

Review Article

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**Recent Advances in Breeding for Abiotic Stress (Drought) Tolerance in Maize**

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**A B S T R A C T**

Globally maize is the third most important crop in the world. The main maize crop is generally grown as rainfed and on marginal lands particularly in hilly terrains of the Kashmir valley. It is situated at longitude and latitude of 73.0-76.2E and 32.50-36.0N respectively. By 2050 demand for maize will double in the developing world, and maize is predicted to become the crop with the greatest production globally, and in the developing world by 2025 (Rosegrant *et al.*, 2008). Heisey and Edmeades (1999) estimated that one quarter of the global maize area is affected by drought in any given year. Since farmers usually plant a single variety in any given field, this implies a need for a good level of drought tolerance in the large majority of hybrids and varieties grown under rainfed conditions. Drought is the major abiotic stress limiting maize production in large part of South and South-East Asia, and many other parts of the world. The drought stress accounted for approximately 16% loss of maize production in lowland tropics (Edmeades *et al.*, 2006). Losses due to drought in lowland tropics averaged 17% (Edmeades *et al.*, 1992), and it reached up to 60% in severely drought-affected regions/seasons (Rosen and Scott, 1992). In India, approximately 2.4 mha (~ 32.4%) of total maize growing areas is prone to face drought or excess moisture stress (AICRP, 2006). Selection and improvement under optimal conditions may not be suitable for the target areas prone to abiotic constraints (Simmonds, 1991). Therefore, in order to achieve improved and stable yields in stress prone environments it is desirable to improve tolerance to major abiotic constraints prevalent in target environment.

**Keywords**

Maize, Drought, Molecular breeding, Anthesis-Silking-Interval, MARS, MABC, MSE's.

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**Introduction**

Globally maize is the third most important crop. It is produced on nearly 100 million hectares in developing countries, with almost 70 % of the total maize production in the developing world coming from low and lower middle income countries (FAOSTAT, 2012). Maize is a versatile crop grown over a wide range of agro climatic zones. In fact, the suitability of maize to diverse environments is unmatched by any other crop.

It is grown from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year and with a growing cycle ranging from 3 to 10 months.

In Jammu and Kashmir, the crop is cultivated over an area of 0.30 million hectares with total production of 0.59 MT and productivity of 1.96 T ha<sup>-1</sup>, whereas, 0.1 million hectare area is under maize in Kashmir with

production of 0.18 MT with an average yield of 1.8 T ha<sup>-1</sup> (Anonymous, 2013-14). The Kashmir Division is agro climatically a typical temperate region. In Kashmir Valley maize is grown as a sole crop at an altitude range of 1850-2300m above mean sea level. Combining ability is an important prerequisite for developing a good economically viable hybrid maize variety. Proper choice of parents is an important criterion in order to exploit hybrid vigour. This key step depends on factors like *per se* performance of the parents and their combining ability. Combining ability is a potent tool in identifying the good combiners for hybridization especially, when a large number of parental lines are available and promising ones are to be selected on the basis of their ability to give superior cross combinations. Besides pin pointing the promising parents to be used in the development of advanced hybrids particularly when the production of such hybrids is not feasible due to some inherent problems in economic hybrid seed production, combining ability analysis has a momentous role in crop improvement as it helps in characterizing the nature and magnitude of genetic effects governing yield and its component traits (Gowhar *et al.*, 2017).

Mexico has the highest diversity of maize germplasm (Ortega, 2000), In the State Jammu and Kashmir(India), maize is second most important crop after rice and is a staple food of some tribal areas such as Gujar and Bakarwall (nomadic race). It is most important crop of United States and Mexico as primary centre of origin. From this centre crop spread to other non-traditional areas and even to temperate belts of world and got established as a new crop. Mexico has the highest diversity of maize germplasm (Ortega, 2000), a large number and high percentage of *campesinos*, and the highest direct, per capita, maize consumption in the world (Warman, 2001). In contrast to the

situation in the United States, where hybrids were essentially introduced in the early 1930s and occupied virtually all maize farmland by 1945, the varied ecology in Mexico has greatly discouraged such universal adoption of hybrids (Frankel *et al.*, 1995). In Mexico, the proportion of maize land surface sown with landraces (80%) is far beyond both the average world value (48.5%) and the average for Latin America (55%; Morris, 2001), excluding Argentina, which now only plants hybrids. In developed countries it is primarily used as a poultry and animal feed, while as in developing regions of the world maize grain is produced for human consumption, besides poultry and animal ration which get the secondary importance.

Development of number of modern high yielding hybrids and synthetic in maize has replaced all the elite landraces and has posed a big threat in terms of loss of valuable genetic variability and potential genetic vulnerability. Genetic diversity created in the farmers' field over millennia complemented by genetic diversity present in the wild relatives of crop provide the raw material for improving crop productivity through plant breeding. These landrace populations are finite and vulnerable to losses due to development of new crop varieties, growing urbanization, natural hazards etc. These valuable resources contribute enormously towards the millennium development goals of food security, poverty alleviation, environmental protection and sustainable development.

### **Biology of maize crop**

Maize (*Zea mays*) belongs to the grass family (Gramineae) and is a tall annual plant with an extensive fibrous root system. It is a cross pollinating species, with the female (ear) and male (tassel) flowers in separate places on the plant. Maize stems resemble bamboo canes.

