

7 Opportunities for Increasing Water Productivity of CGIAR Crops through Plant Breeding and Molecular Biology

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In regions experiencing an absolute or economic shortage of water, there is an urgent need to increase the water productivity (WP) of crops through breeding or natural-resources management. The options for increasing WP through breeding are: (i) to reduce non-transpirational uses of water; (ii) to reduce transpiration without reducing production; (iii) to increase production without increasing transpiration; and (iv) to enhance tolerance of water-related stresses – drought, salinity and waterlogging or submergence. The Green Revolution achieved substantial increases in WP for rice and wheat by reducing the crop duration and increasing the harvest index. Progress in extending these achievements to other Consultative Group on International Agricultural Research (CGIAR)-mandated crops has been considerable and will accelerate following the recent cloning of several of the underlying genes. The success of breeding for greater WP depends heavily on the use of physiological, molecular and genetic methods to identify useful alleles within the genetic resources held by the CGIAR centres, which, in consequence, have a clear comparative advantage in this important enterprise. The complete sequencing of the *Arabidopsis* and rice genomes will provide crucial help in the discovery of genes for other WP-related traits in all mandated crops. It will soon be

possible to enhance WP in popular varieties by DNA-assisted backcrossing, a more efficient breeding strategy than the conventional pedigree method based on phenotypic selection. A major application of molecular breeding is to increasing drought and salt tolerance, particularly at the stress-sensitive flowering stage, with benefits to both water economy and farmers' livelihoods.

The productivity of crops is commonly measured in relation to inputs such as capital, land, energy and labour or fertilizer (Pingali and Heisey, 1999; Socolow, 1999; Ball *et al.*, 2001). In recent years, increasing attention has been given to crop productivity in relation to water consumption (Seckler *et al.*, 1998). WP is an important issue in arid regions that are already experiencing an absolute shortage of water (or are projected to do so in the near future) and in other regions where an economic shortage of water leads to severe competition among water consumers. The magnitude of the problem is illustrated by the fact that agriculture accounts for about 70% of human water use (WMO, 1997). Furthermore, much of the water used in agriculture is lost to the atmosphere as a result of evaporation from the soil and transpiration from leaves, whereas much of the water used for industrial and domestic purposes may be recycled. For every kilogram of grain produced, cereal

plants transpire about 1000 kg of water (Tuong, 1999; Tuong and Bouman, 2002, Chapter 4, this volume).

Seckler *et al.* (1998) presented four options for addressing the limitations imposed on crop production by shortages of water. They were: (i) development of additional water resources and water-storage facilities; (ii) increased productivity of existing water supplies; (iii) regional diversion of water; and (iv) increased importation of food. These options can be interpreted entirely in terms of infrastructural development and water engineering, but their implementation would be more effective and sustainable if accompanied by the breeding of crops with greater WP. Such crops would be valuable both in the intensive cropping systems on which global food security rests (Cassman, 1999) and in the stress-affected ecosystems on which 650 million poor people rely for food and livelihood (Alexandratos, 1999). You (2001) proposed that rice cultivation in China should be reduced in favour of more water-efficient crops and that Chinese rice requirements should be met by imports. However, this proposal does not take into account the steps taken in China to increase the WP of rice through breeding and water management (Dong *et al.*, 2001).

This chapter focuses on the opportunities for increasing crop WP through breeding. The first section takes a general look at water use by plants and by farmers and the implications of these uses for WP at the crop level. The second section discusses the opportunities for increasing WP by focusing on four types of trait. The third section focuses on breeding for drought tolerance and the prospects for improving breeding efficiency through genomics.

Water Use and WP at the Crop Level

WP in different cropping systems

The definition of WP differs at different scales of water management. Dong *et al.* (2001) used three definitions of WP in their study of rice cultivation. The WP per unit of evapotranspiration (WP_{ET}) is the mass of

crop production divided by the total mass of water transpired by the crop and lost from the soil by evaporation. The WP per unit of irrigation water (WP_I) is the crop production divided by the irrigation flow. The WP per unit of gross inflow (WP_G) is the rice production divided by the rain plus irrigation flow. Two related and widely used physiological concepts are water-use efficiency (WUE), defined as crop production per unit transpiration, and transpiration efficiency (A/T), defined as the ratio of photosynthesis (A) to transpiration (T) (Peng *et al.*, 1998). Scientific disciplines differ in the importance they have given to these parameters.

The most appropriate measure of WP varies with the cropping system. For an entirely rain-fed cropping system, in which no attempt is made to trap rainfall on farm for later use, the goal is to maximize WP_G by planting crops and varieties that utilize rainwater fully and efficiently. WP_{ET} is a valuable parameter by which to judge the water efficiency of different crops, varieties and agronomic practices. A comparison between WP_{ET} and WP_G provides information on the availability of rainwater to the crop, as a result of the root structure of the plant or the structure of the soil. In the absence of irrigation, WP_I is irrelevant.

For a rain-fed cropping system with supplementary on-farm irrigation from surface reservoirs or underground aquifers, WP_I will be a sensitive measure of the timeliness of the irrigation, while WP_{ET} will be a guide to the water efficiency of different crops, varieties or agronomic practices. Cabangon *et al.* (2001) found that WP_{ET} for rice increased from 0.48 g kg⁻¹ under dry seeding to 0.53 g kg⁻¹ under wet seeding and to 0.61 g kg⁻¹ under transplanting. As direct rainfall and on-farm irrigation are difficult to divert to off-farm uses, the value of knowing WP here lies in maximizing production rather than making decisions about water use.

The situation is different for a rain-fed cropping system with supplementary off-farm irrigation or for arid-land farming that is continuously dependent on off-farm water. Here it is likely that water will have to be purchased, possibly in competition with other users. WP_I will then help in decision-

making about water allocation. It is important that irrigation water be available at the stages in plant growth (seedling emergence and flowering) when yield is most affected by water shortage. To be sure of supplies at these crucial times, farmers may invest in on-farm storage of off-farm water. Breeders will, of course, wish to develop varieties whose yield is not affected by any water deficit that occurs at these times. They will also wish to make the crop tolerant to low-quality irrigation water (through salt tolerance) and to the waterlogging or flooding that results when rainfall exceeds the sum of evapotranspiration (ET) and seepage.

It is instructive to compare the relevance of WP in the least productive and most productive cropping systems. Poor farmers in rain-fed areas often contend with drought, salinity and waterlogging/flooding. They wish to maximize WP_G , even though they lack the resources to accomplish this objective through water or land management. They look to plant breeding to maximize WP_G through the enhanced tolerance of crops to water-related stresses. One of the most productive cropping systems in the world is the rice–wheat double-cropping system of central and southern China and the Indo-Gangetic plain (Cassman, 1999; Timsina and Connor, 2001). Rice and wheat are grown in summer and winter, respectively. Depending on location, these crops may be largely rain-fed or largely irrigated. Both crops are likely to experience temperature stress and drought at the start and the end of each growing season. In addition, rice may experience submergence in midsummer, and wheat may experience early-season waterlogging and mid-season frost. As provision of irrigation and drainage is expensive, many rice–wheat farmers require cultivars that maximize WP_G through stress tolerance.

WP_{ET} takes into account only water evaporated or transpired and is, therefore, focused on plant behaviour. WP_I and WP_G include not only ET but also water used in other ways for crop production and water that is wasted. In the next two sections, I take a closer look at water use by plants and farmers to gauge opportunities for and limitations on increasing WP.

Water use by plants

Crops and natural vegetation are major users of water. This need arises from four features of plants:

- When plants open the stomata of their leaves to admit atmospheric CO_2 for photosynthesis, they lose water vapour through the same openings, a process known as stomatal transpiration. Many photosynthetic parameters (e.g. electron transport rate, carboxylation efficiency, intrinsic WUE, respiration rate in the light, etc.) are more strongly correlated with stomatal conductance than with water status itself (Medrano *et al.*, 2002).
- Even when stomata are closed, leaves and stems of many species may lose water by transpiration through non-stomatal surfaces.
- Transpiration also serves to cool leaves exposed to high air temperatures, low atmospheric water-vapour pressures or the heating effect of sunlight (Radin *et al.*, 1994).
- Plants use the transpiration stream to transport to the leaves both inorganic nutrients from the soil and a range of chemicals synthesized in the roots, including signal molecules that contribute to whole-plant integration (Peuke *et al.*, 2002).

To satisfy these requirements, plants will transpire in a growing season several hundred times more water than is present in their tissues at any one time.

If plants do not receive enough water to maintain high rates of photosynthesis, total dry-matter accumulation will decline, plant development will be affected and yield will be lost. The extent of yield loss depends on the timing, duration and intensity of the water deficit (Boonjung and Fukai, 1996). It is particularly important that plants gain access to water at the seedling and flowering stages, when yield is most sensitive to water deficit. Poor farmers rely entirely on soil moisture and rainfall to provide water for their crops. They could plant varieties with deep roots (in the uplands) or penetrating roots (in the lowlands) to explore a great vol-

ume of soil – a strategy known as drought avoidance. If the rainy season is short and fairly reliable, farmers may plant short-duration varieties that complete flowering before soil moisture declines at the end of the season – the drought-escape strategy. Some low-land varieties can adapt to a slow onset of drought by modifying their chemical constitution to retain as much water as possible through osmotic adjustment and to protect themselves from irreversible damage during stress – the strategy of drought tolerance. A major challenge for breeders is to produce new varieties that display high yield potential under or after drought stress.

Water use by farmers

Farmers with the financial resources to provide supplemental or continuous irrigation achieve much higher yields than farmers in rain-fed environments (Table 7.1), but whether WP_{ET} increases or decreases with irrigation depends on a variety of factors. WP_{ET} may increase if irrigation is supplied at germination and flowering, when water deficit is most damaging, but WP_{ET} may decline if unnecessary amounts of water are supplied at less sensitive stages of the growth cycle or if significant losses occur through evaporation. Farmers may also use large amounts of water for weed control, for growth of a legume crop as green manure, or for flushing salt and other toxic chemicals from the soil. Other uses include provision of water to moderate high or low temperatures through a microclimatic effect, a practice that tends to be limited to rice because of its ability to tolerate flooding of its root system.

These practices increase yield but tend to reduce WP. Their elimination can be expected to increase WP, provided yield is maintained by using other approaches to control weeds, supplying N fertilizer, etc.

Plant Traits to Exploit for Increased WP

This section discusses four groups of plant traits that can be exploited to enhance WP. They are: (i) traits that reduce the non-transpirational uses of water in agriculture; (ii) traits that reduce the transpiration of water without affecting productivity; (iii) traits that increase production without increasing transpiration; and (iv) tolerance of three water-related stresses (waterlogging/flooding, salinity and drought). In Table 7.2, these traits are accompanied by an estimate (high, medium, low) of the probability that major progress will be made in the next 5 years. The probability is declared to be high where progress has already been made towards identifying regulatory genes. The probability is considered medium where some of the genes of a relevant pathway have been isolated and, therefore, provide an entry point to the identification of regulatory genes. The probability is described as low where few or no relevant data are available.

Traits that minimize non-transpirational uses of water in agriculture

One of the major water-saving innovations in rice production (measured as WP_G rather than WP_{ET}) is the switch from transplanting to direct seeding (Cabangon *et al.*, 2001), but

Table 7.1. A large minority of rice farmers and consumers depend on rain-fed rice production.

