Breeding for Yield Potential and Stress Adaptation in Cereals

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The need to accelerate breeding for increased yield potential and
temperature adaptation to drought and other abiotic stresses is an issue of
increasing urgency. As the population continues to grow rapidly, the
pressure on resources (mainly untouched land and water) is
also increasing, and potential climate change poses further chal-
In this review, we discuss ways to improve the efficiency of crop breeding
by better understanding the physiological basis of crop yield and its response to stresses, with special
emphasis on drought. This is not just because physiology forms
the basis of proper phenotyping, one of the pillars of breeding, but
because a full understanding of physiology is also needed, for example,
to identify the traits associated with molecular breeding approaches
such as marker-assisted selection (MAS) or plant transformation or the way these traits are evaluated. Most of the information in
this review deals with cereals, since they include the world’s main
crops, however, examples from other crops are also included. Topics covered by the review include the conceptual framework for identifying secondary traits associated with yield potential and stress adaptation, and how to measure these secondary traits in practice.
The second part of the review deals with the real role of molecular
breeding for complex traits from a physiological perspective. This
part examines current developments in MAS and quantitative trait
loci (QTL) detection as well as plant transformation. Emphasis is
placed on the current limitations of these molecular approaches to
improving stress adaptation and yield potential. The essay ends by
presenting some ideas regarding future avenues for crop breeding
given the current and possible future challenges, and on a multi-
disciplinary approach where physiological knowledge and proper
phenotyping play a major role.

Keywords breeding, cereals, drought, yield potential

I. YIELD POTENTIAL AND STRESS ADAPTATION

The growing world population together with the lack of ex-
expansion, or even reduction, of available arable lands needed
to maintain agricultural sustainability (Cassman, 2003), impli-
that the relative importance of plant breeding to raise
crop yield potential and adaptiveness is now greater than in
the past (Slafer et al., 1999; Araus et al., 2002). This is not
a minor expectation. Although there is a rising demand for
irrigation and chemical fertilizers, the technological progress
made in cereal cultivation over recent decades has led to a
decline in the cost of cereal production per unit of output.
Without this growth in productivity, many developing coun-
tries would have been forced to further extend cultivation into
marginal lands, thus aggravating the dilemma of how to sust-
ain natural resource bases (IRRI, 1996). In the case of wheat,
yield has been genetically enhanced virtually all over the world
(Calderini et al., 1999). The magnitude of the improvement
has depended upon the environmental conditions of the region
(Figure 1 top). However, when wheat yields are expressed as a
percentage of the mean yield of the trial in which they were as-
sessed, the data appear to converge into a single trend (Figure 1
bottom).

Plant breeding in general, and cereal breeding in particu-
ar, was remarkably successful during the second half of the
20th century, contributing substantially to keeping production
ahead of population growth, in spite of the world population
increasing more rapidly than ever before in the history of hu-
mankind (more than doubling in half a century). Nevertheless,
the current situation regarding crop yields at the world scale is
slightly more complex. In more recent times, yields have not in-
creased at the pace registered from the 1950s to the 1990s (e.g.,
Calderini and Slafer, 1998; Conway and Toenniessen, 1999). As
the population continues to rise along with consumption expec-
tations of new emerging economies, and with it food demand,
food shortages will be unavoidable if agricultural production
 gains do not return to previous rates, at least keeping abreast
of population growth (Khush, 1999). In addition, the increasing
cost of fossil fuels together with the environmental concerns
related to applying large amounts of chemical fertilizers and the
emergence of biofuels which compete with food produc-
tion may further complicate the situation. Moreover, climatic
change, which potentially will lead to increased temperatures and
evapotranspiration losses and eventually decreased rainfall,
is expected to have a particularly negative effect in the agri-
cultural context (The World Bank, 2007) of many developing
regions of Africa and Asia (Rijsberman, 2006; Lobell et al.,
2008). These regions will probably witness a negative impact
on several major crops that are crucial to large food-insecure populations.

REFERENCES
Plant adaptation is a key factor that will determine the future severity of the effects of climatic change on food production. Relatively inexpensive changes, such as shifting planting dates or switching to an existing crop variety, may moderate the negative impact of climatic change. However, improvements in crop productivity to meet the requirement mentioned above will not be easy without further technological breakthroughs that allow yield ceilings to be shifted through the development of new crop varieties (Rosenzweig and Parry, 1994). Expansion of irrigation could be considered another option to counteract the effects of climatic change. In fact, the increase in cereal production in recent decades has been achieved mostly from irrigated land, through the diffusion of improved crop varieties and agronomic practices suitable for specific ecosystems. Agriculture currently uses 75% of the total global consumption of water (Molden, 2007), and in absolute terms consumptive water use in agriculture will rise in coming decades (Falkenmark and Rockström, 2004).

Nevertheless, sustainability concerns constrain the adoption of intensive agronomic practices (Macllwain, 2004), thereby questioning the desirability of the further expansion of irrigation (Araus, 2004). Siltation of reservoirs and canals, lowering of underground water levels, and accumulation of salt in already irrigated soils are now environmental concerns (IRRI, 1996). Moreover, the rising costs of irrigation and the problems of management, cost recovery, and the maintenance of existing systems limit the further expansion of irrigation. Furthermore, new growth sectors, such as industry and tourism, as well as increasing population and urbanization, all compete for water resources.

Abiotic stress factors frequently constrain the growth and productivity of major crop species such as cereals. The single greatest abiotic stress factor that limits crop growth worldwide is water availability (Araus et al., 2002). While genetic increases in yield potential are best expressed in optimum environments, they are also associated with enhanced yields under drought (Trethewan et al., 2002; Araus et al., 2002; Slafer and Araus, 2007) and nitrogen deficiency (Ortiz-Monasterio et al., 1997; Abeledo et al., 2003). These gains are especially relevant given that further large increases in the area under irrigation are not expected, and land deterioration associated with intensive agriculture threatens those areas already irrigated (Araus, 2004).

This review discusses techniques for improving yield potential and adaptiveness to unfavorable environmental conditions of small grain cereals (such as wheat, barley, and rice). It also addresses how knowledge of the physiological mechanisms of these grains can contribute to reaching these goals. The study of crop physiology can assist cereal breeding by: i) improving our understanding of the factors that determine crop yield and adaptation through the syncretic concept of ideotype and, consequently, improve crop simulation models; ii) defining particular “secondary” traits to select (analytical breeding) when choosing parents for crossing or screening in segregating populations; iii) indicating the kind of genetically modified organisms (GMOs) with potential for development and how to test them; and iv) phenotyping associated with marker-assisted selection (MAS). This review also highlights the aspects that may be particularly useful to the national agricultural research systems (NARS) of developing countries, where research budgets are limited and prioritization is required. For this purpose, we will focus on alternatives for evaluating secondary traits in an economical way, and the prospects for the new array of molecular techniques available.

II. ANALYTICAL BREEDING

Genetic improvement can be achieved through selection:

- Directly, for a primary trait (such as grain yield) in a target environment (Ceccarelli and Grando, 1996). This has been referred to as empirical breeding.
- Indirectly, for a secondary trait that must be putatively related to a higher yield potential and/or to improved behavior of the crop when grown in a stressful environment. This is known as analytical or physiological breeding. During the last 50 years, most of the progress in major cereals has been derived from
empirical (also termed conventional or traditional) breeding, which has taken yield as the main trait for selection. Yield increases have been possible through the gradual replacement of traditional tall cultivars by semidwarf and fertilizer-responsive varieties with superior harvest indices. The term “Green Revolution” was coined to describe this process. However, yield is a quantitative inherited trait under multigenic control, and it is characterized by low heritability and a high genotype-by-environment (GE) interaction (Jackson et al., 1996). Hence, as traditional (i.e., empirical) breeding appears to be reaching a plateau; several approaches, which complement traditional with analytical selection methodologies, may be required to further improve grain yields. In this context, analytical breeding, drawing on a physiological understanding of GE interactions, is an option. The multisite testing of elite lines is inevitable, and so the contribution of physiology to interpreting the nature of GE interactions, one of the critical drawbacks that the breeders face, may be crucial for future yield gains.

A. Identifying Physiological Traits

There are diverse ways to assess the phenotypic traits that may help step-up breeding (Araus et al., 2002). One implies the formulation of yield as the combination of distinct independent processes (e.g., agronomical or physiological yield components) (Araus et al., 2002; Reynolds and Tuberosa, 2008). Another approach involves retrospective studies, comparing genotypes released in different eras as a result of previous breeding programs (Araus et al., 2002; Sanguineti et al., 2006).  

1. Primary Determinants of Grain Yield and Drought Adaptation

Grain yield can be expressed as the integrated response of distinct plant processes to a limited resource such as radiation or water. This response involves the following two main steps: the production of photoassimilates, and their further transformation into an economic (usually harvestable) component. An additional factor to consider is the phenological stage of the plant when the limited resource acts.

**Radiation-limited Yield.** Grain yield (GY) can be considered the product of the following:

\[ GY = RAD \times \%RI \times GLD \times RUE \times HI \]

where RAD = incident radiation received per day during the growing season, RI = % of intercepted radiation over crop life cycle, GLD = green leaf duration, RUE = radiation use efficiency, and HI = the harvest index.

The seasonal rate of dry matter accumulation is a function of interception and utilization of incident solar radiation. Differences in the rate of dry matter accumulation can be attributed to differences in light interception caused by variability (i) in maximum LAI (leaf area index) and (ii) in leaf senescence (e.g., ‘stay-green’ in maize) during grain filling, and in canopy-level efficiency of utilization of intercepted radiation as a result of (iii) changes in leaf angle, or (iv) changes in the functionality of leaf photosynthesis during grain filling (Tollenaar and Lee, 2006).

Grain yield can be reduced by the effects of drought on most of these factors (Andrade et al., 1996). Drought during seedling establishment or during the period of leaf area expansion causes a decrease in crop leaf area and radiation interception (%RI). Later on in the growth cycle, water stress reduces the green leaf duration (GLD) from accelerated senescence and radiation use efficiency (RUE) by direct effects on photosynthesis (e.g., Dwyer et al., 1992). This can have direct effects on yield components through induced barrenness, kernel abortion or shrivelled grain, which in turn can reduce the HI.

Reduction in leaf growth caused by a water deficit can be coregulated through several mechanisms, each controlled by a large number of genes. Therefore, it may be naive to look for a single mechanism that accounts for the effect of water deficit on leaf growth, and for the genetic variability of this process (Tardieu, 2006). Nevertheless, and in spite of some controversy (Serraj and Sinclair, 2002), it seems that osmotic adjustment may in some cases facilitate critical growth functions such as root growth, meiosis, and pollen development, thus mitigating some of the most detrimental effects of plant water deficit (Blum, 2005, 2006). Although there is genetic variation in osmotic adjustment, this trait is believed to be controlled by a single gene in wheat and rice (Morgan, 2000).

With regard to photosynthetic efficiency and performance under water stress, clear differences among green tissues have been reported in wheat and other small grain cereals, with ears being better adapted to drought than leaves (Tambussi et al., 2005a). A recent review on this subject can be found in Tambussi et al. (2007).

**Water-limited Yield.** Passioura (1977) proposed a parallel way to consider grain yield in a context of water deficit:

\[ GY = W \times WUE \times HI \]

where: \( W \) = water transpired by the crop and \( WUE = \) water use efficiency, biomass/unit water transpired. \( WUE \) has a somewhat different meaning to water productivity, which is defined at the crop level as the ratio of biomass with economic value (for example grain yield of cereals) compared to the amount of water transpired (Bouman, 2007).

Along the same lines as Passioura’s identity, Blum (2006) summarized the primary factors responsible for superior performance of drought-adapted cereal cultivars in three categories:
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2. Retrospective Studies: Physiological Changes Associated with Genetic Improvement in Grain Yield

The study of contributions made by successful wheat breeding may provide clues to help identify alternatives for breeders to further increase yield. Research into the changes in physiological traits associated with genetic gains in yield potential is essential to improve our understanding of yield-limiting factors and to base future breeding strategies on.

Cultivars released in distinct eras have been compared for yield and morpho-physiological determinants. They have been grown simultaneously under fixed conditions, thereby removing the effects of varying management practices on yield (Slafer et al., 1994) and allowing the comparison of the physiological bases underlying the differences in yield capacity. Most of the traits identified in retrospective analyses are constitutive in nature; that is to say, they are expressed in the absence of stress (Calderini et al., 1999). These attributes are divided into four categories: (i) time to flowering and plant height, (ii) biomass production and partitioning, (iii) main yield components, and (iv) cross-category interactions.

Time to Flowering and Plant Height. The timing of flowering is a major trait related to the adaptation of cultivars to particular areas, thus determining crop performance under prevalent field conditions (e.g., Perry and D’Antuono, 1989; Passioura, 1996, 2002; Richards, 1996a; Slafer and Araus, 1998). This observation explains why time to flowering (phenological adjustment) is one of the first attributes optimized by breeding programs (Slafer, 2003). Consistent changes in this trait are therefore to be expected only in regions in which the lengthening or shortening of the growing season has advantages for adaptation regardless of the cultivars released earlier. A scenario frequently reported in the literature, where the manipulation of time to heading may strongly affect adaptation, is that of regions characterised by a Mediterranean climate; i.e., a hot dry summer and humid temperate winter (Perry and D’Antuono, 1989; Loss and Siddique, 1994; Acevedo et al., 1999). In these environments, the vegetative and early reproductive phases of a crop frequently occur under adequate water availability. However, as the season progresses, drought becomes more intense and frequent water stress occurs during the late reproductive phases. After anthesis, grains fill under severe water and heat stress. The analysis of long-term trends in time to flowering for cultivars released in different eras reveals that, in most cases, there are few or no changes in this trait in regions with climates that differed from the Mediterranean, while reducing time to flowering has been a successful strategy when breeding for environments characterized by terminal stress factors (Álvaro et al., 2008a,b); unless the original landraces on which breeding started to improve yields already had a time to flowering conferring adaptation (e.g., Acreche et al., 2008). This strategy has achieved good results because earliness is probably the most effective means to increase yield in regions where drought commonly occurs during grain filling (Passioura, 1996; Slafer and Whitechurch, 2001). In addition to an adaptive advantage of a reduction in crop duration, this decrease has permitted augmented cropping intensity for cereals such as rice, and has also allowed land to be used for non-cereal crop cultivation in cereal-based farming systems (IRRI, 1996).

