



doi: <https://doi.org/10.20546/ijcrar.2024.1203.007>

## Integration of Conventional Breeding and Molecular Tools for Drought Tolerance in Maize: A Review

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### Abstract

Maize is an important staple crop for food, feed and industry globally. Despite the importance of maize as a principal food crop in developing country, drought is a major constraint that affects maize production, particularly in Sub-Saharan Africa where maize is grown under rainfed condition. Plant breeder has been striving to improve and develop drought tolerance crops. Nevertheless, these efforts still cannot meet the demand of food security due to fast population growth and climatic change. Secondary traits with associated to grain yield, advanced phenotyping, marker assisted selection/QTL, genomic wide association study and genomic selection methods are more expected to help for improvement of drought tolerance in maize. This article discussed the most secondary traits used for selection, plant physiological traits and crossing among drought tolerance elite lines and evaluation their segregants as well as hybrids performance under stress and optimum conditions. Emphasis also given to molecular dissection of drought tolerance complex traits using GWAS and /or gene discovery at hotspot regions for secondary traits, marker-assisted backcrossing and genome selection have been highlighted for increasing genetic gain in grain yield and predicating the breeding value for selection. Thus, further improvement is essential integration of conventional breeding and genomic tools for development of resilience maize varieties for drought tolerance in maize.

### Article Info

*Received: 25 January 2024*

*Accepted: 28 February 2024*

*Available Online: 20 March 2024*

### Keywords

Secondary traits, QTL, GWAS, drought, tolerance, maize.

### Introduction

Maize (*Zea mays* L.) is a worldwide important staple crop for food, feed and industry. Despite the importance of maize as a principal food crop particularly in developing country, its average yield in Africa (2.02 t ha<sup>-1</sup>) is still low as compared to the world average (5.58 t ha<sup>-1</sup>) (FAO, 2021). A significant portion of this yield gap is attributable to biotic and abiotic stresses. Adverse environmental conditions such as drought, salt and high temperature can affect maize growth and considerable yield loss. Among all, drought is a major constraint that

affects crop production across the world and particularly in sub-Saharan Africa where maize is grown under rainfed condition and up to 25% of maize farmland suffers from frequent drought (Lunduka *et al.*, 2019). Climate projections for Eastern and Southern Africa show fluctuation of rainfall and temperature and might be affect agricultural production. It is also expected that by 2050, the air temperatures will increase by an average of 2.1°C and rainfall patterns will dramatically change (Cairns *et al.*, 2013). Changes in rainfall pattern and rising temperature are the major causes of drought and have contributed a considerable decline in crop

productivity including maize (Obidiewu *et al.*, 2015). It is estimated about 15 % to 20 % of maize grain yield lost each year due to drought and even if the losses will increase as frequent drought occurred hence rises of climatic change (Thomson *et al.*, 2005).

Drought affects at any growth maize stages. When drought occurs during the vegetative stage, resulted in reduce leaf area, root spread, stem extension, and metabolic activity. Also, it reduces the chlorophyll content as well light interception resulting in low photosynthesis and subsequently reduction maize yield (Athar and Asharf, 2005). The fact that maize is more sensitive to drought at flowering or occurring between two weeks before and after the silking stage can cause significant yield loss of 20 to 50 % (Nielson, 2007). If drought is severed at flowering and extended to throughout grain filling, it may lead to the complete abortion of ears and the crop becomes barren and / or ears may have fewer kernels. So far plant breeder has been striving to improve and develop drought tolerance crops. Nevertheless, these efforts still cannot meet the demand of food security due to fast population growth and climatic change. Thus, further improvement is essential integration breeding approach both conventional and genomic tools for development of drought resilience maize varieties.