Parameter	Irrigated ecosystem	Rain-fed ecosystem
Production (% of total)	75	25
Land area (% of total)	45	55
Chemical inputs	High	Low
Average yield ($t\ ha^{-1}$)	> 5.0	< 2.3
Consumers (billion)	> 1.2	> 0.8

Table 7.2. Genetic approaches to increasing crop water productivity.

Water-productivity factor	Genetic approach	Probability of major progress in 5 years
Minimize non-transpirational uses of water	Herbicide-resistant crop	Low ^a
	Weed competitiveness	Low
	Heat and cold tolerance at flowering	Medium
	More efficient cooling via evapotranspiration	Medium
	Nitrogen-use efficiency	Medium
	Nitrogen fixation	Low
Reduce transpiration without reducing production	Waxy-cuticle production	Medium
	Rapid stomatal closure	High
	Cooling mechanism for leaves	High
	Rapid canopy closure	Low
	Thicker, more intact Casparian strip	High
Increase production without increasing transpiration	Sustainable production of aerobic rice	Medium
	Short duration, seedling vigour	High
	Higher harvest index	Medium
	C ₄ photosynthesis	Medium
	More photosynthesis per unit water transpired	Low
	More dry matter allocated to grain after stress	Medium
Use cheaper water	Stay-green flag leaf	Medium
	Tolerance of salinity	High
Less water management	Tolerance of waterlogging	Medium
	Tolerance of submergence	High

^aTransgenic mechanism is currently available but its deployment is problematic.

weeds then become a major problem. Herbicide-resistant rice would be a solution, but concerns exist about the spread of herbicide-resistance genes to wild or weedy rice (Rieger *et al.*, 2002). Plants have genetic systems in the nucleus, the mitochondrion and the chloroplast. Insertion of genes for herbicide resistance into the nuclear genome may result in the spread of this trait to wild relatives of crop species, an event that would undermine the value of this trait. As most crop plants show maternal inheritance of plastid DNA ('plastome'), pollen escaping from plants transformed in the plastome will not transmit herbicide-resistance genes to nearby weedy relatives. Daniell *et al.* (1998) reported transformation of the plastome of petunia with the 5-enolpyruvyl Shikimate-3-phosphate (EPSP) synthase gene, which confers resistance to the herbicide glyphosate. Of course, it would still be possible for pollen from weedy relatives to fertilize the transgenic plant and thereby create a par-

tially weedy, herbicide-tolerant hybrid in farmers' fields. Although the rate of this process would be extremely low, given the high self-fertilization rate of elite crop species, further research on eliminating the spread of herbicide resistance will be required before this potentially valuable trait is likely to be widely acceptable. In an alternative approach, the International Rice Research Institute (IRRI) is working with the West Africa Rice Development Association (WARDA) to transfer weed competitiveness from the African cultivated rice *Oryza glaberrima* to the Asian cultivated rice *Oryza sativa*. The basis of this trait appears to be seedling or vegetative vigour.

Green manure is a valuable source of organic nitrogen fertilizer (Ladha and Garrity, 1994). It is derived from short-duration legume crops or the N₂-fixing fern *Azolla*. The use of green manure is frequently advocated but it is often limited by water supply. As water becomes scarcer, it is even less likely

that farmers will use green manure, in spite of its benefits. Chemical N fertilizer is widely available but still expensive for many farmers. N-use efficiency is thus a trait that will be highly valued by farmers. A radical alternative approach is to develop N₂ fixation in non-leguminous crops, such as cereals. IRRRI pursued this approach until recently. It became clear that rice plants are to some extent 'Rhizobium-ready' (Kouchi *et al.*, 1999). *Rhizobium* is closely related to *Agrobacterium*, another bacterium that naturally forms relations with dicotyledons but not with cereals. For many years, attempts to achieve *Agrobacterium*-mediated transformation of cereals were of limited success, but, once acetosyringone from potato was introduced as an activator of *Agrobacterium* virulence genes, cereal transformation by this bacterium became routine (Hiei *et al.*, 1997). Is it possible that a breakthrough of similar simplicity will enable cereals to form a symbiotic relationship with *Rhizobium* and fix their own nitrogen?

Australian rice farmers use an extra depth of floodwater (> 20 cm) to create a warmer microclimate during the flowering stage (Williams and Angus, 1994). Without this effect, cold southerly winds cause considerable pollen sterility in high-N plants. Rice is uniquely suited to this use of water because aerenchyma cells in the stem and the root provide a means of overcoming the root anoxia that makes other crops sensitive to waterlogging and flooding (Dennis *et al.*, 2000). This use of water could be dispensed with and WP₁ could be significantly enhanced if cold tolerance at the reproductive stage could be increased in rice. Several examples of the enhancement of cold tolerance in rice have been reported (Sakamoto *et al.*, 1998; Saijo *et al.*, 2000). This trait would be equally valuable in other crops that suffer from the effect of cold air during flowering, even if no savings in WP₁ were involved.

Traits that reduce the transpiration of water without affecting productivity

Traits that decrease water loss to the air include a waxy cuticle and stomatal closure. It is not clear to what extent the residual

water loss from leaves is due to the permeance of the cuticle or to the incomplete closure of stomata (Riederer and Schreiber, 2001). The water permeances of leaf cuticular membranes from 21 plant species were found to cluster according to life form and climate of origin (Schreiber *et al.*, 1996). The lowest water permeances were observed with evergreen leaves from epiphytic or climbing plants growing naturally in a tropical climate. The next group in the order of increasing cuticular permeance comprised xeromorphic plants typically growing in a Mediterranean-type climate. The group with the highest water permeances comprise deciduous plant species with mesomorphic leaves growing in temperate climates. One of the impediments to defining genes that may be useful in decreasing the water permeance of the cuticle is that the chemistry responsible for determining the permeance has not yet been identified, although cutin polymers, their cross-linking, their esterification and their association with epicuticular waxes have all been implicated (Riederer and Schreiber, 2001).

Much more is known about the controls on stomatal closure in response to drought, salinity, soil compaction and other stresses (Luan, 2002; Roberts *et al.*, 2002). Although it is clear that leaf abscisic acid (ABA) promotes stomatal closure, the role of root-derived ABA as the principal long-distance signalling molecule for stress-responsive stomatal closure has recently been challenged by root-grafting experiments (Holbrook *et al.*, 2002). The ABA-dependent events inside guard cells are emerging, including the coordination afforded in *Arabidopsis* by AtRac1, a small guanosine triphosphatase (GTPase) (Lemichez *et al.*, 2001). AtRac1 inactivation by the protein phosphatase abscisic acid-insensitive 1 (ABI1) is the limiting step in the ABA-triggered signalling cascade leading to stomatal closure.

ABA-mediated stomatal closure is not enough to guarantee drought tolerance. Although it reduces water loss and helps to ensure that the soil-root-xylem-leaf-air hydraulic continuum remains intact, it does commit plants to a period without photosynthesis while radiation continues to be

absorbed, and ABA may have undesirable side-effects on panicle development (Westgate *et al.*, 1996). It is interesting that the recessive *abh1* mutant of *Arabidopsis* shows ABA hypersensitivity and reduced wilting under drought (Hugouvieux *et al.*, 2001). The *ABH1* gene encodes an mRNA cap-binding protein that acts as a modulator of ABA signalling through alteration of transcript levels for early ABA-signalling elements.

Transpiration is regarded as the only productive water outflow at the field level because it contributes to plant growth by promoting photosynthesis and leaf cooling. However, it is possible that only a fraction of transpiration is actually beneficial and the remainder is wasteful. Peng *et al.* (1998) compared the ratio of photosynthesis (A) to transpiration (T) of seven tropical japonica rice varieties developed by IRRI with those of seven of IRRI's indica varieties. The A/T was determined 1 week after flowering for all varieties and throughout the growing season for one genotype from each type. Both A and T were measured on the topmost fully expanded leaves under saturating light with a portable photosynthesis system. Indica varieties had a higher T than the tropical japonica lines. The differences in A between the two types were relatively small and inconsistent across growth stages and years compared with the differences in T. The A/T was 25–30% higher for the tropical japonica than the indica type over 2 years. A lower carbon isotope ($^{13}\text{C}/^{12}\text{C}$) discrimination in a tropical japonica line than in an indica variety confirmed that the improved tropical japonica lines had higher A/T values than the indica varieties. These data indicate that significant variation exists in the rice germplasm for the A/T ratio. It would be important to determine whether a higher A/T translates into a higher WP_{ET} .

The role of transpiration in keeping leaves cool is a potential source of difficulty for breeders. As stomatal transpiration and non-stomatal transpiration decline, is it possible that leaf temperature will rise and inhibit production? One possible way of dealing with this issue is to enhance the heat tolerance of crops by non-transpirational means. Two such approaches are through the

expression of genes for the heat-shock 101 (HS101) proteins (Queitsch *et al.*, 2000) and ascorbate peroxidase (Shi *et al.*, 2001).

Plants also lose water to drying soil. Since this water eventually contributes to evaporation and thereby reduces WP_{ET} , any trait that reduces such water loss is relevant here. Lignin and suberin form the Casparian strip and play important roles in protecting the stele of roots from water loss (Zeier *et al.*, 1999). Some of the key genes regulating the biosynthesis of these hydrophobic molecules are known. Caffeoyl coenzyme A (CoA) O-methyltransferase is a rate-limiting step in the biosynthesis of lignins and suberin (Inoue *et al.*, 1998), and peroxidase activity is essential for later steps in the biosynthesis of these molecules (Roberts and Kolattukudy, 1989). An important step forward will be the identification of transcription factors that upregulate these biosynthetic pathways in response to stress.

Following the lead of agronomists in China and Brazil, IRRI has begun to study the feasibility of establishing a high-input, non-puddled irrigated system for upland rice (Bouman, 2001). Known as 'Han Dao' in China and 'aerobic rice' at IRRI, this system has potential for large water savings, especially on soils with high seepage and percolation rates. Han Dao varieties yield 6–7.5 t ha⁻¹ under flash irrigation in bunded fields in north-east China (Wang and Tang, 2000). It will be necessary to breed new varieties that are adapted to this 'aerobic' ecosystem in the tropics.

Traits that increase production without increasing transpiration

One of the most important traits in this category is the harvest index (HI), the proportion of total above-ground dry matter allocated to the harvested organs (e.g. tubers, fruits or seeds). A higher HI was one of the key traits of the high-yielding modern rice and wheat varieties that contributed to the Green Revolution (Khush, 2001). Manipulation of the HI may be achieved at the level of plant architecture and at the level of carbon allocation. At the architectural level, breeders have

identified dwarfing genes that reduce vegetative biomass of cereals without affecting grain yield. The *sd1* gene for the semi-dwarf trait of rice has been cloned (Sasaki *et al.*, 2002). It encodes an inactive variant of gibberellic acid 20 (GA₂₀) oxidase-2, an enzyme of gibberellin biosynthesis. The wild-type version or allele of this gene is designated *SD1*. Three different mutant *sd1* alleles from rice germplasm prevent GA biosynthesis and confer the semi-dwarf trait. However, another copy of the gene (GA₂₀ oxidase-1) is expressed in flowers and supplies enough GA to promote normal grain filling. The *Rht* dwarfing gene of wheat is a transcription factor in the GA biosynthetic pathway (Peng *et al.*, 1999). As a gain-of-function mutation, it may be useful in reducing the height of other important crop plants and for enhancing WP, because water use is determined by biomass rather than linked to yield.