 Breeders have always selected for reduced height when the initial elite material exceeds a threshold. For plant heights above this threshold (70–100 cm; Richards, 1992; Miralles and Slafer, 1995a), there is no gain in biomass while there is a proportional reduction in the HI Royo et al., 2007). Below this threshold, further gain in the HI does not compensate for the loss in biomass because of extremely poor radiation distribution within the canopy and consequent reductions in RUE (e.g., Miralles and Slafer, 1997). Thus, reducing height to an optimum range increases yield potential (a similar biomass more efficiently partitioned to grains) and simultaneously reduces the risk of lodging. Consequently, plant height was reduced universally through wheat breeding during the 20th century (see a number of cases reviewed by Calderini et al. (1999), and more recent studies: Donmez et al., 2001; Brancourt-Hulmel et al.,

• Capturing more soil water. Thus, where deep soil moisture is available, deep-rooted cultivars have demonstrated a clear yield advantage under drought (Lorens et al., 1987). However, due to the difficulties associated with the study of root architecture, for most breeding programs the role of this architecture and function in drought adaptation has not yet been taken into account (Osmont et al., 2007).

• Economizing water use. For example, vigorous crop establishment is agronomically desirable as it helps to shade the soil and suppress weeds that compete for water. Rapid groundcover is a crucial component and can be achieved by breeding for thinner, wider leaves, a more prostrate growth habit (Richards et al., 2002), lower nitrogen content per unit leaf area (Tambussi et al., 2005b), large seed and embryo size (Aparicio et al., 2002) and longer coleoptiles (Rebetzke et al., 2005).

• Utilizing stem reserves for grain filling under stress. Thus soluble carbohydrate reserves in the stem at the time of anthesis may contribute to superior performance under drought stress (Blum, 1988; Reynolds et al., 2005). Moreover (and even when it is conceptually difficult to support) there are reports indicating that this trait may also increase yield potential (Shearman et al., 2005).

Blum (2006) adds maintaining cellular hydration as a fourth factor. In the past, traits targeted, for example, by plant transformation have dealt mostly with tolerance to cellular dehydration rather than with the maintenance of cell water status (Araus et al., 2003a).
The two main yield components are the number of grains per m² and the averaged individual...
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grain weight. Most experiments analyzing the effects of genetic improvement on yield have considered these components. The vast majority of the studies performed found that, while selecting for higher-yielding cultivars, wheat breeders have consistently increased the number of grains per unit land area and produced either no trend or even a trend towards slightly reduced individual grain weight (see review by Calderini et al., 1999 as well as more recent studies by Donmez et al., 2001; García del Moral et al., 2002; Brancourt-Hulmel et al., 2003; Royo et al., 2007). However, earlier studies have reported a slight increase in this component during the 20th century (Cox et al., 1988) or part of it (Calderini et al., 1995).

The increased number of grains per unit area of modern cultivars compared to their predecessors is attributed to greater survival of floret primordia (Álvaro et al., 2008a), as the number of potential florets per spike is apparently quite similar (e.g., Slafer and Andrade, 1993; Miralles et al., 2002). Thus, the higher survival of floret primordial (a process that occurs during the last half of stem elongation; Kirby, 1988) is the most important factor leading to higher yield potential in modern cultivars (Slafer et al., 1994). This finding is consistent with the observation that semi-dwarfing genes, which contribute significantly to enhanced yields in many breeding programs throughout the world, increase the number of grains per unit land area by augmenting the survival of floret primordial (Miralles et al., 1998; Álvaro et al., 2008a). In fact, the gibberellin-sensitive dwarfing genes Rht-B1b and Rht-D1b, the two most relevant genes from a commercial perspective, reduce plant height by around 18% and simultaneously exert large pleiotropic effects which improve spike fertility (Pflantham et al., 1997; Álvaro et al., 2008a).

Associations between Attributes. The genetic improvement of yield can be understood more mechanistically by inspecting and interpreting the relationships between the main attributes of growth and by partitioning the yield components described above. Studies on the mechanistic bases by which breeding has successfully increased yield may shed light on potential future alternatives.

Genetic improvements in grain yield are almost unequivocally due to gains in the number of grains per unit land area, with no gains and even slight losses in the average weight of the grains, probably as a consequence of the smaller cells in the pericarp induced by insensitivity to GA (Miralles and Slafer, 1995b) and the increased number of small of potential size when grain number is improved (e.g., Acreche and Slafer, 2006). This physiological basis of yield improvement in wheat is so strong that even under conditions characterized by terminal stress grain yield improvement has been associated with increases in grain number per unit land area (e.g., Acreche et al., 2008), mostly due to increases in the number of grains per spike (Royo et al., 2007). Consequently, a negative relationship between grain number per unit land area and the average weight of these grains has frequently been found (Slafer et al., 1996). In fact, this negative relationship is also common when yield is increased by management practices. These observations are generally attributed to greater competition among grains as the number of grains per m² increases; however, we argue that the negative relationship is not competitive in nature (Slafer, 2003). This is because, although wheat breeding has reduced the degree of post-anthesis sink limitation to yield (e.g., Kruk et al., 1997), photosynthetic capacity during grain filling together with the pre-anthesis assimilate reserves are in excess of the demands of the growing grains during post-anthesis (e.g., Slafer and Savin, 1994; Richards, 1996b; Slafer et al., 1996; Borras et al., 2004; Reynolds et al., 2004) even under Mediterranean conditions (e.g., Cartelle et al., 2006).

The high source:sink ratio may be responsible for a down-regulation of photosynthetic capacity during grain filling. For instance, Reynolds et al. (2005) suggested that RUE could be increased by increasing sink-strength (i.e., more grains per unit land area), which is in line with evidence that cultivars with improved sink strength may possess higher photosynthetic rates in post-anthesis (e.g., Calderini et al., 1997). In a recent study, Uddling et al. (2008) showed that this high source:sink ratio more than offsets the direct stimulating effect of elevated CO₂ on post-anthesis photosynthesis. Recently we also found that decreasing post-anthesis sink strength resulted in a reduction in both canopy radiation use efficiency and leaf photosynthesis in wheats released at different eras (Acreche et al., 2007).

The main conclusion from this overall analysis is that, with only a few exceptions, wheat yield is limited by sink strength during grain filling and that further increases in yield depend upon increases in sink strength after anthesis (to either further increase grain number per unit land area or to boost the potential size of the individual grains).

The number of grains per unit land area is determined by various factors, including plants per unit land area, spikes per plant, spikelets per spike, and grains per spikelet. These factors are sensitive to growth conditions from sowing to anthesis (Slafer and Rawson, 1994). However, the number of grains per m² is far more sensitive to changes in growth partitioning over a short window of time immediately before anthesis (coinciding with stem elongation) than to any changes in growth occurring before this time (e.g., Fischer, 1985, 1993; Kirby, 1988; Savin and Slafer, 1991; Abbate et al., 1995; Demotes-Mainard and Jeuffroy, 2001). In all these cases, there is a strong relationship between grain number per m² at maturity and spike dry matter per m² around anthesis (Slafer, 2003). Breeding has increased grain number per m² precisely through the same mechanism: crop growth has not clearly and systematically been affected by breeding for higher yield, but there has been a consistent trend towards reduced plant height (a trait determined during stem elongation), thereby leading to enhanced partitioning towards the growing spikes, which in turn results in an increased spike dry matter associated with more grains per unit land area (e.g., Siddique et al., 1989; Slafer and Andrade, 1993). Studies demonstrating a consistent increase in yield caused by Rht
genes in a wide variety of conditions also report the relevance of this mechanism (e.g., Brooking and Kirby, 1981; Fischer and Stockman, 1986; Miralles et al., 1998; Royo et al., 2007).

In this context, the positive relationship between the number of grains per m² and the HI, found almost without exception when comparing modern and old wheat cultivars, can be explained. The number of grains is simply a reflection of changes in partitioning that occur before anthesis, thereby resulting in lower vegetative biomass.

**Yield Potential versus Stress Adaptation: GE Interaction.**

The limitations of traditional breeding are more evident when selecting for stress (e.g., drought) adaptation because of GE interactions and higher within-site variability, which also diminishes heritability (Richards, 1996a; Araus et al., 2002). Thus, although selecting for yield per se, the targeted environment may be sensitive when stress is uniformly severe (e.g., Ceccarelli and Grando, 1996). This situation is rare in the field. For example, wheat cultivars tested in a particular set of stressful conditions may not show the same performance in another set (Cooper et al., 1997). Moreover, a crossover effect in the yield of genotypes of high and low yield potential when regressed against the environmental index over a wide range of conditions is not frequently found unless the conditions are too extreme. This observation suggests that, in general, genetic types selected under high yielding environments perform better than those with lower yield potential when grown in a wide range of yielding environments (Calderini and Slafer, 1999; Araus et al., 2002; Slafer and Araus, 2007). A recent 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 (Cattivelli et al., 2008). A clear example is provided by the comparison of two barley cultivars released at different times over recent decades (Tambussi et al., 2005b). The more recent cultivar (Graphic) provides greater yields than Kym in a wide range of Mediterranean growing conditions and although GE interaction is observed, no crossover has been identified even for a wide range of yielding environments (Figure 3).

**FIG. 3.** The relationship between grain yield and the Environmental Index for the barley cultivars Graphic (○) and Kym (●). This index was calculated as the average grain yield of the two cultivars in each location of the 23 field trials performed by the Spanish registration office. Each point represents the mean of four replicates (blocks) in each location. Linear regressions and determination coefficients (r²) are shown for each cultivar. Redrawn from Tambussi et al. (2005b).

The more recent cultivar (Graphic) provides greater yields than Kym in a wide range of Mediterranean growing conditions and although GE interaction is observed, no crossover has been identified even for a wide range of yielding environments (Figure 3). Hence Graphic, initially intended for high yielding conditions, is widely cultivated in rain-fed areas in southern Europe. The higher yield of this cultivar under a wide range of conditions is sustained by a constitutively increased plant growth and total photosynthesis during tillering, despite the fact the photosynthetic capacity per unit area of leaves is lower than that of Kym. Moreover, Graphic has a more extensive root system than Kym, thereby improving its water status in the later stages of the crop cycle.

Constitutive whole-plant traits have a major effect on plant water use and the avoidance of plant dehydration under stress. These features largely determine several of the negative relations between yield potential and the capacity to sustain yield under severe water shortage (Blum, 2005). In most dry-land situations where crops depend on unpredictable seasonal rainfall, the maximization of soil moisture use is a crucial component of drought resistance (avoidance), which is generally expressed by a lower WUE (Blum, 2005; Slafer and Araus, 2007), and may explain the positive correlations frequently found under Mediterranean conditions between carbon isotope discrimination (Δ13C, see below) and grain yield (Araus et al., 1998a; 2002, 2003c). However, selection for yield under drought stress has resulted in a dehydration-tolerant phenotype that is rarely compatible with a high yield potential phenotype. If selection strategies were to address factors of stress adaptation in addition to yield under stress, then it may be possible to combine higher yield potential and drought resistance (Blum, 2005). From an historical perspective, compared to genes that control constitutive traits, drought-responsive genes have made a comparatively moderate contribution to the development of drought-resistant cereal cultivars, with the exception perhaps of osmotic adjustment (Blum, 2006), which is not so relevant in maize (Tardieu, 2006).

For maize there is evidence that supports higher tolerance of low resource availability in newer hybrids bred for high yield potential. These hybrids perform better than older ones under stress as a result of parental line involvement which is associated with greater tolerance to high plant density (Duvick, 1997; Tollemar and Lee, 2006). In fact, plant water deficit occurs more readily at high rather than at low crop density, and adaptation to high plant density involves resistance to drought stress when moisture is limited (Tollemar and Wu, 1999). Anthesis-to-silking interval (ASI) under drought has become shorter in modern hybrids and selection has possibly led to an increase in the growth of spikelets and ears and a reduction in final spikelet number (Bänziger et al., 2000). Moreover, ‘stay green’ or the reduction in the rate of leaf senescence during grain filling is one of the traits that most visually differentiates older from newer
hybrids (Duvick et al., 2004a). Changes in constitutive traits, such as plant phenology, are also involved in the distinct responses to limited resources. Older hybrids show a greater yield loss, in part, because they extract most of the plant-available water before entering the critical flowering period (Nissanka et al., 1997; Campos et al., 2004). In temperate maize hybrids, there has also been a significant reduction in tassel size. From 1967 to 1991, tassel dry weight decreased 36% (Duvick and Cassman, 1999). However, tropical inbreds usually still have a relatively large tassel, which eventually may negatively affect the development of ear and silk when the supply of photoassimilates is limited by drought stress (Ribaut et al., 2004; Sawkins et al., 2006). Retrospective studies also show a large hybrid x environment (applied as sowing density) interaction in maize. The GE interaction could be a result of (i) enhanced genetic yield potential of newer hybrids; (ii) increased capacity of newer hybrids to tolerate low resource availability; or (iii) greater general stress tolerance of newer hybrids (Tollenaar and Lee, 2006). The increased yield of new hybrids could be a result of the synergistic effect between augmented yield potential and increased resource availability (Duvick and Cassman, 1999). In general, enhanced yield potential will place a greater demand on all resources, thereby resulting in greater stress frequency unless the higher yield potential is accompanied by an increase in general stress tolerance. In fact, yield stability and general stress tolerance are highly associated and yield stability does not appear to have declined with increasing yield potential (Tollenaar and Lee, 2002; Duvick et al., 2004b).

