

Chapter 7

Improvement of Drought Resistance in Crops: From Conventional Breeding to Genomic Selection

Anna Maria Mastrangelo*, Elisabetta Mazzucotelli*, Davide Guerra, Pasquale De Vita, and Luigi Cattivelli

Abstract Drought stress is the major factor limiting yield and yield stability of crops. To improve plant performances under drought conditions direct selection for yield over multiple locations has traditionally been employed. This approach is hampered by low heritability and high $G \times E$ interaction influenced by differences arising from soil heterogeneity and others environmental factors. The indirect selection using secondary traits has succeeded only in a few cases, due to problems with repeatability and lack of high-throughput phenotyping strategies. During last years, considerable efforts have been directed towards identifying physiological traits associated with yield and drought resistance. With the availability of whole genome sequences, physical maps, genetics and functional genomics tools for many crops, integrated approaches using molecular breeding and genetic engineering offer new opportunities for improving yield in drought prone conditions. The identification of the genetic bases of important physiological traits and the cloning of the genes sustaining yield in drought-prone environments will move the selection toward a “breeding by design” approach that will accumulate an increasing number of useful traits into elite genotypes that, in turn, will result in a reduction of the gap between yield potential and actual yield.

*These authors, in alphabetical order, contributed equally to the work.

A.M. Mastrangelo • P. De Vita
CRA – Cereal Research Centre, S.S. 16km 675, 71122 Foggia, Italy

E. Mazzucotelli • D. Guerra • L. Cattivelli (✉)
CRA – Genomics Research Centre, Via S. Protaso 302, 29017 Fiorenzuola d’Arda (PC), Italy
e-mail: luigi.cattivelli@entecra.it

7.1 The Complexity of Drought Environments and Plant Adaptation

Drought is by far the most important environmental stress in agriculture, causing important crop losses every year. Temperature increase and more erratic rains predicted as consequences of climate change could cause more frequent drought events. As irrigation is not considered a sustainable option to drought everywhere, losses of crop yield under water scarcity require a genetic solution. Furthermore increase in crop demand for food to feed the world population also mandates that future genetic gain in yield both in well watered and stress prone conditions need to be utilized. The development of elite cultivars for drought prone areas has been hampered by difficulties inherent to the complexity of drought environments and to the complexity of plant responses to drought.

A drought event can have different faces. A critical factor is the timing of the drought episode in relation to the crop developmental stage. Drought events are most harmful at flowering, when the reproductive process can be drastically affected by insufficient supply of photosynthates and changes in phytohormones. Furthermore, often drought is not the only stress event, the contemporary presence of different kinds of stresses, like diseases or high temperatures, mineral toxicities or poor nutrition, can exacerbate the effect of water scarcity. The International Maize and Wheat Improvement Center (CIMMYT) defined three main patterns of drought stress: grain-filling stress, pre-flowering stress, and continuous stress (Reynolds et al. 2005). Considering the wheat crop, the first pattern describes the typical water distribution profile of Mediterranean countries and Australian regions, where crop experiences water deficit during grain-filling, while average precipitation exceeds average pan evaporation prior to flowering. The second adverse scenario occurs in South America where drought stress is most likely to occur prior to wheat flowering. The third scenario is experienced by wheat grown after monsoonal rains, such as in regions of South Asia where irrigation water is unavailable, and in some Australian regions. In this context, there is no rainfall during the growth season, so the crop must survive on whatever moisture is stored in the soil profile from the summer rainfall. Nevertheless, unpredictability is the main characteristic of drought, for instance in the Mediterranean environment beside the typical “terminal drought”, early drought during vegetative growth and flowering as well as years with ample water availability during the whole cereal growing season can also occur.

A number of physiological studies have identified many traits whose presence/ expression is associated with plant adaptability to drought prone environments. They span a wide range of vital processes and/or pattern of organ development, some are constitutive, others activated upon exposure to stress. In the 1972, Levitt defined plant responses to stress according to three main categories. The escape strategy is based on the modulation of plant development timesheet on the climate trend to conclude the life cycle before the environmental adversities show up and it is distinct from features influencing resistance to drought. The latter is defined avoidance which is based on maintenance of plant water status through improved

water balance often by means of constitutive adaptations, the most important of which concerns root architecture and leaf morphology, in terms of increased water uptake by deeper roots and/or reduced water loss by increased leaf waxiness. Osmotic adjustment is also a typical physiological mechanism for drought avoidance. Dehydration tolerance involves biochemical mechanisms (e.g. accumulation of compatible solutes to preserve membrane integrity, water-soluble carbohydrates relocation, etc.) activated upon stress exposure to enable the cell to tolerate the negative effects caused by cellular dehydration. At whole plant level, biomass partitioning and other traits associated to plant reproductive biology enable the plants to mitigate the negative effects of drought on yield (Cattivelli et al. 2008).

From the yield point of view the plant behaviour towards limited water and energy resources can be summarized by Passioura's equation $Y = WE \times WUE \times HI$, where WE =Water Extracted from soil, WUE =Water Use Efficiency and HI =Harvest Index. According to this rule, the drought adapted ideotype is an opportunistic plant able to exploit all water resources in the most efficient manner and avoid the stress. It will perfectly balance the need of water of the yield-related physiological processes with the pattern of water supply. Such an ideotype will be characterised by high yield potential and high yield stability (minimum $G \times E$ interaction), ensuring high production both in stress free environment as well as under moderate drought.

The effectiveness of selection for drought related traits is not well defined, due to high $G \times E$ interaction and also there is no single response well correlated with yield under all drought situations. For example, small plant size, reduced leaf area, early maturity and prolonged stomatal closure leading to a reduced total seasonal evapotranspiration can improve plant survival conferring the ability to tolerate extreme stress. Even if this conservative and protective strategy can ensure a minimal yield in dry environments, it can result into reduced yield in stress-free and mild to moderate stress environments. Since rainfall patterns and evaporative demand over a crop cycle show geographic variation, traits conferring drought-adaptation in specific environments may differ. In general, a plant designed to cope with a specific type of drought may under-perform when stress conditions are different. For example, WUE (Water Use Efficiency) is a physiological trait often considered as an important determinant of yield under stress and even as a component of crop drought resistance. Indeed, Rebetzke et al. (2002) reported a successful breeding program for wheat in drought-prone environments, based on the carbon isotope discrimination (Δ) as a surrogate for WUE (Farquhar and Richards 1984). However the association between WUE and plant production looked very elusive for various crops under different growing conditions and depending on soil water availability (Condon et al. 2004). Therefore even if strong relationships between physiological traits associated to drought tolerance and yield are found, they can be limited to narrow environmental conditions within a given crop phenology (Blum 2009). The strong heterogeneous features of drought events can reduce or even overturn the positive effect also of traits associated to increased water availability. For example, root meristems capable of adjusting osmotically at given water potential and deeper roots to improve the extraction of additional moisture from the soil will positively

impact root mass and final yield. Nevertheless, if additional moisture is unavailable at deeper soil layers, a condition quite common in many drought-prone environments (e.g. the Mediterranean basin), growing larger/deeper roots will not be advantageous and might even negatively influence final yield due to excessive partitioning of photosynthates to the roots and the high metabolic cost required for sustaining the functions of a large root system.

7.2 Assessment of Drought Tolerance in Crops

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

7.3 Achievement of the Conventional Breeding in the Last Century

Despite yield in drought-prone environments has a low heritability due to a high $G \times E$ interaction, high intra-site variability and low genetic variation (Blum 1988), conventional breeding has obtained some positive achievements in the last century. Indeed, retrospective analyses show that a genetic gain from 10 to 50 kg ha⁻¹ year⁻¹ has been recorded for cereals and legumes over the last Century in all countries, including those characterized by vast drought-prone regions (Calderini et al. 1995; Calderini and Slafer 1998; Abeledo et al. 2002). Moreover, when yield increases were expressed as percentage, similar values were recorded in environments with different degrees of water stress (Araus et al. 2002). Several experiments have compared large sets of cultivars characterized by different year of release under different water regimes, allowing a direct comparison of the performance of the same cultivars in drought and well watered conditions (Tollenaar and Lee 2002; Araus et al. 2002). For most crops these analyses showed a linear relation between yield and year of release, the slope of which gives an estimate of the genetic improvement. These studies show that conventional breeding had bred cultivars adapted to stressed environments also selecting for high yield in stress free environments which ensure higher heritability (Cattivelli et al. 2008). Very often, modern cultivars are selected based on the absolute performance of the genotypes across a number of different environments. This breeding strategy has contributed to reduced interaction of genotypes with environments, selecting genotypes with better stability across a wide range of locations and years. As a result, very often modern genotypes outperformed the old ones in all test environments including those with moderate drought stress with a stronger responsiveness to improved fertility (De Vita et al. 2010). This suggests that some of the traits selected to improve yield potential also increase yield at least in mild to moderate drought conditions ensuring yield stability.

In barley, a study carried on a sample of the germplasm grown in Europe identified several modern genotypes with high yield potential and minimal $G \times E$ interaction which ranked among the best in both rainfed and irrigated treatments with highly significant correlation between yield in rainfed and irrigated conditions (Rizza et al. 2004). Notably, the authors observed that while old varieties were characterized by low yield in rainfed conditions and by a minimal ability to improve yield when water became available, modern cultivars showed a higher yield in rainfed conditions and strong yield increases in response to irrigation. Moreover, among superior genotypes there was a large predominance of varieties selected in regions usually not affected by drought (e.g. Northern Italy, Sweden, France, and Netherlands), further suggesting that selection under favourable environments allows the identification of genotypes with a wide range of adaptability. Analogous experiments on sugar beet genotypes representing diverse genetic backgrounds grown in drought prone and irrigated conditions led to similar results (Ober et al. 2004).

The comparison of different cultivars allowed the identification of the main morpho-physiological traits modified during selection in association with yield improvement. Many reports suggest that increases in yield in water-limited environments are mainly determined by the inherent yield potential, instead of traits associated to drought adaptation. Under mild to moderate drought conditions, traits maximizing productivity normally expressed in the absence of stress can still sustain a significant yield improvement (Blum 1996; Slafer et al. 2005; Tambussi et al. 2005). This can be explained considering that the main targets of selection (high harvest index in wheat and barley, stay green in maize and sorghum, resistance to pests and diseases, nitrogen use efficiency) are equally beneficial under dry and wet conditions and, often, the best performances for these traits were overriding the differences in drought adaptability. In soybean for example, modern cultivars characterized by high yield under irrigated conditions, also outperformed in rainfed trials, despite the greater capacity of old cultivars to retain water when measured in terms of water potential and stomatal conductance (Frederick et al. 1990, 1991). This rationale can explain the success of wheat and rice varieties bred at CIMMYT and IRRI where selection under stress free environments identified genotypes with high yield in a wide range of conditions including regions with a low yield potential (van Ginkel et al. 1998; Trethowan et al. 2002). Selection for high yield potential alone would therefore be sufficient, however high yield potential, that is the maximum conversion of environmental inputs into grain output, can be considered the ideal genetic background where to introgress relevant adaptive traits for genotypes intended for more stressed environments. Van Ginkel et al. (1998) provided evidence that in wheat yield potential explains only part of the yield variance under drought conditions and that adaptive traits also contribute significantly to performance under drought. In maize, retrospective studies on the genetic yield improvement have underlined that adaptation to stress conditions is the main feature of modern hybrids. Comparison of short-season hybrids representing yield improvement from the late 1950s to the late 1980s showed that genetic yield improvement was 2.5% per year and that most of the genetic yield improvement could be attributed to increased resistance to stress, like high plant population density, weed interference, low night temperatures during the grain-filling period, low soil moisture, and low soil N (Tollenaar 1989; Cattivelli et al. 2008; Tollenaar and Wu 1999; Tollenaar and Lee 2002). Yield improvement is the result of more efficient capture and use of resources, both the seasonal incident radiation as well as nutrients and water. The improved resource capture is associated with increased leaf longevity, a more active root system, and a higher ratio of assimilate supply by the leaf canopy (source) and assimilate demand by the grain (sink) during the grain-filling period. The improved efficiency in resource capture and use of the new hybrids is most clearly evident under stress. Indeed, improvements of resource use under optimum conditions have been small, as leaf photosynthesis, leaf-angle distribution of the canopy, grain chemical composition, and the proportion of dry matter allocated to the grain at maturity (i.e., harvest index) have remained virtually constant. These findings suggest that the new avenues for selection cultivars with high yield potential and high yield stability should associate

both traits for yield per se as well as traits for stress avoidance/tolerance to maximize the exploitation of available resources. Yield potential and adaptive traits can be combined by alternating segregating generations between an optimum moisture location to select for yield potential, and water-stressed sites to add traits contributing to adaptation under drought. This strategy can allow the identification of superior genotypes with broad adaptation capability, yielding well under the relevant type of drought, but also exploiting eventual improved conditions of the target environment. A selection index could be effective to combine information from both environments (Rosielle and Hamblin 1981; Edmeades et al. 1999; Monneveux et al. 2006).