At the level of carbon allocation, a larger HI implies that a greater proportion of carbohydrate has been deposited in the harvested product, e.g. as starch in cereal grains. The ability of seeds or fruit to accumulate carbohydrate depends on their sink strength. There is a growing consensus that a key step is the unloading of sucrose from the phloem through irreversible hydrolysis by the apoplastic invertase of the sink organ (Fridman *et al.*, 2000; Druart *et al.*, 2001; Nguyen-Quoc and Foyer, 2001). Pollen sterility and embryo abortion in drought-stressed cereals are associated with low levels of apoplastic invertase (Zinselmeier *et al.*, 1999; Saini and Westgate, 2000). Pollen-based male sterility was induced in plants by downregulation of the anther apoplastic invertase by the antisense approach (Goetz *et al.*, 2001). The antisense form of the invertase gene contained part of the gene sequence in the reverse orientation. When this antisense construct was introduced into plants, cells accumulated a novel form of mRNA, which bound strongly to normal invertase mRNA molecules, preventing them from directing the synthesis of invertase molecules. Chopra *et al.* (2000) implicated another sucrose-hydrolysing enzyme, sucrose synthase, in determining the sink strength in mung-bean seeds. Unlike apoplastic invertase, sucrose

synthase is cytosolic and catalyses a reversible reaction.

Where the rainy season is reliable but comparatively brief, short duration is a trait that increases WP in crops by permitting drought escape. In such locations, flowering occurs before the onset of terminal drought. In locations with adequate rain throughout the year, short duration can enhance WP by permitting multiple cropping. Although short duration may be selected visually, a molecular understanding is expected to facilitate more precise control of the flowering date. Yamamoto *et al.* (2000) noted that 23 major genes and numerous quantitative trait loci (QTL) for heading date have been reported for rice, a plant with accelerated flowering under short days. A QTL is a genetic locus associated with some fraction of the variance of a quantitative trait, such as heading date. The variance explained by individual QTL varies from large to small. QTL of large effect are few but are readily mapped and potentially useful in breeding. QTL of small effect are numerous and difficult to map with sufficient accuracy for use in breeding. In the case of heading date, however, several QTL of large effect are known. One of such QTL, *Hd1*, was first identified in a segregating population derived from the cross Nipponbare/Kasalath, with the Nipponbare allele reducing the heading date relative to the heading date of Kasalath. *Hd1* has been cloned and shown to encode a zinc-finger transcription factor (Yano *et al.*, 2000). *Hd1* is an allele of the major photoperiod sensitivity gene *Se1*. The Nipponbare allele of this gene, when introduced into rice by transformation, reduced the time to heading under short days from 88 days to 58 days. DNA-based selection may now be used to achieve the same goal. Shorter duration has also been induced in rice by the *Arabidopsis* floral-transcription factor *LEAFY* (He *et al.*, 2000).

The new varieties of the Green Revolution also enhanced WP. Traditional cultivars are of long duration (150–180 days), with flowering triggered by changes in day length (longer day length for winter wheat and shorter day length for summer maize and rice). The development of photoperiod-

insensitive varieties of short to medium duration (90–120 days) enabled crops to increase WP by escaping the late-season drought, which adversely affects flowering and grain development. Shorter duration also permitted double cropping and triple cropping, which make more efficient use of monsoonal floods. By increasing yield and simultaneously reducing crop duration (and therefore the outflows of evapotranspiration, seepage and percolation), the modern varieties of rice have a WP_G that is about three-fold higher than that of traditional varieties (Tuong, 1999; Tuong and Bouman, 2002, Chapter 4, this volume).

In most genotypes of sorghum, drought during grain filling hastens leaf senescence, leading to premature death. Stay-green genotypes, in contrast, retain more green leaf area and continue to fill grain normally under drought conditions (Rosenow *et al.*, 1983). Moreover, there is a positive association between stay-green and grain yield under water-limited environments (Borrell and Douglas, 1996). Although the stay-green trait may involve more transpiration as the leaves remain active longer, they appear to give higher yields without requiring supplemental irrigation, increasing WP_G and perhaps even WP_{ET} . QTL *Stg1* and *Stg2* contribute to this trait; they have been mapped but not isolated as yet (Xu, W. *et al.*, 2000).

In 1999, IRRI held a symposium on the theme of achieving C_4 photosynthesis in rice, a C_3 plant (Sheehy *et al.*, 2000). The rationale was to explore the feasibility of achieving in rice the productivity, N-use efficiency and WUE of C_4 plants, such as maize and sorghum, in a plant producing rice grain. Yeo *et al.* (1994) surveyed photosynthetic gas exchange in 22 of the 23 species of the genus *Oryza*. Some species with the highest assimilation rates were assessed for photorespiratory losses, and these were generally around 30%, i.e. similar to those of *O. sativa* varieties. However, a range of *Oryza rufipogon* accessions had photorespiration rates significantly lower than the *O. sativa* genotypes tested. No species in the genus possessed C_4 photosynthetic metabolism, although some did overlap, with compensation concentrations and phosphoenolpyruvate (PEP) carboxylase

activities reported for C_3 – C_4 intermediate species. Nevertheless, the high level of expression of C_4 maize genes observed in transgenic rice by Ku *et al.* (1999) is indicative of progress using biotechnological tools. Transgenic rice overexpressing maize C_4 -type PEP carboxylase, pyruvate- P_i dikinase or both exhibited superior photosynthetic and yield traits (Ku *et al.*, 2000, 2001). However, these superior traits were associated with reduced stomatal resistance and might, therefore, be associated with enhanced transpiration. Measurements of WP_{ET} for these transgenic plants are clearly highly desirable.

Waterlogging and flooding

Waterlogging and flooding are common in rain-fed ecosystems, especially on soils with poor drainage. They can seriously reduce yield (Dennis *et al.*, 2000) and are among the stresses considered by the Food and Agriculture Organization (FAO) and the International Institute for Applied Statistical Research in their estimates of global arable land area and global productivity (Fischer *et al.*, 2001). Roots obtain oxygen for growth and mineral uptake from air pockets in the soil, but, when roots are partially submerged (waterlogged) or completely submerged (flooded), the anoxic conditions prevent root growth and send signals to the rest of the plant to reduce shoot growth and plant productivity. Plants such as rice are tolerant to waterlogging because of their well-developed aerenchyma tissues in the roots and the stem. Some rice varieties, such as FR13A, are even tolerant of 2 weeks of submergence of the entire plant. In a genetic analysis of rice, Xu and Mackill (1996) localized a major gene for submergence tolerance on chromosome 9 (*Sub1*). Nandi *et al.* (1997) additionally localized minor QTL for submergence tolerance on chromosomes 6, 7, 11 and 12. Xu, K. *et al.* (2000) fine-mapped *Sub1* from FR13A, using a very large mapping population derived from a cross between M202 and a derivative of FR13A. Two markers co-segregated with *Sub1* and others were at a distance of 0.2 centiMorgans (cM) on the genetic map of rice

(or ~60 kbp at its position on the physical map). The high-resolution map should serve as the basis for map-based cloning of this important locus, as it will permit the identification of bacterial artificial chromosome (BAC) clones (~150 kbp) spanning the region. Sripongpangkul *et al.* (2000) mapped a gene for submergence tolerance from the cultivar IR74; it mapped to the same location as *Sub1* and is presumably allelic with it. Dennis *et al.* (2000) discuss options for achieving waterlogging tolerance in wheat and other crops using genetic engineering.

Salinity

Flooding and waterlogging can lead to the salinization of soil above saline groundwater. Salinity arises also from intrusion of sea water in coastal areas and from the use of irrigation water of low quality. About 10% of the global land area is affected by salinity (Szabolcs, 1989) and about 20% of irrigated land is similarly affected (Yeo *et al.*, 1999). Overlapping sets of QTL for salt tolerance have been detected in tomato at germination and at the seedling stage (Foolad, 1999). A major gene and several QTL for salt tolerance have been reported for rice seedlings (Koyama *et al.*, 2001; Gregorio *et al.*, 2002). Many transgenes have been reported to enhance salt tolerance in *Arabidopsis* or crop plants. One of the most promising approaches is the overexpression of the vacuolar Na⁺-H⁺ antiporter NHX1 (Apse *et al.*, 1999; Zhang *et al.*, 2001) or of the vacuolar pyrophosphatase (Gaxiola *et al.*, 2001). The pyrophosphatase helps to energize the antiporter by pumping protons in the vacuole and allowing the antiporter to drive Na⁺ ions into the vacuole in exchange for the protons. Overexpression of transcription factor *DREB1A* under control of the stress-sensitive *rd29A* promoter increases not only salt tolerance but also drought and cold tolerance in *Arabidopsis* (Kasuga *et al.*, 1999). Bennett and Khush (2002) review the discovery of genes conferring salt tolerance.

The reproductive stage is salt-sensitive in most crops. In the case of rice, this stage lasts for about 70 days, from panicle initia-

tion to grain maturity, with flowering occurring at about the thirtieth day. The most salt-sensitive period is about 7–10 days before flowering (Makihara *et al.* 1999a,b). Few germplasm screens have been conducted to rank accessions according to salt tolerance at the reproductive stage, and yet it is at this stage that an episode of stress has its largest and least-reversible effect on yield. It is unfortunate that most studies on salt tolerance focus on survival of vegetative-stage stress rather than on productivity after a season of realistic stress, including reproductive-stage stress.

Drought

Drought is the most common water-related stress experienced by crops. Across the broad spectrum of research on water, four definitions of drought are in common use (Yevjevich *et al.*, 1978). Meteorological drought is defined as an extended period during which precipitation is below normal. Hydrological drought is an extended period during which stream flow and water levels in lakes and reservoirs are below normal. Socio-economic drought is defined as the meteorological and hydrological condition under which less water is available than anticipated and needed for the normal level of social and economic activity of the region. Agricultural drought is due to a shortage of water in the root zone, such that yield is reduced considerably. It is agricultural drought that is the principal concern here.

Agricultural drought may develop at any time of the cropping season. Its impact is usually most severe at the seedling and flowering stages (Boonjung and Fukai, 1996; Zinselmeier *et al.*, 1999; Saini and Westgate, 2000). It may be prevented by supplemental or continuous irrigation except under conditions of hydrological drought. To help the many farmers who do not have access to irrigation, breeders produce varieties that have an enhanced ability to escape, avoid or tolerate drought (Blum, 1988). The remainder of this chapter focuses on new methods for increasing the efficiency of breeding for drought tolerance.

Breeding for Drought Tolerance

Drought tolerance in CGIAR varieties

The CGIAR centres study a total of 22 mandated crops: six cereals, six food legumes, four forage plants and four tuber crops, along with bananas and plantains. Enhancing drought tolerance is a feature of the breeding programmes for all crops except cowpea, yam, banana and plantain (Table 7.3). These exceptions are grown in humid and subhumid environments and the germplasm collections lack adequate levels of drought tolerance for breeding purposes. The centres often have parallel programmes in crop, water and natural-resources management to ensure that the new lines perform well in their target environments. Farmer participation is often prominent in both the breeding programmes and the management programmes.