Beside the occasional interactions between yield potential and stress adaptation discussed above, there may also be interactions between stress adaptation and different stress environments. This may be particularly relevant in the case of drought. Drought is highly variable; there exist different kinds of drought depending, for example, on the severity of the water shortage or the phase in the crop cycle when it occurs (Araus et al., 2002). Therefore drought adaptive features may be yield positive in one environment but yield negative in another. This is the case, for example, of earliness for drought escape in small grain cereals under Mediterranean conditions. A short-duration cultivar, in addition to having its yield potential limited, may be exposed to late freezing episodes that occur around flowering time. Another example is the amount of water available. In barley (again under Mediterranean conditions) the genotypic relationship between Δ13C (see below) measured in kernels and grain yield moves from negative, in very drought prone conditions, to positive, when drought conditions are less severe (i.e., water input improves and/or transpirative demand decreases) (Volotas et al., 1999). Similar trends (although less clear) may be observed for durum wheat (Araus et al., 2003c; Royo et al., 2005). Such a change in the slope of the relationship may be understood by considering that while under drought prone conditions, genotypes which save water (i.e., with higher WUE) are better suited, as drought conditions are less severe (e.g., more water is available), genotypes able to use more water (regardless of whether this is in exchange for a lower WUE) will yield more. For bread wheat, genotypes exhibiting high WUE at the seedling stage (measured as a low Δ13C) are the most productive under severe drought conditions in Australia, where crop growth only relies on the water stored prior to planting (Condon et al., 2004). However, such a relationship disappears when water conditions improve and the amount of water input accumulated during the growth cycle increases.

3. Physiological Avenues for Increasing Yield Potential

As mentioned above, retrospective studies on wheat indicate that the improvement in yield has more often been associated with augmented partitioning of biomass to the grain than with enhanced overall biomass (Austin et al., 1980; Waddington et al., 1986; Sayre et al., 1997; Calderini et al., 1999). Thus plant height is usually negatively related to the HI (Royo et al., 2007). However, there is a minimum height below which yield limitation is evident (Slater et al., 2005). Moreover, given that the HI is estimated to have an upper limit of just over 60% (Austin, 1980), and that this limit is already being approached (Shearman et al., 2005), it is becoming more important than ever to unveil the physiological and genetic bases of RUE and biomass determination if increased yields are to be achieved (Araus et al., 2003b).

Increasing RUE. Rubulose carboxylase oxygenase (Rubisco) enzyme, which catalyzes the assimilation of CO2 by the carboxylation of RuBP in the Calvin Cycle, is the most obvious target for attempts to improve photosynthetic rate and thus RUE. Moreover since Rubisco exhibits a slow catalysis, large amounts are needed to sustain high photosynthetic rates, therefore negatively affecting N use efficiency. Rubisco also catalyzes the wasteful oxygenation of ribulose bisphosphate (RuBP), initiating the process of photorespiration and the consequent release of CO2, NH3 and energy expenditure. The dual specificity of Rubisco seriously impacts upon water use efficiency. Thus stomatal closure leads to a lower internal CO2/O2, making carbon assimilation less efficient; conversely, the unrestricted exchange of gases through fully open stomates would exacerbate water loss. Therefore high temperature and even more drought (the combination of water stress and high temperature) may further increase oxygenase activity of Rubisco relative to the carboxylation of RuBP and therefore photorespiration (Long, 2006; Parry et al., 2007).

Except maize and sorghum, major crops like wheat, rice, barley or soybean have C3 photosynthetic metabolism. There are different avenues to improve assimilation of CO2 subsequently decreasing photorespiratory losses. While one strategy would be to introduce forms of Rubisco with higher relative specificity for CO2, an alternative exists to insert the carbon concentration mechanisms of C4 plants. Such approach would be of greatest benefit under drought or other conditions that lead to stomatal closure and low intercellular CO2 (von Caemmerer, 2003). Other targets for the improvement of photosynthetic rate impact upon the capacity for RuBP regeneration and include...
other Calvin cycle enzymes, principally sedoheptulose bisphosphatase, and components of electron transport and light capture by the photosystems (Parry et al., 2007, 2008).

To date no substantial increase in the carboxylase relative to oxygenase activity or catalytic rate has yet been achieved by the characterization of site-directed mutants and chimaera enzymes for cyanobacterial, algal and bacterial forms of Rubisco (Parry et al., 2003a, 2007, 2008). Alternatively, natural variation, although comparatively small (Galmes et al., 2005), can be exploited to improve Rubisco specificity and thus CO₂ assimilation in crop plants (Parry et al., 2007). Models indicate that replacing native wheat Rubisco with that from *Limonium gibbonii* will give a 12% increase in net photosynthesis and thus greatly increased yield. Although much progress has been made to develop the tools for introducing genes into the nuclear genome of elite cultivars of wheat (Pastori et al., 2001; Shewry and Jones, 2005), transfer of the plastid genome is still slow and limited to model species (Maliga, 2004).

Furthermore the additional complexities of assembling copies of the large and small polypeptide subunits of Rubisco into a functional holoenzyme *in vivo* (requiring high levels of expression, interaction with chaperonins, posttranslational modification and interaction with Rubisco activase) remains a major challenge (Parry et al., 2007).

An alternative approach to overcoming the limitations of Rubisco in C₃ crops is to increase the CO₂ concentration at the catalytic site. For example, IRRI has been developing a research programme on rice on this topic for more than a decade. However, the complexities of C₄ “Kranz” anatomy (Araus et al., 1991) and the segregation of photosynthetic metabolism between the mesophyll and bundle sheath have prevented the introduction of such a system into C₃ plants. The discovery of CO₂ pumps that operate within a single cell of some species (e.g., *Hydrilla verticillata*) has led to a number of biotechnological approaches introducing a single cell CO₂ pump into C₅ crop plants such as rice and wheat (Leegood, 2002). However this approach still requires the coordinated, targeted expression of many transgenes, the assembly of gene products at the correct cellular and subcellular location, together with the appropriate regulation of the component enzymes and transporters. This has yet to be achieved (Leegood, 2002) and, even if successful, additional structural changes may be required to maximize yield potential. The wild plant where this mechanism has been discovered is characterized by a very low rate of growth and in fact this mechanism has developed to allow this species to tolerate extreme growing conditions in its natural environment.

Biotechnological approaches are also being explored to introduce bacterial enzymes to short-circuit the photosynthetic pathway; therefore eliminating the photosynthetic release of NH₃ (Parry et al., 2003b). A pathway which converts chloroplastic glycinate directly to glyceraldehyde without involvement of NH₃ has been introduced into Arabidopsis and releases CO₂ in the chloroplast. This is consistent with an increase in photosynthesis partly due to an increased CO₂ at the catalytic site of Rubisco (Kebeish et al., 2007).

Other approaches to improve photosynthesis, include preventing the decrease in Rubisco activity particularly under high temperature, by increasing concentration and thermal stability of Rubisco activase (Salvucci et al., 2001) and from altering the abundance of the Rubisco by targeting their synthesis or degradation (Andralojc et al., 2002; see also Parry et al., 2008).

Phosphoenolpyruvate carboxylase (PEPC), which serves as the actual CO₂ pump of the C₄ pathway, is specifically expressed in the mesophyll cells of C₄ leaves. Therefore regulation of PEPC expression is another potential target to increase in photosynthesis. A recent study (Engelman et al., 2008) has demonstrated that the proximal region (-570 to -1) of the ppcA1 promoter of *Flaveria trinervia* (C₄) harbors cis-regulatory elements conferring high expression levels in leaf mesophyll cells of transgenic *Flaveria bidentis* (C₄). Genetic manipulation of these sequences and subsequent analyses in transgenic *F. bidentis* will clarify whether they are able to direct high ppcA1 expression levels in C₄ leaves.

**Biomass Increase.** Moderate increases in biomass have started to be reported in spring wheat (Reynolds et al., 1999) and winter bread wheat (Shearman et al., 2005). In some cases, these increases are associated with a small rise in the assimilation rate during the spike growth stage, and a much larger increase in photosynthetic rate during grain filling (Reynolds et al., 2005a). Further experiments, in which grain number was increased artificially in elite lines with a brief light treatment during the rapid spike growth stage, show that these lines have a photosynthetic capacity that exceeds that required to fill the grains they would normally set (Reynolds et al., 2005).

Nevertheless, in maize, gains in yield potential have been accompanied by clear increases in total biomass and the HI has remained steady (Tollenaar and Lee, 2006). The higher dry matter accumulation in newer than in older maize hybrids during the grain-filling period can be attributed, in part, to a longer duration of the grain filling period in the former (Tollenaar and Lee, 2006). However, the silking dates as well as relative maturity do not differ between modern and old hybrids (Cavaleri and Smith, 1985), which suggest that heterosis has not been responsible for this genetic gain (Tollenaar et al., 2004).

**Phenological Adjustment.** An alternative approach to further increase the number of grains per unit land area might be to lengthen the stem elongation phase (hypothesised by Slafer et al., 1996, 2001). The hypothesis is that a longer stem elongation phase results in greater crop growth during this phase, higher spike dry matter at anthesis and subsequently more grains being filled. This hypothesis would be viable only when the length of the stem elongation phase can be manipulated and when the expected higher biomass accumulated during this phase is not counterbalanced by reduced partitioning to the spikes. These manipulations might involve genes responsible for sensitivity to photoperiod or for earliness per se. The hypothetical involvement of photoperiod genes is supported by the fact that the
duration of stem elongation is regulated by photoperiod response (e.g., Slafer and Rawson, 1997, Miralles and Richards, 2000; González et al., 2002, 2003a), while that of earliness per se genes is backed by the existence of genetic variation in minimum duration of stem elongation and the intrinsic earliness of that phase (when grown under long photoperiods after removing vernalization requirements; Slafer, 1996). Details of this hypothetical alternative can be found in Slafer et al. (2001). Briefly, there is clear genetic variation in the duration of stem elongation, even when holding constant the length of the period to anthesis (Slafer and Rawson, 1994; Kernich et al., 1997; Whitechurch et al., 2007a). At least part of this variation may due to sensitivity to photoperiod (Whitechurch et al., 2007a). This sensitivity varies between phenological phases (Slafer and Rawson, 1996; González et al., 2005a). Exposing plots to photoperiod extensions during the stem elongation phase (natural length was maintained before this phase) altered the duration of the phase, which was associated with changes in the number of fertile florets and grains as a result of modifications in spike dry matter at anthesis (González et al., 2003a, b; 2005b, c). These observations suggest that the mechanism by which photoperiod alters the final number of grains per m² is the same as that determined by radiation interception during stem elongation (González et al., 2005b). Therefore, isolating and subsequently manipulating (traditionally or through MAS) the genetic control sensitivity to photoperiod (in this example, or genetic control of earliness per se) during stem elongation might be an effective avenue for increasing yield. Although recent research achievements have provided new insights into this issue (see section IV.A.2.), to date no conclusive evidence for genetic control have been reported (e.g., Whitechurch and Slafer, 2001, 2002; González et al., 2005a).

Nitrogen Metabolism. The doubling of agricultural food production worldwide is partly attributed to a 7-fold increase in the use of nitrogen (N) fertilizers. However, due to environmental and economic (rising cost of fossil fuel) constraints, further increases in yield must be attained through improved nitrogen use efficiency (NUE) rather than more N fertilizer (The World Bank, 2007). This challenge is particularly relevant to cereals, for which large amounts of N fertilizers are required to attain maximum yield and for which NUE is estimated to be well under 50% (Hirel et al., 2007 and references herein). Studies are currently underway on bread wheat, maize, and rice to identify key traits related to plant performance at low N input (Gallais and Coque, 2005; Kichey et al., 2006, 2007) and to localize chromosomal regions and genes involved in tolerance to N deprivation (Laperche et al., 2007). For example, quantitative genetic studies confirmed that in maize variation in the utilization of N, including remobilization at low N input, was greater than the variation of N uptake before or after flowering, whereas the opposite was observed at high N input (Gallais and Coque, 2005). The impact of knock-out mutations on grain yield and its components was examined in maize plants grown under conditions of suboptimal N availability (Martin et al., 2006). These and other results (Kichey et al., 2006; Hirel et al., 2007) strongly suggest that, in maize and probably also wheat (Bernard, et al., 2008), glutamine synthetase (GS) controls grain yield regardless of N conditions. The constitutive nature of this enzyme regardless of N nutrition was also highlighted by the identification of the N-responsive chromosomal region following recurrent selection (Coque and Gallais, 2006).

III. THE PRACTICAL USE OF SECONDARY TRAITS

Putative secondary traits for a breeding program assisted by analytical selection can be used:

- for the selection of parents to be included in the crossing block.
- as direct selection criteria for screening a large number of genotypes (i.e., segregating populations) and/or when the amount of seed available is too small to carry out field trials with replications (i.e., the evaluation of double-haploid lines).

