leaves 28%, and third leaves 27% (Yoshida et al 1972). Crop management practices such as fertilizer application aim to optimize CP_n and yield, mainly by controlling LAI. Optimal LAI is defined as the LAI at which CP_n and crop growth rate (CGR) are maximum, and beyond which CP_n and CGR will decrease. This is because canopy gross photosynthesis (CP_g) increases with an increase in LAI up to a point where CP_n reaches its maximum, whereas canopy respiration increases in proportion to the increase in LAI (Monsi and Saeki 1953). This concept was supported by the study of Murata (1961). The optimal LAI values depend on growth stage, plant type, and PAR (Tanaka et al 1966). Cultivars with erect leaves have a higher optimum LAI than those with horizontal leaves (Yoshida 1981). However, Yoshida et al (1972) questioned the existence of optimal LAI in rice. Cock and Yoshida (1973) reported that canopy respiration increased linearly with CP_n but curvilinearly with LAI. Thus, CP_n and CGR only leveled off but did not decline beyond a certain LAI, which was defined as ceiling LAI (Fig. 4). Yoshida (1981) reported that CGR reached a maximum at an LAI of about 6 for IR8 and about 4 for Peta, beyond which it remained the same. However, if lodging occurs at high LAI,

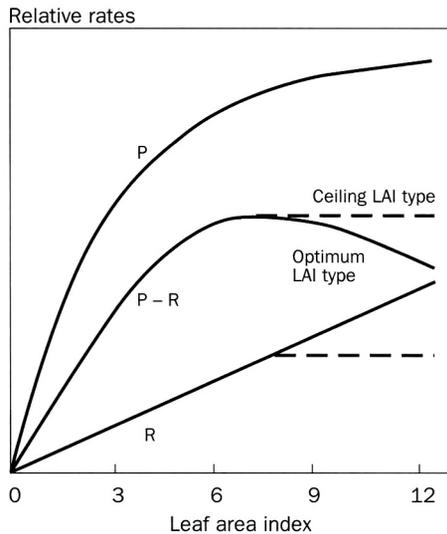


Fig. 4. Schematic explanation of optimum leaf area index (LAI) and ceiling LAI. P represents photosynthesis and R respiration (redrawn from Tanaka 1972).

CP_n and CGR will decrease with an increase in LAI and optimal LAI will appear. Therefore, Tanaka (1983) explained that ceiling LAI existed in the high-yielding cultivars with short and erect leaves (such as IR8) and grown in the dry season, whereas optimal LAI appeared in leafy cultivars (such as Peta) grown in the wet season. Crops with ceiling LAI are easier to manage than those with optimal LAI since excessive N application does not have a negative effect on crops with the ceiling LAI (Tanaka 1983). Critical LAI is defined as the LAI when 95% of PAR is intercepted by the crop canopy (Gifford and Jenkins 1982).

Canopy structure. Monsi and Saeki (1953) stated that rice cultivars with erect, short, and thick leaves have a small light extinction coefficient (K). The value of K is related to the leaf spread, which is determined by the leaf angle and curvature of the leaf blade (Tanaka et al 1966). Tanaka et al (1969) studied the effect of leaf inclination on CP_n . The drooping leaf canopy was created with weights hung on the leaf tips. Light saturation for CP_n was observed in the drooped-leaf plot but not in the control plot with erect leaves. The maximum CP_n of the drooped-leaf plot was 68% of that of the control. Sinclair and Sheehy (1999) argued about the benefit of erect leaves in light interception for a rice crop with LAI greater than 4.2. They stated that the major benefit of erect leaves is to sustain a high LAI for N storage for a high-yielding rice crop. The effect of erect leaves on the proportion of diffused light in the low portion of the canopy and the benefit of erect leaves for reducing photoinhibition under high light intensity (Murchie et al 1999) deserve further investigation.

Cultivars with erect leaves give a higher CP_n only when LAI is larger than 5, whereas cultivars with droopy leaves give a higher CP_n when LAI is less than 3. Therefore, an ideal variety should have a droopy-leaf canopy in the very early vegetative stage to intercept PAR effectively. As the crop grows, a plant community with vertically oriented leaves has better light penetration and a higher CP_n at high LAI. The beneficial effect of erect leaves is pronounced when light intensity is high (Yoshida 1981). Erect leaves have a considerable advantage in rice, some advantage in wheat, but little advantage in maize (Paulsen 1972). It was reported recently that V-shaped leaf blades reduce mutual shading and increase canopy photosynthesis as do erect leaves (Sasahara et al 1992).

Plant and panicle height. The semidwarf plant type reduces susceptibility to lodging at high N inputs and increases HI (Tsunoda 1962). Recent studies, however, claim that the height of semidwarf rice and wheat may limit canopy photosynthesis and biomass production (Kuroda et al 1989, Gent 1995). Under a given LAI, a taller canopy has better ventilation and therefore higher CO_2 concentration inside the canopy than a shorter canopy (Kuroda et al 1989). Similarly, light penetrates better in the tall canopy than in the short one (Kuroda et al 1989).

Panicles that droop below the flag leaves (lower panicles) increase the interception of PAR by leaves and consequently increase canopy photosynthesis (Setter et al 1995). But the adverse effects of lowering the panicles on panicle exertion and panicle diseases need to be investigated. In addition, the panicle contributes to photosynthesis. On the basis of projected area, the P_n of the panicle is 20% of that of the flag leaf and the gross photosynthetic rate of the panicle is 30% of that of the flag leaf (Table 2). On the basis of chlorophyll, the photosynthetic capability of spikelets is similar to that of the flag leaf (Imaizumi et al 1990). It was estimated that panicle photosynthesis contributed 20% to 30% of the dry matter in grain (Imaizumi et al 1990).

Nitrogen concentration. Increased leaf N concentration results in increases in LAI and P_n , therefore enhancing CP_n . Under favorable rice-growing conditions, CP_n is limited by LAI during the vegetative growth stage and by foliage N concentration during the reproductive stage (Schnier et al 1990). The foliage N compensation point (N concentration at which CP_n is zero) increases with LAI (Dingkuhn et al 1990a). CP_n correlates better with foliage N concentration on a dry-

Table 2. Net photosynthetic rate, respiration rate, and gross photosynthetic rate of rice panicles (ranging from 1 to 10 d after flowering) expressed on the basis of projected area in comparison with rice leaves.

Organ	Net photosynthesis	Respiration ($\text{mmol m}^{-2}\text{s}^{-1}$)	Gross photosynthesis
Panicle	5.03	4.04	9.08
Leaf	25.00	2.50	27.50

weight basis than on a leaf-area basis (Dingkuhn et al 1990a). Fertilizer-N application increases CP_g and canopy respiration. Under excessive N application, CP_n may be reduced due to lodging and pest damage. Simulation modeling suggests that a steeper slope of the vertical N Concentration gradient in the leaf canopy with more N present in the uppermost stratum enhances canopy photosynthesis (Dingkuhn et al 1991).

Photosynthesis and yield potential

Various physiological selection criteria have been tried in plant breeding including P_n , CO_2 compensation point, $K_m(CO_2)$ of Rubisco, photorespiration, SLA, and mesophyll cell size. However, breeding for increased yield potential by improving photosynthesis has resulted in little or no success (Gifford and Jenkins 1982). Although genetic variation in P_n has been reported in rice, the relationship between photosynthetic capacity and biomass production is poor (McDonald et al 1974). Zelitch (1982) stated that the lack of a strong positive relationship is due to measurements of P_n rather than biological reasons. Austin (1993) believed that genotype \times ontogeny and genotype \times environment interactive effects on P_n caused poor correlation. Traits that are pleiotropically and negatively related to P_n may offset any gains from higher P_n (Austin 1993).

Austin (1993) argued that no substantial improvement in biomass production could be obtained by selecting for modified canopy morphology because modern varieties are close to the optimum canopy architecture. This suggests that increasing P_n could be the only way to substantially enhance rice yield potential. With a better understanding of limiting processes in photosynthesis, advances in measurement methodology, and the advent of biotechnology, which enables the modification of content or activity of individual enzymes, we should reexamine the possibility of enhancing biomass production by improving P_n .

Does increased P_n lead to increased biomass production and grain yield in rice? During the vegetative and reproductive stages, P_n can be increased easily by increasing leaf N concentration because of the close relationship between leaf N and Rubisco content (Makino et al 1984). Modern rice varieties respond quickly to N application by increasing leaf N concentration because of the high N-absorbing capacity of the root system (Peng and Cassman 1998). High leaf N concentration, in the meantime, increases tiller production and leaf area expansion, which cause mutual shading and an actual reduction in CP_n (Fig. 5). If the increased P_n is not due to increased leaf N concentration or if increased leaf N concentration and P_n do not lead to excessive LAI and tiller production, high P_n should contribute to increased biomass and grain yield. The concern is how to increase P_n without increasing leaf N concentration, or how to increase leaf N concentration without significant increases in tillers and leaf area. The value of P_n can be increased by thicker leaves and by reducing midday depression and

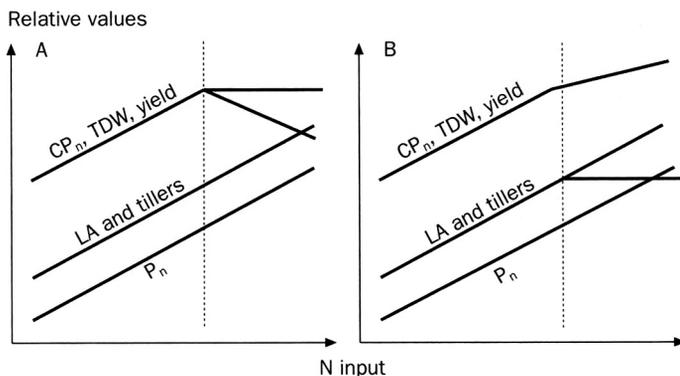


Fig. 5. Effects of nitrogen input on single-leaf net photosynthetic rate (P_n), leaf area (LA), tillering, canopy net photosynthetic rate (CP_n), total dry weight (TDW), and grain yield. Leaf area and tiller production increase proportionally with nitrogen input. This causes the leveling-off or decline in CP_n , TDW, and yield as nitrogen input increases (A). If leaf area and tiller production become insensitive to a further increase in nitrogen input, a continuous increase in CP_n , TDW, and yield can be achieved under high nitrogen input (B).

photoinhibition, which is independent of leaf N concentration. Transgenic C_4 rice may have increased P_n without the increase in leaf N concentration. For the cultivars with moderate tillering capacity and semital stature, leaf area and tiller production are not very sensitive to increased leaf N concentration because tillering is limited by light intensity inside the rice canopy for those cultivars. Genotypic variation in the sensitivity of leaf area and tiller production to leaf N concentration may exist in rice that is independent of plant height.

During the ripening phase, increasing P_n generally results in increased yield as long as the sink is available. Such a yield increase results from an improvement in the percentage of filled spikelets. Increased leaf N concentration results in an increased P_n but not increased LAI and tillers during the grain-filling period. Our study showed that N application at flowering increased leaf N concentration, P_n , and percentage of filled spikelets of a source-limited cultivar. Cook and Evans (1983) reported that the correlation between P_n and relative growth rate was negative in *Oryza* species during the vegetative stage. However, the P_n of the flag leaf after heading was positively correlated with grain yield. Sasaki et al (1986) compared the P_n of 32 varieties bred in Japan between 1882 and 1976. No significant difference in P_n of flag leaves was observed between old, intermediate, and new variety groups at heading time. However, P_n of flag leaves showed a large difference among the groups during grain filling and this difference was correlated with differences in grain yield among the three groups. Ohno (1976) stated that P_n accounted for 30% of the varietal difference in grain yield and attributed the remaining 70% to the differences in LAI and leaf area duration (defined as the integral of LAI over time),

The yield potential of wheat varieties released by the International Maize and Wheat Improvement Center has increased by $0.83\% y^{-1}$ over the past 30 years. This increase was mainly attributed to increased stomatal conductance and canopy temperature depression (Fischer 1994). Irrigated rice has a much higher stomatal density and stomatal conductance than wheat (Chen et al 1990, Teare et al 1971), suggesting that P_n in irrigated rice plants is unlikely to be limited by stomatal conductance. Small differences in carbon isotope discrimination among varieties and over a wide range of N input levels (S. Peng, unpublished data) also suggest that there is little chance to improve P_n by increasing stomatal conductance for irrigated rice. Other options proposed to increase P_n include suppression of photorespiration and reduction of

maintenance respiration (Penning de Vries 1991). There is little evidence that photorespiration can be suppressed in C₃ plants, and although 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. However, carbon dioxide, like methane, diffuses out of the leaf sheaths through the stomata just above the water level.

Horton and Ruban (1992) believe that operational photosynthesis in the field never actually reaches the intrinsic maximum photosynthetic rate (P_{max}). During the course of the day and the entire growing season, photosynthesis operates at P_{max} over a very short period of time. Internal (feedback inhibition or sink limitation) and external (photooxidative stresses or photoinhibition) factors limit attainment of the full potential of photosynthesis. Preliminary studies indicate that alternative dissipative electron transfer pathways, such as the xanthophyll cycle and free radical-scavenger enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase, give plants overall tolerance for photooxidative stresses. The capacity of photoprotection is variable among species (Johnson et al 1993). Tu et al (1995) reported genotypic variation in photoinhibition and midday photosynthetic depression under high light-induced conditions, suggesting scope for improvement by breeding.

Increasing photosynthetic duration is often achieved by delaying the senescence of the flag leaf. Senescence is associated with the degradation of Rubisco and chlorophyll. Rubisco breakdown usually occurs earlier than chlorophyll loss in a senescing leaf (Makino et al 1983). Increased late-season N application protects Rubisco from degradation, which delays flag leaf senescence and increases photosynthetic duration. But delaying senescence of the flag leaf does not always result in greater yield if the sink is limiting. Moreover, delaying senescence of the flag leaf results in a reduction in nutrient translocation from the flag leaf to grain although the quantitative effects remain to be determined.

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Notes

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Future intensification of irrigated rice systems

A. Dobermann

By 2020, average irrigated rice yields must rise by 30% to about 7 t ha⁻¹. This increase appears achievable, but requires that improved germplasm with a yield potential of 12 t ha⁻¹ in the dry season and 8–9 t ha⁻¹ in the wet season become available within the next 10 years. Moreover, significant improvements in soil and crop management are necessary, particularly nutrient and pest management, to lift average farm yields to about 70% of the yield potential. All this must be achieved in an environment where changes in crop management practices will occur, mainly triggered and driven by socioeconomic changes and competition for natural and human resources. Beyond 2020, further yield increases will probably require germplasm with greater radiation-use efficiency approaching that of plants with C₄ photosynthesis.

Irrigated rice is grown in banded, puddled fields with assured irrigation for one or more crops a year on alluvial floodplains, terraces, inland valleys, and deltas in the humid and subhumid subtropics and humid tropics of Asia. Favorable climatic conditions in combination with irrigation facilities allow farmers to grow one to three crops per year in submerged, mostly fertile soil (IRRI 1997). The irrigated rice ecosystem accounts for 55% of the global harvested rice area and 75% (about 420 million t) of the global annual rice production. In this chapter, I will briefly characterize intensive irrigated rice systems and discuss some of the current and future challenges to crop management. More detailed discussions, mostly related to historical changes and issues of sustainability, can be found elsewhere (Cassman and Harwood 1995, Cassman and Pingali 1995a, Greenland 1997).

Historical development

Single-crop irrigated rice systems in Asia date back several thousand years. Double cropping became common in the longer Yangzi River region about 1,000 years ago and triple cropping probably started in the 14th century (Greenland 1997). Naturally occurring sedimentation, nutrient inflow by irrigation, organic residues, biological N₂ fixation, and carbon assimilation by floodwater flora and fauna played an important role in securing the sustainability of these traditional irrigated rice systems (Greenland 1997). Around 1,000 years ago, rice yields in China and Japan were about 1 t ha⁻¹ and it took several hundred years to increase them to 2 t ha⁻¹ (Greenland 1997).

The breeding and widespread adoption of high-yielding varieties in the 1960s led to a rapid intensification in the tropical lowlands of Asia. The release of semidwarf short-duration varieties such as IR8 (1966), IR20 (1969), and IR36 (1976) triggered investments in irrigation infrastructure and allowed farmers to grow two to three rice crops per year. The new varieties had a short growth period and more efficient biomass partitioning, were short-statured and lodging-resistant, and responded well to fertilizer N additions. Tillage and management intensity increased and soils remained submerged for longer periods. The use of external inputs such as fertilizers, water, energy, and pesticides increased and the diversity of rice varieties used in the irrigated systems decreased. By growing two or three short-duration crops per year, each at a higher yield level than before 1965, annual crop nutrient removal increased five- to sevenfold compared with the pre-Green Revolution period. The use of N₂-fixing green manure ceased in many rice areas because its main purpose of providing N was replaced by cheap and less labor-intensive mineral N. To facilitate land preparation for the next crop, farmers started to cut the entire crop and remove or burn the straw (Uexkuell and Beaton 1992). The average grain yield of irrigated rice reached 4.9 t ha⁻¹ in 1991 (Cassman and Pingali 1993a).

Current situation

Area, cropping systems, and yields

Worldwide, about 79 million ha of rice is grown under irrigated conditions (harvested area). East Asia (China, Taiwan, Japan, Korea) accounts for about 43% (34 million ha) of the global irrigated rice area. About 24 million ha of irrigated rice is grown in South Asia and 15 million ha in Southeast Asia. The countries with the largest irrigated rice areas are China (31 million ha), India (19 million ha), Indonesia (7 million ha), and Vietnam (3 million ha).

Examples of intensive rice-based sequential cropping systems in the tropical and subtropical regions are rice-rice, rice-rice-rice, rice-rice-pulses, rice-wheat, and rice-rice-maize (Fig. 1).

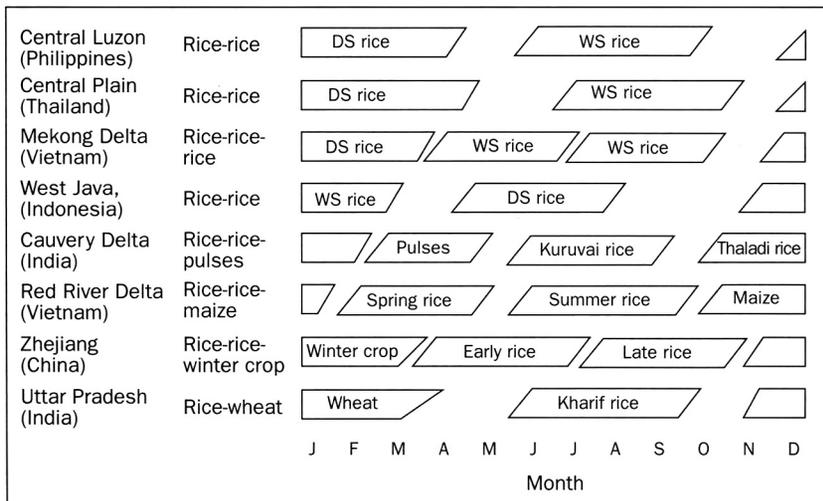


Fig. 1. Cropping systems and the cropping calendar in major irrigated rice areas of South and Southeast Asia. Each site shown represents a large irrigated area with similar intensive rice cropping. DS = dry season, WS = wet season, kuruvai = early monsoon season (Jun-Oct), thaladi = Oct-Feb, kharif = dry season.

Double- and triple-crop monoculture rice systems occupy about 14 million ha. Two to three short-duration crops are typically grown per year, with up to seven crops in two years in parts of the Mekong Delta of Vietnam. Fallow periods between two crops may range from a few days to about 3 mo (Fig. 1). Rice-wheat cropping systems occupy 12 million ha in the Indo-Gangetic floodplain of South Asia (Bangladesh, India, Nepal, and Pakistan) and about 10 million ha in China.

The global average yield of irrigated rice is about $5.2 \text{ t ha}^{-1}\text{crop}^{-1}$, which is equivalent to about 60% of the climate-adjusted yield potential (8.1 t ha^{-1}) of high-yielding varieties across different geographical regions and climatic seasons in Asia (Matthews et al 1995). High yields ($> 5\text{--}6 \text{ t ha}^{-1}$) are obtained in China, Egypt, Japan, Indonesia, Vietnam, and the Republic of Korea, medium yields ($4\text{--}5 \text{ t ha}^{-1}$) in Bangladesh, northwestern and southern India, Lao PDR, Malaysia, Myanmar, the Philippines, Sri Lanka, and Thailand, and low yields ($< 4 \text{ t ha}^{-1}$) in Cambodia, eastern India, Madagascar, Nepal, and Pakistan (IRRI 1997). In the tropics, good rice farmers achieve yields of $7\text{--}8 \text{ t ha}^{-1}\text{crop}^{-1}$ in the dry season and $5\text{--}6 \text{ t ha}^{-1}$ for a wet-season crop.

