

Breeding for abiotic stresses for sustainable agriculture

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Using cereal crops as examples, we review the breeding for tolerance to the abiotic stresses of low nitrogen, drought, salinity and aluminium toxicity. All are already important abiotic stress factors that cause large and widespread yield reductions. Drought will increase in importance with climate change, the area of irrigated land that is salinized continues to increase, and the cost of inorganic N is set to rise. There is good potential for directly breeding for adaptation to low N while retaining an ability to respond to high N conditions. Breeding for drought and salinity tolerance have proven to be difficult, and the complex mechanisms of tolerance are reviewed. Marker-assisted selection for component traits of drought in rice and pearl millet and salinity tolerance in wheat has produced some positive results and the pyramiding of stable quantitative trait locuses controlling component traits may provide a solution. New genomic technologies promise to make progress for breeding tolerance to these two stresses through a more fundamental understanding of underlying processes and identification of the genes responsible. In wheat, there is a great potential of breeding genetic resistance for salinity and aluminium tolerance through the contributions of wild relatives.

Keywords: abiotic stresses; aluminium toxicity; drought; nitrogen; salinity

1. THE IMPORTANCE OF LOW NITROGEN, DROUGHT, SALINITY AND ALUMINIUM TOXICITY

Nitrogen is one of the most important yield-increasing agricultural inputs. In many parts of the world, farmers apply low levels of inorganic and organic N owing to the unavailability of inputs and the use of low-input, risk-avoiding strategies to minimize the cost of crop failure. The large-scale application of inorganic N is essential as it is not possible to supply sufficient organic N to be able to feed the world's population (Smil 1991). However, the cost of inorganic N will increase with rising prices for the energy and feedstock required for its manufacture. Hence, the nitrogen utilization efficiency (NUE) of crop plants is of great strategic importance.

Upland rice (*Oryza sativa* L.) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) are prime examples of cereal crops grown under hot, dry conditions where drought is a major constraint on productivity. Rainfed rice systems have only half the productivity of irrigated systems (Hossain & Narciso 2004) and within the rainfed systems upland rice is grown in the most drought-prone situations. The arid and semi-arid tropics where pearl millet is grown are characterized by high temperatures, high levels of solar radiation, high evaporative demand, and unreliable and irregular rainfall. Although pearl millet has an ability to withstand periods of water-limited conditions and still

produce biomass and grain (Bidinger & Hash 2003), post-flowering drought stress is one of the most common and serious environmental limitations to yield (Mahalakshmi *et al.* 1987).

Salinity (for definitions of saline and sodic soils, see Richards (1954), available as handbook no. 60 at www.ars.usda.gov) has long been a major constraint on crop production, and is increasing in irrigated systems (Szabolcs 1994; Shannon 1997). Approximately 800 Mha are affected by salinity or sodicity (FAO 2000), which can reduce wheat yields by 65% (Quayyum & Malik 1988), leading to increased poverty and reliance on imports.

Approximately 49% of arable land worldwide is affected by acid soils (Waquil & Matzenbacher 2000). Aluminium is only phytotoxic under acid conditions. In Nepal, Ethiopia and Kenya, these acid soils are common where wheat (*Triticum aestivum* L.) is the mainstay of smallholder agriculture. Acid soils afflict nearly half of the wheat production area of Nepal, primarily in the mid-hill areas where production on over 100 000 ha is affected (Sherchan & Gurung 1996). In Brazil, production on 70% of the wheat acreage (approx. 900 000 ha) is affected by acidic soils.

2. BREEDING FOR TOLERANCE TO LOW NITROGEN

(a) Nitrogen use efficiency

NUE is the product of two components: uptake efficiency (UptE) and utilization efficiency (UtlE).

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These are simply defined; $NUE = \text{grain yield} / N \text{ applied}$ (with or without top dressing, i.e. all available N); $UptE = \text{total plant N} / N \text{ applied}$; and $UtlE = \text{grain yield} / \text{total plant N}$. In most cereals, $UptE$ is sufficient for approximately 50% of the applied nitrogen to be taken up by the plant (but decreases as more N is applied) while $UtlE$ results in approximately 75% of the total plant N being in the grain at harvest. Uptake efficiency is more important than utilization efficiency in low N conditions, and vice versa in high N. There is an important link between grain N content (a proxy for protein content) and grain yield. Improved NUE has some incompatibility with high grain protein content as many authors have shown these traits to be negatively correlated (e.g. Anderson & Hoyle 1999 in wheat; Sinebo *et al.* 2004 in Ethiopian barley). However, Guarda *et al.* (2004) point out, using the example of Italian bread wheat cultivars over the period 1900–1994, that a very gradual decline in protein content is accompanied by an appreciable increase in protein production per hectare, and the lower protein percentage is simply a dilution effect from the great increases in carbohydrate yield.

Interestingly, breeding for tolerance to low N may be related to tolerance to other stress factors and vice versa. Bänziger *et al.* (2002) report that tropical maize selected for drought tolerance is also tolerant to low N. They postulate constitutive stress tolerance mechanisms that increase yield and yield stability.

Genetic advances for improved NUE have been assessed in three main ways: retrospective analysis of changes in cultivars over time for their grain yield and NUE; quantitative genetic approaches to analyse parameters that can predict genetic advance (here only analyses that compare these statistics in high (HN) and low N (LN) are reviewed); and comparisons of the results of selection in both high and low N environments.

