Chapter 13
Conventional and Advance Breeding Approaches for Developing Abiotic Stress Tolerant Maize

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13.1 Introduction

Maize (*Zea mays* L.) is a multipurpose cereal crop of the world with wider adaptability to different agro-climatic regions. It is the most versatile food crop being grown on 193.7 million ha area in more than 170 countries across the globe with 1147.6 million ton of production and 5.9 tonnes/ha productivity (Anonymous, 2020a). Maize is the principal staple food crop in large parts of Africa and many parts of developing countries. Globally, maize is predicted to become the crop with greatest production by 2025 (Cairns et al., 2012). In India, during 2019–20, maize is cultivated on area of around 9.0 million ha with production and productivity of 28.07 million tonnes and 3.11 t/ha, respectively (Anonymous, 2020b). The most important use and demand driver of maize in India is poultry feed, which accounts for 47% of total maize consumption. Further, it is an important industrial raw material where more than 3000 products are being made from it providing wide opportunity for value addition. After the wheat and rice, it is the third most important food grain crop in India. Being an international crop, its demand is increasing day by day. However, sustaining maize

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yield stability over the year and locations is the big challenge in the era of climate change.

There are many biotic and abiotic factors which are hindering maize production and productivity worldwide. Different forms of abiotic stresses like drought, cold, heat, water logging, salinity and climate change are some of the major factors that reduce the agriculture crop production considered major threats to global crop production and this is also at the time when supply of basic food required to be enhanced at a significantly higher level (Meena et al., 2017a, 2017b; Zorb et al., 2019). These abiotic stresses (drought, heat, cold waterlogging and salinity) drastically reduce plant biomass, yields and survival of major food crops up to 70% (Meena et al., 2017a, 2017b). Drought stress caused nearly 52% grain yield reduction (Kumar et al., 2016). These stresses threaten the food security worldwide because the crops productivity is not increasing as much as the food demand rising up. The abiotic stresses disturb the plant growth and development and therefore prevent the genotype(s) from realizing their full genetic potential (Waqas et al., 2019). Now, challenges are to reduce the crop and yield losses caused by different abiotic stresses and to meet the increasing food demand. Grain yield in general is a trait which have low heritability under selection leading to slow progress in developing varieties/cultivars with higher yield. Relief from abiotic stresses is possible either by changing/avoiding the stressful environment or by changing the genetic constitution of the plant itself. Several varieties and hybrids in maize were developed and recommended for cultivation using conventional breeding approaches. The recent advent of molecular tools, have revolutionized the genetic analysis of traits for crop plants and provided tremendous opportunities not only to the plant breeders but also to physiologists, agronomists, and biochemist to identify traits of importance in improving tolerance to abiotic stresses. To get more success in achieving target production of maize, conventional breeding requires integration of advance technological tools. To design and implement advanced breeding strategies for developing abiotic stress tolerant maize genotypes, new tools and technologies like doubled haploids (DH), whole genome sequencing, high-density genotyping, high-throughput phenotyping, genomics-assisted breeding (rapid-cycle genomic selection, marker-assisted recurrent selection), and crop modelling play a greater role (Prasanna et al., 2013). During last two decades, the development of new genomics and breeding tools changed the methodology of doing plant breeding. Now new tools and techniques like New breeding techniques like genomic selection and rapid generation advancement, DNA/RNA sequencing, high-throughput genotyping/phenotyping, trait mapping, functional characterization etc. are now available to speed up the breeding process (Nepolean et al., 2018). Thus, the aim of this chapter is to report the important conventional and advance breeding approaches for abiotic stress tolerance with special emphasis on drought and water logging as they are the major ones and affect almost 80% of the total cultivated maize in India.

13.2 Abiotic Stresses in Maize

Abiotic stresses are considered in the form of adversarial effects on developmental phases of plant due to the poor climatic or soil conditions which results in loss of millions of dollars annually (Joshi & Karan, 2013). Abiotic stresses are severely affect plant development and productivity either individually or in combination and finally reduce the grain yield. Some abiotic stresses are interrelated like drought and heat, excess moisture and salinity. The abiotic stresses cause changes in physiological, morphological, molecular and biochemical processes. Major abiotic stresses like drought, heat, cold, waterlogging and salinity induce damage to plant cellular parts of the plant, including maize. There is always a strong need of mitigating abiotic stresses by adopting various scientific strategies including either management practices or breeding climate resilient genotypes. The lateral one is more preferable and sustainable as it provides in-built capacity in genotypes for surviving under various abiotic stresses. Following are the different types of abiotic stresses affecting maize crop.