Yield trends in long-term experiments

In recent years, concern was raised about a long-term yield decline in unfertilized (control) plots as well as in treatments with the “best recommended” fertilizer rates in rice-rice systems at various Philippine experiment stations (Cassman and Pingali 1995b, Flinn et al 1982, Flinn and De Datta 1984, Ponnampereuma 1979) and at some rice-wheat long-term experiments (LTE) in India and Nepal (Brar et al 1998, Nambiar 1994, Nand Ram 1998). *Yield decline* is defined as the decline in measured grain yield of the highest-yielding cultivars per unit land area under constant input levels and management practices during a longer period of time (Dawe and Dobermann 1999).

However, a recent analysis of yield trends in 30 LTE conducted in different countries suggests that yield declines are not as common as previously thought, particularly at yields of $4\text{--}7 \text{ t ha}^{-1}$ (Dawe et al 2000). The strongest yield declines always occurred in LTE conducted at IRRI characterized by high cropping intensity relatively wet fallow periods between crops, and significantly larger initial yields than at other sites. Outside of IRRI, only 4 of 35 data sets had statistically significant yield declines. The average yield trend at IRRI was $-1.51\% \text{ y}^{-1}$ in the dry season (DS) and $-1.27\% \text{ y}^{-1}$ in the wet season (WS), and there were statistically significant yield declines in 6 of 7 data sets. This compares with an average yield trend outside IRRI of $-0.54\% \text{ y}^{-1}$ in the DS and $-0.68\% \text{ y}^{-1}$ in the WS.

Although the yield declines observed in various LTE conducted at IRRI may not be representative for other irrigated rice areas in Asia, their causes must be understood, also with regard to possible consequences for breeding new germplasm with higher yield potential. The key question is whether the yield declines observed in some LTE are caused by inherent soil changes related to intensive rice cropping or whether they are caused by location-specific inappropriate soil and crop management (Cassman et al 1997). Location-specific factors such as soil nutrient depletion (P, K, Zn) due to a negative input-output balance are of less concern because they can be easily corrected. However, there is also evidence for more generic, slow changes in soil quality. One example is the hypothesis of a decline in soil N-supplying capacity caused by changes in the chemical composition of young soil organic matter (Olk et al 1996, 1998). However, a causal relationship between the chemical composition of soil organic matter and soil N-supplying capacity has not been established yet. There is also not enough evidence that a decline in soil N-supplying capacity is common in intensive rice systems.

The complexity of factors affecting grain yield and the lack of detailed historical measurements often complicate the search for causal relationships that may explain the yield trends observed. As an example, Figure 2 shows the yield trends in IRRI's long-term continuous cropping experiment (LTCCE), the longest running experiment of triple-cropped rice in the world. It was begun at IRRI in 1963 to study the feasibility of continuous triple-crop rice systems made possible by short-duration high-yielding varieties and irrigation. More than 100 crops have been harvested since 1963, aiming at sustaining yields close to the yield potential. From 1968 until 1991, trend yields of the highest-yielding variety in this experiment declined at an average annual rate of 1.6% in the dry season, 2.0% in the early wet season, and 1.4% in the late wet season. These are equivalent to cumulative declines of 44%, 58%, and 38% in 24 years (Cassman et al 1995).

Factors such as (1) a change in climatic or genetic yield potential, (2) increased incidence of insect pests and diseases, or (3) deficiencies of P, K, Zn, and Si did not explain the gradual, long-term yield decline in the LTCCE (Cassman et al 1995. Dobermann et al 2000). Our main

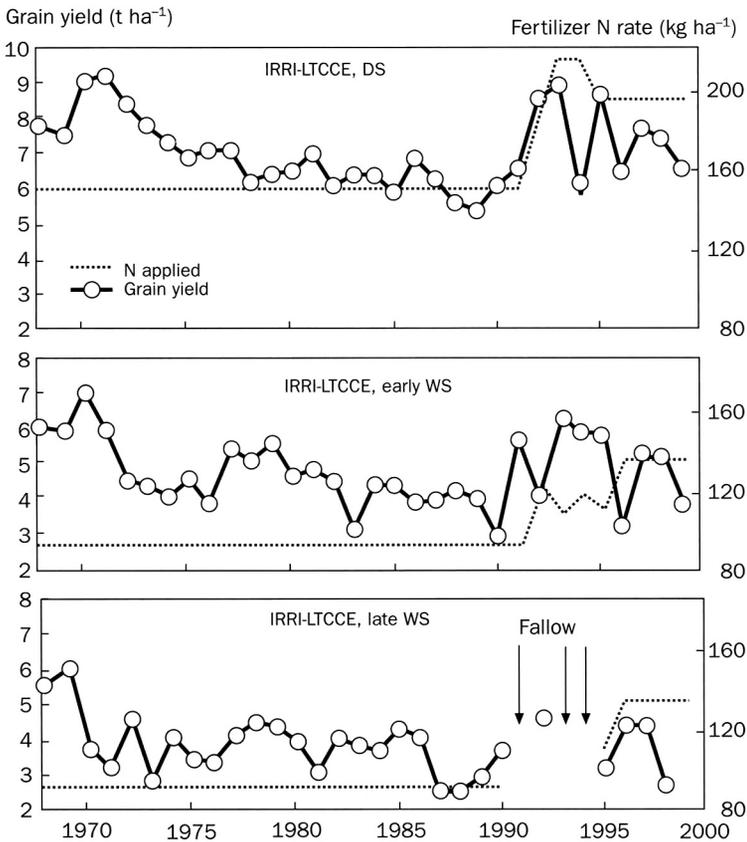


Fig. 2. Rice yield trends in the long-term continuous cropping experiment (LTCCE) at IRRI. Data points and trends shown refer to the average grain yield of three varieties at the highest nitrogen level. In 1991, 1993, and 1994, only two rice crops were grown (dry season: Jan-Apr, wet season: Jul-Oct). Fallow periods are indicated with arrows. DS = dry season, WS = wet season.

hypothesis was that crop N nutrition had decreased over time by (1) increasing losses of fertilizer N, (2) declining N supply to the root system from indigenous sources (soil N mineralization), or (3) declining root N uptake capacity because of abiotic or biotic stresses affecting root health. Therefore, since 1991, N rates were increased, the number of split applications was changed from two to three or four, the timing of N applications was modified, and three fallow periods occurred. From 1991 to 1995, grain yields in the LTCCE increased to levels of 80% to 100% of the simulated yield potential of about 9 to 10 t ha⁻¹ in the DS (Fig. 2), because of the combined effect of higher solar radiation and improved nitrogen nutrition. The latter resulted from a combination of more precise timing of N applications, higher N rates, soil aeration, and improved crop management (Dobermann et al 2000). However, DS yields in the LTTCE started to decline again after 1995 and are now below 8 t ha⁻¹ in the highest N treatment. We are still in the process of understanding this recent yield decline. Preliminary observations suggest that a lower climatic yield potential (El Niño–La Niña cycle during 1997 to 1999) and the reduction in the number of insecticide and fungicide sprays may be involved in this. However, it also seems that the changes in crop management introduced since 1991 did not mitigate the actual causes of the yield decline, which appear to be related to gradual changes in the soil properties that govern N mineralization and N uptake by the crop.

In conclusion, stable yields of 5 to 6 t ha⁻¹ are common in LTE and suggest that intensive rice systems are sustainable at this level. It is worrisome, however, that in only a few LTE have researchers been able to sustain rice yields above levels of 7–8 t ha⁻¹ in the DS or 5–6 t ha⁻¹ in the WS, about 80% of the yield potential of the present generation of modern rice varieties grown under subhumid and humid tropical conditions.

Productivity at the farm level

Rice yields on Asian rice farms continue to grow, but at a slower pace. From 1967 to 1984, rice production in Asia grew at an annual rate of 3.2%, mainly because of yield increases (2.5% y⁻¹). However, growth rates declined to 1.5% y⁻¹ (production) and 1.2% y⁻¹ (yield) during the period 1984–96 (Dawe and Dobermann 1999). This slowdown is partly due to lower rice prices and the slowdown in demand growth due to secular trends in population and per capita consumption of rice (Dawe 1998), but FAO has raised concern about resource degradation and declining yield growth in rice-rice and rice-wheat systems of Asia (FAO 1994 a, b, 1997). Confusion has existed, however, about the proper use of terms and there is little clear evidence of a productivity decline in intensively cropped rice systems, primarily because of a paucity of data.

Productivity decline can also be described in terms of a decline in total factor productivity (TFP) with time, where total factor productivity is the productivity of all inputs taken together. A decline in TFP is not the same as a decline in grain yield or grain production, defined as yield production on a regional, national, or global level. It is possible for TFP to decline while production and yield are increasing if certain inputs, such as fertilizer or machinery, and their costs increase substantially to increase production or yield (Dawe and Dobermann 1999). Concern may only arise regarding the sustainability of the cropping system if TFP declines faster than technologies can be improved. Yields will eventually decline, with potentially important consequences for Asia's food supply. It should also be noted that measures such as TFP cannot be used as the sole indicator of the sustainability of a cropping system because socioeconomic changes (labor cost) may override a possible resource degradation.

Farm surveys conducted in the 1980s and 1990s provided some evidence for stagnating or declining productivity, soil fertility, and resource-use efficiency in intensive rice and wheat areas of the Philippines, China, India, Bangladesh, Nepal, and Pakistan (Ali 1996, Byerlee

1992, Byerlee and Siddiq 1994, Cassman and Pingali 1995b, Huang and Rozelle 1995). It was generally thought that declining soil fertility is a major cause of declining productivity and rice farmers in Asia often claim that they need to apply more N to obtain yields similar to those of 10 or 20 years ago.

The basis for drawing such conclusions is weak, however, because all these studies have in common a lack of thoroughly measured *time series of socioeconomic and biophysical data* as well as methodological problems related to estimating TFP. Recently, long-term productivity trends in two rice bowls of the Philippines were reexamined. Examination of secondary data showed that the survey years were unrepresentative of long-term trends. Correction for this removed the productivity decline reported in an earlier analysis and demonstrated that farmers in these areas are operating close to the technical efficiency barrier (Tiongco and Dawe 2000). More detailed information on these issues is expected from IRRI's multinational project on "Reversing Trends of Declining Productivity in Intensive Irrigated Rice Systems," which began in 1994. We currently collect data sets from 205 farm sites in six countries, which will allow a detailed assessment of TFP, efficiency of different production inputs, and changes in soil quality over time.

Irrespective of a productivity decline or not, data collected in recent years demonstrate that farm-level productivity is often suboptimal and varies greatly among farms and with time. Understanding this variation and managing it has become a key strategy for improving intensive rice systems in Asia.

Table 1 provides an overview of the ranges of some agronomic characteristics in intensive rice systems across Asia. At the farm level, yields in this sample varied from 2 to 10 t ha⁻¹ with an average of 5.2 t ha⁻¹. The yield components in this sample suggest that establishing a large sink size under farm conditions is a challenge for many farmers, as demonstrated by a median number of spikelets of 25,800 m⁻² or 67 panicle⁻¹. Only 20% of the farmers were able to harvest yields of more than 6 t ha⁻¹.

Another key issue, also with regard to growing new varieties with increased yield potential, is how to increase nutrient uptake, particularly that of N during the vegetative and early reproductive growth stages. Our farm data suggest that N efficiency has not changed during the past 30 years, despite voluminous research on N loss mechanisms and different forms of N application in rice. The average apparent recovery efficiency of N (RE N, ΔN uptake per unit N applied) is only 26% and the average agronomic efficiency of N (AE N, Δ grain yield per unit N applied) only 10 kg kg⁻¹ (Table 1). Only about 20% of all farmers in this large sample achieved N efficiencies typically measured in well-managed experiments (RE N >50% and AE N >20 kg kg⁻¹). Nitrogen losses from applied fertilizer average about 60%, assuming that about 10–15% of the fertilizer remains in soil organic fractions. In contrast to low N-recovery efficiencies observed in farmers' fields, irrigated rice has a large capacity for taking up topdressed fertilizer N if it is applied to coincide with crop demand. In field experiments at two sites in the Philippines, maximum uptake rates of 9–12 kg N ha⁻¹ d⁻¹ were measured over a period of 4 d following application of 100 kg N ha⁻¹, with recovery efficiencies of topdressed urea-N of 39–55% at midtillering and 74–78% at panicle initiation (Peng and Cassman 1998).

Of great concern are negative K input-output balances. We estimate that about 80% of the intensive rice fields in Asia have a negative K balance, with an average of about -28 kg K ha⁻¹ crop⁻¹ (Table 1). Such K budgets may vary, however, from -100 to +100 kg K ha⁻¹ crop⁻¹, depending on the fertilizer K rate, the source of irrigation water, straw management, and yield level (Dobermann et al 1998). It is only a matter of time until the indigenous K supply becomes a limiting factor for increasing rice yields, even on the most fertile lowland rice soils in Asia.

Table 1. Ranges of grain yield, fertilizer use, NPK uptake, and N-use efficiency in 205 irrigated rice farms in China, India, Indonesia, the Philippines, Thailand, and Vietnam (see Fig. 1 for a description of these sites). The data set includes transplanted and wet-seeded rice fields. Values shown are for two successive rice crops grown in 1995-96 or 1997 (N = 391).

Factor	Minimum	25% quartile	Median	75% quartile	Maximum
Grain yield (GY, t ha ⁻¹)	1.91	4.01	5.17	5.93	9.93
Harvest index	0.27	0.44	0.49	0.52	0.64
No. of panicles per m ²	50	283	416	575	1,136
Total no. of spikelets per m ²	4,864	21,602	25,786	32,276	80,399
Total no. of spikelets per panicle	19	48	67	94	258
Filled spikelets (%)	57	75	82	87	99
Fertilizer N use (FN, kg ha ⁻¹)	31	86	111	138	270
Fertilizer P use (FP, kg ha ⁻¹)	0	11	18	25	51
Fertilizer K use (FK, kg ha ⁻¹)	0	0	17	46	184
N uptake (UN, kg ha ⁻¹)	27	67	84	105	207
P uptake (UP, kg ha ⁻¹)	6	12	16	19	30
K uptake (UK, kg ha ⁻¹)	27	63	80	101	219
Input-output N balance (kg ha ⁻¹ crop ⁻¹)	-74	-12	2	15	50
Input-output P balance (kg ha ⁻¹ crop ⁻¹)	-22	-3	4	14	42
Input-output K balance (kg ha ⁻¹ crop ⁻¹)	-104	-42	-28	-10	127
Partial productivity of N (PFP, kg kg ⁻¹)	17.2	32.3	44.5	58.6	112.0
Agronomic efficiency of N (AE, kg kg ⁻¹)	0.0	4.4	9.6	17.0	51.8
Recovery efficiency of N (RE, kg kg ⁻¹)	0.00	0.14	0.26	0.43	0.91
Internal efficiency of N (IE, kg kg ⁻¹)	35.4	51.2	57.5	68.0	100.2
Physiological efficiency of N (PE, kg kg ⁻¹)	0.0	21.6	35.0	49.3	80.7

PFP = GY/FN, AE = (GY - GY₀)/FN, RE = (UN - UN₀)/FN, IE = GY/UN, PE = (GY - GY₀)/(UN - UN₀), all using GY in kg ha⁻¹. Symbols without subscript refer to measurements made in the farmers' fertilizer practice. Symbols with subscript refer to measurements made in replicated 0 N plots embedded within the farmers' fields. Source: "Reversing Trends of Declining Productivity" project database, unpublished data.

Recent field observations suggest that several intensive rice areas are approaching this state, particularly in direct-seeded rice areas such as Central Luzon (Philippines), the Mekong Delta (Vietnam), and Central Thailand.

In summary, stable yields of 3–5 t ha⁻¹ in the WS and 5–7 t ha⁻¹ in the DS are common in farmers' fields, but yields average only about 60% of the yield potential of the present generation of modern rice varieties grown under subhumid and humid tropical conditions. Closing the present yield gap is a slow process and requires greater fine-tuning of soil and crop management, which often conflicts with the need to reduce labor and other production costs.

Future changes

General scenario

Most recent estimates, taking into account recent gains in yield, are that rice yields in Asia must increase by about 20% from 1998 to 2010 and 30% from 1998 to 2020 (Table 2). Assuming that (1) irrigated rice has a 75% share of global rice production, (2) the irrigated harvest area does not change significantly over time, and (3) yield increases of about 1.1 % y⁻¹ can also be achieved in rainfed lowland and upland systems, average yields of irrigated rice must then rise from about 5.2 t ha⁻¹ in 1998 to roughly 7 t ha⁻¹ in 2020. This is significantly less than estimated a few years ago, when it was assumed that the average yields of irrigated rice would have to increase by 60% from 4.9 t ha⁻¹ in 1991 to 8 t ha⁻¹ in 2025 (Cassman and Pingali 1995a).

These estimates have considerable uncertainty, but even a yield increase of roughly 30% over a period of 22 years remains a serious challenge. For tropical areas, this represents average yields of about 8 t ha⁻¹ in the DS and 6 t ha⁻¹ in the WS, which is equivalent to about 80% of the yield potential of the presently available germplasm and which is perhaps hard to achieve and sustain as an average. Moreover, such a shift also means that about 30% of all farmers must achieve yields of >8 t ha⁻¹ and 15% >9 t ha⁻¹ in at least one crop per year (Fig. 3).

Farmers will have to learn how to grow such high-yielding crops on a sustainable, resource-efficient, profitable, and environmentally friendly basis, but they are also exposed to many

Table 2. Predicted changes in the global harvested area, yield, and production of rice.

Actual^a	1991	1993	1998			
Area (10 ⁶ ha)	147.5	145.4	150.3			
Yield (t ha ⁻¹)	3.50	3.64	3.75			
Production (10 ⁶ t)	515	530	563			
Predicted^b	2010	2020	1998-2010		1998-2020	
			%	% y⁻¹	%	% y⁻¹
Area (10 ⁶ ha)	151.8	150.0	0.5	0.04	-0.7	-0.03
Yield (t ha ⁻¹)	4.43	4.86	17.6	1.36	28.9	1.16
Demand (10 ⁶ t)	673	729	18.2	1.40	28.0	1.13
Yield of irrigated rice (t ha⁻¹)^c	1991	1998	2010	2020		
	4.9	5.2	6.2	7.0		

^aActual production (FAO statistics, paddy). ^bModified IMPACT model projections (paddy, assuming 1 t paddy = 0.67 t milled rice). ^cActual (1991) and estimated average yield of irrigated rice assuming no change in the irrigated harvest area and increases in rice production from other ecosystems equivalent to the average rate of the project demand growth. Source: D. Dawe, IRRI, November 1999.

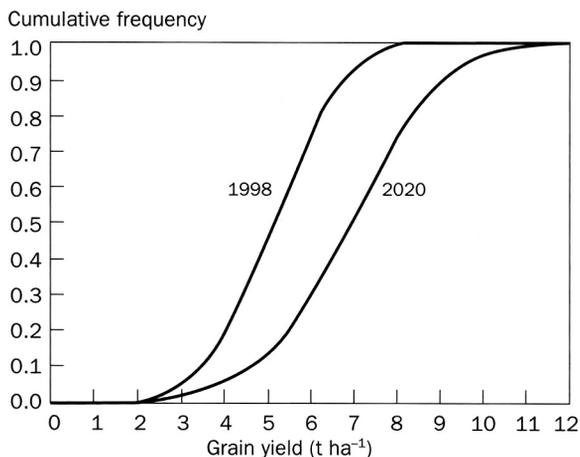


Fig. 3. Hypothetical cumulative frequency distribution of irrigated rice yields in Asia. The curve for 1998 represents a sample of yields measured on 205 rice farms in China, India, Indonesia, Thailand, Vietnam, and the Philippines consisting of two successive harvests on each farm (mean yield = 5.2 t ha⁻¹, CV = 25%). The curve for 2020 assumes an increase in the mean yield to 7.0 t ha⁻¹ and a CV of 25%.

