

*Full Length Research Paper*

# **An overview of genomics assisted improvement of drought tolerance in maize (*Zea mays* L.): QTL approaches**

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Today drought is the worldwide problem that causes the reduction in the crop yield of plants. Maize (*Zea mays* L.) is greatly affected by drought and its production is declining day by day. However there are many techniques accessible today to achieve a better understanding of the multifarious network of plant responses to drought and to maneuver these responses. These techniques comprise the association mapping, mapping of quantitative trait loci (QTLs), studies of gene expression and genetic transformation. Selection based on performance in multi-environment trials (MET) has increased grain yield under drought through increased yield potential and kernel set, rapid silk exertion, and reduced barrenness, though at a lower rate than under optimal conditions. Knowledge of the physiology of drought tolerance has been used to dissect the trait into a series of key processes. This has been complemented by genetic dissection through the identification of QTL associated with these same traits. Both have been used to identify suitable organ- and temporal-specific promoters and structural genes. The present paper will overview both conventional breeding and QTL/MAS approaches towards improvement of maize yield in drought-prone environments and will briefly describe a QTL approach.

**Key Words:** Drought, QTL, Maize, *zea mays*, multi-environment trials, promoters, marker assisted selection, Pakistan.

## **INTRODUCTION**

Maize (*Zea mays* L.) world's leading cereal food crop with added importance for countries like Pakistan where rapidly increasing population has already outstripped the available food supplies. Maize is dual purpose crop such as food for human and feed for livestock and also used as industrial raw material for the manufacture of different products. Maize has highly nutritive value as it contains sugar, and 1.70 % ash (Chaudhary, 1983). The maize 72 % starch, 10 % protein, 4.80 % oil, 9.50 % fiber, 3.0 %

grain is used for making starch, glucose, oil and various other products. It is milled to make food products such as maize bread, corn flakes and cereals. The stalks are used for the manufacture of paper, insulator and card boards while rachis is used for the production of pipes, chemicals, methanol tar and furfural as substrate for mushroom cultivation. Drought is a worldwide phenomenon and is a major production restraint, reducing crop yields. The extent of climate change over the next 20 years and its impact are difficult to predict but it is critical to put research in place now that will be needed in the longer term. Maize is the third most important source of calories for humankind after rice and wheat. Most of the total world maize is grown under rain

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fed conditions, and maize is more inclined to drought than all other cereals excluding rice. Drought is already a major abiotic constraint to global food production. Climate change will further exacerbate this condition. Moreover, the expected increase in the world's population over the next decades will pose huge challenges to food fabrication as the available water per capita decreases. Although several methods have been developed, it remains difficult to judge where water is and whether it will be scarce (Araus *et al.*, 2002; Tester and Bacic, 2005). The FAO considers drought to be the single most common cause of ruthless food shortage in developing countries, with more far-reaching penalty than other causes of food shortage, such as conflicts, flooding and economic negligence (Ribaut *et al.*, 2008). The situation will probably be aggravated further by climate change, resulting in more erratic weather, more frequent and stronger storms, with many regions of the world having higher temperatures and less precipitation (Sivakumar *et al.*, 2005). Although, drought stress is also an issue in temperate regions, certain tropical regions will be affected to a greater extent. However, the overriding global change that will occur during the next 40 years is a massive increase in the world's population (Wallace, 2000). This global change will pose enormous challenges to agriculture, particularly in Africa and Asia. The annual renewable water resources are largely fixed. Climate change may alter these resources to a certain extent, but compared to the changes in the availability of water per capita due to population growth, the impact of climate change on the renewable water resources is small (Wallace, 2000). Without significant improvements in the efficiency of water use in agriculture, the per capita availability of water for food production will decrease drastically. It is difficult to estimate and even more difficult to predict the impact of decreases in the availability of water on global food security.

Critical to these considerations is the question of whether water is scarce or whether it is used inefficiently (Rijsberman, 2006). First of all, it is important to determine how much water is required to produce enough food for today's population. Based on predictions of population growth and information about the renewable freshwater resources, it is possible to estimate the extent to which water is and will be scarce (Wallace, 2000). Several indicators have been developed for this purpose (Gleick, 2002). The Falkenmark indicator or "water stress index" (Falkenmark *et al.*, 1989, 1997) is the most widely used. Based on the estimated demand of water for domestic use for agriculture, environment, industry and the energy sector, Falkenmark *et al.* (1997) proposed 1,700 m<sup>3</sup> of renewable water per capita per year as the threshold below which water stress occurs. Regions experience water scarcity when the water supply is below 1,000 m<sup>3</sup>; below 500 m<sup>3</sup> the scarcity of water is extreme.