The ears are female inflorescences, tightly covered over by several layers of leaves. The apex of the stem ends in the tassel, an inflorescence of male flowers. When the tassel is mature and conditions are suitably warm and dry, anthers on tassel release pollen. Maize pollen is dispersed by wind, most pollen falls within a few meters of the tassel. The kernel of maize has a pericarp of the fruit fused with the seed coat, typical of the grasses. It is close to a multiple fruit in structure, except that the individual fruits never fuse into a single mass. The grains are about the size of peas, and adhere in regular rows. The grain develops in the ears or cobs often one on each stalk; each ear has about 300 to 1000 kernels, weighing between 190 and 300 g per 1000 kernels, in a variable number of rows (12 to 16). Weight depends on genetic, environmental and cultural practices. Grain makes up about 42 % of the dry weight of the plant. The kernels are often white or yellow in colour, although black, red and a mixture of colours is also found. There are a number of grain types, distinguished by differences in the chemical compounds deposited or stored in the kernel.

### **Agricultural drought**

It is the result of soil moisture stress due to imbalance between available soil moisture and evapo-transpiration of a crop. It is usually gradual and progressive. Plants can therefore, adjust at least partly, to the increased soil moisture stress. This situation arises as a consequence of scanty precipitation or its uneven distribution both in space and time. It is also usually referred as soil drought. Relevant definition of agricultural drought appears to be a period of dryness during the crop season, sufficiently prolonged to adversely affect the yield. The extent of yield loss depends on the crop growth stage and the degree of stress.

Water stress is considered as one of the most devastating environmental stresses worldwide as it has rendered large area of agricultural land unproductive around the globe (Avramova *et al.*, 2015; Huang *et al.*, 2015; Langridge and Reynolds, 2015; Obidiegwu *et al.*, 2015; Zhan *et al.*, 2015).

Alterations in rainfall pattern and rising temperature are major causes of drought and have contributed an appreciable decline in crop productivity (Lobell *et al.*, 2011; Langridge and Reynolds, 2015; Obidiegwu *et al.*, 2015). Consequently, considerable agriculture losses occurred because drought-sensitive crops failed to grow under such conditions (Athar and Ashraf, 2009; Huang *et al.*, 2015). It is more likely that increasing population and changing climatic conditions will increase water scarcity, which will cause a further decrease in crop productivity in the world. For example, current trends of climatic changes will increase water scarcity and will reduce maize productivity by 15- 30% (Lobell *et al.*, 2014). Therefore, concrete efforts are required to meet the increasing demand for food for heavily populated geographical areas with water scarcity. In order to achieve this target, it is imperative to understand how plants respond and adapt to water stress.

The inhibition of plant and root growth due to water stress is the earliest growth response, which reduce rate of transpiration thus help in water conservation. However, such effects can reduce the yield up to 60% of maize even if maize plants do not show leaf wilting (Ribaut *et al.*, 2009). Among different plant adaptive strategies to water stress, drought avoidance is one of the most important drought adaptive strategies that can be used for enhancing crop yield under water stress conditions (Blum, 2011a). This can be achieved in a variety of ways, including adjustment of growth rate and growth pattern of shoot and root (Comas *et al.*, 2013).

Thus, extent of drought avoidance or tolerance in plants can be determined by a number of biometric attributes such as leaf number and structure, root length and branching pattern, leaf waxy layer, leaf rolling etc. (Blum, 2011a; Comas *et al.*, 2013). Since crop sensitivity at the germination growth stage governs overall success of a crop, it is advocated that biometric attributes at the early growth stages can be used as indicator for crop performance at later growth stage or as a selection criteria for improving crop resistance against drought (Lobell *et al.*, 2008; Reynolds and Tuberosa, 2008; Blum, 2011b; Comas *et al.*, 2013). This argument can be supported by the fact that several germination and seedling growth indices are frequently used as predictors to appraise drought tolerance in crop plants (Comas *et al.*, 2013; Ayalew *et al.*, 2014; Shamim *et al.*, 2014; Obidiegwu *et al.*, 2015). The variability in morphological attributes that were associated with tolerance for a target environment can be explored by applying strong selection pressures (Kausar *et al.*, 2006; Huang *et al.*, 2015). Thus, exploitation of inter and intraspecific variation of characteristics for tolerance or avoidance for drought provides an efficient and economic mean of crop selection. As such selected species/cultivars can successfully be grown under drought conditions. Maize (*Zea mays* L.) is one of the three significant crops of the world following wheat and rice (Ribaut *et al.*, 2009; Cooper *et al.*, 2014; Lobell *et al.*, 2014; Huang *et al.*, 2015). Despite considerable significance of maize as food, forage and oil, a few studies have been focused on the selection of maize germplasm to appraise its drought or water stress tolerance (Avramova *et al.*, 2015). One of the most plausible techniques to simulate uniform drought includes the use of metabolically inactive compound such as Polyethylene glycol (PEG) which has been widely employed by a number of workers to

study the effects of water stress in different groups of plants (Ashraf *et al.*, 1996; Kausar *et al.*, 2006; Shamim *et al.*, 2014).

