# International Journal of Advanced Research in Biological Sciences ISSN: 2348-8069

www.ijarbs.com

DOI: 10.22192/ijarbs

Coden: IJARQG (USA)

Volume 8, Issue 7 -2021

**Research Article** 

2348-8069

**DOI:** http://dx.doi.org/10.22192/ijarbs.2021.08.07.010

## The Improvement of Maize (Zea mays L.) for Drought Stress Tolerance

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#### Abstract

Maize is one of the important cereals in the world after rice and wheat. It is grown for grain as well as fodder in tropical, subtropical and temperate regions of the World. The reduction of maize productivity under drought stress conditions depends on different factors such as plant development stage, drought intensity and duration of water deficit, and varietal sensitivity to drought stress. Drought stress is one of the major constraints to agriculture where limited water availability for crop irrigation causes a reduction in carbon fixation by the photosynthetic apparatus that result in net yield losses. Plant breeders desire novel and more accurate tools to fast-track breeding programs required for the increasing demands of food and fodder because farmers are facing a changing climate in which drought stress has major impacts on crop damages globally. Crop loss, because of drought stress, has a huge impact on agriculture industry and the economy. Therefore, to prevent economic loss and to make the crop plants more tolerant to drought stress, a variety of approaches such as conventional breeding and molecular/ genetic engineering are being practiced.

Keywords: Genetic engineering, Conventional breeding, Molecular assisted selection, Drought and Stress

#### Introduction

Maize (*Zea mays* L.) is one of the important cereals in the world after rice and wheat. It is grown for grain as well as fodder in tropical, sub-tropical and temperate regions of the World (Majid et al., 2017). The While, the scarcity of water resource (drought) is the single most critical threat for reducing crop productivity under arid and semi-arid environmental condition in the World. The reduction of maize productivity under drought stress conditions depends on different factors such as plant development stage, drought intensity and duration of water deficit, and varietal sensitivity to drought stress (Fiedrick et al., 1989).

Therefore, to increase yield potential of maize under normal and abnormal environments has been inevitable for solving the world hunger at this moment. In line with the aforementioned issue, the aim of the crop breeding programs should to improve yield potential and drought tolerance/resistance maize hybrids under changing climate. Whereas several earlier research findings indicated that water deficit during reproductive stage was more effective (Barutcular et al., 2016b). The reduction of yield due to drought stress at reproductive stage was big-ger than at vegetative and grain filling stages (Khalili et al., 2010).Water stress decreases relative water contents, water potential, growth and yield of various crops (Akram, 2011).

Several indices have been proposed as useful for drought tolerance identification in maize (Moradi et al., 2012). Drought stress is one of the major

constraints to agriculture where limited water availability for crop irrigation causes a reduction in carbon fixation by the photosynthetic apparatus that result in net yield losses (Eldakak et al., 2013). Plant breeders desire novel and more accurate tools to fasttrack breeding programs required for the increasing demands of food and fodder because farmers are facing a changing climate in which drought stress has major impacts on crop damages globally (Abreu et al., 2013). Crop loss, because of drought stress, has a huge impact on agriculture industry and the economy. Therefore, to prevent economic loss and to make the crop plants more tolerant to drought stress (so that crop can thrive even in drought-prone situations), a variety of approaches such as conventional breeding and/or genetic engineering are being practiced. Therefore, the objective of this paper is to review maize improvement for drought stress tolerance.

#### Effect of drought stress in maize

drought is referred to as Strategies that allow plants to mitigate the negative effects of water deficits and can be classified into two broad categories (Levitt, 1972): (i) dehydration avoidance, which encompasses morpho-physiological features (e.g., deep roots, early flowering, etc.) that enable the plant, or parts thereof, to maintain hydration; and (ii) dehydration tolerance involving features that allow the plant to maintain, at least partially, proper functionality even in a dehydrated state. Maize plants may respond differently to drought stress at different crop stages. Poor establishment and bad plant stand are usually the result of soil drying during or after germination. Drought effects on maize include reduced leaf area resulting in incomplete ground cover as well as reduced stem and root expansion due to reduced assimilate fluxes to growing organs. In general, the root/shoot ratio increases slightly under drought stress; however, as the stress becomes more severe, root growth decreases and nutrient uptake from dry soil is sharply reduced (Bänziger et al., 2000).