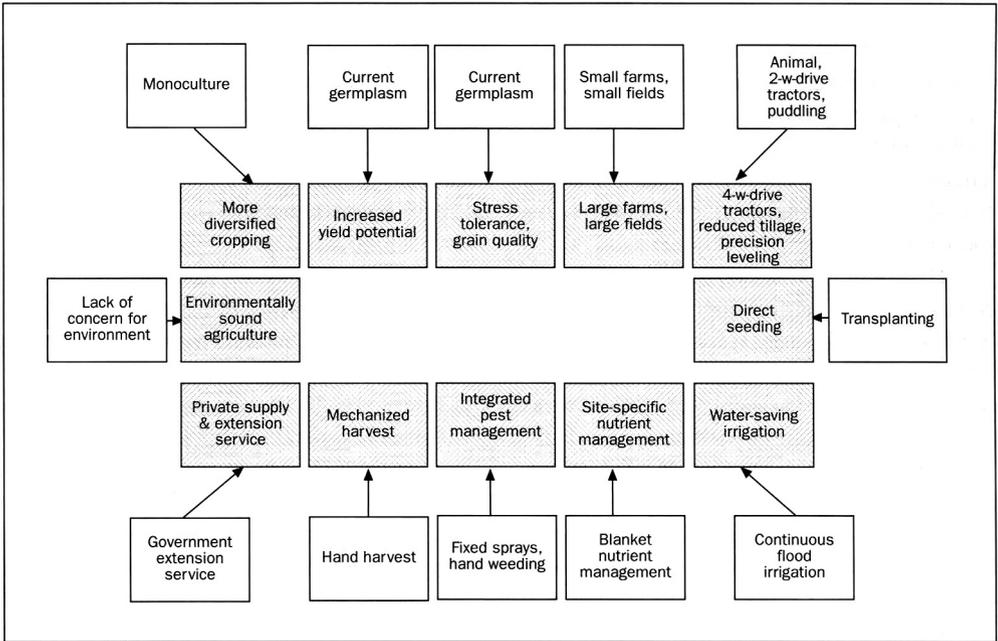


Fig. 4. Possible future changes in crop management of intensive rice systems in Asia. In some areas, some of these changes have already occurred or are in progress.

factors that complicate their actions. Figure 4 is an attempt to illustrate some of the changes in soil and crop management practices that may occur in intensive rice systems during the next two decades. Not all of these changes are expected to occur simultaneously or in all rice-growing regions. Some of them are motivated by the need to increase food production and its efficiency, others are forced by external pressures originating from the nonagricultural sector.

The major scenario is one of technological changes triggered and driven by increasing food demand and labor cost for agriculture, increasing competition for natural resources such as water and land, socioeconomic changes favoring the adoption of mechanized technologies, and increasing pressure to reduce unfavorable environmental impacts. Where feasible, cropping systems will diversify. New germplasm with greater yield potential and improved grain quality will become available, also driven by changing consumer demands of the urban population or the food-processing industry. Locally, we might also see aggregation of fields and farms resulting in further diversification of farm communities. Farm types will range from small subsistence production to larger, highly mechanized commercial farms. Land preparation, crop establishment, and harvest are labor-intensive farm operations in transplanted rice and farmers will increasingly seek ways to reduce costs associated with them. Trends toward direct seeding and mechanization of land preparation and harvest will continue and spread out to more rice-growing areas. Pressure on water resources will lead to various forms of water-saving irrigation. Technologies such as nutrient and pest management are expected to become more knowledge-intensive and site-specific. Mechanized forms of harvest and postharvest operations such as drying will become predominant.

Crop intensification and diversification

Crop intensification by increasing the number of rice crops grown per year continues in parts of Asia, but we expect no significant increase in the total harvest area of irrigated rice. Some gains in the irrigated rice area will be offset by urbanization or crop diversification. Farmers in various parts of tropical Asia, for example, in the Mekong Delta, Vietnam, the Central Plain of Thailand, and Java, Indonesia, started growing three short-duration rice crops per year, but the overall area under triple rice cropping is likely to remain small, perhaps only a few hundred thousand hectares.

Diversifying risk and sources of farm income, changing consumer demands to nonrice products, increasing buying power of the urban population, availability of export markets, and the desire to efficiently spread out farm machinery use are factors that may motivate farmers to shift from rice monoculture to more diverse crop rotations or integrated cereal-livestock systems. Rice monoculture in double- or triple-crop systems, however, will remain the preferred choice in very low-lying areas with heavy clay soils and other soil constraints, for example, in many parts of the Mekong Delta. Experience from rice-wheat systems also suggests that it is often difficult to optimize soil management for both the rice crop and the upland crop in the rotation.

Environmental implications must be considered too. Carbon and N dynamics in intensive rice systems depend largely on the length and frequency of the aerobic and anaerobic phases. Continuous rice double or triple cropping results in significant C and N conservation, even with all aboveground biomass removed from the field (Cassman et al 1998, Sahoo et al 1998). This is different from what occurs in rice-upland crop or pure upland crop systems, where soil organic matter content tends to decline unless large amounts of manure or crop residues are returned to the field. On coarse-textured soils where rice-vegetable cropping is practiced, the excessive use of agrochemicals threatens water quality and human health.

Thus, diversification alone does not necessarily solve the problems associated with intensification in irrigated rice lowlands, although it can be part of an overall solution. An aerated phase long enough to grow an upland crop is justified if the total productivity is maintained or even enhanced, and the quality of the resource base is not adversely affected. Except for a few case studies, no attempt has been made to assess the biophysical and socioeconomic potential for crop diversification in Asia's irrigated lowland rice areas.

Tillage, crop establishment, and water management

The lack of labor and water and the increasing availability of 4-wheel-drive tractors and herbicides will cause changes in soil tillage, crop establishment practices, and water use. Broadcast seeding of presoaked seed has already become widespread in irrigated rice areas with high labor cost or insufficient labor availability, such as southern Vietnam, Thailand, parts of the Philippines, and Malaysia. Further adoption of and changes in this technology will take place. We will probably see expansion to areas where transplanting currently predominates (e.g., Indonesia, India), some more development of row-seeding techniques to reduce high seed rates, and a move toward dry seeding of rice driven by the need to reduce water consumption.

The increasing competition for water among agriculture, industry, and the urban population will force many rice farmers to use less water and increase their water-use efficiency. More than 80% of the developed freshwater resources in Asia are used for irrigation purposes and more than 90% of the total irrigation water is used for rice production. In 2025, per capita available water resources in this area are expected to decline by 15–54% compared with 1990, but agriculture's share of water will diminish at an even faster rate (Guerra et al 1998). The potential for water savings in rice production appears to be large. Examples include reduced

water depth because of better land leveling in direct-sown rice or periodical flush irrigation instead of a continuously flooded field. However, little is known about the degree to which various farm and system interventions can lead to sustainable water savings in the water basin and downstream impacts. Studies on the economic benefits and costs of alternative interventions are also lacking. Although water-saving irrigation may lead to increased water-use efficiency, such practices require a high degree of management and infrastructure at both the farm and system levels and are often associated with increased herbicide use and larger N losses (Guerra et al 1998). Understanding these interactions as a basis for developing technologies that offer the best compromise is one of the most challenging current research areas.

Farm mechanization may also offer new opportunities for manipulating soil organic matter cycling in intensive rice systems. The widespread adoption of combine harvesting will lead to an increase in the amount of straw available as a potential source of organic matter and nutrients. Larger field and farm sizes require more powerful machinery so that the increased availability of 4-wheel tractors will allow dry tillage and crop residue incorporation during the fallow period. Both measures will affect short- and long-term C and N turnover. Compared with the traditional method of wet incorporation of crop residues shortly before planting of the next rice crop, potential beneficial effects of an earlier, dry incorporation include (1) accelerated aerobic decomposition of crop residues leading to increased N availability (Witt et al 1998) and reduced CH₄ emission (R. Wassmann, IRRI, unpublished data), (2) reoxidation of ferrous iron and other reduced substances leading to increased P availability, (3) reduced weed growth, (4) savings in irrigation water during land soaking (Tuong et al 1996), and (5) greater phenol degradation leading to improved qualitative composition of soil organic matter (Olk and Senesi 2000).

We may also see more attempts to replace cost- and time-intensive tillage operations by minimum or even zero tillage for at least one crop per year. The long-term consequences of minimum (zero) tillage for soil quality in intensive rice systems compared with a system with plowing and puddling are largely unknown.

Dynamic nutrient and pest management

With yield approaching potential yield, the demand for nutrients will increase. A balanced supply of all major nutrients becomes increasingly important. Two key issues are (1) What are the nutrient requirements for high-yielding crops? and (2) What are practical nutrient management technologies for yields of 70–80% of the yield potential?

Literature estimates of total nutrient removal per ton of rice grain range from 15 to 24 kg N, from 2 to 11 kg P, and from 16 to 50 kg K (Witt et al 1999) so that the use of single “rule-of-thumb” numbers offers little promise for fine-tuning nutrient management. The internal efficiencies of nutrients such as N, P, and K (IE, kg grain kg⁻¹ nutrient in aboveground plant dry matter) vary greatly depending on the variety, nutrient supply, crop management, and climatic yield potential. Figure 5 illustrates this variation for N. Two borderlines describing the minimum (YNA = maximum accumulation of N in the plant) and maximum (YND = maximum dilution of N in the plant) internal efficiency of N were estimated at 42 and 96 kg grain kg⁻¹ N. In other words, under field conditions, a plant N accumulation of 80 kg N ha⁻¹ can be associated with a yield ranging from 3.4 to 7.7 t ha⁻¹. However, Figure 5 also shows no data points close to the YND line above yields of 8 t ha⁻¹, suggesting that internal nutrient efficiencies are not linearly related to grain yield.

We currently use the empirical model QUEFTS (Janssen et al 1990) for estimating NPK requirements of rice in our most advanced fertilizer recommendation model, which accounts for different yields and for interactions among macronutrients affecting the IE of N, P, and K

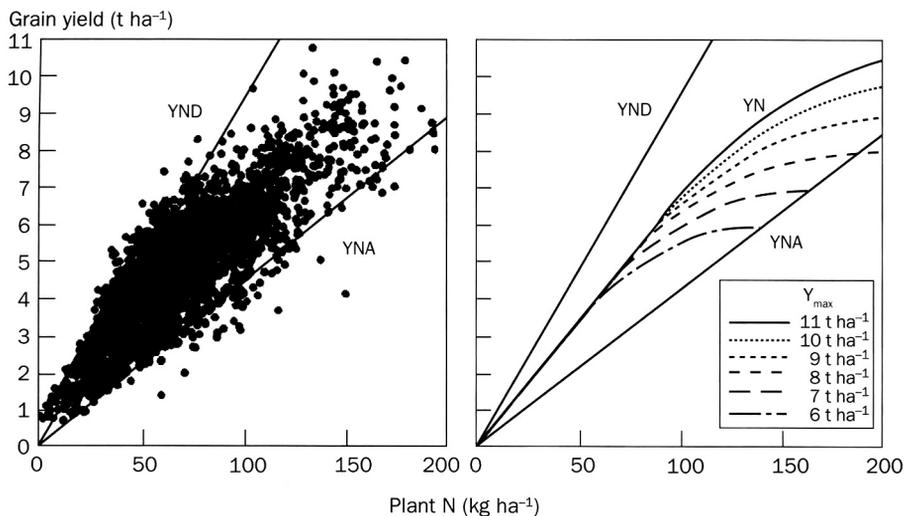


Fig. 5. Relationship between grain yield and accumulation of N in total aboveground plant dry matter at maturity of rice. The line to the left of each figure represents the boundary of maximum dilution of N (YND), whereas the line to the right indicates the boundary of maximum accumulation of N (YNA). The optimal N requirements for a balanced NPK nutrition (YN) were calculated for different yield potential (Y_{\max}) using the QUEFTS model (modified from Witt et al 1999).

(Witt et al 1999). On the condition that plant growth is limited only by nutrient supply, the model assumes a linear increase in grain yield if nutrients are taken up in balanced amounts until yield targets reach about 70% of the climate-adjusted potential yield. Optimal IEs for a balanced nutrition in this yield range are 68 kg grain kg^{-1} N, 385 kg grain kg^{-1} P, and 69 kg grain kg^{-1} K. The model predicts a nonlinear decrease in IEs when yields approach the ceiling (Table 3, Fig. 5). The nutrient requirements of rice as defined by the modified QUEFTS model were much lower than the values commonly used in the literature. Perhaps the major reason is that previous estimates were often derived from experiments conducted on research stations with a high nutrient supply and at yields that are not economical for farmers (Witt et al 1999).

Many questions exist on the nutrient requirements for breaking the yield ceiling. We do not have enough empirical data for yields above 9 t ha^{-1} to use the approach shown in Figure 5. However, a theoretical analysis following the concept of a critical N dilution curve in plants has shown that superior germplasm must be capable of accumulating 144 kg N ha^{-1} in the first 35 to 45 d following transplanting for yields aiming at breaking the current yield ceiling of 10 t ha^{-1} in the tropics (Sheehy et al 1998). How can this be implemented under tropical field conditions? What are the P and K requirements for such yields? The breeding potential for C_3 rice suggests a yield potential of 12–13 t ha^{-1} . To estimate nutrient requirements, can we assume that the same empirical model as used in Figure 5 holds, that is, that the linear range of the relationship between grain yield and plant nutrient accumulation will simply extend further with an increase in yield potential? Is there any potential to breed for germplasm with greater IE of N, P, or K to shift the slope upward, which would offer potential to reduce fertilizer requirements?

Farmers do not aim for yields approaching the ceiling, but increasing farm yields requires increasing nutrient uptake and N-use efficiency. Nitrogen management strategies of high-yielding crops ($>8 \text{ t ha}^{-1}$) differ from those developed for moderate yields (5–6 ha^{-1}) and we

Table 3. Balanced uptake requirements, internal efficiencies (kg grain kg⁻¹ nutrient), and reciprocal internal efficiencies (kg nutrient 1,000 kg⁻¹ grain) of N, P, and K for irrigated lowland rice as calculated by QUEFTS to achieve certain grain yield targets. The grain yield potential was set to 10 t ha⁻¹ (Witt et al 1999).

Yield (t ha ⁻¹)	Required nutrient uptake			Internal efficiency			Reciprocal Internal efficiency		
	N	P	K	N	P	K	N	P	K
	(kg ha ⁻¹)			(kg kg ⁻¹)			(kg 1,000 kg ⁻¹)		
1	15	2.6	15	68	385	69	14.7	2.6	14.5
2	29	5.2	29	68	385	69	14.7	2.6	14.5
3	44	7.8	43	68	385	69	14.7	2.6	14.5
4	59	10.4	58	68	385	69	14.7	2.6	14.5
5	73	13.0	72	68	385	69	14.7	2.6	14.5
6	88	15.6	87	68	385	69	14.7	2.6	14.5
7	104	18.4	103	67	380	68	14.8	2.6	14.7
8	127	22.6	126	63	354	64	15.9	2.8	15.7
9	159	28.2	157	57	319	57	17.6	3.1	17.4
9.5	182	32.2	180	52	295	53	19.2	3.4	19.0
10	243	43.1	240	41	232	42	24.3	4.3	24.1

still have to gain more understanding of this. Increasing nutrient uptake and N-use efficiency at the farm level will require fine-tuning of fertilizer management that goes beyond the blanket recommendations associated with the Green Revolution period or the empirical acquisition of knowledge by farmers. Currently, rice farmers have limited means for adjusting fertilizer rates according to the potential nutrient supply from indigenous sources (soil, water, atmosphere) or to seasonal climatic fluctuations to avoid incorrect rates of application of one or more nutrients.

Real-time N management appears to be necessary for optimizing N nutrition at high yields. We have made considerable progress in using plant-based technologies such as the chlorophyll meter, a simple leaf color chart, or strategies based on tiller counts at critical growth stages (Balasubramaniam et al 1999, Peng et al 1996). Recently, we have proposed an integrated nutrient management strategy, which focuses on managing spatial and temporal variability in nutrient supply in a dynamic, site-specific manner (Dobermann and White 1999, Witt et al 1999, Witt and Dobermann 2000). This concept for site-specific nutrient management (SSNM) combines (1) farm- or field-specific estimates of the potential indigenous nutrient supply, (2) the QUEFTS model for estimating crop nutrient requirements, (3) tools and rules for plant-based adjustment of N rates at critical growth stages, and (4) a model for estimating changes in the soil P and K supply over time as a function of the nutrient balance.

Field testing of this SSNM is ongoing on 205 rice farms in Asia. Currently available data sets indicate increases in grain yield and profit compared with the farmers' practice in about 80% of all cases. Yield increases average about 10%, but can be as large as 15–20% on the best farms where overall crop management is optimal. Particularly encouraging are the large increases in N-use efficiency achieved with this approach. Over a period of 2 y, the average agronomic N efficiency increased from 10 to 15 kg grain kg⁻¹ N applied and the recovery efficiency of applied N increased from 26% to 41%. On 25% of all farms, yields exceeded 6.5 t ha⁻¹, agronomic N efficiency was >20 kg kg⁻¹, and the recovery efficiency of N was >55%. Realistic long-term goals for the SSNM approach appear to be yields of 7–8 t ha⁻¹ in the DS and 5–6 t ha⁻¹ in the WS, an average agronomic N efficiency of 20 kg kg⁻¹ and a recovery efficiency of applied N of 50%.

Table 4 illustrates how such improvements in nutrient management could help to achieve the yield increases of irrigated rice needed by 2020. In the first scenario, we assume that no major improvements in the yield potential can be achieved. In this case, achieving average farm yields of 7 t ha⁻¹ by 2020 would require fertilizer N rates of 236 kg N ha⁻¹ at the present level of N efficiency, but only 127 kg N ha⁻¹ at levels that are feasible with a widespread adoption of a more knowledge-intensive N management such as the SSNM proposed. In the second scenario, we assume that present work on developing new germplasm such as hybrids or the new plant type will be successful so that varieties with a 20% higher yield potential will be widely adopted by farmers within the next 20 years. We also assume that, because of their stronger root system, such varieties extract slightly more indigenous N from the soil and that the greater yield potential slightly increases the internal efficiency of N in the plant. As a result, average yields of 7 t ha⁻¹ would require about 200 kg N ha⁻¹ with no increase in N efficiency, but only 110 kg N ha⁻¹ from fertilizer if the recovery efficiency can be raised to about 50%. Therefore, future yield increases at the farm level should be based on a combination of increasing the yield potential and increasing the efficiency of fertilizer N to avoid risks associated with very large N applications. This is different from the past 30 years, during

Table 4. Hypothetical scenarios for increasing average irrigated rice yields at the farm level to sustain sufficient rice supply. Scenario 1 shows how the yield increases projected until 2020 (Table 2) can be achieved with the presently available rice germplasm by increasing the N rate or N-use efficiency. Scenario 2 shows the combined effect of adoption of new germplasm with a 20% higher yield potential and increasing N-use efficiency.

	No increase in N-use efficiency			Increase in N-use efficiency	
	1998 ^a	2010	2020	2010	2020
Grain yield (GY, t ha ⁻¹)	5.2	6.2	7.0	6.2	7.0
Grain yield with no N applied (GY ₀ , t ha ⁻¹)	4.1	4.1	4.1	4.1	4.1
Indigenous N supply (INS, kg N ha ⁻¹)	55	55	55	55	55
Plant N uptake (UN, kg ha ⁻¹)	85	103	119	103	119
Recovery efficiency of N (RE, kg kg ⁻¹)	0.27	0.27	0.27	0.40	0.50
Fertilizer N required (FN, kg N ha ⁻¹)	110	179	236	121	127
Internal efficiency of N (IE, kg kg ⁻¹)	61	60	59	60	59
Agronomic efficiency of N (AE, kg kg ⁻¹)	9.9	11.7	12.3	17.4	22.8

	No increase in N-use efficiency			Increase in N-use efficiency	
	1998	2010	2020	2010	2020
Grain yield (GY, t ha ⁻¹)	5.2	6.2	7.0	6.2	7.0
Grain yield with no N applied (GY ₀ , t ha ⁻¹)	4.1	4.5	4.5	4.5	4.5
Indigenous N supply (INS, kg N ha ⁻¹)	55	60	60	60	60
Plant N uptake (UN, kg ha ⁻¹)	85	100	115	100	115
Recovery efficiency of N (RE, kg kg ⁻¹)	0.27	0.27	0.27	0.40	0.50
Fertilizer N required (FN, kg N ha ⁻¹)	110	148	203	100	110
Internal efficiency of N (IE, kg kg ⁻¹)	61	62	61	62	61
Agronomic efficiency of N (AE, kg kg ⁻¹)	9.9	11.5	12.3	17.0	22.8

^a1998: current situation. Estimates of farm averages are based on a sample of 205 rice farms in six countries of Asia sampled in 1998. ^bSee Table 1 for definition of N-use efficiencies.

which yield increases were mainly based on new germplasm, irrigation, and increasing the N rate, with little or no improvement in N-use efficiency at the farm level.

Farmers' yields can only rise if crops can be protected from pests. Much progress has been made in characterizing rice pests (Savary and Willocquet 1999) and developing integrated pest management (IPM) for rice (Heong et al 1995). The current IPM approach emphasizes improving host-plant resistance and farmers' knowledge as a basis for changing their perceptions about rice pests, particularly insect pests (Heong et al 1998). However, we do not have a tested IPM concept for high yields of $>8 \text{ t ha}^{-1}$, which is one of the research challenges currently being addressed. This particularly refers to disease control in very dense canopies with a tropical microclimate favoring pathogens such as sheath blight. Other issues are management of weeds in direct-seeded rice, pest resistance, and the question whether the IPM guidelines developed for insect control at moderate yields can also be applied to managing a high-yield crop, during both the vegetative and reproductive growth stages.

Environmentally sound rice farming

Reducing greenhouse gas emissions and preserving surface water and groundwater quality are becoming increasingly important targets of agricultural production. Legislation may impose restrictions on straw burning, total N input-output budgets at the farm level, crop management practices related to nitrate accumulation and leaching, and pesticide use.