A common feature of the breeding programmes is the use of wild relatives of the crop plants as sources of drought tolerance. In its work on the Asian cultivated rice *O. sativa*, IRRI commonly crosses tropical japonica varieties from the uplands and indica varieties from the lowlands in an attempt to produce high-yielding recombinants capable of drought avoidance and drought tolerance (Lafitte *et al.*, 2002). WARDA has introduced genes for drought tolerance into *O. sativa* from the African cultivated rice *O. glaberrima*. The International Center for Agricultural Research in the Dry Areas (ICARDA) has introduced drought tolerance into *Hordeum vulgare* from *Hordeum spontaneum*. *Aegilops tauchii* has been a donor of drought tolerance for *Triticum aestivum* in the wheat programmes of the International Maize and Wheat Improvement Center (CIMMYT) and ICARDA, with *Triticum durum* used as the

Table 7.3. Status of breeding for drought tolerance at CGIAR centres (from <http://www.cgiar.org>).

Crop	Centre breeding for drought tolerance	Tolerant germplasm
<i>Gramineae</i>		
Rice	IRRI: lowland, upland, released; aerobic WARDA: released	Upland tropical japonicas, <i>O. rufipogon</i> , <i>O. glaberrima</i>
Maize	CIMMYT: sub-Saharan Africa, released	Ac7643S5
Wheat	CIMMYT, ICARDA: released	<i>Aegilops tauchii</i>
Barley	ICARDA: released	<i>Hordeum spontaneum</i>
Pearl millet	ICRISAT	PRLT 2/89–33, 863B
Sorghum	ICRISAT	B35, stay-green
<i>Brachiaria</i>	CIAT	Endophytic fungi important
<i>Leguminosae</i>		
Common bean	CIAT: heat	Tepary bean
Chickpea	ICRISAT	ICC4958, large root system
Groundnut	ICRISAT: released	Common
Pigeonpea	ICRISAT	Common
Cowpea	[Needs moist conditions]	None
Soybean	IITA	Northern China germplasm
Lentil	ICARDA: released	Common
Faba bean	ICARDA	Common
Grass-pea	ICARDA: toxin-free variety released	<i>Lathyrus sativus</i>
<i>Others</i>		
Potato	CIP	Andean germplasm
Sweet potato	CIP: tolerant late in season	Common
Cassava	IITA	Northern Brazil germplasm
Yam	[Needs high rainfall]	None
Banana	[Needs high rainfall]	<i>Musa balbisiana</i> (starchy)
Plantain	[Needs high rainfall]	None

bridging species. The International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) has identified donors of drought tolerance in the germplasm of pearl millet (PRLT 2/89–33 and 863B), sorghum (the stay-green line B35) and chickpea (ICC4958, a line with a large root system). CIMMYT's maize-breeding programme has made considerable use of Ac7643S5 as a donor of drought tolerance. Centro Internacional de la Papa (CIP) is enhancing the drought tolerance of potato, using germplasm from the Andes. The International Institute for Tropical Agriculture (IITA) is using germplasm from northern China and north-eastern Brazil for soybean and cassava, respectively.

Drought-tolerant lines for several mandated crops have already been released by the centres for evaluation by collaborating institutes and farmers. The crops include rice (IRRI, WARDA), maize (CIMMYT), wheat (CIMMYT, ICARDA) and barley (ICARDA), among the cereals, and cowpea (IITA), groundnut (ICRISAT) and lentil (ICARDA), among the food legumes. ICARDA has also released important new lines of grass-pea (*Lathyrus sativus*) for drought-affected areas, but here the main achievement was to use mutation to reduce the neurotoxin levels of already drought-tolerant lines. ICARDA also has a drought-tolerance programme for faba bean. CIP and its collaborators have released drought-tolerant lines of sweet potato.

Several CGIAR-mandated crops have been difficult to improve for drought tolerance because of reproductive barriers or absence of suitable donors in the available germplasm. In its programme to enhance the drought tolerance of the common bean, *Phaseolus vulgaris*, CIAT has shown by grafting that the root system of tepary bean (*Phaseolus acutifolius*) confers considerable tolerance of drought and heat. However, attempts to cross the two species have encountered a reproductive barrier that has not yet been breached. Tissue-culture studies are under way along the lines that have proved successful for interspecific crosses of many other species, including rice. Centro Internacional de Agricultura Tropical (CIAT) is also improving the pasture grasses of the

genus *Brachiaria*, especially *B. brizantha*, for which both sexual and apomictic accessions are known. *B. brizantha* is already highly drought-tolerant, but this trait has been attributed in part to the presence of an endophytic fungus, *Acrimonium implicatum*. If the endophyte also proves to be the cause of a serious cattle disease, drought tolerance in this grass might have to be reassessed. Three of IITA's mandated crops – banana and plantain (both with International Network for the Improvement of Banana and Plantain (INIBAP)) and yam – need high rainfall. The enhancement of drought tolerance in these crops is not a priority compared with enhancing resistance to biotic stresses. The drought tolerance of banana increases in proportion to the contribution of *Musa balbisiana* to the genome, but the *M. balbisiana* genome also contributes starchiness, rather than sweetness, to the fruit.

Why is breeding for drought tolerance currently inefficient?

In spite of the success of the CGIAR centres in releasing a number of drought-tolerant varieties, breeding for drought tolerance is a slow, painstaking and inefficient process. This situation arises principally from three problems. First, the variability of drought in terms of its timing during the plant growth cycle means that early-season drought, mid-season drought and terminal drought are essentially different challenges. Secondly, for each time of onset of drought, water deficit affects all tissues and involves multiple responses, during both stress and recovery, leading to complex genetic control of drought tolerance. Thirdly, the screening of germplasm collections or breeding materials for drought tolerance is highly sensitive to environmental conditions (soil chemistry, soil texture and weather). These problems have led to the division of drought-breeding programmes into subprogrammes to match the major types of drought-prone environments.

Completely different approaches are taken in these subprogrammes. Where the wet season is brief but reliable, photo-

period-insensitive, short-duration varieties are favoured to allow the crop to pass the very sensitive flowering stage before the onset of water deficit. Where drought is most likely to occur mid-season, photoperiod sensitivity is used to delay flowering until late in the season, when rains are more reliable. Of course, in this situation, the plants must still survive mid-season drought. In upland rain-fed conditions, deep-rooted varieties can continue to grow by tapping the deeper layers for water (Champoux *et al.*, 1995), while in lowland rain-fed conditions plants may require roots with the capacity to penetrate the hardpan, a layer of compacted soil located about 20–25 cm below the soil surface (Ray *et al.*, 1996). In either situation, when water deficit is finally experienced, desired traits include the abilities to: (i) reduce water loss to the soil and the air; (ii) maintain turgor for an extended period; (iii) survive the loss of turgor; and (iv) protect cells against oxidative damage caused by continued absorption of radiation under conditions where stomatal closure prevents photosynthesis. When water is restored, the plants must be able to recover photosynthetic activity and growth, and they must allocate a large fraction of fixed carbon (either stored from before drought stress or synthesized after stress) to grain, fruit and tuber production (Blum, 1998; Richards, 2000).

When water stress is experienced during flowering, pollen sterility and embryo abortion may occur and crop yield may be greatly reduced (Zinselmeier *et al.*, 1999; Saini and Westgate, 2000). It is not entirely clear whether these problems are due to a direct effect of stress on the flowers or whether they arise indirectly from a diminished supply of carbohydrate from the stressed leaves or a premature supply of ABA from stressed leaves and roots (Westgate *et al.*, 1996). There is considerable genetic variation in rice in the capacity to withstand water deficit at flowering and maintain pollen sterility (J.X. Liu and J. Bennett, unpublished data). Proteomic, microarray and genetic approaches are being taken to identify the pathways that permit tolerance of water stress at flowering.

This long list of desirable traits makes the genetics of drought tolerance very complex. The selection for the desired combination of traits is rendered additionally difficult by interactions between the plants and their environment, including soil chemistry, soil texture and weather. To overcome these problems, breeders must conduct large, replicated field trials or use managed environments, such as greenhouses or drip-irrigated fields. Replicated field trials are difficult to conduct early in the breeding programme, when the number of breeding lines is large and the amount of available seed is small. Managed environments are expensive to create and operate and may not fully represent the target environment.

Breeders are focusing increasingly on yield components, rather than on the yield itself. The rationale behind turning to yield components is that each component tends to be determined during a comparatively brief period of the plant growth cycle and is presumed to be controlled by fewer key genes than yield as a whole. The four commonly used yield components for rice are: (i) panicles per square metre; (ii) spikelets per panicle; (iii) spikelet fertility; and (iv) single-grain weight. Each yield component is most sensitive to drought or salinity at a different time in the growth cycle (Boonjung and Fukai, 1996; Makihara *et al.*, 1999a,b). Component i is affected by stress, principally during germination, seedling development and tillering. Components ii, iii and iv are affected by stress, principally during panicle initiation, flowering and grain filling, respectively.

Boonjung and Fukai (1996) exposed rice to water stress for 23–34 days at different growth stages over two growing seasons. They quantified the effect of stress on the four yield components (Fig. 7.1). When drought occurred during tillering, it reduced yield by up to 30% through reductions in the number of panicles per unit area and the number of spikelets per panicle. When drought occurred during panicle development, anthesis was delayed, the number of spikelets per panicle was reduced by 40% and the percentage of filled grains decreased markedly (to zero in 1 year). A decrease in grain yield of > 80%

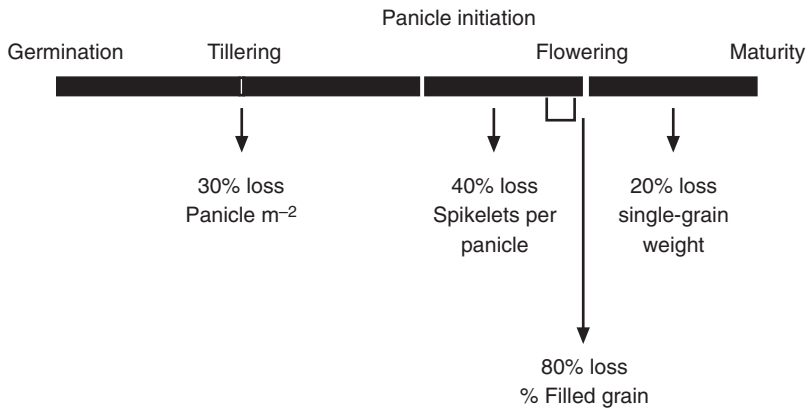


Fig. 7.1. The impact of drought stress on four rice-yield components. Boonjung and Fukai (1996) subjected rice to 3 weeks of water stress at different intervals during the growth cycle and measured the impact on panicles m^{-2} , spikelets per panicle, percentage filled grains and single-grain weight. These components were most severely reduced at tillering, panicle initiation, flowering and grain filling, respectively. The largest impact of stress was recorded at flowering through an 80% reduction in the percentage of fertile grains.

was associated with low dry-matter production during both the drought period and the recovery period. When drought occurred during grain filling, the percentage of filled grains decreased by 60% and individual grain mass decreased by 20%. Makihara *et al.* (1999a,b) obtained similar results for salt stress, but the salt sensitivity of different yield components was genotype-dependent. These observations raise the possibility of simplifying the genetics of drought and salt tolerance by examining the different mechanisms of tolerance at different growth stages for different yield components. The molecular analysis of tolerance would also be made easier because of more precise knowledge of when and from which tissue to extract protein and RNA for analysis. Molecular analysis of stress responsiveness has already begun, using microarrays (Kawasaki *et al.*, 2001; Seki *et al.*, 2001) and proteomics (Moons *et al.*, 1995; Thiellement *et al.*, 1999; Salekdeh *et al.*, 2002a,b). Figure 7.2 illustrates the analytical methods of microarrays and proteomics. Interesting cDNAs are identified by sequencing and interesting proteins by mass spectrometry (Salekdeh *et al.*, 2002a). The genotype \times environment interactions of these mechanisms should be greatly reduced compared with yield as a whole; the genetics is simpler and their duration is shorter.