(b) NUE and the results of long-term plant breeding

Austin *et al.* (1980) showed that in 12 European wheat cultivars genetic gains for grain yield had been made that were expressed in both LN and HN regimes. NUE (for both uptake and utilization) of Italian wheat cultivars improved from 1900 to 1994 under both residual and added N conditions (Guarda *et al.* 2004). Ortiz-Monasterio *et al.* (1997) showed that the grain yield of CIMMYT wheats had improved from 1950 to 1985 under both LN (1.1% yr^{-1}) and HN (1.9% yr^{-1}) conditions. When the transition from tall to semi-dwarf wheats was disregarded by measuring grain yield gains only within the semi-dwarf wheats (i.e. 1962–1985), the gains in LN were higher (1.2% yr^{-1}) but under HN somewhat lower (0.9% yr^{-1}). Gains in NUE were significant and similar in both low and high N in this same period. The improved NUE came from improved uptake efficiency under LN whereas improved utilization efficiency was the most important factor under HN, a result confirmed in European winter wheat by Gouis *et al.* (2000).

(c) Direct or indirect selection for adaptation to low N environments

Low N is a stress environment and heritabilities that determine genetic advance under selection can change with stress. The results are inconsistent. Lower heritabilities in stress environments were found by: Frey (1964) and Atlin & Frey (1990) for low fertility and drought stress in oats; Ud-Din *et al.* (1992) for drought in wheat; and by Quisenberry *et al.* (1980) for low temperature and drought stresses in cotton. In maize, under low N stress conditions broad-sense heritabilities were lower largely owing to lower genotypic variances than owing to increased error variances (Bänziger *et al.* 1997). However, Atlin & Frey (1989) with LN, low P (LP) and late planting stresses in oats found no consistent relationship between heritability and mean yield. Presterl *et al.* (2003) found that in European flint and dent maize breeding materials heritabilities were similar at both LN and HN even though $G \times \text{Location}$ and error effects were higher under LN. Sometimes under stress conditions heritabilities increase. Examples include Pedersen & Rathjan (1981) for low yielding environments in wheat and Zhongzhe *et al.* (2004) for rice under low N.

Indirect selection is the selection in one environment (e.g. in non-stress) to gain a response in another (e.g. a low N stress environment). For indirect selection to be more effective than direct selection, the product of the heritability in the non-target environment and the genetic correlation between the two environments has to be higher than the heritability in the target environment. Such a situation rarely occurs and when indirect selection is reported to be effective it may simply be that the alleles under selection are more or less the same in both environments or because the environments do not differ sufficiently, i.e. in situations when $G \times E$ is low. Hence, Bänziger *et al.* (1997) showed that indirect selection was more efficient than direct selection when the relative yield in the two environments did not differ greatly; only when the relative yield reduction of LN compared with HN exceeded approximately 45% was direct selection in LN statistically more effective (and this occurred even though broad-sense heritabilities were approximately 30% lower under LN). Reductions in yield to this extent (greater than 45%) through limited N may not be typical in temperate areas but are common under tropical conditions. In European maize, Presterl *et al.* (2003) reported a similar result. The efficiency of selection for grain yield at low N through direct selection at LN was 40% higher than indirect selection at HN. As the low and high N environments became more distinct, i.e. the grain yield differences increased, the efficiency of direct selection in LN increased relative to indirect selection.

(d) Results of selection experiments for improved NUE

A selection experiment in rice in segregating generations under zero and applied N showed that h^2 and response for NUE and grain yield were higher in LN. Indirect selection was also effective with selection in

one N regime showing responses in both (ZhongZhe *et al.* 2004).

The most elegant breeding scheme is to select genotypes in early generations that give high grain yield under both high and low N. In CIMMYT Zimbabwe, selection was made in maize for S1 lines that yielded well either with or without N topdressing. These were compared with S1 lines that yielded well under the higher N regime but poorly in the low N environment. S2 lines were derived from the S1 lines and the ability to perform well in both N regimes was inherited (Short 1994).

In wheat, van Ginkel *et al.* (2001) examined selection strategies for improved NUE under low, moderate and high N. Two sets of selection were done; one where the breeders applied visual selection for yield and the other where only natural selection was permitted. The results are reinterpreted here to exclude the results that included breeders' visual selection for yield where few of the selection responses fitted with the N regime under which the selection occurred; the visual selection appears to have overwhelmed selection for adaptation to N regime. When the results of natural selection only are considered (simple bulk harvesting at each generation) then selection in moderate nitrogen was the most effective strategy. It was the only selection regime which resulted in populations that were in the statistically highest yielding group for every N environment for most traits (grain yield, harvest index, biomass, uptake efficiency, utilization efficiency and NUE). This fits well with the evidence that UptE is more important under low N, and UtIE is more important at high N, so that under moderate conditions both traits are positively selected.

3. COMPONENTS AND MECHANISMS OF DROUGHT AND SALINITY STRESS RESISTANCE

Drought and salinity are two of the most complex stress tolerances to breed for as the type (combinations of heat and drought or sodicity and salinity), timing in relation to plant growth stage and intensity of stress can all vary considerably.

There is no unified abiotic stress resistance mechanism for drought at the level of the whole plant or the single gene (Blum 2004). The traits associated with avoidance and tolerance can be constitutive (differing between genotypes) or adaptive (vary with the stage of the life cycle). Drought avoidance and drought tolerance involve different mechanisms and processes, and phenology is the single most important factor influencing whether a plant avoids drought. Drought stress is highly variable in its timing, duration and severity, and this results in high environmental variation and $G \times E$ variation. The whole-plant response to stress is complex because it is determined by component traits that interact and differ in their individual responses to the intensity and duration of water deficits and temperature. The use of managed stress environments can be very effective in breeding for drought tolerance, however, it is important to apply sufficient drought stress intensity to maximize $G \times E$ (Bänziger *et al.* 2006).

