

# Quantitative Trait Loci and Crop Performance under Abiotic Stress: Where Do We Stand?

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The improvement of crop yield has been possible through the indirect manipulation of quantitative trait loci (QTLs) that control heritable variability of the traits and physiological mechanisms that determine biomass production and its partitioning. This article surveys how QTL-based approaches contribute to a better understanding of the genetic basis of crop performance under environmentally constrained conditions and critically analyzes how this knowledge can assist breeders accelerate the release of cultivars better able to cope with abiotic constraints.

Crop performance is the end result of the action of thousands of genes and their interactions with environmental conditions and cultural practices. During the past century, conventional breeding has been very successful in constantly raising the yield potential of crops (Campos et al., 2004; Borlaug and Dowsell, 2005; Duvick, 2005). This was mainly achieved with little or no knowledge of the factors governing the genetic variability exploited by breeders for crop improvement (Blum, 1988; Borlaug, 2007). However, this approach may now be insufficient, because the pressure to provide improvements at a rapid pace will mount if global climate change increases the frequency and severity of abiotic constraints. Heat stress, drought, water-logging, and salinity will probably become more prevalent in certain areas, while there will be an increased demand for agricultural products and reduced availability of agricultural land and natural resources such as water and fertilizers. Consequently, the genetic dissection of the quantitative traits controlling the adaptive response of crops to abiotic stress is a prerequisite to allow cost-effective applications of genomics-based approaches to breeding programs aimed at improving the sustainability and stability of yield under adverse conditions.

## UNVEILING THE NATURE OF QUANTITATIVE VARIABILITY

Robertson (1985) postulated that qualitative mutant alleles and QTL alleles represent the extremes of a possible range of effects, with QTLs resulting from the segregation of natural alleles with smaller effects. The cloning of a number of plant QTLs has confirmed Robertson's hypothesis, in as much as the types of mutations, genes, and metabolic pathways determining quantitative traits are not distinct from those underlying Mendelian traits and in some cases involve the same loci for which a strong mutation was already known (Salvi and Tuberosa, 2005; Bortiri et al., 2006). Examples of the latter are provided by studies of *Arabidopsis* (*Arabidopsis thaliana*) tolerance to water deficit (Masle et al., 2005), salinity (Rus et al., 2006), and aluminum (Al) toxicity (Hoekenga et al., 2006).

## Constitutive versus Adaptive QTLs

QTLs can be categorized according to the stability of their effects across environmental conditions. A "constitutive" QTL is consistently detected across most environments, while an "adaptive" QTL is detected only in specific environmental conditions or increases in expression with the level of an environmental factor (e.g. a QTL that is expressed more strongly with increasing temperature; Vargas et al., 2006). The presence and magnitude of these adaptive QTLs, therefore, vary greatly between experiments, while the sensitivity of the same trait to environmental conditions can be underscored by stable QTLs across a range of environments (see below on modeling). The sensitivity to environmental conditions may be due to the responsiveness of regulation (e.g. transcription) of the QTL gene to an environmental cue. Alternatively, differences in response may have an indirect cause (e.g. genotypes with larger root systems will be less affected by water shortage or nutrient deficit, so genes controlling root development may underpin QTLs defined by abscisic acid [ABA] content, stomatal conductance, or biomass accumulation). Additionally, QTLs that alter flowering time often influence yield under water or nutrient deficit because the duration of the crop life cycle affects the timing and intensity of the stress experienced by the plants (Reynolds and Tuberosa,

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2008). Consequently, the basis for the differences between adaptive and constitutive QTLs is less straightforward than is often assumed, and the unpredictability of the effects shown by most QTLs on yield in different environmental conditions should not be surprising.

### Collecting and Interpreting QTL Data under Environmentally Constrained Conditions

In order to obtain reproducible data and effectively assess the value of a trait, it is of the utmost importance to consider the environment dependence of QTL expression. This aspect is particularly relevant for stress tolerance traits, which are themselves influenced by the intensity of the stress. For example, ABA levels in the xylem sap and in the leaf control stomatal conductance and transpiration and, therefore, the rate of soil and plant dehydration (Iuchi et al., 2001), factors that themselves influence plant ABA levels (Tardieu and Simonneau, 1998; Tardieu, 2003). Accordingly, the inherent capacity of genotypes to accumulate ABA should be assessed by exposing plants to constant and defined levels of evaporative demand and soil water potential using phenotyping platforms that can provide these conditions (Granier et al., 2006; Sadok et al., 2007).

In addition to environment-dependent expression, other factors are likely to hamper the utilization of QTLs for genetic improvement. A desirable QTL allele discovered in non-elite genetic material may not offer any improvement, because the allele may already be ubiquitous in current varieties. Additionally, the effects of the positive allele may not be transferable to elite backgrounds due to unfavorable epistatic interactions (Podlich et al., 2004). With only a few notable exceptions (e.g. *Bo1*, a major boron [B] tolerance QTL in bread wheat [*Triticum aestivum*; Schnurbusch et al., 2007]; *Sub1*, a major submergence tolerance QTL in rice [*Oryza sativa*; Ismail et al., 2008]; and *Vgt1*, a major flowering time QTL in maize [*Zea mays*; Vladutu et al., 1999; Salvi et al., 2007; Ducrocq et al., 2008]), QTLs from a particular genetic background usually show smaller effects or disappear altogether in different backgrounds, even under similar experimental conditions. Consequently, translating the knowledge gained from QTL and other molecular studies into a tangible product for the farmer is by far one of the most daunting challenges faced by the scientific community and needs to be tackled using a multidisciplinary, holistic approach with collaboration among the different stakeholders (Parry et al., 2005; Passioura, 2007; Tuberosa et al., 2007; Xu and Crouch, 2008). Essential, but not sufficient, prerequisites for meeting this challenge are: (1) identification of the main traits that determine crop yield under the prevailing conditions of the target population of environments; (2) identification of QTLs that govern these traits; and (3) implementation of marker-assisted selection (MAS) schemes to exploit the best QTL alleles. A number of reviews have addressed the most important issues to be considered in using QTL approaches for crop improvement (Hospital and Charcosset, 1997; Beavis, 1998;

Hospital et al., 2000; Servin and Hospital, 2002; Servin et al., 2004; Bernardo et al., 2006; Bernardo and Yu, 2007; Dwivedi et al., 2007; Ragot and Lee, 2007; Xu and Crouch, 2008). The next sections describe QTL case studies for each of these three prerequisites and provide a glimpse of how combining accurate and relevant phenotyping with modeling can help deal with the above-mentioned complexity.