The Falkenmark indicator is an intuitive measure and is easy to calculate. However, it does not take into account

the infrastructure, which can change water availability. Variations in water requirements resulting from different lifestyles and climates are not represented either. Therefore, Ohlson (1999) proposed a modified Falkenmark indicator, which accounts for a society's capacity to adapt to water scarcity by technological, economic and other means.

The Water Resources Vulnerability Index (WRVI) (Shiklomanov, 1991; Raskin *et al.*, 1997) compares the annual withdrawal of water with the available water resources. Water withdrawal is defined as the amount of water removed from lakes, rivers and groundwater aquifers to satisfy human needs (Rijsberman, 2006). Water scarcity occurs in regions where 20 to 40% of the available water resources are used; when the withdrawal of water exceeds 40% of that available, water scarcity is severe. The Water Resources Vulnerability Index is more objective than the Falkenmark index but has limitations, too. The analysis does not take into account either how much water can be made available for human use through improved technology, or the proportion of withdrawn water that can be recycled. Moreover, there is no information about possible adaptation of the population to water stress. The International Water Management Institute (IWMI) attempted to overcome these limitations by developing an indicator of physical and economic water scarcity. The IWMI analyzed the proportion of renewable water resources that is available to meet human needs; it differentiated between water consumption (for example, evaporation) and water use, which allows for recycling (return of flow) and considered the adaptation through changes in the infrastructure and management (for example, more efficient irrigation systems). Some countries will not meet the estimated demand for water in 2025 because water is physically scarce, whereas in other countries water is economically scarce. With considerable investments in infrastructure, these countries will be able to meet the estimated demand for water in 2025. Although, the IWMI analysis is more precise than the Falkenmark indicator or the Water Resources Vulnerability Index, it still provides only a rough measure at a national scale.

According to the IWMI, 75% of the world's population lives in areas characterized by physical water scarcity. One billion people live in regions with economic scarcity (IWMI, 2006). Based on the United Nation's medium population projections of 1998, more than 2.8 billion people in 48 countries will face water stress or scarcity by 2025. Arid and semiarid regions account for approximately 30% of the total area of the world (Sivakumar *et al.*, 2005); 40 of the 48 countries in these regions are located in western Asia, North Africa or sub-Saharan Africa. By 2050, 54 countries could face water stress or scarcity, accounting for about 40% of the projected global population of 9.4 billion people (UNESCO, 2002). Based on these predictions, there is an immediate need to increase water use efficiency and

water productivity in agriculture. There is a large potential to increase water productivity by increasing the efficiency of irrigation systems. For example, it has been estimated that almost one third of irrigation water is lost in storage and conveyance (Bos, 1985). Surface runoff and drainage lead to further water loss; thus the efficiency of global irrigation systems is disturbingly low (approximately 37%) (Postel, 1993). Technological improvements, through, for example, the re-use of water, precision irrigation and precision agriculture, drip irrigation, partial root-zone irrigation or deficit irrigation based on measurements of the water status of individual plants or the whole canopy can largely increase irrigation efficiency (Parry et al., 2007).

### Maize yield under drought

The water productivity of crops must also be increased. This is principally vital in rainfed agriculture. The resulting assistance is twofold: On the one hand, food security in rainfed systems will be superior and on the other, the overall water balance will be more encouraging. The more food produced in rainfed systems, the smaller the demand for freshwater resources for irrigation (Wallace, 2000). Genetic improvements (i.e. more drought-tolerant crop varieties) cannot counteract all drought-induced yield losses but, nevertheless, will donate considerably to global food security. Poor farmers in particular can enthusiastically benefit from genetic improvements, which are incorporated in the seed and do not depend on agronomic techniques, on experience in soil and crop management, on the availability of inputs, on infrastructure or access to markets. Passioura (2006) distinguished three factors involved in improving yield under water limited conditions, by developing and growing plants that 1) transpire more water, 2) exchange the transpired water more efficiently for CO<sub>2</sub> in order to produce more biomass, and 3) allocate more biomass to the grains (or to the harvestable part). Considering the plant only, these components can be addressed by altering the morphology and the physiology of the crop. The crop's response to stress depends on numerous traits, many of which are constitutive, but which may also be modified by stress. According to the definition of drought tolerance by Cooper et al. (2006), a drought-tolerant genotype produces higher yields than a drought-susceptible genotype in a variety of water-stressed environments. The ideal genotype combines both a high yield under favorable conditions with tolerance to water stress. The overall goal of breeding for drought tolerance is, therefore, to realize a high maximum yield potential and to reduce the gap between yield potential and yield under stress. Research in plant science must identify drought-tolerance traits and manipulate these traits by "conventional" breeding, marker-assisted breeding or gene transformation, as

