Review

Drought tolerance improvement in crop plants: An integrated view from breeding to genomics

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Abstract

Drought is the most significant environmental stress in agriculture worldwide and improving yield under drought is a major goal of plant breeding. A review of breeding progress pointed out that selection for high yield in stress-free conditions has, to a certain extent, indirectly improved yield in many water-limiting conditions. Further progress requires the introduction of traits that reduce the gap between yield potential and actual yield in drought-prone environments. To achieve this three main approaches can now be exploited: (i) plant physiology has provided new insights and developed new tools to understand the complex network of drought-related traits, (ii) molecular genetics has discovered many QTLs affecting yield under drought or the expression of drought tolerance-related traits, (iii) molecular biology has provided genes useful either as candidate sequences to dissect QTLs or for a transgenic approach. The extent of information that breeders have now offers them new tools for breeding, such as markers for QTLs and single genes for plant transformation. Breeders are thus asked to blend together all knowledge on the traits sustaining yield under drought and to accumulate the most effective QTLs and/or transgenes into elite genotypes without detrimental effects on yield potential. This strategy will lead to new cultivars with high yield potential and high yield stability, that in turn will result in superior performance in dry environments.

Keywords: Drought tolerance; Marker assisted selection; QTL; Stress index; Drought tolerance genes; Plant transformation

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1. Introduction

Drought is by far the most important environmental stress in agriculture and many efforts have been made to improve crop productivity under water-limiting conditions. While natural selection has favoured mechanisms for adaptation and survival, breeding activity has directed selection towards increasing the economic yield of cultivated species. More than 80 years of breeding activities have led to some yield increase in drought environments for many crop plants. Meanwhile, fundamental research has provided significant gains in the understanding of the physiological and molecular responses of plants to water deficits, but there is still a large gap between yields in optimal and stress conditions. Minimizing the 'yield gap' and increasing yield stability under different stress conditions are of strategic importance in guaranteeing food for the future.

The evolution of crops since their domestication has been driven by the selection of desired traits recognized at the phenotypic level. Nevertheless, direct selection for grain yield under water-stressed conditions has been hampered by low heritability, polygenic control, epistasis, significant genotype-by-environment (G × E) interaction and quantitative trait loci (QTLs)-by-environment (QTL × E) interaction (Piepho, 2000). The complexity of drought tolerance mechanisms explains the slow progress in yield improvement in drought-prone environments. In recent years, crop physiology and genomics have led to new insights in drought tolerance providing breeders with new knowledge and tools for plant improvement (Tuberosa and Salvi, 2006). This article aims to provide an overview of the breeding progress in drought tolerance, and highlight future perspectives in plant breeding that could result from the integration of the recent advances in physiology and genomics.

2. Breeding progress in favourable versus drought-prone environments

Increases in yield potential achieved by plant breeding during the last Century have been well documented for numerous crops. Frequently, genetic gain has been studied by comparing in the same field trial the yield of cultivars characterized by different years of release. For most crops a linear relation between yield and year of release was found, the slope of which gives an estimate of the genetic improvement. Comparison of the different cultivars then enabled identification of the main morpho-physiological traits modified during selection in association with yield improvement. For instance, studies carried out on wheat and barley genotypes commonly grown in the last Century showed that the increase in grain yield was directly correlated to an increase of the harvest index from about 30 up to 55% (reviewed by Slafer et al., 1994; Cattivelli et al., 1994), while total biomass accumulation remained almost unchanged over the years due to a concomitant decrease in plant height, a process associated with the introgression in the elite germplasm of a few key genes affecting plant height, mainly Rht (Slafer et al., 1994). In general, a genetic gain from 10 to 50 kg ha\(^{-1}\) year\(^{-1}\) has been recorded for cereals and legumes over the last Century in all countries, including those characterized by vast drought-prone regions.

Information on the genetic gain obtained by comparing cultivars with different year of release is not absolute due to the influence of the environment (mainly water availability) on the performance of the varieties. When grain yield values of wheat and barley were estimated in environments with different levels of water availability, yield progress attributed to genetic improvement was, in absolute terms, higher in the environments characterized by a low level of water stress (Slafer et al., 1994). However, when yield increases were expressed as a percentage, no differences were recorded for environments with different degrees of water stress (Araus et al., 2002). This suggests that some of the traits selected to improve yield also led to yield increases in dry environments.

A number of physiological studies have identified some traits for which presence/expression is associated with plant adaptability to drought-prone environments. Among them, traits, such as small plant size, reduced leaf area, early maturity and prolonged stomatal closure lead to a reduced total seasonal evapotranspiration, and to a reduced yield potential (Fischer and Wood, 1979; Karamanos and Papatheohari, 1999). Depending on the stress conditions (timing and intensity) of the target environments, some adaptive traits can be considered for yield improvement under drought if they enable plants to cope with a stress event that tends to occur every year at the same growth stage. For instance, a good level of earliness is an effective breeding strategy for enhancing yield stability in Mediterranean environments where wheat and barley are exposed to terminal drought stress. In this condition shortening crop duration, a typical escape strategy, can be useful in synchronizing the crop cycle with the most favourable environmental conditions. It is known, however, that extreme earliness leads to yield penalty, with earliness not being correlated to grain yield in Mediterranean environments or fertile conditions (Cattivelli et al., 1994). Late heading and flowering, followed by a short grain-filling period can be associated with higher yield when drought stress is experienced early in the season, during the vegetative phase (van Ginkel et al., 1998). A more general “xerophytic” breeding strategy to improve plant survival through the limitation of evapotranspiration can be applied in extremely harsh environments. Nevertheless, every breeding strategy for drought-prone environments also has to consider that the timing and intensity of the stress events vary significantly from year to year and plants designed to cope with a specific type of drought may under-perform when the stress conditions are different or absent. In a typical Mediterranean environment, years with ample water availability during the main cereal growing season alternate with years in which terminal drought occurs as well as years with early drought during vegetative growth and flowering.

In mild to moderate drought conditions characterized by a wheat/barley grain yield between 2 and 5 mg ha\(^{-1}\), selection for high yield potential has frequently led to some yield improvements under drought conditions (Araus et al., 2002). In these cases the breeders have selected plants characterized by...
high yield potential and high yield stability, with the latter being attributed to a minimal G × E interaction. This implies that traits maximizing productivity normally expressed in the absence of stress, can still sustain a significant yield improvement under mild to moderate drought (Slafer et al., 2005; Tambussi et al., 2005). An example is the success of wheat and rice varieties bred at CIMMYT and IRRI where selection under stress-free environments identified genotypes with high yield in a wide range of conditions including regions with a low yield potential (Troughton et al., 2002).