Among various crop stages, the reproductive stage especially 3-4 weeks bracketing male flowering(anthesis) is the maize crop's most susceptible phase (Grant et al., 1989). Female reproductive structures are more seriously affected than the male flowers (tassels). Extreme sensitivity seems confined to the period 2 to 22 days after anthesis, with a peak at 7 days, and almost complete barrenness can occur if maize plants are stressed in the period from just before tassel emergence to the lagphase of grain-filling (Grant et al., 1989). Unlike other cereals, in maize the male and female flowers areseparated by as much distance as one meter; therefore, pollen and fragile stigmatic tissue have to be exposed to a dry and hostile atmosphere for pollination to occur. As silk growth and early kernel development appear to depend directly on the flow of current photosynthetic products drought-induced decrease in rate of photosynthesis at this stage significantly enhances the sensitivity to the stress (Schussler and Westgate, 1995).

When photosynthesis per plant at flowering is reduced by drought, silk growth is delayed, leading to an increase in the anthesis-silking interval (ASI), and kernel and ear abortion (Bolaños and Edmeades, 1996). Leaf senescence begins from the bottom of the plant (older leaves affected first) and proceeds towards the top of the plant. However, in conditions of high evapotranspiration due to combined heat and drought stress, leaf senescence may also occur at the top of the plant. Known as leaf firing, this further reduces the leaf area for radiation interception. About 2-3 weeks after pollination, once kernels enter the linear phase of grain-filling, they develop the sink strength needed to attract reserve assimilates stored in the stem and husk, apart from accessing current assimilates. If kernels reach this stage, they normally grow to at least 30% of the weight of kernels of unstressed plants, even if drought becomes more severe (Bolaños and Edmeades, 1996).



Fig.1 Effects of drought stress on vegetative and reproductive growth stages of maize(Muhammad et al., 2015)



Fig. 2 Gains from selection in a time series of temperate hybrids grown under drought stress imposed at different stages; A: ears per plant; B: leaf rolling score where 4 is rolled and 8 is flat; C: tassel branch number; D: the coefficient of variation (CV) of stem volume per plant (Edmeades, 2006).

#### Mechanisms of drought resistance in maize

Effects of drought stress are very uncertain and unpredictable because they impair the yield, yield potential and across the years performance. However, selection of genotypes with better yield under drought prevailing conditions is effective tool for combating against drought stress. Heterogeneity in nature of drought stress, variable effects in space and time, degree and severity of stress are further increasing the erratic and unpredictable behavior of stress. Nature has bestowed the plants to adapt for survival and productivity under stressful conditions (Gill et al., 2003). Plants harbor different morphological, physiological and biochemical traits which enable them to adapt or resist under stressful conditions. Resistance can be described as least reduction in yield under drought stress conditions relative to normal water availability. Resistance can be in the form of escape, avoidance and tolerance (Bohnert et al., 1995). In evolutionary reference, drought resistance is described as ability of the varieties or species to survive and reproduce under limited water availability.

In agricultural context, drought resistance is described as ability of th eplants to produce economical yield under limited water availability (Qualset, 1979).Plant mechanisms which contribute to bring least losses in yield under drought prevailing conditions compared to higher yield under normal water availability are also described as drought resistance. Existence of genetic variability among different crop plants and varieties of same species for drought resistance was reported. Yield stability in wheat, maize, rice, barley and sorghum was used to determine the drought resistance (Singh, 2010). Differences in yield, yield stability and level of drought resistance showed that improvement can be made by proper exploitation of this genetic variability. Different sources can be used for improvement of drought resistance in crop plants and some of them are as following; cultivated varieties, land races, wild relatives and development of transgenes. Landraces and wild relative are possible option to get the genes for drought resistance and to incorporatet hem in modern cultivars to improve their status of drought resistance. Zeamaxicana or tripsacum floridanum, wild relative of maize, are tremendous source of novel genes for improvement of tolerance against drought and other stresses (Singh, 2010). Transgenes can be developed if genes for drought resistance are available in non-crossable parents. Existence of significant genetic variability among or

between genetic populationsis prerequisite for genetic improvement.