Salt tolerance is a complex and multigenic trait (Shannon 1997; Flowers 2004). Key traits for salt tolerance in wheat were discussed by Colmer *et al.* (2005): one of the most important is the combination of low rates of Na^+ (high K^+/Na^+ discrimination) and Cl^- uptake and transport to the leaves (salt exclusion; Munns *et al.* 2002). However, in rice, transpirational flow is highly correlated with Na^+ uptake that is an order of magnitude greater than in wheat (Garcia *et al.* 1997), reducing the importance of K^+/Na^+ discrimination in rice.

Salt has both osmotic and salt-specific effects on plants, reviewed by Munns (2002, 2005), impacting at different times. Tolerance also differs with growth stage and environmental conditions (Shannon 1997). Rapid changes in field salinity (Richards 1983) make reliable screening difficult. The effects of these changes are increased by plants preferentially extracting water from less saline areas of the root zone, by drought in rainfed environments (Srivastava & Jana 1984), and by waterlogging in irrigated environments. This can be compounded by additional stresses which vary with time, so cultivar adaptability depends upon tolerance over the growing season (Gregorio *et al.* 2002).

Richards (1992) concluded that because only small areas of a field may be highly saline, selection at low salinity was preferable as most yield comes from the less affected areas. However, his conclusions are related to drying saline fields that are inappropriate for the irrigated conditions of many developing countries, and he did not consider important salinity/waterlogging interactions (Barrett-Lennard 2003). Isla *et al.* (2003) agreed that, for moderately saline soils the best strategy was to breed for yield, but for higher salinity, breeding for yield and salinity tolerance together was important. Performance can also be improved by simultaneously screening for salinity and waterlogging tolerance (Akhtar *et al.* 1994). The selection method should depend upon the combined stresses faced in the target environment, and these often differ between irrigated and rainfed agriculture.

(a) Sources of salt tolerance

Most crops are adversely affected by salinity. In general, cereals are more tolerant than legumes (Reynolds *et al.* 2005). Many wild relatives of crop plants show greater tolerance than their domesticated descendants. Bread wheat is generally regarded as moderately tolerant, durum wheat somewhat less so, while rice is regarded as susceptible (Francois & Maas 1994).

Efforts have long been made to breed crops tolerant to salinity, but with little progress in producing varieties used by farmers (Flowers 2004; Gregorio & Cabuslay 2005). However, tolerant varieties of rice, wheat and other crops have been produced and released by the International Rice Research Institute (IRRI) (Dedolph & Hettel 1997; Senadhira *et al.* 2002), and in India (Mishra 1996; IRRI 1998; CSSRI 1999) and Pakistan (Qureshi *et al.* 1980; Qureshi *et al.* 1996).

Bread wheat shows much variability for salt tolerance among existing cultivars and landraces (Ashraf & McNeilly 1988; Martin *et al.* 1994; Poustini & Siosemardeh 2004). The Rajasthan landrace Kharchia has been a source of tolerance in India for many years (Dhir & Bhatia 1975), resulting in two released

varieties, KRL 1–4 and KRL 19 (Mishra 1996; CSSRI 1999). Variation in Na⁺ and Cl⁻ partitioning occurs between and even within wheat varieties (Rashid *et al.* 1998; Salam *et al.* 1999; Ashraf *et al.* 2005). In tetraploid wheat, lines were identified that have low Na⁺ accumulation and high K⁺/Na⁺ discrimination (Munns *et al.* 2000) and they were used to improve the tolerance of durum wheat (Lindsay *et al.* 2004). Similarly, in rice, Yeo *et al.* (1988) selected lines from within varieties that showed either low or high rates of Na⁺ transport: the high rates were up to three times those of the low. Rice landraces from the saline coastal belt of Bangladesh have recently been identified as potential sources of tolerance (Lisa *et al.* 2004).

Greater K⁺/Na⁺ discrimination at low salinities is shown by hexaploid bread wheat than by tetraploid durum wheat (Wyn Jones *et al.* 1984), and by *Aegilops tauschii* (syn *Aegilops squarrosa* syn *Thinopyrum tauschii*), the D genome ancestor of bread wheat (Gorham 1990).

In *Thinopyrum* species, related to wheat, tolerance is associated with shoot Na⁺ and Cl⁻ exclusion, particularly at higher NaCl concentrations (Gorham 1994). In *Thinopyrum elongatum*, Dvořák *et al.* (1988) and Omielan *et al.* (1991) reported major effects of chromosomes 3, 4 and 7 on ion exclusion. In *Thinopyrum bessarabicum*, group 2 chromosomes carry genes conferring susceptibility to salt, and 5E^b has a major dominant gene or genes for tolerance (Forster *et al.* 1988, Mahmood & Quarrie 1993). Koebner *et al.* (1996) proved the involvement of chromosomes of homoeologous group 5 in salt tolerance in wheat. These effects are closely linked to genes controlling vernalization (group 5) and photoperiod (group 2) response (Taeb *et al.* 1992; Martin *et al.* 1993). Zhong & Dvořák (1995a,b) suggested that Triticeae species generally shared common mechanisms of tolerance to sudden salinity stress.