Screening for yield potential improves yield under drought if yield under drought and yield under well water conditions are positively correlated. This is usually the case for moderate drought conditions. 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 environments is found when drought conditions become extreme. An ideal genotype would combine the highest yield (highest intercept) with the lowest sensitivity to water stress (lowest slope); nevertheless very often a high yield performance under wet and dry conditions of superior genotypes was found to be associated with a high sensitivity to water stress (high slope) (Pantuwan et al. 2002; Ober et al. 2004; Rizza et al. 2004; Pidgeon et al. 2006). The threshold generally valuable for all crops has been identified when stress reduced yield to less than 50% of non-stress yield (Pantuwan et al. 2002), conditions that are rare in commercial agriculture. Recent studies in tropical maize (Banziger et al. 2006), bean (Beebe et al. 2008), and rice (Venuprasad et al. 2007) suggest that under extreme stress conditions, the response to selection for yield in low-yielding environments is greater when selection is performed in the target environment than in a high-yielding environment. These achievements have been obtained with germplasm showing higher genetic variance for yield in low than high yielding environments, probably because association of traits specific for drought tolerance.

Selection in drought stress environments can be achieved either by screening under natural drought stress in the target environments or by screening in managed-stress environments. While the first approach is often less efficient because of the sporadic and unpredictable occurrence of drought events, screening in managed-stress environments, including dry-season trials, rain-out shelters, and drained upper paddies, is more reliable. However in this case, the effectiveness depends on the accuracy of predictions of the performance under natural drought stress, therefore on yield heritability in the selection environment and its genetic correlation with yield under stress in the target environment (Atlin and Frey 1990). In upland rice, the effectiveness of direct selection for yield under severe drought using natural and artificially imposed conditions was recently assessed on segregating populations obtained by crossing high-yield cultivars, developed for irrigated lowland conditions, with drought-tolerant lines adapted to upland conditions (Venuprasad et al. 2007). This study demonstrated that direct selection for yield under stress is likely to be more effective if conducted in a well managed screening environment effectively controlling intra-trial field heterogeneity. Indeed, under these conditions, yield is usually heritable as yield estimated

under non stress conditions (Bernier et al. 2007; Atlin 2004; Venuprasad et al. 2007). Moreover direct selection for yield under drought stress in the dry season was compared with selection for yield under non-stress conditions. Direct selection for yield was effective, notwithstanding yield gains under severe stress occurred only in a population having a highly drought tolerant parent. On the other hand, although yields under stress and non-stress conditions were positively correlated, selection under non-stress conditions resulted in significant gains in stress environments where mean yield was reduced by less than 60%.

7.4 Selection by Secondary Traits

As discussed, traditional breeding for stress adaptation is handicapped since genetic variation and heritability for yield generally decline under stress (Blum 1988). Notwithstanding physiological analyses of yield components and retrospective studies comparing genotypes released in different breeding eras have led to the identification of many traits associated to yield under water scarce conditions, opening the era of the analytical breeding. Provided that these “secondary traits” have higher heritability under stress than yield, they can be exploited to improve selection efficiency during the analytical breeding of new cultivars. These secondary traits should be genetically associated with yield under drought conditions, highly heritable, cheap and easy to measure by accurate phenotyping methods suitable to screen large populations over multiple environments, and should carry no yield penalty under favourable conditions (Ludlov and Muchow 1990). Secondary traits correlated to yield under drought could be used alone to drive the selection, otherwise phenotyping of secondary traits associated to drought-resistance can be combined with yield evaluations to identify higher drought resistant cultivars avoiding penalties for yield potential.

The secondary traits most commonly and successfully used by breeders are those associated to plant phenology, like flowering date and plant height, or disease resistance, which are easily visually determined. Secondary traits for adaptation to drought prone conditions have been just sporadically used, notwithstanding Richards (2006) reported some successful examples, demonstrating that physiological knowledge can result in a more precise targeting of genetic variation. These traits impacted on yield under drought by influencing components of the Passioura's equation both constitutively or in response to drought. Successful examples are based on extended crop duration and osmotic adjustment for wheat to increase the water use and increased axial resistance to water transport and carbon discrimination to increase water use efficiency, while reduced anthesis silking interval (ASI) and delayed leaf senescence have been considered respectively in maize and sorghum to affect harvest index. In maize, the value of secondary traits in breeding for drought resistance has been also demonstrated by examining the genetic correlations with grain yield, applying divergent selection for specific secondary traits, or estimating the correlated response after selecting for grain yield (Edmeades et al. 1992; 1997).

In groundnut, it has been demonstrated that physiological trait-based breeding can help to change the target of selection, effectiveness being the same of the empirical approaches. Indeed, while yield advantage in empirical selections comes largely from greater water uptake ability (probably through deeper root system exploiting more water from the soil), the trait-based approach allowed to largely improve the water use efficiency (Nigam et al. 2005).

Secondary traits for breeding drought tolerant crops can be chosen within three broad strategies: phenology (escape), yield potential and drought resistance depending on the specific features of the target drought environment (stress timing, frequency and intensity). The traits for drought escape can be considered for yield improvement under drought if they allow plants to cope with a predictable severe stress event, usually occurring every year at the same growth stage. The escape traits usually act at phenology level, i.e. flowering time, photoperiod sensitivity and vernalization requirement. Shortening crop duration can be useful to synchronize the crop cycle with the most favourable environmental conditions. Indeed earliness is an effective breeding strategy for enhancing yield stability in Mediterranean environments where wheat and barley are exposed to terminal drought stress or for rice in eastern India, Thailand, and Laos where the monsoon tends to withdraw predictably and sharply. It is known, however, that an extreme earliness leads to yield penalty also in Mediterranean environment and, in fertile conditions, earliness is not correlated with grain yield (Cattivelli et al. 1994). Late heading and flowering, followed by a short grain-filling period can be associated to higher yield when the drought stress is experienced in early season, during the vegetative phase (van Ginkel et al. 1998).

When soil water is no longer available through roots to sustain evapotranspiration, stomata close saving water, but also limiting entry of CO_2 for photosynthesis. Nevertheless high yield requires high stomatal conductance to sustain a great CO_2 fixation. Therefore every response to water scarcity whose effect is a bigger capture of soil water or a better maintaining of plant water status will maximise soil water use thus sustaining transpiration and yield. Therefore breeding for higher yield potential plus traits conferring stress avoidance may be a good option in case of moderate stress conditions. For instance, when rainfall matches the early phase of plant cycle, a vigorous crop establishment is agronomically desirable as it helps to shade the soil thus avoiding loss of water by evaporation directly from the soil and suppressing weed competing for water. Early vigour depends on large seed and embryo size, while wider leaves and a more prostrate growth habit sustains a rapid ground cover. The early vigour can produce assimilates partitioned in a better root system to relieve subsequent moisture stress. To avoid premature exhaustions of soil water, early dry matter could be successfully combined with early flowering. Where soil moisture is still available at maturity or deep soil moisture is available, deep rooted cultivars have demonstrated a clear yield advantage. A vigorous root system could be constitutive or adaptive. Osmotic adjustment (OA) is a frequently cited adaptive mechanism based on the accumulation of solutes whose effect on osmotic pressure helps to maintain a favourable gradient of water potential. It allows plants to maintain water absorption, to sustain cell expansion for root growth or flow of

water into the plant itself from a relatively dry soil for higher photosynthetic rate. Questions remain with respect to the positive contribution of OA in plant productivity (Serraj and Sinclair 2002). Although intraspecific variability for OA and the association between OA and grain yield maintenance under drought stress have been reported in a number of crop species (Chimenti et al. 2006), the correlation between osmotic adjustment and yield increases with increasing drought stress, being positive under severe water deficit when crop survival is threatened but irrelevant for most agriculture conditions (Serraj and Sinclair 2002). A proven benefit of OA is the maintenance of root development, while the increase in water potential gradient develops when yield capacity has been lost and crop survival is threatened as little additional water can be extracted (Serraj and Sinclair 2002).

Leaf permeability also is crucial, as leaves can lose water through cuticle, increasing crop transpiration without an associated benefit in CO₂ fixation. Glauconsness which is caused by the presence of epicuticular wax can prevent these losses (Kersters et al. 2006). Recent insights from physiology studies have indicated a critical role of mesophyll permeability in controlling the movement of CO₂ into the leaf as well as the factors affecting its extent. It is expected that further progress on this topic will suggest specific leaf traits to be modified to increase the availability of CO₂ into the leaf under drought conditions.

The traits discussed above mainly contribute to greater yields through increase in total biomass. Then, biomass is converted into yield based on harvest index (HI). Two factors influence HI: the number of grains and the quantity of assimilate to fill the grains. In all crops, drought events during specific stages of floral development can severely damage seed set, through pollen sterility and abortion of ovary and/or embryos or can prematurely end grain filling. Low water potential around the time of anthesis is particularly damaging in maize and rice. Maize yields failed completely because of excessive delayed ASI which results in ovary abortion and severe embryo abortion (Boyle et al. 1991; Bolanos and Edmeades 1993) when water deficit decreases photosynthate flux to developing organs. In rice sterility increases when drought stress prevents panicle exertion (Saini and Westgate 2000). Moreover drought causes premature leaf senescence thus further limiting photo-assimilation. To allow more assimilate supply to the growing florets, thus reducing the very high rate of floret abortion just before anthesis, an option is the increase of duration of spike growth by a longer stem elongation period (Miralles et al. 2000; Slafer et al 2001). Under post-anthesis stress, the storage of water soluble carbohydrates in the stem of small grain cereals and their subsequent remobilization to grain can directly improve HI. Stay green phenotype resulting in a delay of senescence is therefore an important trait in several crops (e.g. maize, sorghum) exposed to drought during the grain-filling (Campos et al. 2004; Tollenaar and Wu 1999) since it improves assimilate production and storage.

A “xerophytic” breeding strategy which improves plant survival through the limitation of the evapotranspiration can be applied in extremely harsh environments where survival is jeopardised, according to the indicated crossover limit. In such conditions, progress in improving drought tolerance is usually slower than using direct selection for yield in unfavourable environments because several negative correlations between tolerance traits and yield may limit genetic progress using the

indirect selection method (Ceccarelli 1994; Bänzinger et al. 1997; Simmonds 1991). On the other hand, in Australia the comparison of “indirect” (trait) selection with the conventional “direct” (yield) selection method to improve drought resistance in navy bean indicated that seed yield has more $G \times E$ interaction than some yield components such as transpiration, water use efficiency, and HI (Dowkiw et al. 2000).

7.5 Assessment of Secondary Traits

Many secondary traits for breeding in drought prone conditions, like root systems, OA and stomatal conductance, are difficult to measure directly. Nevertheless, as they improve the plant water status, they can be indirectly assessed through other traits correlated to plant water status. As transpiration causes leaf cooling, canopy temperature and its reduction relative to ambient air temperature are an indication of transpiration and thus of stomatal conductance, both factors favoring net photosynthesis and crop duration. Canopy Temperature Depression (CTD) has been used to assess plant water status since it represents an integrated physiological response to drought and high temperature (Amani et al. 1996). An imaging technique, Infrared Red Thermography (IRT), has been used to easily assess whole leaf temperature based on IR for selecting rice genotypes with higher conductance associated with higher yield (Horie et al. 2006).

In plants, the heavy isotope of carbon ^{13}C is discriminated compared to ^{12}C during diffusion and biochemical reactions, as a result, in several C_3 species, the carbon isotope discrimination (Δ) is positively correlated with the ratio of internal leaf CO_2 concentration to ambient CO_2 concentration (C_i/C_a) and negatively associated with transpiration efficiency (TE , which corresponds to WUE at leaf level). Thus, a high C_i/C_a leads to a higher Δ and a lower TE (Farquhar and Richards 1984). Both measure of canopy temperature and carbon discrimination are suitable for large screening, however both present some shortcomings. Several methodological limitations have been found for measure of CTD due to not homogeneous canopy or growing conditions (Royo et al. 2002; Lafitte et al. 2003), while carbon discrimination is limited by the cost of its determination. As $\Delta^{13}\text{C}$ is not applicable to C_4 species due to differences in the photosynthetic pathway, oxygen isotope composition in plant tissues might be an alternative since it reflects evaporative enrichment of leaf water caused by transpiration (Barbour 2007). Isotope discrimination can be applied on leaf or seed material for integrated measures during and after organ growth. To look at the dynamics of photosynthetic fixation as stress occurs, soluble and starch carbohydrate components can also be considered (Ghashghaie et al. 2001; Fresneau et al. 2007). Since the ash content is physiologically associated to carbon discrimination through the control of leaf water content, the mineral or the total ash content of leaves or grains represents a cheaper option to carbon discrimination analysis also used in C_4 plants (Voltas et al. 1998; Araus et al. 2001a).