While plant physiologists are performing intensive work on adaptation to abiotic stress factors and even on ways to increase yield potential, few breeders routinely use the latest physiological criteria in their mainstream breeding programs. One reason for this may be the difficulty in evaluating the response of the selection of secondary traits, this being an essential requirement for their incorporation into these programs. The true value of a given trait can be assessed only by determining the genetic gain in segregating populations following selection. However, many traits are not available in well adapted genotypes and their validation frequently requires the development of appropriate breeding material, which is costly and time consuming (see Royo et al., 2005). Moreover, the evaluation of several of the traits proposed by plant physiologists can be time-consuming—and sometimes even expensive—which is not practical for application to the thousands of entries that comprise the segregating generations of breeding programs. In addition, selection in segregating populations requires screening at the plant level or between very small plots, thus hindering the use of traits that require the assessment of large field plots.

Nevertheless, a number of analytical or indirect selection criteria have been used for decades in breeding programs. Plant height, days to heading or to maturity, photoperiod or vernalization responses, spike length, disease reaction, tillering capacity or grain weight are all examples of characteristics commonly evaluated in wheat and barley breeding programs so as to provide relevant information about the performance of genotypes. The traits chosen for selection purposes must fulfill a set of requirements related to their relevance in terms of crop performance as well as how they can be measured. These points are discussed below.

A. How to Choose a Trait

Three comprehensive manuals have been developed in recent years by CGIAR Centers for cereals such as maize
(Bänziger et al., 2000), wheat (Reynolds et al., 2001) and rice (Fischer et al., 2003). All three are freely accessible. One deals more with how physiology can contribute to crop breeding (Reynolds et al., 2001, http://www.cimmyt.org/research/wheat/map/research_results/wphysio/WPhysiology.pdf) while the other two refer more to the overall context of a given breeding program, where physiological traits can be used (Bänziger et al., 2000; http://www.knowledgebank.irri.org/drought/drought.pdf). Fischer et al., 2003, http://www.knowledgebank.irri.org/drought/drought.pdf).

For a secondary trait to be useful in a program, it must comply with several requirements (Bänziger et al., 2000; Araus et al., 2002; Lafitte et al., 2003; Royo et al., 2005):

1. It must be genetically correlated with grain yield in the environmental conditions of the target environment, that is to say, the relationship with yield must be causal not casual.

2. It should be less affected by environment than grain yield is; that is, it should have greater heritability than yield itself, and so less GE interaction.

3. It must show genetic variability within the species.

4. For breeding programs for stress-prone environments, it should not be associated with poor yields in unstressed environments. Unfortunately, this is the situation of many traits selected because they confer tolerance instead avoidance to a given stress (Araus et al., 2002, 2003a).

5. It must lend itself to rapid and reliable measurement, and measurement should be less expensive than determining yield itself.

6. It must lend itself to assessment in individual plants or in very small plots.

We can predict whether the use of a secondary trait can enhance expected progress in selection by calculating its genetic correlation with yield (point 1) and heritability (point 2). Any trait that fulfills the first three requirements will provide breeders with a useful prediction tool. While this may be enough for a breeding program, for direct confirmation of the value of a trait (validation), several approaches can be adopted, including the development of lines that express the secondary trait well and the assessment of their performance under stress, the evaluation of the genetic gain in segregating populations following selection for the target trait (Royo et al., 2005) and also the identification of co-segregation of quantitative trait loci (QTL) for the trait in question as well as for yield. Once a given trait is chosen as a candidate, a practical way to introduce it into a breeding program (that is, to fulfill point 5) is required. This may be particularly pertinent when evaluating germplasm in segregating populations.

Secondary traits may be particularly suited to improving selection response to stress conditions if they: 1) improve precision (in the case where the heritability of yield is reduced by stress); 2) prevent confounding effects of stress timing on yield (e.g., drought and flowering dates); 3) focus the selection on a specific type of stress, and 4) are cheaper, easier and faster to measure than grain yield.

Moreover, when choosing traits, it is necessary to keep in mind an eco-physiological perspective. In the case of drought, if the target environment is characterized by stress-prone conditions that are so severe that survival is jeopardized, it may be helpful to select for traits conferring survival abilities. In most circumstances, however, the main effect of drought is to reduce grain yield without killing the plant. In this context, breeding for higher yield potential plus traits that confer stress avoidance (i.e., to avoid cell dehydration) may, in general, be a better option (see examples in Araus et al., 2002, 2003a, b). In contrast, and indeed in most cases, trait evaluation should be carried out under field conditions, thereby avoiding experimental conditions (growth chambers, greenhouses, pots) that do not truly reflect a natural agricultural environment. This is also important to discover which genetic factors govern tolerance to stress under actual agricultural conditions.

B. Which Traits to Use in Practice

While many traits have been studied for their use in breeding strategies for drought resistance, there is a general consensus among breeders that only a few are currently recommendable for application in practical breeding programs. For example, CIMMYT (Reynolds et al., 2001) and IRRI (Lafitte et al., 2003) recommend the use of flowering and maturity dates, spike fertility, changes in green biomass (e.g., leaf death score), and low canopy temperature. For maize, Bänziger et al. (2000) at CIMMYT also include forward traits related to flowering (ASI) and senescence (staygreen). The manuals developed by these two institutions include a comprehensive explanation on how to measure these traits. In practical terms, these characteristics are valuable when breeding for higher yield potential and adaptation to a certain degree of stress. Development of modern apparatus and new analytical tools will facilitate future measurements of new physiological traits in the field.

There are numerous categories of phenotyping traits. To date, the most successful (or at least most promising) have been integrative traits, which provide information on the long-term eco-physiological performance of the whole plant or canopy (Araus et al., 2002). Although several research programs have addressed the responsiveness of plant metabolism to drought, application of metabolite measurements to phenotyping has been less successful. However, there are critical periods during the crop cycle (like the flowering period, in which grain setting occurs) where even exposure to short-duration stress may strongly affect yield. In this context, metabolic profiles (e.g., carbohydrate levels, activities of key enzymes, growth regulators) of these stages might provide essential information about the effect of drought on plant development and yield.
1. Phenology

Phenology is the most widely used secondary trait because of ease of measurement (see section II.A.1.) and relatively high heritability (e.g., Bänziger et al., 2000). If the pattern of water deficit is predictable in a given region, selection for a flowering date that does not coincide with the period of water deficit (that is, it exhibits an escape strategy) is a highly effective way to improve drought adaptation (Araus et al., 2002). However, this approach has several limitations, for example, in winter cereals, very early varieties may suffer yield penalty in good seasons, while late-in-season freezing episodes may affect ear fertility.

2. Harvest Index

Ear Fertility: Visual Traits. When stress occurs near flowering, the most sensitive growth stage, the main yield component affected is the percentage of fertile spikelets and thus the number of grains. This trait, highly relevant for any cereal, is critical for rice under water stress (Lafitte et al., 2003) and even more so for maize, in which short ASI is a key selection trait related to kernel number (Bänziger et al., 1999, 2000).

Metabolic Profiles. Several studies have related kernel set to plant growth rate in the period of 10–15 days either side of flowering (e.g., Vega et al., 2001). In addition to the effect of GS on grain yield (Kichey et al., 2006; Hirel et al., 2007), a number of metabolites may be involved in kernel set under stress. Recent research suggests that sucrose serves as a substrate for ovary growth, and that the concentration of this disaccharide is a signal for gene expression (Boyer and McLaughlin, 2007). When its concentration is low, invertase genes are downregulated while genes associated with senescence are up-regulated.

Senescence is a type of cell death program that can be inappropriately activated during drought. In cereals, ethylene has been related to decreases in kernel number (Hays et al., 2007), in addition to reduced plant growth. The role of abscisic acid (ABA) in response to drought has been intensively studied over many years in maize (Tuberosa et al., 2002; Setter, 2006). ABA is widely believed to be a major contributor to the control of plant transpiration and leaf growth (Davies et al., 2002; Tardieu, 2006). Moreover, it is considered that ABA inhibits cell division in the endosperm, and if this occurs at an early development stage, the kernels will abort. Much research effort has centred on the control of biosynthesis and catabolism, the action and role of ABA under water stress (Setter, 2006). The signalling pathways of ABA and ethylene overlap, as demonstrated by the observation that mutants that show affected sensitivity to ABA are allelic with mutants of ethylene sensitivity (Beaudoin et al., 2000). Furthermore, a similar overlapping is observed between the signalling pathways of ABA and sucrose (Leon and Sheen, 2003).

Stem Reserves. Reserves accumulated in the stem by anthesis may play a crucial role under terminal (i.e., end of the crop cycle) stress conditions when photosynthesis is impaired (Blum, 1988; Reynolds et al., 2005). The effect of stem reserves on grain filling and the HI can be evaluated at the canopy level by spraying with chemical desiccants at anthesis (Blum, 1988) and on individual plants by measuring the specific stem weight (dry weight per unit of stem length) or by analyzing total carbohydrate content (Reynolds et al., 2001; Araus et al., 2008; Reynolds and Tuberosa, 2008).

3. Plant Growth and Senescence

Spectroradiometric Techniques. Stress may accelerate leaf senescence. To measure leaf desiccation, symptoms can be visually integrated and translated into a ranking. Also, to check for early senescence of leaves, portable chlorophyll meters are extensively used. In contrast, stress factors, such as drought, strongly impair leaf expansion (Royo et al., 2004) and thus plant growth (Villegas et al., 2001) and further yield (Araus et al., 2002). Therefore, potentially powerful traits for evaluation are total green biomass at a critical plant stage (i.e., anthesis) or its change over time. In practice, a feasible evaluation of total biomass is possible only through indirect methods, for example using spectroradiometers to measure the spectra of light reflected by the canopy both at the crop (Aparicio et al., 2000; Araus et al., 2001; Royo et al., 2003) and at the plant levels (Álvaro et al., 2007). In addition, other physiological characteristics of plants, such as canopy architecture, plant water status, nitrogen concentration and even photosynthetic efficiency are captured in the spectra (Araus et al., 2001; Aparicio et al., 2000; Tambussi et al., 2002; Babar et al., 2006). In many cases, however, the wide range of spectroradiometrical indices have not fulfilled expectations when evaluating field plots for yield and their adaptation to environmental conditions. The scarce use of spectral reflectance measurements as tools for screening in breeding programs can be attributed to several reasons: i) a wide range of variability for the trait of interest must occur within the set of genotypes in order to be detected by the apparatus (Royo et al., 2003); ii) the devices currently available commercially allow measurements only at canopy level, that is to say on medium to large plots, while selection in early segregating generations is based on individual plants grown in small plots; iii) the inadequate use of spectral reflectance indices. Apart from the “classical” vegetation indices (VI) related to green biomass such as the normalized difference vegetation index (NDVI) or the simple ratio (SR), other indices are strongly affected by differences in green biomass (Araus et al., 2001). Therefore, the information provided by indices such as the water index (WI) or the photochemical reflectance index (PRI) is confounded by differences in this trait. Indices other than VI allow the tracking of only physiological changes (i.e., in photosynthetic efficiency, pigment content, and so forth) when differences in biomass are not present across accessions or when used to track changes along time as a response to stress (see Tambussi et al., 2002 for PRI). As an alternative to indices, models constructed using the complete VIS/NIR reflectance spectra have proven accurate in
4. Water Status

**Leaf Porometry.** Transpiration can be divided into two components. One is leaf conductance \( (g_l) \), mostly determined by the degree to which stomata are open, and this parameter depends largely on the water status of the plant. The other is the evaporative demand (which depends on environmental variables such as temperature, relative humidity and wind). Thus \( g_l \) has been used to screen for water status in maize (Nelson et al., 2007), and the current generation of relatively “low-cost” (a few thousand US$) and “easy-to-handle” porometers, such as the Decagon Leaf Porometer SC-1 or the Delta-T AP4, allows rapid (20–30 s) measurement of leaf conductance. However, unless several porometers are used simultaneously, measurement of transpiration may be impractical for a large-scale evaluation purposes.

**Canopy Temperature.** Given that a major role of transpiration is leaf cooling, canopy temperature and its reduction relative to ambient air temperature are an indication of the degree to which transpiration cools leaves under a demanding environmental load. Higher transpiration implies cooler leaves and higher stomatal conductance, both aspects favoring net photosynthesis and crop duration. A relatively lower canopy temperature indicates enhanced capacity to take up soil moisture or to maintain better plant water status. Thus, higher transpiration is a positive trait when selecting for higher yield potential or better adaptation to mild to moderate stress factors (Blum et al., 1982; Reynolds et al., 1999, 2001). The same may be inferred for higher carbon isotope discrimination when this trait is positively correlated with grain yield. Although at first glance canopy temperature may appear to be easy to measure, in practice several methodological limitations are encountered, particularly in Mediterranean drought environments (Araus et al., 2002; Royo et al., 2002) or when the canopy is not homogeneous. In fact, screening by canopy temperature measurements under drought stress can be done only during the vegetative growth stage, after full groundcover has been attained and before inflorescence emergence (Lafitte et al., 2003) at high vapor-pressure deficits in recently irrigated crops and without the presence of wind or clouds (Royo et al., 2005). For other cereals, such as maize, which are characterized by tall plants and more heterogeneous canopies, individual leaves rather than canopy should be measured (Araus et al., 2008).

Thermal imaging is a novel phenotyping technique that allows the observation of spatial temperature patterns associated with transpiration at the canopy or single leaf level (Chaeere et al., 2007, Grant et al., 2007; Möller et al., 2007). While this approach is a very promising phenotyping technique (Figure 4), its implementation will strongly depend on a radical drop in the cost of these cameras (exceed 20,000 US$).