Drought-related Traits

One major approach to understanding and simplifying the genetics of drought tolerance focuses on mapping QTL that condition drought-related physiological traits. Many of the drought-related traits studied at the level of QTL analysis relate to root behaviour. In the case of rice, most mapping populations are derived from intersubspecific crosses between upland tropical japonica cultivars, such as CT9993 and Azucena, and lowland indica cultivars, such as IR62266 and IR64. Key QTL have been mapped for root morphology, root distribution and drought avoidance (Champoux *et al.*, 1995; Price and Tomos, 1997; Yadav *et al.*, 1997; Ali *et al.*, 2000; Courtois *et al.*, 2000; Kamoshita *et al.*, 2002), root penetration ability (Ray *et al.*, 1996; Price *et al.*, 2000; Zheng *et al.*, 2000), osmotic adjustment and dehydration tolerance (Lilley *et al.*, 1996; Zhang *et al.*, 1999), stomatal conductance, leaf rolling and heading date (Price *et al.*, 1997), cell-membrane stability (Tripathy *et al.*, 2000) and ABA accumulation (Quarrie *et al.*, 1997). The contribution of a QTL to the variance of its trait is often quite small, making it difficult to map the QTL with sufficient accuracy for use in marker-assisted selection (MAS) (< 5 cM) or for map-based cloning (< 1 cM) and requiring several QTL to be pyramided to reconstruct the trait to an adequate extent.

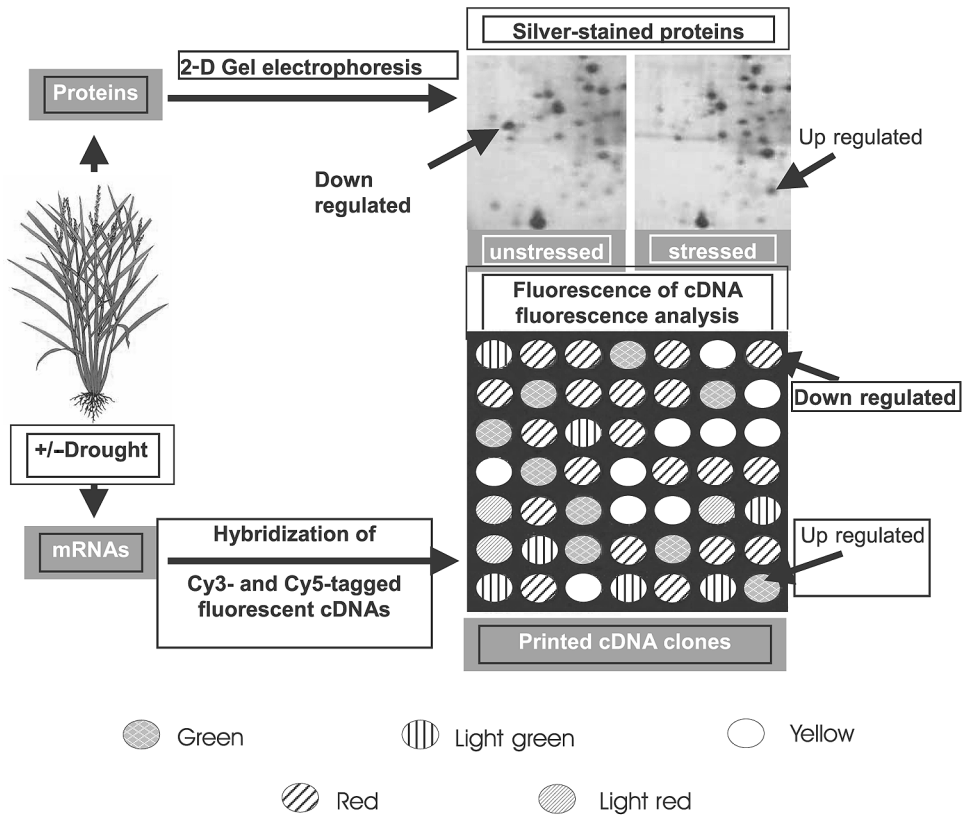


Fig. 7.2. Comparison of gene expression in drought-stressed and unstressed plants through proteomics and microarray analysis. Proteins extracted from plant tissue are separated by two-dimensional electrophoresis (isoelectric focusing, followed by dodecylsulphate-polyacrylamide gel electrophoresis), and then visualized by silver staining and quantified by scanning (Salekdeh *et al.*, 2002a). The most commonly observed changes are changes in abundance, but changes in position as a result of cleavage or phosphorylation are also seen. The mRNA populations extracted from plant tissues are used as a template for cDNA synthesis and concomitant tagging with the fluorophores Cy3 (green, stressed) and Cy5 (red, unstressed). The cDNA populations are hybridized to immobilized arrays of cDNA clones on glass slides, and a fluorescence detector scans over the slide to record hybrid abundance. Both digital and false-colour representations of the hybridization data are recorded (Kawasaki *et al.*, 2001). Green fluorescence shows a cDNA upregulated by stress, red fluorescence is a sign of downregulation by stress and yellow fluorescence indicates that in the cell the cDNA is probably unresponsive to stress.

QTL for drought-related traits have also been reported for other crops, especially cereals. These traits include osmotic adjustment in wheat and barley (Teulat *et al.*, 1998), anthesis silking interval (ASI) in maize (Ribaut *et al.*, 2002) and the stay-green trait in sorghum (Xu, K. *et al.*, 2000). In spite of changes in the chromosome number, the genomes of the cereals display a high degree of synteny, or conservation of gene order, along homologous chromo-

somes (Gale *et al.*, 2001). As a result, it is sometimes possible to predict the genomic location of a gene in one cereal from its known location in another cereal. The frequency of this sort of prediction will increase as comparative mapping improves. Orthologous genes for osmotic adjustment in barley, rice and wheat have been located in syntenic regions of the respective genomes by QTL mapping (Zhang *et al.*, 1999).

ASI is the time difference in days between anthesis in tassels (male) and silking in ears (female). A longer ASI (due to a longer delay in silking) is strongly associated with greater drought sensitivity (Edmeades *et al.*, 1993). At least six QTL for ASI have been identified and together they account for 47% of the total variance of this trait in the mapping population. QTL for ASI are among several markers being considered by CIMMYT for use in molecular breeding for drought tolerance (Ribaut *et al.*, 2002), but they are not effective by themselves. This situation may arise from the fact that, whereas a long ASI is sufficient to confer drought sensitivity (silks develop too late for efficient pollination), a short ASI is not sufficient to confer drought tolerance. As the separate development of tassel and ear in maize has no counterpart in rice, wheat and barley, it might be thought that ASI has no relevance to these cereals, but, to the extent that ASI is a sign of altered carbohydrate allocation to tassel and ear, it may be relevant to the fundamental question of carbohydrate allocation between organs and between spikelets within a panicle in small-grain cereals.

Candidate genes for drought tolerance

In recent years, many drought-responsive genes have been identified in plants, especially in *Arabidopsis thaliana*. These genes have been identified by several approaches:

- Studies on the anabolic and catabolic pathways for metabolites that accumulate in drought-stressed plants (e.g. proline, glycine, betaine, trehalose, ABA).
- Analysis of other mechanisms of drought tolerance.
- Analysis of protein or mRNA changes in response to drought.
- Analysis of signal-transduction pathways.
- Mapping of QTL for drought tolerance, using segregating populations.

All of these methods are being accelerated as a result of the sequencing of the *Arabidopsis* and rice genomes. A sixth method (isolation of mutants) has so far been less successful with drought than with salinity tolerance.

Alleles and pyramids

All accessions of a particular crop species are expected to contain essentially the same genes. Differences in agricultural performance between accessions are thought to be due to allelic differences within the same gene set. Thus, achieving a high level of drought tolerance depends on finding the most appropriate alleles of key genes and combining or pyramiding them together.

One successful method of screening a germplasm collection for the best alleles is to apply standardized phenotyping protocols to the collection and then to conduct detailed genetic analysis of the best performers. Some of these accessions will owe their superior performance to positive alleles at a considerable number of QTL of small effect and are not of interest here. Other accessions will perform well because they contain positive alleles at one or two major genes or QTL of large effect; these alleles are of great interest in crop improvement. The two types of accessions can be distinguished by advanced backcross analysis.

Desirable alleles may also be recovered from accessions that do not perform particularly well in phenotypic tests. Such alleles might be recognized first only after introgression into an elite genetic background by backcrossing (Tanksley and McCouch, 1997). The genetic basis of these effects is beyond the scope of this chapter, but the recipe for success is easily summarized: hard work, attention to detail and luck in the choice of accessions to be screened. Given the very large size of the germplasm collections in most CGIAR centres, it is impractical to screen a whole collection, but DNA fingerprinting could be exploited to select a core germplasm set of the most divergent accessions for closer analysis. The new techniques of diversity array technology (DaRT) (Jaccoud *et al.*, 2001) and targeting induced local lesions in genomes (TILLING) (McCallum *et al.*, 2000) facilitate these steps.

Desirable alleles might also be the product of a carefully designed programme of random or directed mutagenesis. An exquisite example of directed mutagenesis is the use of recombinant DNA technology to change the promoter on the gene encoding the drought response element binding protein (DREB1A)

transcription factor to enhance abiotic stress tolerance in *Arabidopsis* (Kasuga *et al.*, 1999). Naturally occurring alleles and mutant alleles may be moved into new genetic backgrounds by marker-assisted backcrossing, but alleles produced by promoter switching and other forms of recombinant DNA technology must be introduced as transgenes, after which they may also be manipulated with the help of markers (Fig. 7.3). Ideally, allele pyramiding will become possible not only for drought but also for other traits that contribute to WP, such as salinity tolerance and waterlogging/flooding tolerance.

In summary, the alleles that will eventually be pyramided to confer a high level of drought tolerance on crop plants may derive from many different genetic sources. They may take several different forms:

- Precisely mapped major genes or QTL of large effect that confer drought tolerance through a specific mechanism but have not yet been cloned and identified.
- Known genes discovered by map-based cloning of major genes or QTL or through some other approach.

- Novel alleles produced from known genes by mutagenesis or recombinant DNA technology and reintroduced into plants by genetic engineering.

The major genes and major QTL will be pyramided using tightly linked flanking markers, whereas alleles of known genes and novel transgenic alleles will be pyramided through the use of allele-specific DNA probes based on the genes themselves.