Irrigated rice fields are important global sinks of carbon and sources of greenhouse gas emissions. Wetland rice has contributed to the increase in atmospheric methane (CH_4) concentration, which has more than doubled during the past 200 years and continues to rise at a rate of $1\% \text{ y}^{-1}$ (Neue 1993). Recent estimates suggest a global methane emission from flooded rice fields of $40\text{-}50 \text{ Tg CH}_4 \text{ y}^{-1}$ (R. Wassmann, IRRI, personal communication). If intensification is associated with increasing the number of rice crops per year and greater straw recycling, adhering to CH_4 emission quotas might increasingly affect rice production practices. Possible mitigation options for reducing methane emissions from rice fields include reduced length of flooding, temporary drainage, management of organic residues, changes in the kind and application mode of mineral fertilizers, diversification of rice-based cropping systems, and selection of varieties with reduced emission potential (Neue 1993). The objective of reducing methane emissions must be combined with improvements in soil and crop management strategies to achieve a high acceptance.

Nitrous oxide emissions from rice fields occur as a result of nitrification-denitrification during periods of alternating wetting and drying. Emissions are usually small in irrigated rice systems with good water control and small to moderate inputs of fresh organic material (Bronson et al 1997a,b). If intensification of rice agriculture involves more crop diversification, N_2O emissions and NO_3^- leaching may become greater problems. Nitrate leaching and contamination of groundwater are well-known problems in rainfed rice areas or rice-upland crop systems on coarse-textured soils (Buresh et al 1989). In heavy-textured lowland soils with poor drainage, N leaching losses are usually below 10% of the applied fertilizer N (Koyama 1981), but more research is needed to study the importance of leaching in intensive rice systems.

Because of enhanced decomposition of pesticide residues in tropical flooded rice soil, small amounts of pesticides applied at recommended rates and intervals do not persist beyond the crop growing period and rarely have a detrimental effect on microbial populations or their activities (Roger 1996). The available information is insufficient, however, on possible detrimental effects of pesticides on microorganisms and water quality, particularly in areas with heavy pesticide use.

Conclusions

Farmers in many irrigated rice areas are operating at moderate to high yields, but there is still some scope for increasing rice yields through improved technologies for soil and crop management. Increasing rice production by reducing the gap between farmers with low and high yields requires managing variability, with greater demand for knowledge and precision. However, future incremental yield increases and increases in technical efficiencies will be small and will require new germplasm with increased yield potential, high grain quality, and multiple resistance to pests.

By 2010, average irrigated rice yields must rise by about 20% to at least 6 t ha⁻¹, that is, DS yields of 7 t ha⁻¹ and WS yields of 5 t ha⁻¹. This appears feasible with the current generation of modern rice varieties, provided that (1) the harvest area of irrigated rice remains constant and (2) improvements in crop management can be implemented on the majority of rice farms. Nitrogen-use efficiency must rise to average levels of AE N > 15 kg grain kg⁻¹ N and RE N > 40%, mainly by more knowledge-intensive, dynamic forms of season- and location-specific nutrient and pest management.

By 2020, average irrigated rice yields must rise by about 30% to at least 7 t ha⁻¹, that is, DS yields of 8 t ha⁻¹ and WS yields of 6 t ha⁻¹. This will be feasible if (1) the harvest area of irrigated rice remains constant, (2) the yield potential of C₃ rice can be increased to about 11–12 t ha⁻¹ in the DS and 8–9 t ha⁻¹ in the WS, (3) water does not become a yield-limiting factor, and (4) precise crop management enables average farmers to achieve about 65–70% of the potential yield on a sustainable and economical basis. The more precise crop management technologies must be water-saving, labor-efficient, environmentally friendly, and attractive to farmers. Nitrogen-use efficiency must rise to average levels of AE N > 30 kg grain kg⁻¹ N and RE N > 50%. New approaches for IPM at high levels will be required to minimize yield losses from pests.

Although it appears feasible to produce enough rice by 2020 through the ongoing improvement of C₃-photosynthesis rice in combination with more precise soil and crop management, there is no reason to slow down investments in research. Improved C₃ germplasm must enter farmers' fields within the next 10 years. Widespread adoption of more efficient water, nutrient, and pest management technologies remains a much more difficult task than promoting the adoption of new germplasm. Failure to improve crop management at the farm level will increase the pressure to breed new germplasm with higher yield potential. Because further yield increases will probably require germplasm exceeding the limits of C₃ rice to continue feeding Asia beyond 2020, strategic investments in this area should be made in the near future.

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Rate-limiting steps in nitrogen acquisition by rice in flooded soil

G.J.D. Kirk

Rice can absorb fertilizer N rapidly from rice-field floodwater, but much N is lost from the floodwater by gaseous emission. Uptake of N from the soil, on the other hand, is often limited by root uptake properties. Modeling and experimental work have shown that rates of N transport through the flooded soil to root surfaces will generally not be limiting. Using the short-lived tracer ^{13}N , measurements have been made of the kinetics of N absorption and assimilation by rice and their regulation, and of interactions between NH_4^+ and NO_3^- uptake. Rice roots are particularly efficient in absorbing and assimilating NO_3^- and NH_4^+ absorption and assimilation are stimulated by NO_3^- . Calculations indicate that the uptake of NO_3^- formed in the rice rhizosphere by root-released O_2 may be more important than previously thought, with beneficial consequences for overall N efficiency. In some soils, root-induced acidification of the rhizosphere resulting from excess absorption of cations over anions by roots, and from oxidation of mobile reductants, can impede access of NH_4^+ to roots by depressing HCO_3^- . Absorption of NH_4^+ is also sensitive to inhibition by organic acids in the soil solution formed under strongly reducing conditions. Implications of C_4 photosynthesis for rice N nutrition are discussed.

Plant growth and yield depend on the acquisition not only of carbohydrates through photosynthesis but also of mineral elements through absorption and assimilation, and complex interactions occur between the sources of carbohydrates and mineral elements via the plant as their common sink. The mineral nutrition of the crop is therefore an essential consideration in redesigning rice photosynthesis. Of the mineral elements, nitrogen is the most important. It is required in large amounts—several percent of dry matter (Peng, this volume)—and limits grain yield and quality when in short supply.

Rice crops in flooded soil absorb nitrogen from both the floodwater and the soil. Absorption of N fertilizer broadcast into the rice-field floodwater is very rapid if the fertilizer application is timed carefully to match the plant's demand. However, the N that is not absorbed rapidly is lost by gaseous emission. Consequently, N fertilizer tends to be used very inefficiently and the average recovery in irrigated rice across Asia is less than 30% (IRRI 1998). The rapid uptake of N from the floodwater is due to surface roots in the floodwater and in the soil near the floodwater. These roots differ morphologically and physiologically from roots in the anoxic soil bulk. The dynamics of N in the soil bulk, where NH_4^+ is the principal form of plant-available N, is very different from that in the floodwater and soil surface. Figure 1 shows the main processes governing N dynamics in flooded soils. The work described here was carried out to discover if there were possibilities to improve the acquisition of N from the soil, whether by breeding or agronomic management.

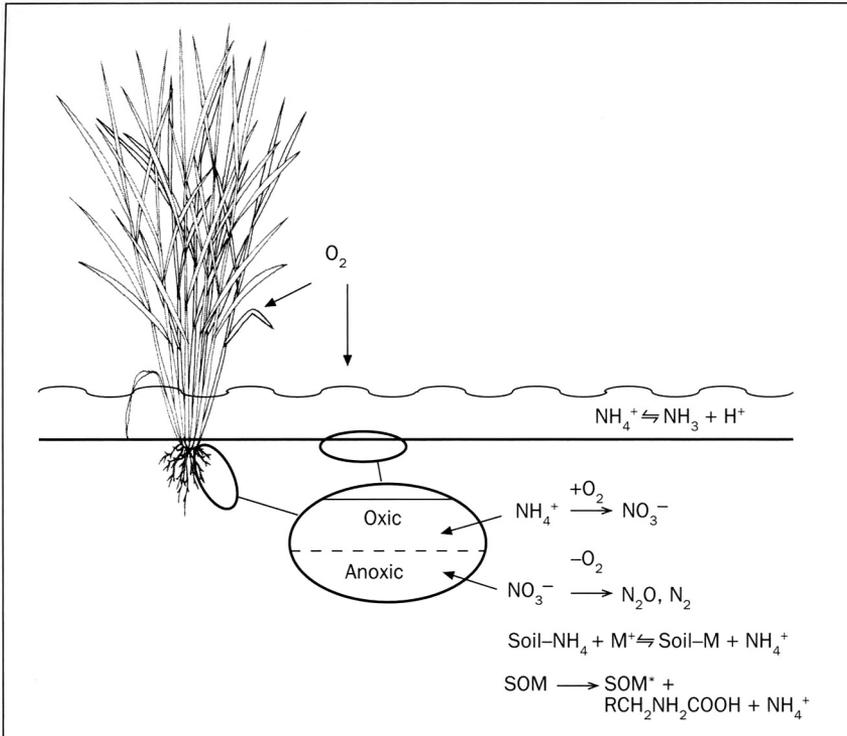


Fig. 1. Nitrogen transformations in flooded rice soils. Soil- NH_4 , Soil-M = NH_4^+ , cation M^+ adsorbed on soil solid, and SOM = soil organic matter.

Importance of root properties versus N transport through the soil

Kirk and Solivas (1997) developed a mathematical model with which to calculate how far rates of N acquisition by rice growing in flooded soil were limited by root uptake properties compared with N transport through the soil to the absorbing roots, and they used the model to analyze experimental results. The calculation was based on

- (1) all the N being absorbed as NH_4^+ , which is in general the main form of plant-available N in flooded soils;
- (2) the rate of uptake per unit root length for a given concentration of NH_4^+ at the root surface being maximal, as indicated by a Michaelis-Menten relation derived from measurements with plants grown hydroponically under moderately N-deficient conditions (Kronzucker et al 1998a);
- (3) the average concentrations of NH_4^+ in the soil solution at different times, which were measured;
- (4) the diffusion coefficient of NH_4^+ in the soil, which was also measured.

The calculations showed that for uptake of N mixed in the soil it would have been necessary for the whole of the measured root length to have been active in uptake to achieve the measured uptake rates.

This is somewhat surprising. In upland crops, the total root length is generally far larger than necessary to account for N uptake. A difference between flooded and upland soils is that the concentrations of N as NH_4^+ in the soil solution of flooded soils are much smaller than

those as NO_3^- in the soil solution of upland soils. In most aerobic arable soils, little NO_3^- is adsorbed on soil particles, whereas in flooded rice soils a large part of added NH_4^+ is adsorbed and may be slowly immobilized within clay lattices. Consequently, the concentration of NH_4^+ in the soil solution will be one to two orders of magnitude smaller than that of NO_3^- for an equal quantity of N, and will approach K_m values for root transport systems. Thus, uptake per unit root length will tend to be smaller. Nonetheless, Kirk and Solivas's calculations indicate an unlikely lack of margin for error in the root length.

A possible explanation is that species other than NH_4^+ , such as NO_3^- and amino acids, are also being absorbed. Uptake of NO_3^- by rice is discussed below. Transporters for amino acids have been found in plant roots, and concentrations of amino acids in the soil solution in flooded soils can be appreciable. To assess the contributions of different N species to N uptake, it is necessary to know the kinetics of absorption into roots as well as rates of delivery of the different N species through the soil to root surfaces.

Uptake of N species other than ammonium

Kronzucker et al (1998a,b, 1999, 2000) have studied the kinetics and regulation of NH_4^+ and NO_3^- absorption by rice roots in hydroponic cultures using the short-lived isotope ^{13}N . They found that the lowland rice cultivar used in their experiments, IR72, adapted to flooded-soil conditions, was exceptionally efficient in absorbing NO_3^- . This raises the possibility that rice growing in flooded soil may absorb significant amounts of NO_3^- formed by nitrification of NH_4^+ in the rhizosphere where oxygen diffuses out of the roots into the soil. This is important because (1) this N is otherwise lost through denitrification in the soil bulk, and (2) plant growth and yield are generally improved when plants absorb their nitrogen as a mixture of NO_3^- and NH_4^+ compared with either on its own.

Three lines of evidence point to unusually efficient NO_3^- absorption. First, the steady-state influx of NO_3^- and NH_4^+ followed Michaelis–Menten kinetics over the relevant concentration range, and V_{\max} for NO_3^- was some 30% larger than that for NH_4^+ and K_m 50% smaller. Second, induction of the root NO_3^- transporters following resupply to plants deprived of NO_3^- for 24 h was exceptionally rapid, peaking within 2 h. For comparison, in white spruce, which is not well adapted to NO_3^- , full induction takes several days, and, in barley, which is considered one of the most efficient NO_3^- users, full induction takes up to 23 h. Third, subcellular pool sizes and fluxes estimated from the kinetics of ^{13}N efflux out of labeled roots indicated highly efficient NO_3^- use. While similar proportions of incoming NH_4^+ and NO_3^- were channelled into assimilation and to the vacuole, the proportion of NO_3^- translocated to the shoot was larger and that lost through efflux out of roots smaller.

When NO_3^- and NH_4^+ were provided together in the nutrient solution at the same total N concentration (100 μM , i.e., $[\text{NO}_3^-] = [\text{NH}_4^+] = 50 \mu\text{M}$), NO_3^- influx, accumulation, and metabolism were repressed. However, plasma membrane fluxes of NH_4^+ , cytosolic NH_4^+ accumulation, and NH_4^+ assimilation were larger than with solely NH_4^+ at 100 μM , and NH_4^+ efflux was smaller. Because very little free NH_4^+ is translocated to the shoot, enhanced translocation of ^{13}N derived from $^{13}\text{NH}_4^+$ in the presence of NO_3^- indicates that NH_4^+ assimilation was stimulated by NO_3^- . As a result, net N acquisition and translocation to the shoot were much larger than when NO_3^- or NH_4^+ was provided alone.

Based on the above observations, Kirk and Kronzucker (2000) developed a model to assess how far rice growing in flooded soil can absorb NO_3^- formed in the rhizosphere from root-released O_2 and the consequences for the efficiency of N uptake. The model allows for the following processes:

1. Transport of O₂ away from a root and its consumption in microbial processes—in addition to nitrification—and nonmicrobial processes, especially reaction with mobile reductants such as ferrous iron.
2. Transport of ammonium toward the root and its consumption in nitrification and uptake at the root surface.
3. Transport of nitrate formed from ammonium toward the root and its consumption in denitrification and uptake by the root.

With realistic flooded rice-soil conditions, the model shows that substantial quantities of N may be absorbed as NO₃⁻—on the order of 30% of total N uptake.

The calculations are sensitive to the input parameters governing O₂ release from the root. This in turn is sensitive to both the rate of delivery of O₃ through the root and the rate of consumption in the external medium. The maximum nitrification rate is some function of the maximum overall rate of microbial O₂ consumption, both being functions of the supply of oxidizable organic substrates. The maximum rate of denitrification will also depend on the supply of organic substrates and will be related to the maximum rate of microbial O₂ consumption. Thus, the rates of nitrification and denitrification are very sensitive to reducing conditions in the soil as particularly influenced by water management and organic matter management.

The assumption that only uptake of ammonium is important in flooded rice is therefore no longer tenable. Research is needed to link processes in the plant governing N absorption and assimilation to processes in the soil governing the delivery of N species to root surfaces under field conditions. A more quantitative understanding is needed of how the dynamics of N species in the soil solution—NH₄⁺, NO₃⁻, and amino acids—is affected by microbial and nonmicrobial processes over the range of conditions under which rice is grown. Simple methods are also needed to measure the different N species absorbed by roots under field conditions, for example, based on plant N transporters, which are becoming increasingly well characterized at the molecular level.

Other effects of soil conditions

Conditions in the soil near rice roots are in some circumstances very different from those in the anoxic soil bulk. Rice absorbing nitrogen as ammonium will take up a considerable excess of cations (NH₄⁺, K⁺, Ca²⁺, Mg²⁺) over anions (H₂PO₄⁻, SO₄²⁻, Cl⁻) and will balance this excess by exporting protons from the roots, tending to acidify the rhizosphere soil. If nitrogen is absorbed as NO₃⁻ rather than NH₄⁺, the acid-base change in the rhizosphere will be the same because, although the root exports 2 mol less H⁺ for each mol of NO₃⁻ replacing a mol of NH₄⁺, 2 mol of H⁺ are formed in the nitrification of each mol of NH₄⁺. Rice also absorbs large quantities of silicon, shoot contents typically exceeding 5%; but because Si is present in soil solutions almost entirely as Si(OH)₄ (pK₁ = 9.46), it is probable that the bulk of the Si is absorbed as the uncharged Si(OH)₄ molecule. Protons are also produced in the oxidation of other mobile reductants in the rhizosphere, such as ferrous iron: $4 \text{ Fe}^{2+} + \text{O}_2 + 10 \text{ H}_2\text{O} \rightarrow 4 \text{ Fe(OH)}_3 + 8 \text{ H}^+$.

As a result of these processes, the pH in the rhizosphere can be as much as 2 units lower than that in the soil bulk (Begg et al 1994, Kirk and Bajita 1995, Saleque and Kirk 1995). However, in most fertile lowland rice soils, the movement of acidity away from the root is fast because of the high soil water content and hence high solute diffusion coefficients, and because

of the neutral soil pH and high CO_2 pressure and hence high concentration of HCO_3^- . Consequently, the pH change at the root surface will tend to be small, probably < 0.5 units. Where large pH changes arise, for example, in sandy soils with small pH buffer powers or soils with large Fe^{2+} concentrations and therefore high rates of acidity generation in the rhizosphere, there may be impaired movement of NH_4^+ and other nutrient cations to root surfaces. This is because the main anion balancing cations in the soil solution in anaerobic soils is generally HCO_3^- , and the concentration of HCO_3^- will fall as the pH falls below neutral, so that the concentration of NH_4^+ and other cations in solution and hence their mobility must also fall. Root-induced changes in the rhizosphere will also affect microbial processes.

Root function may also be impaired by toxins generated in anaerobic soil respiration, such as organic acids. Continuing experiments at IRRI on the effects of long-term intensive irrigated rice production on soil conditions and rice growth have shown a long-term change in soil conditions—evidently associated with more strongly reducing conditions—which has deleterious effects on rice growth (Dobermann and Witt 2000). The change manifests itself as impaired uptake of soil N and can be offset by more careful timing of N fertilizer additions. Older cultivars, such as IR8, are apparently more susceptible to the effects of the changed conditions than modern cultivars, such as IR72 (S. Peng, unpublished results). We are currently testing a hypothesis that the impaired N uptake is due to larger concentrations of organic acids in the soil solution formed under the more strongly reducing conditions, and that modern cultivars bred on the IRRI farm are better adapted to the larger concentrations. Initial results show that NH_4^+ uptake by IR8 is far more susceptible to inhibition by organic acids than IR72. Inhibition of NH_4^+ uptake by IR8 grown in nutrient cultures was several times that of IR72 with concentrations of ferulic acid $< 100 \mu\text{M}$. Total concentrations of water-soluble phenolic acids in the long-term experiments at the IRRI farm are in the range of 100–2,000 μM , and are somewhat larger in rice-rice-rice than rice-rice experiments, and at the IRRI farm in general compared with less wet environments.

Implications of C_4 photosynthesis for rice N nutrition

Plants with C_4 photosynthesis have inherently smaller N contents than C_3 plants, implying a smaller N uptake requirement. However, a corollary of this is that they may contain insufficient stored N in their tissues during the crop grain-filling stages—when absorption of new N is in decline—to support grain production. Sheehy (this volume) calculates that a C_4 rice plant with sufficient photosynthetic capacity to produce 15 t ha^{-1} of grain would contain only enough N to produce 0.8% N in the grain, whereas a typical high-yielding rice has 1.4% N in the grain. The shortfall is some 90 kg N ha^{-1} . An implication is that in C_4 rice it would be critical to improve N storage or N absorption during grain filling, or both. Nitrogen absorption tends to decline during the reproductive stages as soil N is depleted and the root system senesces. Keeping the root system operating would require attention to the processes limiting soil N availability described above. Maintaining an adequate oxygen supply to the roots as the root system degrades and the soil sink for O_2 increases is likely to be a critical aspect.

Alternatively, C_4 rice plants may find a role in rainfed environments where water deficit often limits production and the lower water requirement associated with C_4 photosynthesis would be valuable (Sheehy, this volume). Because very high yields are not sought, the inherently low N requirement would be beneficial.

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Reflections

Genetic modification and agriculture

P.L. Mitchell and J.E. Sheehy

Potential benefits of genetically modified (GM) crops include improved food quality such as vitamin or mineral contents, improved crop physiology for higher yields or growth in harsh environments, herbicide resistance to allow better weed control with broad-spectrum herbicides, and pest resistance. Genetic engineering in rice is illustrated by case studies on the dwarfing gene and basmati rice, and on the possibility of producing a rice plant with C_4 photosynthesis. We conclude that the potential benefits of GM crops, especially to poor farmers in developing countries, justify investment in genetic engineering research, which must include full risk assessment. Risk assessments of GM crops must be based on scientific research because science offers objectivity even if not certainty. There is a need for fair comparisons with conventional crops in the context of the farming system. We discuss the public concerns about GM crops in terms of the safety of food, effects on the environment, and the promotion of GM crops by multinational companies that control the technologies or the crops.