(b) Marker-assisted selection

Molecular marker technologies can be used to dissect defined quantitative traits by quantitative trait locus (QTL) mapping so that individual loci can be targeted in marker-assisted selection (MAS). Usually, QTL mapping studies are a prerequisite for MAS which can be used to pyramid several different QTLs. Marker-assisted backcrossing (MABC) combines 'foreground' selection of donor alleles linked to QTLs and 'background' selection of recurrent parent alleles in the BC₂ and later generations. Advanced backcross methods were developed by Tanksley & Nelson (1996) that allow simultaneous analysis and introgression. Despite advances such as the replacement of the 1980s RFLP markers by PCR-based markers such as simple sequence repeats or microsatellites (SSRs) that permit multistage selection which increases the cost-effectiveness of MAS, the introgression of multiple QTLs into elite varieties is an expensive and laborious process. In the case of stress-related QTLs, the results of MAS are limited (Tuberosa *et al.* 2002; Steele *et al.* 2006) owing to difficulties such as QTLs that have epistatic interactions and do not contribute significantly in a novel genetic background. For effective MAS programmes, QTLs must be expressed and beneficial in new genetic

backgrounds and need to be stable across different mapping populations and environments.

(c) Drought tolerance in rice

Rice researchers are currently seeking QTLs that are stable across environments and that co-segregate with improved yield. Lafitte *et al.* (2004) found co-segregation between QTLs for yield components under drought and for traits such as leaf rolling and drying in a population from Azucena/Bala. In a Vandana/Way Rarem population, there was co-location for QTLs for grain yield under drought with QTLs for maturity, panicle number and plant height (Bernier *et al.* 2007). Overall, there were stronger associations with maturity (avoidance) than with traits that are considered as important for plant-water relations (tolerance).

Much genetic analysis has been done in the past 20 years to identify QTLs for traits related to drought resistance (Nguyen *et al.* 1997). A high-density map for a cross between an upland (CT9993) and a lowland variety (IR62266) revealed QTLs across the genome for osmotic adjustment (OA) and root physiological and morphological traits. This map has been used to locate expressed genes and identify putative candidates for these traits (Nguyen *et al.* 2004; Yang *et al.* 2004).

Roots have been the focus of many physiological and QTL mapping studies aimed at improving drought tolerance (e.g. Moons *et al.* 1995; Tuberosa *et al.* 2003). In rice, some of the many QTLs for roots are common across different genetic backgrounds (Li *et al.* 2005a). A QTL for root length and thickness on chromosome 9 has been mapped in several populations and is expressed across a range of environments. It was the only one of the four target root QTLs that significantly increased root length when introgressed into a novel genetic background (Steele *et al.* 2006). Root density and root thickness QTLs show poor alignment with QTLs for non-root-related drought avoidance traits; however, root penetration QTLs do co-segregate with them, particularly leaf drying QTLs (Price *et al.* 2002; Lafitte *et al.* 2004). Many root QTLs show strong interactions with the environment, in particular the physical properties of the soil (Yue *et al.* 2002; Cairns *et al.* 2004). Owing to considerable G × E, their effects on productivity under stress in the field are very difficult to determine and disappointingly few root QTLs have been found to be related to yield.

Steele *et al.* (2004) tested an alternative method to simultaneously identify molecular markers linked to agronomic performance under stress and transfer them to elite rice varieties. Termed marker-evaluated selection (MES), this approach used a very large segregating population derived from a wide cross between the upland variety Kalinga III and the irrigated variety IR64. The population was selected for overall agronomic performance in several target stress environments over many generations and the products from the selection were evaluated with markers. Varieties selected in upland ecosystems (e.g. Ashoka 228) outperformed the parent Kalinga III. In the lines adapted to upland environments alleles from Kalinga III were inherited at more than 95% of the loci tested. This shift was most apparent where selection pressures for drought resistance were strongest. The approach has led to the

identification of molecular markers at the rare genomic regions donated by the lowland parent that are present in the selected upland varieties (Steele *et al.* 2005, unpublished data). Analysis of the extent of linkage disequilibrium is currently being used to detect the size of these introgressed regions. The findings complement those of Lafitte *et al.* (2006) who demonstrated that the yield of IR64 under drought can be improved by backcrossing with selection under stress.

The range of approaches available to identify markers for selection in rice has broadened as a result of the availability of the full genome sequence (International Rice Genome Sequencing Project 2005; Shen *et al.* 2004). Map-based cloning (cloning of genes at QTL) bridges the gap between QTL analysis and genomics and has recently led to the isolation of a gene for a yield QTL in rice (Ashikari *et al.* 2005), but the effect of this QTL under stress has not yet been reported.

(d) Drought tolerance in pearl millet

Two mapping populations based on hybrid parents with known differential response to stress were created at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), India. One of the parents of each of the two populations (PRLT 2/89-33 and 863B) was mainly derived from landrace material from Togo and Ghana that has enhanced tolerance to post-flowering drought stress but poor seedling thermotolerance. For each population, the other parent of each population (H 77/833-2 and ICMB 841, respectively) had poor drought tolerance, but both are widely used in India for the commercial production of pearl millet hybrids. A linkage map for each population was generated using molecular markers developed at the John Innes Centre (Liu *et al.* 1994; Qi *et al.* 2004). Progeny of these two mapping populations were evaluated for agronomic and physiological traits under both irrigated control and a wide range of post-flowering drought stress conditions. This led to the identification of a major genomic region on linkage group 2 (LG2) in both populations and in all environments that was associated either with grain yield, or with the ability to maintain grain yield, under drought conditions (Yadav *et al.* 2002, 2004). Delay in leaf rolling also mapped to this region. A number of minor QTL for both yield and yield components were identified, in some but not all phenotyping environments, on LG1, LG5 and LG6 associated with maintenance of grain yield under drought conditions and which co-mapped for osmotic adjustment and leaf senescence during drought stress (Yadav *et al.*, 1997, 1998, 1999, unpublished data). In most cases, the drought-tolerant parent contributed the positive allele. Co-mapping of QTL for individual traits and grain yield under stress suggested a linkage between the ability to maintain grain yield under terminal stress and the ability to maintain both panicle harvest index (primarily grain filling) and harvest index, as well as confirming the benefits of drought escape achieved through early flowering.