### CASE STUDIES IN QTL MAPPING AND MAS FOR TOLERANCE TO ABIOTIC STRESSES

During their entire life cycles, crops are exposed to multiple abiotic stresses that limit yield. The ushering in of the genomics era has allowed dissection of the physiological and molecular traits underlying stress tolerance mechanisms to an unprecedented level (Bohnert et al., 2006; Tuberosa and Salvi, 2006; Jenks et al., 2007; Vij and Tyagi, 2007). To date, hundreds of studies and reviews have reported QTLs that may play a role in mitigating the negative effects of abiotic stresses. However, MAS has contributed very little to the release of improved cultivars with greater tolerance to abiotic stresses, and this is perhaps partly because, with only a few exceptions (LeDeaux et al., 2006; MacMillan et al., 2006; Ribaut et al., 2007; Welcker et al., 2007), interactions between multiple stresses are seldom considered. Therefore, promising strategies to improve performance under water-limiting conditions involve MAS of genes and QTLs for tolerance to abiotic and biotic factors that impair root growth and function, such as nematodes in soybean (*Glycine max*; Ha et al., 2007) and bread wheat (Langridge, 2005; Zwart et al., 2006), high B in barley (*Hordeum vulgare*) and bread wheat (Langridge, 2005; Schnurbusch et al., 2007; Sutton et al., 2007), and Al toxicity in rice (Nguyen et al., 2003) and sorghum (*Sorghum bicolor*; Magalhaes et al., 2007). A common feature of cereal responses to drought, heat, and cold stresses near flowering and during early stages of seed growth is a reduction of reproductive fertility. This loss of fertility has been attributed to a range of metabolic causes (e.g. insufficient supply of photosynthates, excessive accumulation of ABA, changes in the cell cycle following diverse signalings, etc.) acting during gametogenesis and/or during the early stages of grain growth (Zinselmeier et al., 1999, 2002; Saini and Westgate 2000; McLaughlin and Boyer, 2004a, 2004b, 2007; Fresneau et al., 2007). The QTL approach provides an opportunity to dissect out the genetic and physiological components affecting source-sink relationships under abiotic stress (Prioul et al., 1997, 1999; Pelleschi et al., 2006; Miralles and Slafer, 2007; Welcker et al., 2007).

#### QTLs for Drought Tolerance

Among the different abiotic stresses, drought is by far the most complex and devastating on a global scale (Pennisi, 2008). Improving the tolerance of crops to drought, compared with other abiotic stresses, re-

quires a broader interdisciplinary approach, involving an understanding of the factors (e.g. availability of water during the crop cycle) determining yield in a particular target population of environments. Drought tolerance is one of the most ambiguous concepts in the literature (Tardieu, 2003; Blum, 2005), and its definition is strongly dependent on the phenotyping methods used. Remarkable levels of dehydration tolerance have been obtained under laboratory conditions using genetic engineering strategies designed to manipulate a variety of processes, including cell protection mechanisms (Garg et al., 2002; Capell and Christou, 2004; Jenks et al., 2007; Nelson et al., 2007), detoxification of reactive oxygen species that accumulate under stress (Lee et al., 2007; Yang et al., 2007; Zhu et al., 2007), and hormone balance to facilitate an avoidance strategy (Iuchi et al., 2001; Hsieh et al., 2002; Rivero et al., 2007). Nevertheless, there is at present little evidence that characteristics enabling survival under the drastic water stresses typically imposed on the tested transgenic lines will provide any yield advantage under the milder stress conditions usually experienced in commercially productive fields. In contrast, exploitation of natural variation for drought-related traits has resulted in slow but unequivocal progress in crop performance (Rebetzke et al., 2002; Ribaut et al., 2004; Reynolds and Tuberosa, 2008).

Biomass accumulation is intrinsically linked to transpiration, because stomatal aperture and leaf area determine the rate of both photosynthesis and transpiration. Therefore, there is an inherent contradiction between biomass accumulation and stress avoidance via a reduction of transpiration. The genetic progress can only optimize the terms of this conflict by taking different, and in many cases opposite, strategies, such as: (1) maintaining or even increasing transpiration rate, thereby increasing the biomass accumulation but also the risk of crop failure following excessive soil dehydration; (2) maintaining growth and biomass accumulation under low water status, again with a maintained biomass accumulation and a consequent increased risk of excessive soil dehydration; (3) conversely, reducing the risk of total yield loss by decreasing cumulated transpiration, for instance by reducing the duration of the crop cycle, leaf area, or stomatal conductance; and (4) increasing the amount of harvested biomass per unit of transpired water (water use efficiency [WUE]), which often coincides with the conservative strategy described in (3).

The maintenance of transpiration rate under water deficit is most often achieved by improving the size, architecture, or hydraulic conductance of the root system (Price et al., 2002; Steele et al., 2006; Maurel et al., 2008). This is observed when an improved root system increases access to soil moisture. In contrast, when roots grow in a limited volume of soil because of physical (e.g. hard layer due to compaction) or chemical (e.g. acid soil) barriers, a larger and/or deeper root system can be detrimental, because depletion of soil moisture occurs more rapidly, thereby causing severe

stress at the end of the season (Tardieu et al., 1992), and because the assimilates invested in roots would be better invested in other organs. Accordingly, while a number of genetic studies of root systems have shown positive associations between yield and certain root features (see below), some programs to improve yield under water deficit have resulted in a reduced root biomass (Bolaños and Edmeades, 1993; Bruce et al., 2002) or a decreased water conductivity of the roots (Richards and Passioura, 1989).

A number of studies have reported QTLs for root architecture and have investigated their effects on yield under varying moisture regimes in rice (MacMillan et al., 2006; Steele et al., 2006, 2007; Yue et al., 2006) and maize (Tuberosa et al., 2002, 2003; Landi et al., 2007). In rice, following the identification of four major QTLs influencing root traits (Courtois et al., 2000), marker-assisted backcrossing (MABC) was used to introgress the alleles for greater root length from Azucena into Kalinga III, an upland variety (Steele et al., 2006, 2007). In maize, a major QTL originally reported for leaf ABA concentration (Tuberosa et al., 1998) was later shown to affect root size and architecture (Giuliani et al., 2005b) and grain yield (Landi et al., 2007). Following its isogenization (Landi et al., 2005), the positional cloning of this QTL is under way at present (S. Salvi, unpublished data).

Another strategy to maintain transpiration is to avoid leaf senescence ("stay-green" trait), thereby increasing the accumulated photosynthesis over the crop life cycle (Borrell et al., 2001). As above, this strategy is adequate for soils with appreciable water reserves but may otherwise cause severe stress at the end of the growth season due to increased transpiration (Hammer et al., 2006). In sorghum, four major QTLs that control stay-green and grain yield (*Stg1–Stg4*) have been identified (Harris et al., 2007). Near-isogenic lines (NILs) for these *Stg* QTLs have been derived, providing an opportunity for a detailed analysis of stay-green physiology and positional cloning of the underlying genes.

The maintenance of growth and biomass accumulation under water deficit allows better light interception by leaves, thereby increasing photosynthesis but also transpiration rate and soil water depletion. Therefore, it is an appropriate strategy in many cases, but not for severe terminal water deficits. The decline in leaf growth rate with water deficit is a very sensitive response, resulting in decreased photosynthesis and transpiration rate. A high degree of genetic variability in sensitivity has been observed in maize, with a cessation of nocturnal leaf growth occurring in a range from  $-0.8$  to  $-1.6$  MPa (Welcker et al., 2007), and also in rice (J. Cairns, unpublished data). In three maize mapping populations, QTLs of leaf growth sensitivity to water deficit largely overlapped with QTLs for leaf responses to evaporative demand, suggesting hydraulic mechanisms (Reymond et al., 2003; Sadok et al., 2007; Welcker et al., 2007). It is noteworthy that in one mapping population, half of the QTLs for sensitivity of

leaf growth overlapped with those for silk growth (Welcker et al., 2007), suggesting that mechanisms favoring expansive growth may affect multiple organs.