Stress breeders suggested that elite breeding populations have very low frequencies of stress resistant alleles so, first of all these populations must be evaluated (Blum 1988). Significant genotypes  $\times$  environment interaction (GEI) in response of drought stress showed that substantial

genetic variation exists in breeding population. Breeders recommend that selection of breeding population for improvement is perquisite (Hallauer and Miranda 1988). Most importantly genetic variation for kernel yield under stress and stress free conditions is conducive. Elite breeding genotypes or cultivars have significant variability for drought resistance related traits which should be used for improvement of drought resistance on priority basis. So yield is primary trait associated with drought resistance whereas, appropriate secondary traits which confer drought resistance are selected as selection criteria if following assumptions are full filled; trait should be genetically correlated with yield, should have higher heritability, should be of stable nature, easy to measure and traits should be correlated with yield losses under normal prevailing conditions (Edmeades, 2008).

Most of secondary traits do not full fill all of these prerequisite criteria however, effective secondary maize traits associated with drought resistance are; leaf rolling, stay green, shorter anthesissilking interval, cob barrenness (number of kernels perear), root system, increased leaf erectness, kernel weight and low canopy temperature(Edmeades et al., 2000). Genetic variability in secondary traits of maize i.e. yield components and physiological traits can also be exploited to accelerate the improvement in yield under drought stress. Numerous factors contributed to the increase in resistance of maize germplasma gainst drought stress i.e. high plant density in field during development of in bredlines, prevalence of drought and heat stress in nurseries with insufficient water availability, use of high yielding and stable progenitors for breeding program andmulti-location testing of material (Tollenaar and Lee 2011). Major breeding objectives are to cultivate maize hybrids with greater yield potential, stable yield and improved grain traits for user whereas an additional ondem and objective is to produce the hybrids with enhanced resistance against adversaries.

New genotypes of maize developed through keeping in view the abovementioned objectives, would overcome the water deficiency by lowering the yield penalty. This implies that all maize hybrids should have significant level of drought resistance (Kitchen et al., 1999). Heterosis acts as important mechanism for stress tolerance, as maize hybrids give higher yield even under drought stress relative to maize varieties (Blum 1988). In maize, kernel yield is critically determined during flowering and early grain development (Claassen and Shaw 1970).Drought resistance is not heritable plant trait but numerous mechanisms are involvedin conferring the resistance in different ways. These mechanisms are classified into three different types: drought escape, drought avoidance and drought tolerance (Fig. 3).



Fig. 3 Mechanism of drought resistance in maize with background traits like, morphological, physiological, biochemical and molecular traits (Muhammad et al., 2015)

# Breeding approaches of maize improvement for drought tolerance

Management of drought stress to reduce the yield losses in crop plants is practiced in different forms across the world. Saving irrigation water with the help of different water management practices, exploitation of agronomic practices to improve cropper formance under drought stress condition, and development of drought-tolerant germplasm are the main tools which are exploited by agronomist and breeder. Water saving and cultural practices are found to be inconvenient, expensive, and requiring special skills. Development of drought-resistant germplasm is proved to be effective, efficient, and feasible approach for improving yield in drought prevailing territories (Athar and Ashraf 2009). Different strategic characters are improved by numerous biological approaches which enable the plants to escape, avoid, and tolerate the drought stress. Screening of germplasm for assessment of tolerant variants, development of tolerant genotypes through conventional breeding, mutation breeding, molecular breeding, and transgenic approaches are possible options which are working and can further be employed for further improvement.



Fig. 4 Biological/breeding approaches for improvement of drought resistance in maize

#### Screening for drought tolerant Maize germplasm

Evolutionary pathway has diverged the biological diversity at different levels of organization e.g., development of eukaryotes from prokaryotes followed by diversion toward development of plants, animals, fungi, bacteria, viruses, and other creations. Extensive prevailing diversity is categorized into different taxonomic levels like, species, genus, order, class, phylum, and kingdom. Species are further comprised of large number of varieties, strains, cultivars, and lines. Different groups of populations even within species have genetic differences for numerous parameters. Drought resistance is one of the aspects for which lot of genetic difference sare present within species. These genetic differences could be assessed by screening for drought stress resistance. Earliness is critical parameter which enables the plants to escape the drought stress. Development of extensive root system and prevention of water loss enabled the plants to

avoid drought stress (Athar and Ashraf, 2009). Mainte nance of normal physiological mechanism with satisfactory yield brings drought tolerance in plants. Genetic variability among different genotypes of maize is present for drought escape, avoidance, and tolerance which could be retrieved by suitable screening of germplasm. Different characteristic parameters which are strongly linked with these three mechanisms are focused in screening of germplasm. Screening of maize germplasm could be done in growth room under controlled conditions and in field under natural conditions. Parameters used for screening of germplasm must be associated with grain yield because higher grain yield is ultimate objective of screening. Traits which are affected severely by drought stress are targeted in screening.