MABC is being used to introgress these QTLs into inbred hybrid parental lines for the subsequent production of improved hybrids (Hash *et al.* 2000). Employing MABC to enhance the drought tolerance of proven parental lines allows the breeder to concentrate

on improving drought tolerance *per se*, with the knowledge that the recurrent parents are otherwise fully acceptable to the seed industry (Hash *et al.* 2000).

An initial evaluation of the QTL on LG2 from PRLT 2/89-33 associated with drought tolerance was made by comparing hybrids made with topcross pollinators bred from progenies selected from the original mapping population for presence of the tolerant allele at the target QTL compared with those selected by field performance in the phenotyping environments (Bidinger *et al.* 2005). A set of 36 topcross hybrids was evaluated in 21 field environments, which included both non-stressed and drought-stressed treatments during the flowering and grain filling stages. The QTL-based hybrids were significantly, but modestly, higher yielding in a series of both absolute and partial terminal stress environments. However, this gain under stress was achieved at the cost of a lower yield in the non-stressed evaluation environments. This particular pattern of adaptation in the QTL-based hybrids was consistent with their general phenotype—early flowering, limited effective basal tillering, low biomass and a high harvest index—which resembled that of the drought-tolerant parent of the original mapping population. The results thus confirmed the effectiveness of the drought tolerance QTL on LG2, but suggested that it may enhance drought tolerance by favouring a particular phenotype with adaptation to terminal stress. In later work in a different genetic background (described below), the effectiveness of the QTL was confirmed but these phenotypic effects were no longer obvious.

MABC was used to produce a set of near-isogenic versions of H77/833-2 with and without the LG2 drought tolerance QTL from the donor parent PRLT 2/89-33. BC₄F₃ progenies from selected BC₄F₂ plants homozygous for various portions of the LG2 target region were crossed to each of five different seed parents, and the resulting hybrids were evaluated under a range of moisture regimes (non-stressed control, early onset, medium-onset and late-onset terminal drought stress). The hybrids exhibited a large variation in yield component expression and yield response to the moisture regimes, but there was a consistent yield advantage in hybrids carrying markers for the target QTL (Serraj *et al.* 2005). Hybrids produced on the lines with the QTL yielded up to 21% more grain under post-flowering drought tolerance with no adverse effect on grain yield under fully irrigated control conditions. Several of these introgression lines had a significant, positive general combining ability (across all test-crosses) for grain yield under terminal stress, which was associated with a higher panicle harvest index. Interestingly, this superior grain yield performance of the introgression line hybrids was often accompanied by increased biomass yields and reduced grain harvest indices; hybrids of the donor parent had reduced biomass yield and increased grain harvest index that contributed to their higher grain yield under non-stressed and terminal drought conditions. Thus, these marker-assisted breeding products appear to have greater value for breeding dual-purpose (grain and stover) hybrids for both water-limited and assured moisture conditions than either of their parental lines.

(e) Salt and aluminium tolerance

Pyramiding of traits has long been advocated to improve salt tolerance (Yeo & Flowers 1986) and MAS makes this more feasible. In wheat, a trait of enhanced K^+/Na^+ discrimination at low salinity was found to be located on chromosome 4DL (Gorham *et al.* 1987). This was controlled by a single gene (*Kna1*; Dvořák & Gorham 1992), and mapped as both a quantitative and a qualitative trait (Dubcovsky *et al.* 1996). QTLs for salt tolerance have been located on chromosome 5A (Semikhodskii *et al.* 1997), homoeologous to two yield QTL clusters on 5B and 5D in a different population grown under saline conditions (Quarrie *et al.* 2005).

In rice, Flowers *et al.* (2000) identified AFLP markers for ion transport and selectivity. In the tolerant variety Pokkali, a major gene, possibly *SalT* (Causse *et al.* 1994), has been mapped (IRRI 1998), as were QTLs governing high K^+ and low Na^+ absorption, and high K^+/Na^+ ratio (Gregorio *et al.* 2002). A common major QTL was found on chromosome 1 for three traits associated with salt tolerance (IRRI 1998). Other markers have been identified for traits associated with productivity in saline environments, although many are associated with tolerance to submergence, and micronutrient deficiency or toxicity (Gregorio *et al.* 2002). Other QTLs for salt tolerance have been mapped by Koyama *et al.* (2001), Lin *et al.* (2004) and Takehisa *et al.* (2004).

Overall, relatively few QTLs have been identified for salt tolerance. This could either be because the traits are actually determined by a limited number of loci, or that the genes associated with the traits were clustered on particular chromosomes (Flowers 2004). One QTL for salt tolerance on rice chromosome 1 has been fine mapped and the gene identified as a sodium transporter (Ren *et al.* 2005). It is not yet clear whether it is responsible for other QTLs on chromosome 1, or if there is a cluster of relevant genes in this region.