### **Plants response to drought stress**

In agricultural context, drought is a situation when the water availability to plant is less than what is required to sustain its growth and development or drought is a situation when inadequate moisture in the soil at particular time to meet the needs of the plant. Plants use different mechanism to cope with the drought. These strategies (mechanism) include; drought escape strategies i.e plants accelerate their flowering time before onset drought season and early maturation (Deikman *et al.*, 2011). For examples, breeders evaluate and select maize germplasm that can escape drought via early flowering. However, yield penalty can be occurred when too early and / or when high rain falls at time of maturity of crop. Drought avoidance also other strategy when plants improve water use efficiency through closing stomata and increasing the thickness of the leaf cuticle or improving water uptake via developing root system. Plants also adapt metabolism mechanism to minimize water loss such as osmotic adjustment through active accumulation of solutes in the cell and helps for retention of water and limiting turgor lose (Paillard *et al.*, 2003; Ribaut *et al.*, 2009; Gowda *et al.*, 2011). Traits like

relative water, proline and chlorophyll contents are also the most reliable parameters for drought tolerance in plants (Rahul *et al.*, 2018). Drought escape and avoidance mechanisms are associated to morphological traits that can be exploited from diverse germplasm and selected under drought prone areas. Therefore, the objective of this paper was to review the statuses of maize breeding under drought condition by integration of conventional breeding and biotechnical tools.

### **Importance of secondary traits under drought tolerance in maize breeding**

Grain yield under stress condition is often the primary and complex trait for selection. Evaluation and selection can be applied under direct stress, optimum and or at same time under both conditions. However, selection under stress condition require multi-locations and over season hence direct selection under drought tolerance for grain yield likely low heritability (Venuprasad *et al.*, 2007; Ziyomo *et al.*, 2013). Considering secondary traits such as anthesis-silking interval (ASI), era per plant, leaf senescence, stay green, chlorophyll content and other morphological traits are genetically associated with grain yield, heritable and increase selection efficiency of those traits under drought stress conditions (Lafitte *et al.*, 2003; Banziger *et al.*, 2006). When drought coincided at anthesis silking interval (ASI) it affects embryo development and early grain filling, the yield loss is estimated about 45-60 % (Campos *et al.*, 2006; Kumar *et al.*, 2015). Delayed ASI means the negative correlation between ASI and grain yield. Banziger *et al.*, (2006) reported that 90 % decreased in yield as ASI increased from -0.4 to 10 days. On other hand, the shorter ASI led to the positive association between ASI and grain yield and, then, indirectly ears per plant and kernel per row selected. Monneveux *et al.*, (2005) reported that ears per plant showed significant correlated with grain yield across drought ( $r=0.96$ ), Low-N ( $r=0.66$ ) and optimum ( $r=0.83$ ) environmental conditions.

Delayed leaf senescence and high chlorophyll contents are associated with the stay-green and key traits for drought tolerance. Stay green retain the moisture and help better in photosynthesis during grain filling stages (Lee and Tollenaar, 2007). Thus, the plants survive with the existing soil moisture and maximize its grain filling efficiency. Consequently, breeder selects such genotypes that capable to convert the sources to sink rapidly. For example, leaf growth and ASI are the secondary traits that determinants of source and sink strengths of maize via their relations with light interception and yield,

respectively. In addition, ASI, stay green and root architecture are the most important secondary traits to impart drought tolerance and contributed indirectly to yield (Nepolean *et al.*, 2013). Thus, indirect selection through secondary traits that associated with grain yield can improve the efficiency of maize breeding under drought condition. Therefore, secondary traits are highly significant that associated to grain yield and to be considered for selection under drought stress in maize breeding program.

### **Field management for drought stress**

Germplasm under drought tolerance requires careful management to expose genetic variation in order to select grain yield and secondary traits. Maize germplasm is evaluated and selected under managed drought stress (using irrigation system) and under drought prone areas.