demonstrated here for maize. Duvick (2005) gave a comprehensive overview of the contribution of breeding to the improvement of yield in temperate maize. About 50% of the on-farm increase in the yield of hybrid maize during the last 60 to 70 years has been achieved through genetic improvements, mainly by the better tolerance to stress that has been incorporated into newer hybrids. The increase in yield was accompanied by changes in a number of morphological and physiological traits, for example plant height, the anthesis-to-silking interval (ASI), staygreen, lodging resistance and more efficient photosynthesis, as well as an improved photosynthetic rate after stress events (Duvick, 2005). These advances were achieved mainly by selection in rainfed nurseries, which are seldom prone to drought and in which the plants were grown at high planting densities. This measure is especially efficient in maize breeding maize has evolved from a single-plant ecosystem, whereas small-grain cereals have undergone continuous and considerable intra- and interspecific competition. Therefore, selection at high planting densities improves the tolerance of maize to intraspecific competition and suboptimal light conditions in the canopy. Large populations were grown under these conditions and inbred lines with stable yields were recycled (Bruce et al., 2002). Considerable progress in the genetics of tropical maize was also achieved, leading to a higher yield potential and improved drought tolerance (Heisey and Edmeades, 1999; Bänziger et al., 2000; Monneveux et al., 2006b). Breeding for drought tolerance at flowering, the developmental stage, at which maize is most susceptible to drought (Claassen and Shaw, 1970; Westgate and Boyer, 1985), has identified key secondary traits for grain yield, with the anthesis-to-silking interval being the most prominent one. The ASI was very useful in identifying genotypes with a high level of stress tolerance; there was considerable genetic variability in ASI, the ASI was negatively associated with grain yield, it was a good predictor of hybrid performance, and it was easy and cheap to record (Edmeades, 1997). However, as a consequence of continuous selection for a short ASI, the association between grain yield (grain abortion) and ASI decreased over time (Monneveux et al., 2006a). Once the ASI of elite germplasm is sufficiently short, a further reduction is unlikely to generate significant genetic gains (Byrne et al., 1995). Hence, it is necessary to identify novel secondary traits in order to predict the performance of maize genotypes under drought. Further progress in drought tolerance will probably depend on changes in the area architecture, which has a strong impact on the partitioning of assimilates and the radiation use efficiency (Ribaut et al., 2008); however the architecture of the root system and physiological mechanisms that confer drought tolerance will play a significant role also. Based on the progress in improving drought tolerance of tropical maize, the ideotype of a drought-tolerant maize plant is characterized by short

plants, reduced leaf area (especially on the upper part of the stem), small tassels, thick stems, erect leaves, delayed senescence, deeper rooting (with less lateral branching), better grain filling capacity, robust growth of spikelets and kernels and good osmotic adjustment at low water potential (Ribaut et al., 2008).

