Breeding for Drought Tolerance in Maize
(Zea mays L.)

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Abstract
Drought, like many other environmental stresses, has adverse effects on crop yield including maize (Zea mays L.). Low water availability is one of the major causes for maize yield reductions affecting the majority of the farmed regions around the world. Therefore, the development of drought-tolerant lines becomes increasingly more important. In maize, a major effect of water stress is a delay in silking, resulting in an increase in the anthesis-silking interval, which is an important cause of yield failures. Diverse strategies are used by breeding programs to improve drought tolerance. Conventional breeding has improved the drought tolerance of temperate maize hybrids and the use of managed drought environments, accurate phenotyping, and the identification and deployment of secondary traits has been effective in improving the drought tolerance of tropical maize populations and hybrids as well. The contribution of molecular biology will be potential to identify key genes involved in metabolic pathways related to the stress response. Functional genomics, reverse and forward genetics, and comparative genomics are all being deployed with a view to achieving these goals. However, a multidisciplinary approach, which ties together breeding, physiology and molecular genetics, can bring a synergistic understanding to the response of maize to water deficit and improve the breeding efficiency.

Keywords
Maize (Zea mays L.), Drought Stress, Anthesis-Silking Interval, Breeding

1. Introduction
Maize (Zea mays L.) along with wheat and rice provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries where one-third of children are malnourished [1] [2]. By 2050, the demand for maize in the developing
The world will be almost double to the current demand [3]. However, an estimated 15% to 20% of maize grain yield is lost each year due to drought and such losses may further increase as droughts become more frequent and severe because of climate change [4]. Irrigation is not an option for large numbers of farmers and there is limited potential for any expansion of irrigation in developing countries [5].

The use of genetics to improve drought tolerance and provide yield stability is an important part of the solution to stabilizing global production. That is why the development of maize varieties with enhanced tolerance to drought stress and higher water use efficiency (WUE) has become a high priority goal for major breeding programs, both in the private and public sectors. The breeding programs improve drought tolerance via diverse strategies such as recurrent selection and evaluation of segregating population under managed and multi-location drought-stress environment, use of secondary traits for selection under drought condition, genomic-based approach and transgenic technology. Understanding the nature of drought response in maize and some major strategies used for improving drought stress-tolerant maize lines will provide opportunities to improve the breeding process.

Although other reviews on plant breeding for drought tolerance are available [6]-[9], this review will focus on the major strategies or approaches used by major breeding programs for improving drought tolerance in maize.

2. Maize Responses to Drought

Drought is a multidimensional stress, affecting plants at various levels of their organization over space and time, so that the physiological responses to it are complex and often unpredictable. However, in maize, a major effect of water stress is a delay in silking, resulting in an increase in the anthesis-silking interval (ASI), which is an important cause of yield failures [10]. In fact, typical visual symptoms of drought stress in maize are a change in color from green to green-gray, and rolling of the lower leaves followed by those in the upper canopy. At the same time stomas are closing, photosynthesis is being sharply reduced and growth is slowing. When stress coincides with the 7 - 10 days period prior to flowering, ear growth will slow more than tassel growth and there is a delay in silk emergence relative to pollen shed, giving rise to an interval between anther extrusion and silk exposure [11]. This ASI was shown to be highly correlated with grain yield, in particular kernel number and ear number per plant [10]. At the same time leaf senescence begins at the base of the plant and spreads upwards to the ear. Severe stress at flowering may lead to the complete abortion of ears and the plant becomes barren. Drought-affected ears typically have fewer kernels that will be poorly filled if drought extends throughout grain filling [12].

In many cropping environments, drought stress increases in severity at the end of the growing season. In this situation, assimilate which has accumulated to a high level in the maize stem is re-mobilized, and contributes to yield [13]. From the root to the aerial tissues the action of abscisic acid involves interactions with other growth regulators, particularly ethylene and reactive oxygen species [14]. Moreover, the development of a root system capable of accessing water far down the soil profile is a valuable trait in drought-
affected environments [15]. Many species, including maize, respond to water deficit by redirecting growth and dry matter accumulation away from the shoot to the root [16].