Screening large number of germplasm during off season (winter) with application of irrigation can be conducted under managed strategy. The germplasm is induced at flowering time that means drought stress coincided with anthesis and silk emergence named as severe stress (Banziger *et al.*, 2000) and onwards supplementary irrigation should be applied. Similarly, Chandel *et al.*, (2012) evaluated 190 maize inbred lines for different morphological parameters; ASI, plant height, leaf senescence, leaf rolling, and grain yield/plant under limiting irrigation vs normal irrigated crop (control) at different growth stages, *viz*: at knee height, grain filling, before flowering and both at flowering and grain filling.

### **Breeding scheme**

#### **Traits based crossing strategy and hybrids maize evaluation under managed and drought stress**

Maize breeding is a step wise selection producer to identify the best performing progenies. The first step is identifying stress adaptive parents and then, strategic crossing among parents with inclusive target traits (Reynolds and Tuberosa, 2008; Reynolds *et al.*, 2009).

When crossing among parents, traits include: ASI, ear per plant, stay green/ or Leaf senescence, root structure, tassel structure, water use efficiency, stomata conductance and others. This means that identifying the candidate parents for target traits crossing should be given to top priority in terms of secondary traits incorporating for genetic gain under stress and random stress conditions.

### **Source of germplasm for drought tolerance and crossing strategy**

The source of germplasm potentially holding allelic variation and diverse that promoting drought tolerance. The divers 'sources include adapted elite lines, derived from introgression, multi-parent population, parental lines carrying favorable alleles and other source that maximize genetic diversity within and opposite heterotic groups. Crossing bi-parental would be started for inbred line development with targeting traits. For example: drought tolerance x drought tolerance for most secondary traits, drought tolerance x Low N tolerance, drought tolerance x disease resistance and drought tolerance x susceptible for inbred lines development. Notice that the current trend of inbred line development in maize is through pedigree method, modified single seed decent, back crossing and double haploid (DH). DH is the fast inbred line development since it enhances for the production of homozygous lines and genetic gain within short period of time (Segui-Simmarro, 2015).

### **Evaluation of inbred lines and hybrids under managed and drought stress**

The goal of maize breeding for drought tolerance is 1) to reduce the gap between yields in optimal and stress condition and 2) improve yield stability for a range of stress conditions. This can be employed through evaluating wide array of maize segregants under drought stress vs optimum conditions at flowering and grain filling stage is the first step in selection process. This means that several segregating populations screened under managed drought tolerance as well optimum and evaluated for performance. The selected progenies advanced to next stages. Several studies conducted on segergant families and cycled open pollinated varieties. One hundred maize inbred lines were evaluated under contrasting soil moisture and 15 inbred lines showed high tolerance to drought. Also, tolerance inbred lines showed shorter ASI, low canopy temperature, lower drought susceptibility index, higher chlorophyll content and comparatively higher grain yield when subjected to drought stress (Shadakshari and Shanthakumar, 2015).

Similarly, eleven inbred lines were evaluated under drought and heat over two years and two of inbred lines revealed that it relatively high-water content and greatly maintain vegetative growth and alleviate damage to reproductive tissues under drought conditions (Chen *et al.*, 2012). Likewise, Malook *et al.*, (2016) reported that a high heritability and genetic advance was found for

plant height, TKW, grain rows per ear and grain yield per plant as well as high specific combining ability suggested that some parental lines showed superior to develop higher yield maize hybrids under stress condition. Most studies showed that physiological traits such as stomata conductance, chlorophyll content, transpiration rate, leaf water potential and relative water content used to improve breeding for stress tolerance. Dordas *et al.*, (2018) reported that good association physiological characteristics with grain yield under stress condition for inbred lines than hybrids and these traits are strongly help for selection of adaptive parents. Similarly, correlation of secondary traits with grain yield under stress condition conducted.