Important causes for agricultural drought are:

- Inadequate precipitation
- Erratic distribution
- Long dry spells in the monsoon
- Late onset of monsoon
- Early withdrawal of monsoon
- Lack of proper soil and crop management

Conventional drought stress tolerance breeding has yielded significant dividends in maize (Bänziger *et al.*, 2006). Conventional breeding for drought tolerance has resulted in gains of up to 144 kg ha<sup>-1</sup>yr<sup>-1</sup> in tropical maize when stress was imposed at flowering (Edmeades *et al.*, 1999). In temperate maize, the rate of breeding progress has been estimated at 73 kg ha<sup>-1</sup>yr<sup>-1</sup> for mild stress (Zhang *et al.*, 1987), 146 kg when the stress was imposed at the flowering stage, and 76 kg ha<sup>-1</sup>yr<sup>-1</sup> when the stress was imposed during mid-grain filling stage (Campos *et al.*, 2004). Success in breeding drought tolerant tropical maize has been largely attributed with the application of proven drought breeding methodologies in managed stress screening (Bänziger *et al.*, 2006). While drought negatively affects all stages of maize growth and production, the reproductive stage, particularly between tassel emergence and early grain-filling, is the most sensitive to drought stress (Grant *et al.*, 1989). Drought stress during this period results in a significant reduction in grain yield, associated with a reduction in kernel size (Bolaños and Edmeades, 1993). The susceptibility of maize to drought stress is generally attributed to its separation of male and female flowers (Grant *et al.*, 1989). While silking is delayed under drought stress, there is little effect on the timing of pollen shed. Comparisons of the responses of male and female reproductive

tissues under drought stress confirmed female tissues to be the most sensitive. Westgate and Boyer (1986) compared the response of male and female reproductive tissues and found silk water potential to follow changes in leaf water potential, while pollen water potential remained unchanged. The results of their experiments indicated stigmatic tissues were in moderate hydraulic contact with vegetative tissue. Using stem infusions of sucrose solution, showed that the effects of drought at flowering could be partially alleviated; suggesting silk delay may be a symptom of limited assimilates supply rather than a primary cause of bareness. The delay in silking results in decreased male-female flowering synchrony or increased anthesis-silking interval (ASI). CIMMYT initiated a drought breeding program for maize using the elite lowlan (Bolanos and Edmeades, 1993). A recurrent selection approach was applied to increase the frequency of alleles conferring tolerance. Evaluations were conducted under managed drought stress imposed at flowering with selection for grain yield, increased flowering synchrony and delayed leaf senescence (Bolaños and Edmeades, 1993a).

### **Morphological changes**

If we talk about the morphological changes, there are changes in the cell elongation, stimulation of cell division and alternation in cell differentiation status (Potters *et al.*, 2007). So therefore there is negative effect on the plant growth and development through the arrest of the cell cycle machinery (Peres *et al.*, 2007). In plant tissues, water potential and content are maintained to increasing uptake or limiting loss, so they are in balance. So these balances are achieved by the morphological traits and their development, which is parallel to decrease the photosynthesis rate (Lawlor, 2002). So therefore decreasing the CO<sub>2</sub> and water loss from the leaves will affect the mesophyll metabolism (Parry *et al.*, 2002).

But if we look on long term, there is also root and shoot growth effects which leads to increased growth, tissue water storage capacity effect and therefore there is change in root growth to maximize water uptake are most crucial for crop plants (Verslues *et al.*, 2006).

### **Physiological changes**

Drought stress affects some key physiological traits:

#### **Abscisic acid (ABA) accumulates**

ABA is generated mainly in the roots, where it stimulates growth. It passes to leaves (and grain to a much lesser degree) where it causes leaf rolling, stomatal closure and accelerates leaf senescence. This happens even before hydraulic mechanisms reduce leaf turgor (Zhang *et al.*, 1987). It seems likely that it is this “root signal” that causes the plant to reduce water loss. Thus, ABA is a plant growth regulator that helps the plant to survive drought stress but does not seem to contribute to production under drought. ABA passes as well to the grain, where it contributes to the abortion of tip grains during grain filling.

**Under mild to moderate stress, cell expansion is inhibited:** This manifest itself in reduced leaf area expansion, followed by reduced silk growth, then reduced stem elongation, and finally reduced root growth, as stress intensifies.

**Under severe drought stress, cell division is inhibited**

So even if the stress is alleviated the affected organs lack the cells for full expansion.

**Osmotic adjustment:** Most species are able to form osmotically active substances in the

cytoplasm and vacuole, in response to drought stress. This allows the plant to take up more soil water and maintains turgor and cell function for a longer time under drought. Osmotic adjustment is particularly apparent in sorghum, wheat and rice (the increase in negativity in  $\psi_s$  is from 1 to 1.7 MPa), but is much less in maize (0.3 to 0.5 MPa) (Bolaños and Edmeades 1993).

**Accumulation of proline has often been observed under severe drought:** It may act as an osmolyte or protect protein structures, as turgor is lost.

**Photo-oxidation of chlorophyll:** Drought affects Photosystem 2 more than Photosystem 1 in the photosynthetic mechanism. They become uncoupled, resulting in free, high-energy electrons in the leaf. Uncoupled electron transport leads to photo-oxidation of chlorophyll and loss of photosynthetic capacity. A very obvious bleaching of leaves exposed directly to the sun under drought stress can be observed.

**Enzyme activity is in general reduced under drought:** For example, the conversion of sucrose to starch in the grain decreases because the activity of acid invertase—a key enzyme that converts sucrose to hexose sugars—diminishes (Westgate 1997; Zinselmeier *et al.*, 1995).