### Drought research on maize

The importance of these traits can be illustrated for two tropical maize inbred lines that were used to develop a segregating mapping population of recombinant inbred lines (RILs) at the International Maize and Wheat Improvement Center (CIMMYT) in Mexico. CML444 was developed at CIMMYT in the 1990s. It has a squashed phenotype with strong, erectophilic, dark green leaves and produces high yields under drought, despite a reasonably low drought-tolerance index (DTI; defined as the yield under severe stress divided by the yield under non-stress conditions) as a result of its high yield potential under optimal growing conditions. SC-Malawi was developed in Zimbabwe in the 1960s and has long, horizontal, light-green leaves, short internodes at higher positions on the stem, a relatively low yield under stress but, due to its low yield potential, it has a high DTI. Several field experiments conducted at CIMMYT in Mexico and Zimbabwe with drought stress at flowering and under well-watered conditions showed that CML444 performed better than SC-Malawi because of better constitutive traits conferring high plant vigor across water regimes (Messmer *et al.*, 2009). Experiments conducted at the Swiss Federal Institute of Technology (ETH), Zurich, showed that young CML444 plants (up to the 5-leaf stage) produce a deeper root system than that of SC-Malawi and extract more water from deeper soil layers. At the 8-leaf stage, CML444 also produces longer axile roots, essential for a greater vertical (and horizontal) distribution of the root system than SC-Malawi (Hund et al., 2009). Similar differences between these lines were also detected at the 2-leaf stage in a high-throughput phenotyping platform for root traits (Trachsel et al., 2009). These apparently constitutive differences in the architecture of the root system may also be expressed in the field and may be associated with the good drought tolerance of CML444. However, the relationship between root traits and drought tolerance of maize is still unclear. Recurrent selection in tropical maize populations has reduced root biomass and inbred lines with poor early root development had higher yields under drought than inbred lines with vigorous early development of roots (Bruce et al., 2002). However, the root system of the latter could have reached deeper soil layers (Hund et al., 2008).

Further root studies were done by Ruta et al. (2010) at the 2-leaf stage in the high throughput phenotyping platform for root traits (see above) with RILs of another

CIMMYT mapping population, derived from the cross between Ac7643 (P1) and Ac77290/TZSRW (P2). P1 has a short ASI and a relatively high yield under drought, in contrast to P2 (Ribaut et al., 1996). Quantitative trait loci (QTLs) for the shoot-to-root weight ratio and leaf area-to-root length ratio collocated with QTLs for ear number under water stress; this could be an intriguing indicator that the well-known adaptive mechanism, favoring root development over shoot development after the onset of drought, enables the plant to produce a more stable yield under drought in the field. An increase in the growth of axile roots seemed to be linked to a decrease in ASI; the alleles of the drought-tolerant parent, P1, increased the number of crown and seminal roots, while the alleles of P2 increased the ASI at both loci. This is an important indication that the genes responsible for early proliferation and elongation of axile roots may be active at later growth stages, too. These QTLs collocated with QTLs for root pulling force from another marker population (Sanguinetti et al., 1999; Tuberosa et al., 2002b); thus it is expected that there is a relationship between the number of axile roots of seedlings and a well-structured architecture of the root system at later stages.

### Gene expression

Unlike a typical Northern blot that detects the expression of individual messenger RNA only, gene expression profiling technology allows the measurement of steady state messenger RNA levels of thousands of genes at a time. There are many different types of gene expression platforms (Lockhart and Winzeler, 2000), but they all have a common theme: to provide a genome-wide view of the state of gene expression. This technology permits the exploration of responses of gene networks to water deficits while still maintaining the resolution to measure the transcriptional activity of individual genes in tissue sampled at a particular developmental stage in stressed versus unstressed plants (Bohnert et al., 2001; Seki et al., 2001; Wenqiong et al., 2002; Yu and Setter, 2003). We have used both cDNA- and oligonucleotidebased gene-expression profiling platforms to examine the effects of drought stress on maize yields (Habben et al., 2001; Zinselmeier et al., 2002). A considerable difference was observed in gene expression profiles between plants drought stressed in a limited rooting volume in the greenhouse (buckets) versus those stressed in an unlimited rooting volume in the field. Briefly, both bucket and field grown plants were drought stressed around flowering and the overall gene expression in female reproductive tissues was compared. Bucketgrown plants reached a water-deficit status sufficient to completely inhibit photosynthesis within 4 days, whereas field-grown plants required 21 days to reach a similar level of water stress. When assessed using a high-density maize

oligonucleotide array, far fewer (<2% versus 27%) genes showed differential expression in maize ear tissue under a gradually developing stress than under a sudden stress. Since we need to target our research effort to the production environment, more emphasis has been placed on field-based profiling experiments, using managed stress environments (as detailed above) to generate realistic changes in gene expression in tolerant and susceptible germplasm. Other 'omic' technologies can also be used to characterize the response of maize genotypes to drought. These include proteomics and metabolomics that generate global profiles of proteins and metabolites, respectively. These high technologies allow researchers to assemble a large database of molecular responses from field grown plants in a short period of time, and this can be used to determine key pathways associated with drought response. Other commercial companies have reported similar types of investigation (Johnson, 2004).