The expansive growth of reproductive organs is also subject to genetic variation. In maize, the growth rate of the silks differs between genotypes, resulting in differences in the relative timing of male and female flowering (anthesis-silking interval [ASI]), which is typically increased by water deficit and negatively correlated with yield (Duvick, 2005). Phenotypic selection under well-managed stress environments for low ASI has produced large genetic gains and has resulted in significant impacts (Bolaños and Edmeades, 1996; Ribaut et al., 2004). Five QTL alleles for short ASI were introgressed through MABC from a drought-tolerant donor to an elite, drought-susceptible line. Under severe drought, the selected lines clearly outyielded the unselected control. However, this advantage decreased at a lower stress intensity and vanished when stress curtailed yield less than 40% (Ribaut and Ragot, 2007).

Among the yield components in grain crops, seed weight usually has a large impact under terminal drought. In pearl millet (*Pennisetum americanum*), an extensive data set allowed the identification of three major QTLs for grain yield with low QTL  $\times$  environment interactions across a range of postflowering moisture environments, and selection of these positive alleles by MAS could be useful in breeding programs (Bidinger et al., 2007). Major QTLs for seed weight and grain yield across diverse moisture conditions have also been identified in rice (Wang et al., 2006; Bernier et al., 2007) and durum wheat (*Triticum durum*; Maccaferri et al., 2008).

The increase in WUE (i.e. the amount of biomass produced per unit of transpired water; Bacon, 2004) may seem to be the ideal candidate mechanism for drought-prone environments. However, increasing WUE has been regarded as a conservative strategy for crops involving reduced transpiration, because it can have a reduced yield benefit under moderately favorable environments and even cause a yield penalty under the most favorable conditions (Rebetzke et al., 2002; Condon et al., 2004). Most studies of WUE are based on the discrimination of the lighter and prevalent isotope of carbon in CO<sub>2</sub> (<sup>12</sup>C over <sup>13</sup>C), which is linked to WUE both by statistical relations and by a physical theory (Farquhar et al., 1989). Mapping the discrimination trait has enabled the identification of WUE QTLs in *Brassica oleracea* (Hall et al., 2005), rice (Laza et al., 2006), barley (Teulat et al., 2002), and wheat (Spielmeyer et al., 2007). The use of carbon isotope discrimination per se (rather than genetic markers linked to QTLs) in wheat grown in Australian environments, where water must be used conservatively to allow the crop to complete its life cycle, has led to the release of two cultivars (Condon et al., 2004).

The above paragraphs suggest that the maintenance of biomass accumulation under water deficit should be considered as an optimization process between transpiration, biomass accumulation, and its partition-

ing between root and shoot, rather than as a tolerance process per se. It follows that a given QTL can have positive, null, or negative additive effects depending on the drought scenario (Chapman et al., 2003; Vargas et al., 2006). This complication has considerably slowed the utilization of QTL data for breeding. Additionally, the complexity of predicting the performance of a particular genotype when considering multiple QTLs suggests a role for modeling.

### QTLs for Salinity Tolerance

Salinity toxicity in crops is caused by the presence of high levels of soluble salts in the soil solution, namely the Na<sup>+</sup> cation and probably to a lesser extent Cl<sup>-</sup>. Salinity affects approximately 830 million hectares worldwide and is becoming an increasing problem in regions where saline water is used for irrigation (Rengasamy, 2006). Salt tolerance has often been found to be associated with lower accumulation of sodium (Na) in the shoot, but not always. In bread wheat, shoot Na accumulation and salt tolerance were not correlated, suggesting that tissue tolerance would be a better selection criterion for salinity tolerance (Genc et al., 2007). Tissue tolerance in leaves would be identified by mapping a locus that controls salt tolerance but not shoot Na accumulation. Tissue tolerance is likely achieved by sequestration of toxic Na in the vacuole. Using Na accumulation and other measures of salt tolerance, major and minor QTLs have been mapped in various crop species (Flowers and Flowers, 2005; Jenks et al., 2007). As Na accumulation is heavily dependent on transpiration rate, salinity tolerance could depend on growing medium and growth stage. Selection for salt tolerance has been performed in hydroponics, where transpiration is low. However, at least in wheat, the results of salinity tolerance measurements obtained in hydroponics were in good agreement with those of soil-based screens (Genc et al., 2007).

Recently, members of the HKT (for high-affinity K<sup>+</sup> transporter) family of K<sup>+</sup> and Na<sup>+</sup> transporters (Platten et al., 2006; Rodríguez-Navarro and Rubio, 2006) were demonstrated or implicated to control natural variation in salinity tolerance at a number of loci in rice, wheat, and Arabidopsis. Two loci, *Nax1* and *Nax2*, controlling shoot Na accumulation were identified by QTL mapping in durum wheat (genome AABB; Munns et al., 2003; James et al., 2006). Both exclusion genes represent introgressions from an accession of *Triticum monococcum* (genome AA). The Na exclusion genes have also been introgressed into backgrounds of five cultivars of bread wheat (genome AABBDD) by using durum  $\times$  bread wheat interspecific crosses and MABC, and the resulting lines are currently under field evaluation in three Australian states (Colmer et al., 2006; R. James and R. Munns, personal communication). *TmHKT7-A2* is the candidate for *Nax1* on chromosome arm 2AL (Huang et al., 2006). *TmHKT1;5-A* (*HKT8*) is the candidate for *Nax2* on chromosome arm 5AL, and the corresponding homolog in the D genome (*TaHKT1;5-D*) is

the candidate for the *Kna1* gene on chromosome arm 4DL, which is responsible for the superior salt tolerance of bread wheat compared with durum wheat (Byrt et al., 2007). The apparent ortholog of the *TaHKT1;5* genes in rice (*OsHKT1;5*) affects Na accumulation and salt tolerance controlled by a mapped QTL on rice chromosome 1 (Ren et al., 2005). An *HKT1* homolog in Arabidopsis (*AtHKT1*) has been mapped to a natural QTL (Rus et al., 2006), and its contribution to shoot Na exclusion and salinity tolerance in this species was demonstrated with an insertion mutant (Sunarpi et al., 2005). Plant HKT transporters reduce shoot Na accumulation by facilitating the unloading of Na from the xylem (Ren et al., 2005; Sunarpi et al., 2005). *Nax1* promotes Na retention in the leaf sheath relative to the leaf blade, whereas *Nax2* and *Kna1* do not affect this relative accumulation, suggesting that the former promotes xylem unloading of Na in the leaf sheath as well as in the roots (James et al., 2006).

### QTLs for Submergence and Anoxia Tolerance

In rice alone, submergence stress periodically affects approximately 15 million hectares of rain-fed lowland areas in Asia, causing annual losses of up to U.S. \$1 billion (Xu et al., 2006). *Sub1*, a major QTL on chromosome 9, has enabled the use of MABC to improve submergence tolerance of Swarna, a cultivar widely grown in flood-prone regions (Neeraja et al., 2007). In backcrosses between a submergence-tolerant donor and Swarna, molecular markers were used to select for both introgression of *Sub1* and recovery of the recurrent parent background. A double recombinant line was identified that was homozygous for all Swarna-type alleles except for an approximately 2.3- to 3.4-Mb region harboring *Sub1*. The results showed that the Swarna could be efficiently converted to a submergence-tolerant variety in three backcross generations over a period of only 2 to 3 years. Markers have been developed for introgressing *Sub1* into six other popular varieties to develop a wider range of submergence-tolerant varieties to meet the needs of farmers in flood-prone regions (Ismail et al., 2008; Septiningsih et al., 2008). Field testing of the six NILs (with and without *Sub1* introgression) showed that *Sub1* is effective in all target environments and is independent of the recurrent parent background, with no effect on agronomic or quality aspects of these varieties under normal shallow conditions. Relative to the intolerant lines, *Sub1* NILs had severalfold greater yield when flooding occurred for 12 to 18 d during the vegetative stage (A. Ismail, personal communication). It is expected that cultivation of these new varieties will significantly increase yield and food security for the local farmers. Positional cloning revealed a cluster of three putative ethylene response factor genes (*Sub1A*, *Sub1B*, and *Sub1C*) responsible for controlling the phenotype at the *Sub1* locus (Xu et al., 2006). While *Sub1B* and *Sub1C* were found to be invariably present in the *Sub1* region of all rice accessions analyzed, the presence of *Sub1A*