### **Major abiotic stress factors affecting maize production**

Drought and low N are the two major abiotic stress factors affecting maize production in sub-Saharan Africa. In Zimbabwe the major maize producers are small scale farmers who are mainly located in dry regions of the country with low soil inherent fertility. Initial efforts in the National Breeding Programme were towards breeding maize varieties for high rainfall regions with optimum

fertilisation. It is therefore important that efforts are made towards improving new maize varieties for tolerance to these stresses. Currently few drought and low N tolerant maize varieties have been released by the National Breeding Programme. Maize yields are mostly affected by drought through reduction of the growing season and erratic mid-season dry spells that take place at any time during the growth of the crop (Edmeades *et al.*, 1994). Maize is mainly susceptible to drought stress that takes place just before and during flowering when its yield potential is determined (Malosetti *et al.*, 2007). Drought is a water deficit in the plant's environment that has the potential to reduce crop yield (Cooper *et al.*, 2006). It has devastating economical and sociological effects. Drought incidents are predicted to increase due to long term effects of global warming (Cook *et al.*, 2007). It is difficult to forecast manifestation of natural drought making it challenging or almost impossible to differentiate between stress and non-stress agricultural systems (Cooper *et al.*, 2006). In the semi-arid tropics the effect of drought is intensified by extremely erratic rainfall, high temperatures, high levels of solar radiation and poor soil productiveness.

### **Effects of drought on maize**

Maize inflorescence consists of separate male and female flowers making it more vulnerable to drought stress during flowering time (Grant *et al.*, 1989). Tassel development and pollen shed in maize are less sensitive to fluctuations in moisture availability compared to silk growth. The allocation of nutrients to ears, ovules and silks is reduced under drought as a result of the dominance effects of the apical tassel. Silk emergence in relation to male flowering is delayed when drought takes place just before flowering and this result in and increased anthesis silking interval (Bolanos and Edmeades, 1993a). When the

anthesis silking interval is lengthened the pollen might arrive when silks have dried up (Bassetti and Westgate, 1993) or after ovaries have used up their starch reserves (Saini and Westgate, 2000; Zinselmeier *et al.*, 2000). This scenario results in retarded ear and silk growth and accelerated kernel and ear abortion (Edmeades *et al.*, 1993). The maize crop has been found to be more susceptible to moisture stress one week before to two weeks after flowering (Grant *et al.*, 1989). Grain abortion normally takes place during the first 2-3 weeks after the emergence of silks (Westgate *et al.*, 1991). It is intensified by any stress that decreases canopy photosynthesis and movement of assimilates to the developing ear. This scenario results in the growing ear being deprived of the necessary nutrients (Stevens, 2008). Therefore the amount of assimilates reduces to below threshold levels required to sustain grain development and growth (Edmeades and Tollenaar *et al.*, 1992). The decrease in photosynthesis can be due to a decrease in radiation interception associated with increased leaf rolling (Bolanos *et al.*, 1993). Reduction in photosynthetic rate decreases the volume of nutrients available for distribution to the sink organs. The amount of stress that drought imposes on the maize crop results in modifications of photosynthetic pigments and constituents. It also causes damage to photosynthetic organs (Fu and Huang, 2001) and the Calvin cycle enzyme activity is reduced. Carbohydrate metabolism activity in the plant's reproductive organs is also negatively affected (Liu *et al.*, 2004). Maize is more vulnerable to drought compared to sorghum as a result of its shallow root system, enlarged leaf surface area, increased transpiration rate, slower grain development rate and extended grain filling period.

### **Breeding for drought tolerance in maize**

Maize is considered the most susceptible cereal to drought stress, with the exception of

rice (Banziger and Araus, 2007). Maize yields remain below 2 t ha<sup>-1</sup> in most countries in sub-Saharan Africa and yields vary from year to year (FAO, 2011). Maize is the staple food crop of importance to over 300 million people in eastern and southern African countries (Heisey and Edmeades, 1999). Rainfall distribution and amount have been found to have a direct effect on maize productivity in these two regions. In southern Africa the 2002/03 drought left about 14 million people exposed to starvation and the food deficit was 3.3 MT (World Food Programme, 2003). The World Food Programme was expected to provide food aid to 7.8 million people in five East African countries (Somalia, Ethiopia, Kenya and Uganda) as a result of consecutive seasons of drought (World Food Programme, 2009).

East Africa experienced a severe drought in 2011 that left more than 10 million people relying on food aid (World Food Programme, 2011). Therefore, in order for farmers to realise increased and stable yields and for seed merchants to be in a position to market a variety widely, it is critical that drought tolerance is incorporated in maize breeding strategies (Campos *et al.*, 2004). Hence, improvement or development of maize genotypes with high and constant yields under drought stress conditions is essential. Among abiotic stresses, breeding for drought tolerance is one of the most challenging endeavours, because selected germplasm ought to perform exceptionally well not only under drought stress but also under optimum conditions. Since water is a scarce resource, improving varieties for drought tolerance is an important approach in reducing this problem. It is important in breeding for drought tolerance to consider breeding for other stress factors as well (Beebe *et al.*, 2008). Progress in breeding for drought tolerance has been slow as a result of the complex nature of the trait and an improved