13.2.1 Drought

Drought stress is the major abiotic stress followed by waterlogging casing remarkable yield reduction during rainfed crop. Maize production as mainly dependent on rainfall, its yield fluctuates more widely due to drought as compared to rice and wheat (Prasanna, 2016) that is why drought is considered as the major constraint and destabilizing factor in maize grain production. Physiological responses to drought are complex and are unpredictable as drought stress affects the crop in differential way at different growth levels of the plant. Moisture stress during vegetative stage reduced plant height, dry stover yield, grain yield and its components traits. However, the major effect of drought in maize is delayed silking which results in widening of ASI which is the major reason of reduced kernel setting in maize ears. The Highest reduction was recorded in kernels per ear from 11.26 to 54.59% (Singh et al., 2009). The probable mechanisms in maize in response to drought include redirecting of growth and accumulation of dry matter in roots (Hsiao & Xu, 2000) and osmotic adjustments. At the time of moisture stress, the water potentials and turgor are reduced enough which affect the normal functioning and well-being of the plants (Zhu, 2002). The excessive desiccation of water from the plant systems lead to blockage of various important metabolic pathways and hence may lead to plant death. Maize being principally grown as rainfed crop is more prone to both low as well as high moisture stresses (Kumar et al., 2016). The effect of these abiotic stresses in maize can be very easily seen in the form of variable yield level in different genotypes. In nature, there are several in-built mechanisms which results in combating of all these stress effects and therefore help in plant survival. These mechanisms and traits are needs to be explored through scientific breeding strategies.

13.2.2 Waterlogging

Waterlogging is a condition when water accumulates in excess of the plant requirement for long period and hinders the different process of plant. Waterlogging occurred due to inadequate rainfall distribution either enhancing number of rainy days or high intensity of daily rains. Waterlogging is the natural phenomena in Southeast Asia including eastern India. In these areas waterlogging and floods affects nearly 18% maize growing areas which reduce nearly 25–30% annual production (Cairns et al., 2012). The intensity of losses caused by flooding or waterlogging on plant differ from one stage to other. (Mano et al., 2002). Water-logging causes depletion of oxygen as rate of oxygen diffusion is slower in water than in air which subsequently reduces the oxygen availability in plant roots due to the imbalance between slow diffusion and rapid consumption (Erdmann et al., 1986). Oxidative stress induced by waterlogging is encountered by various mechanisms including enhanced availability of soluble sugars, greater activity of glycolytic pathway, aerenchyma formation, and anti-oxidant defence mechanism. During deficiency of oxygen in plant ethylene reported to play an important role in changing the plants mechanism (Hossain & Uddin, 2011). One of the water-logging adaptive traits in maize is the development of brace roots in tolerant genotypes that provide anchorage the maize plant.

13.2.3 Heat Stress

During growing period, increasing temperature disrupt the crop production system in two ways, firstly, by affecting the pollination resulted in reduced grain development and secondly by enhancing the growth rate thereby reducing the grain filling duration in maize (White & Reynolds, 2003). The optimum temperature for maximum grain filling in maize is 25–32 °C above which germination capacity of pollen is reduced on silk resulting in fewer grain development (Basra, 2000) and production of starch is also reduced affecting the kernel development (Singletary et al., 1994). Reproductive stage in maize is most vulnerable to elevated temperatures and when supplemented with drought conditions lead to significant yield losses (Cairns et al., 2013). The impact of heat becomes more severe when it occurs with drought stress. Metabolic activities at cellular level are altered by high temperature stress. Heat stress affects the biomass accumulation, net photosynthesis, leaf area, and seed test weight (Cheikh & Jones, 2006).

13.2.4 Cold Stress

The maize crop is very sensitive to cold, therefore, its cultivation in temperate region is limited. The temperatures between 18 and 27 °C during the day and around 14 °C during the night is most suitable for growth of maize plant. The temperature below 10 °C reduce the growth of the plant and further reduction in temperature below 5 °C cause chilling injury to leaf tissues. Prolong duration of low or chilling temperature slow down the plant metabolism and affects various processes. In maize low temperatures for long duration slow the cell cycle and reduce the cell division in susceptible genotypes (Rymen et al., 2007). Cold stress also affects the reproductive development of maize plant. It mitigates the initiation of floral development and reduce the rate of tassel branch meristem development and also reduce the branches and spikelets of tassel (Bechoux et al., 2000). In maize exposure to chilling stress reduce the rate of development and cell division and leading to which decreased leaf area and plant growth. A reduction of 20% in leaf size was reported in cold stress affected maize (Rymen et al., 2007). During the reproductive developmental stage cold stress induce abnormalities in reproductive system, which lead to failure of fertilization. Cold stress also leads to low pollen formation and extreme cold for longer duration during floral development leads to complete failure of pollen development. The low pollen availability for fertilization or complete pollen non availability results partial seed setting or complete failure of kernel setting in maize ears.