was variable. Among those *indica* varieties that possess this gene, two alleles were revealed: a tolerance-specific allele named *Sub1A-1* and an intolerance-specific allele named *Sub1A-2*. Overexpression of *Sub1A-1* in a submergence-intolerant *O. sativa* ssp. *japonica* conferred enhanced tolerance to the plants, down-regulation of *Sub1C*, and up-regulation of *Alcohol dehydrogenase1* (*Adh1*), thus indicating that *Sub1A-1* is a primary determinant of submergence tolerance (Xu et al., 2006). MABC was also used to pyramid a submergence-tolerant QTL allele together with three genes for tolerance to different biotic stresses into KDML105, an *indica* cultivar widely grown in Thailand (Toojinda et al., 2005). These two reports clearly demonstrate the effectiveness of MAS for introgressing agronomically valuable QTL alleles into elite material.

### QTLs for Low- and High-Temperature Tolerance

Heat stress often accompanies drought stress, and the two interact in the way they affect plants. Excessive heat perturbs many cellular and developmental processes and directly affects grain production by reducing fertility and grain quality (Barnabás et al., 2008). Male gametophyte development is the facet of reproduction that appears most prone to disruption by drought, heat, and cold stresses (Saini, 1997; Mamun et al., 2006). However, it is unclear at present whether there is any common basis to the sensitivity of male reproduction to the various stresses. The genetic basis of heat stress tolerance in crop plants is poorly understood. In wheat, two QTLs were identified that controlled grain-filling duration, a trait thought to be correlated with heat tolerance (Yang et al., 2002). In maize, QTLs were identified that controlled pollen heat tolerance (germinability and pollen tube growth), a factor influencing heat-induced sterility (Frova and Sari-Gorla, 1994). Studies investigating multiple parameters related to heat tolerance in wheat provided evidence for genetic variability and multiple tolerance mechanisms (Dhanda and Munjal, 2006). Mutants for seven "hot" loci in Arabidopsis, which are compromised in their ability to acquire thermotolerance, may further our understanding of heat tolerance in crop species (Hong et al., 2003).

Maize and rice originate from tropical/subtropical regions and are relatively cold sensitive. Chilling leads to poor seedling establishment and, at booting stage, results in reduced fertility due mainly to the arrest of microspore development. QTLs controlling sensitivity to chilling have been identified in maize seedlings (Hund et al., 2005; Jompuk et al., 2005; Presterl et al., 2007), in sorghum seedlings (Knoll and Ejeta, 2008), and in rice at the seedling and booting stages (Andaya and Tai, 2006; Kuroki et al., 2007; Lou et al., 2007). In cultivated tomato (*Solanum lycopersicum*), a QTL allele introgressed from a wild relative (*Solanum hirsutum*) increased chilling tolerance (Goodstal et al., 2005). Crops sown in the fall may experience subfreezing temperatures at the vegetative stage during winter.

Freezing damage arises from dehydration and membrane damage caused by the growth of ice crystals. Full expression of frost tolerance at the vegetative stage typically requires a prior period of acclimation during which plants are exposed to low, nonfreezing temperatures (Xin and Browse, 2000). Loci controlling vegetative frost tolerance have been identified at corresponding positions across the Triticeae species at two locations on the long arms of group 5 chromosomes. The proximal loci (*Fr-1*) are close to or coincident with the *Vrn-1* loci involved in flowering response to vernalization. Clusters of *C-REPEAT BINDING FACTOR* (*CBF*) genes map at the distal (*Fr-2*) loci and represent likely candidates for the controlling genes (Galiba et al., 2005; Båga et al., 2007; Francia et al., 2007). *CBFs*, also known as dehydration-responsive element-binding 1 factors, are cold-responsive transcription factors with likely roles in coordinating cold responses leading to cold/freezing tolerance in plants (Shinozaki and Yamaguchi-Shinozaki, 2000). A *CBF* gene (*CBF2*) also maps at a freezing-tolerant QTL in *Arabidopsis* and appears to be the controlling gene (Alonso-Blanco et al., 2005). In many parts of the world, frost at flowering time can also cause sterility or shriveled grains in cereals and other crops, leading to sporadic episodes of severe losses (Fuller et al., 2007). QTLs for reproductive frost tolerance have been reported in barley, near the *Fr-H1* locus, and on chromosome arm 2HL (Reinheimer et al., 2004). However, efforts to breed for reproductive frost tolerance have met with comparatively little success, most likely due to the lack of sufficient genetic variation, the confounding effects of tiller developmental stage on susceptibility, and the dependence on frost simulation chambers for efficient phenotyping. QTLs for winter hardiness have also been identified in lentil (*Lens culinaris*; Kahraman et al., 2004), rapeseed (*Brassica napus*; Asghari et al., 2007), and ryegrass (*Lolium perenne*; Xiong et al., 2007).

#### QTLs for Tolerance to Mineral Toxicities and Deficiencies

Plant growth is severely impaired in soils containing toxic or insufficient concentrations of particular minerals (Ismail et al., 2007). Soil acidity (pH < 5.0), a characteristic of over half of the world's arable land, poses a serious limitation to crop production worldwide, principally through the effect of promoting Al toxicity.

##### Aluminum Toxicity Tolerance

Al is the most abundant metallic element in the earth's crust. At acid pH, the phytotoxic  $Al^{3+}$  cation is released into the soil solution, where it inhibits root growth, hindering the ability of plants to acquire water and nutrients. Liming and use of tolerant crop varieties can be used to treat soil acidity (Kochian et al., 2005). Major loci and QTLs controlling Al tolerance have been identified in alfalfa (*Medicago sativa*;

Narasimhamoorthy et al., 2007), soybean (Bianchi-Hall et al., 2000), rice (Xue et al., 2007), sorghum (Magalhaes et al., 2007), maize (Ninamango-Cárdenas et al., 2003), barley (Wang et al., 2007), wheat (Raman et al., 2005), oat (*Avena sativa*; Wight et al., 2006), and rye (*Secale cereale*; Matos et al., 2005). Comparative mapping has indicated possible homologies between Al tolerance loci in different cereal species (Kochian et al., 2005). The most commonly documented mechanism of Al tolerance is the Al-activated extrusion of Al-chelating anions such as malate and citrate from the root tips and subsequent formation of nontoxic Al complexes in the apoplast or rhizosphere. The four recently cloned Al tolerance genes all encode organic anion transporters involved in this tolerance mechanism. Malate transporters belonging to the novel plant-specific Al-activated malate transporter 1 (ALMT1) family of proteins are encoded by Al tolerance genes in wheat and *Arabidopsis* (Sasaki et al., 2004; Hoekenga et al., 2006), and citrate transporters belonging to the multidrug and toxic compound extrusion (MATE) family of membrane transporters are encoded by Al tolerance genes in sorghum and barley (Furukawa et al., 2007; Magalhaes et al., 2007). A cluster of *ALMT1* genes also controls Al tolerance in rye (Collins et al., 2008). mRNA expression levels of the tolerance genes cloned from wheat, barley, and sorghum showed strong positive correlations with the tolerance and organic acid secretion levels in collections of diverse genotypes of these species (Raman et al., 2005; Furukawa et al., 2007; Magalhaes et al., 2007; Wang et al., 2007). At least for the major cloned Al tolerance gene in sorghum, multiple tolerance alleles conferring a range of tolerance levels seem to exist (Caniato et al., 2007). Therefore, mRNA expression levels of these cloned tolerance genes may be a more reliable and quantitative indicator of Al tolerance than a marker based on the sequence of an individual tolerance allele and, therefore, may serve as a better means of identifying sources of Al tolerance for use in breeding.