Approximately 15 million years before the evolution of humans, at a time when the atmospheric concentration of carbon dioxide was low, several plants mutated: changing the cellular arrangement of their leaves, and their biochemistry. These changes proved to have an evolutionary advantage in hotter climates, probably because of the improved ability of those plants to capture carbon dioxide in photosynthesis. The descendents of those plants, such as maize, have the C_4 photosynthetic pathway. In common with the ancestors of the C_4 plants, rice has the C_3 photosynthetic pathway. Whether rice ever mutated to form C_4 pathways cannot be known: all we can say is that if such mutations occurred they did not confer an obvious evolutionary advantage on rice (Sage, this volume). In agriculture, plants are cultivated in systems invented by humans and some of the properties that made plants successful in evolutionary terms are not necessarily advantageous in the plant communities of agricultural systems. In this workshop, we discussed whether it would be possible to imitate nature and produce a C_4 rice, and what advantages such a novel plant would offer in terms of yield and management of natural resources. Given our present state of knowledge, transferring genes from maize to rice seems to be a logical way of exploring some of the issues, and this involves the use of the biotechnological tools currently available.

Genetic engineering is mentioned many times in this book, both as a research tool to understand crop physiology (e.g., Sharkey et al, this volume) and as a means of manipulating rice germplasm to introduce desirable features (e.g., Ku et al, this volume). The participants at the workshop were aware of some adverse public reaction to genetic engineering in agriculture and that this could affect the stance of the Consultative Group on International Agricultural

Research (CGIAR) and of organizations funding international agricultural research. Part of the general discussion was devoted to this topic with the objective of addressing the issue of the potential benefits of genetic engineering of crops, in the context of public concerns about genetically modified crops.

This is not the place for a full review, which can be found elsewhere. The Nuffield Council on Bioethics (1999) presents a thorough and authoritative analysis. The CGIAR and the United States National Academy of Sciences convened a conference in Washington, D.C., in October 1999 with the title "Ensuring Food Security, Protecting the Environment, Reducing Poverty in Developing Countries: Can Biotechnology Help?" This international conference on biotechnology covered all the issues, and a full report has now been published (CGIAR 2000). The case for the essential role of genetic engineering in raising the yield potential of tropical staple food crops has been made by Lipton (2000). In conjunction with the current positive trends in third-world population, increasing yield offers the chance to eliminate rural poverty in two generations, provided that international agricultural research makes the best use of genetic engineering. Puonti-Kaerlas et al (1999) provide a concise account of the need to increase food production, the role that genetic engineering of crops can play, and the work carried out in Switzerland on rice and cassava. Crawley (1999), Beringer (2000), and Hails (2000) discuss the role of ecological research in evaluating the risks of genetically modified crops, against the background of public concerns in the United Kingdom.

The terms genetically modified (GM) and conventional crop have become current and are used here: GM crops are those produced with one or more genes transferred by genetic engineering, other crops are conventional. Of course, all crops have been genetically modified for millennia by selection, unconscious rather than deliberate, and by breeding in the past hundred years. The Nuffield Council on Bioethics (1999) concluded that genetic engineering could not be regarded as more unnatural than conventional plant breeding. Those who regard DNA as sacrosanct are forgetting that nature does not: mutations occur in all organisms, recombination is part of sexual reproduction, and some viruses and a few bacteria such as *Agrobacterium tumefaciens* add their DNA at least temporarily to that of the plant.

Commercial planting of GM crops started in the United States and acceptance by the public was probably associated with the generally high degree of trust in the U.S. Department of Agriculture (Bruhn 1999). This may be changing as the public controversy in Europe has reopened the question in the U.S. In the U.K., the Ministry of Agriculture, Fisheries, and Food (MAFF) does not have the same degree of credibility, mainly because of past uncertainties over bovine spongiform encephalopathy (BSE) and the safety of beef (Crawley 1999), and also because MAFF is thought to favor the interests of farmers over consumers. In the countries of the European Union, the issue has become muddled with free trade in agricultural produce and the desire of many consumers to have conventional and GM products kept separate, and labeled at the point of sale. Labeling of GM food is being introduced now in Japan. Consumers in Australia are tending to ask: Why take the risk with GM crops when the food supply is perfectly adequate? Overall, in the developed world, there is general apprehension concerning GM crops. The concerns of the developing world seem to be different: these countries want to develop their own programs and do not want the technology thrust upon them by the forces of multinational business and free trade. Public concerns fall into three areas, probably in this order of priority:

1. The safety of GM food.
2. The effects of GM crops on the environment.
3. The use of GM crops by multinational companies operating in developing countries.

Science and risk assessment of GM crops

Most scientists involved in agricultural research were surprised by the public reaction to GM crops. These crops were the outcome of several decades of research in biotechnology, which has proved to be a useful tool in understanding crop growth and improving plant breeding, culminating in the genetic enhancement of existing cultivars. It is important that public concerns be addressed by rational debate. Scientists must be aware of the concerns and be prepared to explain their work, and should not exaggerate benefits nor deny risks. Conventional crops can also pose hazards to the environment and to food safety, and GM crops and conventional crops each have to be evaluated in the context of the farming system.

Understanding the distinction between hazard and risk will aid rational debate about GM crops. A hazard is any harmful event that could happen: risk is a measure of harm actually occurring, a combination of the nature and severity of the hazard and the frequency or probability of occurrence. The hazard in crossing the Atlantic Ocean by boat is death by drowning. The risk is minuscule on an ocean liner, appreciable on a yacht, and much greater if crossing alone in a rowboat, where the risk can be reduced by wearing a life jacket, keeping in radio contact with rescue services, etc. Hazards are easy to identify with a little knowledge and some imagination. Risks are hard to quantify because that requires a good understanding of the system and reliable data covering a range of conditions. For example, finding the effect of pollen from GM maize on caterpillars of the monarch butterfly (Losey et al 1999) identified the hazard but did not quantify the risk to the butterfly population (Bruhn 1999, Crawley 1999). The hazards of GM crops provide attractive material for newspapers but discussing the risks needs more research, careful analysis, and lengthy exposition.

Risks can be compared, and weighed against benefits. It seems to be human nature to underestimate familiar risks (e.g., road traffic accidents) and to fear greatly rare or novel risks. In developed countries with a secure food supply, GM food, such as from a crop with herbicide tolerance, is perceived as offering no direct benefit to the consumer but a finite risk, albeit small. A rational response is to avoid all risk since no loss of benefit occurs. Compare the use of mobile telephones, where the risk of brain damage from radiation has not been demonstrated conclusively but has received much publicity. Mobile telephones are popular because the benefits to users are large and judged worth the risk.

The public has a right to expect the risks arising from GM crops to be minimized. Scientific research provides the basis for the risk assessment. Standards of scrutiny and acceptability have to be set by politicians in collaboration with the expert scientific community. Ecologists have the necessary theory and can carry out the field experiments needed to discover the interactions among crops, weeds, diseases, pests, and other organisms, and to generate parameter values for models. Models embody the mechanisms of interactions and allow exploration of many different conditions (Crawley 1999). It will never be possible to carry out all possible experiments, so the sceptics can never be convinced by experimental results alone, but a range of experiments with modeling will greatly reduce the chance of unforeseen events.

We live in an uncertain world in which science helps us to minimize the adverse consequences of foreseeable or unavoidable hazards, but complete safety cannot be assured by science. Since the scientific method operates by falsification of hypotheses, it is incapable of offering definitive proof for anything, merely an accumulation of evidence. Nevertheless, "even if science cannot offer certainty it can strive to offer objectivity" (Crawley 1999). We all have to accept the lack of black and white answers to questions and learn to deal competently with the shades of gray.

Science underpins the technologies that enhance the quality of modern life: the triumphs of medicine, the convenience of digital technologies, and the usefulness of high-speed communications are clear to all. Nonetheless, much more obscure is agricultural science and the technology on which it rests. There is little understanding of the triumphs of modern intensive agriculture and the Green Revolution in feeding a hugely increased world population from more or less constant cultivated area. The emergence of biotechnological tools that enable genetic changes to be made to crop plants more efficiently has created unease in the minds of the public. When viewed from the vantage point of history, biotechnology will no doubt be seen as comparable with the electronics industry. It will change the way we live and work, but in an acceptable and often highly desirable manner.

Potential benefits of GM crops

Despite the exaggerated claims sometimes made, GM crops are not the answer to all problems of food production. They offer some potential ways of increasing yield in some environments, improving food quality in some crops, improving crop performance in the difficult environments of developing countries, or introducing resistance to pests and diseases more effectively. Moreover, GM crops will not solve any food production problem once and for all, just as improved conventional crops have not. New pests, diseases, and weeds appear, old ones evolve resistance to control measures, and still higher yields are required with less use of fertilizer or irrigation. Sustainable productive agriculture needs continual support from research—nature does not owe us a living, certainly not a population of six billion for any degree of food security. There have been misleadingly optimistic claims for the benefits of GM crops, especially those from biotechnological companies with obvious financial interests. Unfortunately, this has contributed to an uncritical rejection of GM crops instead of recognition that GM crops, like conventional ones, should be assessed on their merits, within the context of the farming system.

Public attention has been captured by the early commercial GM crops, which happened to be those constructed for resistance to a herbicide. It is important to remember the whole range of possible GM crops, some of which offer much greater direct benefit to consumers (Jones 1999, Nuffield Council on Bioethics 1999). The following list is in approximate order of decreasing benefit to consumers and then of benefit to farmers, ending with a benefit to plant breeders.

1. Industrial uses. Crops producing oil for industrial use or plastics have direct benefit to consumers without any worry about their safety as food.
2. Food quality. A good example close to realization is rice containing β -carotene, a vitamin A precursor, in the grain. This required the introduction of genes for three enzymes and their expression in the grain where β -carotene is not usually made (Guerinot 2000, Ye et al 2000). This improvement in rice quality could alleviate vitamin A deficiency among poor rice-eating communities more effectively than other means, and the cultivars produced will be made freely available. Similar work is being carried out to increase the iron content of rice, and it may be possible to remove allergens or poisons from some foods, such as cyanogenic glucosides from cassava (Puonti-Kaerlas et al 1999). Tomatoes containing a gene that slows ripening have been introduced, but here benefit to consumers is perceived to be less than that to retailers. Finally, there is the possibility of producing crops such as banana that express genes for vaccines. This could ensure complete coverage of the community through a staple food but raises the issue of medication without individual consent, similar to the controversy

in developed countries over fluoridation of water supplies to reduce dental decay in the population overall.

3. Crop physiology. Crops with increased tolerance for drought, low temperature, and saline soil, or dwarfed, or with improved photosynthesis (e.g., C_4 pathways) are being produced or considered. Consumer benefits are indirect through lower prices but farmers could benefit greatly from better productivity and an increased choice of crops for their land. Crops of signal plants, in which an introduced gene produces a visible change when the plant experiences a shortage of water or nutrients, offer the possibility of more precise application of scarce or costly resources.
4. Herbicide resistance. This has proved to be the easiest attribute to introduce to a GM crop and is especially valuable in broadleaf crops such as soybean, in which herbicides selective against broadleaf weeds are not available. The use of a broad-spectrum herbicide in a herbicide-resistant crop may permit minimal tillage agronomy, thus reducing energy use and soil erosion. The benefit is to farmers and only indirectly to consumers.
5. Pest resistance. The best known example is *Bt* maize, which expresses the gene for the toxin from *Bacillus thuringiensis*, active against lepidopteran larvae and useful against the corn borer. Several types of *Bt* rice have been produced and are being evaluated (IRRI 1997a).
6. Nonviable seeds. AGM crop without viable seeds is neutral to consumers and may be negative to farmers, but of positive benefit to the seed company in protecting its intellectual property rights simply and cheaply. There might be a positive benefit to the environment since GM crops of this kind could not persist as weeds, and, if the mechanism was nonviable pollen, there would be no risk of cross-pollination.
7. Plant breeding. One advantage of GM crops is the speed and precision with which desirable traits can be introduced, once the genes have been identified and cloned as DNA. Conventional breeding requires crossing to introduce the desirable trait from another cultivar or sexually compatible species, followed by lengthy backcrossing to reassemble, more or less, the original genome plus the introduced genes.

If it had been possible to introduce GM crops in the sequence above, then there would probably have been less controversy and quicker public acceptance. By far the largest areas of GM crops are those with herbicide resistance, followed by pest resistance, with improved food quality at less than 1% by area (Christian Aid 1999). As a wider range of GM crops become available, these proportions will change. Public perception may also change as the benefits to consumers become more apparent and if experience shows the risks to be small and controllable.

Concerns about GM crops

Food safety

Consumers are right to be concerned that food should be safe and wholesome. All countries have mechanisms to ensure this, especially for novel food from GM crops (Beever and Kemp 2000). Regulatory authorities have accepted food from GM crops if it was “substantially equivalent” to food from conventional crops, although the validity of this concept has been questioned (Millstone et al 1999). The context in which to evaluate GM food is that food crops naturally contain many secondary metabolites, some of them poisonous. Plants have evolved defenses against herbivores, such as cyanogenic glucosides or unusual amino acids, and these have not been completely eliminated by selection and breeding in all crops. For example.

consumers are aware that raw cassava or kidney beans are poisonous but perfectly nutritious after correct cooking. Food from GM and conventional crops is unlikely to differ as greatly as this; and, if it did, the routine testing would identify the problem (Jones 1999, Beever and Kemp 2000). All cultivars are likely to vary in composition as food so there is a case for thorough testing of all new cultivars. Conventional breeding often uses crosses with wild relatives, which can introduce genes for toxins and allergens in unpredictable ways. Occasionally, this produces lines with undesirable characteristics as food, which, once detected, are not released as commercial cultivars. A related example is a GM soybean for animal feed containing desirable sulfur-rich amino acids produced by genes from Brazil nut. The nut protein was found in safety checks to be an allergen in humans, so the breeding line was not used in case this soybean might ever be diverted into human food (Jones 1999).

The use in the transformation method of DNA from viruses and bacteria, and antibiotic resistance as a marker, has also attracted public attention. There is the hazard of further movement of viral or bacterial DNA, or transferring antibiotic resistance, from the transgenic crop to other organisms, for example, bacteria in the gut flora or in the soil. Beever and Kemp (2000) have shown that the risk is extremely small. The small amounts of DNA present in food are digested enzymatically and the DNA of a transferred gene would not suffer any different fate. No plant gene has ever been found in the genome of animals. Fragments of bacterial DNA occasionally found in mammalian cells seem to be a result of the normal immune response. A long-term route to better public acceptance would be to minimize the use of nonplant promoters and of antibiotic resistance as a marker. Advances in the technology and careful selection of methods will thus address public concerns in this area, assuming that the public cannot be otherwise assured that the risks are already acceptably small.

Environmental effects

There has been much debate about whether GM crops are good or bad for the environment, especially whether herbicide-resistant crops encourage the use of less or more herbicide. Effects on the environment can be evaluated only by considering the whole farming system: it is not the type of cultivar but how it is grown that matters. Neither GM nor conventional crops are intrinsically good or bad. The aim of productive agriculture is to channel as much as possible of the resources of solar radiation, carbon dioxide, water, and mineral nutrients into wanted parts of the crop, and to minimize diversions into weeds, pests, and diseases. The art of environmentally sensitive farming is to do this while minimizing the effects on other organisms, which can be encouraged in the crop and elsewhere on the farm (field margins, uncultivated land, water courses, wooded areas, etc.), and minimizing pollution such as pesticides and nitrate in groundwater. Cultivars that are more productive, regardless of their origin, will reduce the need to maintain or extend the world's cropped area, allowing alternative land uses including conservation of biodiversity.

Integrated pest management (IPM) can make a contribution to environmentally sensitive farming using GM or conventional crops. Crops with resistance to pests are an important part of IPM and genetic engineering can introduce resistance quickly and selectively, such as using *Bt* toxins. Pests will continue to overcome crop resistance whether this is introduced by genetic engineering or conventional breeding. Consequently, rotations of crops or of cultivars with different patterns of resistance continue to be necessary to manage pest damage at the farm and regional scale. Research on how *Bt* rice should be deployed to maintain its resistance to stem borers is being carried out well before any release to farmers (IRRI 1997a). The finding that caterpillars of the monarch butterfly were poisoned by eating pollen from *Bt* maize (Losey et al

1999) attracted attention and is a classic case of laboratory results not being interpreted in context. The caterpillars are not active when maize pollen is shed and little pollen in fact lands on the food plant (Bruhn 1999). The hazard to the monarch butterfly exists but the actual risk is minimal. Not surprisingly, any caterpillar that ingests the *Bt* toxin, a potent insecticide, is likely to die, but *Bt* maize restricts the toxin to the plant (or to the part to be protected alone, in probable future developments) in contrast to spraying the whole field.

Research is needed to establish whether the transferred genes in GM crops can escape into other plants (Dale 1992, Crawley 1999, Beringer 2000, Hails 2000). Once the transferred gene has become stably incorporated into the crop plant genome, a prerequisite for development of a commercial cultivar, the escape of this gene is no more likely than any other. The hazard of cross-pollination can be identified in terms of the presence, in and around the field containing a GM crop, of sexually compatible species, which will usually be closely related, or of other cultivars growing as weeds, perhaps from a previous crop. The risk must be quantified as the frequency of occurrence of hybrids in the field, and then as the likelihood that the hybrids or their progeny (selfed F_2 or backcrosses) will be favored in that environment if they contain the gene from the GM crop. Many genes of agricultural interest probably have little value in the wild so would not be selected for even if they transferred to a wild plant. For instance, dwarfing genes have been spread around the world in wheat and rice apparently without escaping to any wild species: indeed, they are useful only in an agricultural context where competition from taller weeds can be eliminated.

Promotion of GM crops by multinational companies

Research and development on GM crops is a long-term and costly investment: therefore, where it is undertaken with private capital, the companies seek a good return, including new markets in developing countries. Several recent takeovers in the agricultural industry have produced fewer and larger multinational companies that are dominating markets in seeds, fertilizers, and pesticides in some countries. Aggressive marketing in developing countries that could lead to poor farmers becoming reliant on the products of one company and abandoning traditional cultivars and agronomy is undesirable (Oxfam 1999, Christian Aid 1999). Together with this go more general concerns about capitalism and globalization. The introduction of GM crops can make it easier to link products, such as a herbicide and a cultivar resistant to it, but aggressive marketing can also be carried out with improved conventional cultivars, fertilizers, and pesticides. Most of the genuine problems identified by Oxfam (1999) and Christian Aid (1999) involve these confounding issues and cannot be associated with GM crops as such.

There will always be a need for appropriate government regulation of the activities of businesses and, most important, for effective agricultural extension work. Farmers must be educated about the cultivars and agronomic practices available and enabled to make well-informed decisions about trying them out. They must be encouraged to seek improvements incrementally and to be suspicious of “get-rich-quick” schemes, while retaining the option to revert to traditional crops and systems if the new ones are not successful in the long run. The policy of achieving a yield increase, followed by consolidation for a year or two, then attempting the next change for a further yield increase has been demonstrated successfully in eastern India in an extension project (IRRI 2000b). Just as there is a continuing need for research to maintain the sustainability of productive agriculture, so extension work must also be maintained.

The possibility of patenting transgenic organisms and the processes used to produce them have received much attention, along with the restrictions placed on the use of GM crops, particularly saving seed to plant in the next season. In developed countries, plant breeders’

rights already exist so that patenting is an extension of this. In principle, the application of intellectual property rights (IPR) to crops is similar to the familiar situation with computer software. Purchase confers the right to use and to make a backup copy but not to make further copies or to distribute the software. Moreover, farmers (and many gardeners) are perfectly used to crops for which it is not worthwhile to save seed for the next season but essential to purchase anew: F₁ hybrids, such as maize and rice; biennial crops in which the harvest occurs before flowering, such as sugar beet; or crops that accumulate viruses where fresh stock, free of virus, is required at intervals, such as potato and strawberry. The “terminator” gene whereby crops would not produce viable seed has attracted particular opprobrium, but it is a logical method of protecting IPR in some cases, analogous to the anticopying mechanisms sought by the entertainment industry for compact discs and video tapes. It is the business of extension work to ensure that farmers are aware of the terms of the contract; if seed saving is important for economic or security reasons, then any alleged improvement in crop yield or pest resistance must be weighed against this. (It was announced in October 1999 that commercial use of the terminator gene was not being pursued.)