understanding of the fundamental mechanisms of drought would hasten progress in breeding for the trait (Ribaut *et al.*, 2002). In an effort to improve maize productivity, maize breeders have exerted enormous efforts to breed hybrids with drought tolerance (Bruce *et al.*, 2002). The efficiency in selection of germplasm for drought tolerance can be improved through use of managed drought environments. This can be done during the off-season (winter) with the use of controlled irrigation whereby the occurrence, extent and amount of drought stress on the crop are controlled (Banziger *et al.*, 2000). As a result of significant G x E interaction, it is important that genotypes screened for drought tolerance are evaluated in the target locations before they are incorporated as parents in the breeding programmes. Although progress in drought tolerance can be achieved through conventional selection methods, trials must usually be replicated across a large number of locations and across several years before the expression of the trait can be conclusively identified. Stevens (2008) reported that CIMMYT has been involved in breeding for drought tolerance in maize over the past three decades. A unique selection programme aimed at improving tolerance to drought using the lowland tropical maize populations Tuxpeño Sequía, LaPosta Sequia and Pool 26 Sequia was started at CIMMYT in the 1970s. Average maize yields under drought conditions were 126 kg ha<sup>-1</sup> per cycle (Stevens, 2008). Progress in selection for drought tolerance of Tuxpeño Sequía resulted in average breeding gain of 108 kg ha<sup>-1</sup> yr<sup>-1</sup> with yield levels ranging from 1-8 t ha<sup>-1</sup> (Bolanos and Edmeades, 1993a). A significant gain of 9.4% for ears per plant and reduction of anthesis silking interval from 34.2 days in the first cycle to 9.8 days in cycle eight were further reported by Bolanos and Edmeades (1993a). Bolanos and Edmeades (1993b) reported an increase in anthesis silking interval to 18.8 days under severe

stress. The three populations outperformed their original cycles of selection and check varieties under drought conditions and yields ranged from 1.0-4.5 Mg ha<sup>-1</sup> and 5.8-10.4 Mg ha<sup>-1</sup> under drought and optimum conditions respectively (Edmeades *et al.*, 1999). The authors further reported yield gains ranging from 0.08 - 0.29 Mg ha<sup>-1</sup> cycle<sup>-1</sup> under drought conditions and 0.04 - 0.18 Mg ha<sup>-1</sup> cycle<sup>-1</sup> under optimum conditions. Campos *et al.*, (2004) reported 146 kg ha<sup>-1</sup> gains annually when drought stress was introduced at flowering and 76 kg ha<sup>-1</sup> when stress was introduced at mid-grain filling stage. Drought tolerant germplasm was introgressed into local African germplasm, producing varieties with stable and superior performance in southern and eastern Africa. Superior maize OPVs with yield performance 18 comparable to commercial hybrids under moderate to severe moisture stress were also produced. Grain yield is considered the primary trait for selection under drought stress conditions. Nonetheless, reduced heritability and variance of yield components make selection based only on grain yield inefficient (Stevens, 2008). The major strategy in breeding for drought tolerance in maize has been direct selection for high yield. Grain yield under severe mid and late season moisture stress has been improved by 30-50% in three late maturing maize populations through recurrent selection at rates of up to 12% per selection cycle (Edmeades *et al.*, 1999). It has been reported that use of both secondary traits and grain yield in improving germplasm for drought stress tolerance has resulted in significant selection progress (Mhike *et al.*, 2011).

#### **Suitable secondary traits used in selection for drought tolerance**

Appropriate secondary traits selected for under drought stress should be genetically related to grain yield, have high heritability

estimates, be consistent and easy to select for and not too expensive. These traits must furthermore be measurable at or before flowering so that undesirable parents are selected against and these traits should not be accompanied by yield loss under optimum environments (Edmeades *et al.*, 1998). Critical secondary traits under drought identified at CIMMYT and Pioneer Hi-Bred include reduced prolificacy, anthesis silking interval, stay green and to a lesser extent leaf rolling (Banziger *et al.*, 2000). Anthesis silking interval is measured as the number of days between silk emergence and pollen shedding and increases under drought stress as a result of retarded ear and silk growth (Bolanos and Edmeades, 1993b). Work done at CIMMYT has revealed that the heritability estimate for anthesis silking interval is related to or greater than the heritability for grain yield. A high negative correlation of anthesis silking interval with grain yield and other related traits such as kernel number and number of ears per plant has been reported. Anthesis silking interval and ears per plant have been widely used in breeding for stress tolerance in maize (Bolanos and Edmeades, 1993b; Banziger *et al.*, 2000). These two traits have shown good genetic variability under drought stress conditions and high heritability 19 The variation in number of kernels has a major effect on maize grain yield under drought (Bolanos and Edmeades, 1996). Bolanos and Edmeades (1993a) observed a 90% drop in yield as anthesis silking interval increased from -0.4-10 days, whilst Du Plessis and Dijkhuis (1967) reported 82% drop in grain yield as anthesis silking interval increased from 0-28 days. In genotypes selected for short anthesis silking intervals and increased grain yield under drought the bulk of the carbohydrates are channeled towards development of the ear and less towards the growth of tassels and vegetative organs (Edmeades *et al.*, 1993). In tropical maize gains in selection have been

linked with improved synchronisation in silking and pollen shedding, reduced barrenness, reduced tassel size, increased harvest index, delayed leaf senescence and reduced root length density in the upper soil profile with no alterations in water uptake or biomass (Bolanos and Edmeades, 1993a; b; Bolanos *et al.*, 1993; Chapman and Edmeades, 1999). Genotypes are selected under managed drought stress based on grain yield performance and appropriate secondary traits.