Chlorophyll fluorescence emissions from intact plants are easily and rapidly measured, and have been shown to be directly related to photosynthetic activity

(Baker and Rosenqvist 2004). The fluorescence parameter Fq'/Fm' , which estimates the quantum yield of photosystem II photochemistry, has been shown to be directly related to the rates of linear electron transport and CO_2 assimilation in leaves (Baker and Oxborough 2004). Consequently, this parameter potentially provides an effective tool for rapidly screening for differences in photosynthesis and thus assimilation, or for differences in the response to stresses like drought. As photosynthetic performance across the area of individual leaves can be extremely heterogeneous, especially for stressed plants, to screen for effects on leaf photosynthesis using fluorescence on the whole leaf imaging instruments using charge coupled device (CCD) cameras should be adopted (Nedbal and Whitmarsh 2004; Oxborough 2004).

The leaf water use efficiency (assimilation/transpiration) is a critical component of yield under drought, but it is generally effective in breeding practise when its increase is associated to an increase in assimilation, while traits reducing the transpiration have often negative consequences on yield. By coupling fluorescence and thermal imaging techniques it is now possible to rapidly evaluate both assimilation and stomatal conductance for high-throughput genotype screening (Chaerle et al. 2007).

Spectroradiometers measure the spectra of light reflected by the canopy both at the crop (Aparicio et al. 2000; Araus et al. 2001b; Royo et al. 2003) and at the plant levels (Alvaro et al. 2007). As drought strongly impairs leaf expansion (Royo et al. 2004) and thus plant growth (Villegas et al. 2001), these instruments can therefore be used to indirectly evaluate total green biomass at a critical plant stage (i.e., anthesis) or its change over time. 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. 2001b; Aparicio et al. 2000; Tambussi et al. 2002; Babar et al. 2006). A wide range of spectroradiometrical indices have been identified to evaluate field plots for yield and their adaptation to environmental conditions, the most famous being the “classical” vegetation indices (VI) related to green biomass such as the normalized difference vegetation index (NDVI) or the simple ratio (SR). However, up to now spectral reflectance measurements such as the water index (WI) or the photochemical reflectance index (PRI) have been scarcely used in breeding programs particularly due to the strongly influence of differences in green biomass (Araus et al. 2001b).

A secondary trait routinely evaluated is leaf color acquired by portable chlorophyll meters such as the Minolta SPAD ([http://www.specmeters.com/Chlorophyll Meters/Minolta SPAD 502 Meter.html](http://www.specmeters.com/Chlorophyll%20Meters/Minolta%20SPAD%20502%20Meter.html)) which measures the total chlorophyll content. Besides the physiological significance of the trait per se, total chlorophyll is an indicator of early senescence such that caused by drought stress (Araus et al. 1997; Rharrabti et al. 2001), as well as N fertilization, protein content of grains and it is positively correlated with yield in wheat (Rharrabti et al. 2001).

Recently, the development of modern phenomics technologies based on high throughput analysis using visible, near infra-red, far infra-red and fluorescence imaging, allows us to reduce the “phenotyping bottleneck” which has, until now, limited our ability to capitalize on substantial investments already made in plant functional genomics and modern breeding technologies (Tester and Langridge 2010).

7.6 Molecular Markers-Assisted Genetic Improvement

Molecular markers have the capability of revealing polymorphisms in the nucleotide sequence and allowing therefore discrimination between different alleles at a given locus. Such information can be used for a number of studies aimed to confirm identity between parents and progeny, to determine evolutionary relationships and genetic distances and to construct genetic and physical maps to localize genes or genomic regions responsible for the expression of a trait of interest. Once an association between a molecular marker and the gene(s) responsible for a trait of interest has been established, it can be employed in a plant breeding program in order to significantly increase the speed and the effectiveness of selection (Marker Assisted Selection – MAS). The fact that they are largely unaffected by environmental factors and developmental stages makes them even more suitable for this purpose.

Molecular markers characteristics and applications have been recently and extensively reviewed (Korzun 2002; Röder et al. 2004; Francia et al. 2005; Varshney et al. 2005; Khlestkina and Salina 2006; Borrelli et al. 2009); virtually, every kind of molecular marker can be used for MAS; nevertheless, they should meet the five main requirements for utilization in MAS defined by Collard and Mackill (2008): flexible and high-throughput detection methods, low quantity and quality of DNA required, low cost per assay, tight link to target loci and a high level of polymorphism in breeding material. PCR-based markers, particularly microsatellites (SSRs), and Single Nucleotide Polymorphisms (SNPs), are much more suitable for use in MAS programs than other techniques, SNPs are expected to become the best choice in the next years.

7.6.1 SNP and Genomic Selection

The new DNA sequencing technologies, made available in the last years, rapidly are producing huge amounts of sequences with a number of applications including genome resequencing and polymorphism detection, mutation mapping, DNA methylation and histone modification studies, transcriptome sequencing, gene discovery, alternative splicing identification, small RNA profiling and DNA-protein interactions (Lister et al. 2009; Delseny et al. 2010). Currently, there are three widely deployed deep sequencing platforms, Genome Sequencer FLX from 454 Life Sciences/Roche, Illumina Genome Analyzer, and Applied Biosystems SOLiD. Each instrument essentially massively parallelizes individual reactions, sequencing hundreds of thousands to hundreds of millions of bases in distinct, relatively short (50–400 bases) DNA fragments, in a single run. The development of new molecular markers in particular is taking advantage of these new technologies that offer therefore a new perspective to applications in the field of genetic improvement of crops. To benefit fully from this major breakthrough in genome sequencing, bioinformatic tools that analyze huge amounts of sequences for the automated design of molecular markers have been developed.

Single nucleotide polymorphism (SNP) markers are increasingly becoming the marker system of choice. They occur in virtually unlimited numbers: several millions of SNPs have been developed based on the sequence of the human genome (Frazer et al. 2007), and similar amounts of SNPs are expected to be produced also in crops in the next future.

Even if the use of SNPs in plants is still in its infancy with respect to results obtained in animal species, an increasing number of large-scale genetic variant discovery initiatives are being undertaken by means of next-generation sequencing platforms, allowing for drastically quicker and cheaper variant discovery, and leading towards a far more comprehensive view of the genome (Ganal et al. 2009). All SNP detection approaches are based on resequencing of different genotypes to determine allelic variants at the highest number of loci as possible. Large projects aimed to identify a very high number of SNPs have been undertaken for crops. About 2,000 and more than 125,000 SNPs were identified by 454 resequencing in two independent projects in maize with a rate of about 85% of validated SNPs (Barbazuk et al. 2007; Gore et al. 2009). In rice, the reference sequences of cultivars Nipponbare and 93-11 were utilised to identify SNPs and to map them by resequencing a set of 150 recombinant inbred lines derived by the cross between the two varieties on an Illumina Genome Analyzer (Huang et al. 2009). New platforms are now available for the rapid genotypization of individuals with SNPs. The Infinium assay by Illumina (http://www.illumina.com/technology/beadarray_technology.ilmn) can provide the analysis with several hundred thousands SNPs in a single assay. The KBiosciences Competitive Allele Specific PCR SNP genotyping system (KASPar) is a novel homogeneous fluorescent genotyping system (<http://www.kbioscience.co.uk>) providing very fast and cheap analyses.

The possibility to develop and analyze so huge numbers of SNPs in crop species opens new perspectives for the use of these molecular markers to accelerate selection of improved genotypes. While the current MAS strategies use only markers for which a significant association with a trait has been identified, a new method called genomic selection and developed by Meuwissen et al. (2001) predicts breeding values using data deriving from a huge number of molecular markers with a high coverage of the genome. Genomic selection uses all marker data as predictors of performance and consequently delivers more accurate predictions with respect to current MAS approaches. Marker effects are first estimated on a training population for which genotypic data (with very dense marker coverage) and a deeply accurate phenotypic evaluation are available. Based on these data, prediction models for marker effects are developed in order to estimate the genomic breeding value of any individual in breeding programs for which only genotypic data are available. Simulation studies have shown that genomic selection can lead to high correlations between predicted and true breeding value over several generations without repeated phenotyping (Meuwissen et al. 2001; Habier et al. 2007). Therefore, genomic selection can result in lower costs and increased rates of genetic gain (Zhong et al. 2009).

7.6.2 *Identification of Phenotype-Associated Markers*

The search for molecular markers strictly associated with a locus controlling important phenotypic traits is a key objective in molecular genetics and can be carried out with methods based on segregation mapping, genomic introgression, and association mapping (Morgante and Salamini 2003). Segregation mapping is based on the availability of large mapping populations such as F₂s, Recombinant Inbred Lines (RILs), Backcross Inbred Lines (BILs) or Doubled Haploid Lines (DHLs). These segregating populations are used to develop linkage maps in which the relative position of hundreds of molecular markers is indicated on each chromosome. Then, the gene(s) and QTL(s) position can be determined through the combination of phenotypic characteristics and segregation data of molecular markers. A plethora of studies have been carried out in order to identify genomic regions involved in determining high yield in stress and non stress environment in crops. The wide range of physiological and biochemical mechanisms involved in dehydration response explains the complexity of plant response to drought, for which a high number of QTLs widespread on many chromosomes have been found (Cattivelli et al. 2002, 2008). QTLs for yield and yield-related traits most frequently account for between 2% and 10% of the total phenotypic variation; major QTLs with R² values higher than 15% have seldom been described, especially when evaluating segregating materials obtained from elite accessions (Quarrie et al. 2005; Dilbirli et al. 2006). In durum wheat, Maccaferri et al. (2008) identified 16 QTLs in which two major QTLs on chromosome arms 2BL and 3BS of durum wheat affecting grain yield and showing significant effects in multiple environments (rain-fed and irrigated). Some recent papers have reviewed in details the QTLs identified for traits related to drought stress tolerance (Maccaferri et al. 2009; Ashraf 2009), furthermore, for many crop plants information on drought-related QTL findings have been collected in open source databases, such as GRAMENE (<http://www.gramene.org/>) or GRAINGENES (<http://wheat.pw.usda.gov/GG2/quickquery.shtml#qtls>). In particular, Courtois et al. (2009) extracted information from about 60 papers published between 1995 and 2007 and compiled a database containing QTLs for drought resistance traits and for 29 root parameters. The data describe 2,137 root and drought QTLs, out of which 675 for root traits detected in 12 mapping populations.

Once identified, a QTL needs to be validated in order to provide reliable QTL-associated markers for breeding. Near Isogenic Lines (NILs) can be produced backcrossing a donor plant with a recurrent parent. As each NIL carries only one target QTL in the genomic background of the recurrent parent, behaving as a single Mendelian factor, the effect of the target QTL can be precisely evaluated, and the gene/QTL finely mapped and isolated (Salvi and Tuberosa 2005).

The whole QTL analysis process, from the construction of the primary mapping population to the achievement of NILs, is labour intensive and requires many generations and years. An alternative and effective approach for identification of target QTLs is the development of Introgression Lines (ILs), a set of lines obtained through the marker assisted backcross of a donor parent, usually a wild relative, with a recurrent

parent. After 2–3 backcross generations, each line will contain a specific genomic region of donor parent into the recurrent genetic background and the whole set of ILs is organized to represent the whole donor genome (Zamir 2001). Because the identification of QTLs using ILs does not require a pre-existing linkage map, this is a more user-friendly method for practical breeding works. Different examples are available, in which genomic segments carrying QTLs for drought resistance traits from *O. rufipogon* were identified in a set of introgression lines in rice (Zhou et al. 2006; Zhang et al. 2006). Xu et al. (2005) evaluated a large set of 254 introgression lines in an elite indica genetic background for grain yield and related traits under the irrigated and drought conditions in two consecutive years. They found 36 QTLs, some being expressed in both treatments, and other specific of the irrigated or drought condition. Once a QTL has been identified, the IL carrying the region of interest can be used for fine mapping and cloning of QTL/gene as well as donor line in breeding programmes.

Introgression of useful alleles from wild species has highlighted the problem of the effectiveness of a gene in a particular genetic background. It is infact necessary to confirm that QTLs discovered in a given mapping population will improve drought tolerance when introduced into high yielding elite genotypes. This is particularly difficult when the traits are governed by interaction with other genes and/or environment. In these cases the effect of the QTL alleles can differ depending on the genetic structure of the current germplasm set in the breeding program (Wade 2002), and the continuous change of the genetic background during the breeding process can lead to a change in the value of the QTL allele that is transferred by MAS. The “Mapping As-You-Go” strategy (Podlich et al. 2004) involves repeated re-estimation and validation of the QTL effects throughout the breeding process to ensure that they remain relevant throughout. This method results in substantial increases in MAS efficiency compared with standard approaches based on the evaluation of the QTL effects only at the beginning of the breeding program, particularly when epistasis and/or genotype-environment interactions play a significant role.