Applications of MAS for salt tolerance are few. A Na^+ exclusion trait was mapped in durum wheat in a population from a cross between a low Na^+ landrace and wheat cv. Tamaroi (Lindsay *et al.* 2004). An SSR marker for this QTL, closely linked to a locus, *Nax1*, on chromosome 2AL which accounts for 38% of the phenotypic variation, has been used for marker-assisted selection and, in glasshouse trials, improved grain yields by 20% under moderate salinity (Husain *et al.* 2003).

For tolerance to aluminium toxicity, plant breeders in Brazil have used MAS to incorporate a tolerance gene found on chromosome 4D into modern cultivars (Lagos *et al.* 1991; Riede & Anderson 1996).

4. THE POTENTIAL OF 'OMICS' TECHNOLOGIES FOR ABIOTIC STRESS BREEDING

The success of MAS for pyramiding QTLs could be improved by targeting the genes controlling the traits rather than linked marker loci, so that linkage drag is reduced. Finding functional genes within a QTL is complicated because the QTL confidence intervals can span several hundred genes. Bennett & Khush (2003) concluded that only major QTLs could be mapped with enough precision, and efforts should be on screening collections for major QTLs rather than

trying to identify minor QTLs from a single donor. However, Price (2006) notes that in some cases map-based positional cloning of small QTLs (with low heritability) is now a realistic possibility. Integrated genomics studies are possible due to the availability of DNA sequences in databases, and complete physical maps for several plant species. Here we briefly review the expanding range of techniques available that can identify the molecular processes involved and reveal vast amounts of information about gene expression, gene function and gene interactions during stress adaptation.

Gene expression studies that were, until recently, limited to only a few genes at once (Skriver & Mundy 1991) can today be performed with oligonucleotide and cDNA microarrays on multiple genes at the same time to produce expression profiles specific to the environments in which they are assayed. They can identify candidate genes or proteins for specified physiological or adaptive processes (Edmeades *et al.* 2004; Riccardi *et al.* 2004). Expression profiling to understand responses to applied drought and salinity stresses have been used in barley (Ozturk *et al.* 2002) and rice (Kawasaki *et al.* 2001). They have given knowledge of many osmotically induced genes, allowing the possibility of deciding on gene combinations and modifications for the engineering of signalling and metabolism, and so to improve tolerance (Hasegawa *et al.* 2000).

Many genes responsive to abiotic stresses have been identified in expression experiments in *Arabidopsis* (Seki *et al.* 2002, 2004; Bray 2004). Such experiments produce long lists of candidate genes and their putative functions. For example, Seki *et al.* (2002) listed 53 genes induced by cold, 277 by drought and 194 by salt stress. The elucidated or hypothetical function of the genes reveals two major gene classes. One class encodes proteins that are directly involved in biochemical, physiological or morphological adaptive changes, for example, stress decreases the expression of genes for cell expansion (Bray 2004). The other class encodes proteins that are involved in the regulation of biochemical pathways. As more gene and protein sequences become available in public databases, the information revealed in any expression array experiment becomes more meaningful. For practical use in breeding, the choice has to be narrowed by interpreting the roles of these genes or proteins in relation to abiotic stress by employing hypotheses on what are the underlying physiological traits.

The isolation of component genes for root QTLs could be speeded through expression studies. In rice, Yang *et al.* (2004) identified 66 transcripts that were differentially expressed in different types of root tissue of the upland variety Azucena under water deficit. Some of them were not expressed in the lowland variety IR1552 and four mapped to intervals containing QTL for root growth under water deficit in this population. Although it is possible to detect QTL using large-scale expression analysis, the lack of precision in most QTL maps means that, by chance, differentially expressed genes will be found in the region of QTLs that are not the cause of the quantitative variation between the parents (Hazen *et al.* 2004).

Knowledge of the rice proteome is relatively advanced compared with other crop plants (Komatsu & Tanaka 2005). Using silver-stained two-dimensional gels, Salekdeh *et al.* (2002) compared several thousand leaf proteins from two rice varieties. Of 42 proteins with significant quantitative and/or qualitative changes on drought and rewatering, eight were excised, digested, ionized and analysed to find the masses of peptide fragments (peptide mass fingerprints). This enabled their functions to be predicted.

The metabolic basis of salt adaptation has recently been reviewed (e.g. Hasegawa *et al.* 2000; Wang *et al.* 2003b). However, few studies have revealed transcripts unambiguously related to salt tolerance (Munns 2005). Often studies are not designed to identify the genes conferring tolerance under natural conditions: for example, high NaCl concentrations applied in one dose, without Ca²⁺ to mitigate the shock, make it unlikely that genes controlling Na⁺ or K⁺ transport will be discovered. Such a disregard of the mechanisms of tolerance means that much work has identified tolerance to osmotic stress rather than to salinity.

5. TRANSGENICS

Gene function can be assigned either through the classical forward genetics approach of starting with a phenotype and identifying the genes responsible, or reverse genetics that starts with a DNA sequence and identifies its biological role. In either case, transgenic technologies allow the genetics to be verified by confirming the action of single genes the over expression of which can lead to increased tolerance to abiotic stresses. A substantial proportion of transgene studies lack any rigorous measurement of either the environmental stress imposed or the resulting tissue water status (Jones 2007).