Canopy temperature depression ( $r=0.61$ ) and total chlorophyll present in leaves/ SPAD reading ( $r= 0.50$ ) showed positive correlation while leaf rolling score ( $r=-0.49^{**}$ ), leaf senescence score ( $r=-0.57$ ) and anthesis silking interval ( $r= -0.15$ ) showed negative correlation (Parajuli *et al.*, 2018). This means that increasing leaf roiling and subsequent leaf dry at early and non-nicking of ASI result in decreasing grain yield.

After evaluation of several inbred lines, promising lines are selected based on their tolerance. The selected S3 (F4) stages progenies and inbred lines should be crossed at least by two opposite testers and their crosses evaluated under managed drought tolerance for direct selection and evaluated at broad array of environment under drought prone areas. The hybrids maize that selected under stress might be reduced to 50% of yield potential at least (i.e., if yield under optimum conditions is around 7 t/ha, yield under stress likely about 3.5 t/ha) (Banziger *et al.*, 2006). Forty drought tolerance hybrids evaluated under well-watered, combined drought and heat over three years and found that significant difference among hybrid for grain yield and, the yield showed positive correlated with secondary traits (Meseka *et al.*, 2018). Among all, three hybrids produced high grain yield under three conditions. Likewise, forty-nine maize hybrids that developed via Line by tester evaluated under drought stress, low nitrogen and optimum conditions, and showed the drought stress reduced grain yield and plant height. The authors also noticed that further maize improvement for grain yield is required under stress and optimum conditions (Ertiro *et al.*, 2017).

Development of early maize maturity at moisture areas also conducted. Escaping strategy is the fact that plants accelerate their flowering time before onset drought

season. For examples, Melkasa maize breeders in Ethiopia evaluated and selected maize germplasm that can escape drought via means of early flowering.

So far Melkassa1, Melkassa1Q, Melkassa4 (OPV) and hybrid maize (MH130) were released and commercialized at low moisture stress area which receives annual rainfall < 800 mm in Ethiopia. Similarly, Monneveux *et al.*, (2005) evaluated CIMMYT maize populations; under drought, low N, and optimal conditions showed that yield gain per cycle and significant increase in terms of ears per plant and the number of grains per ear.

### **Marker Assisted selections (MAS), Genomic Selection (GS) and Genomic wide association study (GWAS) for drought tolerance in maize**

Molecular marker or DNA marker is defined as a particular segment of DNA sequence on known chromosome location for particular genes or specific trait. Currently, SSR; SNP and DArT markers are commonly used in plant breeding for crop improvement.

Advanced markers such as SSR, SNP and genome-based selection (GBS) are useful for different traits study, contribute for selection and to enhance maize improvement for drought tolerance. Maize genomic assisted breeding can be 1) Marker assisted selection (MAS): - molecular markers are strongly associated with traits of interest. 2) Genomic Selection (GS) depends on models and dens of genetic markers distributed across the whole genome and phenotyping the training population and then, to predicate genetic gain and estimated breeding values (Meuwissen *et al.*, 2001; Varshney *et al.*, 2014).

### **QTL Mapping and Marker-Assisted Selection (MAS)**

Understanding the genetic and physiological basis of secondary traits are useful information during selection for drought tolerance in crops. Quantitative traits locus (QTL) is link to phenotypic data (traits measurement) and genotype data using molecular marker that attempt to explain the genetic basis of variation in traits. QTL mapping is including the development of mapping population for drought tolerance related traits, identification of markers, genotyping the mapping population with polymorphic markers, constriction of genetic map and phenotyping traits. Finally, QTL mapping using genotyped and phenotypic data (Chamarthi *et al.*, 2011).