The rationale of this breeding strategy is also supported by several retrospective studies where the yield of large sets of cultivars was evaluated in parallel fields under different water regimes, thus enabling a direct comparison of the performance of the same cultivars in drought and non-drought (usually irrigated) conditions. When 89 barley genotypes representing a sample of the germplasm grown in Europe were evaluated in Southern Italy in rainfed and irrigated conditions, eight genotypes with high yield potential and minimal G × E interaction were identified. They ranked among the best in both rainfed and irrigated treatments and, although considerably reduced in the absence of supplementary irrigation, their yield was superior under all tested conditions (Rizza et al., 2004). Old varieties were characterized by low yield in rainfed conditions and by a minimal ability to improve yield when water became available. On the other hand, modern cultivars showed a higher yield in rainfed conditions and strong yield increases in response to irrigation. A highly significant correlation ($r = 0.73^{***}$) between yield in rainfed and irrigated conditions was found (Rizza et al., 2004), suggesting that, in a typical Mediterranean environment, selection based on the absolute performance of the genotypes across environments is more successful than selecting for the minimum yield decrease under stress with respect to favourable conditions. Notably, among the genotypes with superior yielding capacity there was a large predominance of varieties selected in regions usually not affected by drought stress (e.g. Northern Italy, Sweden, France, The Netherlands) demonstrating that selection under favourable environments also has positive effects when plants are grown in stressed environments.

A corresponding experiment including 46 sugar beet genotypes representing different genetic backgrounds grown in drought and irrigated conditions led to similar results (Ober et al., 2004). Sugar beet genotypes with high yielding capacity when irrigated also tended to perform well under drought (correlation coefficient $r = 0.64^{***}$), while the genotypes with minimum yield loss under stress did not belong to the group of high yielding genotypes in either irrigated or drought conditions.

Comparison between old and recent maize hybrids showed that selection for high yield potential is intimately linked to selection for stress resistance; with tolerance to weed interference, low soil nitrogen and low soil moisture among the key factors that sustained the genetic gain in maize (Tollenar and Wu, 1999; Tollenaar and Lee, 2002). In soybean, modern cultivars characterized by high yield under irrigated conditions, also outperformed older cultivars in rainfed trials, despite the greater capacity of the latter to retain water measured in terms of water potential and stomatal conductance (Frederick et al., 1990, 1991).

Many reports suggest that, during the last Century, yield in water-limited environments was mainly determined by the inherent yield potential. This can be explained by considering that the main targets of selection (high harvest index in wheat and barley, stay green in maize and sorghum, resistance to pests and diseases, nitrogen use efficiency) are equally beneficial under dry and wet conditions and, often, the best performances for these traits were overriding the differences in drought adaptability. Nevertheless, examples where selection for stress resistance was effective in improving yield in limited environments are also known (Bänziger et al., 1999; Morgan, 2000).

Further progress will depend on the introduction in high yielding genotypes of traits able to improve drought tolerance without detrimental effects on yield potential, thus reducing the gap between yield potential and yield in drought-prone environments. This goal can be achieved via the identification of drought tolerance-related traits and the subsequent manipulation of the corresponding genes using marker assisted selection (MAS) and/or gene transformation.

3. Drought tolerance assessment

A crucial aspect in all studies dedicated to drought tolerance is the assessment of the degree of drought tolerance of different genotypes. In many studies the identification of tolerant and susceptible cultivars is based on few physiological measures related to drought response. The difficulty in identifying a physiological parameter as a reliable indicator of yield in dry conditions has suggested that yield performance over a range of environments should be used as the main indicator for drought tolerance (Voltas et al., 2005). Furthermore, a number of regression techniques of yield against environmental indices as independent variables were developed to evaluate genotype adaptability. Several indices were proposed to describe yield performance of a given genotype under stress and non-stress conditions or in comparison with the average yield or the yield of a superior genotype (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Fischer and Maurer, 1978; Soika et al., 1981; Lin and Binn, 1988; Yadav and Bhatnagar, 2001). Some authors have expressed the yielding capacity with regard to an environment-related physiological trait, such as canopy temperature or water potential. To estimate the water stress experienced by crop plants Idso et al. (1981) suggested a “crop water stress index” (CWSI) derived from the increase in average canopy temperature in relation to that of a well-watered reference plot and evaluated by infrared thermometry. In a study on *Triticum aestivum* and *Vicia faba* water potential index (WPI) was suggested as a measure of the total water stress experienced by a crop in a given environment for a specific time interval (Karamanos and Papatheohari, 1999). Other approaches aim to quantify the degree of drought based on specific environmental factors (such as weather, soil water availability, etc.) in order to provide a measure that is
Independent of the G × E interaction. Araus et al. (2003) found that yield was well correlated with water input under different water stress conditions. Motzo et al. (2001) proposed a seasonal water stress index based on soil-plant-atmosphere interaction, where stress was quantified as 1 - (fraction of transpirable soil water). Rizza et al. (2004) proposed an integrated “water stress index” (WSI), based on a simple soil water balance and the integrated reduction of plant transpiration relative to potential transpiration. They proposed that yield potential and adaptability of cultivars be related to water stress by means of the intercept and slope of a linear regression of yield versus WSI.

By providing yield analysis as a function of an environmental index these different approaches enabled comparison of genotype performances under different degrees of water limitation. With studies quantifying yield potential, yield stability, and susceptibility/resistance to stress, an improvement of yield in drought-prone environments can now be attempted. An ideal genotype would combine the highest yield (highest intercept) with the lowest sensitivity to water stress (lowest slope); nevertheless very often a high yield performance under wet and dry conditions of superior genotypes was found to be associated with a high sensitivity to water stress (high intercept and high slope) (Pantuwan et al., 2002; Ober et al., 2004; Rizza et al., 2004; Pidgeon et al., 2006).