13.2.5 Salinity Stress

Salinity is the excess quantity of dissolvable salts present in the soil which affects various growth and developmental process of plant. The low rainfall in a area, poor soil and water management and high rate of evapotranspiration are some of the causes for salinity of soil (Munns, 2002). During salinity stress external water potential is low which results high osmotic stress. Salinity stress also caused ion toxicity sodium and/or chloride. Due to salinity plant face imbalance of essential nutrients as the salinity caused the interference in absorption and transport of the nutrients. Salinity leads to stunted maize plants growth causing wilting of plants at a salinity level 0.25 M NaCl or more (Menezes-Benavente et al., 2004). The germination, seedling and early plant developmental stages are more sensitive to salinity. High amount of sodium and chloride reduce the absorption of potassium, nitrogen, magnesium, calcium, and iron. The deficiency of Phosphorus enhanced the salt tolerance in maize plants as it reduces the Sodium (Na^+) accumulation and selective absorption of K^+ over Na^+ (Tang et al., 2019). To enhance and sustain the maize production in salinity prone areas development of salinity tolerant genotypes are essentially required (Farooq et al., 2015).

Most of the abiotic stresses are polygenic in nature and show large amount of G × E interaction. Due to higher interaction and effect of environments they show low heritability and small number of potentials gains during selection in crop improvement programme. Screening is required to be done at multiple stages and locations which are very critical and sensitive for particular stress. Further, screening must be coinciding with high and uniform level of stresses across the field which is it is unpredictable and therefore have low success rate.

13.3 Current Understanding of Abiotic Stresses Responses in Maize

Various abiotic stresses like low moisture, waterlogging, high salt and high temperature, influence the yield of maize crop. While the maize plant has developed strategies to cope with each, certain common set of genes have been deciphered that respond to all stresses. Li et al. (2017) studied genes expressed differentially upon treatment of maize seedlings (third leaf) with four types of stresses, viz., low moisture, high salt, low and high temperatures. The authors reported 167 genes that responded to all the four stresses. These common genes also included transcription factors (TFs), ten of them were always up-regulated, while the two of them were always down-regulated in response to the stresses. Apart from transcription factors, the common set of genes includes those which regulated abscisic acid (ABA) biosynthesis, and very-long-chain fatty acid and lipid signaling. While the differentially expressed genes belonged to various TF families, viz., ERF, MYB, bZIP, bHLH, WRKY, and NAC; Ethylene Responsive Factor (ERF) family TFs, constituted the largest TF family that responded to the stresses. Most of the TFs, belonging to bZIP (basic leucine zipper-type) family were implicated in abiotic stress in maize. These constitute an important reservoir of molecular factors, which can be potentially used in genome edited/cis- or trans-genic plants, for imparting resistance to multiple stresses in maize.

Hussain et al. (2018) implicated the physiological and biochemical processes that are common between drought and cold stress tolerance in maize. Among the processes that maize shares when responding to low moisture and low temperature stresses, are stomatal closure, production of reactive oxygen species (ROS), regulation of dehydrins, calcium signaling, loss of turgor etc. There are certain responses that are unique to each stress and not expressed in the other. Adee et al. (2016) reported that the incorporation of traits that collectively constitute drought tolerance (DT) in maize does not result in yield penalty in low stress/high yield environments. DT hybrids were able to conserve water for the critical early and late stages of reproduction. The authors found that in environments with low evapotranspiration or those under full irrigation regardless of the evapotranspiration potential, non-DT hybrids show significant improved performance. However, in poor yield environments, DT hybrids show better yield than non-DT counterparts. Cooper et al. (2014) reported differential water usage by DT maize hybrids as compared to non-DT hybrids. Hence, it appears that differential usage of resources leads to stress tolerance in one genotype, while resulting in susceptibility to stress in a different genotype. The physiological and biochemical responses to stress often entail consequent mechanisms that have the potential to reduce yield, while protecting the plant from stress condition. For example, stomatal closure can lead to reduced photosynthesis. ROS generation can lead to oxidative damage. Loss of turgor can lead to poor growth. All these may translate to altered plant-nutrient relationships, impaired flowering and pollination, and overall yield penalty. The genotypes that are able to balance intensity and duration of molecular phenomena leading to stress tolerance, preventing the corrective mechanisms from cascading to events that reduce yield, would constitute stress tolerant material. Adee et al. (2016) report that the DT hybrids showed less phenotypic plasticity than non-DT hybrids. This means that the expressions of individual characteristics of a DT maize genotype change to a lesser extent by drought environment, when compared to a non-DT genotype. Therefore, the ability to respond, but also to balance the response, preventing initiated molecular process to translate to yield reduction, through different physiological and biochemical mechanisms, would be helpful in maintaining plant yield under stressed environments.