##### Tolerance to Zinc Deficiency

Zinc (Zn) deficiency is one of the most widespread crop micronutrient deficiencies and is capable of causing severe yield reductions. Zn deficiency is also a common problem of human nutrition in developing countries and in vegetarians, making it desirable to generate foods with higher Zn content. In wheat and common bean (*Phaseolus vulgaris*), the ability to grow well on Zn-deficient soils was correlated with high Zn content in the grain, suggesting that the two traits can be selected together (Graham et al., 1999; Gelin et al., 2007). In wheat and bean grain, Zn content is often positively correlated with the accumulation of iron, another nutrient important for human nutrition (Graham et al., 1999; Gelin et al., 2007). Wissuwa et al. (2006) identified two major QTLs for Zn efficiency in rice. Interestingly, loci controlling Zn efficiency in

solution culture and a flooded field site did not coincide. One possible explanation is that mechanisms to increase the bioavailability of Zn, such as exudation of organic acids or phytosiderophores from the roots, behaved differently in these two situations (Wissuwa et al., 2006). In some wheat genotypes, relief from Zn deficiency in an arid environment was only achieved with the concomitant application of water, indicating that soil moisture may be important in facilitating the acquisition of Zn by certain mechanisms (Bagci et al., 2007). QTLs influencing Zn content in bean seeds have been described (Gelin et al., 2007). However, loci controlling the ability of crop plants to tolerate Zn-deficient soils have, to our knowledge, not yet been reported.

#### **Tolerance to Low and High Boron**

B is an essential micronutrient that can be present in either limiting or toxic concentrations in cultivated soils. B-toxic soils occur naturally in arid and semiarid regions such as Australia and can arise as a consequence of using irrigation water high in B. Loci for B toxicity tolerance have been identified by QTL mapping in barley and wheat (Jefferies et al., 1999, 2000). B tolerance may be associated with a reduction in shoot B accumulation, root stunting, or leaf symptoms, depending on the locus, suggesting that a variety of natural B tolerance mechanisms exist. A major B toxicity tolerance gene in wheat (*Bo1*) has been credited as being an important determinant of yield in cultivars that perform well in B-toxic regions of southern Australia. An easy-to-use PCR marker (Schnurbusch et al., 2007) is facilitating MAS of *Bo1* in breeding programs (T. Schnurbusch and P. Langridge, personal communication). In barley, linkage to undesirable genes may have hampered the successful utilization of strong B toxicity tolerance derived from an Algerian landrace (Eglington et al., 2004). However, the recent fine-mapping and cloning of the major B tolerance QTL allele from this source should now provide opportunities to overcome any such limitations (Sutton et al., 2007). Good progress has been made in identifying genes controlling B transport in and out of plants using *Arabidopsis* (Takano et al., 2006; Miwa et al., 2007), and the cloned barley B tolerance gene is a homolog of one of these genes. In rapeseed, QTLs have been identified for B efficiency, providing the opportunity to use MAS to select for this trait. Comparisons with B tolerance QTLs and genomic sequences in the corresponding regions of the closely related *Arabidopsis* genome should assist in the fine-mapping and ultimately the positional cloning of these rapeseed QTLs (Xu et al., 2001; Zeng et al., 2008).

#### **QTLs for Tolerance to Low Nutrients**

The increase in crop yields during the past century is attributed to the selection of genotypes with a higher yield potential and to the parallel increase in the application of fertilizers, particularly nitrogen (N; Borlaug and Dowsell, 2005). The sharp increase in energy cost

has also made N fertilizer more expensive. Phosphorus (P) deficiency can also be a major factor limiting crop yield, particularly in low-input agricultural systems, including those of many developing countries. P presents a more critical situation for the long term, as it is a nonrenewable fertilizer that cannot be synthesized artificially like N. P reserves are expected to be completely depleted by the end of this century. Leaching of P and N into surface and sea water also causes environmental problems, including algal blooms. Clearly, increasing the efficiency with which crops utilize N and P represents an urgent priority for ensuring cost-effective and sustainable agriculture for the future.

#### **Nitrogen Use Efficiency**

In temperate maize, Gallais and coworkers (Bertin and Gallais, 2000, 2001; Hirel et al., 2001; Gallais and Hirel, 2004; Coque and Gallais, 2006; Hirel et al., 2007) identified a set of QTLs for N use efficiency, grain yield, and its components at high and low N levels. QTLs mapped in clusters and those identified under low N were generally a subset of those identified under high N, except for grain protein content, for which a higher number of QTLs were detected in low N. A number of genes that encode enzymes involved in N and C metabolism were close to QTLs for vegetative development and for grain yield and its components (Gallais and Hirel, 2004). These included genes for Gln synthetase (GS; Glu-ammonia ligase), Suc-P synthase, Suc synthase, and invertase ( $\beta$ -fructofuranosidase). The most notable outcomes of these studies were the colocalization of a major grain yield QTL on chromosome 5 with the gene encoding cytosolic GS (*gln4* locus) and the correlation between the expression levels of the *gln4* alleles and the contributions of the respective QTL alleles at this locus. Other candidate genes encoding enzymes involved in N metabolism that colocalized with N use efficiency QTLs were the two GS genes (*gln1* and *gln2*) on chromosome 1 and the GS gene (*gln3*) on chromosome 4. Collectively, these results suggest that increased productivity in maize genotypes under low N may be due to their ability to accumulate nitrate in the leaves during vegetative growth and to efficiently remobilize the stored nitrate during grain filling.

In tropical maize, QTLs for grain yield and secondary traits under varying N and water supply were identified by Ribaut et al. (2007). A mapping population previously developed for identifying QTLs under water-limited conditions was evaluated under varying N and water regimes. Two of the eight grain yield QTLs identified under low N conditions were also detected under high N conditions. Five QTLs were stable across the two low N environments and five colocalized with QTLs identified for ASI or for the number of ears per plant under low N conditions. These results suggest that both ASI and the number of ears per plant can be selected for simultaneously to improve the performance of maize under low N and drought stress (Ribaut et al., 2007).

In wheat, a QTL meta-analysis and factorial regression were deployed to investigate QTL  $\times$  N interactions, revealing influences of the three major phenological trait loci, *Ppd-D1*, *Rht-B1*, and *B1*, on N-related QTLs (Laperche et al., 2007). Additionally, QTL clusters for GS activity on wheat chromosomes 2A and 4A coincided with the location of GS and GSr genes, respectively, and although QTL alleles for higher GS activity were associated with higher grain N, they showed little or no effects on grain yield components (Habash et al., 2007). QTLs for tolerance to low N have also been described in Arabidopsis (Loudet et al., 2003), rice (Lian et al., 2005), and barley (Mickelson et al., 2003).