Besides linkage map studies, an emerging approach for the identification of genes underlying phenotypic variations in complex traits is represented by linkage disequilibrium (LD, i.e. non random association between alleles at linked loci) mapping (Syvänen 2005). LD infers associations between genotypes (haplotypes) and phenotypic variations examining the genetic polymorphisms that have been generated into different genetic backgrounds through thousands of generations of recombination. A whole genome may be scanned to identify regions that are associated with a particular phenotype by using molecular markers, covering the whole genome. Otherwise, alleles at a few selected candidate genes may be tested for association with a phenotype (Rafalski 2002). Candidate genes for a trait of interest are usually referred to sequences whose expression profile or protein function can be associated to the trait and whose position on the genome co-maps with a QTL controlling the trait. The extent of LD is affected by many factors, including the population history and the frequency of recombination in the examined genome segment. In plant species where population bottlenecks are common, such as sugarcane and sugarbeet, LD extending for several cM was found (Jannoo et al. 1999; Kraft et al. 2000). On the contrary, maize studies have shown that LD decays within 1,500 bp distance, allowing successful

identification of QTLs associated with variation in flowering date (Thornsberry et al. 2001). LD has been successfully employed in finding marker-trait associations for yield and yield stability in a collection of 146 modern two-row spring barley germ-plasm: the association was evaluated on a mean of 15 environments for each variety in Northern Europe (Kraakman et al. 2004), and for kernel morphology and milling quality in a selected sample of 95 cultivars of soft winter wheat (Brescghello and Sorrells 2006), indicating that association mapping approaches can be a viable alternative to classical QTL approaches based on segregating population studies. Association mapping has been also employed to study drought resistance determinants, in particular in sunflower and *Pinus taeda* (Kane and Rieseberg 2007; Gonzalez-Martinez et al. 2006). 46 SNPs from 41 disease and abiotic stress-inducible genes were tested for their genetic association with carbon isotope discrimination with 961 clones by Gonzalez-Martinez et al. (2008). Two particularly promising candidates for their genetic effects on carbon discrimination were *dhn-1*, involved in stabilization of cell structures, and *lp5-like*, a glycine rich protein putatively related to cell wall reinforcement proteins, both of which already shown to be water-deficit inducible.

LD estimates can also be used to determine target marker densities for genomic selection, as dense marker coverage is needed to maximize the number of QTLs in LD with at least one marker, thereby also maximizing the number of QTLs whose effects will be captured by markers (Heffner et al. 2009).

7.6.3 Critical Factors for Success of MAS

The success of a MAS breeding programme depends first of all on the nature of the trait to be improved. While the transfer of monogenic traits is simple and well documented in literature (Campbell et al. 2002; Bonafede et al. 2007), MAS programmes for traits with a complex genetic basis are much more difficult due to the high cost, the genotype \times environment and gene-gene (i.e. epistasis) interactions, trade-offs between different organization levels, which in turn, result in a low efficiency, as the case of breeding for drought resistance. An important factor is also the degree of association between the markers and the major gene(s) or quantitative trait loci (QTLs) of interest; the closer are the marker and the gene, the lower is the probability of marker and gene being separated by recombination. If the gene controlling the trait has been isolated, it is possible to design a so called “perfect” marker directly within the gene; in this way the association between the gene and the marker cannot be lost.

The high interaction of $G \times E$ is one of main constraints in application of MAS for improved drought resistance. The ability of an organism with a given genotype to change its phenotype in response to changes in the environment is known as phenotypic plasticity (Schlichting 1986; Pigliucci 2005). When QTLs are detected in a stressed environment vs. optimal conditions they are considered as related to adaptive traits, while little attention is paid to phenotypic plasticity. Reymond et al. (2003, 2004) have proposed a new approach in which QTLs are identified through the correlation between the molecular data and the parameters describing the

response curve of the trait to the environmental conditions, rather than the raw phenotypic data per se. This approach allows the characterization of the genotype per se rather than its behaviour in a given environment. The authors have successfully applied this method to the evaluation of leaf elongation rate (LER) in response to temperature, evaporative demand and soil water status. Response curves of LER to meristem temperature, water vapor pressure difference, and soil water status were established in 100 RILs of maize in six experiments carried out in the field or in the greenhouse, and a QTL analysis was carried out on the slopes of these responses. Most QTLs were specific of one response only.

Another critical factor for traits explained by a number of QTLs, each of them controlling a part of the observed variability, is an accurate phenotyping. Capacity for precise phenotyping under reliable conditions probably represents the most limiting factor for the genetic analysis of quantitative traits. Phenotypic measurements are needed, giving a good precision level on a large number of genotypes (Cattivelli et al. 2008). Designs of laboratory or field experiments defining stress combinations pertinent to specific target crops and their environment should take into account as many environmental factors of the target environment as possible, including macro- and micro-environmental factors (Salekdeh et al. 2009). To achieve a careful phenotyping, it is also important to control timing, intensity and duration of stress. To reduce the signal-to-noise ratio in field based experiments there is a need to select research plots with low spatial variability in soil properties, good management of nutrients, weeds and pests, and experimental design that should control within-replica variability (Edmeades et al. 2004).

Uniformity of conditions in both control and stressed plots is a key factor in screening large number of accessions for a conditional trait like drought tolerance. This problem is particularly important when accessions with different morpho-phenological traits are compared, making difficult to apply the same intensity of stress to a specific stage of the crop lifecycle. For this reason, there is great interest in methods that reduce systematically the number of accessions to be screened without significant loss of diversity in traits of interest. The use of taxonomical, geographical and morphological descriptors, as well as molecular markers, can help in constituting mini-core collections composed by a lower number of individuals, but retaining the genetic diversity for the trait of interest. Bidirectional selective genotyping is another method of reducing the number of lines in a mapping population that requires genotyping and refined phenotyping. A previously detected large-effect QTL for yield under drought stress in rice (Bernier et al. 2007) was confirmed in a 436-line population by selective genotyping just 20 lines at each phenotypic extreme of the population (Bhatnagar-Mathur et al. 2008).

7.6.4 MAS for Drought Tolerance

Environmental variability and low stability of QTLs for grain yield make difficult the choice of genomic regions to be transferred by MAS to improve grain yield in

drought prone environments. This is the main reason why the MAS-mediated improvement of grain yield in drought prone environments has yield limited success by transferring QTLs for yield. MAS programs for drought-related morphological and physiological traits may represent a better strategy for improvement of drought resistance. During the last 15 years, the application of QTL analysis allowed the identification of many chromosome regions involved in control of variation in almost all the physiological, morphological and developmental changes observed during plant growth in water-limiting conditions. Particular attention has been paid to: (i) genetic variation of the OA (Teulat et al. 1998; Robin et al. 2003), (ii) genetic bases of phenological traits – e.g. stay green phenotype (Sanchez et al. 2002; Jiang et al. 2004; Verma et al. 2004) and anther-silking interval (ASI increases in response to drought) (Hall et al. 1982; Ribaut et al. 1996); (iii) the ability of the roots to exploit deep soil moisture to meet evapotranspirational demand (Johnson et al. 2009; Nguyen et al. 2004); (iv) the limitation of water-use by reduction of leaf area and shortening of growth period (Anyia and Herzog 2004); (v) isotope discrimination (Martin et al. 1989; Juenger et al. 2005; Saranga et al. 2004); (vi) the limitation of non-stomatal water loss from leaves – e.g. through the cuticle (Lafitte and Courtois 2002) and (vii) the response of leaf elongation rate to soil moisture and evaporative demand (Reymond et al. 2003). Comparative analysis of QTL results clearly shows that chromosomal regions determining variation in agronomic and physiological drought-related traits cover a large proportion of the whole genome.

Examples of successful MAS programmes for water stress-related traits have been reported for different species and with different outcomes. In a marker-assisted backcross experiment carried out in maize, the drought tolerant line Ac7643, donor parent, and the drought susceptible line CML247, recurrent parent, characterized by a very large male-female flowering asynchrony interval, were crossed. Five QTLs for ASI, located on different chromosomes and co-localized with QTLs for yield components, were selected, each of them explaining >5% of the phenotypic variance for ASI. Plants with the highest proportion of CML247 alleles at 60 markers distributed throughout the genome, and with good agronomic traits and yield performance were selected in two backcrosses. When compared with controls, hybrids obtained from these selected lines, even if with no significant differences in grain yield under mild water stress and well watered conditions, showed a consistently higher mean grain yield under severe water stress conditions, the best five MAS-derived hybrids yielding at least 50% more than control hybrids (Ribaut and Ragot 2007).

In rice, MAS was used to transfer several QTLs for root depth from the japonica upland cultivar “Azucena”, adapted to rainfed conditions, to the lowland indica variety “IR64”. MAS selected lines showed a greater root mass in low rainfall trials (Courtois et al. 2003; Steele et al. 2006). More recently, four Near-Isogenic Lines (NIL) derived from a previous study (Steele et al. 2006), were evaluated in field trials for 3 years and showed to excel as compared to parents not only for development of root apparatus, but also grain yield (Steele et al. 2007). All these efforts resulted in the release of a highly drought tolerant variety, Birsas Vikas Dhan 111 in India, characterized by early maturity, high drought tolerance and high grain yield with good grain quality (Steele 2009).

Isolating the gene(s) behind a QTL and designing a marker within it (them) could represent the best approach to pyramid different QTLs into the same genotype through MAS. For instance, QTLs explaining some grain yield components have also been cloned in rice, as grain number (Gn1a) (Ashikari et al. 2005) and grain size (GS3) (Fan et al. 2006). Candidate genes can be identified by association mapping studies, by confirming the co-segregation between the gene and QTLs for drought resistance or by analysing with bioinformatic tools the genes present in QTL-underlined genomic regions. A study in *Arabidopsis* has led to the cloning of the ERECTA gene, a sequence beyond a QTL for transpiration efficiency (Masle et al. 2005). Sequencing of entire genome of crop species is expected to provide a huge opportunity to clone QTL for drought-related traits replacing the previous approach based on the construction of functional genetic maps with candidate genes to identify the genetic determinants of QTLs based on cosegregation between the candidate and the QTL (e.g. Zheng et al. 2003; Nguyen et al. 2004; Diab et al. 2004; Tondelli et al. 2006).

7.7 Transgenic-Assisted Genetic Enhancement

Another possible breeding practice consists of the introduction of relevant genes via genetic transformation. Molecular biology studies have identified a number of genes involved in plant response to drought. Usually, expression studies, carried out at level of one/few genes or of the whole transcriptome, are utilised to identify genes whose transcription profile is regulated by the dehydration stress.

Plant transformation provides new variability and a wide array of novel genes not previously accessible to plant breeders, also from related plants or other species even in other taxonomic phyla, which can be easily inserted and functionally expressed into agronomic background in a single event, without associated deleterious genes (Sharma et al. 2002). This process also allows changing the level and the spatial and temporal pattern of transgene expression by choosing suitable promoters, constitutive or inducible, or adding introns into the constructs (Sahrawat et al. 2003). The engineered genetic enhancement may solve many plant breeding problems particularly when the gene controlling the trait of interest is not present or is very difficult to access in the gene pool of the major commercial crops. Pest, disease and herbicide resistance provide examples in which transgenic crops have been obtained and successfully adopted worldwide (Borrelli et al. 2009). Anyway, in the case of more complex traits, transgenic events are at a pre-commercial phase and many more are still at laboratory level and most likely few of them will be successfully validated in field trials.

A drought stress event triggers at cellular level the activation of signal transduction pathways in which transcription factors, RNA-interacting proteins and protein kinase/phosphatases play a key role and which in turn regulate the expression of a number of downstream stress-related genes involved in different metabolic and physiological pathways, as free-radicals and toxic-compounds scavenging, osmolyte

accumulation, water and ion uptake and transport (Apse and Blumwald 2002; Wang et al. 2003; Zhang et al. 2004; Vinocur and Altman 2005; Cattivelli et al. 2008). On the basis of this knowledge it is possible to develop transgenic plants in which the general stress response is up regulated, or in which specific metabolic or physiological processes related to stress tolerance are enhanced (Parry et al. 2005; Bohnert et al. 2006; Umezawa et al. 2006).

An example of metabolic engineering successful in several plants subjected to stress consists of the design of transgenic plants for higher osmolyte contents (Wang et al. 2003). In particular, an improvement of tolerance to different abiotic stresses was observed in transgenic rice lines showing stress-inducible accumulation of trehalose (Garg et al. 2002), in wheat showing a small increase in mannitol (Abebe et al. 2003), tobacco and maize plants with increased content of glycine betaine (Shen et al. 2002; Quan et al. 2004; Zhang et al. 2008), and in soybean, petunia and tobacco plants with enhanced accumulation of proline (Ronde et al. 2004; Yamada et al. 2005; Gubis et al. 2007). Other strategies to broaden stress tolerance in plants provided for controlling the amount of reactive oxygen species (ROS – Ashraf 2009), maintaining energy homeostasis under stress conditions (De Block et al. 2005), or, specifically for water deficit, for the production of phenotype with decreased conductance and higher water use efficiency by over expression of a maize NADP-malic enzyme (Laporte et al. 2002), by regulating the ABA synthesis and response (Wang et al. 2005; Hu et al. 2006), by improving the root system (Gaxiola et al. 2001; Park et al. 2005) or delaying water stress induced leaf senescence (Rivero et al. 2007).