#### Selection for high yield potential

High yield potential is a constitutive trait that often gives increased yield under moderate levels of drought. In such conditions, the likelihood of spillovers from one environment to another can be estimated through the genetic correlation between yields of the same cultivars grown in those two environments (Bänziger et al., 2000). Spill-overs can be expected when the genetic correlation (rG) between yields in stressed and well-watered sites is positive and significant. If rG is weak/non-significant, selection for yield potential alone does not contribute much to drought tolerance.

#### **Selection for earliness**

A major goal of breeding is to develop cultivars that can escape drought by being sufficiently early maturing so as to complete their life cycle within a given favorable season length. Selection for earliness matches the phenology of the crop to the pattern of water availability/rainfall. Since the time from sowing to flowering or physiological maturity is a highly heritable trait, selection for earliness can be easily accomplished. However, earliness carries a yield "penalty," especially when moisture availability is optimal. Under those circumstances, the yield of an early maturing cultivar is limited by the amount of radiation the cultivar can capture normally less than the radiation captured by later maturing cultivars (Bänziger et al., 2000).

#### **Selection for drought tolerance**

In the tropics, a successful maize cultivar must be able to withstand year-to-year variation in rainfall and associated moisture deficits at critical growth stages. Drought tolerant cultivars are characterized by minimal yield losses when soil moisture availability is significantly reduced. Except at the seedling stage, traits that increase plant survival but not production are of little value in selection (Bänziger et al., 2000). To minimize yield losses under drought without significantly compromising yield under optimal moisture conditions, selection for grain yield along with stress-adaptive secondary traits under drought stress should be coupled with selection for high yield potential and desirable agronomic traits.

#### **Conventional Breeding Strategies**

Creation of genetic variability and novel gene combination through intercrossing of targeted parents is one of the practices used to develop tolerant genotypes. Intercrossing followed by appropriate selection scheme enables to develop an ideotype plant that is suitable for environment specific cultivation (Bänziger et al.2000). Higher genetic variability, high heritability, and higher selection intensity empower the breeder to make appropriate selection in the germplasm (Falconer1989). Breeding strategies for development of drought-resistant germplasm are economical and effective tool for combating the global issue of water deficiency (Subbarao et al. 2005). Existence of genetic variability at generic, specific, and varietal levels act as raw material for selection and breeding against drought stress(Serraj et al. 2005a).

Maize breeders have to focus on large number of traits for improvement of drought resistance as it is well known that single trait could not improve the resistance because plant responses interact with each other in complex fashion. Gene pyramiding, efficient and systematic breeding method, can effectively improve the drought tolerance by incorporation of large number of favorable traits in one genotype. Morphological and physiological parameters which prevent water loss, improve water use efficiency, and economic yield must be focused for pyramiding as recommended by (Subbarao et al., 2005). Early vigor, rapid establishment, structural and functional traits of roots, osmo protection, stomatal conductance, and leaf characteristics are suggested by (Parry et al., 2005) as key parameters for improvement of drought tolerance. Development of early maturing varieties is important tool for escaping terminal drought stress by completing the life cycle before the onset of drought stress. So, earliness could also be incorporated by breeding to escape terminal drought (Athar and Ashraf 2009). Breeding for development of genotypes which are efficient water user (collect more quantity of water and loose less) could be effective to avoid the harmful effects of drought stress. Structural and functional traits of roots and stomata should be focus of breeders for development of drought avoiding genotypes.

Breeding efforts for improvement of drought tolerance concentrated on the traits which maintain normal physiological mechanisms (osmolytes, antioxidants, plant growth regulators, stress-responsive proteins, and transcription factors) and economic yield (yield and yield components) of crop plant (Bänziger et al. 2000).Breeders collect large number of germplasm with variable origin; initially, selections (screening phase) are made on yield and yield component basis; after reducing the number of genotypes by selection then selections are made for drought resistance (testing phase). Quantitative inheritance, low heritability, and higher genotype into environment interaction proved as barriers for quick improvement of yield under drought stress (Babu et al. 2003). Assessment of yield limiting traits with the help of morphological, physiological, biochemical, and molecular techniques could supplement the conventional breeding methods for improvement of yield (Cattivelli et al. 2008).Conventional breeding methods rely on

conducting multi location, multiyear and multiseas on yield trials for evaluation of stability in the performance against drought stress (Babu et al. 2003). Yield and yield components are primary target traits to be focused for crop improvement against drought stress.