### Phosphorus Use Efficiency

Because of the low mobility of P in the soil, extensive work has been carried out to investigate the effects of QTLs for root architecture on P uptake. Relevant QTLs have been identified in Arabidopsis (Reymond et al., 2006), common bean (Liao et al., 2004; Beebe et al., 2006; Ochoa et al., 2006), soybean (Kuang et al., 2005; Li et al., 2005), rice (Wissuwa et al., 2002; Shimizu et al., 2004), maize (Kaeppler et al., 2000; Zhu et al., 2005, 2006), and wheat (Su et al., 2006).

A common feature of the above-mentioned studies is that the QTL alleles for high P efficiency were associated with greater root surface area due to an increase in either root mass or root hair density. Additionally, QTLs for P uptake in bean were found to influence H<sup>+</sup> and total acid exudation from the root (Yan et al., 2004), two processes capable of mobilizing soil-bound P through soil P desorption or mineralization. In rice, MABC of the beneficial allele at *Pup1*, a major QTL on chromosome 12 for P uptake efficiency that was mapped to a 3-cM interval (Wissuwa et al., 2002), provided a 3- to 4-fold increase in P uptake (Wissuwa et al., 2005). A segregation analysis using several pairs of contrasting NILs at the *Pup1* locus confirmed that *Pup1* cosegregated with external uptake efficiency but not with seedling root growth (Wissuwa et al., 2005). Although fine mapping had delimited *Pup1* to a relatively small interval (approximately 240 kb), the large number (>60) of genes in this region has so far prevented the identification of the *Pup1* controlling gene (Ismail et al., 2008).

### CLONING QTLs: WHY BOTHER?

Although QTL cloning remains a cumbersome procedure, several reasons justify such a daunting undertaking. The sequence of a cloned QTL gene offers the perfect marker for MAS and provides the template for the identification of potentially superior allelic variants in the crop species or wild progenitors via EcoTILLING (Till et al., 2007). Modifying the expression of the gene by genetic engineering could also provide superior tolerance. Putatively identified and

cloned genes relating to stress tolerance loci are listed in Table I. The cloning of further genes and expression QTLs relating to changes in mRNA levels under abiotic stress will shed further light on the relative importance of cis- and trans-regulation in governing the expression of key structural genes and their impact on yield (Potokina et al., 2008). Positional cloning has been the approach most frequently used to clone QTLs (Salvi and Tuberosa, 2005; Bortiri et al., 2006). Because accurate phenotyping is the most critical factor for fully dissecting QTLs, positional cloning is largely limited to traits with high heritability and to QTLs with large effects that can be easily Mendelized. Accordingly, QTLs with major effects on flowering time have been positionally cloned in rice (Yano et al., 2000; Takahashi et al., 2001; Kojima et al., 2002; Doi et al., 2004) and maize (Salvi et al., 2007). Early-flowering alleles are of interest for providing escape from late-season drought episodes.

The candidate gene approach is better suited for QTL cloning when there are genes known to control the same or a similar trait in another species. For example, homologs of the *ALMT1* and *HKT1* genes that control Al and salinity tolerance in cereals were found to determine the same traits in Arabidopsis (Ren et al., 2005; Hoekenga et al., 2006; Huang et al., 2006; Rus et al., 2006; Byrt et al., 2007). Another possibility is to consider candidates on the basis of a specific biochemical activity rather than biological function. For example, the barley *Bor1* B tolerance gene, although homologous with a group of B efflux transporters that had been known to be involved in B uptake in Arabidopsis (B efficiency), limits B uptake because it has a spatial expression pattern within roots that differs from that of the Arabidopsis homolog (Sutton et al., 2007). The candidate gene approach appears to be most successful when a specific biochemical activity underlying the tolerance trait is known (e.g. transport of an element or compound, as in the above examples). Unfortunately, it is likely to be of limited use in the cloning of QTLs for yield maintenance under drought and drought-associated physiological traits, because detailed information about the underlying biochemistry of these traits is seldom available. There are some loci that remain uncloned using a candidate gene approach, despite the availability of likely candidate genes. For example, homologs of B transporters have been mapped in wheat, but none coincided with the *Bo1* locus that controls B accumulation and tolerance (T. Schnurbusch and P. Langridge, unpublished data). In such cases, proceeding by a noncandidate gene (forward genetics) approach may reveal a novel gene function.

For the cloned and putatively identified plant genes relating to abiotic stress tolerance (Table I), and related traits, a difference in the level of transcription, rather than differences in protein sequence and function, appears to be the most common basis for the functional difference between naturally occurring alleles. Therefore, allelic differences in mRNA expression

**Table 1.** Putative and cloned genes relating to stress tolerance loci

Loci represented are those defined either by natural allelic variation or by segregation in interspecific crosses.

QTL/Gene	Locus	Species	Phenotype	Gene Product and Function	Basis for Allelic Difference	References
<i>AtHKT1;1</i>	No name	Arabidopsis	Na tolerance	Na <sup>+</sup> transporter; facilitates Na <sup>+</sup> unloading from xylem, leading to reduced Na <sup>+</sup> accumulation in the shoots	Alleles allowing higher shoot Na <sup>+</sup> accumulation produce lower root mRNA levels, correlated with deletions in a 5' repeat structure	Rus et al. (2006)
<i>TmHKT1;5-A</i>	<i>Nax2</i>	<i>T. monococcum</i>	Na tolerance	Na <sup>+</sup> transporter, as above	The A genomes of durum and bread wheat lack a copy of the gene	James et al. (2006); Byrt et al. (2007)
<i>TaHKT1;5-D</i>	<i>Kna1</i>	Bread wheat	Na tolerance	Na <sup>+</sup> transporter, as above	No allelic variation known (locus was mapped using deletions and interspecific recombination)	James et al. (2006); Byrt et al. (2007)
<i>OsHKT1;5</i>	<i>Skc1</i>	Rice	Na tolerance	Na <sup>+</sup> transporter, as above	Differences in protein sequence	Ren et al. (2005)
<i>TmHKT7-A2</i>	<i>Nax1</i>	<i>T. monococcum</i>	Na tolerance	Na <sup>+</sup> transporter; facilitates Na <sup>+</sup> unloading from xylem in roots and leaf sheaths, leading to reduced Na <sup>+</sup> accumulation in the shoots, especially in the leaf blades	Tolerance allele produces mRNA in the roots and leaf sheaths; intolerance durum wheat allele produces no detectable transcript	Huang et al. (2006)
<i>Sub1A</i>	<i>Sub1A</i>	Rice	Submergence tolerance	Member of the ERF subfamily of <i>APETALA2</i> -like transcription factors; may positively regulate submergence acclimation responses (e.g. involving <i>Adh</i> )	Tolerance haplotypes contain a strongly submergence-induced allele of the <i>Sub1A</i> gene copy	Xu et al. (2006)
<i>CBF2</i>	<i>FTQ4</i>	Arabidopsis	Freezing tolerance	CBF transcription factors are induced by cold stress and activate expression of other genes	Intolerance allele produces a lower amount of transcript, correlated with a deletion in the promoter	Alonso-Blanco et al. (2005)
<i>CBF</i> gene cluster	<i>Fr-H2</i>	Barley	Freezing tolerance	See above	Not known	Francia et al. (2007)
<i>CBF</i> gene cluster	<i>Fr-A2</i>	<i>T. monococcum</i> , bread wheat	Freezing tolerance	See above	Not known	Miller et al. (2006); Båga et al. (2007)
<i>AtALMT1</i>	No name	Arabidopsis	Al toxicity tolerance	Malate efflux transporter; malate is pumped out of the root and binds/detoxifies Al <sup>3+</sup>	Not known	Hoekenga et al. (2006)
<i>TaALMT1</i>	<i>Alt<sub>BH</sub></i>	Bread wheat	Al toxicity tolerance	Malate efflux transporter, as above	Tolerant allele produces higher constitutive mRNA levels; expression is positively associated with the copy number of a promoter repeat element	Sasaki et al. (2004, 2006)
<i>ScALMT1-M39.1</i> , <i>ScALMT1-M39.2</i> , <i>ScALMT1-1</i>	<i>Alt4</i>	Rye	Al toxicity tolerance	Malate efflux transporter (putative), as above	Al-inducible root mRNA levels are higher in the tolerant lines, plus the intolerant line has inefficient mRNA splicing and possibly defective protein	Fontecha et al. (2007); Collins et al. (2008)