Many examples are also available demonstrating that it is possible improving the resistance to drought stress by acting on genes involved in signal transduction and regulation of the expression of downstream genes. Transgenic plants with over-expression of OsMAPK5, a mitogen-activated protein (MAP) kinase as well as of OsCDPK7, a calcium-dependent protein kinase, resulted in enhanced levels of stress-responsive genes allowing a more sustained stress tolerance (Saijo et al. 2000; Yang et al. 2001; Xiong and Yang 2003).

Among the best characterized transcription factors acting in response to abiotic stresses are the members of the DREB/CBF gene family as well as sequences coding for proteins with basic leucine-zipper (bZIP), MYC and MYB DNA binding domains (Abe et al. 1997, 2003; Jacoby et al. 2002; Dong et al. 2003; Dubouzet et al. 2003; Shen et al. 2003; Li et al. 2005; Agarwal et al. 2006; Hu et al. 2006; Ito et al. 2006; Olsen et al. 2006; Sakuma et al. 2006; Kim et al. 2007). Many crops were engineered with genes encoding the DREB/CBF transcription factors (tomato – Hsieh et al. 2002; rice – Dubouzet et al. 2003; Oh et al. 2005; Ito et al. 2006 and wheat – Pellegrineschi et al. 2004), showing increased stress tolerance as well as the over induction of downstream stress related genes. A common constraint encountered with plants over expressing drought-related genes and transcription factors in particular, consisted of abnormalities in plant growth and development in absence of the stress factor when transformed plants were realized with the transgene under the control of constitutive promoters. This problem is usually solved by utilising a stress-inducible promoter.

The majority of these studies were carried out in controlled conditions, but it is necessary to perform field trials in order to collect data on the real effect of the transgene on drought resistance and on yield in stress conditions. Under water limited environments both in controlled and field trials, transgenic maize plants with increased expression of the transcription factor ZmNFYB2 showed tolerance to water stress when evaluated using stress-related parameters such as chlorophyll content, stomatal conductance, leaf temperature, reduced wilting, and maintenance of photosynthesis (Nelson et al. 2007). Encouraging field trial data are available for also transgenic rice plants over-expressing the rice stress responsive transcription factor SNAC1 (Hu et al. 2006).

The involvement of the farnesyltransferase (ERA1) gene in the regulation of ABA sensing and water stress tolerance was assessed by molecular studies, but field trials carried out for 3 years on Brassica carrying an ERA1 antisense construct have shown that, under moderate water stress conditions, a significant reduction in stomatal conductance and water transpiration was found at flowering, and the yield of the transgenics was significantly higher than the control (Wang et al. 2005). Water stress could accelerate leaf senescence leading to a decrease in canopy size, loss in photosynthesis and reduced yields. Transgenic tobacco plants expressing a gene encoding isopentenyltransferase showed reduced drought-induced leaf senescence and resulted in improved water stress tolerance, with a minimal yield loss when watered with only 30% of the amount of water used under control conditions (Rivero et al. 2007).

Improved biomass productivity and water use efficiency under water deficit conditions were detected in transgenic wheat constitutively expressing the barley HVA1 gene, encoding a member of the group 3 late embryogenesis abundant (LEA) proteins. When these plants were tested in nine field experiments over six cropping seasons (the largest field test for transgenic plants with improved tolerance to water stress published so far) the data have shown that the HVA1 protein confers a significant protection from water stress (Bahieldin et al. 2005). Very promising results have been showed also for genes coding for aquaporins. In field tests, tomato plants overexpressing the gene SITIP2 developed significantly higher (26%) plant biomass than the control plants and this was also associated with a significant (up to 21%) increment of fruit yield under relatively mild and more severe regimens of water deficiency stress (Sade et al. 2009). Finally, Jeong et al. (2010) performed a 2-years field trials with rice transgenic plants overexpressing the gene OsNAC10, a transcription factor containing the NAC domain, under the control of the constitutive promoter GOS2 and the root-specific promoter RCc3. While the transgene under the control of the constitutive promoter did not improve grain yield in field with respect to wild types in both normal and drought conditions, the transgene under the control of the root-specific promoter was effective in increase the plant tolerance to drought, high salinity and low temperature at the vegetative stage. More importantly, the RCc3:OsNAC10 plants showed significantly enhanced drought tolerance at the reproductive stage, increasing grain yield by 25–42% and 5–14% over controls in the field under drought and normal conditions, respectively.

In the last decade many differently engineered plants have been proposed and tested for improved performance under abiotic stresses. Even though about 3,000 patents and patent applications emerge in CAMBIA's BiOS patent database by searching for "drought tolerance" and "drought resistance", however a large proportion is concerned with metabolic or stress-induced genes having doubtful functional significance under field conditions, as indicated by Passioura (2007). The evaluation of "drought tolerance" of transgenic plants has often been based on survival capacity, with very limited analyses of the transgene effects on yield potential. In general, the majority of abiotic stress studies are performed under controlled conditions, where a single constrain is applied as 'shock' treatment, and do not reflect the real conditions that occur in the field where the crops are routinely subjected to a combination of different constrains. Then the response of plants in field cannot be directly inferred from the response to each of different stresses applied individually (reviewed by Mittler 2006). This gap might explain why some transgenic plants, that showed enhanced tolerance to a particular stress when analysed in controlled conditions, failed to show this tolerance when tested in field (Borrelli et al. 2009). Very recently, the agriculture company Monsanto announced that its first-generation drought-tolerant corn has moved to the fourth, and final, phase before an anticipated market launch early next decade (<http://monsanto.mediaroom.com/index.php?s=43&item=676>). In field trials drought-tolerant corn have shown 6–10% yield enhancement in some of the key drought-prone areas in the United States.

7.8 Conventional Versus Molecular Breeding

To be advantageous with respect to classical phenotypic selection, MAS should either (i) provide an increased genetic gain per unit of time, which is the case when MAS permits selection at DNA level at an early stage in the development and/or when it permits reduction of the generation interval, or (ii) provide an increased genetic gain per unit of cost (i.e. molecular genotyping cheaper than phenotypic evaluation) (Hospital 2009).

A direct comparison of MAS vs CB (Conventional Breeding) for yield and yield-components improvements was run in cucumber (Robbins and Staub 2009). Four inbred lines were intermated and then maternal bulks were used to create four base populations for recurrent mass selection. Both MAS (genotyping at 18 marker loci) and CB (open-field evaluations) were practiced for yield indirectly by selecting for four yield-component traits that are quantitatively inherited with 2–6 quantitative trait loci per trait. These traits included multiple lateral branching, gynoeceous sex expression (gynoecey), earliness, and fruit length to diameter ratio. Both MAS and CB provided improvements in all traits under selection in at least one population, except for earliness, which did not respond to MAS. Generally, CB was most effective for gynoecey, earliness, and fruit length to diameter ratio, while MAS was most effective for multiple lateral branching and provided the only increase in yield (fruits

per plant). Therefore, both MAS and CB were useful for multi-trait improvement, but their effectiveness depended upon the traits and populations under selection.

Reliable information regarding comparative advantage of MAS over CB in breeding is scarcely available. There is a certain agreement in affirming that the cost of MAS is much lower than those of CB depending on the choice of markers. In a recent study, Abalo et al. (2009) carried out a comparative study in order to determine the efficiency of both methods in breeding for maize streak virus (MSV) resistance in Uganda. They found that, although both breeding approaches were effective in generating MSV-resistant lines, disease incidence was higher in populations under CS (79%) than MAS (62%). However, an equal number of lines generated by MAS and CS displayed high yield potential and MVS resistance in testcrosses. In this evaluation, MAS was cheaper than CS by 26%, and authors concluded that when laboratory facilities are already established, MAS would be recommended in breeding for MSV resistance (Abalo et al. 2009). Anyway, with the new technologies for high-throughput SNP development and analysis, costs of genotypization are expected to decrease significantly in the next future. In this scenario, the limiting step for marker assisted selection will be the identification of markers strongly linked to traits of interest and robust prediction models for genomic selection.

7.9 Concluding Remarks

Thanks to new molecular technologies, breeders now have new perspectives for plant improvement. Markers for single loci and QTLs as well as genes for plant transformation will provide an opportunity to integrate classical phenotype-based selection with selection on the basis of genotype. Based on all knowledge of the traits sustaining yield and drought resistance, randomly dispersed QTLs, transgenes or both can be accumulated into elite genotypes in a “breeding by design” strategy (Peleman and Van der Voort 2003). The understanding of the genetic bases of all agronomic traits and the analysis of allele variations at the corresponding loci would enable the breeders to design new ideotypes first *in silico*, then to construct the new genotypes *in planta*. For instance a combination of different approaches has been followed to develop new rice cultivars referred to as “Green Super Rice”, possessing resistance to multiple insects and diseases, high nutrient efficiency, and drought resistance, promising to greatly reduce the consumption of pesticides, chemical fertilizers, and water (Zhang 2007). Nevertheless, the polygenic basis of complex traits and the difficulty to follow minor QTLs with molecular markers strongly limit the application of MAS to traits related to drought resistance. Furthermore, the biparental mapping populations used in most QTL studies do not readily translate to breeding applications. However, high throughput sequencing has made marker discovery affordable for most crop species, and the continued reduction of genotyping costs will facilitate dense genome wide marker coverage for all crop species. The availability of large SNP panels for crops on one hand will accelerate the QTL discovery and transfer in MAS programs already in course; on

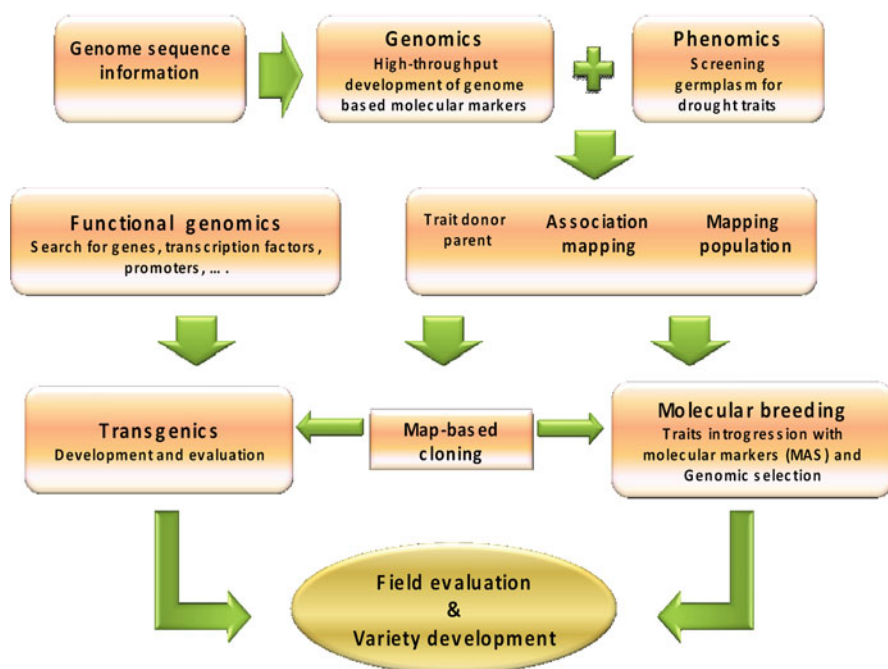


Fig. 7.1 Flowchart illustrating the relationships among the different genomic tools available to identify superior alleles for plant improvement

the other hand it will make possible genomic selection to accelerate the breeding cycle, and introduce greater flexibility in the relationship between phenotypic evaluation and selection (Fig. 7.1).

Acknowledgments This work was supported by Ministero dell'Università e della Ricerca (MiUR) of Italy, special grant AGROGEN, and Ministero delle Politiche Agricole Alimentari e Forestali (MiPAAF), special grant ESPLORA.