**Stable Isotopes.** Other putative traits, while potentially useful, are less widely accepted, despite being very promising. This is the case for discrimination against the stable isotope \( \Delta^{13}C \), which is limited by the cost of its determination. In recent years, the CSIRO Plant Industry Division has released the two first commercial wheat varieties (Drysdale in 2002, and Rees in 2003) selected for high transpiration efficiency using \( \Delta^{13}C \) as the selection criterion (Richards, 2006). These varieties are cultivated under rain-fed conditions and rely solely upon soil moisture accumulated before planting. These varieties have been selected based on their low \( \Delta^{13}C \) (and thus high transpiration efficiency), measured at tillering under favorable conditions, thereby fitting with what has been postulated for this trait. However, for Mediterranean environments, \( \Delta^{13}C \) (particularly when measured in mature grains) is often positively correlated with grain yield (Araus et al., 1998a, 2003c; Villegas et al., 2000; Condon et al., 2004). One of the reasons for this positive relationship is that a genotype exhibiting higher \( \Delta^{13}C \) shows greater capacity to maintain a better water status and thus more open stomata (see Araus et al., 2002 and Condon et al., 2004).

Due to differences in the photosynthetic pathway, \( \Delta^{13}C \) is not applicable to species with \( C_4 \) metabolism, such as maize (Monneveux et al., 2007). In this context oxygen isotope composition (\( \delta^{18}O \)) in plant tissues might be an alternative since it reflects (among other effects) evaporative enrichment of leaf water caused by transpiration (Barbour, 2007; Farquhar et al., 2007). Accordingly, Barbour et al. (2000) proposed the use of \( \delta^{18}O \) in plant matter as an integrative indicator of genetic differences in stomatal conductance in bread wheat and its application to drought conditions has been further assessed (Ferrio et al.,

FIG. 4. Thermal imaging displaying irrigated (left) and rain-fed (right) plots of durum wheat at Tel Hadya (ICARDA, headquarters), Syria. Courtesy of Dr. Miloudi Nachit and Hani Hazamm.
A recent study also found a relationship between δ^{18}O and plant transpiration for groundnut and rice (Sheshshayee et al., 2005). Thus, δ^{18}O offers the possibility to integrate water use of crops (both C_4 and C_3, as it is independent from photosynthesis) over the crop cycle. However, the expense of δ^{18}O analysis continues to prevent its widespread use in breeding programs. Natural abundance of the stable isotope 15N, usually expressed as a composition (δ^{15}N) has also been proposed as a suitable trait for identifying genotypic performance of cereals under abiotic stresses, in this case salinity (see Yousfi et al., 2008 and references therein).

Although most other traits, particularly those that are expensive or difficult to measure, cannot yet be recommended as part of an ongoing breeding program, some can be used for the selection of parents. Also, QTLs can be mapped for traits such as root characteristics (Tuberosa et al., 2003). Nevertheless the relationship between these loci and drought resistance is not well established (Mackill et al., 2003). Other characteristics such as chlorophyll fluorescence for trait evaluation have been proposed (Baker and Rosenqvist, 2004) and new technical approaches to remote sensing measurement of this trait have been developed (Chaerle et al., 2007). Nevertheless, the application of chlorophyll fluorescence in the field may be strongly limited by crop phenology (Araus et al., 1998b).

5. How to Measure These Traits Inexpensively  

**Carbon Isotope Discrimination.** Given the relatively high costs associated with carbon isotopic analysis (over 10 US$ per sample), several surrogate approaches that are much cheaper, faster, and easier to handle have been proposed. The option most studied has been the use of mineral or simply the total ash content of leaves (Masle et al., 1992; Mayland et al., 1993; Araus et al., 1998a) or grains (Febrero et al., 1994; Araus et al., 1998a, Voltas et al., 1998). Moreover, this approach can be used in C_4 plants such as maize. Another promising alternative approach relies on the estimation of Δ^{13}C through the Near Infrared Spectroscopy (NIRS) technique (Clark et al., 1995; Ferrio et al., 2001), which has the further advantage of being nondestructive.

**Leaf Color.** Leaf color is an extensively used trait because of the speed and ease of use of portable chlorophyll meters such as the Minolta SPAD (http://www.specmeters.com/Chlorophyll_Meters/Minolta_SPAD_502_Meter.html) and also the physiological significance of the trait per se. Total chlorophyll content has been extensively used for managing N fertilization. It provides a high-quality, standardized tool for N management. It may be also useful for the screening of the protein content of wheat grains (Rharrabti et al., 2001). Moreover, since total chlorophyll content is an indicator of early senescence, it is positively correlated with wheat yield (Araus et al., 1997; Rharrabti et al., 2001), and indeed SPAD measurements are routinely taken in breeding programs. However, the cost of a portable chlorophyll meter (at best over 1,500 US$) makes this device unaffordable for many breeding programs in developing countries. Thus, IRRI in collaboration with the NARS, has developed a multi-panel leaf color chart developed and calibrated for use with rice throughout Asia (Shukla et al., 2004; IRRI 2005).

**Biomass Assessment.** In the past, field spectroradiometers that measure the spectrum of light reflected by the canopy were expensive devices (over 20,000 US$). However, prices have now dropped. Designed initially for nitrogen management, a simple and easy-to-handle spectroradiometer such as GreenSeeker (http://www.ntechindustries.com) has become a potentially useful device for plant breeding purposes (Marti et al., 2007). This device gives only the basic spectroradiometric indices of green biomass, such as NDVI. In fact, these single indices are the most useful for routine breeding activities. Moreover, as the GreenSeeker includes its own radiation source, it can be used regardless of the atmospheric conditions (sunny/cloudy day) and, more importantly, its cost is comparable to that of a SPAD (under 3,500 US$).

Conventional digital cameras are inexpensive and can be used for standard procedures in a number of agricultural applications, including plant breeding. Through adequate processing of the information in digital pictures, it is possible to evaluate total green biomass much more cost-effectively than with land-based portable spectroradiometers (Casadesús et al., 2007). In addition, digital pictures may also provide information that is not currently acquired through spectral reflectance measurements, such as the degree of soil covered by the crop, the percentage of yellow leaves (Figure 5; Casadesús et al., 2005) or even yield components such as the number of spikes per unit land area.

C. When to Use the Traits

Grain yield is the primary trait for selection in breeding programs aimed at increasing yield potential and at achieving adaptation to stress-prone environments. When breeding for drought adaptation, the conceptual model used considers yield under drought to be a function of (1) yield potential, (2) the flowering date (which indicates whether the crop will tolerate drought stress), and (3) traits that provide drought-resistance.

Several examples on how to implement indirect selection for physiological traits for drought resistance in a number of cereals are illustrated in Fischer et al. (2003). Most breeders select strongly for traits other than yield in the early segregating generations, and perform yield testing only at later stages, when a certain level of homozygosity has been achieved and a sufficient amount of seed is available. While acknowledging the relevance of secondary traits, they are not part of the selection schemes of most breeding programs because of the reasons given previously. The decision to advance or reject a genotype is often complex, and in practical terms breeders most often use a system of multiple cut-offs. The usual approach consists of carrying out the selection in early generations in stress-free environments, in
order to optimize the expression of desired traits in the plant and maximize, at the same time, the heritability and response to selection. In early generations, breeders select genotypes that presumably achieve the levels required for the primary traits evaluated in segregating populations (resistance to diseases, plant type, plant height, growth cycle, spike fertility, etc.), choosing only those with potential good score expression in the next generation, when they will be tested again. Quality is frequently evaluated early at family level, in order to detect crosses with desirable characteristics. In early generations, it may also be worth evaluating some additional physiological traits that provide an indication of yield potential or crop adaptation to abiotic stress factors.

At further stages, in more advanced generations, multi-site field experiments are conducted to study the adaptation of lines to the target environments. The combination of yield data with data on secondary traits in environments ranging from well-watered to severely water-stressed allows us to ascertain the adaptability of genotypes to a wide range of conditions, thus facilitating more reliable decisions by breeders. At this level, selection is based mainly on the main goals of the program, which usually focus on relevant commercial traits. However, data on secondary traits may be decisive at this stage in interpreting and explaining GE interactions, mostly when the heritability of the secondary traits is higher than that of yield, and the genetic correlation of these traits with yield in the target environment is high. Whatever the breeding strategy deployed with secondary trait selection, indices help to identify the best individuals for the next breeding cycle on the basis of the phenotypic values observed for several traits of each candidate individual (Bänziger et al., 2000; Cerón-Rojas et al., 2006). Backcrossing is the easiest and most effective way of producing commercial cultivars with a secondary trait in inbreeding species. Apart from its widespread use in conventional breeding, it is also commonly employed when selection is based on molecular markers, and it is thus known as marker-assisted backcross selection (Ribaut and Ragot, 2007).

IV. MOLECULAR BREEDING: PANACEA OR MIRAGE?

In what follows, we will discuss the importance of new technological developments derived from molecular biology for plant breeding purposes. Due to space limitation, only two main aspects will be considered: the use of molecular markers as a tool for selection, and transformation (genetic modification).

A. Molecular Markers

In the last two decades molecular markers have proved to be useful for genome characterization and breeding. DNA fingerprinting has become a practical tool for plant genotyping while DNA markers can be used to screen large numbers of entries for a particular trait with improved efficiency and effectiveness, thus assisting the conventional breeding process. Once a link between a molecular marker and the characteristic to be selected has been established, screening can be done in the laboratory, thereby removing the influence of environmental conditions during the selection, a major constraint in the conventional breeding of traits influenced by drought. To date, many QTLs have been identified and mapped. Thus, for example, Diab et al. (2008) have recently identified QTLs for canopy temperature, photosynthesis-related traits and water status index, including differentially expressed sequences and candidate
Differentially expressed sequence tags in durum wheat that co-segregate with canopy temperature depression, grain carbon isotope discrimination, and chlorophyll content in several environments. Extracted from Diab et al. (2008).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Locus</th>
<th>Gene product</th>
<th>Chr</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canopy temperature depression</td>
<td>AF519805</td>
<td>Protein kinase</td>
<td>2A</td>
<td>Late planting</td>
</tr>
<tr>
<td></td>
<td>BM815946</td>
<td>Unknown</td>
<td>4B</td>
<td>Late planting</td>
</tr>
<tr>
<td></td>
<td>BM816306</td>
<td>Oxalate oxidase</td>
<td>4B</td>
<td>Late planting</td>
</tr>
<tr>
<td></td>
<td>BM816414</td>
<td>Early flowering protein 1</td>
<td>5B</td>
<td>Rain-fed</td>
</tr>
<tr>
<td></td>
<td>D13042</td>
<td>Protein kinase</td>
<td>6B</td>
<td>Late planting</td>
</tr>
<tr>
<td></td>
<td>BM816648</td>
<td>Arginine decarboxylase 2</td>
<td>7B</td>
<td>Late planting</td>
</tr>
<tr>
<td>Carbon isotope discrimination</td>
<td>BQ740214</td>
<td>Unknown</td>
<td>4B</td>
<td>Rain-fed</td>
</tr>
<tr>
<td></td>
<td>BM816121</td>
<td>Stearoyl-CoA desaturase</td>
<td>4B</td>
<td>Rain-fed</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>BM816370</td>
<td>Alcohol dehydrogenase</td>
<td>2A</td>
<td>Irrigation</td>
</tr>
<tr>
<td></td>
<td>AF519805</td>
<td>Protein kinase</td>
<td>2A</td>
<td>Late planting</td>
</tr>
<tr>
<td></td>
<td>BM816640</td>
<td>Phosphoprotein phosphatase</td>
<td>4B</td>
<td>Irrigation</td>
</tr>
<tr>
<td></td>
<td>BM816474</td>
<td>Cathepsin B</td>
<td>5A</td>
<td>Irrigation</td>
</tr>
<tr>
<td></td>
<td>AP210723</td>
<td>Fructan fructosyltransferase</td>
<td>6A</td>
<td>Irrigation</td>
</tr>
<tr>
<td></td>
<td>BM816608</td>
<td>Glutathione oxidase</td>
<td>6B</td>
<td>Rain-fed</td>
</tr>
</tbody>
</table>

Although a high-density genetic map may be available, several critical issues must be addressed for an effective implementation of MAS:

- a close linkage between molecular markers and the gene or QTL of interest (Landjeva et al., 2007). Thus, when the markers are completely linked to the desired trait allele, they are called ‘functional markers or perfect markers’ (FMs, generated from gene sequence data), to be distinguished from the ‘random markers’ (RMs generated from an anonymous region of the genome) (Varshney et al., 2005).
- the validation of the marker-trait association across distinct genetic backgrounds (Sharp et al., 2001; Landgride and Chalmers, 2004),
- the availability of markers to analyse a huge number of plants in a time-adequate and cost-effective manner (Koebner and Summers, 2003; Landjeva et al., 2007).

Although a large number of molecular markers for genes of interest have been reported for example in wheat, few are close enough to the target genes to be efficient in MAS or the QTL peak is not positioned with enough accuracy. Moreover, several markers developed in a given genetic background are ineffective in others (Gupta et al., 2006; Snape et al., 2006). The main reasons for the delay in deploying MAS in wheat breeding are: i) the high cost of its application (Bonnett et al., 2005; Kuchel et al., 2005; William et al., 2007); ii) inadequate quality of markers with respect to their predictive and/or diagnostic values; iii) the need for specific genetic materials (cytogenetic stocks, near isogenic lines, mutant lines) and facilities for large-scale phenotyping and dissection of complex quantitative traits (Landjeva et al., 2007); and iv) the unwillingness of wheat researchers to share information and germplasm (William et al., 2007).

A critical review on the identification and application of molecular markers in plant breeding can be found in Landgride and Chalmers (2004). One of the criticisms directed at studies performed to detect QTLs associated with specific traits is that the agronomic value of the parental lines of the mapping populations is usually poor, as they are chosen mainly on the basis of their divergence for the target traits, or the populations are readily available. Thus, when the QTL allele of the best parental line is introgressed into the best performing cultivar, its agronomic value often declines (Tuberosa and Salvi, 2006; Collins et al., 2008). Moreover, the methods used for QTL analysis and the size of the mapping populations are usually inappropriate for an efficient and precise detection of epistatic QTLs (Tuberosa and Salvi, 2006).