Secondary traits are equally important for breeding against drought stress. Secondary traits which have strong correlation with grain yield, stable in nature, easy to measure, higher itability, and improve yield are preferred even under normal environmental conditions (Edmeades et al. 2001). Worth of secondary traits is realized through selection indices, heritability in progenies, and genetic association. Development of near isogenic lines and synthetics helped the breeder to know the association of targeted secondary trait with economic yield (Bänziger et al. 2000). Interspecific and intervarietal differences are present for water use efficiency indifferent crop plants and impairment of this trait is among early drought responses. Water use efficiency is genetically governed trait and its higher value depicts drought tolerance. Water use efficiency is reduced under drought stress either due to sustained biomass production or higher water losses. Higher ratio for biomass production to transpired water, partitioning of biomass toward economical part, reduced water loss, and increased water uptake are the components of water use efficiency which could be focused for breeding against drought stress (Farooq et al., 2009).

Improvement in drought tolerance is complex due to polygenic nature and low frequency of alleles for tolerance in maize. Open-pollinated varieties (OPV) and hybrid products are targets in maize for improvement of drought tolerance. Improving locally adapted germplasm, improving tolerant exotic germplasm for adaptability, and development of new breeding population through introgression are the recommended options for drought tolerance improvement in maize. Development of source population and evaluation of that population are subcomponents of introgression. Selection and development of source population must be done on the basis of following characteristics; general adaptability, grain color, grain texture, maturity, disease resistance, abiotic stress tolerance, heterotic pattern, heterotic response, combining ability, and other value added traits. Evaluation of developed population could be done through line evaluations, hybridization, diallels of local or exotic populations or lines, population x local tester top cross, and line xlocal tester topcross. Intrapopulation improvement for drought tolerance could bedone through individual plant selection, per se performance, test crosses using individual plants, halfsib progenies and parental testers (Bänziger et al. 2000). It is well admitted that conventional breeding approaches are effectively involved in the improvement of drought tolerance in maize. These techniques are effective because evaluation is made under field conditions and interaction with environment is well considered.

Mutation-assisted breeding along with modern molecular techniques are effective in generation and identification of desired mutations. Mutation breeding being nontransgenic approach is safe and secure strategy for crop improvement. Ideotype for droughttolerant maize genotype can be developed through mutation-assisted breeding, so there is need to explore the otential of mutations for maize improvement.

Trait	Heritability	Correlation with yield	Selection objective	Target growth
Grain yield	Medium to low under flowering stress, medium during grain filling stress	High Positive	Increase economic yield	Flowering and grain development
Ears per plant	High and increasing With stress intensity	High	More ears per plant or low barrenness	Flowering stage
Anthesis-silking interval (ASI)	Medium under normal, high level under severestress	High under Stress	Reduced or negative ASI	Flowering stage

Table 1 Secondary traits targeted for drought tolerance improvement through conventional breeding (Recommended by CIMMYT; Bänziger et al. 2000)

Tassel size	Medium to high	Medium	Smaller tassel with fewer branches	Could be measured under normal and stress conditions
leaf senescence	Medium	Medium under grain filling stress	Delayed leaf senescence or stay-green property	Grain filling Stage
Leaf rolling	Medium to high	Medium to Low	Unrolled leaves	Flowering stress

#### Int. J. Adv. Res. Biol. Sci. (2021). 8(7): 90-102

#### Marker-assisted and genomic-assisted breeding

Effectiveness of conventional breeding is reduced due to low heritability of traits infield, high-field management cost, seasonal variability, time and space issues and higher genotype into environment interaction. So, marker-assisted selection becomes effective tool because DNA present within cell is independent of environmental and managerial effects. Pace of crop improvement increases by markerassisted breeding because these are based on cellular DNA. Numerous DNA markers are available but plant breeder need ideal markers which must have following characteristics features and must be strongly linked with trait controlling genes, co-dominant inheritance, and PCR based; marker should depict large variability for traits, polymorphic in nature, abundant in genome, and easy to amplify (Varshney 2010). Marker-assisted selection is used for numerous tasks. Genetic distance between parents, prediction of heterotic potential of hybrids, and selection of inbred lines to be used as parent could be made effectively by fingerprinting of inbred lines. Line conversion in maize can also be done through marker-assisted backcrossing by transferring the one desired trait coding gene from donor line to recipient line. Number of generations for backcrossing and probability of linkage drag is reduced significantly in cases of marker-assisted to backcrossing comparative conventional backcrossing (Bänziger et al. 2000).