### Transgenic analysis

When genes and pathways with potential impact upon a trait have been identified, their utility is normally tested by using transformation to introduce the candidate genes into a target genotype one at a time. This step provides a targeted means of altering the plant phenotype, increases the heritability of what is normally a quantitative trait, and provides a gene that is dominant in action and therefore easy to manipulate through conventional breeding. Creation and testing of transgenic events, once a slow process, has become much higher throughput because of improvements in vectors and in plantlet regeneration.

A significant portion of Pioneer's drought research uses transgenic analysis to test hypotheses regarding the role of key processes imparting drought tolerance. The literature contains numerous studies on genes that when ectopically expressed (that is, expressed differentially in time and/or tissue) in model plants induce drought tolerance in one form or another. Maize orthologs of such "drought" genes can then be identified from databases, attached to several different promoters, and reintroduced to maize to assess the effects of different patterns of expression on drought tolerance. In many instances the putative drought tolerant ortholog does not produce the same result observed in the model plant, or causes an unacceptable level of yield reduction in the absence of stress. This may be because most candidate genes have come from dicotyledonous model plants (for example, *Arabidopsis thaliana* (L.) Heynh.), or that gene expression was originally assessed in plants subject to sudden drought shocks, or because effects in the absence of stress were not assessed in model systems.

Many transgenes putatively associated with drought tolerance do not directly affect the trait of interest, but rather depend in a complex manner on a chain of

metabolic responses. Thus, for example, increased grain yield (the primary trait) may be linked to a gene modulating assimilate flux, which itself is affected by a plant hormone. The lack of consistent transgene performance, however, may also be due to the long history of intensive improvement in this species. As a consequence, transgenic drought-tolerant phenotypes that is relatively easy to generate in a non-selected plant like *Arabidopsis*, or in older maize cultivars, are relatively less evident in today's elite hybrids.

### Marker-assisted breeding

Drought tolerance is an intricate and multigenic trait, although single genes, controlling, for example, the time of flowering or osmotic adjustment (OA), may also be involved in the plant response to drought. A large amount of QTL data from segregating populations has been gathered over the last two decades. The genetic control of target traits differed between plants under drought-stressed and well watered conditions. Therefore, breeding for drought tolerance will be efficient when conducted under stress (Messmer *et al.*, submitted). QTLs identified under stress tend to cluster in genomic regions related to drought tolerance, whereas those identified under well-watered conditions are related to plant performance. Nevertheless, clusters of QTLs are also found in both water regimes and are probably involved in the broad adaptation of plants (Ribaut *et al.*, 2008). However, Tuberosa *et al.* (2002a) compiled a single genetic map of QTLs from various studies and found that drought related QTLs were dispersed throughout the maize genome. As QTLs are specific to their original genetic background and the effect of single genes on target traits is usually weak, the number of successful marker-assisted breeding (MAB) experiments for improved drought tolerance is limited. This draws attention to the development of new MAB strategies, which go beyond marker assisted back-crossing or marker-assisted recurrent selection. MAB strategies based on genomic regions involved in the drought response across germplasm and environments, as identified through meta-analyses, would enable the use of the large amount of genomic data and would circumvent the need to identify QTLs in each target cross. The possibility of linkage drag and the lack of contrasting alleles may limit such approaches (Ribaut *et al.*, 2008). A logical extension would lead to "breeding by design" (Peleman and van der Voort, 2003) with the objective of stacking the favorable alleles from several parental lines at each of a large number of loci under selection. Most of the information on drought tolerance is based on single genotypes and experimental mapping populations. Association studies, however, are more powerful in identifying useful allelic variation than QTL mapping, because the former explore the results of many

generations of recombination and selection (Syvänen, 2005). Informative markers, resulting from association mapping, are usually functional in diverse genetic material and are suitable for MAB. In maize, however, the low level of linkage disequilibrium compared to other species, poses methodological and technological challenges for association mapping. Combining QTL mapping and Association mapping has great potential for maize breeding. The overall goal of both approaches should be to investigate the effect of quantitative traits on yield across different levels of water supply in order to identify and combine alleles that increase crop productivity. As stated by Ribaut et al. (2008), a key consideration should be the need to explore alternative segregating populations and approaches to selection, which can take advantage of the increasing ability to define the alleles that are the most desirable at multiple target loci, rather than focusing only on the role of markers in conventional breeding. With the genomic tools available, it is now possible to study the expression of thousands of genes.