Another potentially valuable stress tolerance mechanism is the osmotic adjustment (OA) because it involves the active accumulation of solutes in the cell [14]. This accumulation enables the retention of water during episodes of low external water potential, limiting turgor loss and damage from cell shrinkage. Under more prolonged or severe moisture deficit, these solutes also are implicated in the stabilization of various macromolecular structures.

Finally, the stomata respond dynamically to changes in the environment and play a vital role in limiting water loss during drought. Maize is an “isohydric” species, so called because the signaling and response system is sensitive to a decrease in water potential, limiting water loss in the early phases of water deficit, so that leaf water potential can be maintained at the well-watered level until more advanced stages of drought [17]. The sensitivity of maize stomata also protects xylem from cavitation [18], and under high vapor pressure deficit, it curtails water loss in the middle of the day, so that photosynthesis and transpiration is restricted to the cooler morning periods when WUE is higher [14].

Many of these mechanisms favor survival but may have limited value in enhancing grain yield under a level of water stress that varies spatially and across seasons.

3. Selection and Evaluation of Segregating Population under Managed and Multi-Location Drought-Stress Environments

Breeding for drought tolerance in maize is a complex task, not least because drought can affect the crop at any stage of development. The choice of a selection strategy is critical to breeding for stress tolerance. Probably the most widely used strategy is to select for yield under non-stressed conditions, and then evaluate those selections at many sites with variable moisture availability or “random stress” [19]. Underlying assumptions of this approach are that genes for drought tolerance are present in elite high yielding material, even after the number of genotypes has been narrowed to the few evaluated under random stress, and that selection under optimum growing conditions can also increase performance in sub-optimum conditions [20]. Moreover, hybrids usually yield better than varieties under drought with heterosis acting as an important source of stress tolerance [6].

The choice of the testing environment(s) is critical to the rate of achievable genetic gain. Ideally, the selection environment should mirror the target environment in rainfall distribution, physical and chemical soil properties, water distribution profiles and potential evapotranspiration rates, otherwise significant genotype × environment (G × E) interactions will result in much of the gain achieved in the selection environment not being reproduced in the target environment [14]. The use of uniform soils, along with rigorous experimental design and appropriate statistical analysis will together maximize the precision of genotypic means [14]. Multi-location evaluation is necessary to estimate the importance of G × E. It is especially critical in the context of breeding
for drought tolerance, where a consequence of lowered plant vigor is a higher responsiveness to environmental variation.

This methodology was used to develop drought tolerant versions of several elite lowland tropical populations [21]-[26]. For example, Monneveux et al. evaluated direct and correlated responses to recurrent selection for drought tolerance in two CIMMYT maize populations, DTP1 and DTP2, adapted to the lowland and mid-altitude tropics [25]. Cycles C0, C3, and C6 of DTP1 and C0, C3, C5 and C9 of DTP2 were evaluated under drought, low N, and optimal conditions. Evaluation for drought tolerance was based on replicated trials at one or two water stress levels during a rain-free period using controlled irrigation. Severe water stress was induced during flowering and grain filling such that average grain yield in these trials was reduced 15% to 30% relative to unstressed yields. The same germplasm was also grown under well-watered conditions. This selection was based on an index involving grain yield under drought and well-watered conditions and ASI, barrenness, leaf senescence, and leaf rolling under drought. They observed a significant yield gains in both populations under drought conditions. Yield gain from C0 to C6 cycles was 0.20 and 0.12 Mg·ha⁻¹ Cycle⁻¹ (16.5% and 10.1% Cycle⁻¹) for the white and yellow versions, respectively. This gain was explained by a significant increase of both the number of ears per plant and the number of grains per ear. Yield gain, however, was less than that under drought, and was associated mainly with an increase in the number of grains per ear. They also reported that selection for drought tolerance did not affect yield and the number of grains per ear under optimal conditions but did increase 1000-kernel weight in DTP1. Ears per plant were significantly correlated with grain yield across cultivars among three environments (Figure 1). Moreover, they found that effects of recurrent selection on dry matter partitioning at maturity showed a significant increase in ear weight and a significant decrease in tassel, stem, and leaf weight in DTP1 when evaluated under drought [25].