(Table continues on following page.)

**Table 1.** (Continued from previous page.)

QTL/Gene	Locus	Species	Phenotype	Gene Product and Function	Basis for Allelic Difference	References
<i>HvMATE</i> / <i>HvAACT1</i>	<i>Alp</i>	Barley	Al toxicity tolerance	Citrate efflux transporter; citrate is pumped out of the root and binds/detoxifies Al <sup>3+</sup>	Tolerant allele produces higher root mRNA levels	Furukawa et al. (2007); Wang et al. (2007)
<i>SbMATE</i>	<i>Alt<sub>Sb</sub></i>	Sorghum	Al toxicity tolerance	Citrate efflux transporter, as above	Tolerant allele produces higher Al-induced root mRNA levels; expression is positively correlated with the size of a 5' MITE insertion	Magalhaes et al. (2007)
<i>Bot1</i>	No name	Barley	B toxicity tolerance	B efflux transporter; leads to B efflux from the root and reduced B accumulation in the shoots	Tolerant allele produces higher constitutive mRNA levels and shows a 4-fold gene duplication	Sutton et al. (2007)

levels are likely to remain a very useful criterion when identifying candidate genes. Transcriptome profiling from model or target crop species can also be used to identify candidates (Giuliani et al., 2005a; Guo et al., 2007; Poroyko et al., 2007). However, the power of this information alone in QTL cloning is limited by the large number of genes that usually show expression differences related to stress treatment, genotype, or source tissue. One example of where such information has led to the identification of a stress tolerance QTL is provided by the *CBF* genes. Interest in *CBF* genes arose from observations that they are induced by stress, regulate other stress-responsive genes, and confer tolerance when overexpressed in transgenic plants (Shinozaki and Yamaguchi-Shinozaki, 2000), while more direct genetic evidence for a contribution of these genes to cold tolerance in wild-type plants was obtained only later by QTL mapping and mutant analysis (Alonso-Blanco et al., 2005; Båga et al., 2007; Francia et al., 2007; Novillo et al., 2007). Integration of information obtained from different genomic approaches, including protein-protein interaction studies and transcriptome, proteome, and metabolome profiling, will allow better understanding of regulatory networks in plants (Gutierrez et al., 2005) and better prediction of genes likely to be critical in determining phenotypes.

The set of candidates can be narrowed by knowledge of the genes present in a QTL interval/region defined by linkage/association mapping. Gene content can be known directly from genomic sequence when available. In unsequenced genomes, most of the gene content of an interval can be predicted by exploiting the partial conservation in gene content and order that exists between many crops and sequenced species (e.g. between rapeseed and the *Arabidopsis* sequence and between the large-genome cereals barley and wheat and the sequences of rice and the model grass *Brachypodium distachyon*). Once the corresponding interval in the sequenced genome is identified by mapping of homologous flanking

genes, the genes within the sequenced interval can be scrutinized for likely candidates as well as used for targeted marker generation to further delimit the interval. While the wheat *HKT7* gene and barley and sorghum *MATE* genes were colinear with rice, rye, and wheat *ALMT1* genes, barley *Bot1* and wheat *HKT1;5* genes were not, despite good overall levels of colinearity directly around these loci (Huang et al., 2006; Byrt et al., 2007; Furukawa et al., 2007; Magalhaes et al., 2007; Sutton et al., 2007; Wang et al., 2007; Collins et al., 2008). Further cases will need to be tested to verify whether or not abiotic stress tolerance genes break colinearity more often than other types of genes.

#### COMBINING QTLS, PHENOTYPING, AND MODELING

The above sections illustrate the complexity of adaptive traits. Component traits may vary with environmental conditions within a time frame of some seconds (e.g. stomatal conductance or tissue water potential) to several weeks (e.g. no. of lateral roots). The genetic analysis of such highly variable traits needs a strategy to cope with the temporal variability of phenotypes. One strategy involves interpreting networks of field trials using a statistical method that calculates QTL × environment interactions (Malosetti et al., 2006). A second strategy involves identifying intrinsic characteristics of each genotype relating to its interaction with particular environmental conditions. This requires the development of models able to identify these variables and to simulate the behavior of genotypes in a large range of environmental conditions. Ecophysiological models calculate quantitative phenotypic traits (e.g. transpiration rate, expansion rate of organs, or biomass accumulation) from environmental inputs such as organ temperature, light irradiance, and soil water potential. It is assumed that the network of gene regulation is coordinated in such a

way that a plant reacts in a predictable way to a given environmental condition, giving way to a “meta-mechanism” at the whole plant level (Tardieu, 2003).

For example, an approach has been developed for the identification and genetic analysis of the sensitivity of maize leaf growth to environmental conditions (Reymond et al., 2003; Sadok et al., 2007; Chenu et al., 2008). First, it involved establishing response curves of leaf elongation rate to temperature, evaporative demand, and soil water status, which were stable characteristics of the genotypes in different experimental conditions. The parameters of these responses were calculated for lines of mapping populations and were then analyzed genetically (Reymond et al., 2003; Welcker et al., 2007). This allowed the simulation of leaf growth in novel inbred lines as defined by their QTL alleles (Reymond et al., 2003; Sadok et al., 2007). This approach, therefore, allows the identification of QTLs of plant responses that, in principle, should not include a genotype-environment interaction. It theoretically allows one to predict the performance of any “virtual genotype” with a given combination of alleles in any climatic scenario. This possibility is a promising avenue, but is limited at present to very simple traits and genetic systems.

More integrative models simulate crop development as a function of environmental conditions. Consequently, they allow for the evaluation of the effects of individual traits on the seasonal dynamics of crop water use and carbon assimilation (Chapman et al., 2003; Yin et al., 2004). However, their algorithms remain relatively crude, so the effects of genes or QTLs usually cannot be simulated at the crop level except for constitutive traits (e.g. phenology; Chapman et al., 2003; Yin et al., 2005), for binary traits related to environmental triggers (e.g. flowering response to photoperiod; Hoogenboom et al., 2004), or when QTL models at the organ level can be combined with crop models (Chenu et al., 2008). Their main function until now has been to evaluate whether a given trait will have a positive effect over a long series of climatic scenarios. For instance, Hammer et al. (2005) simulated the effect of stay-green, a trait considered to confer drought tolerance, across 547 location-season combinations. As expected, this trait had a positive effect under midseason or terminal stress but a negative effect under severe terminal stress. The value of this approach is to quantify the potential value of a trait in a given environment by considering current and projected climatic patterns. Additionally, the same approach allows us to virtually gauge the effect of a QTL and/or a QTL combination and, consequently, to elaborate and test long-term selection schemes that cannot otherwise be compared in the “real world.”