Many drought-responsive genes have been identified and manipulated during the last decade, including the examples of genes involved in the biosynthesis of osmolytes, genes related to scavenging active oxygen, or genes encoding molecular chaperons, signaling molecules, and transporters or transcription factors (Araus *et al.*, 2003; Wang *et al.*, 2003; Parry *et al.*, 2005). Transcription factors may be suitable for the genetic engineering of complex traits, unless they activate additional genes that have a negative effect on crop performance. Stress-inducible promoters could circumvent this limitation by avoiding negative effects on crop performance under nonstress conditions and, at the same time, allow for improved drought tolerance under stress conditions. However, most of the drought responsive genes identified thus far are related to primary metabolism and protect and maintain the structure and function of cells at low water potential (Parry *et al.*, 2005). Protection of the primary metabolism is required only when plant tissues are dehydrated under severe drought stress. Hence, genes protecting the primary metabolism mainly contribute to plant survival rather than to productivity and may even have a negative effect on productivity. Moreover, most of the genetic transformations were done with model species and/ or in the laboratory. Only a few of these transformations have been validated in first-phase trials under field conditions (Dunwell, 2000). Nevertheless, there are examples of successful genetically engineered crops (Cattivelli *et al.*, 2008): transgenic wheat, expressing the DREB1A transcription factor (Pellegrineschi *et al.*, 2004), rice plants, over expressing the SNAC1 transcription factor (Hu *et al.*, 2006), and rice plants with ectopic expression of OsCDPK7, a calcium-dependent protein kinase (Saijo *et al.*, 2000).

Transpiration efficiency depends mainly on the

photosynthetic pathway. C<sub>4</sub> plants, such as maize and sorghum, produce about twice to three times as much biomass per unit of transpired water as C<sub>3</sub> plants, mainly because they concentrate CO<sub>2</sub> at the carboxylation sites. Moreover, their optimum temperature for growth is higher; hence C<sub>4</sub> plants produce higher yields in warm climates. Increasing mesophyll conductance for CO<sub>2</sub>, as a means of improving photosynthesis at a given stomata conductance, is not yet possible, but may become possible, because aquaporins seem to play a role in CO<sub>2</sub> conductance (Uehlein *et al.*, 2003; Hanba *et al.*, 2004; Parry *et al.*, 2005). Introducing a form of Rubisco with higher specificity to CO<sub>2</sub> could increase the photosynthetic efficiency of C<sub>3</sub> crops. Recent work on *Arabidopsis* showed that this might be possible in the future: The ERECTA gene resulted in higher water-use efficiency, irrespective of water availability (Masle *et al.*, 2005). Genes that simultaneously increase water-use efficiency and photosynthesis have great potential for improving the drought tolerance of crops, but their usefulness under realistic field conditions has yet to be proven. Thus far, improving water-use efficiency, a key component of improving the water productivity of crops, is usually accompanied by a lower rate of photosynthesis and lower yield (Parry *et al.*, 2005). Finally, at the 50th Annual Maize Genetics Conference in Washington D.C. in 2008, Monsanto presented a “proof of concept” from the private domain, illustrating the enhancement of drought tolerance of maize by genetic transformation. Monsanto scientists permanently activated a maize gene equivalent to the *Arabidopsis* NF-YB1 gene. Transformed maize plants produced yields that were about 10 to 15% higher under a variety of stress conditions, but the yield increase did not exceed that of natural variation.

### Conventional plant breeding

Selection for yield and yield stability has been at the core of most maize breeding programs. The significant breeding gains in temperate maize under drought stress is mainly attributed to the use of rainfed breeding nurseries with high plant densities and large scale multi-location testing (Banziger and Araus, 2007). The newer hybrids, selected in the last decade performed better under stress than older ones due to involvement of parental lines selected to tolerate higher planting densities (Tollenaar and Lee, 2006). It is well established that plant water and nutrients deficit occurs more readily at high rather than in low planting densities. Therefore, the adaptation to high plant density involves drought stress tolerance in cases when soil moisture is limited.