Table 1
Physiological traits relevant for response to drought conditions

<table>
<thead>
<tr>
<th>Plant traits</th>
<th>Effects relevant for yield</th>
<th>Modulation under stress</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal conductance/leaf temperature</td>
<td>More/less rapid water consumption. Leaf temperature reflects the evaporation and hence is a function of stomatal conductance.</td>
<td>Stomatal resistance increases under stress</td>
<td>Jones (1999), Lawlor and Cornic (2002)</td>
</tr>
<tr>
<td>Photosynthetic capacity</td>
<td>Modulation of concentration of Calvin cycle enzymes and elements of the light reactions</td>
<td>Reduction under stress</td>
<td>Lawlor and Cornic (2002)</td>
</tr>
<tr>
<td>Timing of phenological phases</td>
<td>Early/late flowering. Maturity and growth duration, synchrony of silk emergence and anthesis, reduced grain number</td>
<td>Wheat and barley advanced flowering, rice delayed, maize asynchrony</td>
<td>Slafet al. (2005), Richards (2006)</td>
</tr>
<tr>
<td>Anthesis-silking interval (ASI) in maize</td>
<td>ASI is negatively associated with yield in drought conditions.</td>
<td>Drought stress at flowering causes a delay in silk emergence relative to anthesis</td>
<td>Bolanos and Edmeades (1993), Edmeades et al. (2000)</td>
</tr>
<tr>
<td>Starch availability during ovary/embryo development</td>
<td>A reduced starch availability leads to abortion, reduced grain number</td>
<td>Inhibition of photosynthetic activity reduces starch availability</td>
<td>Boyer and Westgate (2004)</td>
</tr>
<tr>
<td>Partitioning and stem reserve utilization</td>
<td>Lower/higher remobilization of reserves from stems for grain-filling, effecting kernel weight</td>
<td>Compensation of reduced current leaf photosynthesis by increased remobilization</td>
<td>Blum (1988), Slafet al. (2005)</td>
</tr>
<tr>
<td>Stay green</td>
<td>Delayed senescence</td>
<td>Reduced under stress (wilting, senescence, abscission)</td>
<td>Rajcan and Tollenaar (1999), Walter and Shurr (2005)</td>
</tr>
<tr>
<td>Single plant leaf area</td>
<td>Plant size and related productivity</td>
<td>Reduced total mass but increased root/shoot ratio, growth into wet soil layers, regrowth on stress release</td>
<td>Hoad et al. (2001), Sharp et al. (2004)</td>
</tr>
<tr>
<td>Rooting depth</td>
<td>Higher/lower tapping of soil water resources</td>
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<td></td>
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<tr>
<td>Cuticular resistance and surface roughness</td>
<td>Higher or lower water loss, modification of boundary layer and reflectance</td>
<td></td>
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<tr>
<td>Photosynthetic pathway</td>
<td>C4/C3/CAM, higher WUE and greater heat tolerance of C4 and CAM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmotic adjustment</td>
<td>Accumulation of solutes: ions, sugars, poly-sugars, amino acids, glycinebetaine</td>
<td>Slow response to water potential</td>
<td>Serraj and Sinclair (2002)</td>
</tr>
<tr>
<td>Membrane composition</td>
<td>Increased membrane stability and changes in aquaporine function</td>
<td>Regulation in response to water potential changes</td>
<td>Tyerman et al. (2002)</td>
</tr>
<tr>
<td>Antioxidative defense</td>
<td>Protection against active oxygen species</td>
<td>Acclimation of defence systems</td>
<td>Reddy et al. (2004)</td>
</tr>
<tr>
<td>Accumulation of stress-related proteins</td>
<td>Involved in the protection of cellular structure and protein activities</td>
<td>Accumulated under stress</td>
<td>Ramanjulu and Bartels (2002), Cattivelli et al. (2002)</td>
</tr>
</tbody>
</table>

4. Physiological bases for yield under drought

The physiologically relevant integrators of drought effects are the water content and the water potential of plant tissues (Jones, 2007). They in turn depend on the relative fluxes of water through the plant within the soil-plant-atmosphere continuum. Thus, apart from the resistances and water storage capacities of the plant, it is the gradient of water vapour pressure from leaf to air, and the soil water content and potential that impose conditions of drought on the plant. Once a drop in water potential develops, responses of a wide range of physiological processes are induced. Some of these responses are directly triggered by the changing water status of the tissues while others are brought about by plant hormones that are signalling changes in water status (Chaves et al., 2003). Physiological traits relevant for the responses to water deficits and/or modified by water deficits span a wide range of vital processes (Table 1). As a consequence, it can be expected that there is no single response pattern that is highly correlated with yield under all drought environments.

The different crop developmental stages show different sensitivity to drought stress. In wheat most of the floret primordia that reach the fertile floret stage become grains after anthesis. The number of fertile florets or grains per m², the most
relevant component in ensuring high yield in drought conditions (Slafer and Whitechurch, 2001), is determined during stem elongation, a few weeks before anthesis. An extended duration of the stem elongation phase without a change in the timing of anthesis has been proposed as a physiological determinant of an increase in the number of grains per m² without altering the amount of water used by the crop (Slafer et al., 2005). In maize a close synchrony between pollen shed at anthesis and silk emergence is required for high kernel set and a negative relationship exists between final kernel number and the extent of anthesis-silking interval (ASI). Drought stress at flowering causes a delay in silk emergence relative to anthesis (Bolanos and Edmeades, 1993). Meiosis, anthesis, and male and female fertility are all extremely susceptible to drought stress and their failure directly affects the kernel number, thus leading to a significant yield penalty. In maize ovary abortion is greatest when drought is experienced a few days before pollination (Boyer and Westgate, 2004). Water deficit around pollination increases the frequency of kernel abortion in maize due to a lack of photosynthate and to the disruption of carbohydrate metabolism in ovaries (Zinselmieier et al., 1995). In the absence of stress, the sucrose, converted by invertase into hexoses, is the source for starch deposition in the ovaries and ovary development. Water deficit inhibits photosynthesis and, therefore, the photosynthetic flux decreases, a condition associated with a loss in invertase activity and a depletion of starch and sugars in the developing maize ovaries and embryos. When drought treated maize plants were fed with sucrose to ensure the photosynthate flux even when photosynthesis was inhibited, ovary abortion was largely prevented suggesting that sugar starvation is the main reason sustaining ovary abortion (Zinselmieier et al., 1999; Boyer and Westgate, 2004). Carbohydrate metabolism is also a key factor in ensuring male fertility; in wheat it has been shown that the disruption of starch deposition in pollen grains due to losses of invertase activity, leads to increased male sterility (Dorion et al., 1996). In rice, low water potentials around the time of anthesis may lead to a failure of anther dehiscence which leads to male sterility (Saini and Westgate, 2000). In maize, water deficit later in development tends to reduce kernel size rather than number, and size is mainly determined by photosynthetic activity and available photosynthetic reserves (Boyer and Westgate, 2004; Saini and Westgate, 2000).