### **Managed drought**

Managed drought stress screening is usually done off-season (winter) with the use of irrigation. Drought stress on genotypes is induced either at flowering or at grain filling stage. At intermediate stress level average grain yield is targeted to reduce by 15-30% of yields expected under optimum conditions and the stress will be targeting grain filling. A yield reduction of 30-60% of yields realised under optimum conditions is targeted for severe stress levels and the stress affects both flowering and grain filling (Banziger *et al.*, 2000). Under severe stress, irrigation is scheduled such that drought stress coincides with anthesis and silk emergence, but supplementary irrigation is applied 14 days after the end of pollen shedding in order to facilitate adequate grain filling of the formed grain. In intermediate stress, drought stress is timed to coincide with grain filling. It is important to ensure that irrigation is uniformly applied before onset of stress as this will result in stress levels being uniform in all genotypes, more constant plant performance and eventually improved breeding progress.

### **Breeding strategies**

Maize is the most world grown crop in the America, Asia etc. Hybrid maize, because of

its high grain yield as a result of heterosis (hybrid) is preferred by farmers over conventional varieties. When a region notes a deficiency in its water supply then a drought condition occurs. This occurs when a region receives consistently below average precipitation. It can have a substantial impact on the ecosystem and agriculture of affected region. Drought is important due to instability of national maize grain yields and of food supply and economy of small-scale maize based farming systems in the tropics. Water shortage affect maize yields throughout the crop cycle, but most severely at flowering and to a lesser degree at establishment. There are some techniques which are used to improve the maize in drought tolerance conditions, such as;

### **Improvement of drought tolerance through Conventional Breeding**

Population Improvement for Drought Tolerance in Tropical Maize.

Hybrid Improvement for Drought Tolerance in Temperate Maize.

### **Molecular breeding approaches**

Marker-Assisted Back Cross (MABC) Approaches.

The Marker-Assisted Recurrent Selection (MARS) Approaches.

### **Genetic gains**

### **Improvement of drought tolerance through conventional breeding**

**Population improvement for drought tolerance in tropical maize:** Improvement in drought tolerance maize on flowering stage in CIMMYT (International Maize and Wheat Improvement Center) is using recurrent

selection to improve under drought tolerance condition. So in this way, the grain yield of maize increased between 3.8% and 12.6% (Edmeades *et al.*, 1999; Bolanos and Edmeadas, 1993a). Due to this, there will be increases the EPP (Ear per plant) and HI (Harvest Index) and reduce the ASI (Anthesis-silking interval), leaf senescence, plant height, stem biomass, time to anthesis and tassel primary branch number, and a small but significant increase in grain yield, EPP (Ear per plant), kernel weight per fertile ear and individual kernel weight was also be achieved under WW (well-water) conditions (Westgate, 1997). Increase the yield of grain in drought tolerance has improved in the specific conditions and low fertility conditions, two common adaptive stress involved (Bänziger *et al.*, 1999). A common genetic basis between drought tolerance and better performance under low N conditions has been confirmed through QTL analysis, and QTL common for both abiotic stresses were identified for ASI (Anthesis-silking interval) and EPP(Ear per plant) (Ribaut *et al.*, 2007).

### **Hybrid improvement for drought tolerance in temperate maize**

To improve the hybrid seed for drought tolerance, the seed industry has succeeded in efficient testing to improve mechanization and imposing high selection intensities (Coors, 1999). These hybrid evaluation are designed as such to maximize the adaptation and stability under different environment cultural practices such as planting density, planting date, drought stress, fertilizer input, tillage and crop rotation etc; climatically and edaphically. Most of recent improvement in hybrid performance is due to a greater tolerance to abiotic stress, particularly in situations where high planting densities are used (Duvick *et al.*, 2004; Tollenaar and Wu, 1999)

## **Stress management**

The key to breeding for both drought and low N tolerance is to manage stress. In the case of drought this is done by conducting experiments partly or entirely in the dry season and managing the stress through irrigation. In the case of low N, this is done by conducting experiments in fields that are depleted of N. The objective of such experiments is to measure the genotypic drought tolerance or to measure the genotypic low N tolerance.

Timing, intensity, and uniformity of the stress are factors to consider in stress management.

Timing should be such that the growth stages targeted are susceptible to the stress, have a high probability of being affected by that stress in the target environment, and involve tolerance-related traits that can be modified through breeding.

Stress intensity should be severe enough so that traits become important for yield distinct from those which affect yield under non-stressed conditions.

Uniformity: If the stress is uniform over space and time, genetic differences will be easier to observe and progress will be greater.

## **Secondary traits that help to identify drought tolerance**

The following traits are recommended for use in a drought breeding program. They are listed in order of decreasing importance.

### **Grain yield**

Heritability: medium under grain filling stress, medium to low under flowering stress.

Relationship with grain yield: high.

Selection: for increased grain yield.

Stress type: to be measured under flowering or grain filling drought stress.

Measurement: shelled, adjusted for grain moisture.

Remarks: Shelling percentage varies considerably under drought. Grain weight, not ear weight, should be used for calculating grain yield.

### **Ears per plant**

Heritability: high and increasing with stress intensity.

Relationship with grain yield: high under flowering stress.

Selection: for more ears per plant (i.e., less barrenness).

Stress type: to be measured under flowering drought stress; heritability and genetic variance is largest when flowering stress is intense enough so that ears per plant average 0.3 to 0.7 across the entire experiment.