References

- Abalo G, Tongona P, Derera J, Edema R (2009) A comparative analysis of conventional and marker-assisted selection methods in breeding maize streak virus resistance in maize. *Crop Sci* 49:509–520
- Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K (1997) Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid regulated gene expression. *Plant Cell* 9:1859–1868
- Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signalling. *Plant Cell* 15:63–78
- Abebe T, Guenzi AC, Martin B, Cushman JC (2003) Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. *Plant Physiol* 131:1748–1755

- Abeledo LG, Calderoni DF, Slafer GA (2002) Genetic improvement of barley yield potential and its physiological determinants in Argentina (1944–1998). *Euphytica* 130:325–334
- Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant Cell Rep* 25:1263–1274
- Alvaro F, García del Moral LF, Royo C (2007) Usefulness of remote sensing for the assessment of growth traits in individual cereal plants grown in the field. *Int J Remote Sens* 28:2497–2512
- Amani I, Fischer RA, Reynolds MP (1996) Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. *J Agron Crop Sci* 176:119–129
- Anyia AO, Herzog H (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *Eur J Agron* 20:327–339
- Aparicio N, Villegas D, Casadesus J, Araus JL, Royo C (2000) Spectral reflectance indices for assessing durum wheat biomass, green area, and yield under Mediterranean conditions. *Agron J* 92:83–91
- Apse MP, Blumwald E (2002) Engineering salt tolerance in plants. *Curr Opin Biotechnol* 13:146–150
- Araus JL, Amaro T, Zuhair Y, Nachit MM (1997) Effect of leaf structure and water status on carbon isotope discrimination in field-grown durum wheat. *Plant Cell Environ* 20:1484–1494
- Araus JL, Casadesus J, Bort J (2001a) Recent tools for the screening of physiological traits determining yield. In: Reynolds MP, Ortiz-Monasterio JI, McNab A (eds) *Application of physiology in wheat breeding*. CIMMYT, Mexico DF
- Araus JL, Casadesus J, Asbati A, Nachit MM (2001b) Basis of the relationship between ash content in the flag leaf and carbon isotope discrimination in kernels of durum wheat. *Photosynthetica* 39:591–596
- Araus JL, Slafer GA, Reynolds MP, Royo C (2002) Plant breeding and drought in C3 cereals: what should we breed for? *Ann Bot* 89:925–940
- Araus JL, Villegas D, Aparicio N, García del Moral LF, El Hani Rharrabi Y, Ferrio JP, Royo C (2003) Environmental factors determining carbon isotope discrimination and yield in durum wheat under Mediterranean conditions. *Crop Sci* 43:170–180
- Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H, Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. *Science* 309:741–745
- Ashraf M (2009) Inducing drought tolerance in plants: recent advances. *Biotechnol Adv*. doi:10.1016/j.biotechadv.2009.11.005
- Atlin GN (2004) Improving drought tolerance by selecting for yield. In: Fischer KS, Lafitte R, Fukai S, Atlin G, Hardy B (eds) *Breeding rice for drought-prone environments*. International Rice Research Institute, Los Banos
- Atlin GN, Frey KJ (1990) Selecting oat lines for yield in low-productivity environments. *Crop Sci* 30:556–561
- Babar MA, van Ginkel M, Klatt A, Prasad B, Reynolds MP (2006) The potential of using spectral reflectance indices to estimate yield in wheat grown under reduced irrigation. *Euphytica* 150:155–172
- Bahieldin A, Mahfouz HT, Eissa HF, Saleh OM, Ramadan AM, Ahmed IA, Dyer WE, El-Itriby HA, Madkour MA (2005) Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance. *Physiol Plant* 123:421–427
- Baker NR, Oxborough K (Chlorophyll fluorescence as a probe of photosynthetic productivity) Chlorophyll fluorescence as a probe of photosynthetic productivity. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a fluorescence: a signature of photosynthesis*. Springer, Dordrecht
- Baker NR, Rosenquist E (2004) Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. *J Exp Bot* 55:1607–1621
- Bänziger M, Betran FJ, Lafitte HR (1997) Efficiency of high nitrogen and water stress on biomass accumulation, nitrogen uptake, and seed yield of maize. *Field Crop Res* 19:297–311
- Bänziger M, Setimela PS, Hodson D, Vivek B (2006) Breeding for improved abiotic stress tolerance in maize adapted to southern Africa. *Agric Water Manage* 80:212–224
- Barbazuk WB, Emrich S, Schnable PS (2007) SNP mining from maize 454 EST sequences. *Cold Spring Harbor Protocols*; 2007. doi:10.1101/pdb.prot4786

- Barbour MM (2007) Stable oxygen isotope composition of plant tissue: a review. *Funct Plant Biol* 34:83–94
- Beebe SE, Rao IM, Cajiao C, Grajales M (2008) Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. *Crop Sci* 48:582–592
- Bernier J, Kumar A, Venuprasad R, Spaner D, Atlin G (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci* 47:507–518
- Bhatnagar-Mathur P, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. *Plant Cell Rep* 27:411–424
- Blum A (1988) Plant breeding for stress environments. CRC Press, Boca Raton
- Blum A (1996) Constitutive traits affecting plant performance under stress. In: Bänziger M, Mickelson HR, Peña-Valdivia CB, Edmeades GO (eds) Developing drought- and low nitrogen-tolerant maize. CIMMYT, Mexico DF, pp 131–135
- Blum A (2005) Drought resistance, water use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive? *Aust J Agric Res* 56:1159–1168
- Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crop Res* 112:119–123
- Bohnert HJ, Gong Q, Li P, Ma S (2006) Unraveling abiotic stress tolerance mechanisms – getting genomics going. *Curr Opin Plant Biol* 9:180–188
- Bolaños J, Edmeades GO (1993) Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass, and radiation utilization. *Field Crop Res* 31:233–252
- Bonafede M, Kong L, Tranquilli G, Ohm H, Dubcovsky J (2007) Reduction of a *Triticum monococcum* chromosome segment carrying the softness genes *pinA* and *pinB* translocated to bread wheat. *Crop Sci* 47:821–826
- Borrelli GM, De Vita P, Mastrangelo AM, Cattivelli L (2009) Integrated views in plant breeding: modern approaches for an old topic. In: Sadras VO, Calderini DF (eds) Applied crop physiology: boundaries with genetic improvement and agronomy. Part 3 – Crop physiology, genetic improvement, and agronomy. Elsevier, Amsterdam
- Boyle MG, Boyer JS, Morgan PW (1991) Stem infusion of liquid culture medium prevents reproductive failure of maize at low water potential. *Crop Sci* 31:1246–1252
- Breseghele F, Sorrells ME (2006) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetics* 172:1165–1177
- Calderini DF, Slafer GA (1998) Changes in yield and yield stability in wheat during the 20th century. *Field Crop Res* 57:335–347
- Calderini DF, Dreccer MF, Slafer GA (1995) Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest. *Trends Plant Breed* 114:108–112
- Campbell MA, Fitzgerald HA, Ronald PC (2002) Engineering pathogen resistance in crop plants. *Transgenic Res* 11:599–613
- Campos H, Cooper M, Habben JE, Edmeades GO, Schussler JR (2004) Improving drought tolerance in maize: a view from industry. *Field Crop Res* 90:19–34
- Cattivelli L, Delogu G, Terzi V, Stanca AM (1994) Progress in barley breeding. In: Slafer GA (ed) Genetic improvement of field crops. Marcel Dekker, New York, pp 95–181
- Cattivelli L, Baldi P, Crosatti C, Di Fonzo N, Faccioli P, Grossi M, Mastrangelo AM, Pecchioni N, Stanca AM (2002) Chromosome regions and stress-related sequences involved in resistance to abiotic stress in *Triticaceae*. *Plant Mol Biol* 48:649–665
- Cattivelli L, Rizza F, Badeck F-W, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. *Field Crop Res* 105:1–14
- Ceccarelli S (1994) Specific adaptation and breeding for marginal conditions. *Euphytica* 77:205–219
- Chaerle K, Leinonen L, Jones HG, Van Der Straeten D (2007) Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging. *J Exp Bot* 58:773–784
- Chimentì CA, Marcantonio M, Hall AJ (2006) Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. *Field Crop Res* 95:305–315

- Collard BCY, Mackill DJ (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos Trans R Soc B* 363:557–572
- Condon AG, Richards RA, Rebetzke GJ, Farquhar GD (2004) Breeding for high water-use efficiency. *J Exp Bot* 55:2447–2460
- Courtois B, Shen L, Petalcorin W, Sarandang S, Mauleon R, Li Z (2003) Location QTLs controlling constitutive root traits in the rice population IAC 165×Co39. *Euphytica* 134:335–345
- Courtois B, Ahmadi N, Khowaja F, Price AH, Rami JF, Frouin J, Hamelin C, Ruiz M (2009) Rice root genetic architecture: meta-analysis from a drought QTL database. *Rice* 2:115–128
- De Block M, Verduyn C, De Brouwer D, Cornelissen M (2005) Poly(ADP-ribose) polymerase in plants affects energy homeostasis, cell death and stress tolerance. *Plant J* 41:95–106
- De Vita P, Mastrangelo AM, Matteu L, Mazzucotelli E, Virzi N, Palumbo M, Lo Storto M, Rizza F, Cattivelli L (2010) Genetic improvement effects on yield stability in durum wheat genotypes grown in Italy. *Field Crop Res* 119:68–77
- Delseny M, Han B, Hsing YI (2010) High throughput DNA sequencing: the new sequencing revolution. *Plant Sci* 179:407–422
- Diab AA, Teulat B, This D, Ozturk NZ, Bensch D, Sorrells ME (2004) Identification of drought-inducible genes and differentially expressed sequence tags in barley. *Theor Appl Genet* 109:1417–1425
- Dilbirli M, Erayman M, Campbell BT, Randhawa HS, Baenziger PS (2006) High-density mapping and comparative analysis of agronomically important traits on wheat chromosome 3A. *Genomics* 88:74–87
- Dong J, Chen C, Chen Z (2003) Expression profiles of the Arabidopsis WRKY gene superfamily during plant defense response. *Plant Mol Biol* 51:21–37
- Dowkiw A, Wright GC, Cruickshank A, Redden R (2000) Indirect selection for drought resistance: a pilot study. *ACIAR Food Legume Newslett* 31:4–10
- Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *Plant J* 33:751–763
- Edmeades GO, Bolanos J, Lafitte HR (1992) Progress in breeding for drought tolerance in maize. In: Wilkinson D (ed) Proceedings of the 47th annual corn and sorghum industry research conference, Chicago. American Seed Trade Association, Chicago, pp 93–111
- Edmeades GO, Bänziger M, Beck D, Bolaños J, Ortega A (1997) Development and per se performance of CIMMYT maize populations as drought-tolerant sources. In: Edmeades GO et al (eds) Developing drought and low-N tolerant maize. CIMMYT, Mexico DF, pp 254–262
- Edmeades GO, Bolanos J, Chapman SC, Lafitte HR, Bänziger M (1999) Selection improves drought tolerance in tropical maize populations: I. Gains in biomass, grain yield and harvest index. *Crop Sci* 39:1306–1315
- Edmeades GO, McMaster GS, White JW, Campos H (2004) Genomics and physiologist: bridging the gap between genes and crop response. *Field Crop Res* 90:5–18
- Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor Appl Genet* 112:1164–1171
- Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust J Plant Physiol* 11:539–552
- Finlay KW, Wilkinson GN (1963) The analysis of adaptation in a plant breeding programme. *Aust J Agric Res* 14:742–754
- Fischer RA, Maurer R (1978) Drought resistance in spring wheat cultivars. I. Grain yield response. *Aust J Agric Res* 29:897–912
- Francia E, Tacconi G, Crosatti C, Barabaschi D, Bulgarelli D, Dall'Aglio E, Valè G (2005) Marker assisted selection in crop plants. *Plant Cell Tissue Organ Cult* 82:317–342
- Frederick JR, Woolley JT, Hesketh JD, Peters DB (1990) Seed yield and agronomic traits of old and modern soybean cultivars under irrigation and soil water-deficit. *Field Crop Res* 27:71–82