Figure 1. Relationship between ears per plant and grain yield across cultivars within and across environments [25]. *, **, and *** Significant at P = 0.05, 0.01, and 0.001, respectively.
In the same study, Monneveux et al. observed a positive correlation between tassel and stem weight per plant across cultivars, both at anthesis and maturity (Figure 2) [25]. There was also a significant negative correlation between ear weight and tassel weight at anthesis (Figure 3).

**Figure 2.** Relationship between tassel and stem dry weight at anthesis and maturity across cultivars, under drought conditions [25]. **, and *** Significant at P = 0.01 and 0.001, respectively.

**Figure 3.** Relationship between ear and tassel dry weight at anthesis across cultivars, under drought conditions [25]. **Significant at P = 0.01.
In another study using similar methods, Edmeades et al. observed an increase in grain yield under drought of 0.26 Mg ha\(^{-1}\) or 12.6% Cycle\(^{-1}\) following S1 recurrent selection using a selection intensity of 5 to 10%, and a lower rate of improvement (0.08 Mg ha\(^{-1}\) or 3.8% Cycle\(^{-1}\)) following fullsib recurrent selection with a selection intensity of 26 to 32% [24]. They also reported a small but significant increase in grain yield, ears per plant, kernel number per fertile ear, and individual kernel weight under well-watered conditions. Bänziger et al. evaluated contrasting selection cycles of several of these populations under low N and observed similar yield gains under low N as well [27]. They suggested that common mechanisms were responsible for increased partitioning of assimilates to the developing ear and for increased yields under both types of stress.

Despite success achieved using selection and evaluation under managed and multilocation drought-stress environment, the effectiveness of this method is however, largely unknown. For example, using the same approach, Magorokosho et al. compared two maize populations, ZM601 and ZM607 for drought tolerance during flowering, the most drought-vulnerable period for the maize plant [19]. The results did not show much difference in drought tolerance between ZM601 and ZM607. Differences between population means for grain yield, ASI, and number of ears per plant (EPP) were small.

4. Secondary Traits for Selection under Drought Conditions

Secondary traits are those other than economic yield itself which can provide a measure of plant performance [28]. An ideal secondary trait would be genetically correlated with grain yield in the target environment, genetically variable, have a high level of heritability, be simple, cheap, non-destructive and fast to assay, be stable throughout the measurement period and would not be associated with any yield loss under non-stressed conditions [14]. Under drought stress conditions, breeding progress is impeded by a significant level of G × E (both with respect to cropping season and location). Given the poor heritability of grain yield under drought stress conditions, genetic progress is hard to achieve via direct selection. However, because under drought, both the heritability (h\(^2\)) of at least some secondary traits remains high and the genetic correlation between grain yield and these traits increases significantly, recourse to indirect selection becomes an attractive strategy [28] [30]. Selection based on secondary traits which reflect the direct effects of drought can improve the response, since it avoids the confounding effects of other stresses, such as poor soil fertility, micronutrient deficiency and pathogen presence [14]. Application of this strategy has generated genetic gains under a range of environmental conditions.

In fact, after evaluation of a total of 3509 inbred progenies (S1 to S3 level) in 50 separate yield trials under two or three water regimes, Bolaños and Edmeades reported a general tendency to decrease with increasing moisture stress in the heritability for grain yield from around 0.60 in well-watered environments to values of 0.40 or less at very low yield levels [29]. The h\(^2\) of kernels ear\(^{-1}\) and weight kernel\(^{-1}\) was around 0.60 under well-watered conditions, but also decreased with increasing stress. In contrast, the heritability for ASI and ears plant either increased or remained fairly constant with in-
creasing moisture stress and declining yield levels. The $h^2$ for days to anthesis remained fairly constant across all moisture regimes. They also suggesting that earlier-flowering progenies were associated with high grain yields and similarly, a short ASI was linked to high grain yield under stress. This perception is reinforced by findings of Magoro-Kosho et al. who found that a phenotypic correlation between grain yield and ASI were small ($r = -0.08$ to $-0.21^*$) under adequate moisture conditions and became much larger ($r = -0.40^{**}$ to $-0.43^{**}$) at moisture-stressed sites [19]. Similarly, the relationship between ears per plant (EPP) and grain yield, also became stronger with increasing moisture stress (from 0.08 to 0.24** without stress, to 0.40** to 0.45** with moisture stress).