A number of genes have been characterized and tagged with molecular markers; however, some of these markers are based on RFLPs, which may be unfeasible for large-scale MAS given their high cost and the low level of polymorphisms that they can detect. Most of the PCR-based markers are also impractical as the level of polymorphism detected by some of them is insufficient and most of the marker types are not amenable for automation.

Given their abundance, high informativeness, appropriateness for automation and balanced cost, microsatellites (SSR) are a practical option for MAS (Landjeva et al., 2007). The development of new DNA extraction protocols and high-throughput genotyping equipment has resulted in a substantial reduction of the cost of MAS (Dubcovsky, 2004). It is now
possible to run molecular markers through commercial labs at prices unthinkable only a few years ago (e.g., 5000 AFLP in barley for less than 30 US$). The assay cost including DNA extraction, PCR amplification and gel-based separation has been estimated at $1.09 (Australian) in Adelaide and $1 US at CIMMYT (William et al., 2007). However, while the cost per assay has fallen dramatically, the cost of running an advanced molecular marker lab is rising because of the need for sophisticated DNA analysis, detection systems and robotics. A new generation of molecular markers, requiring the detection of single nucleotide polymorphisms (SNP), promises high-throughput assays of entire breeding populations at relatively low cost, along with the potential for high levels of multiplexing (Dubcovsky, 2004). Applications based on SNP marker systems in wheat are several years away, while for maize and rice robust SNP-based assay platforms are already available (William et al., 2007).

Molecular markers could be particularly useful for introgressing genes whose expression is highly affected by the environment, traits of complex inheritance, traits of economic relevance in cases where biological assays are unreliable and/or not cost effective, genes for disease-resistance, as well as for gene pyramiding. An additional advantage of the introduction of MAS into breeding programmes is that multiple traits (e.g., disease-resistance and grain quality) can be manipulated by means of the same technology (Dubcovsky, 2004).

MAS has been used in a modest but increasing number of cases, and is most likely to be useful when genetic variability is obscure, phenotypes are difficult or expensive to evaluate, or where detectable variation is a result of complex interactions of many genes and/or gene products (Nelson et al., 2004). The greatest achievement of MAS has been to accelerate the transfer of one or a few donor genes into a recipient genotype in backcross selection (Baenziger et al., 2006).

To summarize, MAS could confer an advantage:

- When measuring the phenotype accurately is expensive or difficult (e.g., due to insufficient amount of grain, lack of disease pressure or recessive inheritance).
- When multiple genes conferring a similar phenotype are being combined.
- When there is a need to rapidly remove donor chromosome segments in a backcrossing program.

An additional positive aspect of MAS in comparison with genetic modification is that the genes identified reside at their natural chromosomal locations, thereby minimizing the possibility of gene silencing. Furthermore, from a pragmatic viewpoint, the fact that the resulting cultivars are not transgenic implies that they are not rejected by society (Dubcovsky, 2004).

1. Is MAS Already an Alternative for Complex Traits?

Although complex traits are the most difficult to handle during a breeding program, they are responsible for most breeding progress in critical features such as yield, yield stability, and adaptation (Goodman, 2004; Nelson et al., 2004). Breeding progress continues to increase yield, with additional gains made for disease resistance, maturity, and production efficiency. Virtually all gains have resulted from the modification of polygenic factors not readily handled by the molecular procedures currently available. The contribution of molecular genetics to routine breeding practices will be limited until methodological limitations are overcome (Goodman, 2004). Successful use of MAS requires FMs linked to traits of interest. Consequently, the application of MAS for inherited traits can be justified only when it replaces more expensive or tedious assays, or results in increased precision in the identification of desired genotypes (Cooper et al., 2004). Examples of ideal markers (in wheat) include genes for gluten strength, starch properties, hardness, vernalization genes, and the Lr genes for resistance to leaf rust (Dubcovsky, 2004). More recently 3B- and 5A-QTL alleles have been pyramided for fusarium head blight (FHB) resistance in wheat (Miedaner et al., 2006). Almost one decade ago markers linked with genes Sr38/Lr37/Yr17 and Sr39 had already been implemented by the National Cereal Rust Control Program at the University of Sydney (Australia) to accumulate several rust resistance genes in wheat lines (Eagles et al., 2001). A QTL associated to cereal cyst nematode resistance has been identified on chromosome 1B of wheat (Williams et al., 2006). QTL associated with dough strength and loaf volume have been identified on chromosome 2A and a significant QTL associated with loaf volume and crumb quality has been identified on chromosome 3A of bread wheat (Kuchel et al., 2006). A candidate gene controlling aluminium tolerance in barley has been mapped on the long arm of chromosome 4H of barley (Wang et al., 2007). A Russian wheat aphid resistance gene Dn7 has been identified on chromosome 1RS/1BL translocation (Lapitan et al., 2007). Studies to identify genes related to morphophysiological traits have been published recently. Kuraparthty et al. (2007, 2008) mapped the tiller inhibition (tin3) gene that influences shoot architecture in chromosome 3A of Triticum monococcum. In addition, a number of significant QTL have recently been identified for coleoptile growth in bread wheat, many of them mapping directly to the Rht-B1 or Rht-D1 dwarfing gene loci (Rebetzke et al., 2007). A 192-bp allele at the microsatellite marker GwM261 locus has been proposed for the identification of the Rht8 dwarfing allele in wheat cultivars derived from the Japanese landrace ‘Akakomugi’ or a Nazareno Strampelli wheat ancestor (Ellis et al., 2007).

However, as pointed out above the most relevant traits (yield, stress adaptation, etc.) are governed by multiple genes, each producing a relatively small individual effect. These ‘quantitative traits’ are the most difficult to study and improve. Markers to be used must be robust and reliable. MAS for these traits using anonymous QTL-associated markers is more challenging than initially projected because of the imprecise localization of QTL and inconsistent QTL expression (Slafer et al., 2005). Thus, many QTL indicated in mapping population studies are cross-specific, subject to GE interaction effects or illusory (Araus et
al., 2003a; Slafer et al., 2005). Other reasons that have limited
the use of markers in practical plant breeding are the com-
plexity of the genomes of the graminaceous crops, its rapid
evolution and its heterogeneity, even within species (Sorrels,
2007), the lack of confidence in published information (Kuchel
et al., 2003) and other aspects mentioned before (William et al.,
2007). However, a QTL on chromosome 7AL, expressed mainly
under stress conditions, has been found in bread wheat and is
associated with differences in grains per ear and biomass (Quar-
rrie et al., 2005, 2006). A QTL for flag leaf senescence under
drought-stress was detected on chromosome 2B of bread wheat
(Verna et al., 2004), while five candidate genes and ten differ-
etially expressed sequences have been associated with QTLs
for drought tolerance of barley seedlings under field conditions
(Diab et al., 2004). Moreover, potential chromosome segments
for use in MAS to improve yield and yield components have
recently been identified in spring wheat (Cuthbert et al., 2008).
Nevertheless, many of these results still need to be validated.
Collins et al. (2008) report a list (Table 1 in their paper) of
putative and cloned genes relating to stress tolerance loci, but
(perhaps not surprisingly) for stresses other than drought.

QTL mapping ultimately aims to identify multiple QTLs for
transfer into more productive breeding lines. However, MAS
is not effective when QTLs exceed three or four, as the popu-
lation sizes required for MAS increase rapidly as more QTLs
are included. For example, measuring the adaptation to drought
environments in terms of grain yield of a large number of geno-
diversity in collections of diverse germplasm (Remington et al.,
2003), the lack of confidence in published information (Kuchel
et al., 2003) and other aspects mentioned before (William et al.,
2007). The QTLs associated with earliness per se have been
molecular markers in breeding programs calls for the integration of a range of complementary
disciplines and multidisciplinary teams (Reece and Haribau,
2007).

In summary, cautious optimism has been voiced about the
use of MAS for complex traits. Although molecular markers
have been successfully associated with QTLs, these associations
have had very limited practicality in plant breeding programs.
Long-term efforts are required to examine the nature of complex
traits at the molecular level before their selection by molecular
markers can be fully realized.

2. MAS and Phenology

MAS may be pertinent in a number of circumstances: for ex-
ample for manipulating phenology when breeding for adaptation
to Mediterranean rain-fed environments. The main genetic fac-
tors regulating time to heading in wheat have been identified
and can be divided into three main groups: 1) photoperiod sen-
sitivity (Ppd genes); 2) vernalization requirement (Vrn genes);
and 3) intrinsic earliness (Eps genes). Within the three types of
factors, Hanoq et al. (2004) found up to 13 significant QTLs in
bread wheat. Given the synteny between the genomes of several
cereal species, it is considered that there are about 25 loci con-
trolling the duration of plant cycle (including Vrn, Ppd and Eps),
which remain to be mapped in wheat (Snape et al., 2001). The
genomes of the three systems regulating the flowering date have
pleiotropic effects, as shown in numerous studies. Three major
loci for photoperiod sensitivity have been mapped in wheat: Ppd-
A1 and Ppd-B1, located in the short arm of the chromosomes
2A and 2B respectively (Snape et al., 1996), with dominant
alleles conferring low sensitivity to photoperiod. Although the
biochemical process by which the photoperiod influences floral
initiation remains to be elucidated, there seems involved two
forms of a certain photo-reversible phytochrome, which con-
verts from one form to the other during the day/night cycles
and interacts with the circadian rhythms (Thomas, 1993), for
the synthesis of a product which would promote the flower-
ing and would be translocated to the apex (Boyd et al., 2003).
Detection of Ppd genes by means of microsatellites is under
development and to date there are markers for Ppd-B1 (Mohler
et al., 2004; Goncharov and Watanabe, 2005). Recently, genes
of response to photoperiod have been cloned in barley (Turner
et al., 2005). The QTLs associated with earliness per se have been
located in chromosomes 2B (Scarth and Law, 1983), 3A, 4A,
4B, 6B (Hoogendoorn, 1985), 7B (Sourdille et al., 2000) and 5B
...
(Tóth et al., 2003). Low response to vernalization requirement or absence of this requirement is basically under the control of the dominant alleles Vrn-A1, Vrn-B1 and Vrn-B4, located in the chromosomes 5A, 5B (Worland, 1996) and 7B (Snape et al., 2001), respectively. An exact molecular marker is available for Vrn-A1 (Sherman et al., 2004), while for Vrn-B1 and Vrn-B4 this kind of marker is expected to be found in the near future (Van Beem et al., 2005). Genes of response to vernalization have been cloned in common wheat (Yan et al., 2003). In barley, variation in vernalization requirement is determined by the Vrn-H1, Vrn-H2 and Vrn-H3 loci, but the range of allelic variation is largely unexplored (Cockram et al., 2003). Recently, multiple genes or QTLs affecting flowering (phase transition, photoperiod and vernalization), inflorescence architecture and grain development have been mapped and cloned, and gene expression research has provided new information about the nature of complex genetic networks involved in the expression of these traits (Dwivedi et al., 2008). In addition, MAS may facilitate the identification of diverse cytoplasmic male sterility (CMS) in hybrid breeding and some Rf (fertility restorer) genes cloned in maize, rice and sorghum (Dwivedi et al., 2008).

B. Widening the Genetic Basis: The Role of Biotechnology

A germplasm strategy is also required for breeding for yield potential, and even more so for stress adaptation. Most breeders focus only on the elite gene pool, which reflects decades of crossing, selection and recombination of modern breeding, in addition to thousands of years of crossing and recombination in the primary gene pool of wheat (see Araus et al., 2007b, for wheat). Apart from disease-resistance genes, the primary gene pool usually has a surplus of variation for yield and performance under drought and for secondary traits (R. Richards, personal communication). However, apart from the limitations inherent to conventional breeding practices discussed above, difficulty in introducing desired traits from distant related species has been an additional factor. To overcome this limitation, more emphasis should be given to the use of new genetic variability, particularly through the genetic development of new parents for crosses, thereby introducing desired traits into the gene pool after a period of pre-breeding activity, which may involve effective pyramiding and recurrent selection. In this context, wider crosses (involving several species or even genera) and mutagenesis would be appropriate if the introduction of broad genetic variability is envisaged. Not only do wild ancestors usually show 75% more variation than the derived domestic crop (Schaal, 2002), they also show traits for survival and adaptation to drought conditions. Alleles for better performance to drought under field conditions have been found in wild barley (Hordeum spontaneum), the closest relative of cultivated barley (H. vulgare) (Baum et al., 2003; Talamé et al., 2004) and exotic germplasm has considerable potential to improve drought adaptive mechanisms in wheat (Reynolds et al., 2007).

It is not within the scope of this review to discuss the array of techniques available to address the problems inherent to the introduction of this natural variability. The use of broad-based genetic diversity in breeding will benefit from MAS, which will help follow specific genes from unadapted relatives and/or distinct plant species as they are bred into elite varieties (Dubcovsky, 2004). However, as the number of loci selected increases, particularly if the donor parent is a related wild species, linked deleterious alleles may become a problem (Varshney et al., 2005).

As a step toward increasing genetic variability and thus adaptation, major drought-related QTLs can be considered for cloning (Tuberosa and Salvi, 2006) in order to manipulate the target trait more directly by plant transformation. The combination of QTL maps with maps enriched with genes putatively associated with the target trait or with fully annotated genomic sequences (functional or genetic maps) may help to identify candidate genes for the QTLs and to elucidate their functional role (Varshney et al., 2005). The role of candidate genes can also be ascertained by the EcoTILLING technique (Till et al., 2007).