## THE WAY AHEAD

Genomics-assisted improvement of abiotic stress tolerance of crops will increasingly rely on the QTL

approach. The systematic dissection of QTLs governing the naturally occurring variation relevant for crop yield and its sustainability will facilitate a more targeted and effective tailoring of cultivars with an improved performance under abiotic constraints (Baum et al., 2007; Dwivedi et al., 2007; Mackill, 2007; Xu and Crouch, 2008). Nonetheless, it is difficult to predict to what extent QTL-based MAS will affect breeding practices aimed at improving tolerance to abiotic stresses. The first tangible examples of such effects have been presented here.

The identification and cloning of QTLs may be facilitated in the future by a number of factors, namely: (1) the constant improvement of molecular platforms (e.g. single nucleotide polymorphism profiling, “-omics” profiling, yeast one- or two-hybrid screens, chromatin immunoprecipitation, tiling arrays, etc. [Varshney and Tuberosa, 2007]) and mapping approaches (e.g. association mapping [Ersoz et al., 2007]); (2) new types of genetic materials (e.g. multiparental schemes [Yu et al., 2008] and advanced backcross mapping populations using wild relatives [Grandillo et al., 2007]); (3) progress in bioinformatics (e.g. functional maps [Gardiner et al., 2004; Falque et al., 2005] and comparative mapping [Salse and Feuillet, 2007]); (4) accurate, high-throughput phenotyping (Granier et al., 2006; Sadok et al., 2007) coupled with modeling that allows the prediction of allele functions in new environments (Tardieu, 2003; Hammer et al., 2006; Cooper et al., 2007); and (5) increasing availability of sequence information (e.g. “deep” sequencing of DNA and mRNA [Chen et al., 2007; Torres et al., 2008]) and tools for functionally testing candidate genes (e.g. TILLING [Till et al., 2007] and RNA interference [Lukens and Zhan, 2007]). In polyploid species, high-resolution mapping using radiation-induced deletion lines (Kalavacharla et al., 2006) offers a useful route to cloning QTLs in regions that are low in polymorphism or recombination. In maize, a substantial advancement in QTL discovery and cloning has been made possible through the adoption of the nested-association mapping (NAM) system (Yu et al., 2008). Combining QTL mapping based on 25 biparental recombinant inbred line populations (25 founders crossed with a common tester) with association mapping based on a panel of approximately 300 lines, Buckler and coworkers (Yu et al., 2008) assembled the most powerful QTL dissection platform devised for any species. The first results obtained using this NAM platform have validated the value of this strategy (Buckler, 2008), the only limiting factor being the phenotyping of approximately 5,000 lines required to take full advantage of the scheme. As in general association mapping, the mapping resolution offered by NAM largely depends on the linkage disequilibrium (LD) among the founder individuals. Given the diversity of these 25 maize founders and the rapid LD decay (approximately 2 kb), a high mapping resolution is expected for NAM. With the NAM strategy, the improvement in resolution due to the rapid

decay of LD is thus fully exploited while avoiding its drawbacks (e.g. the need for strong candidate genes or a large no. of markers) by projecting the genomic information from the founders to the recombinant inbred lines (Yu et al., 2008). However, if the effects of the QTLs are too small, combining them by MAS to achieve a significant improvement may be impractical and would constrain the potential to achieve yield gain at other loci. In such a case, it may be preferable to simply select for the phenotype or yield itself rather than to attempt to manipulate the trait by MAS at multiple loci (Bernardo et al., 2006).

Arabidopsis and other model plants (e.g. *Brachypodium*, *Medicago truncatula*, etc.) will continue to offer insights into the genetic and biochemical basis of adaptation to abiotic stress (Bohnert et al., 2006) and will provide opportunities to verify the function of candidate genes. Attractive features of Arabidopsis as a model are the availability of a well-annotated sequence, a wealth of genetic materials and -omics data, a very short life cycle, and the possibility to phenotype at a fraction of the cost required for crops. An interesting example is the identification in Arabidopsis of QTLs controlling the plasticity of root architecture (Loudet et al., 2005; Gerald et al., 2006; Sergeeva et al., 2006), a crucial factor for optimizing crop performance under a number of abiotic constraints (de Dorlodot et al., 2007). Equally worthy of exploration will be the identification of QTLs regulating signal transduction in response to environmental cues (Shinozaki and Yamaguchi-Shinozaki, 2007) and the ensuing modifications in gene expression (Sunkar et al., 2007). To what extent this knowledge will affect the release of better performing crops will depend on our capacity to identify the crops' orthologs at the relevant loci and to properly evaluate their effects in crops under stressed conditions (Nelson et al., 2007).

We anticipate a growing interest in wild relatives of crops and landraces in an attempt to identify superior alleles among those that the domestication bottleneck and modern agriculture have left behind (Tanksley and McCouch, 1997; Grandillo et al., 2007; Lippman et al., 2007; Feuillet et al., 2008). Advanced backcross QTL analysis (Tanksley and Nelson, 1996) will be instrumental in identifying agronomically superior alleles not present within the elite germplasm. To this end, introgression libraries based on wild relatives and/or landraces as donors provide a wealth of valuable opportunities (Gur et al., 2004). A QTL approach based on wide crosses will facilitate gene discovery and will shed light on the mechanisms that allow plants to adapt to harsh environments.

Likewise, a model-based approach following a simplified framework of the components interacting at the physiological and developmental levels could facilitate the identification of the most promising entry variables (Tardieu, 2003; Hammer et al., 2006; Cooper et al., 2007), that is, the genes/QTLs to preferentially act upon. Particularly challenging examples are provided by gene interactions at the transcriptional and

posttranscriptional levels, metabolic fluxes, key developmental steps, and their integrated responses to environmental cues. Increasing evidence suggests an important role of epigenesis (Matzke et al., 2007; Perruc et al., 2007) and RNA interference (Chinnusamy et al., 2007; Sunkar et al., 2007) in the regulation of gene expression under stress conditions. High-throughput proteomic approaches promise to shed light on the complexities of posttranslational events and how these may influence functional variability (Briggs et al., 2008). Although reverse genetics approaches are likely to become increasingly popular as more annotated sequences become available, a complete understanding of natural variation will only come by studying functional differences between natural alleles, including variation in noncoding regions (Magalhaes et al., 2007; Salvi et al., 2007). Therefore, while the candidate gene approach should be explored whenever the opportunity arises, forward genetics approaches, such as positional cloning of natural QTLs, will continue to play a prevailing role in providing quantum leaps in our understanding of the molecular control of stress tolerance. Ultimately, integration of QTL information into a breeding pipeline aimed at improving tolerance to abiotic stresses will best be achieved within a multidisciplinary context able to provide the operational framework required to correctly link the stress-responsive mechanisms of crops with the functional variation of the relevant networks at the cellular and molecular levels.

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