The discovery of genes for enhancing WP is not an isolated activity. It forms part of a breeding and resources-management programme that begins and ends with farmers (Fig. 7.4). The overall starting-point for such a programme is participatory rural appraisal, involving a wide spectrum of stakeholders. One task of the stakeholders is to determine how much of the responsibility for increasing WP should be shouldered by breeding and how much by natural-resources management, especially water management. Poor farmers will not be able to afford most management options and will look more to breeding for solutions. Intractable traits, such as drought tolerance, require detailed physiological, bio-

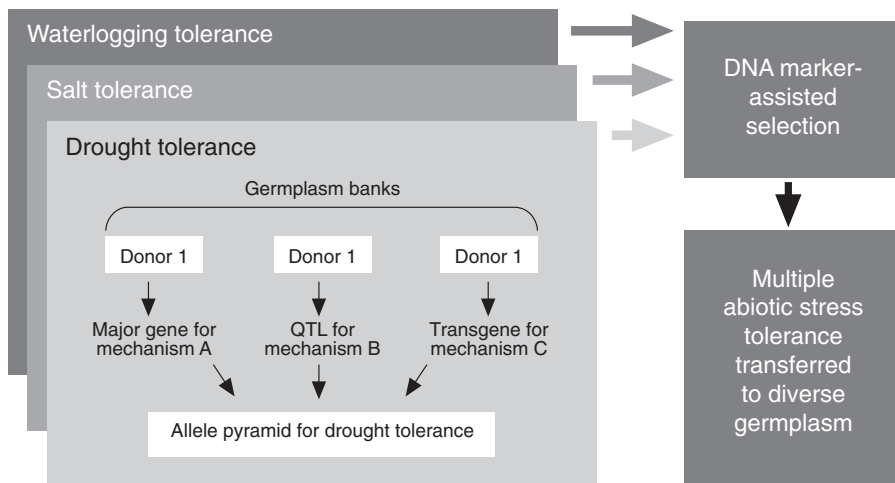


Fig. 7.3. Identification and use of genes conferring tolerance of drought, salt or waterlogging. Tolerance of an abiotic stress involves several distinct molecular or cellular mechanisms. Mapping of the corresponding major genes or major quantitative trait loci (QTL) allows the use of DNA markers to backcross these mechanisms into popular varieties that are sensitive to stress. The sequencing of plant genomes increases the probability that the underlying genes can be isolated and used directly to search for superior alleles. The isolated genes may also be modified (e.g. by promoter switching) to create entirely novel alleles suitable for reintroduction into plants as transgenes. Several different donors may have to contribute genes before an adequate level of tolerance can be assembled through allele pyramiding. The initial assembly of the pyramid and its subsequent transfer to popular varieties both depend on the development of a robust set of molecular markers.

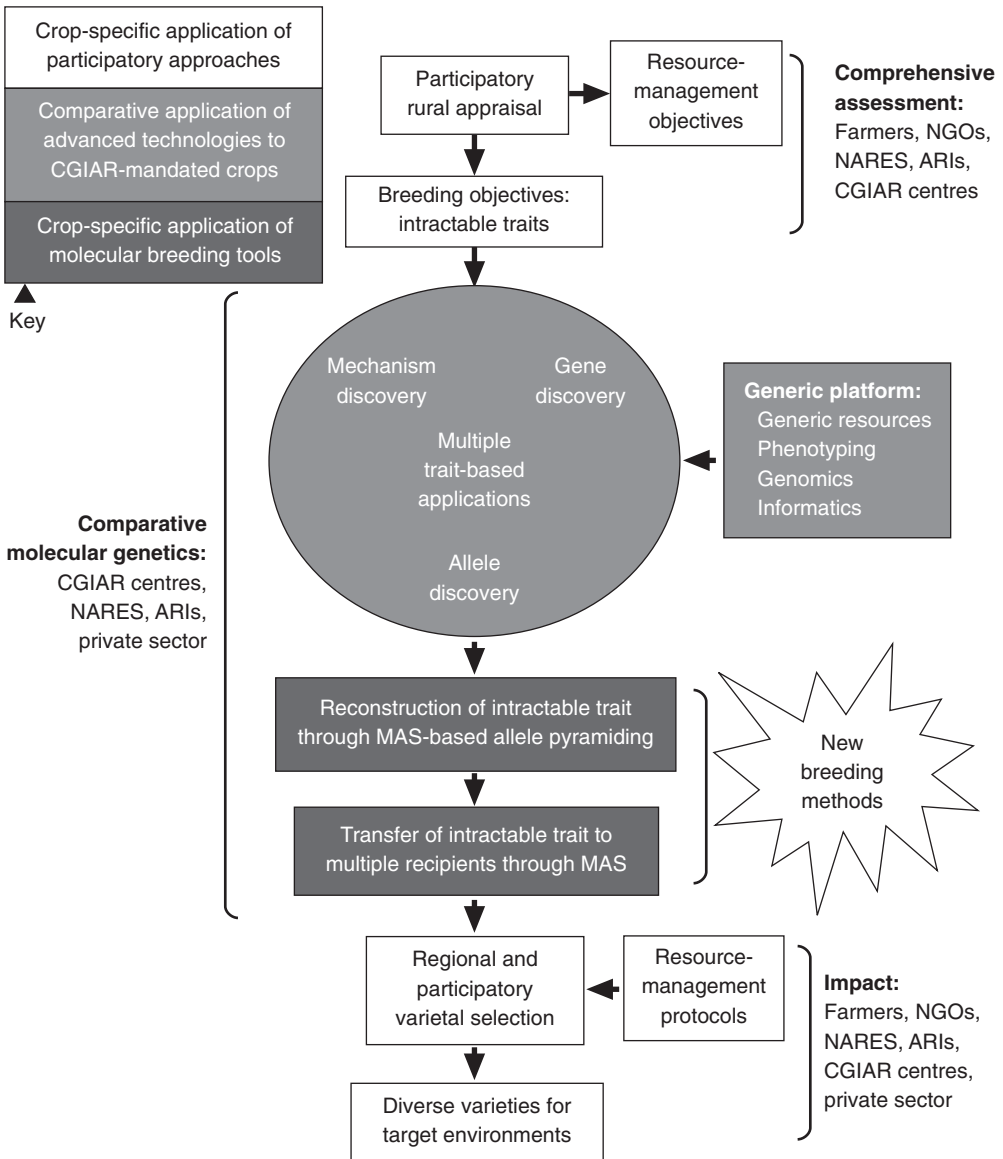


Fig. 7.4. A comprehensive programme of gene and allele discovery to enhance water productivity across the CGIAR-mandated crops. Crop-specific rural appraisal identifies the breeding objectives for key intractable traits, such as drought and salinity, in different target environments. The comparative molecular genetics of those traits helps the discovery process and allows orphan crops to benefit from genomic analysis of the major cereals and *Arabidopsis*. The prerequisite for discovery is a platform consisting of genetic resources (including mutants and mapping populations), standardized phenotyping protocols, genomic resources and tools and bioinformatics. Repeated application of the resource platform allows multiple traits to be analysed, simplified and improved in multiple crops. Marker-assisted selection (MAS) replaces field-based selection and thereby accelerates the enhancement of water productivity. NGOs, non-government organizations; NARES, National Agricultural Research and Extension System; ARIs, Advanced Research Institutes; CGIAR, Consultative Group on International Agricultural Research.

chemical and molecular study, but the order of discovery of mechanisms, genes and alleles will vary depending on the point of entry into the analysis. These steps are therefore depicted as a circle in Fig. 7.4. Feeding into this circular discovery process is a generic technical platform consisting of genetic resources, phenotyping, genomics and informatics. The platform is generic in the sense that all of its components (with the possible exception of phenotyping) are largely independent of whichever traits are under investigation.

The new breeding activities highlighted in Fig. 7.4 were shown in more detail in Fig. 7.3. The primary function of these activities is to show the efficacy of the pyramided set of alleles developed to enhance the intractable trait in question. In this proof-of-concept stage, breeders would use robust molecular markers to backcross the set of alleles into only a limited set of varieties. At the final stages of the process, when a wider range of stakeholders become directly involved again, the set of alleles is backcrossed into a much larger number of locally popular varieties by local breeders. Participatory varietal selection can be included if it can increase the likelihood of uptake of the new variety.

Future Prospects: Linkage between Challenge Programmes

Water will be the most important challenge for agriculture over the next century. Can we breed plants that use water more efficiently? If the answer is no, the increasing demand for water from non-agricultural sectors will leave food security permanently in jeopardy. If the answer is yes, the prospects will be bright for achieving long-term food security, even in resource-poor, highly populous developing countries. The decisive factor will be how quickly the new crop varieties can be generated and released to farmers. The speed of this process will be determined by the strength of the linkage between scientists working on water and scientists working on gene discovery and the determination of these scientists to achieve significant gains in WP over a wide range of crops.

The new challenge programmes of the CGIAR offer a way of linking the water-research community and the genomic community across the major crops. WP is one of the themes of the Water and Food Challenge Program (WFCP), and water-related stresses are high-priority issues for the Genetic Resources Challenge Program (GRCP). Figure 7.4 shows how these two challenge programmes could be linked in relation to intractable problems, such as drought, salinity, waterlogging and submergence. WFCP has a comparative advantage over GRCP in conducting the rural appraisals needed to identify target environments, set objectives for breeding and resource management and evaluate and disseminate new varieties. GRCP has a comparative advantage over WFCP in discovering key genes and alleles in the germplasm collections of the CGIAR centres and combining the alleles to produce unprecedented levels of stress tolerance and greatly enhanced WP for all crops.

This strategy begins and ends with farmer participation. However, more research is needed to find the best ways of integrating participation into breeding programmes. Participatory rural appraisal is essential in defining the environmental adaptability expected of a new variety, the traits required for uptake of the variety by farmers and integrating breeding and natural-resources management to ensure that the variety and its management are consistent with the local environment and the resources of the farmers and their community. The current comprehensive assessment on water resources provides an opportunity to refine appraisal techniques and set the stage for the challenge programmes.