The cross-talk of different stresses and the elucidation of common genes that respond to multiple stresses, raises the possibility of utilizing advanced tools of genetic manipulation for stress management. Determination of key regulators at the hierarchical top of stress-responsive metabolic pathways would lead to identification of molecular factors that could be deployed to impart stress tolerance. In addition to genotype-specific molecular mechanisms, another physiological phenomenon, stress memory, can be used to impart stress tolerance. In some cases, when a plant faces a stress, it becomes primed to handle another episode of that or some other stress. The plant develops a stress memory and keeps a repertoire of molecules in the form of epigenetic marks, lingering proteins, stalled RNA polymerase, which respond to a future episode of stress in a faster and effective way (Ding et al., 2012; Herman & Sultan, 2011; Schneider et al., 2019). Stress memory can also be utilized for stress management in maize. On the basis of understanding of abiotic stress effective breeding strategies to be formulated to develop stress tolerance maize genotype. The steps involved in stress tolerance breeding and various breeding approached have been shown in Fig. 13.1.

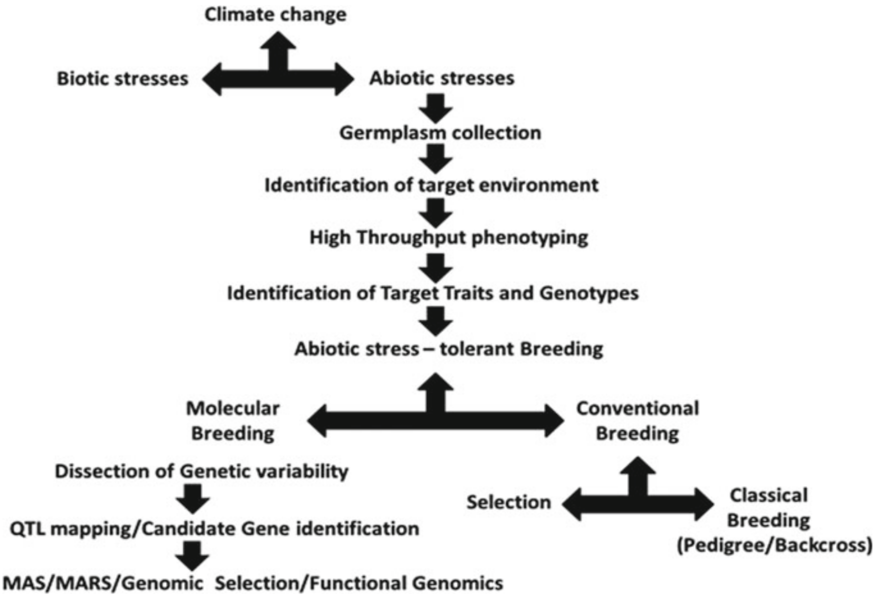

Fig. 13.1 A schematic representation of stress tolerance breeding approaches in maize

13.4 Conventional Breeding Approaches for Abiotic Stress Tolerance in Maize

A combined approach of cultural practices and genetic improvement should be followed to mitigate the negative effect of abiotic stresses on maize productivity. Selection for stress-adaptive traits for waterlogging tolerance, like early brace root development found helpful. Recurrent selection for several cycles results in the development of stress tolerant inbred lines in maize. Genetic improvement though conventional methods involve development of cultivars tolerant to abiotic stresses using various population improvement methods to enhance yield. Adjustment and modification in agronomic practices such as change in planting date, plant density and soil and water management minimize the stress effects but development of plant genotype with improved genetic make-up is the best substitute to fight and beat the abiotic stress without sacrificing yield. Physiological and genetic studies indicate that most stress traits are governed by polygenes which are highly influenced by the environment. Under these circumstances recurrent selection of their modifications can be used to develop stress tolerance population. The simple steps which breeders follow to develop abiotic stress tolerant cultivars include:

(a) Creation of variability: by intercrossing parents possessing desirable characteristics breeders produce new gene combinations and useful variability among genotypes or it is introduced by bringing new germplasm lines from other breeding programs.
(b) Selection: genotypes performing best in the target environment are selected and used in the further breeding programs.