Several studies have addressed yield under drought stress as a function of single physiological traits in attempts to understand which metabolic processes and/or morpho-physiological traits are crucial in ensuring high yield performance under a wide range of environments. Fischer et al. (1998) studied representative semi-dwarf spring wheat cultivars for changes in stomatal conductance associated with selection progress. They found that stomatal conductance and maximal rates of photosynthesis were positively correlated with increased yields of advanced cultivars, while leaf temperatures were negatively correlated. Looking at the relationships between conductance and leaf water potential in modern wheat cultivars, Siddique et al. (1990) suggested that wheat varieties may be “opportunistic” in relation to available water, having high rates of leaf conductance when soil moisture was favourable, and markedly reduced leaf conductance when soil moisture was limiting. Old cultivars, on the other hand, were characterized by a “conservative strategy” with lower leaf conductance even at high soil moisture. An effect of developmental plasticity affecting yield response under drought stress was shown by the fact that modern cultivars used less water in the pre-anthesis period and had more water available in the post-anthesis period (Siddique et al., 1990).

Stay green is an important trait in several crops (e.g. maize, rice, sorghum). Stay green plants are characterized by a post-flowering drought resistance phenotype that gives plants resistance to premature senescence, stalk rot, and lodging when subjected to drought during grain-filling. Stay green has been extensively used in plant breeding to improve yield potential and yield stability in all environments, including drought-prone areas (Campos et al., 2004; Tollenar and Wu, 1999). The improvement of grain-filling capacity under terminal drought could also be achieved by increasing the mobilization of the vegetative reserves from stems to ears (Blum, 1988). Selection for stem reserve contribution to grain-filling was extensively carried out in cereals (Blum, 1988), however, this mechanism can only be useful in environments where terminal drought is the main recurrent problem.

Osmotic adjustment (OA) is a key mechanism enabling plants under drought to maintain water absorption and cell turgor pressure, thus contributing to sustained higher photosynthetic rate and expansion growth. A general analysis of OA in wheat under several drought stress conditions has shown that osmoregulation can be an effective selection criterion for drought tolerance, and that it has a role in reducing drought-dependent yield loss especially when water deficit occurs during the reproductive growth stage (Morgan, 1983; Moiuddin et al., 2005). Nevertheless, a number of contrasting reports on the role of OA have been published. For instance, the recent study of Turner et al. (2007) concluded that differences in OA were not associated with yield benefits in a population of chickpea advanced breeding lines developed from a cross between cultivars with high and low OA. A comparative analysis of many studies dedicated to OA has suggested that OA cannot be considered equally useful in all crops and/or drought conditions, but that a general positive association between yield and OA can be found under severe water stress where yields tend to be low (Serraj and Sinclair, 2002).

A further example of a successful breeding program for dry environments, based on a physiological trait, was reported by Rebetzke et al. (2002). They used carbon isotope discrimination (Δ) as a surrogate for water use efficiency to select wheat lines with high water use efficiency in drought-prone environments. During photosynthesis plants discriminate against the heavy isotope of carbon (¹³C) and, as a result, in several C₃ species, Δ is positively correlated with the ratio of internal leaf CO₂ concentration to ambient CO₂ concentration (Cᵢ/Cₐ) and negatively associated with transpiration efficiency. Thus, a high Cᵢ/Cₐ leads to a higher Δ and a lower transpiration efficiency (Farquhar and Richards, 1984). In wheat, when employing divergent selection, the resulting low Δ lines had
increased aboveground biomass and kernel weight. Yield was increased by about 2% under mild stress conditions and up to about 10% under the driest conditions (Rebetzke et al., 2002).

5. Molecular markers to dissect drought tolerance-related traits

Molecular markers can be used to explore germplasm through segregation and association mapping to identify useful alleles in both cultivated varieties and wild relatives. Although association mapping is intrinsically more powerful than ‘classical’ genetic linkage mapping because it scrutinizes the results of thousands of generations of recombination and selection (Syvänen, 2005), most of the data available up to date on drought tolerance are based on segregation mapping and QTL analysis. Many efforts have been dedicated to understanding the genetic basis of physiological traits conferring advantages in dry environments, while less attention has been given to understanding the high yield stability in dry and wet environments.

Drought tolerance is a typical quantitative trait, however single genes, such as those controlling flowering time, plant height, ear type and OA, may have important roles in adaptation to drought-prone environments (Forster et al., 2004). For instance, a single gene influencing OA in wheat was mapped on the short arm of chromosome 7A (or gene—Morgan and Tan, 1996), and a breeding program for the or gene was shown to increase yield under reduced water supply conditions (Morgan, 2000).

During the last 10 years, the application of QTL analysis provided unprecedented opportunities to identify chromosome regions controlling variations in almost all the physiological, morphological and developmental changes observed during plant growth in water-limiting conditions. Particular attention has been paid to: (i) genetic variation of the OA (Teulat et al., 1998; Robin et al., 2003), (ii) genetic bases of phenological traits—e.g. stay green phenotype (Sanchez et al., 2002; Jiang et al., 2004; Verma et al., 2004); (iii) the ability of the roots to exploit deep soil moisture to meet evapotranspirational demand (Johnson et al., 2000; Nguyen et al., 2004); (iv) the limitation of water-use by reduction of leaf area and shortening of growth period (Aniya and Herzog, 2004); (v) isotope discrimination (Martin et al., 1989; Juenger et al., 2005; Saranga et al., 2004); (vi) the limitation of non-stomatal water loss from leaves—e.g. through the cuticle (Lafitte and Courtois, 2002) and (vii) the response of leaf elongation rate to soil moisture and evaporative demand (Reymond et al., 2003).

Comparative analysis of QTL results clearly shows that chromosomal regions determining variation in agronomic and physiological drought-related traits cover a large proportion of the whole genome. For many crop plants information on drought-related QTL findings have been collected in open source databases, such as GRAMENE (http://www.grame-ne.org/) or GRAINGENES (http://wheat.pw.usda.gov/GG2/).