The application throughout the world of a system of IPR that is suitable for developed countries is not desirable. The protection offered by plant breeders' rights may be the appropriate course for developing countries. Rice germplasm has been freely shared and cultivars made available without restriction on their use, whether saving seed for the next season or for further breeding. To continue the analogy with computer software, rice germplasm is akin to shareware, available to anyone and with modifications welcomed provided that the improved code is in turn made readily available. The problem of IPR and plant germplasm is rightly being addressed by the CGIAR and by IRRI through conferences and workshops (CGIAR 2000, IRRI 2000a). It is laudable that work in Switzerland on transgenic rice is funded with the intention that cultivars ultimately produced will be made freely available (Puonti-Kaerlas et al 1999, Ye et al 2000).

Genetic engineering in rice

Work at IRRI

The use of biotechnology in rice research is summarized in the *Rice almanac* (IRRI 1997b). Techniques such as embryo rescue and selection aided by molecular markers are contributing to conventional plant breeding. Genetic engineering is the component of biotechnology that offers the greatest opportunities for manipulating rice germplasm with the ultimate aim of producing new cultivars. Researchers at IRRI are involved in most lines of research in genetic engineering of rice (IRRI 1997b, 2000b), either directly or through collaboration with institutions around the world. The thorough research on *Bt* rice is noteworthy for its attempt to set a positive precedent for GM rice (IRRI 1997a). The debate on IPR of rice germplasm (IRRI 2000a) has been mentioned above. The role of IRRI is not to promote but to evaluate genetic engineering for the benefit of the poor people of the world (director general's opening address to the International Rice Research Conference on 31 March 2000 at IRRI). It is essential that research on GM rice be undertaken primarily by the public sector because private companies tend to concentrate their efforts on GM crops that can be marketed profitably in the developed countries, with the technology protected by patents. Public-sector work will include the risk assessments necessary before GM crops are approved for release to farmers. We consider briefly below two possibilities for genetic engineering in rice to outline the sorts of arguments that may help to allay public concerns.

Case study 1: the dwarfing gene and basmati rice

Dwarfing genes have been used in plant breeding for several decades and the shorter cultivars of wheat and rice contributed greatly to the Green Revolution. The dwarfing gene has recently been identified and isolated in terms of DNA and shown to be orthologous in rice, wheat, maize, and *Arctidopsis thaliana* (Peng et al 1999), that is, it is a piece of DNA with essentially the same sequence of bases. Dwarf plants respond less to their endogenous gibberellin, so stem elongation is restricted. When the gene was introduced into a cultivar of basmati rice, it exerted its dwarfing effect and is expected to increase yield in the same way as all dwarf cultivars from conventional breeding: the shorter stem increases harvest index, and the cultivar is less prone to lodging, so there is a better use of nitrogen fertilizer. Previous attempts to dwarf basmati rice by conventional breeding had been largely unsuccessful because the genome disrupted by crossing was never reassembled in backcrosses to produce the high-quality properties for which basmati rice is prized. The precise, delicate introduction of a single gene using genetic engineering did not disrupt the genome and maintained grain quality. Research continues at the John Innes Centre, Norwich, U.K., to establish that grain yield is indeed increased.

This case is a good example for considering the risks and benefits of a GM crop. The transformation technique used a particle gun rather than *Agrobacterium tumefaciens*, thus minimizing concern over the introduction of nonplant DNA. No marker gene, for example, antibiotic resistance, was required since the transformed plants could be identified by their response to applied gibberellin. The dwarfing gene was controlled by a maize promoter but perhaps a rice promoter could be used in the future to minimize the use of DNA from any other species. The dwarfing gene exerts its effect during stem elongation of the crop and is not expressed in the seed. The introduced piece of DNA would be consumed in food, in minute quantities, just as the same DNA has been eaten in modern wheat and rice cultivars for a generation with no ill effects. Given the maintenance of grain quality in the GM basmati rice, there will unlikely be much difference between the conventional and GM rice grains identifiable at the level of food components, so concerns about the safety of the GM food should be minimal. Consumers should benefit from lower prices as farmers benefit from higher yields. A crop more resistant to lodging will encourage greater use of nitrogen fertilizer but that would also occur with a modern cultivar produced conventionally. If the cultivar eventually produced is made available to basmati rice growers without restrictions, and there are no other adverse socioeconomic factors, then this GM crop could bring real benefits to communities in northern India with no discernable risk to consumers and little risk to the environment.

Case study 2: rice with C₄ photosynthesis

During evolution, C₄ photosynthesis has arisen independently many times in several families of flowering plants, in response to a combination of global low carbon dioxide concentration and local high temperatures during the growing season (Ehleringer et al 1997). Within the grasses, C₄ photosynthesis occurs in the subfamilies Arundoideae, Chloridoideae, and Panicoideae but not in the Pooideae (mainly north temperate) or in the Bambusoideae (mainly tropical and subtropical, often in shady or wet habitats), which contains rice (Clayton and Renvoize 1986). From the point of view of grain yield, water use, or nitrogen use, C₄ photosynthesis could be a benefit to rice grown as a crop although it has not evolved naturally in rice or in any closely related species. The prevalence of C₄ weeds of rice, including mimic weeds such as *Echinochloa oryzoides*, suggests that C₄ photosynthesis is compatible with growth in tropical irrigated conditions. Since evolution has not provided a C₄ rice, it is left to scientists to imitate nature using genetic engineering. If C₄ rice does have the benefits expected, then it

could contribute to breeding high-yielding cultivars, or cultivars with a better use of water and nitrogen. Such cultivars would be available for release to farmers only after exhaustive testing appropriate for GM crops.

A C_4 rice would contain transferred genes for enzymes of the C_4 pathway and genes controlling leaf development, assuming that Kranz anatomy proves to be essential. As Beever and Kemp (2000) point out, adding a few genes to the 20,000 to 50,000 genes of the typical plant genome makes a negligible increase in the amount of DNA. The grain would certainly contain the transferred genes but the DNA would be digested in the normal way. The proteins encoded by the transferred genes are unlikely to be produced in large amounts in the grain, especially if promoters are chosen that restrict expression to the leaves. Testing the grain for composition and any allergenic effects is routine for GM crops. The improved nitrogen economy of a C_4 rice may produce grain of low protein content but then so do many current conventional cultivars when grown with low inputs of fertilizers.

Rice cultivars may cross with closely related weed species and particularly with weedy varieties of *Oryza sativa*. Experiments are necessary to establish the frequency of formation of hybrids and whether they and their progeny (selfed F_2 or backcrosses) perform better as weeds once they contain some or all of the C_4 genes. No release of a C_4 rice cultivar would be contemplated without a thorough environmental risk assessment.

If a C_4 rice were developed under the current system of public-sector research, within the CGIAR and national agricultural research systems, then breeding lines would be made available on the current terms for sharing rice germplasm. Could such a large and long-term project, also somewhat speculative, be supported entirely by the public sector? If not, then private-sector funding would be required and some form of IPR may become essential to ensure a reasonable return, given the amount of capital invested and the financial risk incurred. The effects of a C_4 rice cultivar on the farming practices and productivity of poor farmers in the developing world will probably be no different from those of any other improved cultivar. Farmers need to assess the costs and benefits in their conditions, with the help of agricultural extension services. It is unlikely that C_4 rice will solve the world's food production problems in one stroke but neither will it be disastrous for poor farmers.

A C_4 rice could bring benefits to hundreds of millions of poor and undernourished people, through greater yields or yields maintained with less water or less fertilizer. The environmental risks can be assessed and strategies adopted for minimizing them. In terms of food safety, we think that making a rice with maize-like photosynthesis would be no more harmful than mixing rice crispies and corn flakes in the same breakfast bowl.

Conclusions

Genetic engineering is an additional tool for the plant breeder: it supplements but does not replace existing methods. What genetic engineering does offer is a precise and relatively quick introduction of desired traits into a breeding line. Some features could not be introduced in any other way, such as maize genes into rice or vice versa, or the ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) with higher specificity for carbon dioxide from red algae, which could improve photosynthesis (Mann 1999). Scientists must constantly listen to public concerns and be prepared to explain the technology, the contest in which it is to be applied, and the possible outcomes with an assessment of risks and benefits (Bruhn 1999). Many of the concerns about GM crops are greatly lessened when separated from confounding issues, such as aggressive marketing by multinational companies, IPR, and free trade, and when put in the context of the farming system and compared with conventional crops on a fair basis.

The CGIAR is in a good position to promote discussion of the risks and benefits of GM crops, their possible role in developing countries, and the problems of controlling IPR for the benefit of poor farmers. The conference held in October 1999 is an example of this (CGIAR 2000). It is unfortunate that concerns about the confounding issues have led such influential charities as Oxfam and Christian Aid to oppose GM crops (Oxfam 1999, Christian Aid 1999), although Oxfam does concede that there could be long-term benefit to poor farmers with properly directed funding. Puonti-Kaerlas et al (1999) provide several examples of research currently funded to produce GM crops of direct benefit to poor farmers. The Nuffield Council on Bioethics (1999) concluded that donor aid should be used for application of biotechnology in developing countries that want it. Considering the problems of feeding the world population in the coming decades, there is a moral imperative to investigate thoroughly the potential of GM crops; it would be immoral to refuse their use without good reason. Lipton (2000) sees genetic modification, if applied to the right crops (tropical staples), the right traits (primarily yield potential), and in the right way (without knowledge locked up in IPR, with germplasm freely available), as essential to progress in eliminating poverty. We hope that funders of agricultural research will not be inhibited by adverse public opinion on GM crops, some of which is ill-informed, but will vigorously defend their investment in this research by rational analysis of the risks and benefits. We are confident that improvements in photosynthesis brought about by genetic engineering would not be harmful in any way.

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Notes

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Discussion: opportunities for redesigning rice photosynthesis

P.L. Mitchell and J.E. Sheehy

The statistics of world population are of great concern: 1.3 billion when the oldest continuous agricultural experiments were set up in 1843 in the U.K. at Rothamsted Experimental Station, 6 billion reached in 1999, and a predicted 8 billion in 30–40 years' time. Perhaps as many as 1 billion will be malnourished and denied a long and fulfilled life, with a high proportion of these in Asia, where rice is the principal cereal. Some of this poverty and misery can be alleviated by increasing rice yields. High yields do reduce poverty, directly and indirectly, and contribute to general economic development (Dawe, this volume). The chance to start a strategic process for increasing rice yield must be seized. Possible courses for the future must be charted soon so that those working at IRRI 10–15 years from now will be close to introducing high-yielding and resource-efficient cultivars, with any improved agronomic practices, to achieve the required 50% increase in yield by 2050.

It should be remembered that there are limits to yield, set by the laws of thermodynamics and mass conservation. At the limit, the demands by the crop for the dilute resources (carbon dioxide, soil nutrients) that become fixed and concentrated in dry matter cannot exceed the maximum physical rate at which they can be supplied, either by mass transport or diffusion. Within the crop, there are limits to the rates of processes that produce dry matter and assemble it into further cells, tissues, and organs. Since our understanding of the processes is based on existing species and cultivars, the limits to yield must be calculated, guided by general laws of physics and chemistry used in a biological setting.

Two papers in this volume (Mitchell and Sheehy, Evans and von Caemmerer) have established that, if yield is to be increased, then so must the radiation conversion factor (RCF = radiation-use efficiency, RUE), which is the amount of aboveground dry matter produced from unit PAR intercepted (so not a dimensionless efficiency). There is evidence that rice can reach a value of 2.6 g MJ⁻¹ and calculations suggest that 2.9 g MJ⁻¹ is the limit for rice. This is not sufficient: 3.3 g MJ⁻¹ is required to produce the increase in yield we need. In Mitchell et al (1998). RCF is given as

$$\varepsilon = \frac{P_g - R_s - m_a W}{I_{\text{int}}} \quad (1)$$

where ε is the radiation conversion factor (g MJ⁻¹; total dry matter, intercepted PAR), P_g is gross photosynthesis (g m⁻² d⁻¹), R_s is growth (synthesis) respiration (g m⁻² d⁻¹), m_a is the

coefficient for maintenance respiration ($\text{g g}^{-1} \text{d}^{-1}$), W is total dry matter (g m^{-2}), I_{int} is the intercepted photosynthetically active radiation (PAR; $\text{MJ m}^{-2} \text{d}^{-1}$), and assuming that photosynthesis and respiration are measured in terms of dry matter.

Because opportunities for reducing respiration are limited, photosynthesis must be improved. Introducing C_4 biochemistry is one option and improving C_3 photosynthesis is the other. The benefit of this increase in photosynthesis must cross the interface between the chloroplast and the rest of the plant, at the point of action of the enzyme sucrose-phosphate synthase (Sharkey et al, this volume), and contribute to increasing canopy photosynthesis, growth, and yield (Mitchell and Sheehy, Evans and von Caemmerer, this volume).

There is no reason to think that crop plants have evolved or have been selected to the best possible state. For example, the photosynthetic mechanism of green plants evolved in an atmosphere richer in carbon dioxide but with less oxygen than today. The C_4 pathway evolved much later during a period of low carbon dioxide concentration (Ehleringer et al 1997, Sage, this volume). During the past 10,000 years since the last glaciation, the concentration of carbon dioxide has been stable so that plants presumably became adapted to 280 ppmv, the preindustrial concentration. The limited adaptation of short-lived plants, or merely the acclimation of long-lived trees, must have occurred in response to rising concentrations of the past 250 years, but plants cannot be considered to be fully adapted to the present 370 ppmv carbon dioxide. Thinking of crop growth as analogous to the manufacturing industry may be illuminating. The modern trend in industrial management is away from the maintenance of high stocks of components to ensure continuous production (just in case) and toward securing the appropriate flow of components and minimal stocks (just in time). Do plants operate on a just-in-case basis of acquiring and conserving resources whenever they are available to survive varying and unpredictable growing conditions? Since agronomy provides crops with better conditions, including minimal or smoother changes in resource availability, plant breeding can afford to aim for plants with a just-in-time physiology. A better understanding of fundamental plant physiology and the use of genetic engineering offer great opportunities for breeding high-yielding cultivars well suited to productive environments such as the irrigated tropics and subtropics.

This chapter summarizes current knowledge and makes recommendations for research. The aim is to identify research that will have a fundamental effect on yield potential through improvements to photosynthesis, and thus attract funding. It is expected that such research would be undertaken by partnerships between IRRI and research institutes around the world with the necessary expertise. Some projects will require substantial funds but others may not. A small amount of money directed at a particular topic where there is scientific interest and enthusiasm could be most productive in certain cases.

At the workshop, the general discussion was divided into several themes, largely determined by the discussions during the previous three days of presentations. Despite “the incorrigible nature of man to hold different opinions on speculative subjects” (Trevelyan 1942), there was a good measure of agreement on what were the important topics and what lines of research might be profitably pursued. A general theme was the need for the highest possible standards (protocols and measurements) in scientific work, ranging from field-based agronomy to molecular biology in the laboratory. Several topics were noted that did not involve photosynthesis but where parallel research is essential if the full benefits of improved photosynthesis are to be realized. Finally, background research on some basic topics may contribute to the ultimate success of research on rice photosynthesis.

Improving C₃ photosynthesis

The maximum rate of leaf photosynthesis of rice in the normal atmospheric concentration of carbon dioxide and saturated for PAR is 25–32 mmol CO₂ m⁻² leaf s⁻¹ (Yoshida 1981, Peng et al 1995). This is higher than wheat at 16–25 mmol m⁻² s⁻¹ (Fischer 1983) but much lower than maize at up to 55 mmol m⁻² s⁻¹ (Larcher 1995). Recent work at IRRI and the University of Sheffield (Murchie et al 1999, Horton and Murchie, this volume) has shown that the rate of leaf photosynthesis in rice can sometimes be less than expected in apparently good conditions, particularly when high PAR is combined with high temperature, as in a tropical dry season. What is less clear is the quantitative effect of suboptimal photosynthesis because little is known of the duration of these extreme conditions in the field when allowance is made for the angle of the leaf. Incident PAR at meteorological stations is recorded on a horizontal surface. Erect leaves receive lower solar radiation per unit area, especially when the sun is high in the sky, thus reducing the heat load and reducing the chances of photosynthesis being saturated for PAR. In addition, erect leaves in the upper canopy allow more PAR to penetrate to lower leaves. The more equitable distribution of PAR throughout the canopy should mean that more leaves are operating in the middle of the photosynthesis versus PAR curve where both quantum yield (determining the initial slope) and maximum rate (asymptote) have some influence on the rate of photosynthesis actually occurring. A C₄ rice can be envisaged with the appropriate biochemistry but requiring more energy than a true C₄ plant so that quantum yield would not be increased: such a rice plant is unlikely to have increased RCF, or increased yield insofar as that depends on RCF. Recent analysis (Sinclair and Sheehy 1999), however, suggests that the importance of canopies with erect leaves is not so much in total canopy photosynthesis but in maintaining live leaves lower in the canopy. Nitrogen is taken from these lower leaves during the ripening stage and transferred to the filling grain, while the upper leaves continue to produce carbohydrates.

Carbohydrate production depends on gas exchange through the stomata. Rice stomata are distributed equally on the upper and lower surfaces; this is important for leaves that are more or less erect. The significance of the much higher stomatal frequency and smaller aperture in rice (400–600 stomata mm⁻², aperture 1.5 μm) compared with wheat (40 stomata mm⁻², aperture 6 μm) is not clear; rice and wheat are not closely related within the grass family so perhaps this merely reflects phylogeny. Stomatal frequency must set an upper limit to conductance when all the stomata are at maximum aperture but it bears no simple relation to conductance in the usual range of conditions. Stomatal function has been less studied in rice, probably because it was thought to be less important in a crop that is usually irrigated. Transpiration rates up to 9 mm d⁻¹ have been recorded at IRRI in the dry season (O'Toole and Tomar 1982), and significant transpiration occurs despite the high humidity over a tropical paddy field. Stomata do not close at high temperature, as occurs in some species. Transpiration cools the leaves to below air temperature but the high flux of water is not important in bringing nutrients to the roots by mass flow (Robinson 1994, Kirk, this volume); diffusion in the soil is sufficient. A high rate of transpiration in irrigated rice, provided that it can be sustained by the rather small proportion of the plant that is roots and by adequate hydraulic conductance of the stem and leaves, would not be a problem if it were associated with greater photosynthesis. The reverse is true for upland and rainfed lowland rice, for which the amount of carbon fixed per unit of transpiration is important.

While stomatal conductance can be measured on a portion of a leaf, it is difficult to integrate up to the canopy. For a complete canopy, the magnitude of the depression of canopy temperature, measured by infrared thermometry, is associated with yield in wheat (Fischer 1983) and in rice

(Horie 2000). This could be a useful screening tool, whether it is empirical or mechanistic, in that more transpiration indicates higher stomatal conductance and therefore more photosynthesis and growth and yield. In this connection, the observation of higher stomatal conductance in rice transformed with maize C_4 genes (Ku et al, this volume) is most interesting.