Multi-environment trials, conducted at >100 to >1000 locations, exposed new hybrids frequently to drought conditions, and selection for yield stability applied consistent selection pressure on drought tolerance related traits (Banziger and Araus, 2007). Compared to hybrids from previous decades, recent and more drought

tolerant hybrids showed shorter Anthesis-silking interval (ASI) under drought, increased leaf longevity and more erect leaves; also decreased apical dominance as tassel size decreased and an increased grain sink size through decreased plant-to-plant variability and fewer barren plants (Campos et al., 2004). Changes in constitutive traits, such as plant phenology, are also involved in the distinct responses to limited resources. Drought susceptibility of older hybrids was also associated with faster water extraction in the -1 for hybrids released between 1930 and 1990, and slightly less than under optimal conditions. Improvement in the drought breeding methodology by using more severe drought stressed upper soil layers (Campos et al., 2004). Using trials exposed to different weather conditions in different years, Duvick (1997) estimated the rate of breeding progress under mild drought to be 0.85% year conditions imposed at different stages of development, led to increase of breeding progress under flowering drought to about 2.0 to 2.5% year<sup>-1</sup> as compared to plants under unstressed conditions (Campos et al., 2004). Inclusion of managed drought screening at early breeding stage, careful and uniform management of timing and intensity of drought stress during selection, and use of secondary traits in addition to grain yield, resulted more recently, in significant larger selection gains under random stress conditions than those expressed by equivalent genotypes selected through multiplication testing. The average differences between hybrids selected under managed drought stress conditions and conventionally selected hybrids were 19% at average grain yields of 3 t ha<sup>-1</sup> (Banziger et al., 2006). Despite the shorter breeding history, yield gains of 3.8 to 6.3% year<sup>-1</sup> under drought and slightly less under well-watered conditions were reported for tropical maize, and those were mainly associated with increased flowering synchronization, fewer barren plants, a smaller tassel size, a greater harvest index, delayed leaf senescence and, in one population, a reduced root density in the upper soil profile, but no changes in water uptake or biomass were observed (Banziger and Araus, 2007). (Hammer et al., 2009) used a crop model framework to evaluate the potential contributions of component traits to the long term genetic gain of corn yields in the US corn-belt. The study revealed that the historical corn yield trend and its association with higher plant density was more likely related to change in root system architecture than to change in leaf erectness. Results from simulations conducted for hypothetical hybrids that varied in root system characteristics were found to be consistent with a set of field experiments that reported yield response to density for hybrids released over the past 20 years.

### **QTL mapping and marker assisted selection (MAS)**

First attempts to apply QTL analysis to obtain genetic

insights into the drought tolerance response in maize were reported by Lebreton et al. (1995). Since then, a number of QTL regulating important morphophysiological traits and grain yield in water limited conditions have been identified in maize. An updated compilation of mapped QTL and major genes associated with abiotic stress tolerance including drought in maize and other plants is available at <http://www.plantstress.com/biotech/index.asp?Flag=1>.

Khavkin and Coe (1997) hypothesized that many apparent QTL of major effect in maize are in fact clusters of genes (for example, homeotic genes and other genes encoding for transcription factors), regulating development and that many plant reactions to abiotic stresses rely on such gene clusters. Drought tolerance QTLs studies in maize and other crops, and the strategies for their use in marker assisted selection (MAS) in breeding programs have been extensively discussed in several comprehensive and recent reviews (Araus et al., 2008; Collins et al., 2008; Tuberosa et al., 2009). While the genetic dissection of performance in drought prone environments has greatly benefited from the use of DNA markers (Messmer et al., 2009; Szalma et al., 2007, Tuberosa et al., 2009), up to now few applications have emerged in practical maize breeding programs. Reasons include the complex genetic basis and influence of genetic background, developmental stage and environment on QTL effects (Tuberosa et al., 2002), inadequate phenotyping, time and cost considerations in fine mapping QTLs, and gene-by-gene effects (Campos et al., 2004). Other major problem is that in many of the previous drought QTL studies, there has been a tendency to examine crosses between lines that were not agronomically elite (landraces or exotic materials) and/or did not exhibit extreme differences in yield under stress. QTL are often germplasm-specific and the costs for applying MAS for many QTL of small effect may be greater than those of conventional cross-breeding (Xu et al., 2009). MAS for drought-related traits based on genetic mapping information should preferably target "major" QTL with a considerable effect, consistent across germplasm and with a limited interaction with the profile of water availability. In maize, however, QTL studies in the past have not identified any QTL with sufficiently large effects to be effectively used in MAS programs (Xu et al., 2009). Recently a conceptual framework for molecular breeding towards drought tolerance based on the Passioura equation of expressing yield as the product of water use (WU), water use efficiency (WUE) and harvest index (HI) was proposed (Salekdeh et al., 2009). Its implementation in QTL and omics studies will greatly improve the uniformity of the information provided for each QTL experiment and will facilitate detection of QTLs that are stable across multiple populations and environments by using software tools like CMTV (Sawkins et al., 2004) and MetaQTL. Two approaches are commonly applied for detecting