### QTL mapped for secondary traits for drought tolerance in maize

Drought tolerance is a complex quantitative trait in maize that controlled by many genes and it also affected by genotype x environment interaction. Some secondary traits are expressed under drought tolerance and their genetic basis dissected using marker. Several QTLs that regulate morpho-physiological traits and grain yield under water limited conditions have been identified in maize. Several QTLs were detected across six chromosomes for ASI, ear per plant, stay green, plant height ear ratio under stress and water regime (Wange *et al.*, 2012; Almeida *et al.*, 2014). The identified QTLs for these traits had additive and non-additive effect meanwhile drought stress reduced grain yield, whereas morpho-physiological traits were stable or even increased variance. Out of detected QTLs, 65 % of QTL were identified under water stress suggesting that the relevance of secondary traits for breeding under drought tolerance. For most secondary traits, 29 QTL detected for ear per plant, 80 QTL for stay green (leaf senescence and chlorophyll content) in three maize populations under both conditions. Similarly, several QTL identified under both conditions for deferent traits and common QTLs shared (Li *et al.*, 2016). Likewise, 9 QTL detected for ASI under both conditions and showed non additive effect. Almeida *et al.*, (2013) reported QTL detected for relation to GY with ASI under different water regimes in three biparental maize population. Also, Zhao *et al.*, (2018) found 62 QTLs for major agronomic traits and out of these, 9 of each QTL detected for ASI and ear length. Out of these, 75.4% (52 QTLs) detected under water-stressed conditions, suggesting that high possibility for maize improvement under stress conditions. Traits were located on different chromosomes under different conditions suggesting that different genes influence different physiological processes which contribute to different morphological traits expression under the different conditions.

It was interesting that clustered QTLs were found on chromosome 3 that harbored QTL (hotspot region) for most morpho-physiological traits as well the location of two important candidate genes (Almeida *et al.*, 2014) and consequently useful for marker assisted introgression for drought tolerance in tropical maize. Similarly, co-located QTLs were found on chromosomes 1, 3 and 5 for ear height and ASI (Yang *et al.*, 2008). It is suggesting that the same gene for controlling different traits or locus has pleiotropic effect that controls multiple traits. Clustered QTLs also detected for both stress and watered

conditions. Likewise, overlapped QTLs found for leaf growth and ASI in inbred lines of maize under water stress condition, suggesting that a high leaf elongation rate conferred a short ASI, indicating a high silk elongation (Ribaut *et al.*, 2009). Similarly, Zhao *et al.*, (2018) also identified 36 meta-QTLs across 26 populations under stress and watered conditions using a meta-analysis, and several candidate genes identified through fine mapping. Furthermore, a cluster of QTLs detected on chromosome 3 and flanked by the markers for plant height, TKW and grain yield per plant and these may be used to enhance maize drought tolerance by marker assisted selection (MAS) for water limited environments (Chene *et al.*, 2012). The clustered QTLs in maize also suggested that a cluster of genes such as homeotic genes and other genes encoding for transcription factors, regulating development and that many plant response to drought stresses rely on such gene's clustered.

### Genomic Selection (GS) for drought tolerance in maize

Several QTLs have been identified for drought tolerance in maize and those QTLs have been used to improve stress tolerance through marker-assisted breeding. The Marker assisted selection focus on major QTLs of load score greater or equal to three score values. However, minor QTLs are not part of the selection process that leads to a loss of genetic gain. To overcome this limitation, Genomic selection (GS) has been proposed (Meuwissen *et al.*, 2001) to capture the small effect of genes and genetic gain efficiency over MAS. GS reduced the selection time when compared to the phenotypic selection for almost traits in the different crops including maize (Lorenzana and Bernardo, 2009). GS contributed appreciable genetic gain for grain yield under drought stress tolerance in tropical maize germplasm (Beyene *et al.*, 2015). Prediction accuracy of GS among the full-sibs was more accurate than unrelated crosses (Riedelsheimer *et al.*, 2013)