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References

- Alexandratos, N. (1999) World food and agriculture: outlook for the medium and longer term. *Proceedings of the National Academy of Sciences USA* 96, 5908–5914.
- Ali, M.L., Pathan, M.S., Zhang, J., Bai, G., Sarkarung, S. and Nguyen, H.T. (2000) Mapping QTL for root traits in a recombinant inbred population from two indica ecotypes in rice. *Theoretical and Applied Genetics* 101, 756–766.
- Apse, M.P., Aharon, G.S., Snedden, W.A. and Blumwald, E. (1999) Salt tolerance conferred by overexpression of a vacuolar Na⁺/H⁺ antiport in *Arabidopsis*. *Science* 285, 1256–1258.
- Ball, V.E., Butault, J.P. and Nehring, R. (2001) *U.S. Agriculture, 1960–96: a Multilateral Comparison of Total Factor Productivity*. ERS Technical Bulletin No. 1895, US Department of Agriculture, Washington, DC, 56 pp.
- Bennett, J. and Khush, G.S. (2002) Plant breeding for salt tolerance. *Journal of Crop Production* 7, 1–25.
- Blum, A. (1988) *Plant Breeding for Stress Environments*. CRC Press, Boca Raton, Florida, 223 pp.
- Blum, A. (1998) Improving wheat grain filling under stress by stem reserve mobilization. *Euphytica* 100, 77–83.
- Boonjung, H. and Fukai, S. (1996) Effects of soil water deficit at different growth stages on rice growth and yield under upland conditions. 2. Phenology, biomass production and yield. *Field Crops Research* 48, 47–55.
- Borrell, A.K. and Douglas, A.C.L. (1996) Maintaining green leaf area in grain sorghum increases yield in a water-limited environment. In: Foale, M.A., Henzell, R.G. and Kneipp, J.F. (eds) *Proceedings of the Third Australian Sorghum Conference*. Occasional Publication No. 93, Australian Institute of Agricultural Science, Melbourne, Australia, pp. 315–322.
- Bouman, B.A.M. (2001) Water-efficient management strategies in rice production. *International Rice Research Notes* 16(2), 17–22.
- Cabangon, R.J., Castillo, E.G., Bao, L.X., Lu, G.A., Wang, G.H., Cui, Y.L., Tuong, T.P., Bouman, B.A.M., Li, Y.H., Chen, C.D. and Wang, J.Z. (2001) Impact of alternate wetting and drying irrigation on rice growth and resource-use efficiency. In: Barker, R., Li, Y.H. and Tuong, T.P. (eds) *Water-saving Irrigation for Rice: Proceedings of an International Workshop*. International Water Management Institute, Colombo, Sri Lanka, pp. 55–79.
- Cassman, K.G. (1999) Ecological intensification of cereal production systems: yield potential, soil quality, and precision agriculture. *Proceedings of the National Academy of Sciences USA* 96, 5952–5959.
- Champoux, M.C., Wang, G., Sarkarung, S., Mackill, D.J., O'Toole, J.C., Huang, N. and McCouch, S.R. (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. *Theoretical and Applied Genetics* 90, 969–981.
- Chopra, J., Kaur, N. and Gupta, A.K. (2000) Ontogenic changes in enzymes of carbon metabolism in relation to carbohydrate status in developing mungbean reproductive structures. *Phytochemistry* 53, 539–548.
- Courtois, B., McLaren, G., Sinha, P.K., Prasad, K., Yadav, R. and Shen, L. (2000) Mapping QTL associated with drought avoidance in upland rice. *Molecular Breeding* 6, 55–66.
- Daniell, H., Datta, R., Varma, S., Gray, S. and Lee, S.B. (1998) Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nature Biotechnology* 16, 345–348.
- Dennis, E.S., Dolferus, R., Ellis, M., Rahman, M., Wu, Y., Hoeren, F.U., Grover, A., Ismond, K.P., Good, A.G. and Peacock, W.J. (2000) Molecular strategies for improving waterlogging tolerance in plants. *Journal of Experimental Botany* 51, 89–97.
- Dong, B., Loeve, R., Li, Y.H., Chen, C.D., Deng, L. and Molden, D. (2001) Water productivity in the Zhanghe irrigation system: issues of scale. In: Barker, R., Li, Y.H. and Tuong, T.P. (eds) *Water-saving Irrigation for Rice*. International Water Management Institute, Colombo, Sri Lanka, pp. 97–115.
- Druart, N., De Roover, J., Van den Ende, W., Goupil, P., Van Laere, A. and Rambour, S. (2001) Sucrose assimilation during early developmental stages of chicory (*Cichorium intybus* L.) plants. *Planta* 212, 436–443.
- Edmeades, G.O., Bolanos, J., Hernandez, M. and Bello, S. (1993) Causes for silk delay in a lowland tropical maize population. *Crop Science* 33, 1029–1035.
- Fischer, G., Shah, M., van Velthuisen, H. and Nachtergaele, F.O. (2001) *Global Agro-ecological Assessment for Agriculture in the 21st Century*. International Institute for Applied Systems Analysis, Laxenburg, Austria, 33 pp.
- Foolad, M.R. (1999) Comparison of salt tolerance during seed germination and vegetative growth in tomato by QTL mapping. *Genome* 42, 727–734.

- Fridman, E., Pleban, T. and Zamir, D. (2000) A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. *Proceedings of the National Academy of Sciences USA* 97, 4718–4723.
- Gale, M., Moore, G. and Devos, K. (2001) Rice – the pivotal genome in cereal comparative genetics. *Novartis Foundation Symposium* 236, 46–53.
- Gaxiola, R.A. et al. (2001) Drought- and salt-tolerant plants result from overexpression of the AVP1 H⁺-pump. *Proceedings of the National Academy of Sciences USA* 98, 11,444–11,449.
- Goetz, M., Godt, D.E., Guivarc'h, A., Kahmann, U., Chriqui, D. and Roitsch, T. (2001) Induction of male sterility in plants by metabolic engineering of the carbohydrate supply. *Proceedings of the National Academy of Sciences USA* 98, 6522–6527.
- Gregorio, G.B., Senadhira, D., Mendoza, R.D., Manigbas, N.L., Roxas, N.L. and Guerta, C.Q. (2002) Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crops Research* 76, 91–101.
- He, Z., Zhu, Q., Dabi, T., Li, D., Weigel, D. and Lamb, C. (2000) Transformation of rice with the *Arabidopsis* floral regulator LEAFY causes early heading. *Transgenic Research* 9, 223–227.
- Hiei, Y., Komari, T. and Kubo, T. (1997) Transformation of rice mediated by *Agrobacterium tumefaciens*. *Plant Molecular Biology* 35, 205–218.
- Holbrook, N.M., Shashidhar, V.R., James, R.A. and Munns, R. (2002) Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. *Journal of Experimental Botany* 53, 1503–1514.
- Hugouvieux, V., Kwak, J.M. and Schroeder, J.I. (2001) An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in *Arabidopsis*. *Cell* 106, 477–487.
- Inoue, K., Sewalt, V.J., Murray, G.B., Ni, W., Sturzer, C. and Dixon, R.A. (1998) Developmental expression and substrate specificities of alfalfa caffeic acid 3-O-methyltransferase and caffeoyl coenzyme A 3-O-methyltransferase in relation to lignification. *Plant Physiology* 117, 761–770.
- Jaccoud, D., Peng, K., Feinstein, D. and Kilian, A. (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Research* 29, E25.
- Kamoshita, A., Zhang, J., Sciopongco, J., Sarkarung, S., Nguyen, H.T. and Wade, L.J. (2002) Effects of phenotyping environment on identification of quantitative trait loci for rice root morphology under anaerobic conditions *Crop Science* 42, 255–265.
- Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nature Biotechnology* 17, 287–291.
- Kawasaki, S., Borchert, C., Deyholos, M., Wang, H., Brazille, S., Kawai, K., Galbraith, D. and Bohnert, H.J. (2001) Gene expression profiles during the initial phase of salt stress in rice. *Plant Cell* 13, 889–906
- Khush, G.S. (2001) Green revolution: the way forward. *Nature Reviews of Genetics* 2, 815–822.
- Kouchi, H., Takane, K., So, R.B., Ladha, J.K. and Reddy, P.M. (1999) Rice ENOD40: isolation and expression analysis in rice and transgenic soybean root nodules. *Plant Journal* 18, 121–129.
- Koyama, M.L., Levesley, A., Koebner, R.M.A., Flowers, T.J. and Yeo, A.R. (2001) Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiology* 125, 406–422.
- Ku, M.S.B., Agarie, S., Nomura, M., Fukayama, H., Tsuchida, K., Ono, K., Hirose, S., Toki, S., Miyao, M. and Matsuoka, M. (1999) High-level expression of maize phosphoenolpyruvate carboxylase in transgenic rice plants. *Nature Biotechnology* 17, 76–80.
- Ku, M.S.B., Cho, D., Ranade, U., Hsu, T.-P., Li, X., Jiao, D.-M., Ehleringer, J., Miyao, M. and Matsuoka, M. (2000) Photosynthetic performance of transgenic rice plants overexpressing maize C₄ photosynthesis enzymes. In: Sheehy, J.E., Mitchell, P.L. and Hardy, B. (eds) *Redesigning Rice Photosynthesis to Increase Yield*. International Rice Research Institute, Manila, Philippines, pp. 193–204.
- Ku, M.S.B., Cho, D., Li, X., Jiao, D.-M., Pinto, M., Miyao, M. and Matsuoka, M. (2001) Introduction of genes encoding C₄ photosynthesis enzymes into rice plants: physiological consequences. In: Goodie, J.A. and Chadwick, D. (eds) *Rice Biotechnology: Improving Yield, Stress Tolerance and Grain Quality*. John Wiley & Sons, Chichester, pp. 100–116.
- Ladha, J.K. and Garrity, D.P. (1994) *Green Manure Production Systems for Asian Lowlands*. International Rice Research Institute, Manila, Philippines, 194 pp.
- Lafitte, H.R., Courtois, B. and Atlin, G.N. (2002) The International Rice Research Institute's experience in field screening for drought tolerance and implications for breeding. In: Saxena, N.P. and O'Toole, J.C. (eds) *Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice*. International Crop Research Institute for the Semi-Arid Tropics, Patancheru, India, pp. 25–40.

- Lemichez, E., Wu, Y., Sanchez, J.P., Mettouchi, A., Mathur, J. and Chua, N.H. (2001) Inactivation of AtRac1 by abscisic acid is essential for stomatal closure. *Genes and Development* 15, 1808–1816.
- Lilley, J.M., Ludlow, M.M., McCouch, S.R. and O'Toole, J.C. (1996) Locating QTL for osmotic adjustment and dehydration tolerance in rice. *Journal of Experimental Botany* 47, 1427–1436.
- Luan, S. (2002) Signaling drought in guard cells. *Plant Cell Environment* 25, 229–237.
- McCallum, C.M., Comai, L., Greene, E.A. and Henikoff, S. (2000) Targeting induced local lesions IN genomes (TILLING) for plant functional genomics. *Plant Physiology* 123, 439–442.
- Makihara, D., Tsuda, M., Morita, M., Hirai, Y. and Kuroda, T. (1999a) Effect of salinity on the growth and development of rice (*Oryza sativa* L.) varieties. *Japanese Journal of Tropical Agriculture* 43, 285–294.
- Makihara, D., Tsuda, M., Hirai, Y. and Kuroda, T. (1999b) Effects of saline irrigation at various growth stages on rice yield. *Japanese Journal of Crop Science* 68, 487–494.
- Medrano, H., Escalona, J.M., Bota, J., Gulias, J. and Flexas, J. (2002) Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of Botany* 89, 895–905.
- Moons, A., Bauw, G., Prinsen, E., Van Montagu, M. and Van der Straeten, D. (1995) Molecular and physiological responses to abscisic acid and salts in roots of salt-sensitive and salt-tolerant Indica rice varieties. *Plant Physiology* 107, 177–186.
- Nandi, S., Subudhi, P.K., Senadhira, D., Manigbas, N.L., Sen-Mandi, S. and Huang, N. (1997) Mapping QTL for submergence tolerance in rice by AFLP analysis and selective genotyping. *Molecular and General Genetics* 255, 1–8.
- Nguyen-Quoc, B. and Foyer, C.H. (2001) A role for 'futile cycles' involving invertase and sucrose synthase in sucrose metabolism of tomato fruit. *Journal of Experimental Botany* 52, 881–889.
- Peng, J., Richards, D.E., Hartley, N.M., Murphy, G.P., Devos, K.M., Flintham, J.E., Beales, J., Fish, L.J., Worland, A.J., Pelica, F., Sudhakar, D., Christou, P., Snape, J.W., Gale, M.D. and Harberd, N.P. (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400, 256–261.
- Peng, S.B., Laza, R.C., Khush, G.S., Sanico, A.L., Visperas, R.M. and Garcia, F.V. (1998) Transpiration efficiencies of indica and improved tropical japonica rice grown under irrigated conditions. *Euphytica* 103, 103–108.
- Peuke, A.D., Jeschke, W.D. and Hartung, W. (2002) Flows of elements, ions and abscisic acid in *Ricinus communis* and site of nitrate reduction under potassium limitation. *Journal of Experimental Botany* 53, 241–250.
- Pingali, P.L. and Heisey, P.W. (1999) *Cereal Crop Productivity in Developing Countries: Past Trends and Future Prospects*. Working Paper 99–03, CIMMYT, Mexico D.F., Mexico.
- Price, A.H. and Tomos, A.D. (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.). II. Mapping quantitative trait loci using molecular markers. *Theoretical and Applied Genetics* 95, 143–152.
- Price, A.H., Young, E.M. and Tomos, A.D. (1997) Quantitative trait loci associated with stomatal conductance, leaf rolling and heading date mapped in upland rice (*Oryza sativa*). *New Phytologist* 137, 83–91.
- Price, A.H., Steele, K.A., Moore, B.J., Barraclough, P.B. and Clark, L.J. (2000) A combined RFLP and AFLP linkage map of upland rice (*Oryza sativa* L.) used to identify QTL for root-penetration ability. *Theoretical and Applied Genetics* 100, 49–56.
- Quarrie, S.A., Laurie, D.A., Zhu, J., Lebreton, C., Semikhodskii, A., Steed, A., Witsenboer, H. and Calestani, C. (1997) QTL analysis to study the association between leaf size and abscisic acid accumulation in droughted rice leaves and comparisons across cereals. *Plant Molecular Biology* 35, 155–165.
- Queitsch, C., Hong, S.W., Vierling, E. and Lindquist S. (2000) Heat shock protein 101 plays a crucial role in thermotolerance in *Arabidopsis*. *Plant Cell* 12, 479–492.
- Radin, J.W., Lu, Z., Percy, R.G. and Zeiger, E. (1994) Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. *Proceedings of the National Academy of Sciences USA* 91, 7217–7221.
- Ray, J.D., Yu, L., McCouch, S.R., Champoux, M.C., Wang, G. and Nguyen, H.T. (1996) Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 92, 627–636.
- Ribaut, J.M., Banziger, M. and Hoisington, D. (2002) Genetic dissection and plant improvement under abiotic stress conditions: drought tolerance in maize as an example. In: *JIRCAS Working Report*. Tankuba, Japan, pp. 85–92.
- Richards, R.A. (2000) Selectable traits to increase crop photosynthesis and yield of grain crops. *Journal of Experimental Botany* 51, 447–458.