The association between variation in drought-related quantitative traits and, ultimately, the effects of these traits on yield in drought and favourable environments is the main goal for present and future research. Looking for the coincidence of loci for specific traits and loci for yield under drought stress and in stress-free environments, it is possible to test more precisely whether a specific trait is of significance in improving drought tolerance and yield potential. For example in rice, QTLs for plant yield under drought were coincident with QTLs for root traits and OA (Babu et al., 2003). Several major loci for yield under different environmental regimes were mapped along with QTLs for late senescence of the flag leaf in winter wheat (Verma et al., 2004). Similarly, Lanceras et al. (2004) found that favourable alleles for yield components were located in a region of rice chromosome 1 where QTLs for many drought-related traits (root dry weight, relative water content, leaf rolling and leaf drying) were previously identified (Zhang et al., 2001). These results may suggest that selection for drought tolerance could become more efficient thanks to the availability of handle markers tightly linked to loci for stress related traits. Marker diagnostics of individual QTLs represents an important surrogate for physiological trait measurements, and may ultimately improve breeding efficiency through MAS. However, we also need to consider that favourable alleles for drought tolerance and for yield components detected in QTL studies could be contributed by the two contrasting parents (Lanceras et al., 2004). Therefore, it is possible that selection for drought tolerance using these regions as targets may incur a penalty on grain yield by decreasing the yield components.

One of the possible disadvantages of molecular markers is that the genetic linkage between a specific random DNA marker and a target locus allele, established by QTL studies, can be broken by genetic recombination, although a QTL/gene can be tagged by two flanking markers to reduce the recombination risk. Furthermore an accurate QTL mapping usually resulting in a small QTL interval, is also a pre-requisite to improve MAS-QTL efficiency. These intrinsic difficulties together with the polygenic nature of drought tolerance and the interaction with the environment, makes MAS for drought tolerant QTLs extremely difficult (Francia et al., 2005) due to the number of genes involved and the interactions among them (epistasis). The fact that numerous genes are involved in the expression of polygenic traits means that the individual genes generally have small effects on the plant phenotype. This implies that several regions (i.e. QTLs) must be manipulated at the same time in order to obtain a significant impact, and that the effect of individual regions is not readily identifiable. Replicated field tests are needed in order to accurately characterize the effects of QTLs and to evaluate their stability across environments. Although significant QTL effects can be detected across a range of environments, the evaluation of the QTL × E interactions remains a major constraint on the efficiency of MAS (Beavis and Keim, 1996).

The contribution of genomics-assisted breeding to the development of drought-resistant cultivars has so far been marginal and only few significant examples of MAS for traits associated with drought tolerance have been reported. Due to the high cost of molecular marker technology and the relative low efficiency in MAS for QTL, particularly for traits with a strong G × E interaction and epistatic effects, the cost
The effectiveness of QTL-MAS versus standard breeding strategies in commercial programs still needs to be accurately evaluated (Barker et al., 2005). Ribaut and Ragot (2007) used a marker assisted backcross in maize to introgress the favourable alleles at five QTLs explaining about 38% of the total phenotypic variance for the interval between anthers and silks extrusion (ASI—Bolanos and Edmeades, 1996), a trait negatively associated with yield in drought conditions. Grain yield of the best maize hybrids selected with molecular markers for four generations was, on average, 50% higher than control hybrids under severe water stress conditions. It is worth noting that no yield penalty was observed under well-watered conditions. Similarly, when a pearl millet major QTL for grain yield under terminal drought stress was transferred into a drought sensitive genotype the genotypes carrying the introgression at the target QTL showed a consistent grain yield advantage (Serraj et al., 2005). In rice, MAS was used to transfer several QTLs for deep roots from the japonica upland cultivar “Azucena”, adapted to rainfall conditions, to the lowland indica variety “IR64”. MAS selected lines showed a greater root mass and higher yield in drought-stressed trials (Courtois et al., 2003). In sorghum, molecular markers were exploited to develop near isogenic lines each containing one of four stay green QTLs previously identified (Harris et al., 2007). Favourable alleles in each of the four loci contributed to the lower rate of leaf senescence under post-anthesis water deficit.

An important step towards the application of molecular markers in breeding for drought tolerance is the cloning of DNA sequences underlying QTLs. To date most plant QTLs have been cloned by the positional cloning approach, although alternative strategies based on candidate genes and linkage disequilibrium may represent an interesting shortcut to QTL cloning (Salvi and Tuberosa, 2005). A candidate gene for drought tolerance usually refers to a sequence for which the expression profile or protein function can be associated with the stress response/adaptation process, and the position on the genome is linked with QTLs. Candidate genes can be identified from the literature, by mapping of known stress responsive genes (Tondelli et al., 2006), or using bioinformatic analysis of all genes present in QTL-underlined genomic regions.

So far no QTL for drought tolerance has been cloned in crop species, although a recent study in Arabidopsis has led to the cloning of the ERECTA gene, a sequence beyond a QTL for transpiration efficiency (Masle et al., 2005). In plants with large genomes, the generation of molecular-linkage maps based on candidate genes (molecular-function maps) is one way to identify the genetic determinants of QTLs—i.e. functional markers (Causse et al., 2004; Chen et al., 2001), in spite of the time-consuming fine mapping. This candidate gene strategy shows promise to bridge the gap between quantitative genetic and molecular genetic approaches to study complex traits and, for example, has been applied to find genes potentially involved in barley and rice drought tolerance (Zheng et al., 2003; Nguyen et al., 2004; Diab et al., 2004; Tondelli et al., 2006). The identification of the QTL corresponding genes will also provide the best markers for MAS, those designed to directly tag the different alleles of the drought-related genes (QTN—quantitative trait nucleotide).

In most QTL studies the work has not been extended beyond their detection for a given trait under drought. The development of consensus QTL maps generated from a number of crosses is an important step towards the identification of regions commonly associated with drought tolerance. A major challenge remaining is to confirm that QTLs discovered in a given mapping population will improve drought tolerance when introduced into high yielding elite genotypes. This is particularly difficult when the traits are governed by “context dependent” gene effects (i.e. interaction with other genes and/or environment). In these cases the value of the QTL alleles can differ depending on the genetic structure of the current germplasm set in the breeding program (Wade, 2002). Under these conditions, the value of a given QTL allele can change during selection due to changes in the background effects at any given time in the breeding process. As a consequence, when the background effects are important, the stacking of desirable alleles by MAS becomes inadequate because the initial target combination of alleles may no longer be the best target, or even a relevant target, for increasing trait performance in subsequent breeding cycles. The “Mapping As-You-Go” strategy (Podlich et al., 2004) involves repeated re-estimation and validation of the QTL effects throughout the breeding process to ensure that they remain relevant throughout. This method results in substantial increases in MAS efficiency compared with standard approaches based on the evaluation of the QTL effects only at the beginning of the breeding program, particularly when epistasis and/or genotype × environment interactions play a significant role.