In general, stomata exhibit a feedback response (Jones 1992) so that they maintain the intercellular concentration of carbon dioxide (C_i) at about 70% of the atmospheric concentration (C_a) in C_3 plants, that is, $C_i/C_a = 0.7$. Rice seems to be no exception. In C_4 plants, $C_i/C_a = 0.4$. This information can be used with predicted increases in atmospheric carbon dioxide concentration to calculate the benefit of C_4 photosynthesis relative to that of C_3 photosynthesis in the future. Given that it will take many years to produce a C_4 rice, will rising carbon dioxide make it more suitable for droughted than irrigated conditions? The shape of the curve of leaf photosynthetic rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$) against the concentration of carbon dioxide in the intercellular spaces of the leaf (C_i , ppmv), often called the A/C_i curve, depends on the photosynthetic pathway (Fig. 1). The C_4 curve has a rapid increase in A as C_i increases but turns abruptly to approach an asymptote after about 100 ppmv. The C_3 curve is offset from the origin (carbon dioxide compensation point typically 50 ppmv), rises more slowly, and approaches an asymptote gradually. These differences arise from the initial fixation of carbon dioxide by phosphoenolpyruvate carboxylase (PEPcase) and the absence of photorespiration in C_4 plants. The curves can be modeled using biochemical properties of ribulose-1,5-bisphosphate carboxylase–oxygenase (Rubisco) and PEPcase. The curves start to converge beyond a C_i of 250 ppmv and cross at around 600–700 ppmv. At these high concentrations of carbon dioxide, there is no benefit of the C_4 pathways to leaf photosynthesis in terms of response to carbon dioxide concentration. The atmospheric concentration of carbon dioxide in the late 18th century (preindustrial) is believed to have been 280 ppmv, the value in 2000 was taken as 370 ppmv, and predictions from the Intergovernmental Panel on Climate Change (IPCC) 1992 emission

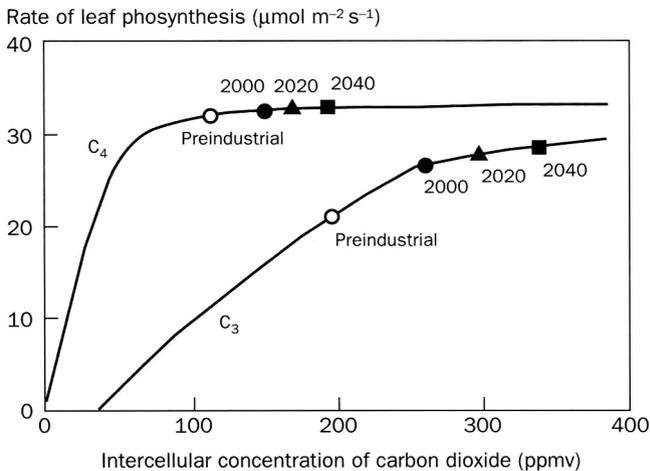


Fig. 1. Rate of leaf photosynthesis as a function of the concentration of carbon dioxide in the intercellular spaces of the leaf. The curves were redrawn from Figure 2 of Long (1999), which was based on biochemical models of leaf photosynthesis for typical C_3 and C_4 leaves, when photosynthesis is saturated by PAR and with leaf temperature of 25 °C. The points on the curves represent the intercellular concentrations (C_i) at the dates shown and assuming that C_i is 70% of atmospheric concentration for C_3 plants and 40% for C_4 plants (Jones 1992).

scenario a (with minor revisions in 1994) are 422 ppmv in 2020 and 481 ppmv in 2040 (Schimel et al 1996, Figure 2.3). Using a C_1/C_a of 0.7 for C_3 and 0.4 for C_4 , the values of C_1 for the late 18th century, 2000, 2020, and 2040 were computed and rates of photosynthesis measured from the curves in Figure 1. The improvement of C_4 leaf photosynthesis over C_3 was 51% in the late 18th century, 22% in 2000, 18% in 2020, and 14% in 2040. Using the extreme emission scenarios (IPCC 1992 [revised 1994] scenario c: 407 ppmv in 2020 and 437 ppmv in 2040; scenario e: 430 ppmv in 2020 and 510 ppmv in 2040) makes little difference since the curves have gentle slopes in the regions concerned: the C_4 improvement is 17–19% in 2020 and 14–17% in 2040. (It is noteworthy that the C_4 benefits were much greater at preindustrial carbon dioxide concentrations, perhaps an artifact of this simple calculation. The curves probably have the correct shape since properties of the photosynthetic enzymes are highly conserved but the assumptions of C_1/C_a of 0.7 for C_3 and 0.4 for C_4 may be inappropriate.) The curves in Figure 1 are for 25 °C leaf temperature but 30 °C is more relevant to tropical rice. Since photorespiration increases with temperature so that net photosynthesis decreases, the C_3 curve for 30 °C is expected to be a similar shape but lower. This makes the benefits of C_4 photosynthesis slightly larger than the figure calculated here but with the same trend over time.

Ehleringer et al (1997) used a model based on quantum yield to compare the rates of C_3 and C_4 canopy photosynthesis in carbon dioxide concentrations estimated for past geological periods. On a graph of atmospheric concentration of carbon dioxide against daytime temperature during the growing season (their Figure 2), they demarcated regions—combinations of carbon dioxide concentration and temperature—where C_3 or C_4 photosynthesis was favored, on either side of a crossover temperature at which C_3 and C_4 quantum yields were equal at a particular carbon dioxide concentration. The line for the monocot NADP-malic enzyme (NADP-ME) subtype of C_4 photosynthesis has a larger region on the graph where C_4 photosynthesis is favored compared with other C_4 subtypes. This is an additional argument for considering this subtype (which includes maize) for genetic engineering in rice, in addition to its comparative biochemical simplicity (Leegood et al 1997) and high productivity. The monocot NADP-ME subtype is favored at carbon dioxide concentrations up to 340 ppmv at 20 °C daytime temperature, up to 430 ppmv at 25 °C, and up to 540 ppmv at 30 °C. Thus, the advantage of a tropical C_4 rice would remain until 540 ppmv, which is predicted for 2058. In one extreme scenario, 540 ppmv is never reached; in the other, it would be reached in 2046, according to Schimel et al (1996).

In conclusion, the rising atmospheric concentration of carbon dioxide should not be a deterrent from constructing a C_4 rice. The benefits of C_4 leaf photosynthesis in irrigated rice will decrease with time but not greatly in the next 20 years. Even in 2040, a benefit of 14% will be worthwhile, provided that it does indeed translate fully into canopy photosynthesis, crop growth, and grain yield. Improved nitrogen use (see later, “Nitrogen in C_4 Rice”) would be unaffected by changes in carbon dioxide concentration. Water use will improve as carbon dioxide concentration increases, and this is of greatest importance for upland and rainfed lowland rice. In 2040, a C_4 rice would probably lose much less water by transpiration than a C_3 rice for the same rate of photosynthesis. (There is a well-known saying among upland rice researchers that the best kind of upland rice would be maize, for exactly this reason, improved water use of C_4 photosynthesis.)

The following recommendations not only contribute to improving C_3 photosynthesis but also establish more firmly the base from which a C_4 rice could be constructed.

1. Research on canopy photosynthesis, transpiration, and micrometeorology must be continued and enhanced. Answers to the following questions are required: What are the responses of rice stomata to vapor pressure deficit, temperature, and carbon dioxide

concentration? What is the frequency of occurrence of leaf temperature or received solar radiation, or combinations, so high that photosynthesis is reduced? Is there a reliable relationship between depression of canopy temperature and yield as has been found for wheat? Measurements of canopy photosynthesis and transpiration in rice are difficult because the fluxes of carbon dioxide and water from the soil, irrigation water, and aerenchyma must be taken into account. Enclosures for use above porous soil with positive pressure to minimize soil efflux have been devised (Woodward and Sheehy 1983; full details in Stiles 1977) and may be adaptable to rice. Other approaches are possible: fluorescence techniques combined with a model of stomatal conductance (Edwards et al, this volume), eddy correlation (covariance) for field-scale measurements, or stable isotope (^{13}C) discrimination. Stomatal responses are critical for upland and rainfed lowland rice crops (20% of global production) that experience periods of water shortage.

2. If manipulation of stomatal conductance can be shown to be a route to increasing yield, then some transgenic approaches can be explored. For example, there are antisense techniques usable on abscisic acid receptors for turning down the sensitivity of stomata to water shortage.
3. It is assumed that losses through photorespiration are high in tropical rice (Mitchell and Sheehy, this volume). Ultimately, this arises from the relative carboxylase and oxygenase activities of Rubisco, summarized by the relative specificity for carbon dioxide, S_r , or by the parameter G^* , the intercellular concentration of carbon dioxide at which gains by photosynthesis and losses by photorespiration are equal, that is, the compensation point without concurrent leaf respiration. (The values of S_r and G^* are related by the concentration of oxygen around Rubisco, which for the usual atmospheric concentration can be taken as constant, and a factor of two arising from only one carbon dioxide being produced from two oxygenations by Rubisco.) Variation in G^* of rice species and cultivars has not been examined (Evans and von Caemmerer, and Sage, this volume). It is known that the S_r of Rubisco in certain red algae is much higher than that of Rubisco in higher plants (Uemara et al 1997). A long-term prospect for improving C_3 photosynthesis would be to introduce Rubisco with better properties by genetic engineering (Watson and Tabita 1997, Mann 1999).
4. High rates of leaf photosynthesis are often observed in leaves with low specific leaf area (i.e., less area per unit dry matter, a "thicker" leaf). There is usually a negative correlation between leaf photosynthesis per unit area and specific leaf area (SLA). Plants that are grown as spaced individuals with high photosynthesis per unit leaf area and low SLA increase their SLA, probably because of mutual shading, when grown at close spacing in crop communities. Consequently, the high rates of leaf photosynthesis are lost as a result of competition for light. Community-tolerant rice plants would be desirable in the drive for higher leaf photosynthesis.

Improving primary partitioning

Primary partitioning covers the metabolism between triose phosphate, which is the output of the Calvin cycle, and the sucrose and amino acids exported in the phloem from source leaves (Lunn and Hatch 1995, Sharkey et al, this volume). It includes the synthesis and breakdown of assimilatory starch, the cycling of phosphate between the chloroplast and cytosol, the synthesis of sucrose and amino acids, and the fixation of carbon dioxide by PEPcase to replenish the intermediates in the Krebs cycle drawn off for amino acid skeletons (the anaplerotic role of

PEPcase). Cycling of phosphate between the chloroplast and cytosol is essential for continuous photosynthesis, which can be limited by the rate of phosphate cycling in some circumstances (Sharkey et al 1986). In rice growing in a controlled environment, feedback inhibition of photosynthesis and lack of response to an elevated concentration of carbon dioxide have been attributed to limitation of the rate of use of triose phosphate (Winder et al 1998).

As photosynthesis increases during the early morning, production exceeds translocation and carbohydrates start to accumulate in the leaf as assimilatory or transitory starch, most of which is translocated during the night. The synthesis of sucrose is a critical control point between the production of carbohydrate and its translocation or temporary storage in the leaf (Sharkey et al, this volume). The regulation of carbon metabolism occurs at several scales to link the variable output of photosynthesis (day and night, and within a day dependent on incident PAR, stomatal conductance, etc.) and the more or less constant rate of translocation of sucrose and its use in sinks (Geiger and Servaites 1994). Plant breeding, whether conventional or including genetic engineering, must maintain or improve the regulation of the balance between sources and sinks.

Rice, like other cereals and grasses in general, is classed as a low-starch plant because it accumulates soluble sugars and rather little starch, in contrast to high-starch plants such as tobacco, tomato, and sugar beet (Thorne and Giaquinta 1984, Pollock and Lloyd 1987). For example, Winder et al (1998) found that after 5 h of photosynthesis the carbohydrate content, as hexose equivalents, of the flag leaf was 96% sucrose and about 1% starch with the rest as glucose and fructose. During the night, about two-thirds of the sucrose disappears from the leaf. It was assumed that most of the sucrose was stored in the vacuole. Cytosolic concentrations of sucrose could be inhibiting photosynthesis. Rice does not produce fructans, which are common soluble storage sugars in wheat, barley, and most grasses of the Pooideae subfamily (Hendry 1993). In the pooid grasses, the sequestration of sugars as fructans into the vacuole seems to be an effective means of minimizing feedback inhibition of photosynthesis (Pollock 1997, p. 330). Although sucrose is the main assimilatory storage carbohydrate in the leaf blades of rice, starch is accumulated in the leaf sheaths and mobilized some days or weeks later to supplement current photosynthesis during grain filling.

Carbohydrate and nitrogen metabolism are linked in primary partitioning by the anaplerotic role of PEPcase (Osaki and Shinano, this volume), which fixes up to 5% of carbon dioxide in C₃ plants as shown by measurements of carbon isotope discrimination (Farquhar et al 1989). Carbon dioxide is added to phospho*enol*pyruvate (PEP), which is available from glycolysis. The carbon dioxide fixed into a four-carbon organic acid has no direct route back to carbohydrate metabolism, which is based on three-carbon and six-carbon compounds in the cytosol, unless it is decarboxylated, which means that there is no net fixation of carbon. Instead, it enters the Krebs cycle and hence amino acids and other compounds (Hill 1997). The activities of PEPcase and of sucrose-phosphate synthase (SPS) appear to be coordinated (Champigny and Foyer 1992). Phosphorylation of each enzyme in the presence of light and nitrate activates PEPcase and inactivates SPS, thus reducing carbon flow to carbohydrate (sucrose) and increasing it to amino acids (via the anaplerotic pathway). Nitrate arriving in the leaf seems to be the trigger mechanism although the actual effector of the phosphorylation is probably a derivative of nitrate (Champigny and Foyer 1992). This may provide some explanation for the effectiveness of nitrate as a nitrogen source for rice (Kirk, this volume) when ammonium was expected to be the sole source from a reduced, anaerobic soil.

Primary partitioning appears to be a rather neglected part of crop physiology. The recommendations are for general and wide-ranging research that will contribute to improving photosynthesis by either the C₃ or C₄ pathway.

1. There is a link between photosynthesis and yield but it is subtle and may depend more on signaling than bulk movement of materials (Sharkey et al, this volume). A better understanding is required of the fluxes from photosynthesis, fluxes to and from assimilatory starch and sugars, and fluxes to translocation and use for new biomass or for long-term storage. Is the greater use of soluble sugars instead of starch in rice limiting photosynthesis, for example, by high sucrose concentration in the cytosol, or by a maximum rate of transport into the vacuole, or by storage capacity in the vacuole? Would rice perform better if assimilatory carbohydrate was mostly starch in the chloroplast instead of sucrose in the vacuole?
2. In primary partitioning, PEPcase has a pivotal role in both C_3 and C_4 plants in directing carbon skeletons to amino acids, and in producing malate for guard cell osmoregulation and movements. It is noteworthy that much of the effect of enhanced PEPcase activity in transgenic rice was attributed to wider stomatal aperture and higher conductance (Ku et al, this volume). What are the effects of manipulating the amount or activity of PEPcase, whether throughout the plant, or in leaves only or in guard cells only?
3. How general is the finding of Winder et al (1998) for the japonica rice cultivar T309 in "moderate conditions of temperature and [PAR]" that photosynthesis is limited by the rate of triose phosphate use? Can this be demonstrated in the field? How can this be reconciled with those experiments that show increased growth of rice in elevated concentrations of carbon dioxide? If this feedback inhibition of photosynthesis and lack of response to elevated carbon dioxide are widespread among rice cultivars and occur in many conditions, then in what ways can genetic engineering contribute to improving primary partitioning? This will be necessary for rice to respond to the rising atmospheric concentration of carbon dioxide. or to increased production of triose phosphate from C_4 photosynthesis of a transgenic rice.

Rice transgenic for maize C_4 enzymes

The introduction of maize genes for enzymes in the C_4 pathway and their expression in rice have been reported by Ku et al (1999, this volume). Excitement must be tempered with caution. Many transgenic plants had been produced with a wide range of overexpression of the genes. Not all amounts of overexpression will be beneficial to the plant and many may have unacceptable agricultural properties. Ku's work is promising but preliminary and rigorous characterization of the transgenic phenotypes is eagerly awaited. Then the work must be reproduced in other hands, with indica rice, and when grown in the tropics.

It is possible that the rice transgenic for maize C_4 enzymes produced so far will show no consistent improvement when grown in the field as a crop. It is essential that the basic properties of transgenic lines be stable and well characterized before even thinking of breeding for commercial cultivars. More analysis is needed of the existing lines, especially of the T_3 and later generations, when stable lines have been produced, free of the quasi-heterosis that often accompanies the T_0 and T_1 generations. (Note on terminology. Just as conventional breeding denotes the generations as P, F_1 , F_2 , etc., so plants regenerated from tissue culture are R_0 , R_1 , etc., and transgenic plants are T_0 , T_1 , etc. The R_0 and T_0 generations are those plants recovered from tissue culture; R_1 and T_1 are plants produced from their seed. The T_0 generation is the plants that were selected by the marker gene, or some other means, as carrying the gene of interest.)

All Ku's work has used japonica rice; Kitaake and Nipponbare are cultivars currently grown commercially in Japan but they do not have a high photosynthetic capacity. The next

step is to use indica rice and IR72 is the obvious choice: it is well characterized, has a high rate of photosynthesis and much resistance to pests and diseases, and gives high yields. Other candidates that Ku has discussed with co-workers in China include their local cultivars and hybrids. The genes to be transferred are those for PEPcase and pyruvate, orthophosphate dikinase (PPDK), individually and in combination; other genes available from *M. Matsuoka* could be used as well. Direct transfer, that is, creating transgenic plants anew, would be quicker than trying to hybridize indica with transgenic japonica especially as transformation techniques have been established for IR72 (Datta et al 1997).

The following recommendations form a sequence: only if the first stage is successful are subsequent stages worth pursuing. Ideally, this work would be carried out by two or three research groups in different locations.

1. Rigorous physiological characterization of the current transgenic rice plants, in the laboratory and glasshouse, is required. Without Kranz anatomy, it is unclear how increased activity of the enzymes of the C_4 pathway can produce C_4 -like photosynthesis. Indeed, it seems unlikely that PEPcase, or PEPcase with PPDK, could produce C_4 -like photosynthesis: a decarboxylating enzyme, such as NADP-ME, and the relevant transporters of intermediates across membranes are also necessary to close the C_4 cycle.
2. If the transgenic rice plants do have demonstrably different phenotypes, explicable in terms of altered biochemistry attributable to the transgene, then field experiments with stable T_3 homozygotes should be carried out. These should be at sites where reliable field measurements can be made, and adjacent to laboratories for measurements on samples taken from the field. The safety regulations permitting experiments with transgenic plants in the field are expected to be in place soon in several Asian countries.
3. If the current transgenic rice plants do have altered, stable phenotypes exhibiting enhanced photosynthesis, growth, and yield, then extension of the work to other cultivars is justified. Carry out transformation of indica rice with the genes for PEPcase, PPDK, and NADP-ME, individually and together. This is a five-year project allowing for the production of stable T_3 lines before physiological characterization, which should follow the sequence of recommendations 1 and 2 above.

Single-cell systems of C_4 photosynthesis

Hydrilla verticillata (L. f.) Royle is a submerged aquatic angiosperm, a monotypic genus in the Hydrocharitaceae, a family of such plants (Mabberley 1987). It is classed as a C_4 plant despite the absence of Kranz anatomy, and is the only C_4 monocot that is not a grass or sedge (Sage et al 1999). When the C_4 pathway is induced, the concentration of carbon dioxide in the chloroplasts is greatly increased and inhibition of photosynthesis by oxygen is reduced (Reiskind et al 1997): this is associated with increases in the amounts and activities of PEPcase in the cytosol, and of NADP-ME and PPDK in the chloroplasts (Magnin et al 1997). Thus, *Hydrilla* operates a C_4 mechanism within a cell, requiring only increased activities of three enzymes (and the relevant metabolite transporters, presumably), making it an attractive system to copy for a C_4 rice. However, the physiological ecology of aquatic plants is highly specialized. Concentrations of inorganic carbon (carbon dioxide and bicarbonate) in water are generally low, especially at temperatures above 35 °C when high rates of photorespiration can occur. A C_4 mechanism may be essential to scavenge carbon dioxide in these conditions and it also operates at night, presumably using adenosine triphosphate (ATP) from respiration (Reiskind et al 1997).

Recently, another plant has been reported to operate the C_4 mechanism within single cells (Freitag and Stichler 2000). The chenopod *Borszczowia aralocaspica* possesses elongated cells in the leaf radially arranged around a central zone of tissue containing the vascular bundles. The elongated cells are closely packed, without intercellular air spaces, at the inner end where they have abundant, large chloroplasts. The outer two-thirds of the cells have the appearance of mesophyll cells, with normal air spaces. The general appearance is of Kranz anatomy apart from the lack of a cell wall between the two parts of the elongated cell. The C_4 mechanism is inferred from $\delta^{13}C$ measurements. Biochemical confirmation and ultrastructural examination to detect any membrane between the two parts of the cell have yet to be carried out. Since *Borszczowia* is a terrestrial plant, its C_4 system might be suitable for a C_4 rice. However, the “poor evolutionary success” (Freitag and Stichler 2000) of *Borszczowia*—a monotypic genus restricted to the saline soils of central Asia—may indicate that this C_4 system is not especially advantageous.

Mechanisms for concentrating carbon dioxide around Rubisco are widespread in algae (Badger and Spalding 2000) and also occur in hornworts (Anthocerotae), a small group of bryophytes (Smith and Griffiths 2000). In these organisms, Rubisco is restricted to a protein body, the pyrenoid, in the chloroplast, and various combinations of bicarbonate and carbon dioxide transporters and localizations of carbonic anhydrase ensure that inorganic carbon is actively moved into the compartment around Rubisco and converted there to carbon dioxide. Study of these systems will contribute to understanding the requirements of a carbon-concentrating mechanism that could perhaps be introduced into a higher plant by genetic engineering.

The key question about *Hydrilla* is: How is carbon dioxide prevented from leaking out of the chloroplast? In conventional C_4 systems, the rate of PEPcase carboxylation is equal to the rate of Rubisco carboxylation plus a leak from the bundle sheath back to the mesophyll. The leakage does not seem to be related to the thickness of the wall of the bundle sheath cell, its suberization in some species, or to chloroplast position, which varies with C_4 subtype (centripetal or centrifugal, i.e., whether chloroplasts in the bundle sheath cells are clustered at the side of the cell nearer to or farther from the vascular bundle). It is believed that leakage is minimized by separating initial and final fixation in two types of cells, by lack of carbonic anhydrase in the bundle sheath cells (so that carbon dioxide is not rapidly converted to bicarbonate), and by a long liquid pathway through the mesophyll cells radially arranged around the bundle sheath. None of these features are present in *Hydrilla*, so does it have high resistance to carbon dioxide diffusion in the chloroplast membrane, or does it rely on the slow rate of diffusion in the surrounding water?