Tolerance is complex feature governed by large number of traits, and these traits are controlled by large number of chromosomal regions known as quantitative trait loci (QTLs). Parents with contrasting phenotypic expression are crossed to develops egregating progenies. Segregating populations are screened with the help of DNA markers like, RAPD, RFLP, AFLP, SSR, and SNPs. Markers linked with specific traits are then identified with bioinformatics tools. Exploitation of DNA-based markers for identification of QTL mapping linked with morphological, physiological, and biochemical traits could be targeted by breeder for drought resistance improvement. After identification of QTLs linked with traits, drought tolerance can be improved by introgression of these QTLs into modern promising cultivars. Marker-assisted selection(MAS) based on trait-linked QTLs proved to be effective for dissecting quantitative traits into unit genetic components and assisting plant breeder to make appropriate-targeted selection (Hussain, 2006).Linkage mapping and association studies through association mapping and approach are effective for candidate gene identification of QTLs.

Most of QTL studies in literature are based on segregation mapping but association mapping is most vigorous tool than segregation mapping (Syvänen, 2005). Monogenic traits like plant height, osmotic adjustment, flowering time, and ear development are more adaptive traits for drought tolerance. Genetic diversity for numerous morphological, physiological, biochemical, and molecular drought responsive traits in maize is reported. So, genetic variability could be exploited for improvement of drought tolerance in maize through marker-assisted selection. MAS proved even more efficient tool if markers are strongly linked with stress-responsive traits. Anthesissilking interval (ASI) is very important trait in maize; lower ASI value is associated with drought tolerance. CIMMYT identified six QTLs linked with ASI, which are located on chromosome number 1, 2, 5, 6, 8, and 10 of maize genome. These QTLs are contributing 50 % phenotypic variability of ASI and are stable across the years and water regimes (Bänziger et al. 2000). Additive QTLs for ear length and kernel weight, whereas epistatic QTLs for kernel number per row are observed in maize recombinant inbred lines (RILs). Genetic background of QTLs is changed (additive to apistatic and vice versa) under different water treatments for some maize traits.

Existence of additive and epistatic QTLs in maize shows that expression pattern oftraits is diverse and nature of drought tolerance is very complex (Lu et al. 2006).obtained from MAS made the breeding programs more effective by following ways; selection can be made at early generations and number of generations required for conventional breeding approach are reduced; accuracy of selection is highly increased (Phelps et al. 1996). Depending on the targeted locus, site of amplification, level of conservation, type of primers used, and breeding objectives large number of DNA-based markers are being used, some of them are enlisted here: random amplified polymorphic DNA (RAPD), selective amplification of microsatellite polymorphic loci (SAMPL), restriction fragment length polymorphism(RFLP), sequence characterized amplified regions (SCAR), expressed sequence tags (EST), simple sequence repeats (SSR), inter-simple repeat(ISSR), sequence single nucleotide polymorphism (SNP), sequence specific amplification polymorphisms (S-SAP), sequence tagged site (STS), sequence tagged microsatellite site (STMS), singleprimer amplification reactions (SPAR), site-selected insertion PCR (SSI), single-stranded conformational polymorphism(SSCP), short-tandem repeats (STR), diversity arrays technology (DART), and variable number tandem repeat (VNTR) (Semagn et al. 2006).

Research efforts in maize were focused on the development of microsatellite markers for germplasm analysis and genetic mapping. Gene mapping is helpful in providing the information about specific locus of genes and number of genes governing the traits. (Dubey et al., 2009) targeted the 24 accessions of tropical maize for assessment of drought-linked SSR markers. They found that UMC1042.DUPSSR12. UMC1056, BNLG1866, UMC1069. DUP13. BNLG1028, UMC1962, and C1344 SSR markers were linked with drought responses (Tuberosa et al., 2002a) and (Sawkins et al., 2006) identified the QTLs in maize, which were linkedwith drought. Introgression breeding in maize, introgression of transgenes, conversion of simple or complex traits, and markerassisted recurrent selection (MARS)were breeding perspectives in maize for which markers were used with special focus to drought stress (Sawkins et al., 2006). Theoretically MAS is known to improve drought tolerance but practically contribution of MAS in release of high yielding drought-tolerant genotype is nonsignificant (Reynolds and Tuberosa 2008). So, focus should be targeted that markers linked with

drought tolerance should also be linked with higher yield potential for getting two fold benefits.