6. Genes and metabolites conferring drought tolerance

New chances to further improve yield and/or yield stability under limiting conditions come from the last 10 years’ progress in the identification of the genetic determinants of the physiological responses related to stress tolerance. Adaptation of plants to drought and to the consequent cellular dehydration induces an active plant molecular response. This response significantly improves the tolerance to negative constraints and it is to a great extent under transcriptional control. Many stress-related genes have been isolated and characterized in the last two decades in a variety of crop species (Cattivelli et al., 2002; Ramanjulu and Bartels, 2002), however, the complexity of the whole molecular response to drought in crop plants has only recently been revealed by large transcriptome analyses (Hazen et al., 2005; Buchanan et al., 2005; Kolipara et al., 2002). Molecular analysis in Arabidopsis has sketched the complex network constituting cell communication during drought response. From model plants, genetic information is being moved to crops exploiting genome synteny, taking advantages of conserved molecular pathways, including those controlling stress tolerance. Following this approach the regulatory components of the drought response are being searched and identified in crop plants (Li et al., 2005; Shen et al., 2003; Kizis and Pages, 2002; Marère et al., 2004).
Transgenic plants have been developed either to up-regulate the general stress response or to reproduce specific metabolic or physiological processes previously shown to be related to drought tolerance by classical physiological studies (Table 2). Transcription factors as well as components of the signal transduction pathways that coordinate expression of downstream regulators are thought to be optimal targets for engineering of complex traits, such as stress tolerance. Successful examples are transgenic crops engineered with genes encoding the DREBs/CBFs transcription factors (tomato—Hsieh et al., 2002; rice—Dubouzet et al., 2003; Ito et al., 2006 and wheat—Pellegrineschi et al., 2004). The transgenic plants showed increased stress tolerance as well as the over induction of downstream stress related genes and/or higher levels of soluble sugars and proline. A recent report has shown that rice plants overexpressing the SNAC1 (stress-responsive NAC1) transcription factor showed improved drought tolerance and yield potential under field conditions. The leaves of SNAC1-overexpressing plants lost water more slowly showing an increased stomatal closure and ABA sensitivity (Hu et al., 2006). Ectopic expression of a stress induced rice gene encoding a calcium-dependent protein kinase (OsCDPK7) also resulted in enhanced levels of stress-responsive genes that contribute to improved salt and drought tolerance (Saijo et al., 2000).

Notably, not all transcription factors involved in drought signal transduction are suitable targets for biotechnology. DREB2 plays a major role in drought-related gene expression, nevertheless, when overexpressed in transgenic plants, DREB2 caused only a weak induction of the downstream genes and did not result in enhanced stress tolerance, probably because of lack of post-translational modifications (Sakuma et al., 2006). On the contrary, CBF3/DREB1A in transgenic rice increased drought and salinity tolerance without affecting growth undesirably (Oh et al., 2005). Overexpression of transcription factors may also activate additional non-stress-related genes that adversely affect the normal agronomic characteristics of a crop, producing deleterious effects on the phenotype and thus yield (Wang et al., 2003). Common detrimental effects due to constitutive expression of regulative factors are growth retardation, reduced fruit and seed numbers and fresh weight of transgenic plants under normal conditions. Alternative strategies based on stress-inducible promoters cause minimal negative effects under normal growth conditions and enhance

Table 2
Relevant examples of genes conferring drought tolerance

<table>
<thead>
<tr>
<th>Genes</th>
<th>Function</th>
<th>Mechanism of action</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DREB/CBFs; ABF3</td>
<td>Stress induced transcription factors</td>
<td>Enhanced expression of downstream stress related genes confers drought/cold/salt tolerance. Constitutively overexpression can lead to stunting growth</td>
<td>Oh et al. (2005), Ito et al. (2006)</td>
</tr>
<tr>
<td>SNAC1</td>
<td>Stress induced transcription factor</td>
<td>SNAC1 expression reduces water loss increasing stomatal sensitivity to ABA</td>
<td>Hu et al. (2006)</td>
</tr>
<tr>
<td>OsCDPK7</td>
<td>Stress induced Ca-dependent protein kinase</td>
<td>Enhanced expression of stress responsive genes</td>
<td>Saijo et al. (2000)</td>
</tr>
<tr>
<td>Farnesyl-transferase (ER1)</td>
<td>Negative-regulator of ABA sensing</td>
<td>Down-regulation of farnesyltransferase enhances the plant’s response to ABA and drought tolerance reducing stomatal conductance</td>
<td>Wang et al. (2005)</td>
</tr>
<tr>
<td>Mn- SOD</td>
<td>Mn-superoxide dismutase</td>
<td>Overexpression improves stress tolerance also in field conditions</td>
<td>McKersie et al. (1996)</td>
</tr>
<tr>
<td>AVP1</td>
<td>Vacuolar H+-pyrophosphatase</td>
<td>Overexpression facilitate auxin fluxes leading to increased root growth</td>
<td>Gaxiola et al. (2001), Park et al. (2005)</td>
</tr>
<tr>
<td>HVA1; OsLEA3</td>
<td>Stress induced LEA proteins</td>
<td>Over-accumulation of LEA increases drought tolerance also in field conditions</td>
<td>Bahieldin et al. (2005), Xiao et al. (2007)</td>
</tr>
<tr>
<td>ERECTA</td>
<td>A putative leucine-rich repeat receptor-like kinase is a major contributor to a locus for Δ on Arabidopsis chromosome 2</td>
<td>ERECTA acts as a regulator of transpiration efficiency with effects on stomatal density, epidermal cell expansion, mesophyll cell proliferation and cell–cell contact</td>
<td>Masle et al. (2005)</td>
</tr>
<tr>
<td>otsA and otsB</td>
<td>Escherichia coli trehalose biosynthetic genes</td>
<td>Increased trehalose accumulation correlates with higher soluble carbohydrate levels, elevated photosynthetic capacity and increased tolerance to photo-oxidative damage</td>
<td>Garg et al. (2002)</td>
</tr>
<tr>
<td>P5CS</td>
<td>θ-Pyrroline-5-carboxylate synthetase</td>
<td>Enhanced accumulation of proline</td>
<td>Kavi Kishor et al. (1995), Zhu et al. (1998)</td>
</tr>
<tr>
<td>mID</td>
<td>Mannitol-1-phosphate dehydrogenase</td>
<td>Mannitol accumulation leads to increased osmotolerance</td>
<td>Abebe et al. (2003)</td>
</tr>
<tr>
<td>GF14B</td>
<td>14-3-3 protein</td>
<td>Lines overexpressing GF14B have a “stay green” phenotype, improved water stress tolerance and higher photosynthetic rates under water deficit conditions</td>
<td>Yan et al. (2004)</td>
</tr>
<tr>
<td>NADP-Me</td>
<td>NADP-malic enzyme</td>
<td>The overexpression decreased stomatal conductance and improves WUE</td>
<td>Laporte et al. (2002)</td>
</tr>
</tbody>
</table>
stress tolerance, although it remains to be established if the threshold stress under which the promoter is active corresponds well to stress levels in target environments (Garg et al., 2002; Wang et al., 2005).