A second question concerns the quantum yield. If *Hydrilla* is scavenging carbon dioxide, then large amounts of ATP are required; as a result, the quantum yield could be small, especially when some inevitable leakage is included. *Hydrilla* is a submerged aquatic and it may be that its C_4 -in-one-cell system does not work out of water, which provides the final slow diffusion barrier reducing leakage from the concentrated carbon dioxide in the chloroplast. In a warm, densely vegetated lake but with abundant solar radiation, a low quantum yield is probably the price to pay for survival and growth, albeit slow.

If these suppositions are true, then *Hydrilla* is less promising as a system to copy for a C_4 rice. It may well be significant that no terrestrial plant has the *Hydrilla* type of C_4 mechanism—evolution has not selected for this probably because it does not work out of water. Keeley (1998) argues that the Kranz-less C_4 photosynthesis of the submerged leaves in species of the grass genus *Orcuttia* is only possible because the water provides a high resistance to leakage of carbon dioxide. The following recommendations emphasize the need for further basic

information about *Hydrilla* and *Borszczowia* before their C₄ mechanisms are copied in C₃ plants.

1. The “gold standard” ¹⁴C pulse-chase labeling experiments should be carried out to confirm the C₄ pathways in *Hydrilla* and *Borszczowia*.
2. The rate of carbon dioxide leakage out of chloroplasts should be quantified, and any mechanism by which this is minimized should be identified.
3. The ratio of photosystems I and II, and electron fluxes, should be measured to establish how much ATP is being synthesized to run the C₄ mechanism in *Hydrilla*. Ultimately, the quantum yield should be measured. This will be difficult and interpretation may not be simple because of concurrent processes in addition to absorption of PAR and fixation of carbon dioxide.
4. A theoretical analysis of the *Hydrilla* and *Borszczowia* systems should be carried out to synthesize the data available from measurements and to suggest new critical experiments. If a model can be constructed, it should be run in a terrestrial setting to see if a C₄ rice with this system is viable.

Kranz anatomy

Kranz anatomy is a conspicuous feature in productive C₄ plants that a C₄ rice should emulate. The types of Kranz anatomy and their development and molecular biology are reviewed by Dengler and Nelson (1999), Sheen (1999), and Dengler and Taylor (2000). C₄ leaves have an anatomical differentiation into mesophyll and bundle sheath cells, with closely spaced vascular bundles so that each mesophyll cell is typically adjacent to a bundle sheath cell with abundant plasmodesmatal connections. This is matched by biochemical differentiation that separates initial fixation of carbon dioxide in the mesophyll cells from final fixation in the bundle sheath cells and shares the reduction of phosphoglyceric acid between the two cell types (Leegood et al 1997). Kranz anatomy appears to be essential for a productive C₄ system, despite the interest in the *Hydrilla* C₄-in-one-cell system. Is Kranz anatomy in the leaf compatible with aerenchymal pathways, which are important in rice to allow oxygen access to the roots? Kellogg (1999), in considering the multiple evolution of C₄ photosynthesis, argues that Kranz anatomy is the central problem and the area of greatest ignorance. Once Kranz anatomy is established, the C₄ pathways, which seem to evolve easily, will follow to produce a C₄ species.

Much less is known about the genes controlling leaf development than those coding for enzymes of the C₄ pathway. Rice already has bundle sheath cells with some chloroplasts. The problem is to obtain more veins, and more photosynthetic activity in bundle sheath cells, but these have limited access to intercellular air space, thus restricting photosynthesis. Selecting for C₄ structure without C₄ biochemistry that enables bundle sheath cells to work is the problem, but evolution does sometimes have to progress from peak to peak across a valley. Little is known about the genetic control of vein pattern formation although a few mutants with altered vein spacing have recently been described (Carland et al 1999). Whether the genes identified by these mutations can be manipulated to produce a C₄ vein pattern is not known. The maize *bsd* mutations have identified genes controlling some aspects of bundle sheath and mesophyll differentiation (Langdale and Kidner 1994), but there are currently no good candidates for genes controlling the development of Kranz anatomy.

The recommendations below are for more general and longer-term research than those for other major topics.

1. An approach suitable for a location with plenty of field space is to study the molecular genetics of *Panicum*, as has been done for *Flaveria*. Cross C₃ and C₄ species in the

genus, self-pollinate the F₁ progeny, and, in the F₂, select for plants with a high degree of Kranz anatomy development, especially complete development. These lines can be backcrossed to C₃ plants with continued selection for Kranz anatomy over several generations. It is necessary to maintain many independent lines; therefore, much space is required over many years. With *Panicum*, more or less all the rice gene markers can be used, owing to the high synteny of grasses, so 5,000–6,000 markers would probably be available. This makes it feasible to identify the genes necessary for Kranz anatomy. Wide crossing between species is a well-developed technique in cereals, using embryo rescue for example. A Japanese plant breeder has already made C₃ × C₄ crosses in *Panicum* and obtained F₂ plants with or without Kranz anatomy (M. Matsuoka, personal communication), so this is a base upon which to build.

2. Look for relevant genes elsewhere by mutagenesis, for example, the *bsd* mutants (Dengler and Nelson 1999) and the pinhead mutant in *Arabidopsis*. Of interest are genes controlling tissue patterns, such as vein spacing, cellular differentiation, and chloroplast replication. Genetic analyses in maize and *Arabidopsis* will be the primary sources of mutations identifying these genes. Are these genes present in rice, as pinhead and *bsd1* are? What is different in these mutants is the program of expression and the changes in regulation, especially temporal. Understanding these differences should suggest methods of introducing Kranz anatomy by genetic engineering.
3. The new technologies of functional genomics are being used to study how all genes in a plant are expressed during growth and development. Comparisons of grain development among cereals may identify genes useful for yield enhancement. A comparison of total gene expression in rice and maize leaves will identify differences in genes and their programs of expression. Some of these differences will be associated with C₄ leaf anatomy. Study of the inducible C₄ system in *Eleocharis vivipara* (see “Background research,” below) with functional genomics could also be fruitful.

Nitrogen in C₄ rice

When annual crops are grown with the minimum nitrogen (i.e., no luxury uptake) to ensure maximal metabolic activity, the nitrogen concentration of the biomass (aboveground dry matter) declines during crop growth (Greenwood et al 1990). This is because an increasing fraction of the crop is composed of structural or storage organs with low nitrogen concentration. Rice is no exception and one conclusion drawn was that the rice crop must take up 50% of its total nitrogen by the time that it acquires 25% of its final biomass (Sheehy et al 1998). Osaki and Shinano (this volume) found that high-yielding crops (receiving 300 kg N ha⁻¹) take up 50% of their total nitrogen after flowering, that is, the ripening phase, thus emphasizing the importance of root activity, and carbohydrate production to feed it, throughout crop duration. Greenwood et al (1990) found that C₃ and C₄ crops fitted curves of the same shape but with different values for the parameters such that the concentration in C₄ crops was always less than that of C₃ crops at the same biomass. This arises because C₄ crops make better use of nitrogen in photosynthesis: the leaves contain less soluble protein because the reduced amount of Rubisco more than compensates for the extra amounts of enzymes of the C₄ pathway (Schmitt and Edwards 1981, Field and Mooney 1986).

During vegetative growth of rice, a positive relationship is found between crop nitrogen content and number of spikelets produced. During grain filling, nitrogen from senescing leaves is transferred to the grain. If there is less nitrogen, because the concentration in leaves of a C₄ rice is lower, then fewer spikelets may be produced, grain filling could be limited by nitrogen

supply, or grains with lower protein content may be produced. Since rice protein content of around 8% is considered low among cereals, there is concern that it should be at least maintained in future high-yielding cultivars. The Greenwood curves are for percentage nitrogen but if a C_4 rice produced more biomass then perhaps crop nitrogen content (kg ha^{-1} , biomass \times %N) could be maintained. Calculations show that this effect is in the correct direction but is not large enough given the shape of the Greenwood curve. To obtain the same nitrogen content (264 kg ha^{-1}) as a C_3 rice yielding 12.5 t ha^{-1} , a C_4 rice would have to produce aboveground dry matter of 42 t ha^{-1} (yield 24 t ha^{-1}), which is not feasible.

The nitrogen concentration of rice grain and straw is variable depending on the cultivar, the yield, and growth conditions, especially nitrogen supply (data supplied by S. Peng). Grain of IR72 in experiments with applied fertilizer in the range of $0\text{--}225 \text{ kg N ha}^{-1}$ varied from 0.7% to 1.48% nitrogen, a nominal protein content of 4.2–8.8% using the standard conversion factor at IRRI of 5.95. A Chinese cultivar yielded 15.2 t ha^{-1} and 1.21% nitrogen (7.2% protein) at Yunnan, China, but 8.6 t ha^{-1} with 1.54% nitrogen (9.2% protein) at IRRI. The nitrogen concentration of the straw was lower (0.42%) at Yunnan than at IRRI (0.75%), so the high-yielding crop extracts more nitrogen from the straw although it still does not attain the protein content of the lower-yielding crop.

One advantage of having lower nitrogen concentration in C_4 plants is that it allows more leaf area to be developed for unit nitrogen and thus full canopy is produced sooner (for the same total amount of nitrogen). Early rapid growth is a general advantage and helps weed control. Luxury nitrogen can be accumulated once the canopy is complete. A plant could be designed to do this: good use of nitrogen at lower concentration to make a larger or earlier canopy followed by further nitrogen accumulation.

Experimental crops grown in elevated carbon dioxide concentration usually have decreased percentage nitrogen through dilution by the larger amounts of soluble (nonstructural) carbohydrate. The results from such experiments may shed light on what happens to spikelet number and grain filling when the percentage nitrogen is lower. Unfortunately, interpretation is difficult even when the relevant data have been recorded and published. For example, experiments at IRRI in the field with open-top chambers (Ziska et al 1997) certainly, showed that elevated carbon dioxide increased yield, by 2 t ha^{-1} , but it was from a depressed value (6 t ha^{-1}) inside the chambers with current carbon dioxide concentration. In the field outside, the yield was 9 t ha^{-1} , so the chamber effect alone was reducing yield substantially. The chamber effect could interact with any effects of carbon dioxide on growth. Earlier work by Yoshida (1973) did not have a chamber effect on yield but was done before climate change was of interest and high carbon dioxide treatments were applied only at individual stages of the crop to study physiological changes. Some of the treatments resulted in higher yields.

Studying the nitrogen economy of species of *Echinochloa* could be revealing. These species have C_4 photosynthesis; some are rice mimic weeds, such as *Echinochloa oryzoides*, and others are minor cereal crops, such as *Echinochloa fruentacea*. If grown in the same way as a rice crop to seed harvest, could they be used to estimate the effect on nitrogen concentration? One objection raised was that rice was a highly developed crop so that its harvest index, for example, would be totally different from that of the C_4 weeds; one might as well grow maize for comparison. This was countered by pointing out that the maize canopy was different in scale and architecture; maize was not aquatic, and root growth was different. The point about the C_4 rice mimics is that all those features are similar to those of rice.

It cannot be known at present whether a C_4 rice would maintain the nitrogen concentration of its C_3 origin or not. If a C_4 rice had C_4 anatomy but the same nitrogen content per unit leaf area (less Rubisco but more of the C_4 pathway enzymes) as C_3 rice, then does the low nitrogen

content of a C₄ plant become less of a problem? One can always go above the Greenwood curve, luxury nitrogen uptake from the point of view of the current metabolism, but making the nitrogen available for grain filling. Osaki and Shinano (this volume) pointed out the key role of PEPcase in linking carbohydrate and nitrogen metabolism through its anaplerotic role in topping up the Krebs cycle. In the various forms of C₄ rice to be produced, this role is likely to be disrupted, and unexpected and possibly negative effects can be expected.

Given the doubts that experimental work could answer quickly the concerns about nitrogen content of a C₄ rice, the recommendations below are mostly about modeling nitrogen in the crop.

1. The calculations made so far have used empirical relationships with limited confidence possible in the predictions. More data are required on nitrogen compounds, pools, and fluxes in the plant to link with nitrogen in the soil and fertilizer application in a system of integrated nutrient management.
2. The equivalences between (1) nitrogenous compounds that occur in rice, (2) fractions that can be extracted routinely from plant material and measured, and (3) components (pools) in models need to be established.
3. A model of nitrogen fluxes in the plant and how they change during crop growth should be produced.
4. Comparative experimental work on rice and its C₄ mimic weeds may provide insights into the nitrogen pools and fluxes of tropical aquatic plants, and discover the differences between C₃ and C₄ plants.

Standards for research in agricultural science

One general recommendation to emerge from the workshop is the need to strive continually for the highest possible standards of observation, experimentation, analysis, and reporting in scientific work. In no sense should the science underpinning agriculture be seen as second class or requiring lower standards than the best science pursued for its own sake. It is particularly difficult to collect reliable and relevant field measurements and greater efforts should be made to produce better data for rice. The discussions after presentations during the workshop were notable for the frank criticism of the precision and relevance of measurements and for the ready acceptance of suggestions for improvement. The following list (components of the recommendation) is not exhaustive and the examples are for illustration and not condemnation.

1. Always provide full descriptions of the crop and measurement conditions. What may seem obvious or normal procedure at the time, so not worth recording, may be obscure to readers less familiar with the system. Omission of details can reduce the value of the results. For example, Yeo et al (1994) did not report the temperature at which they measured quantum yield, making it difficult to compare their results with those of Ehleringer and Pearcy (1983).
2. Do not neglect the theoretical analysis underpinning experimental work. Conocono et al (1998) measured starch and soluble sugars in rice leaves at intervals during the day and claimed that the difference between dawn and dusk measurements could be used to estimate the daily total of canopy photosynthesis, which is difficult to measure. But unlike Servaites et al (1989), they did not analyze the system to take account of continuous loss of soluble sugars during the day and the effect this would have on the dusk measurement. The difference between dawn and dusk is not a simple accumulation but a sum of translocation, which is constant (Geiger and Servaites 1994), and of

accumulation, which varies in rate depending on the PAR received by the canopy and on stomatal conductance.

3. The best method (the gold standard) for establishing that C₄ photosynthesis is occurring is by ¹⁴C pulse-chase labeling. The label is first seen in C₄ organic acids, then decreases in these and occurs in C₃ compounds. Ideally, this method should be used on *Hydrilla* to confirm unequivocally the other evidence for a C₄ pathway.
4. In transgenic work, obtain the T₃ generation. at least, before undertaking detailed physiological characterization, or before any thought of using the transgenic line in breeding. All sorts of curious and unstable features, including quasi-heterosis, may be present in the T₀ and T₁ generations, which could deceive with an apparent increased growth and yield that turns out to be unconnected with the transferred gene. Producing a stable T₃ generation is the first task and inevitably takes 2–3 years.
5. Remember that what is measured in a physiological investigation may not be relevant to the plant in the field. Not only in quantum physics does the observer perturb the system so as to make it difficult to measure true values! Leaf temperature recorded in a gas exchange chamber fastened to a leaf is relevant to photosynthesis measured in the chamber but can differ greatly from the temperature of the leaf before enclosure. The chamber alters the heat exchange of the leaf in many ways. For irrigated tropical rice, leaf temperature is often 3–4 degrees below air temperature from the cooling effect of transpiration (typically up to 30 °C), but temperatures close to 40 °C can be recorded in gas exchange chambers (Horton and Murchie, this volume).
6. Modeling work must use the best current understanding of the system. For example, use of the most recent figures for the stoichiometry of photosynthesis changed the conclusions reached by Mitchell and Sheehy (this volume) in an earlier draft.

Parallel research

These topics require research to back up improvements made in rice photosynthesis.

1. *Lodging*. The analysis of Setter et al (1994) and the recommendations of Cassman (1994, p. 135) are still valid. Little work seems to have been done since then and lodging continues to reduce yields and inhibit full use of nitrogen fertilizer.
2. *Harvest index*. Austin et al (1980) are sometimes cited as evidence for a maximum harvest index of 0.6 for wheat or even for cereals in general. Their calculations were of a harvest index for winter wheat if aboveground dry matter remained constant but half the stem weight was transferred to grain. The equivalent “what-if” calculation has not been carried out for rice but could be valuable if combined with consideration of the dry matter and strength required of stems to resist lodging.
3. *High temperature and fertility*. Rice spikelets show a sigmoid response curve to air temperature from 100% fertility at 33 °C to 0% at 40 °C (Horie et al 1995). Since 33 °C air temperature does occur at IRRI, rice crops are on the threshold of declining fertility with temperature. It is unclear how the high-temperature sterility can be avoided, or whether there is variation in tolerance among rice varieties from various hot climates. Since the high-temperature effect is not likely to involve photosynthesis, this topic is placed in parallel research, and will assume particular importance if tropical air temperatures around the dates of rice anthesis rise as part of global warming.
4. *Nitrogen supply to high-yielding crops*. Greenland (1997) has shown that long-term sustainable yields of irrigated rice cannot exceed about 1 t ha⁻¹ without the use of

fertilizers. Given a grain yield and harvest index, the desired nitrogen content of grain (e.g., 1.4% for 8.3% protein), and a lower limit for the nitrogen content of straw (probably in the range of 0.4–0.6%), then the aboveground nitrogen demand of the rice crop can be computed (Sheehy 2000). Subtracting the total supplied from the soil, water, and biological fixation leaves a large amount to be provided as fertilizer. With 50% fertilizer recovery, twice this amount must be applied to the field, in the region of 250–500 kg N ha⁻¹. Forms of fertilizer and methods and timings of application that greatly enhance recovery by minimizing losses (volatilization or in drainage) are urgently required.

Background research

It cannot be claimed that the following topics are likely to make a contribution to improving rice photosynthesis in the short term. Nevertheless, this basic research builds the foundation of knowledge from which advances may arise in the future.

1. *Physiological ecology of aquatic plants.* Little seems to be known about the physiological ecology of wetland plants, especially, the behavior of stomata. Research is difficult in tropical wetlands because of the harsh environment for fieldwork, for personnel, and for instruments. Understanding the performance of natural wetland vegetation will put the physiological ecology of rice in context. Is rice a typical tropical wetland plant or has cultivation for several thousand years introduced modifications?
2. *Comparative study of Echinochloa.* The rice mimic weeds in this C₄ genus have a physiological ecology similar to that of rice but there are differences apart from photosynthetic pathway. For example, hydrophobic bristles (stiff hairs) on the leaf lamina and sheath of rice retain an air layer between the plant surface and the water through which oxygen moves by mass flow downward (known as snorkeling). *Echinochloa* is not thought to do this. What are the tissue nitrogen concentrations in *Echinochloa* and do they limit seed formation?
3. *Unusual C₄ plants.* Apart from *Hydrilla*, the family Hydrocharitaceae is poorly studied. It contains *Vallisneria spiralis*, which fixes 20% of carbon into C₄ acids and is classed as a C₃-C₄ intermediate (Sage et al 1999), and the family may have other C₄ oddities, including species switching between C₃ and C₄. *Eleocharis vivipara* is a sedge with photosynthetic culms, which has been studied in detail by Ueno et al (1988) and Ueno (1996a,b). The submerged culms are C₃ but the emergent culms are C₄ with the Kranz anatomy characteristic of sedges (Dengler and Nelson 1999, p. 140). Numerous aquatic plants in vernal pools have been studied by Keeley (1999), who classified *Eleocharis acicularis* as a C₃ + C₄ species because initial fixation of carbon dioxide occurs by both pathways when the plant is submerged, early in its life cycle. The terrestrial leaves, however, are C₃, thought to be a response to the greater availability of carbon dioxide in air than in the water of the vegetated vernal pool. *Orcuttia* has been shown to have two types of anatomy: the terrestrial leaves are C₄ with Kranz anatomy, but submerged leaves have C₄ biochemistry without Kranz anatomy (Keeley 1998). The implication is that C₄ photosynthesis without Kranz anatomy is possible only underwater. Study of these species will increase knowledge of the natural range of methods of concentrating carbon dioxide and could aid the design of artificial systems to be constructed with genetic engineering.

Envoi

Nearly 30 recommendations for research are made above. Setting priorities among topics so diverse in subject, scope, and time-scale would be difficult. Several general directions of research can be identified, however, given the overall aim of improving rice photosynthesis as a major contributor to increasing yields of rice. First, improving C_3 photosynthesis and primary partitioning will be useful for rice as a C_3 plant and enable it to benefit from rising atmospheric carbon dioxide concentration and from any success in constructing a C_4 rice. Second, construction of a C_4 rice is an ambitious and long-term project requiring high investment but offering high returns. Kranz anatomy is almost certainly necessary as an ultimate goal. Rice transgenic with maize C_4 enzymes or rice with a C_4 -in-one-cell system is worth pursuing in case simpler options can be found more quickly, or incidental benefits can be exploited. Third, research on photosynthesis itself must be complemented by other research, on nitrogen and lodging in particular, to enable the benefits of improved photosynthesis to be fully exploited in a rice crop by farmers.

We hope that researchers, institutions, and funding organizations will find attractive and productive topics for research among the recommendations made above. In the coming decades, relief from hunger and poverty for a substantial fraction of Earth's population, and food security and political stability for everyone, will depend, in part, on success in research to increase the yield of rice.

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