13.5 Selection Approaches

Breeding varieties or hybrids having tolerant to drought or water logging tolerance is different from normal breeding methods in the sense that additional features are required along with the yield which contributes in survival under aberrant situation. The breeding strategies required are dependent on types of cultivar required, the nature of inheritance and type of gene action prevails for the traits conferring abiotic stress tolerance. Conventional breeding has made significant contribution in the development and deployment of stress resilient genotypes since long back. Around 60–65% of the total maize area in India is under improved maize hybrids, off this around 30% is specifically occupied by the single cross hybrids. Generally maize hybrids are having better roots system, strong stalk, high yield, uniform middle cobs placement, better brace roots, low ASI, uniform plant stand, better germination, stay green traits, better photosynthetic ability and high nutrient use efficiency etc. Therefore, they may be having better adaptability under drought as well as water logging stresses conditions. However, further there is huge amount of genetic variability available, which may be exploited by developing and selecting diverse inbred lines and or hybrids having in-built tolerance to drought and water logging stresses. This will be helpful in increasing the efficiency as well as effectiveness of stress resilient breeding programme. Direct selection for abiotic stress tolerance under field conditions sometimes may not be effective due to quantitative inheritance and involvement of environmental variance. Therefore, the more appropriate technique to identify abiotic stress tolerant plants is to evaluate the breeding materials in managed stress environment and select the genotypes with higher yield potential. Inbred lines can be developed by selfing desirable plants in a source population continuously for five to six generations by following ear to row selection. The tolerance of inbred lines for abiotic stress can be better judge by evaluating their test crosses performance developed using a susceptible tester under managed abiotic stress conditions. The hybrids developed from tolerant inbred lines will increase the chance of getting stress resilient hybrids, however its tolerance need to be established by evaluating in suitable experimental trials. Sometimes, if we have good yielding hybrids can directly be use for screening under drought and water logging trials. The suitable hybrids may be selected on the basis of their yield potential under the trials and best one can be recommended for further testing and cultivation in stress prone ecologies. Wherever there is no access of hybrids seeds, the improved synthetic/composites may be developed and evaluate under stress conditions. The stress tolerant superior combinations can also be identified in the basis of selection indices reported that Some selection indices such as Geometric Mean Productivity, Stress Tolerance Index, Harmonic Mean, Stress Susceptibility Index, and Yield Index were found more accurate criteria for selection of waterlogging-tolerant genotypes (Singh et al., 2017). The frequency of favorable alleles for abiotic stresses tolerance in the cultivars can be increases by adopting different ways of population improvements like recurrent selection methods. The conventional breeding approaches followed to develop stress tolerant genotypes include:

(a) Use of recurrent selection method and evaluation of segregating lines under managed stress and multi-location environment.

The most critical step in developing stress tolerant cultivar is the choice of selection strategy. Selection for yield under non-stress/optimum environment and horizontal evaluation of those selections under multiple locations trial with variable stress level is the most widely used selection strategy (Magorokosho & Tongoona, 2003). Again, the rate of improvement or genetic gain is dependent on the choice of testing environment(s). Ideally, the screening environment must have same environmental conditions as target environment with respect to climatic conditions like physical properties and nature of soils, rainfall distribution, temperature etc. (Ribaut et al., 2009).

(b) Selection of secondary traits under stress environment

Besides yield there are certain traits which give information of the performance of the plant are considered as secondary traits (Lafitte et al., 2003). The ideal secondary trait should have high association with grain yield under selection as well as target environment, additionally the trait should also have high heritability, genetically variable, simple, non-destructive and fast to measure and not related with any yield reduction under optimum environment. As grain yield under stress condition has poor heritability, genetic improvement or genetic gain may be less through direct selection. In this case, indirect selection via secondary traits is an attractive strategy because during stress conditions, at least some of the traits show high heritability with increased genetic correlation (Lafitte et al., 2003).

The early/late generation testing for stress adaptive traits can be followed depending upon the nature of gene action. Early generation evaluation and selection is preferable for the traits having additive gene action. In maize water-logging tolerance reported to be controlled by additive-dominance genetic model, and with more additive gene effects. Under such conditions reciprocal recurrent selection will be a good breeding approach for enhancing water-logging tolerance (Zaidi et al., 2010). The 12 cycles of recurrent selection method were found effective for the development of waterlogging stress tolerant genotypes with 20% yield advantage (Cairns et al., 2012).

13.5.1 Morphological Traits for Drought and Water Logging Stress Tolerance

Conventional breeding focuses mostly on characterization of the germplasm for stress tolerance related traits in managed stress. Field breeding strategies can be effective when there is knowledge available about nature of gene action involved in the inheritance of target traits. Understanding of inheritance of morphological and physiological traits associated with abiotic stress tolerance is very important. Understanding of yield and morpho-physiological basis of drought tolerance is a pre-requisite for making effective selection strategy for genotypes and breeding for water stress conditions. The study of heterotic expression of the parents would help in the development of desirable genotypes e.g. highly heterotic combination can also be possible when one or both the parents have poor general combining ability (Singh et al., 2010). Major traits associated with maize drought stress tolerance, escape and avoidance are deeper root system, short ASI, erect leaves, earliness, protogyny, stay green, low canopy temperature, high chlorophyll content stability, non-barrenness, osmotic adjustment, pubescence, waxy leaves, more proline, and glycine-betaine accumulation in specific tissue of plant. Similarly, high chlorophyll content stability, low mortality rate of the seedlings, deeper root system, well developed brace roots system, adventitious root formation, short anthesis silking interval (ASI), lodging tolerance, possession of a highly porous aerenchyma, better stalk and middle ear placement are the some of the important traits for water logging tolerance in maize. Screening for all these at various stages can help to identify the materials which can survive better under drought and water logging condition. The seedling, reproductive and grain development stages are the most sensitive to majority of the abiotic stresses in maize. Abiotic stresses conditions in the field results in increased of anthesis silking interval (ASI), respiration, canopy temperature, leaf senescence/rolling, decreases of root development, LAI, photosynthesis, biomass production, and hence ultimately the yield. It also results in heavy tassel firing, nutrient deficiency-N & K, plant mortality, lodging, pest and diseases attack, barrenness due to effect on effective pollinations and seed setting. All these results in significant decrease of plant yield.