Metabolic engineering for increasing osmolyte contents was successful in several plants subjected to stress (Wang et al., 2003), although real advantages of such a strategy are always a subject of debate (Serraj and Sinclair, 2002). Given that the target compounds did not achieve levels sufficient to sustain a role in OA, chaperone-like activity and scavenging of reactive oxygen species were proposed as alternative functions in plant protection during stress exposure. The first example of metabolic engineering for drought tolerance was the overproduction of proline in transgenic tobacco (Kavi Kishor et al., 1995) and rice (Zhu et al., 1998), resulting in an enhanced biomass under stress conditions. Garg et al. (2002) developed drought tolerant transgenic rice lines showing tissue- or stress-inducible accumulation of trehalose, which accounted for higher soluble carbohydrate levels, a higher capacity of photosynthesis and a concomitant decrease in photo-oxidative damage, and more favourable mineral balance under both stress and non-stress conditions, without negative effects. A significant improvement of wheat tolerance to water deficit was also achieved by Abebe et al. (2003), through the ectopic expression of the mannitol-1-phosphate dehydrogenase (mtlD) gene that caused a small increase in the level of mannitol.

An emerging strategy to broaden stress tolerance in plants is to maintain energy homeostasis under stress conditions. In fact, it is known that the high energy consumption of the plant’s stress response increases respiration rate with a linked production of reactive oxygen species (Tiwari et al., 2002). De Block et al. (2005) obtained Brassica napus plants tolerant to multiple stresses by preventing over activation of mitochondrial respiration and high energy consumption related to stress-inducible poly(ADP-ribose)ylation activity.

A “stay green-like” phenotype was obtained when the Arabidopsis gene GF14 lambda encoding a 14-3-3 protein was introduced into cotton plants. The transgenic plants showed improved water stress tolerance and a higher photosynthesis rate due to an altered stomatal conductance under water deficit conditions (Yan et al., 2004). Since an earlier-than-normal stomatal closure in a crop is considered a positive trait to improve water use efficiency in drought environments (Sinclair and Muchow, 2001), developing transgenic plants with a drought-avoidance phenotype represents a possible strategy for crop improvement. A phenotype with decreased conductance and higher water use efficiency was obtained in tobacco plants overexpressing a maize NADP-malic enzyme, the primary decarboxylating enzyme in C4 photosynthesis (Laporte et al., 2002). The implication of abscisic acid hormone as a molecular signal in drought activated pathways and in the control of stomatal closure makes ABA synthesis and response a possible target for improving drought tolerance. When a farnesyl-transferase acting as a negative-regulator of ABA sensing was down-regulated in a drought inducible manner in Brassica napus the transgenic plants showed enhanced ABA sensitivity, as well as a significant reduction in stomatal conductance and transpiration under drought stress conditions. Furthermore, transgenic plants were more resistant to water deficit-induced seed abortion during flowering (Wang et al., 2005).

A more robust root system enables plants to take up greater amounts of water during water deficit stress, resulting in a more favourable plant water status and less injury. Although this consideration has been obvious for many years, only recent studies have found the way to increase root size through single gene transformation. The gene coding for the vacuolar H+-pyrophosphatase (H+-PPase)-AVP1-plays an important role in root development through the facilitation of auxin fluxes. Overexpression of AVP1 in Arabidopsis and tomato resulted in more pyrophosphate-driven cation transport into root vacuolar fractions, an increased root biomass, and an enhanced recovery of plants from an episode of soil water deficit stress (Gaxiola et al., 2001; Park et al., 2005).

7. Field phenotyping

A comprehensive and careful field evaluation of mapping populations and transgenic plants is urgently needed in order to provide reliable information on the effectiveness of QTLs, candidate genes and transgenes. Due to the multigenic nature of drought tolerance, the introduction of a single gene or QTL into an elite germplasm may result in a subtle phenotypic effect or yield increase. Capacity for precise phenotyping under reliable conditions probably represents the most limiting factor for the progress of genomic studies on drought tolerance. Often field experiments designed to evaluate genetic differences in drought tolerance are faced with contrasting requirements. There is a need for a high precision because the differences may be small and subtle, and detailed physiological measurements (i.e. evaluation of the photosynthetic activity) are difficult when a large numbers of genotypes are involved; the QTL studies for instance are based on segregating populations with hundreds of progenies in the same trial. To achieve an accurate phenotyping it is also important to control stress levels and timing. The intensity of drought stress is often different from year to year and within fields because of variations in soil composition which determine the capability of the soil to retain water. To reduce the signal-to-noise ratio in field based experiments there is a need to select research plots with low spatial variability in soil properties. Application of nutrients and the control of weeds/pests should be carried out precisely and uniformly; experimental design should control within-replica variability (Edmeades et al., 2004). Variations in rainfall amount and distribution strongly influence the level and timing of the stress; the use of rain shelters and supplementary irrigation can help to control the stress conditions and improve the quality of the phenotyping work.

In the last decade many differently engineered plants have been proposed and tested for improved performance under drought. Nevertheless, in many reports desiccation and salt stresses applied are ‘shock’ treatments, while for most crops drought tends to develop slowly as the soil dries. The evaluation of drought tolerance of transgenic plants has often been based
on survival capacity, with very limited analyses of the transgene effects on yield potential. Furthermore, the assessment of plant water status with different methods, often with a visual score, makes the comparison among different reports difficult. A comparative evaluation of all transgenic plants available for drought tolerance would provide important information for the exploitation of transgenic work in breeding programs.