The transgenic approaches and some of the many experiments designed to study the expression of stress-related genes have been reviewed by Coraggio & Tuberosa (2004) and Bajaj & Mohanty (2005). The majority of transgenes used for stress tolerance are transcription factors that upregulate or downregulate the expression of other genes. Shinozaki *et al.* (2003) reviewed the functional analysis of many genes for transcription factors and have revealed a complex hierarchy of signalling networks. The pathways (there are at least four) are divided into two classes; those responsive to abscisic acid (ABA) and those that are not.

Much of this work has been carried out in *Arabidopsis* with the dehydration-responsive element-binding (DREB) transcription factors of the ABA-dependent pathway IV that was first associated with improved cold tolerance (Gilmour *et al.* 2004). As well as with constitutive promoters, these have been inserted under the control of an *Arabidopsis* dehydration-responsive promoter rd29A. Similar DREB genes and promoters have been found in crop plants including rice (Dubouzet *et al.* 2003), maize (Qin *et al.* 2004) and soybean (Li *et al.* 2005b). Use of the stress inducible promoter rd29A in conjunction with DREB1 may enhance drought tolerance in tobacco (Kasuga *et al.* 2004) and wheat (Pellegrineschi *et al.* 2004). Field trials are currently underway in Mexico to test

transgenic wheat (with the *Arabidopsis* DREB1A under the control of rd29A) that has a more branched root phenotype and developed more ears under drought stress than control lines under greenhouse conditions. However, Pellegrineschi *et al.* (2004) have noted that the presence of the transgene delays germination, and they have yet to determine whether the gene expression can be 'switched-off' when the plants return to non-stress conditions.

Genes for aquaporins (water channel proteins) and late embryogenesis abundant proteins (LEA; proteins with several predicted functions) have been tested in transgenic studies addressing drought and salinity, but much more work is necessary before we know whether they will be useful to breeders. A maize LEA that was over expressed in *Arabidopsis* is reported to have improved salinity tolerance (Figueras *et al.* 2004) and a barley LEA expressed in two rice varieties may enhance growth under either salt or drought stress (Rohila *et al.* 2002; Babu *et al.* 2004).

Much has been written about improving salt tolerance through gene over expression (Winicov 1998; Bohnert *et al.* 2001) but there has been little effect so far on performance in saline field conditions, and papers that report on improvements in salinity tolerance are generally restricted to glasshouse trials (e.g. improved salinity tolerance in cotton (He *et al.* 2005)). Flowers (2004) argues that it is unlikely that crop tolerance could be manipulated by altering the activities of one or two genes, despite many claims to the contrary. Over 700 patents have been granted invoking salt tolerance (Munns 2005) and candidate genes for tolerance traits are listed, but their use to produce cultivars for farmers has been very slow. Very few published reports have compared yield of field-grown transformed and 'wild-type' plants under saline and non-saline conditions, and most could not predict field tolerance as they were conducted under conditions where there was little or no transpiration (Flowers 2004). Any improvement in tolerance is often at the expense of productivity under non-saline conditions. More care in the design of experiments would substantially increase the value of these trials.

6. WIDE HYBRIDIZATION FOR SALINITY AND ALUMINIUM TOLERANCE

Although early work on increasing salt-tolerance in wheat concentrated on screening existing varieties (e.g. Epstein *et al.* 1979; Qureshi *et al.* 1990), the tolerance of wheat relatives to salinity and other stresses has been known for many years. In rice, wild relatives are an important source of resistance to abiotic stresses (Brar & Khush 1997), although resistance to salinity is less common (Akbar *et al.* 1986; Yeo *et al.* 1990) and only one source is known: it could be transferred from the tetraploid Asian wild rice *Porteresia coarctata* to *O. sativa* through DNA-based techniques or bridge crossing (Lath *et al.* 2004).

The enhanced K⁺/Na⁺ discrimination of *A. tauschii* (Gorham *et al.* 1987) has been exploited (Mujeeb-Kazi *et al.* 1996) to incorporate traits into durum wheat by producing synthetic hexaploids with superior K⁺/Na⁺ discrimination in saline hydroponic culture

(Pritchard *et al.* 2002). Direct crossing between *A. tauschii* and bread wheat has been reported several times (e.g. Cox *et al.* 1990, 1991), although in the field these have not done as well as salt-tolerant hexaploid wheats (Acevedo *et al.* 2003). Similar work on *A. tauschii* introgression has been carried out in Australia (Schachtman *et al.* 1991, 1992) and, in particular, using *Aegilops cylindrica*, in Pakistan (e.g. Farooq *et al.* 1992a,b, 1995). The *Kna1* locus from bread wheat was also successfully introgressed into chromosome 4B of *T. turgidum* using homoeologous recombination (Dvořák *et al.* 1994; Luo *et al.* 1996). The lines containing *Kna1* had higher grain yields and lower K^+/Na^+ ratios than the parents or lines without it, in saline field trials.