Measurement: count the number of ears with at least one fully developed grain and divide by the number of harvested plants.

### **Anthesis-silking interval (ASI)**

Heritability: medium, maintaining a reasonably high level under severe flowering stress.

Relationship with grain yield: high under flowering stress.

Selection is for a reduced or even negative ASI.

Stress type: to be measured under flowering drought stress; heritability and genetic variance is the largest when flowering stress

is intense enough so that ASI averages 4 to 5 days across the entire experiment.

Measurement: determine the number of days from sowing until 50% of the plants have extruded anthers (anthesis date, AD), and the number of days from sowing until 50% of the plants show silks (silking date, SD); calculate:  $ASI = SD - AD$ .

### **Leaf senescence**

Heritability: medium.

Relationship with grain yield: medium under grain-filling stress.

Selection: for delayed leaf senescence (stay-green).

Stress type: grain filling stress.

Measurement: score on a scale from 0 to 10, dividing the percentage of estimated total leaf area that is dead by 10.

1 = 10% dead leaf area 6 = 60% dead leaf area

2 = 20% dead leaf area 7 = 70% dead leaf area

3 = 30% dead leaf area 8 = 80% dead leaf area

4 = 40% dead leaf area 9 = 90% dead leaf area

5 = 50% dead leaf area 10 = 100% dead leaf area

Remarks: Leaf senescence should be scored on 2-3 occasions 7-10 days apart during the latter part of grain filling.

### **Tassel size**

Heritability: medium to high.

Relationship with grain yield: medium under flowering stress.

Selection: for a smaller tassel with fewer branches.

Stress type: this is the only trait that can be measured under well-watered conditions but is indicative of drought tolerance at flowering stage.

Measurement: score on a scale from 1 (few branches, small tassel) to 5 (many branches, large tassel).

Remarks: Advisable only with lines that have an inbreeding degree of at least S1; more difficult to determine with full vigor material. Two independent scores are recommended.

### **Leaf rolling**

Heritability: medium to high.

Relationship with grain yield: medium to low.

Selection: for unrolled leaves.

Stress type: flowering stress.

Measurement: score plots on a scale from 1 to 5.

1 = unrolled, turgid 4 = rolled leaf rim covers part of leaf blade

2 = leaf rim starts to roll 5 = leaf is rolled like an onion

3 = leaf has a the shape of a V

Remarks: To be measured before flowering when leaves are still more upright; leaves are less likely to roll after flowering when they become more lax and thicker. Two to three scores are recommended.

### **Additional remarks**

The more high quality information available, the better the likelihood of determining the drought tolerance of a genotype.

Even if only grain yield is measured, anthesis date needs to be known so that drought escaping genotypes can be distinguished from drought-tolerant ones.

With consistent selection for short ASI under flowering stress, the frequency of male-sterile genotypes may increase, because delayed anther extrusion may be confused with a short ASI. This is especially important when evaluating inbred lines under drought.

Many other secondary traits for drought tolerance were evaluated by CIMMYT but proved to be of low heritability, among them: leaf and stem elongation rate, canopy temperature, leaf photo-oxidation, leaf chlorophyll concentration, predawn leaf water potential, and seedling survival under drought.

Other traits evaluated by CIMMYT were heritable, but proved to have no relationship with grain yield under drought: osmotic adjustment, leaf erectness

### **Molecular breeding approaches:**

A few examples of new technologies are given below;

#### **Marker-Assisted Back-Cross (MABC) approaches**

Ribaut and Ragot (2007), they describe a successful experiment, in which favorable alleles were expressed in five genomic regions in plants under limited water conditions. The plants are selected from MB (Molecular Breeding) BC2F3 families and crossed with two testers and evaluated phenotypically. Under severe condition of WS, the yield would be decreased 60-80% as compared to WW conditions.

On observation under mild stress condition, less than 50% yield is reduced, no difference was observed between MABC and control hybrids. No yield was observed under WW conditions. So under this demonstrated that genetic gains can be achieved by introgressing drought QTL.

#### **The Marker-Assisted Recurrent Selection (MARS) approaches:**

The advantage of MARS is higher as compared to phenotypic selection, although the use of MARS has enjoyed only in public sector, only limited success in public sector (Ragot *et al.*, 2000; Johnson, 2004; Crosbie *et al.*, 2006).

For example, (Ragot *et al.*, 2000) he identify QTL in bi parental maize population and then applied a genetic index where is involving agronomic performance (grain yield, and moisture at harvest) and adapted to abiotic stress (early vigor under cold conditions). Similarly other scientist (Eathington *et al.*, 2005) he demonstrated that the rate of genetic gain achieved through MARS was about twice that possible using in the phenotypic selection. So there are several accounts in which at least one of parental lines of commercial maize hybrids was derived via MARS.