13.5.2 Screening Technology for Drought and Water Logging Stress Tolerance in Maize

Choosing of appropriate selection sites and critical growth stage of plant for abiotic stress screening in maize is the crucial step. The sowing should be done in such a way that the sensitive stage must be coinciding with the environmental factors which favour drought and water logging stress. Under climate changing scenario, the 80% of maize rainfed area is mostly facing both drought and water logging stress frequently during the crop duration. Therefore, it is desirable to breed for genotypes having combined drought and water logging stress tolerance. Field Phenotyping should be done preferably in stress prone sites. The soil texture, soil moisture content and proper field leveling are the important point's needs to be taken care while selecting field for screening. Screening for drought stress at flowering stage can be done by withholding irrigation at least 10 days before initiation of flowering and the same can be retain for next 7–10 days during flowering. After that the irrigation can be resumed. Similarly, the stress at early grain filling stage can be induced by restricting the irrigation in trials immediately when the pollination over. The same may be sustained at least for 17–20 days.

It is very much important to have proper categorization of genotypes for their flowering and maturity. It is strongly recommended to have separate trials set on the basis of maturity. The genotypes with similar flowering/maturity groups can be merge to constitute a single trial for evaluation. These points are valid for any types of abiotic or biotic stresses screening. Evaluation under high plant density is a one of the good approaches to select genotypes for abiotic stresses tolerance. The rainout shelter facilities can be availed to evaluate the genotypes for drought. Similarly, screening for water logging stress at seedling stage can be done on flat beds by stagnating water up to minimum level of around 5 cm above the ground surface completely for ten days. Proper bunding should be done around the field to avoid flow of water. Seedling, pre flowering, flowering and grain filling stages are the more vulnerable stages for drought as well as water logging stress. Among all, flowering for drought and seedlings for water logging stress are the most crucial stages, where the occurrence of these stresses can cause maximum yield losses.

13.6 Introgression and Utilization of Distant and Wide Germplasm for Abiotic Stress Tolerance

The genetic variability which reduced over a period of time during artificial selection can be enhanced through the utilization of exotic wild germplasm into the existing germplasm available with any breeding programme for abiotic stress tolerance (Xu et al., 2009). Use of maize wild relatives in pre-breeding programme can also contribute significantly for developing stress resilient germplasm. Various gene banks have conserved approximately 0.05 million maize accessions (Hoisington et al., 1999). CIMMYT also conserved approximately 28,000 accessions http://www.cimmyt.org/germplasm-bank/). Such a huge maize germplasm consisting of pools populations and wild relatives may be the source of large unexploited genetic diversity as well as source of new traits and alleles. This germplasm can also be utilized to develop base populations or broaden the genetic base to exploit beneficial genetic variation. It is necessary to develop trait-specific core set for fully utilization of the genetic variability present in the wild and cultivated maize germplasm. Genetic diversity plays a greater role in the development of stress tolerance pools and populations. The trait contributing towards divergence also equally important. In a study Singh et al. (2020) reported that 100 kernel weight (39.45%) followed by days to anthesis (22.64%) contributed maximum towards total genetic divergence as per cent. Phenotypic characterization of diverse maize germplasm for specific abiotic stress will help in understanding the genetic variability and to develop a stress specific core set. This core set could be exploited through genomic approaches to identify novel genes. A stress specific abiotic stress tolerant population can be developed using genotypes tolerant to that specific stress. For example, a drought-specific pools were developed using anthesis–silking interval, leaf rolling and leaf senescence characters (Monneveu et al., 2006). Teosinte a wild relative of maize has many subspecies of *Zea mays* and some species identified as good source of useful source for improvement of abiotic stress tolerant traits e.g., teosinte *Z. luxurians* and *Z. mays* ssp *huehuetenangensis* can be used as source of genes for adventitious root formation under extreme waterlogging. Introgression lines of maize were developed genomic regions from teosinte (*Z. mays* ssp *nicaraguanensis*) (Mano & Omori, 2007).

13.7 Use of Molecular Markers and Genomic Tools in Abiotic Stresses Tolerance Breeding

Various genetic studies indicated that in general abiotic stress tolerance traits are governed by quantitative traits loci (QTLs). Marker assisted selection methods are effective and save time in breeding programme under high heritability of the trait and also when the cost of phenotypic evaluation of the genotypes is high. MAS methods are also beneficial under the conditions when environmental effects are significant and heritability is low.