- Riederer, M. and Schreiber, L. (2001) Protecting against water loss: analysis of the barrier properties of plant cuticles. *Journal of Experimental Botany* 52, 2023–2032.
- Rieger, M.A., Lamond, M., Preston, C., Powles, S.B. and Roush, R.T. (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296, 2386–2388.
- Roberts, E. and Kolatukudy, P.E. (1989) Molecular cloning, nucleotide sequence and abscisic acid induction of a suberization-associated highly anionic peroxidase. *Molecular and General Genetics* 217, 223–232.
- Roberts, J.A., Hussain, A., Taylor, I.B. and Black, C.R. (2002) Use of mutants to study long-distance signaling in response to compacted soil. *Journal of Experimental Botany* 53, 45–50.
- Rosenow, D.T., Quisenberry, J.E., Wendt, C.W. and Clark, L.E. (1983) Drought-tolerant sorghum and cotton germplasm. *Agricultural Water Management* 7, 207–222.
- Saijo, Y., Hata, S., Kyojuka, J., Shimamoto, K. and Izui, K. (2000) Over-expression of a single Ca²⁺-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant Journal* 23, 319–327.
- Saini, H.S. and Westgate, M.E. (2000) Reproductive development in grain crops during drought. *Advances in Agronomy* 68, 59–96.
- Sakamoto, A., Alia and Murata, N. (1998) Metabolic engineering of rice leading to biosynthesis of glycinebetaine and tolerance to salt and cold. *Plant Molecular Biology* 38, 1011–1019.
- Salekdeh, G.H., Siopongco, J., Wade, L.J., Ghareyazie, B. and Bennett, J. (2002a) A proteomic approach to analyzing drought- and salt-responsiveness in rice. *Field Crops Research* 76, 199–219.
- Salekdeh, G.H., Siopongco, J., Wade, L.J., Ghareyazie, B. and Bennett, J. (2002b) Proteomic analysis of rice leaves during drought stress and recovery. *Proteomics* 2, 1131–1145.
- Sasaki, A., Ashikari, M., Ueguchi-Tanaka, M., Itoh, H., Nishimura, A., Datta, S., Ishiyama, K., Saito, T., Kobayashi, M., Khush, G.S., Kitano, H. and Matsuoka, M. (2002) Green revolution: a mutant gibberellin-synthesis gene in rice. *Nature* 416, 701–702.
- Schreiber, L., Kirsch, T. and Riederer, M. (1996) Transport properties of cuticular waxes: ecophysiological relevance for cuticular transpiration. In: Rennenberg, H., Eschrich, W. and Ziegler, H. (eds) *Trees: Contributions to Modern Tree Physiology*. SPB Academic Publishers, Amsterdam, pp. 19–27.
- Seckler, D., Upali, A., Molden, D., de Silva, R. and Barker, R. (1998) *World Water Demand and Supply, 1990 to 2025: Scenarios and Issues*. Research Report 19, International Water Management Institute, Colombo, Sri Lanka, 40 pp.
- Seki, M., Narusaka, M., Abe, H., Kasuga, M., Yamaguchi-Shinozaki, K., Caminci, P., Hayashizaki, P.Y. and Shinozaki, K. (2001) Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. *Plant Cell* 13, 61–72.
- Sheehy, J.E., Mitchell, P.L. and Hardy, B. (eds) (2000) *Redesigning Rice Photosynthesis to Increase Yield*. International Rice Research Institute, Manila, Philippines, 293 pp.
- Shi, W.M., Muramoto, Y., Ueda, A. and Takabe, T. (2001) Cloning of peroxisomal ascorbate peroxidase gene from barley and enhanced thermotolerance by overexpressing in *Arabidopsis thaliana*. *Gene* 273, 23–27.
- Socolow, R.H. (1999) Nitrogen management and the future of food: lessons from the management of energy and carbon. *Proceedings of the National Academy of Sciences USA* 96, 6001–6008.
- Sripongpangkul, K., Posa, G.B.T., Senadhira, D.S., Brar, D., Huang, N., Khush, G.S. and Li, Z.K. (2000) Genes/QTL affecting flood tolerance in rice. *Theoretical and Applied Genetics* 101, 1074–1081.
- Szabolcs, I. (1989) *Salt-affected Soils*. CRC Press, Boca Raton, Florida, 274 pp.
- Tanksley, S.D. and McCouch, S.R. (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277, 1063–1066.
- Teulat, B., This, D., Khairallah, M., Borries, C., Ragot, C., Sourdil, P., Leroy, P., Monneveux, P. and Charrier, A. (1998) Several QTL involved in osmotic-adjustment trait variation in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 96, 688–698.
- Thiellement, H., Bahrman, N., Damerval, C., Plomion, C., Rossignol, M., Santoni, V., de Vienne, D. and Zivy, M. (1999) Proteomics for genetic and physiological studies in plants. *Electrophoresis* 20, 2013–2026.
- Timsina, J. and Connor, D.J. (2001) Productivity and management of rice–wheat cropping systems: issues and challenges. *Field Crops Research* 69, 93–132.
- Tripathy, J.N., Zhang, J., Robin, S., Nguyen, T.T. and Nguyen, H.T. (2000) QTL for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theoretical and Applied Genetics* 100, 1197–1202.
- Tuong, T.P. (1999) Productive water use in rice production: opportunities and limitations. *Journal of Crop Production* 2, 241–264.

- Wang, H.Q. and Tang, S.X. (2000) Upland rice production in China: its past, today and future. Paper presented at the Aerobic Rice Workshop, 7–8 September 2000, IRRI, Los Baños, Philippines.
- Westgate, M.E., Passioura, J.B. and Munns, R. (1996) Water status and ABA content of floral organs in drought-stressed wheat. *Australian Journal of Plant Physiology* 23, 763–772.
- Williams, R.L. and Angus, J.F. (1994) Deep floodwater protects high-nitrogen rice crops from low-temperature damage. *Australian Journal of Experimental Agriculture* 34, 927.
- WMO (1997) *Comprehensive Assessment of the Freshwater Resources of the World*. World Meteorological Organization, Geneva, Switzerland, 52 pp.
- Xu, K. and Mackill, D.J. (1996) A major gene for submergence tolerance mapped on rice chromosome 9. *Molecular Breeding* 2, 219–224.
- Xu, K., Xu, X., Ronald, P.C. and Mackill, D.J. (2000) A high-resolution linkage map of the vicinity of the rice submergence tolerance locus *Sub1*. *Molecular and General Genetics* 263, 681–689.
- Xu, W., Subudhi, P.K., Crasta, O.R., Rosenow, D.T., Mullet, J.E. and Nguyen, H.T. (2000) Molecular mapping of QTL conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). *Genome* 43, 461–469.
- Yadav, R., Courtois, B., Huang, N. and McLaren, G. (1997) Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theoretical and Applied Genetics* 94, 619–632.
- Yamamoto, T., Lin, H.X., Sasaki, T. and Yano, M. (2000) Identification of heading date quantitative trait locus *Hd6* and characterization of its epistatic interactions with *Hd2* in rice using advanced back-cross progeny. *Genetics* 154, 885–891.
- Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamamoto, K., Umehara, Y., Nagamura, Y. and Sasaki, T. (2000) *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. *Plant Cell* 12, 2473–2484.
- Yeo, A.R., Flowers, S.A., Rao, G., Welfare, K., Senanayake, N. and Flowers, T.J. (1999) Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. *Plant Cell and Environment* 22, 559–565.
- Yeo, M.E., Yeo, A.R. and Flowers, T.J. (1994) Photosynthesis and photorespiration in the genus *Oryza*. *Journal of Experimental Botany* 45, 553–560.
- Yevjevich, V., Hall, W.A. and Salas, J.D. (eds) (1978) *Drought Research Needs. Proceedings of Conference on Drought Research Needs. Colorado State University, Fort Collins, Colorado, 12–15 December 1977*. Water Resources Publications, Fort Collins, Colorado, 288 pp.
- You, S.C. (2001) Agricultural adaptation of climate change in China. *Journal of Experimental Botany (China)* 13, 192–197.
- Zeier, J., Ruel, K., Ryser, U. and Schreiber, L. (1999) Chemical analysis and immunolocalisation of lignin and suberin in endodermal and hypodermal/rhizodermal cell walls of developing maize (*Zea mays* L.) primary roots. *Planta* 209, 1–12.
- Zhang, H.X., Hodson, J.N., Williams, J.P. and Blumwald, E. (2001) Engineering salt-tolerant *Brassica* plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. *Proceedings of the National Academy of Sciences USA* 98, 12832–12836.
- Zhang, J.X., Nguyen, H.T. and Blum, A. (1999) Genetic analysis of osmotic adjustment in crop plants. *Journal of Experimental Botany* 332, 291–302.
- Zheng, H.G., Babu, R.C., Pathan, M.S., Ali, L., Huang, N., Courtois, B. and Nguyen, H.T. (2000) Quantitative trait loci for root-penetration ability and root thickness in rice: comparison of genetic backgrounds. *Genome* 43, 53–61.
- Zinselmeier, C., Jeong, B.R. and Boyer, J.S. (1999) Starch and the control of kernel number in maize at low water potentials. *Plant Physiology* 121, 25–35.