- Frederick JR, Woolley JT, Hesketh JD, Peters DB (1991) Water deficit development in old and new soybean cultivars. *Agron J* 82:76–81
- Fresneau C, Ghashghaie J, Cornic G (2007) Drought effect on nitrate reductase and sucrose-phosphate synthase activities in wheat (*Triticum durum* L.): role of leaf internal CO₂. *J Exp Bot* 58:2983–2992
- Ganal MW, Altmann T, Röder MS (2009) SNP identification in crop plants. *Curr Opin Plant Biol* 12:211–217
- Garg A, Kim J, Owens T, Ranwala A, Choi Y, Kochian L, Wu R (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc Natl Acad Sci USA* 99:15898–15903
- Gaxiola RA, Li J, Undurraga S, Dang LM, Allen GJ, Alper SL, Fink GR (2001) Drought- and salt-tolerant plants result from overexpression of the AVP1 H⁺-pump. *Proc Natl Acad Sci USA* 98:11444–11449
- Ghashghaie J, Duranceau M, Badeck F-W, Adeline MT, Cornic G, Deléens E (2001) Delta-13 C of CO₂ respired in the dark in relation to delta-13 C of leaf metabolites: comparison between *Nicotiana sylvestris* and *Helianthus annuus* under drought. *Plant Cell Environ* 24:505–515
- González-Martínez SC, Ersoz E, Brown GR, Wheeler NC, Neale DB (2006) DNA sequence variation and selection of tag single-nucleotide polymorphisms at candidate genes for drought-stress response in *Pinus taeda* L. *Genetics* 172:1915–1926
- González-Martínez SC, Huber D, Ersoz E, Davis JM, Neale DB (2008) Association genetics in *Pinus taeda* L. II. Carbon isotope discrimination. *Heredity* 101:19–26
- Gore MA, Chia J-M, Elshire RJ, Sun Q, Ersoz ES, Hurwitz BL, Peiffer JA, McMullen MD, Grills GS, Ross-Ibarra J, Ware DH, Buckler ES (2009) A first-generation haplotype Map of maize. *Science* 326:1115–1119
- Gubis J, Vaňková R, Červená V, Dragúňová M, Hudcovicová M, Lichtnerová H, Dokupil T, Jureková Z (2007) Transformed tobacco plants with increased tolerance to drought. *S Afr J Bot* 73:505–511
- Habier D, Fernando RL, Dekkers JCM (2007) The impact of genetic relationship information on genome-assisted breeding values. *Genetics* 177:2389–2397
- Hall AJ, Vilella F, Trapani N, Chimenti C (1982) The effect of water stress and genotype on the dynamics of pollen shedding and silking in maize. *Field Crop Res* 5:349–363
- Heffner EL, Sorrells ME, Jannink J-L (2009) Genomic selection for crop improvement. *Crop Sci* 49:1–12
- Horie T, Matsuura S, Takai T, Kuwasaki K, Ohsumi A, Shiraiwa T (2006) Genotypic difference in canopy diffusive conductance measured by a new remote-sensing method and its association with the difference in rice yield potential. *Plant Cell Environ* 29:653–660
- Hospital F (2009) Challenges for effective marker-assisted selection in plants. *Genetica* 136:303–310
- Hsieh TH, Lee JT, Chang YY, Chan MT (2002) Tomato plants ectopically expressing Arabidopsis CBF1 show enhanced resistance to water deficit stress. *Plant Physiol* 130:618–626
- Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc Natl Acad Sci USA* 103:12987–12992
- Huang X, Feng Q, Qian Q et al (2009) High-throughput genotyping by whole-genome resequencing. *Genome Res* 19:1068–1076
- Idso SB, Reginato R, Reicosky D, Hatfield J (1981) Determining soil induced plant water potential depression in alfalfa by means of infrared thermometer. *Agron J* 73:826–830
- International HapMap Consortium, Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, Belmont JW, Boudreau A, Hardenbols P et al (2007) A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449:851–861
- Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional Analysis of Rice DREB1/CBF-Type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol* 47:1–13
- Jacoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in Arabidopsis. *Trends Plant Sci* 7:106–111

- Jannoo N, Grivet L, Dookun A, D'Hont A, Glaszmann JC (1999) Linkage disequilibrium among modern sugarcane cultivars. *Theor Appl Genet* 99:1053–1060
- Jeong JS, Kim YS, Baek KH, Jung H, Ha SH, Choi YD, Kim M, Reuzeau C, Kim JK (2010) Root specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. *Plant Physiol.* doi:10.1104/pp. 110.154773
- Jiang GH, He YQ, Xu CG, Li XH, Zhang Q (2004) The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an indica by japonica cross. *Theor Appl Genet* 108:688–698
- Johnson WC, Jackson LE, Ochoa O, van Wijk R, Peleman J, St Clair DA, Michelmore RW (2000) A shallow-rooted crop and its wild progenitor differ at loci determining root architecture and deep soil water extraction. *Theor Appl Genet* 101:1066–1073
- Juenger TE, McKay JK, Hausmann N, Keurentjes JJB, Sen S, Stowe KA, Dawson TE, Simms EL, Richards JH (2005) Identification and characterization of QTL underlying whole plant physiology in *Arabidopsis thaliana*: d13C, stomatal conductance and transpiration efficiency. *Plant Cell Environ* 28:697–708
- Kane NC, Rieseberg LH (2007) Selective sweeps reveal candidate genes for adaptation to drought and salt tolerance in common sunflower, *Helianthus annuus*. *Genetics* 175:1823–1834
- Karamanos AJ, Papatheohari AY (1999) Assessment of drought resistance of crop genotypes by means of the Water Potential Index. *Crop Sci* 39:1792–1797
- Kerstiens G, Schreiber L, Lenzian KJ (2006) Quantification of cuticular permeability in genetically modified plants. *J Exp Bot* 57:2547–2552
- Khlestkina EK, Salina EA (2006) SNP markers: methods of analysis, ways of development, and comparison on an example of common wheat. *Russ J Genet* 42:585–594
- Kim M-J, Lim G-H, Kim E-S, Ko C-B, Yang K-Y, Jeong J-A, Lee M-C, Kim CS (2007) Abiotic and biotic stress tolerance in *Arabidopsis* overexpressing the Multiprotein bridging factors 1a (MBF1a) transcriptional coactivator gene. *Biochem Biophys Res Commun* 354:440–446
- Korzun V (2002) Use of molecular markers in cereal breeding. *Cell Mol Biol Lett* 7:811–820
- Kraakman ATW, Niks RE, Van den Berg PMMM, Stam P, Van Eeuwijk FA (2004) Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. *Genetics* 168:435–446
- Kraft T, Hansen M, Nilsson NO (2000) Linkage disequilibrium and fingerprinting in sugar beet. *Theor Appl Genet* 101:323–326
- Lafitte HR, Courtois B (2002) Interpreting cultivar x environment interactions for yield in upland rice assigning value to drought-adaptive traits. *Crop Sci* 42:1409–1420
- Lafitte R, Blum A, Atlin G (2003) Using secondary traits to help identify drought-tolerant genotypes. In: Fischer KS, Lafitte R, Fukai S, Atlin G (eds) *Breeding rice for drought-prone environments*. International Rice Research Institute, Los Banos
- Laporte MM, Shen B, Tarczynski MC (2002) Engineering for drought avoidance: expression of maize NADP-malic enzyme in tobacco results in altered stomatal function. *J Exp Bot* 53:699–705
- Levitt J (1972) *Responses of plants to environmental stresses*. Academic, New York
- Li XP, Tain AG, Luo GZ, Gong ZZ, Zhang JS, Chan SY (2005) Soybean DRE-binding transcription factors that are responsive to abiotic stresses. *Theor Appl Genet* 110:1355–1362
- Lin CS, Binn MR (1988) A superiority measure of cultivar performance for cultivar x location data. *Can J Plant Sci* 68:193–198
- Lister R, Gregory BD, Ecker JR (2009) Next is now: new technologies for sequencing of genomes, transcriptomes, and beyond. *Curr Opin Plant Biol* 12:107–118
- Ludlow MM, Muchow RC (1990) A critical evaluation of traits for improving crop yields in water-limited environments. *Adv Agron* 43:107–152
- Maccaferri M, Sanguineti MC, Corneti S, Araus Ortega JL, Ben Salern M, Bort J, DeAmbrogio E, del Moral LG, Demontis A, El-Ahmed A, Maalouf F, Machlab H, Martos V, Moragues M, Motawaj J, Nachit MM, Nserallah N, Ouabbou H, Royo C, Slama A, Tuberosa R (2008) Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. *Genetics* 178:489–511

- Maccaferri M, Sanguineti MC, Giuliani S, Tuberosa R (2009) Genomics of tolerance to abiotic stress in the Triticeae. In: Feuillet C, Muehlbauer G (eds) Plant genetics and genomics: crops and models, vol 7, Genetics and genomics of the Triticeae. Springer, Dordrecht, pp 481–558
- Martin B, Nienhuis J, King G, Schaeffer A (1989) Restriction fragment length polymorphism associated with water use efficiency in tomato. *Science* 243:1725–1728
- Masle J, Gilmore SR, Farquhar GD (2005) The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*. *Nature* 436:866–870
- Meuwissen TH, Hayes BJ, Goddard ME (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157:1819–1829
- Miralles DJ, Richards R, Slafer GA (2000) Duration of the stem elongation period influences the number of fertile florets in wheat and barley. *Aust J Plant Physiol* 27:931–940
- Mittler R (2006) Abiotic stress, the field environment and stress combination. *Trends Plant Sci* 11:15–19
- Monneveux P, Sánchez C, Beck D, Edmeades GO (2006) Drought tolerance improvement in tropical maize source populations. Evidence of progress. *Crop Sci* 46:180–191
- Morgante M, Salamini F (2003) From plant genomics to breeding practice. *Curr Opin Biotechnol* 14:214–219
- Motzo R, Giunta F, Deidda M (2001) Factors affecting the genotype x environment interaction in spring triticale grown in a Mediterranean environment. *Euphytica* 121:317–324
- Nedbal L, Whitmarsh J (2004) Chlorophyll fluorescence imaging of leaves and fruits. In: Papageorgiou GC, Govindjee (eds) Chlorophyll fluorescence: a signature of photosynthesis. Kluwer Academic Publishers, Dordrecht
- Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni PP, Donnarummo MG, Hinchey BS, Kumimoto RW, Maszle DR, Canales RD, Krolkowski KA, Dotson SB, Gutterson N, Ratcliffe OJ, Heard JE (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. *Proc Natl Acad Sci USA* 104:16450–16455
- Nguyen TT, Klueva N, Chamareck V, Aarti A, Magpantay G, Millena AC, Pathan MS, Nguyen HT (2004) Saturation mapping of QTL regions and identification of putative candidate genes for drought tolerance in rice. *Mol Genet Genomics* 272:35–46
- Nigam SN, Chandra S, Sridevi KR, Bhukta M, Reddy AGS, Rachaputi NR, Wright GC, Reddy PV, Deshmukh MP, Mathur RK, Basu MS, Vasundhara S, Varman PV, Nagda AK (2005) Efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut. *Ann Appl Biol* 146:433–439
- Ober ES, Clark CJA, Le Bloa M, Royal A, Jaggard KW, Pidgeon JD (2004) Assessing the genetic resources to improve drought tolerance in sugar beet: agronomic traits of diverse genotypes under droughted and irrigated conditions. *Field Crop Res* 90:213–234
- Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim Y-K, Kim NY-K, Nahm BH, Kim J-K (2005) *Arabidopsis* CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. *Plant Physiol* 138:341–351
- Olsen AN, Ernst HA, Leggio LL, Skriver K (2006) NAC transcription factors: structurally distinct, functionally diverse. *Trends Plant Sci* 10:79–87
- Oxborough K (2004) Using chlorophyll a fluorescence imaging to monitor photosynthetic performance. In: Papageorgiou GC, Govindjee (eds) Chlorophyll a fluorescence: a signature of photosynthesis. Springer, Dordrecht, pp 409–428
- Pantuwan G, Fukai S, Cooper M, Rajatasereekul S, O'Toole JC (2002) Yield response of rice (*Oryza sativa* L.) genotypes to different types of drought under rainfed lowlands. Part1. Grain yield and yield components. *Field Crop Res* 73:153–168
- Park S, Li J, Pittman JK, Berkowitz GA, Yang H, Undurraga S, Morris J, Hirschi KD, Gaxiola RA (2005) Up-regulation of a H⁺-pyrophosphatase (H⁺-PPase) as a strategy to engineer drought-resistant crop plants. *Proc Natl Acad Sci USA* 102:18830–18835
- Parry MAJ, Flexas J, Medrano H (2005) Prospects for crop production under drought: research priorities and future directions. *Ann Appl Biol* 147:211–226

- Passioura JB (1977) Grain yield, harvest index and water use of wheat. *J Aust Inst Agric Sci* 43:117–120
- Passioura JB (2007) The drought environment: physical, biological and agricultural perspectives. *J Exp Bot* 58:113–117
- Peleman JD, Van der Voort JR (2003) Breeding by design. *Trends Plant Sci* 8:330–334
- Pellegrineschi A, Reynolds M, Pacheco M, Brito RM, Almeraya R, Yamaguchi-Shinozaki K, Hoisington D (2004) Stress-induced expression in wheat of the *Arabidopsis thaliana* DREB1A gene delays water stress symptoms under greenhouse conditions. *Genome* 47:493–500
- Pidgeon JD, Ober ES, Qi A, Clark CJA, Royal A, Jaggard KW (2006) Using multi-environment sugar beet variety trials to screen for drought tolerance. *Field Crop Res* 95:268–279
- Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? *Trends Ecol Evol* 20:481–486
- Podlich DW, Winkler CR, Cooper M (2004) Mapping as You Go: an effective approach for marker-assisted selection of complex traits. *Crop Science* 44:1560–1571
- Quan R, Shang M, Zhang H, Zhao Y, Zhang J (2004) Engineering of enhanced glycinebetaine synthesis improves drought tolerance in maize. *Plant Biotechnol J* 2:477–486
- Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessimbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti MC, Hollington PA, Aragues R, Royo A, Dodig D (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring \times SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theor Appl Genet* 110:865–880
- Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. *Curr Opin Plant Biol* 5:94–100
- Rebetzke GJ, Condon AG, Richards RA, Farquhar GD (2002) Selection for reduced carbon-isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. *Crop Sci* 42:739–745
- Reymond M, Muller B, Leonardi A, Charcosset A, Tardieu F (2003) Combining QTL analysis and an ecophysiological model to analyse the genetic variability of the responses of maize leaf growth to temperature and water deficit. *Plant Physiol* 131:664–675
- Reymond M, Muller B, Tardieu F (2004) Dealing with the genotype \times environment interaction via a modelling approach: a comparison of QTLs of maize leaf length or width with QTLs of model parameters. *J Exp Bot* 55:2461–2472
- Reynolds MP, Mujeeb-Kazi A, Sawkins M (2005) Prospects for utilizing plant-adaptive mechanisms to improve wheat and other crops in drought and salinity-prone environments. *Ann Appl Biol* 146:239–259
- Rharrabi Y, Villegas D, Garcia del Moral LF, Aparicio N, Elhani S, Royo C (2001) Environmental and genetic determination of protein content and grain yield in durum wheat under Mediterranean conditions. *Plant Breed* 120:381–388
- Ribaut JM, Ragot M (2007) Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. *J Exp Bot* 58:351–360
- Ribaut JM, Hoisington D, Deutsch JA, Jiang C, Gonz  les-de-Le  n D (1996) Identification of quantitative trait loci under drought conditions in tropical maize. 1. Flowering parameters and the anthesis-silking interval. *Theor Appl Genet* 92:905–914
- Richards RA (2006) Physiological traits used in the breeding of new cultivars for water-scarce environments. *Agric Water Manage* 80:197–211
- Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, Blumwald E (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proc Natl Acad Sci USA* 104:19631–19636
- Rizza F, Badeck FW, Cattivelli L, Li Destri O, Di Fonzo N, Stanca AM (2004) Use of a water stress index to identify barley genotypes adapted to rainfed and irrigated conditions. *Crop Sci* 44:2127–2137
- Robbins MD, Staub JE (2009) Comparative analysis of marker-assisted and phenotypic selection for yield components in cucumber. *Theor Appl Genet* 119:621–634