13.7.1 Marker Assisted Selection (MAS) and QTL Mapping

Marker assisted selection is a method wherein target trait is selected based on the marker(s) linked to that trait. These markers can be morphological, biochemical or DNA/RNA. Marker-assisted selection (MAS) has become a common method of molecular breeding, where traits are controlled by a small number of major genes. Several markers have been developed and being used in crop improvement. In maize simple sequence repeat (SSR) and single nucleotide polymorphisms (SNPs) markers are being used extensively. Due the availability in large numbers, simple in use and more effectiveness of SSR markers these were used by many maize researchers.

The achievements through MAS technique for abiotic stress breeding depends on the identification of precise Quantitative Trait Loci (QTLs) for the target traits and their introgression. Extensive QTL mapping for drought and waterlogging tolerance was studies by many maize researchers. Five QTL from a drought-tolerant donor line Ac7643 were transferred to susceptible inbred CML247. Zaidi et al. (2015) identified five QTL for grain yield and 13 QTLs for secondary traits associated with waterlogging tolerance in maize.

13.7.2 Marker Assisted Recurrent Selection (MARS)

Marker assisted recurrent selection (MARS) is a breeding method which by the use of molecular markers assembles the desirable alleles in a population. This scheme involve the genotypic selection and intercrossing among best select individuals. This method increases the efficiency of recurrent selection. As this technique used to accumulate the favourable alleles of stress tolerant genes in a population hence, the improved populations can be used as source population to develop next stage of inbred lines with superior performance in terms of abiotic stress traits. In maize MARS was successfully utilized to improve the quantitative traits of drought tolerance. In a study MARS was used to increase the genetic gain for drought tolerance with respect to mean number of combinations of favorable alleles from 114 to 124 in three cycle of population improvement (Abdulmalik et al., 2017) The MARS was also used to increase the frequency of favourable alleles for drought tolerance from 0.510 to 0.515 in C0 to C_2 cycle of population. (Bankole et al., 2017). At CIMMYT researchers achieved remarkable success in developing early generation yellow drought tolerant maize inbreeds using MARS technique.

13.7.3 Rapid Cycle Genomic Selection

Rapid cycle genomic selection (RCGS) improves the genetic gains by decreasing period of selection cycle in stress tolerance breeding programme in maize. RCGS has added advantage over conventional breeding methods in terms of cost and time as three generations per year can be taken under this method. The hybrids derived from the RCGS improved population were yielded 7.3% yield gain than conventional breeding method. Rapid cycle genomic selection (RCGS) was reported an effective breeding technique in multi-parental maize populations for conserving genetic diversity in short period and obtaining high genetic gains (Zhang et al., 2017a, 2017b). In a recent study the realised genetic gain using RCGS in two maize populations MYS-1 and MYS-2 was 0.110 t/ha per year and 0.135 t/ha per year, respectively, under drought stress (Das et al., 2020). Without compromising genetic diversity, the RCGS can also be used as an effective breeding strategy for increasing genetic gains.

13.8 Accelerated Line Development Using Double Haploid (DH) Technology

Development of high yielding maize hybrid or synthetic varieties essentially required homozygous inbred lines as parental lines. The inbred line development is an important component of maize breeding programs. The line development in maize through the conventional pedigree and bulk involve continuous selfing upto 7–8 generations to get the completely almost homozygous inbred lines. This is which is time consuming process. Now doubled haploid (DH) technology has emerged as a powerful technology and become an effective option to the conventional line development method in maize. DH lines can be developed by both in vitro and in vivo methods. However, in maize in *vivo* methods have been found more reliable and are being used for production of in large number of lines of DH lines (Chaikam et al., 2019). By using the facility of off-season nurseries DH technology can produce the homozygous inbred lines in one year in comparison to conventional inbreeding which require three to four years for continuous selfing. Therefore, DH technology is the fastest and efficient method of fully homozygous line development in maize. DH technology not only useful to derive homozygous lines but it can also be used to explore the genetic diversity in maize landraces. (Brauner et al., 2019). The maize breeding programs of some developed countries of Europe, North America, and China already started in vivo DH line development (Molenaar & Melchinger, 2019). CIMMYT, also made the DH technology accessible to public and private organizations. The development of haploid inducer lines with high haploid inducer rate (HIR) opened the way for in *vivo* DH lines development. The In vivo DH technology being followed by may multinational maize companies. After the development of first haploid inducer line "Stock 6," which has the haploid inducer rate (HIR) of 1–3%. Several other inducers were developed using Stock 6 with good field performance and HIR. Most of these inducers like UH400, RWS and PHI were adapted to temperate environments. Earlier CIMMYT has developed temperate inducer lines with HIR 6–9% but recently CIMMYT has developed second-generation Tropically Adapted Inducer Lines (TAILs) with higher HIR (9–14%) and better field performance (Chaikam et al., 2018).