The basic steps in Genomic selection includes: development of training population that means large number of populations should be genotypes with many numbers of markers as well as phenotyped for important traits, application of appropriate genomic models and then, predicate the genetic gain as well as genome estimated breeding values (Meuwissen *et al.*, 2001). Genomic selection is the application of all markers (high density markers) covering the whole genome so that incorporates all the available marker information

simultaneously into a model to predict the breeding value and genetic value of progenies for selection (Lorenz, 2013). Different genomic selection models (RR, LASSO, Bayesian B approaches and non-linear regressions) have been examined in diverse panels of crops to determine breeding values (Heslot *et al.*, 2012; Nepolean *et al.*, 2013). For example, Shikha *et al.*, (2017) tested the breeding values of 240 maize subtropical lines phenotyped for drought at different environments using 29,619 SNPs to predict the accuracies of seven genomic selection models were tested agronomic traits. Out of seven genomic models, Bayes B has been shown to have the highest level of prediction accuracy for data sets. It was also highlighted that several SNPs associated with different physiological functions.

Likewise, Beyene *et al.*, (2015) reported that average yield gain achieved ~ 0.08 t/ha per cycle for eight biparental population under drought condition and emphasized that genomic selection is more effective than pedigree-based conventional phenotypic selection to increase genetic gains in grain yield under drought stress in maize breeding. Similarly, Vivek *et al.*, (2017) reported that under drought condition and across locations, genetic gain per cycle for phenotypic selection ranged from 0.34 to 0.48 t ha<sup>-1</sup>, while that of GS ranged from 0.53 to 0.55 t ha. In addition, genetic gain per cycle for phenotypic selection of ASI showed a range between -0.05 and 0.5 day, while that of Genomic selection ranged from -0.33 to 0.25. Thus, Genomic estimated breeding value-enabled selection of superior phenotypes (without the target drought stress) resulted in rapid genetic gains for drought tolerance.

### **Genomic Wide Association Study (GWAS) for drought tolerance in maize**

Genome-wide association studies (GWAS) have been used widely to analyze the genetic control of complex traits for drought tolerance in maize. Wang *et al.*, (2016) reported that 206 significant SNPs were associated with 115 candidate genes for drought tolerance and related to grain yield, its related and secondary traits.

Moreover, 279 inbred lines was evaluated under field irrigated vs stress conditions and insights into the genetic basis of drought tolerance at the flowering using GWAS and revealed that associated drought tolerance genes, candidate genes and favorable alleles for variation provide insight into the genetic basis of drought tolerance at flowering stage in maize (Zheng *et al.*, 2022). Similarly diverse tropical maize line panel used to

study the association of SNP with root traits and other physiological traits revealed that genes governing several functional traits (Zaidi *et al.*, 2016)

### **Conclusion**

Drought is one of the adverse environmental conditions that affect crop production including maize. However, plants use different mechanism to cope with the drought such as escape strategies, drought avoidance and osmotic adjustment. Taken to account this situation, maize breeder' should consider the important secondary traits for selection under drought stress that directly associated to grain yield.

Several QTLs regulating important morpho-physiological traits and grain yield under managed and optimum conditions have been identified in maize. Some QTLs were found on separated chromosomes whereas, clustered QTLs were found on different chromosome that harbored QTL (hotspot region) for most morpho-physiological traits. Genomic selection to predict the breeding value as well as genetic value of progenies for selection in maize under drought stress condition. In addition, GWAS also revealed a strong association of candidate genes for secondary and physiological traits.

### **Future out look**

Classical maize breeding is applying in most research institute and some drought tolerance variety commercialized. In addition, integration with molecular breeding also applied. However, molecular marker assisted selection and gene editing remained on shelf. Thus, should be incorporated in maize breeding program to develop resilience cultivar that adapted to fluctuation of environment. Finally, integrated physiology, genomics and breeding approaches should be required to accelerate selection efficiency and genetic gain that contribute to maize improvement for drought tolerance.

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**How to cite this article:**

Belay Garoma. 2024. Integration of Conventional Breeding and Molecular Tools for Drought Tolerance in Maize: Review. *Int.J.Curr.Res.Aca.Rev.* 12(3), 55-63. doi: <https://doi.org/10.20546/ijcrar.2024.1203.007>