- Robin S, Pathan MS, Courtois B, Lafitte R, Carandang S, Lanceras S, Amante M, Nguyen HT, Li Z (2003) Mapping osmotic adjustment in an advanced back-cross inbred population of rice. *Theor Appl Genet* 107:1288–1296
- Röder MS, Huang X-Q, Ganal MW (2004) Wheat microsatellites: potential and implications. In: Lörz H, Wenzel G (eds) *Molecular marker systems in plant breeding and crop improvement*, vol 55, *Biotechnology in agriculture and forestry*. Springer, Berlin/Heidelberg, pp 255–266
- Ronde JAD, Cress WA, Krugerd GHJ, Strasser RJ, Van Staden J (2004) Photosynthetic response of transgenic soybean plants, containing an Arabidopsis P5CR gene, during heat and drought stress. *J Plant Physiol* 161:1211–1224
- Rosielle AA, Hamblin J (1981) Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Sci* 21:943–946
- Royo C, Villegas D, Garcia del Moral LF, Elhani S, Aparicio N, Rharrabti Y, Araus JL (2002) Comparative performance of carbon isotope discrimination and canopy temperature depression as predictors of genotypes differences in durum wheat yield in Spain. *Aust J Agric Res* 53:561–569
- Royo C, Aparicio N, Villegas D, Casadesus J, Monneveux P, Araus JL (2003) Usefulness of spectral reflectance indices as durum wheat yield predictors under contrasting Mediterranean conditions. *Int J Remote Sens* 24:4403–4419
- Royo C, Aparicio N, Blanco R, Villegas D (2004) Leaf and green area development of durum wheat genotypes grown under Mediterranean conditions. *Eur J Agron* 20:419–430
- Sade N, Vinocur BJ, Diber A, Shatil A, Ronen G, Nissan H, Wallach R, Karchi H, Moshelion M (2009) Improving plant stress tolerance and yield production: is the tonoplast aquaporin S1TIP2;2 a key to isohydric to anisohydric conversion? *New Phytol* 181:651–661
- Sahrawat AK, Becker D, Lütticke S, Lörz H (2003) Genetic improvement of wheat via alien gene transfer, an assessment. *Plant Sci* 165:1147–1168
- Saijo Y, Hata S, Kyojuka J, Shimamoto K, Izui K (2000) Over-expression of a single Ca²⁺-dependent protein kinase confers both cold and salt/drought tolerance on rice plants. *Plant J* 23:319–327
- Saini HS, Westgate ME (2000) Reproductive development in grain crops during drought. *Adv Agron* 68:59–96
- Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell* 18:1292–1309
- Salekdeh GH, Reynolds M, Bennett J, Boyer J (2009) Conceptual framework for drought phenotyping during molecular breeding. *Trends Plant Sci* 14:488–496
- Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. *Trends Plant Sci* 10:297–304
- Sanchez AC, Subudhi PK, Rosenow DT, Nguyen HT (2002) Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). *Plant Mol Biol* 48:713–726
- Saranga Y, Jiang C-X, Wright RJ, Yakir D, Paterson AH (2004) Genetic dissection of cotton physiological responses to arid conditions and their inter-relationships with productivity. *Plant Cell Environ* 27:263–277
- Schlichting CD (1986) The evolution of phenotypic plasticity in plants. *Annu Rev Ecol Syst* 17:667–693
- Serraj R, Sinclair TR (2002) Osmolyte accumulation: can it really increase crop yield under drought conditions? *Plant Cell Environ* 25:333–341
- Sharma HC, Crouch JH, Sharma KK, Seetharama N, Hash CT (2002) Applications of biotechnology for crop improvement: prospects and constraints. *Plant Sci* 163:381–395
- Shen YG, Du BX, Zhang WK, Zhang JS, Chen SY (2002) AhCMO, regulated by stresses in *Atriplex hortensis*, can improve drought tolerance in transgenic tobacco. *Theor Appl Genet* 105:815–821
- Shen YG, Zhang WK, He SJ, Zhang JS, Liu Q, Chen SY (2003) An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration and ABA stress. *Theor Appl Genet* 106:923–930
- Simmonds NW (1991) Selection for local adaptation in a plant breeding programme. *Theor Appl Genet* 82:363–367

- Slafer GA, Abeledo LG, Miralles DJ, Gonzalez FG, Whitechurch EM (2001) Photoperiod sensitivity during stem elongation as an avenue to raise potential yield in wheat. *Euphytica* 119:191–197
- Slafer GA, Araus JL, Royo C, Del Moral LFG (2005) Promising ecophysiological traits for genetic improvement of cereal yields in Mediterranean environments. *Ann Appl Biol* 146:61–70
- Soika RE, Stolzy LH, Fischer RA (1981) Seasonal drought response of selected wheat cultivars. *Agron J* 73:838–845
- Steele K (2009) Novel upland rice variety bred using marker-assisted selection and client-oriented breeding released in Jharkhand. Bangor University, India
- Steele KA, Price AH, Shashidhar HE, Witcombe JR (2006) Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theor Appl Genet* 112:208–221
- Steele KA, Virk DS, Kumar R, Prasad SC, Witcombe JR (2007) Field evaluation of upland rice lines selected for QTLs controlling root traits. *Field Crop Res* 101:180–186
- Syvänen AC (2005) Toward genome-wide SNP genotyping. *Nat Genet* 37:S5–S10
- Tambussi EA, Casadesus J, Munne-Bosch S, Araus JL (2002) Photoprotection in water stressed plants of durum wheat (*Triticum turgidum* L. var. *durum*): changes in chlorophyll fluorescence, spectral signature and photosynthetic pigments. *Funct Plant Biol* 29:35–44
- Tambussi EA, Nogues S, Ferrio P, Voltas J, Araus JL (2005) Does higher yield potential improve barley performance in Mediterranean conditions? A case of study. *Field Crop Res* 91:149–160
- Tester M, Langridge P (2010) Breeding technologies to increase crop production in a changing world. *Science* 327:818–822
- Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A (1998) Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare* L.). *Theor Appl Genet* 96:688–698
- Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, Buckler ES (2001) Dwarf8 polymorphisms associate with variation in flowering time. *Nat Genet* 28:286–289
- Tollenaar M (1989) Genetic improvement in grain yield of commercial maize hybrids grown in Ontario from 1959 to 1988. *Crop Sci* 29:1365–1371
- Tollenaar M, Lee EA (2002) Yield stability and stress tolerance in maize. *Field Crop Res* 75:161–169
- Tollenaar M, Wu J (1999) Yield in temperate maize is attributable to greater stress tolerance. *Crop Sci* 39:1597–1604
- Tondelli A, Francia E, Barabaschi D, Aprile A, Skinner JS, Stockinger EJ, Stanca AM, Pecchioni N (2006) Mapping regulatory genes as candidates for cold and drought stress tolerance in barley. *Theor Appl Genet* 112:445–454
- Trethowan RM, van Ginkel M, Rajaram S (2002) Progress in breeding wheat for yield and adaptation in global drought affected environments. *Crop Sci* 42:1441–1446
- Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Curr Opin Biotechnol* 17:113–122
- van Ginkel M, Calhoun DS, Gebeyehu G, MirandaA Tian-you C, Pargas Lara R, Trethowan RM, Sayre K, Crossa J, Rajaram S (1998) Plant traits related to yield of wheat in early, late, or continuous drought conditions. *Euphytica* 100:109–121
- Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. *Trends Biotechnol* 23:48–55
- Venuprasad R, Lafitte HR, Atlina GN (2007) Response to direct selection for grain yield under drought stress in rice. *Crop Sci* 47:285–293
- Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW (2004) Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. *Euphytica* 135:255–263
- Villegas D, Aparicio N, Blanco R, Royo C (2001) Biomass accumulation and main stem elongation of durum wheat grown under Mediterranean conditions. *Ann Bot* 88:617–627

- Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr Opin Biotechnol* 16:123–132
- Voltas J, Romagosa I, Muñoz P, Araus JL (1998) Mineral accumulation, carbon isotope discrimination and indirect selection for grain yield in two-rowed barley grown under semiarid conditions. *Eur J Agron* 9:147–155
- Voltas J, Lopez-Corcoles H, Borrás G (2005) Use of biplot analysis and factorial regression for the investigation of superior genotypes in multi-environment trials. *Eur J Agron* 22:309–324
- Wade MJ (2002) A gene's eye view of epistasis, selection, and speciation. *J Evol Biol* 15:337–346
- Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperature: towards genetic engineering for stress tolerance. *Planta* 218:1–14
- Wang Y, Ying J, Kuzma M, Chalifoux M, Sample A, McArthur C, Uchacz T, Sarvas C, Wan J, Tennis DT, McCourt P, Huang Y (2005) Molecular tailoring of farnesylation for plant drought tolerance and yield protection. *Plant J* 43:413–424
- Xiong L, Yang Y (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell* 15:745–759
- Xu JL, Lafitte HR, Gao YM, Fu BY, Torres R, Li ZK (2005) QTLs for drought escape and tolerance identified in a set of random introgression lines of rice. *Theor Appl Genet* 111:1642–1650
- Yadav OP, Bhatnagar SK (2001) Evaluation of indices for identification of pearl millet cultivars adapted to stress and non-stress conditions. *Field Crop Res* 70:201–208
- Yamada M, Morishita H, Urano K, Shiozaki N, Yamaguchi-Shinozaki K, Shinozaki K, Yoshida Y (2005) Effects of free proline accumulation in petunias under drought stress. *J Exp Bot* 56:1975–1981
- Yang KY, Liu Y, Zhang S (2001) Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. *Proc Natl Acad Sci USA* 98:741–746
- Zamir D (2001) Improving plant breeding with exotic genetic libraries. *Nat Rev Genet* 2:983–989
- Zhang Q (2007) Strategies for developing Green Super Rice. *Proc Natl Acad Sci USA* 104:16402–16409
- Zhang JZ, Creelman RA, Zhu JK (2004) From laboratory to field. Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. *Plant Physiol* 135:615–621
- Zhang X, Zhou S, Fu Y, Su Z, Wang X, Sun C (2006) Identification of a drought tolerant introgression line derived from Dongxiang common wild rice (*O. rufipogon* Griff.). *Plant Mol Biol* 62:247–259
- Zhang GH, Su Q, An LJ, Wu S (2008) Characterization and expression of a vacuolar Na⁺/H⁺ antiporter gene from the monocot halophyte *Aeluropus litoralis*. *Plant Physiol Biochem* 46:117–126
- Zheng BS, Yang L, Zhang WP, Mao CZ, Wu YR, Yi KK, Liu FY, Wu P (2003) Mapping QTLs and candidate genes for rice root traits under different water-supply conditions and comparative analysis across three populations. *Theor Appl Genet* 107:1505–1515
- Zhong S, Dekkers JCM, Fernando RL, Jannink JL (2009) Factors affecting accuracy from genomic selection in populations derived from multiple inbred lines: a barley case study. *Genetics* 182:355–364
- Zhou SX, Tian F, Zhu ZF, Fu YC, Wang XK, Sun CQ (2006) Identification of quantitative trait loci controlling drought tolerance at seedling stage in Chinese Dongxiang common wild rice (*Oryza rufipogon* Griff.). *Acta Genet Sin* 33:551–558