#### **The way forward**

##### **Expected rates of progress**

The recent substantial investment by the Gates Foundation in developing and disseminating drought tolerant maize for sub-Saharan Africa has provided a tremendous impetus to stabilizing and improving maize production in this drought-prone region where maize forms a critically important part of the diet. This builds on a solid research effort led by CIMMYT spanning 35 years. Research of

this nature is a relatively slow process, but there are real prospects of increasing the rate of improvement using new techniques. The use of MAS to increase the rate of genetic gain in both the DTMA and WEMA Projects could double the rate of genetic gain, and the availability of a transgene boosting grain yield under drought throughout the crop season opens exciting possibilities. These three approaches – conventional selection, MAS and genetic modification – will likely be additive in effect. The first two provide the prospect of steady improvement over time, and the 15% improvement offered by Monsanto's transgene could be matched by ~3-5 years of conventional + marker-aided selection. The transgene provides a one-off boost to yields obtained by MAS. However, if technology providers such as Monsanto, Pioneer, Syngenta or BASF are persuaded to release newly developed transgenes providing a similar boost to grain yield every 5 years or so, and if their effects are also additive (a good possibility with a complex trait like drought tolerance), then the cumulative effects of transgenes, MAS and conventional selection for drought tolerance can generate very significant improvements in grain yield. There are large investments being made in the development of genetically modified crops by the private sector in the USA and Europe, and these are being matched by public sector investments in China, India, Brazil and the USA. The recent announcement of a US\$3.5 billion investment in genetically modified crops in China over the next decade is the most recent tangible example of this commitment.

### **Managed Drought Stress Environments, MSEs**

Reliable drought phenotyping requires MSEs where drought stress is controlled and applied at the designated timing and intensity. The value of MSEs for efficient drought selection

in maize has been consistently demonstrated over the past 20 years. Progress can be made using multi-location testing at randomly selected sites in the target population of environments, but only if it is on a very large scale. For less developed countries this is not an efficient way of improving yield under water-limiting conditions. A further investment in centres of excellence in phenotyping for drought tolerance in the less developed world seems fully justified. This opens up the possibility of improving a range of crops for drought tolerance in addition to maize at the same location. It is an initiative that would boost operational efficiency, and should be seriously considered by the donor community.

### **New genetic variation, new methods**

The lack of intraspecific genetic variation for stay green under terminal drought stress, and for root depth management in maize has been documented. Transgenic sources of new variation for these traits will likely be required, along with a careful physiological evaluation of the whole-plant effects of such transgenes. Multiple genes contained in single constructs allow for efficient stacking of traits. New molecular methods are under experimentation such as the use of mini chromosomes where a single heritable piece of the plant's own DNA that includes the centromere region is used to deliver several genes simultaneously. Small RNA fragments are emerging as powerful control elements of stress response in plants.

### **Agronomic interventions**

Improved crop management methods can complement the use of drought tolerant hybrids and contribute significantly to increasing and stabilizing yields under rainfed conditions or under irrigation where water supply is limited. Ensuring that planting

densities are optimal, tillage is minimal, weeds are controlled and adequate fertilizer is applied at the right growth stage all increase water use efficiency (WUE).

Water supply to the crop can be increased by water harvesting methods and the use of mulch. Where irrigation is in short supply, deficit irrigation, or the application of water at less than the potential evapotranspiration rate, can increase WUE at little cost to yield. Partial root drying, where dry and wet regimes are alternated under irrigation to reduce water applied can elicit a drought-adaptive response and may save up to 25% of the water normally applied.

### **Regional regulatory and release initiatives**

There is considerable potential for regional harmonization of regulatory procedures in regions like sub-Saharan Africa. If deregulation of a specific transgene has been approved by one country based on a thorough evaluation using standard protocols, this should normally be sufficient to deregulate that same construct and event when used in the same species in other countries in the region. Release of improved varieties and hybrids could be harmonized in a similar manner across countries sharing common agro ecologies, e.g., West Africa.

### **Smith Hazel selection index for the improvement of maize inbred lines under water stress conditions**

Drought is one of the major abiotic stress affecting maize production. In order to detect drought tolerant maize inbred lines, an experiment with hundred homozygous inbred lines was conducted during Kharief 2013 and 2014 in factorial randomized block design with two replications, under different moisture management condition. Results showed diversity among the lines in response

to moisture management conditions. Assessing lines as per the selection indices lead to identification of few lines *viz.*, KDM-361A, CM-129, KDM-372, KDM-331, KDM-1051, KDM-402, KDM-463, KDM-717, KDM-912A, KDM-932A, KDM-343A, KDM-961, KDM-918A, KDM-1156 and KDM-1236 as drought tolerant ones. Highest genetic advance was observed for maturity traits, plant height, leaf relative water content and chlorophyll content before flowering, germination per cent, primary root length and fresh root weight therefore, they should be used for selecting elite lines for drought related traits (Asima *et al.*, 2017).

In conclusion, 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 identify key genes involved in metabolic pathways related to stress response *e.g.*, the factors involved in kernel development. Armed with better understanding of the physiological mechanisms and the genetic basis of the response of maize to drought, it should become increasingly feasible to identify, transfer and select key genes and alleles to build genotype with much improved tolerance to drought. It is only recently that carefully selected or managed abiotic stress screening approaches have been more widely used for assessing the stress tolerance of crop genotypes. Our results suggest that simultaneous selection for tolerance and resistance to abiotic and biotic stresses, while also monitoring performance under high potential conditions, can result in significant breeding progress in target environments where combinations of those stresses occur and particularly at lower yield levels. While

assessing the effectiveness of various selection conditions for breeding progress in the target environment is inherently difficult, as large and long-term breeding investments are involved, breeders may have been too concerned with keeping heritability high while ignoring the need to adequately representing the target environment during selection. Managed stress screening approaches provide an opportunity to keep heritability high and adequately representing abiotic stress factors that are relevant in the target environment. 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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