Few reports have been published on transgenic plants tested for drought tolerance in field trials, although more trials are presently carried out by private breeding companies and a first generation of drought tolerant corn varieties is expected within the coming years (Campos et al., 2004). Obviously, only field trials under real stress conditions allow for conclusive remarks on stress tolerance and yield performance of a genotype. Transgenic wheat plants from six independent transgenic events overexpressing the barley HVA1 gene were tested under irrigated and rainfed conditions over six seasons (Bahieldin et al., 2005). Although the effect of the transgene was changing from year to year, the field trials showed that the HVA1 gene has the potential to confer drought stress protection in field conditions. Results from 3 consecutive years of field trials with transgenic Brassica napus carrying a down-regulation of a farnesyl-transferase acting as a negative regulator of ABA sensing suggested that with adequate water, transgenic plants produced the same amount of seed as the parental control, while under moderate drought stress conditions at flowering seed yield of transgenic canola was significantly higher than the control (Wang et al., 2005). Encouraging field trial data have also been published for alfalfa plants overexpressing the superoxide dismutase gene (McKersie et al., 1996), and for rice overexpressing the stress responsive NAC1 transcription factor (Hu et al., 2006) as well as the stress responsive OsLEA3 gene (Xiao et al., 2007). The report showed that the higher yield under drought conditions achieved by transgenic OsLEA3 rice, was due to increased spikelet fertility. These phenotypic data as well as the fact that the OsLEA3 gene is located within the interval of QTL controlling spikelet fertility under drought conditions illustrate the key role of ovary fertility in drought tolerance (Xiao et al., 2007).

Even if the use of biotechnology in agriculture offers great potential benefits to farmers, the effectiveness of a transgenic-based breeding strategy is nowadays hampered by non-biological constraints, related to the commercialisation of transgenic crops, particularly in Europe. To generate commercially useful products scientists must increasingly integrate social, legal, and economic issues as well as technical issues from the earliest stages of project design. The commercial success of these products will depend upon the development of regulatory frameworks that are clearly defined and scientifically based, and upon public acceptance of transgenic plant products (McElroy, 1999).

8. Future directions

When phenotypic selection was the only tool available to improve yield under drought, the improvements in crop yield observed were likely due to an increase in yield potential through the unconscious pyramiding of yield-related traits or loci. Research in the last three decades has opened up three main approaches: (i) plant physiology provided new tools to understand the complex network of drought-related traits and several drought-related traits useful to improve selection efficiency have been proposed (see Table 1); (ii) molecular genomics has led to the discovery of a large number of loci affecting yield under drought or the expression of drought tolerance-related traits; and (iii) molecular biology has provided genes that are either useful as candidate sequences to dissect QTLs or for transgenic approaches (see Table 2). The integration of molecular genetics with physiology is leading to the identification of the most relevant loci controlling drought tolerance and drought-related traits. Routine cloning of the genes underlying the QTLs is still a long way off, but it will, ultimately, provide simple markers for an effective MAS.

Nevertheless MAS for drought tolerance will not be an easy task because dozens of QTLs for drought-related traits have been identified. Selecting which QTLs/traits follow with MAS is now crucial. The improvement of drought tolerance should not be achieved with a parallel limitation of yield potential. Hence, drought tolerance traits should be tested in both stressed and non-stressed environments before being introduced in a MAS breeding program. QTLs for drought-related traits coincident with QTLs for yield potential should be considered as priority targets for MAS.

The success of any selection process relies on the availability of superior alleles for the target trait. Most QTLs for drought tolerance, rather than being chosen for their overall agronomic value, have been identified in segregating populations derived from parental lines chosen to maximize the differences in the target traits. Typically, a segregating population from a cross between modern and old varieties allows the identification of many QTLs, nevertheless a majority of the positive QTL alleles might derive from the modern parental line and therefore are already present in the best performing cultivars. A chance to find new useful alleles is represented by the exploitation of wild germplasm. During the domestication process, wild plants carrying promising traits were cultivated, leading to locally adapted landraces. These lost many undesirable alleles and useful alleles became enriched in the cultivated gene pool ( Tanksley and McCouch, 1997). Many studies have demonstrated the value of alleles originating from non-cultivar germplasm (Tanksley and Nelson, 1996; Tanksley and McCouch, 1997), showing that centuries of selective breeding have thrown away useful alleles in addition to many useless ones. The effect of a given QTL/locus can also be influenced by the background of the genotypes used in the breeding program epistatic interactions among QTLs, for example, might hamper the development of an efficient MAS program. Notably, when four genomic segments carrying QTLs for root length were transferred from the rice cultivar “Azucena” into “Kalinga III”, only one of them significantly increased root length in the new genetic background (Steele et al., 2006).

Transgenic breeding will also have a role in the future and the possibility of cloning stress-related QTLs will enable the
simultaneous engineering of multiple genes governing quantitative traits. However, the scarcity of field trials for drought tolerant transgenic plants does not allow for final conclusions. New transgenic plants where the gene is introduced into elite genotypes have to be tested under optimal as well as drought conditions to evaluate the impact of the transgenes on yield potential and stress tolerance.

Compared with ten years ago, breeders now have new perspectives for plant improvement for drought. First, they will soon have new tools, such as markers for QTLs and single genes for plant transformation that will provide an opportunity to move the selection from phenotype to genotype. Second, they will have a new role, to blend together all knowledge of the traits sustaining yield under drought and to accumulate randomly dispersed QTLs and/or transgenes into elite genotypes. This so called “breeding by design” strategy (Peleman and Van der Voort, 2003) will lead to new highly yielding cultivars able to improve performance in both high and low yielding environments. A significant example showing how the integration of stress physiology and genomics can lead to an integrated view of plant breeding is represented by the studies on transpiration efficiency. During drought stress plants generally coordinate photosynthesis and transpiration, although significant genetic variation in transpiration efficiency has been identified both between and within species (Rebetzke et al., 2001). After the demonstration that Δ of plant matter is a reliable and sensitive marker negatively related to variation in transpiration efficiency (Martin et al., 1989), several selection programs based on Δ were carried out (Rebetzke et al., 2002; Juenger et al., 2005) and new wheat cultivars, Drysdale and Rees, with improved water use efficiency have already been released in Australia. The isolation of a gene, ERECTA, that regulates transpiration efficiency in Arabidopsis (Masle et al., 2005), and the transcriptional analysis of wheat genotypes with contrasting transpiration efficiency (Xue et al., 2006) are providing the molecular bases of the isotopic discrimination parameter. Hence, in the near future, an integrated approach for transpiration efficiency could involve the use of the physiological test (analysis of Δ), the markers for QTLs controlling Δ, and the ERECTA gene as well as of other genes either to search for allelic variations in the germplasm or as a tool for plant transformation. Every day, it becomes more evident that successful breeding for stable high yield under drought conditions will only be possible when a true integration of traditional breeding with physiology and genomics is achieved. Thus, to face drought stress and to achieve sufficient grain yield in the future requires a multidisciplinary approach based on plant genomics, physiology and modeling (Yin et al., 2004; Wollenweber et al., 2005).

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