C. Plant Transformation

Genetic transformation allows the introduction of characteristics that are apparently absent from a particular gene pool. With two decades behind it, this technique has provided the first transgenic commercial crops, which were introduced more than 10 years ago (1997). Several potential avenues to improve drought adaptation through transformation are described below. Beside all the methodological limitations discussed later, almost none of these avenues involve transforming for any of the secondary traits described above in this review, but frequently for traits related to cell dehydration. While for most of these examples there is no evidence that this could be a useful approach increasing either water use, WUE and/or HI, we have included them to illustrate the current situation and the limitations it faces.

1. Increasing the Amount of Cell Protective Substances

The sugar alcohol mannitol can act as a compatible solute by decreasing the osmotic potential in the cytoplasm. Wheat plants do not have the capacity to synthesize mannitol. However, when the bacterial gene encoding the key enzyme to allow mannitol synthesis is inserted into wheat plants, with expression driven constitutively, it causes severe stunting of plants and sterility (Abebe et al., 2003). This example involves manipulation of processes involved in some of the responses to severe stress, related more to processes of damage limitation (i.e., that is, tolerance to cell dehydration), rather than damage prevention (i.e., prevention of cell dehydration).

Phosphatidylinositol-specific phospholipase C (PI-PLC) plays a crucial role in a variety of physiological processes in plants, including drought tolerance. Transgenic corn expressing ZmPLC1 transgenes (which encode a PI-PLC and upregulate the expression in corn roots under dehydration conditions) exhibit
improved water status, less cell damage and higher grain yield than the wild-type under pot conditions (Wang et al., 2008). Improved drought tolerance has been also reported in transgenic corn plants expressing a glutamate dehydrogenase gene (Lightfoot et al., 2007) as well as in transgenic rice plants (Figure 6) expressing the Datura stramonium adc gene (Capell et al., 2004). However, while this phenotype, which is not uncommon in transgenic plants, is usually attributed to slower water use and therefore better water relations, the stunted nature of these transgenic plants (Figure 6) strongly suggests that it is likely to be yield negative. Carotenoids protect plants from photooxidative stress. In recent years, metabolic engineering efforts have also been undertaken with the aim to improve plant resistance to abiotic stress through overproduction of carotenoids (Giuliano et al., 2008).

2. Stress Inducible Regulatory Genes

Since regulatory genes are critical components of plant response to environmental stress, considerable research effort has addressed the search for DREB-like (drought responsible element) genes in cereal crops such as bread wheat (Pellegrineschi et al., 2004), rice (Qin et al., 2007b; Nakashima et al., 2007), durum wheat (Latini et al., 2007), corn (Qin et al., 2007a; Al-Abed et al., 2007) and pearl millet (Agarwal et al., 2007), as well as in soybean (Chen et al., 2007). Thus stress inducible regulatory genes that encode proteins which act as transcription factors (e.g., DREB-type) have been validated through overexpression in transgenic plants, which show stress-tolerant phenotypes (Yamaguchi-Shinozaki and Shinozaki, 2006). In spite the fact that most of these studies have been performed in phenotyping conditions that are far from real, results suggest a consistent presence and role of DREB-like genes in abiotic stress responses across species. This finding suggests that transcription factors are ubiquitous in higher plants, and reinforces the value of using plant model systems for identifying useful genes that can be implemented in the germplasm enhancement of staple foods (Chen and Zhu, 2004; Ortiz et al., 2007). Encouraged by these findings, researchers are also searching for other families of transcription factors in crop species to enhance performance in stressful environments [e.g., NAM, ATAF and CUC (NAC), Hu et al., 2006]. Particularly attractive is the single, dominant nature of the transgene, which makes the transfer and maintenance of this kind of system in any cultivar much easier than conventional sources based on polygenes. However, transgenic plants overexpressing this kind of transcription factors often exhibit growth retardation and low reproductive yields in the absence of stress (Ito et al., 2006). On the other hand, evaluation under closer-to-real field conditions has concluded that DREB1A in wheat delays development in transgenic plants but does not result in greater biomass accumulation or better grain yields than the control under water stress (Ortiz et al., 2007).

3. Suppression of Drought-Induced Senescence

Senescence is a type of cell death program that can be inappropriately activated during drought. It has been hypothesized that drought tolerance can be enhanced by delaying drought-induced leaf senescence. In cereals, ethylene has been associated with decreases in kernel number (Hays et al., 2007), in addition to reduced plant growth. In fact, in recent years, 1-methylocyclopropene (under the trade mark of Invinsa) has been commercialized, for cereals and other major crops. This compound binds to ethylene receptor sites in plants, thereby reducing the negative effects of ethylene. Recently, Rivero et al. (2007) generated transgenic tobacco plants expressing an isopentenyltransferase gene driven by a stress- and maturation-induced promoter. The suppression of drought-induced leaf senescence resulted in outstanding drought tolerance in pot plants as shown by, among other responses, vigorous growth after withdrawal of water, which killed the control plants.

4. Genes Affecting WUE

Masle et al. (2005) have reported the isolation of a gene that regulates transpiration efficiency, ERECTA. A putative leucine-rich repeat receptor-like kinase (LRR-RLK) known for its effects on florescence development, ERECTA is a major contributor to a locus for Δ13C on Arabidopsis chromosome 2. Mechanisms include, but are not limited to, effects on stomatal density, epidermal cell expansion, mesophyll cell proliferation and cell–cell contact. More recently, Karaba et al. (2007) described that expression of the Arabidopsis HARDY (HRD) gene in rice pot plants improves water use efficiency by enhancing photosynthetic assimilation and reducing transpiration. These drought-tolerant, low-water-consuming rice plants exhibited increased shoot biomass under well watered conditions and an adaptive increase in root biomass under drought stress. HRD, an AP2/ERF-like transcription factor, identified by a gain-of-function Arabidopsis mutant hrld-D with roots showing enhanced strength, branching, and cortical cells, exhibited drought resistance and salt tolerance, accompanied by enhanced expression of genes associated with abiotic stress. HRD overexpression in Arabidopsis produced thicker leaves with more chloroplast-bearing mesophyll cells, and in rice, leaves registered an increase in biomass and bundle sheath cells, which probably contributed to enhanced photosynthesis assimilation and efficiency. However, the above approaches aimed at increasing WUE involve changes in leaf anatomy (e.g., thicker and more densely packed mesophyll) which may be negatively associated with leaf expansion and thus yield.

5. Reproductive Traits

Engineering cytoplasmic male sterility for hybrid breeding may be a way to increase yield potential and adaptation to adverse conditions (Dwivedi et al., 2008). Thus, several mechanisms have been reported for creating de novo CMS trait in cereals. However, severe phenotypic alterations, as a result of interference with plant metabolism and development, on top of
other concerns have precluded their use in agriculture (Goetz et al., 2001; Pelletier and Budar, 2007).

6. Limitations of Current Approaches

Despite the large number of reviews published outlining the possibilities offered by transformation for inducing stress resistance, to date no conclusive results have been attained due to fundamental errors in the design of experiments and/or incomplete knowledge of the basic physiology of abiotic stress tolerance (Flowers, 2004; Tester and Bacic, 2005).

Most of these recent studies referred to above addressed dehydration tolerance in plants grown in pots in growth chambers or in greenhouses. These conditions do not provide a true reflection of those in the field and therefore do not provide an effective prescreening step for economic drought tolerance (Araus et al., 2003a). Stress tolerance and avoidance are part of the stress resistance strategies deployed by plants. However, while the former may offer a significant ecological advantage by preventing plant death, its role in agriculture and breeding for higher yields is irrelevant or even negative, thereby limiting potential yield (Araus et al., 2003a). Breeding for tolerance traits is only worthwhile when yield stability in highly drought-prone environments can be ensured; however, breeding for these traits inevitably implies a trade-off at the expense of yield potential.

Similarly, a more preemptive strategy to tackle abiotic stress could imply processes involved in the early detection of and response to stress. This approach also has the benefit of potentially facilitating the coordinated response to a stress factor (Tester and Bacic, 2005) as well as the possibility of generating plants with catch-all alterations involving the signalling pathways and their early responses, which are common to several abiotic stress factors (Seki et al., 2003). This approach could be followed by altering expression levels of high-level transcription factors involved in the early responses to stress (Dubouzet et al., 2003). Unfortunately, as discussed above, many of the alterations produced through transformation appear to be accompanied by a growth and/or yield penalty in conditions of reduced stress. A strategy to avoid this outcome would be to use an inducible promoter to drive the expression of these genes in response to stress (Kasuga et al., 1999; Su and Wu, 2004).
Nevertheless, to date, and after more than 10 years of research, there has not been enough progress and the prospects for success do not seem to be very encouraging. However, some recent studies look more promising. One of them reports that expression of certain CCAAT-binding transcription factors in plants confers significant tolerance to drought, thereby resulting in increased yield (Nelson et al., 2007). Under water-limited conditions, transgenic maize plants with increased expression of ZmNF-YB2 [an orthologous maize transcription factor from the nuclear factor Y(NF-Y) family] show tolerance to drought, as observed from the field responses of a number of stress-related traits, including chlorophyll content, stomatal conductance, leaf temperature, reduced wilting and maintenance of photosynthesis. In a different study, expression of related cold shock proteins from different bacteria, which may work as RNA chaperons, also conferred stress tolerance in maize (Castiglioni et al., 2008). More importantly, preliminary results from these two studies indicate yield increases under moderate (but not severe) water stress under field conditions (Nelson et al., 2007; Castiglioni et al., 2008). Interestingly, the authors of both studies are employees of a private company (Monsanto) which recently have started a collaborative project sponsored by the Bill and Melinda Gates Foundation aimed to improve drought adaptation of maize for Africa. These two studies illustrate how crucial it is to ensure accurate and precise testing of plant performance under suitable water deficit profiles and other stress conditions, which resemble the real conditions the plant may experience in the field as much as possible. It is essential to rapidly move on to examine the impact of transgenics on plant growth and grain yield in replicated field experiments under various appropriate drought stress profiles and also to determine the stages of growth and the genetic backgrounds in which these genes exert their most significant effect. Moreover, for a transgenic approach to be successful under drought (or any other approach for that matter, it must be demonstrated that the gene or the QTL increases water use, water use efficiency and/or HI in real field conditions.

D. Prospects of Molecular Breeding: Is a New Green Revolution Dawning?

It has been proposed that recent progress in the field of plant molecular biology and plant genomics has the potential to trigger a new Green Revolution. However, these discoveries should be implemented in new cultivars in order to realize this potential (Dubcovsky, 2004). Thus, examples of successful integration of MAS into applied breeding are rare, especially for screening for drought tolerance (Mackill et al., 2003) and the same can be said for plant transformation. Moreover, like the introduction of many other technologies, the popularity of MAS will depend on the economic benefits it confers over conventional selection methods (Dekkers and Hospital, 2002). To date, in only a few cases has a rigorous cost-benefit analysis been presented (e.g., Dreher et al., 2003).

Molecular genetics will not make a great contribution to routine breeding practices until molecular techniques allow the manipulation of complex traits. Overcoming the handling of these traits is not likely to occur until more interdisciplinary efforts are made that combine the expertise of molecular biologists with that of conventional breeders and crop physiologists (Araus et al., 2003a; Slafer, 2003; Sinclair et al., 2004; Slafer et al., 2005; Wollenweber et al., 2005; Tuberosa et al., 2007a, b).

At this stage, little is known about interactions between genes, the so-called “gene-networks.” Accurate gene-to-phenotype models will depend on improved understanding of these intergenic interactions. Global gene expression studies will be valuable in addressing issues that have been intractable until now. For this purpose, in addition, a greater understanding of whole plant physiology and yield, and the functional interactions and properties of genes (Cooper et al., 2004) will be required.

A highly relevant outcome of the new genomic revolution is that most information is publicly available. Therefore, competitiveness will not be determined by access to information, but rather by the speed at which technologies are introduced into breeding programs. Thus, public breeding programs with the expertise to successfully apply MAS technologies face both a challenge and a fantastic opportunity (Dubcovsky, 2004). In addition, the extent to which genetic associations revealed in model systems will be applicable to economically relevant plants is unclear (Havey, 2004). In fact, it is difficult to publish, and therefore assess, the opposite scenario in which genes identified in model systems are not associated with similar traits in these crops (Havey, 2004).

Plant breeding is unlikely to be radically altered by genetic engineering despite progress in genomics. New traits will ultimately be added to today’s breeding goals, but most are likely to require several decades of development (Goodman, 2004). Many consider that the future of plant breeding lies in genomics, relying on claims that molecular genetics has revolutionized the time frame for product development (c.f. Bingham, 1983: “Seldom has it been pointed out that it is going to take as long to breed a molecular engineering gene into a successful cultivar as it takes for a natural gene”) or that it will provide simple solutions to complex problems (Theil, 2001). These claims are often made with little knowledge of the problems encountered when selecting and testing germplasm, GE interactions, or even epistasis (Araus et al., 2003a; Goodman, 2004) in addition to regulatory requirements. These claims are often accepted by management teams that employ breeders who are fully aware that such rapid solutions will not reach farmers’ fields for well over a decade (Goodman, 2004). It is untrue that a new transgenic cultivar can be routinely developed, tested, and deployed within a decade (Goodman and Carson, 2000) and unlikely that the minimal 15-year lag time between gene discovery and seed sales to farmers can be reduced. Rather, political decisions are likely to increase this lag, especially in Europe. Thus new developments in molecular genetics must promise, for example,
a 20 to 30% improvement in yield. However, in practical terms this promise implies no more than a 2% increase in yield per year (Goodman, 2004). New genetic and genomic tools will enhance, but not substitute, the conventional breeding evaluation process (Varshney et al., 2005; Varshney and Tuberosa, 2007).