In an earlier study using similar methods, Ziyomo and Bernardo observed a significant decrease in $h^2$ for grain yield under drought (0.60 in the control experiment and 0.37 under drought) but not for ASI and leaf senescence [31]. They observed a higher $h^2$ and genetic variance for ASI and leaf senescence under drought stress. Furthermore, in this drought experiments, the strongest genetic correlation was observed between grain yield and ASI (−0.77). In summary, compared with direct selection for grain yield under drought, indirect selection based on grain yield in the control experiments had a relative efficiency of 0.78, indirect selection based on ASI had a relative efficiency of 1.04, and indirect selection based on leaf senescence had a relative efficiency of 0.98.

These results indicate that ASI, leaf senescence and EPP are useful secondary traits for the selection of grain yield at moisture stressed sites. Therefore, the use of this strategy can increase selection efficiency.

5. Genomic-Based Approach

Drought tolerance is a complex quantitative trait controlled by many genes, and is one of the most difficult traits to study and characterize. Compared to conventional approaches, genomics offers unprecedented opportunities for dissecting quantitative traits into their single genetic determinants, the so-called quantitative trait loci (QTL), thus paving the way to marker-assisted selection (MAS) and, eventually, cloning of QTLs and their direct manipulation via genetic engineering [32]. Therefore, it is possible to identify major QTLs regulating specific drought responses and it will provide an efficient way to improve drought tolerance in maize germplasm [33]. The increasing number of studies reporting QTLs for drought-related traits and yield in drought-stressed crops indicates a growing interest in this approach [10] [33]-[37].

In fact, using a modeling approach combined with field measurements, Ribaut et al. identified a common QTLs for both leaf growth and ASI in a recombinant inbred line population evaluated under water stress conditions [14]. For all common QTL the allele conferring high leaf elongation rate conferred a short ASI, indicating a high silk elongation rate. They also observed unsurprisingly that drought-related QTL are dispersed throughout the maize genome. In another study, Sari-Gorla et al. performed a linkage analysis between the expression of male and female flowering time, ASI, plant height and molecular markers [10]. The experiment was carried out under two environmental
conditions, well-watered and water-stressed, on a maize population of 142 recombinant inbred lines obtained by selfing the F1 between lines B73 and H99 and genotyped for a total of 153 loci. Linkage analysis revealed that, for male flowering time and plant height, most of the QTLs detected were the same under control and stress conditions. In contrast, with respect to female flowering time and ASI diverse QTLs appeared to be expressed either under control conditions or under stress. All of the QTLs conferring tolerance to drought were located in different chromosome regions. They suggested that plant tolerance, in its different components, is not attributable to the presence of favorable allelic combinations controlling the trait but is based on physiological characteristics not directly associated with the control of the character. Zhu et al. also found five, five, six, four, and five QTLs for ASI, plant height, grain yield, ear height, and ear setting under full irrigation condition, respectively, and four, seven, six, four, and four QTLs for ASI, plant height, grain yield, ear height, and ear setting under severe late stress conditions, respectively with similar method [33].

The universal QTLs information generated in these studies will aid in undertaking an integrated breeding strategy for further genetic studies in drought tolerance improvement in maize.

6. Transgenic Technology

Traditional breeding techniques contribute considerably to the popularization and application of drought-resistant lines and cultivars, but the limitations are the long breeding cycle and the extensive time consumption. Thanks to rapid progress in biotechnology and genome sequencing, there is now a diverse choice of tools for the identification of candidates for genes involved in specific processes, including the response to drought. Many drought tolerance candidate genes have been documented in the literature [34]-[41], but few of them have been validated either via reverse genetics or transgenic approaches, or by the demonstration that directed selection under field conditions in diverse genetic backgrounds produces a clear genetic gain [14].