quantitative trait loci (QTLs), - linkage mapping and association mapping - but both have limitations, such as the low resolution of linkage mapping and the low power of association mapping for detecting rare alleles. McMullen et al. (2009) combined the advantages of both approaches by devising a nested association mapping (NAM) approach. They derived the NAM resource by crossing a common reference maize strain with 25 diverse lines to maximize the allelic diversity captured by NAM, and this process produced 5000 recombinant inbred lines (RILs). In a second paper Buckler et al. (2009) used the NAM resource to study the genetic variation in flowering time. The authors concluded that differences in flowering time are caused by the cumulative effects of many small-effect QTLs, with few epistatic or environmental effects and with no single QTL explaining a variation greater than 2 days. The NAM resource is expected to be an important genetic tool that will allow precise high resolution mapping and dissection of maize QTLs. However, it also has its limitations. The parent strains will need to be sequenced and their genetic polymorphisms will need to be projected onto the NAM lines, and precise phenotyping of 5000 RILs for drought tolerance related traits is not an easy task. Most of the previous drought tolerance QTL studies explored the natural variation existing in maize and many of them used one parent that has no breeding value to maximize the phenotype variation for the trait of interest, thus limiting the utility of the detected QTLs in the MAS breeding programs. To overcome some of these disadvantages, the QTL approach taken in the frame of NSF funded project DO02-105 "Centre for sustainable development of plant and animal genomics" will explore the genetic variation generated by chemical mutagenesis (Kostova et al., 2006) in an elite breeding line. For this purpose we have generated a DH RIL population from a cross between a parental line that was extensively used in the US Corn Belt breeding programs in the past, and a mutant parental line. The later was developed from the first parental line by chemical mutagenesis treatment of dry seeds to induce variation, followed recurrent reciprocal breeding. Compared to the initial elite inbred, the mutant line showed significantly higher yield stability under very variable (especially with respect to water availability) environmental conditions in Bulgaria. Particularly, high yield potential under combined low N and water stress conditions and great yield plasticity at different population densities was shown. In the frame of the project the DH population consisting of 190 RILs will be genotyped using SSR. When necessary other DNA markers and phenotypes in multiple environments with varying water regime, including several environments with managed drought stress at flowering and grain filling stages, will be studied. As evidence is accumulated on the crucial importance of root characteristics for drought tolerance in maize (Ruta et al., 2009, Trachsel et al., 2009; Tuerosa et al., 2009), in a separate experiments

root traits of the same population will be measured in laboratory hydroponics condition to evaluate the importance of root trait QTL for the field drought tolerance of this particular population.

## Conclusion

Today, marker-assisted breeding is becoming the standard breeding approach. An increasing body of information on the genetic control of target traits and on physiological mechanisms conferring drought tolerance is available. Biotechnology has opened-up new possibilities for creating and managing genetic variation, which is the key to crop improvement. These technologies will evolve rapidly and can greatly facilitate further progress in research into drought. The merits of biotechnology should, however, not be exaggerated. A multidisciplinary approach, combining quantitative genetics, genomics, physiology and breeding is probably the best route for improving the drought tolerance of crops, for enhancing water productivity, and, thus, for ensuring food security. Future progress will greatly depend on improved genetics. However, the agronomical context, as well as socio-economic factors and policy, must be taken into account; they influence to a large extent whether farmers adopt improved varieties and whether they can minimize the gap between yield potential and on-farm yield.

This integration of quantitative knowledge arising from diverse, but complementary disciplines will allow researchers to more fully understand genes associated with drought tolerance in maize, and more precisely forecast the penalty of modulating expression levels of those genes.

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