Finally, the economic value of agriculture to a growing world population must not be underestimated. Agriculture is a priority for the overpopulated, young and hungry nations that make up most of the world. The imperative to modernize agricultural research is clear (Martienssen, 2004). Thus genomics can provide a road map for the next generation of agricultural and breeding research, but it cannot replace the geneticist or the plant breeder (Martienssen, 2004). Moreover, it is generally expected that new genomic applications will become available as the price of technology continues to drop and as a greater understanding of plant genomes leads to new insights into their manipulation.

E. Research Priorities: Plant Breeding versus Genomics

Public breeding programs are currently facing tremendous difficulties (Knight, 2003). The example of the United States illustrates how policy makers follow the whims of fashion. The limited investment by federal funding agencies into public wheat breeding programs (i.e., practical application) is difficult to understand in the light of the large investment made by the same funding agencies into wheat molecular genetics and wheat genomics (Dubcovsky, 2004). As Goodman (2004), states “if better crop performance such as yield is the ultimate goal, in forty years from now, traditional plant breeding methods would have been the best investment for today’s dollar.” For developing countries, sensibly prioritizing funding allocation is even more crucial. Because needs are urgent, relatively less systematic breeding has been done and small investments in breeding can provide rapid gains in even a few years. It is a social concern, therefore, if international research centers, whose mandate is to assist the poorest countries, invest in genomics at the expense of plant breeding (Symposium discussion, 2004). In a few decades, entire genome sequences may be available for most economically relevant crops. However, sequences per se do not improve yield per hectare or resistance to major crop pests (Symposium discussion, 2004). To date, genetic improvements in domesticated crops have used empirically obtained phenotypic data, pedigree information and selection. Prediction methods based on data routinely collected by plant breeding programs are far from exhausted.

1. Training and Funding for Genomics-Assisted Plant Breeding

The integration of genomics and breeding relies on a connection between laboratory and field research. Field observation and breeding experience may be the only way to identify the right issues, even with a plethora of genomic information at hand (Symposium discussion, 2004). Knowledge of basic agronomy and biology, population genetics, statistics, and experimental design are critical for analyzing field data; incorporating data provided by molecular markers and genomics; and then relating all this to plant phenotype. Students involved in plant breeding are required to study statistics, plant physiology, molecular biology, and other disciplines; however, it is difficult to find a reciprocal situation: a molecular genetics student with enough statistical training to study quantitative plant breeding.

2. Plant Comparative Genomics and Its Contribution to ‘Orphan’ Crops

In addition to a small number of well-known major global crops such as rice, wheat and maize, many other crops have a significant relevance regionally or locally for nutrition and income in poor regions, including crops such as plantain and bananas; root and tuber crops such as cassava, sweet potato, and yam; millets such as pearl millet, finger millet and foxtail millet; legumes such as cowpeas, groundnut and Bambara groundnut; and tree crops. Moreover, indigenous crops such as teff, quinoa, and many types of vegetables are critical for food security and nutrition at regional and local scales (Nelson et al., 2004). There is a large discrepancy between the potential role of these crops in improving food security and livelihoods, and the low levels of investments they have received. One reason for this may be that research on orphan crops appears to have relatively low returns when measured by gross economic and welfare impact, a view that stems largely from inadequate measurement. The use of alternative metrics, e.g., human capital development, cropping system stability, the promotion of genetic diversity, all of which increase the capacity of agricultural systems to withstand major biotic, abiotic, policy- or economic-induced shock, provides even greater incentives to fund orphan crop germplasm improvement (Conway, 1997; Nelson et al., 2004).

Genes involved in many biochemical pathways and processes are similar across the plant kingdom. Functions, such as gene regulation, general metabolism, nutrient acquisition, disease resistance, general defense, flowering time, and flower development, are largely conserved across taxa (Nelson et al., 2004). Comparative mapping studies reveal that gene order is conserved for chromosomal segments among grass species (Bennetzen and Freeling 1998; Gale and Devos 1998; Devos and Gale 2000). Though weaker, chromosomal colinearity is detectable between monocots and dicots (Bennetzen 2000; Goff et al., 2002; Van Buuren et al., 2002).

Rice is the ideal cereal for molecular work because of its small genome (30 and more than 10 times smaller than the genomes of bread wheat and barley, respectively). The high degree of synteny in the genomes of major cereals make the results obtained in model species transferable to closely related genomes of other cereals. Moreover, advances in crop genomics provide an opportunity for efficient transfer of information systems from model species and major crops to orphan crops (Naylor et al., 2004). As a result, relatively small investments in the transfer of advanced science from major crops to larger sets of orphan crops may potentially result in disproportionately high payoffs in terms of crop production, yield stability, and food.
FIG. 7. QTL likelihood curves of the LOD score of grain yield under stress for Chromosome 12. The SSR marker locations are listed on the Y axis. The black horizontal line indicates the significance threshold of LOD score 13.8 to detect putative QTLs. The vertical dotted lines indicate the position of qtl12.1 (after Bernier et al., 2007).

security in less developed countries (Nelson et al., 2004). It is important to emphasize that investment in genomics for a given species is only likely to be useful when accompanied by a strong conventional breeding effort, and unfortunately, this prerequisite is too often not fulfilled (Nelson et al., 2004; Collins et al., 2008).

Success in the improvement of orphan crops will depend on investment but also on the appropriate integration of knowledge gained (Naylor et al., 2004). Integration starts by linking advanced science with plant breeding and seed programs. While this link is determinant, so too is that between plant breeding, farmers, delivery systems, and consumers. The successful application of genomics depends on transferring advances in science to downstream delivery efforts. For the poorest countries, this integration may take years to achieve (Nelson et al., 2004).

V. THE WAY AHEAD

Breeding is just half of the equation for more productive and sustainable crops, the other half is agronomical management. The progress that has been achieved for grain yield has resulted from combining improved genetics with appropriate crop management strategies (Slafer and Andrade, 1991; Cooper et al., 2004).

The wide range of new developments in fields such as genomics, molecular biology, physiology, double haploids, and statistical analysis provides an opportunity for breeders to make even greater contributions in the future. However, to ensure that future breeding investments also have high returns, breeding programs must assess these new technologies to determine the suitability of their introduction (Brennan and Martin, 2007). Thus those technologies that have low capital investment requirements, such as physiological selection and improved statistical analysis, are likely to be more widely adopted in the early years than more capital-intensive or higher-cost technologies. Ultimately, technologies such as MAS will be incorporated into most programs, at least for quality and pest- and disease-related traits. At present, many seed companies are already routinely and massively using MAS for pest-, disease- and quality-related traits. In fact, commercial breeding programs have reported twice the rate of genetic gain through molecular breeding (which is wider than just using MAS) over phenotypic selection (Eathington, 2005; Crosbie et al., 2006; Ragot and Lee, 2007). Although specific information on these successes of molecular breeding is limited, the first maize hybrids developed by Monsanto through this approach entered the U.S. commercial portfolio in the cropping season of 2006 and it is estimated that by 2010, over 12% of the commercial crop in the U.S. will be derived from molecular breeding (Fraley, 2006).

Nevertheless, the use of MAS for improving complex traits remains a challenge for crop breeders (Sorrels, 2007), at least in the public sector. While the genetic dissection of major crop performance in drought-prone environments has greatly benefited from the use of DNA markers (Tuberosa and Salvi, 2006; Ribaut and Ragot, 2007; Maccaferri et al., 2008), it has not been implementation in real breeding. Thus QTLs are often
Features of QTLs for durum wheat grain yield based on the averaged data of 16 environments. Adapted from Maccaferri et al. (2008).

<table>
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<tr>
<th>Trait QTL</th>
<th>Flanking markers</th>
<th>CIM QTL analysis</th>
<th>MIM QTL analysis</th>
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<td>LOD Peak position</td>
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<td>(cM)</td>
<td>(%)</td>
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<tr>
<td>Grain</td>
<td>QYld.idw-2B † Xgwm1027-Xwmc361</td>
<td>8.9</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>QYld.idw-3B † Xbarc133-Xgwm493</td>
<td>10.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>QYld.idw-7B Xgwm569-Xbarc1005</td>
<td>3.0</td>
<td>0</td>
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<tr>
<td>Q idw-2B × Q idw-3B</td>
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<td>Multiple R²</td>
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QTLs for grain yield identified on the averaged data of 16 environments using the CIM (LOD > 2.5) and MIM analyses. For each QTL, the flanking markers, the peak LOD score, the chromosome position of the LOD peak, the R² value, the additive effect, and the (LOD-1) QTL supporting interval as from the CIM analysis are reported. For each QTL, the chromosome position of the LOD peak, the R² value, the additive effect as from the MIM analysis are also reported. The significant epistatic interactions between QTLs and the corresponding additive x additive effect as estimated in the MIM analysis are also indicated in italic.

† Additive effect for grain yield (q ha⁻¹) computed as half of the difference between the mean phenotypic values of the RILs homozygous for the Svevo and Kofa alleles [(Svevo – Kofa) / 2].

† QTLs influencing more than one trait in a range of environments.

The identification of major QTLs should be viewed as the first step of a longer process aimed at identifying and isolating the underlying molecular cause at the sequence level (i.e., polymorphism) of the functional variation revealed by QTL analysis. The cloning of the sequence underpinning the QTL will allow us to characterize and manipulate more precisely the available germplasm for the type of alleles present at the target QTL (Salvi and Tuberosa, 2005). One of the most common ways to validate the function of a structural or regulatory gene in the control of a physiological or an agronomic trait is to partially or totally inhibit its expression through a reverse genetics approach to further examine the impact of this inhibition on plant phenotype. This can be achieved using a variety of molecular tools such as antisense RNA, RNA interference (RNAi) and by screening mutants in targeting induced local lesions in genomes (TILLING) or insertion mutagenesis (Mutator transposons) populations (Tuberosa and Salvi, 2006). Among these approaches, TILLING is the most widely utilized. TILLING is a powerful reverse genetic technique that employs a mismatch-specific endonuclease to detect induced or natural DNA polymorphisms and allow for a genome-wide functional screening (McCallum et al., 2000; Stemple, 2004; Till et al., 2004; 2007). This facilitates the high-throughput screening for variation in the trait of interest including a knock-out mutation of the candidate gene of interest (Tuberosa and Salvi, 2006). For example a TILLING platform has been established in maize (see http://genome.purdue.edu/maizetilling/) and barley (Talamé et al., 2008; http://www.distagenomics.unibo.it/TILLMore/index.htm) that represents a valuable tool to validate the role of drought-related candidate genes (Tuberosa et al., 2007b). Moreover TILLING, like any other mutagenic approach, may skip the societal concerns of transgenics.
catch-all alterations involving the signalling pathways and early responses that are common to several abiotic stress factors such as heat, cold, salinity or water scarcity, has attracted research attention worldwide (Tester and Bacic, 2005). Nevertheless, the use of transgenics to provide enhanced grain yield under drought stress is still experimental in nature. Despite the increasing number of studies reporting QTLs for drought-related traits and yield under moisture scarcity, and the progress generated by new genomic platforms, sequencing and bioinformatics, the overall contribution of genomics-assisted breeding to the release of varieties with improved yield under water-limited conditions has so far been marginal (Tuberosa and Salvi, 2006). Several approaches may help to maximize the success of this strategy: optimizing both the transformation and screening methods and accounting for gene epistasis (with distinct genetic backgrounds and with different sources of drought tolerance) and genotype-by-environment interaction (Bhalla, 2006; Kirigwi, 2007; Ortiz et al., 2007).

There is a continuous need to integrate disciplines, such as structural genomics, transcriptomics, proteomics and metabolomics, with plant physiology and plant breeding (Varshney et al., 2005). Thus recent advances in transcriptomics, proteomics and metabolomics will allow researchers to address drought tolerance through an integrated approach based on the measurement of the concerted expression of thousands of genes and their products (Tuberosa and Salvi, 2006). A review of the recent progress in proteomics related to drought stress in major crops can be found in Salekdek and Komatsu (2007). The impact of metabolic engineering on breeding programs has been reviewed by Harrigan et al. (2007). In any case molecular markers will become less important as sequence information and proteomic and metabolite profiling increase.

Conventional breeding still offers an opportunity for significant and predictable incremental improvements in the drought tolerance of new maize cultivars (Bänziger and Araus, 2007; Reynolds and Tuberosa, 2008). For example, in this crop significant progress in grain yield under drought stress has been made through selection in multi-environment trials for component traits such as kernel set, rapid silk exertion, and reduced barrenness (Campos et al., 2004). Moreover, appropriate stress management may be more effective for selection than a conventional large multi-trial testing scheme. Thus Bänziger et al. (2006) reported that hybrids selected under managed stress using similar protocols have significantly out-yielded commercial hybrids in southern and eastern Africa, particularly under severe and moderate water-stress conditions.

As the InterDrought-II Organizing Committee (Araus et al. 2007a) conclude, ‘a huge gap remains between the molecular level science and the interpretation and application of this knowledge at the whole plant level in the field. If we are to advance practical solutions for drought-prone farming, there is increasing demand for cross-talk between the component disciplines which contribute to the understanding and amelioration of plant stress responses.’ In addition to the considerations described above, there remains one constant that will not change; namely, that breeding progress depends on accurate selection of rare genotypes that show new or improved attributes as a result of superior combinations of alleles at multiple loci, in the context of a target set of environments (Sorrels, 2007). This implies that precise phenotyping will remain one of the cornerstones of future breeding. This said, however, breeding progress may be jeopardized by restrictions on the exchange of materials and information (Sorrels, 2007).

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