However, some reports have showed potential results. For example, Quan et al. reported that glycine betaine plays an important role in some plants, including maize, in conditions of abiotic stress, but different maize varieties vary in their capacity to accumulate glycine betaine [42]. An elite maize inbred line DH4866 was transformed with the betA gene from *Escherichia coli* encoding choline dehydrogenase, a key enzyme in the biosynthesis of glycine betaine from choline. The transgenic maize plants accumulated higher levels of glycine betaine and exhibited higher tolerance to drought stress than wild-type plants (non-transgenic) at germination and the young seedling stage. Most importantly, the grain yield of transgenic plants was significantly higher than that of wild-type plants after drought treatment. They suggested that enhanced glycine betaine accumulation in transgenic maize provides greater protection of the integrity of the cell membrane and greater activity of enzymes compared with wild-type plants in conditions of drought stress.

In a most recent study, Liu et al. cloned maize *ZmSDD1* and dissected its functions...
and performance to drought stress [43]. Genetically engineered overexpression of ZmSDD1 was employed to transform maize and cultivate new drought-resistant lines. Under an optical microscope, in the visual field × 100 with the adaxial ends, 71 and 51 stomata were found in wild types (WTs) (Figure 4(A-a)) and transgenic plants (TPs) (Figure 4(A-b)), respectively, with the same number of stomata with the abaxial ends, 71 in WTs (Figure 4(A-e)) and 51 in TPs (Figure 4(A-f)). In the fields × 200 of adaxial and abaxial ends, 21 stomata in WTs (Figure 4(A-c) and Figure 4(A-g)) and 15 stomata in TPs (Figure 4(A-d) and Figure 4(A-h)) were observed. A stomatal density of 90/mm² was determined in WT, whereas it was 63/mm² in TP, with a reduction of 30% in TP (Figure 4(B)). The ZmSDD1 expression level in TPs was 6.68 times higher than that in WTs (Figure 4(C-a)); the expression rate of TMM related to the stomatal development signaling pathway was augmented by 4.29 times (Figure 4(C-b)), while the degrees of expression of MAPK3 and MAPK6 increased by 12.21 (Figure 4(C-c)) and 11.16 (Figure 4(C-d)) times, respectively. These results indicate that the reduction of

**Figure 4.** Reduction of stomatal density in TP plants with overexpression of ZmSDD1 [43]. (A) WT and TP maize under optical microscopy; (B) Stomata numbers in TP and WT maize; (C) Genes correlated with stomatal development were regulated by overexpression of ZmSDD1 (a ZmSDD1, b TMM, c MAPK3, d MAPK6). Data represent mean values ± SD from three independent experiments. Asterisks indicate statistical significance (**P < 0.01, Student’s t test) of differences between transgenic and WT plants (WT wild type, TP transgenic plant).
stomatal density in the TPs might be attributed to the regulation of $ZmSDD1$. Therefore, the transpiration is reduced in TPs compared with WTs.

In the same study, The TP and WT maize groups were drought-treated 3 times during 45 days, and the survival rates were observed and summarized. The results showed that 19 TP plants survived and 1 plant died, constituting a survival rate of 95 %. In contrast, only 3 WT plants survived, while 17 plants died, forming a survival rate of 15 %. Therefore, the transgenic maize exhibited a survival rate 80 %, higher than that of WT [43]. These results indicate that, in contrast to the effect on WTs, the drought tolerance of TPs was significantly enhanced.

7. Conclusion and Perspectives

Breeding for drought tolerance in maize is a complex task, not least because drought can affect the crop at any stage of development. Many breeders have focused on alleviating the effects of drought at flowering and during grain filling because maize is most vulnerable to drought at these stages. There is no consensus about the best strategy to use in breeding maize for drought tolerance, and some researchers recommend a combination of two or more of the above mentioned approaches. However, a selection index combining secondary traits such as ASI and EPP with grain yield should result in faster improvement of grain yield under drought stress than selection for grain yield alone. It is desirable that more breeding programs use high-priority abiotic stresses in their mainstream breeding program, so that more experience on breeding approaches that effectively target stress environments can be gained. Such insights are particularly relevant for breeders in low-income countries that target production conditions that are stressed due to both biophysical and socio-economic reasons.

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