In literature, few cases had been reported which showed the involvement of MAS in development of drought tolerance cultivars with higher yield potential (Athar and Ashraf 2009). introgression of five QTLs in maize has increased50 % yield comparative to standard hybrids under drought stress, and no vield losses were observed under normal water availability (Ribaut and Ragot 2007). Introgression of yield-linked QTLs in pearl millet improved the grain yield in drought sensitive genotypes (Serrajet al. 2005b). Staygreen character of sorghum is improved by introgression of QTLs (Harris et al. 2007).QTL mapping enabled the breeders to identify the chromosomal regions linked with different plant traits. Effect of genetic background, complex genetic basis, stage of plant growth and development, environment  $\times$ QTL interaction (Tuberosa et al. 2002b), gene by gene effects, inadequate phenotyping, cost, and skill issues are limiting the effectiveness of QTL mapping (Xu et al.,2009). OTL identification, validation in different populations or under different environments followed by their proper manipulation in breeding program could bemuch more effective for real-sense improvement against drought stress. There isstill gap which must be filled for getting more benefits from marker-based selection. Functional genomics and transcriptomics are recently used for extensive understanding of plant responses against stresses.

Identification of candidate gene, followed by characterization, and determination of transcriptomic responses through microarray or whole genome sequencing help to clearly highlight the tolerance mechanisms. Drought responsive candidate genes are identified by imposition of drought stress on stressresponsive genotypes followed by ESTs generation from either normalized or nonnormalized cDNA library. Public databases are being exploited for retrieval of drought stress-responsive candidate genes in major crops like wheat, maize, barley, and rice (Sreenivasulu et al. 2007). Transcript profiling is used for identification of candidate genes through assessment of differential gene expressions in a specific tissue at different times(Hampton et al. 2010). Transcript profiling can be done through cDNAamplified fragment length polymorphism (cDNA-AFLP), PCR-based differential display PCR (DDRT-PCR) analysis, digital expression analysis based on of ESTs,cDNA oligonucleotide counts and microarrays, serial analysis of gene expression

(SAGE)technique, SuperSAGE, and next generation sequencing (Mir et al.2012).

Among these techniques, next generation sequencing (NGS)-based techniques are most preferred for routine transcript profiling of main crops for identification of drought-tolerant candidate gene followed by exploitation of that gene through genomics and marker-assisted breeding. After identification of major QTLs, contributing to drought tolerance, these are validated in target population. Following validation, these QTLs could be exploited through their introgression into high yielding and drought susceptible (recipient parent) from low yielding and drought-tolerant parent (donor parent), this technique is known as marker-assisted backcrossing (MABC). BirsaVikasDhan 111 (PY 84), a rice variety, was developed through marker-assisted backcrossing in India having improved drought tolerance(Steele et al., 2007). Complexity of mechanism of drought tolerance act as barrier for substantial exploitation of MABC e.g., almost 10 % phenotypic variability was explained by identified OTLs in maize (Xu et al., 2009). These findings conclude that extensively large population size is mandatory for achieving satisfactory improvement through MABC.MABC acts as effective tool when traits are governed by single or few genes but in case of drought tolerance which is very complex feature and governed by large number of genes, this technique becomes least effective. Marker-assisted recurrent selection (MARS) has capability to deal with complex traits like drought tolerance and involves the inter-mating of selected accessions in each recurrent cycle (Ribautand Ragot 2007).

Population improvement is adequately accomplished by MARS because MAS is practiced in each selection cycle followed by interbreeding of selected individuals, which validates and increases the frequency of desired genes in target population (Eathington et al. 2007). MARS is being used for drought tolerance improvement in different crops e.g., wheat, chickpea, cowpea, and sorghum (Mir et al., 2012). Plenty of work needed to be done for improvement of drought tolerance in maize through MARS.Genome selection (GS) and genome-wide selection (GWS) are important molecular techniques for improvement of drought tolerance in crop plants bydeveloping superiorly drought-tolerant genotypes. Unlikely of MARS, genome selection is done through genome-wide marker genotyping. GS has numerous advantages over other techniques like, reduced selection time, increased annual gain from selection, and reduced phenotyping frequency (Rutkoski et al.2010). Initiative for exploration of GS potential in different crops has been taken butits application for improvement of drought tolerance (Mir et al. 2012) especially in maize is lacking. Exploration of GS in maize for the improvement of drought tolerance is very important.