Three perennial species (two diploid: *T. bessarabicum*, *T. elongatum* and one decaploid, *Thinopyrum ponticum*) are important sources of salt tolerance for wheat breeding (Gorham *et al.* 1985; McGuire & Dvořák 1981). Dvořák *et al.* (1985) hybridized *T. ponticum* with wheat and selected a salt-tolerant line with a low number of *Thinopyrum* chromosomes. Chinese Spring wheat was crossed with *T. elongatum*: the resultant 56-chromosome amphiploid produced more grain and dry matter per plant than Chinese Spring in saline field conditions (Dvořák & Ross 1986; Omielan *et al.* 1991), and is also tolerant to saline hypoxia (Akhtar *et al.* 1994). Gorham *et al.* (1985) found that *T. bessarabicum* withstood prolonged exposure to high salinity. They also (Gorham *et al.* 1986b) found variation in ion accumulation within and between many perennial Triticeae (reviewed in detail by King *et al.* (1997a)). Proceeding through amphiploids (Forster & Miller 1985; Gorham *et al.* 1986a; Forster *et al.* 1987), addition (Forster *et al.* 1988) and substitution lines (King *et al.* 1996), tolerance genes from *T. bessarabicum* were introduced into Chinese Spring. Field testing in Spain confirmed the high tolerance of the material (Gorham *et al.* 1997), but so far the segment of the 5E^b chromosome carrying tolerance has not been introduced into wheat without the linked deleterious genes. However, Wang *et al.* (2003a) have registered salt-tolerant germ plasm possessing alien diversity from hexaploid *T. bessarabicum* (*Thinopyrum junceum*), possessing similar tolerance to Kharchia, although there is some doubt as to the screening process upon which the tolerance was based (Colmer *et al.* 2005).

Another route could be to develop the amphiploids as a new salt-tolerant crop, 'tritipyrum', in the same way as triticale (Gregory 1987). Several tetraploid wheats, with chromosome 4D substituted for 4B, were hybridized with *T. bessarabicum* (King *et al.* 1997b). Most were wheat-like, but with speltoid ears and a brittle rachis, although considerably more tolerant than their wheat parents. This would be a long process, but is thought to have potential (King *et al.* 1997b).

The diploid goat-grass *Aegilops uniaristata* (NN; $2x=14$) is tolerant of aluminium during its growth cycle (Berzonsky & Kimber 1986), and such techniques were employed to add its 3N chromosome to the wheat complement, and subsequently to substitute it for the homoeologous wheat group 3 chromosomes (Miller *et al.* 1993). The parental tolerance was expressed in both the addition and the three

substitutions (Miller *et al.* 1997). However, 3N carries deleterious genes such as the dominant spike neck break so wheat/3N recombinants were created to try and remove it. Six lines were derived through a cytogenetic protocol which exploits a deficiency of the *Ph1* homologous pairing control locus of wheat to induce recombination with the alien 3N chromosome (Iqbal *et al.* 2000a,b). Following fast neutron irradiation of wheat/3N substitution hemizygotes another four lines were selected with deletions that had removed the deleterious gene for spike neck break. Tolerance in these lines was assessed and they were entered into a CIMMYT wheat backcross breeding programme using recurrent parents from targeted countries with acid soils.

Both *Aegilops uniaristata* itself and the 3N introgression lines into wheat apparently do not exclude or excrete aluminium, but instead isolate it in root tissue. They survive apparently by continually producing fresh healthy roots. Aluminium susceptible wheat control 'Chinese Spring' (CS), tolerant wheat control 'CS(3D)3N substitution', together with the ten wheat/3N recombinants, were grown to maturity with varying levels of Al^{+++} . Background variation due to the limited number of backcross generations ruled out comparison with controls, so in Al were compared to themselves in the zero Al^{+++} control. Three of the *Ph1*-derived recombinants and one derived via fast neutron irradiation were judged to be tolerant on the basis of multiple traits including biomass and grain yield.

The small number of recombinants meant that statistical linkage could not be determined for the existing translocations. Initially, 46 group 3 chromosome-specific microsatellite (SSR) markers were identified from over 170 screened via DNA extracted from leaf tissue. Eleven of these proved to have discrete 3N specific products, but only five were reliable in repeated use. Another group 3 chromosome-specific marker, *barc 1040*, was later tried and this exhibited the best association with tolerance.

7. CONCLUSIONS

The simplest approach to breeding for stress tolerance is also the most effective. It is to select for yield which is the integrating trait and to carry out the selection in a representative stress environment. It can be enhanced by the use of screens for stresses such as low N, drought or salinity where the stresses are carefully managed (Bänziger *et al.* 2006; Lafitte *et al.* 2006) and by carefully choosing parents of crosses so that various physiological traits can be pyramided (Yeo & Flowers 1986). This implies a reduction in the number of crosses that are made so that larger populations can be employed (Witcombe & Virk 2001), an approach that has been effective in the breeding of stress tolerant, widely adapted rice (Joshi *et al.* 2007). Marker-assisted selection can be effective in enhancing efficiency but, at least so far, selection for markers linked to component traits of low heritability has not produced predicted outcomes. The introgression of QTLs for yield under stress is proving to be a more successful strategy in pearl millet (Serraj *et al.* 2005). Fine-mapping and map-based cloning of QTLs (Price 2006) will allow

them to be used more effectively in breeding by eliminating the effects of unwanted linked alleles. MAS needs to be fine-tuned so that precise combinations of alleles can be combined for maximum effect. Transgenic and functional genomics approaches hold tremendous promise for the future assuming that realistic, evidence-based approaches to their employment emerge for informative assessment of stress tolerance. Researchers should not neglect 'epigenetic' differences in gene expression that arise during development and are subsequently retained through mitosis; such mechanisms allow organisms to respond to the environment through changes in gene expression patterns (Jaenisch & Bird 2003). Future breeding programmes that follow a 'systems biology' approach to integrate omics datasets with experimental results from both controlled and field studies will be the most informative. In the meantime, wide hybridization (with backcrossing to the most well-adapted parent) with selection in the target environment offers the best opportunity for widening the genetic base of stress resistance for sustainable agriculture.

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