Fig. 5 Projected cumulative yield gain over a 19 year period in maize being selected for drought tolerance using conventional selection methods (50 Kg/ha/yr), [marker-aided selection(MAS) + conventional] (75 kg/ha/yr)) and [one transgene introduced every 8 year + MAS + conventional

Each transgene added boosts yield by a cumulative 5%. Effects of each intervention are considered additive.

# Transgenic maize development for drought stress tolerance

Genetically complex nature of drought tolerance makes the transgenic development even more complex than for monogenic traits. Exploitation of signal transduction cascades, transcription factors, or transformation with numerous genes regulates the pivotal processes. But current research work is focused on single-gene transformation. Signal transduction pathways are activated by stress responses which resultantly regulate the cascades of adaptations. These signaling pathways can be modified or tailored using tool of genetic engineering. Development of drought-tolerant transgenic crop basically involves the incorporation of one or more genes from other donor source/sources in target crop to modify the signaling and subsequent events (Vinocur and Altman 2005). Genes which can be manipulated in genetic engineering are categorized into four main classes: (1) genes involved intranscriptional and signal transduction pathways, (2) genes involved in protection of cellular membranes and biosynthesis of stress-responsive proteins, (3) genes involved in uptake of ions and water-like ion transporters and aquaporins (Wanget al. 2003), and (4) genes involved in cellular metabolism e.g., free amino acids, proline, soluble sugars, polyols, and glycinebetaine (Vinocur and Altman 2005).

Transgenic constitutive upregulation of transcription factors (TFs) improve drought tolerance but these TFs also upregulate other genes which impair normal plant growth and development resultantly reduced economic vield (Wang et al.2003). Alternative to TFs, stressinduced promoters could be exploited for improvement of drought tolerance because their side effects are far less than TFs(Athar and Ashraf 2009). NADP-malic enzyme, kev enzyme of C4 photosynthesis, from maize was transgened in tobacco which reduced stomatal conductance and improved water use efficiency (Laporte et al. 2002). So functional sustainability of this enzyme in maize under drought stress definitely will improve the tolerance by improving water use efficiency.

### Conclusion

Drought is major abiotic stress which affects numerous crop plants at different levels of growth and development. Effects of drought stress on maize are prevalent from germination to harvest maturity. Germination percent, germination potential, germination rate, seedling establishment and seedling vigor are disturbed by drought at early growth stages. Growth and development of vegetative parts of maize are seriously affected by diminished cell division and cell proliferation which clarified that cell cycle is critically dependent on water status of plants. Plant height, stem diameter, plant biomass, leaf area and root development are disturbed in maize by drought stress. Reproductive stage in maize is more critically impaired by drought stress. Development of tassel and ear, pollination, fertilization, embryo development, endosperm development and grain filling are seriously affected by drought stress inmaize. All genotypes of maize are not equally affected by drought stress due to high level of variability in genetic background of this crop. Different mechanisms have been evolved in maize like other crops which enable them to effectively survive under drought stress. Drought escape, drought avoidance and drought tolerance are different mechanisms which work under the heading of drought resistance.

Different strategies are used for improvement of maize against drought stress e.g. breeding strategies. Breeding approaches deal with manipulation of genetic background of maize for improvement against drought stress. Breeding strategies are preferred due to long term and economical effectiveness. Breeding approach is practiced in different forms. Available germplasm has lot of genetic variability which can be exploited for higher drought tolerance through effective screening tools. Conventional breeding, marker assisted and genomic assisted breeding and development of drought tolerant transgenic maize are numerous strategies which are enlisted under the heading of breeding strategies. There are few gaps in effectiveness of breeding techniques which must be filled under the umbrella of modern technology for the improvement of drought tolerance of maize in such a way that it can fully combat with drought stress. Lots of novel breeding and evaluation techniques have been developed in recent past and practical application of other techniques will further help to cope the problem of drought stress by development of more drought tolerant maize genotypes for resolution of food security.

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DOI:10.22192/ijarbs.2021.08.07.010

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	Quick Response Code		

How to cite this article:

Zewdu Asrat. (2021). The Improvement of Maize (*Zea mays* L.) for Drought Stress Tolerance. Int. J. Adv. Res. Biol. Sci. 8(7): 90–102. DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.07.010