13.9 Rapid Generation Advancement (RGA)

Conventional breeding programme takes much time to complete any breeding cycle of crop improvement. Rapid generation advancement (RGA) and speed breeding are the most promising innovations for increasing the rate of genetic gain and reducing breeding cycle time (Cobb et al., 2019). RGA was first developed by Goulden (1939) and later modified by Grafius (1965). Its latest destination came in the form of speed breeding (Watson et al., 2018). Using different strategies like manipulation of growing environment, nutrients, use of hormones etc., many generations per year can be obtained in RGA method. The RGA not only reduce the generation cycle but also reduce the cost of getting new recombinant as compared to conventional pedigree breeding. RGA technique in maize needs to be standardized to harness its role in abiotic stress tolerant hybrid maize breeding. Specialized facilities like greenhouse, growth champers needs to be developed for rapid generation advancement, to achieve the desired cycle time and generation per year.

13.10 Precision and High Thorough-Put Phenotyping

Phenotyping is an important part of the stress breeding which provide much understanding and information to the breeders of stress tolerance programme. The main purpose of any plant breeding programme is to develop new genotypes which perform better and yield more than the genotype being cultivated in the target environment. Most of the abiotic stress tolerance traits are controlled by quantitative genes, where heritability is poor therefore to enhance the heritability by improving accuracy and achieve more genetic gain, precision phenotyping techniques are required. Advance techniques are required which may accelerate genetic gains by improving selection efficiency. Techniques are also required for automatic monitoring of nutrient and plant health status. In recent years high throughput phenotyping techniques has drown major attention of researchers to develop many protocols for improving desirable plant traits. In phenotyping work of plant breeding, several hundreds or thousands small plots need to be screened for abiotic stress, where use of high precision tools and fast result machines are always required to get instant results. Reliable and automation, high-throughput phenotypic technologies are being considered more and more important tools to get higher genetic gain in stress breeding programmes. Newly developed high-throughput and nondestructive techniques can be used under controlled stress environment for phenotyping of the primary and secondary traits associated with of plant architecture. Ear photometric and imaging techniques are now used for phenotyping of the ear traits in maize. The techniques are available that either used to phenotype the whole or specific part/s of the plant. Proximal phenotyping which done through the ground based small vehicle and sensors mounted on it. They are used to measure the plant canopy temperature, leaf area, chlorophyll fluorescence, nitrogen content, and plant height. Smartphone-based apps and platform were developed for plant phenotyping using pictures and images (Confalonieri et al., 2017). Next generation advanced techniques of automation, robotics, high accuracy sensors, and imaging may provide other opportunities for high-throughput plant phenotyping (HTPP) (Gennaro et al., 2017). Many HTPPs in growth chamber or greenhouse have been developed using digital and advanced technologies like high-throughput, high precision and automation (Zhang et al., 2018).

13.11 Scope of Advanced Genetic Manipulation Technologies in Developing Stress Resilient Maize

As described earlier, identification of molecular initiators and regulators for control of metabolic process leading to stress tolerance is a pre-requisite for designing advanced stress management strategies. Studies have indicated various molecules that play an important role in single- or multiple-stresses. Kimotho et al. (2019) have reviewed the various transcriptional factors that work in ABA-dependent and ABA-independent pathways, and that can be used to imparting tolerance to drought, salinity, heat and cold stresses. Gene manipulation of the key transcription factors can be done to increase or decrease their expression, alter the nature of expression by changing promoter DNA, making the expression respond to a particular regulator etc. This can be done in cis- or trans- approach, depending on whether the gene from a maize itself or from some other species has been taken, respectively. Gene editing involves deliberately introducing knock-in/knock-out mutations, where either the amino acid sequence if changed or the protein expression is shut down. Although many systems exist for introducing specific changes in DNA, one system CRISPR/Cas9 has gained popularity. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. These are genetic elements, which originate from viral genomes and have got incorporated into the bacterial genome. Endonuclease proteins are associated with these genetic elements, referred to as CRISPR-associated proteins or Cas. The RNA of viral DNA incorporated into bacterial genome, directs Cas proteins to act like specific endonuclease. Whenever, a viral genome is encountered in the cell, bacteria respond by engaging the Cas protein specific for that virus, resulting in cleavage of viral nucleic acid. This phenomenon has been used to design plasmid containing CRISPR Cas systems for precise editing of plant genomes (Fig. 13.2a).

Zhang et al. (2018) identified HKT1 family sodium transporter, as a key factor for imparting salinity tolerance in maize. HKT1 transporter, expressed primarily in root cells, is responsible for exclusion of sodium from the xylem sap. This reduces the flow of sodium to shoot, thereby helping in tolerating saline conditions. The authors used CRISPR/Cas 9 system to create a loss-of-function mutation for HKT1, demonstrating retarded performance of edited plants in saline soil. Shi et al. (2019) reported generation of variants in ARGOS8 gene through CRISP/Cas, which resulted in drought tolerance. ARGOS8 is a negative regulator of ethylene biosynthesis, its

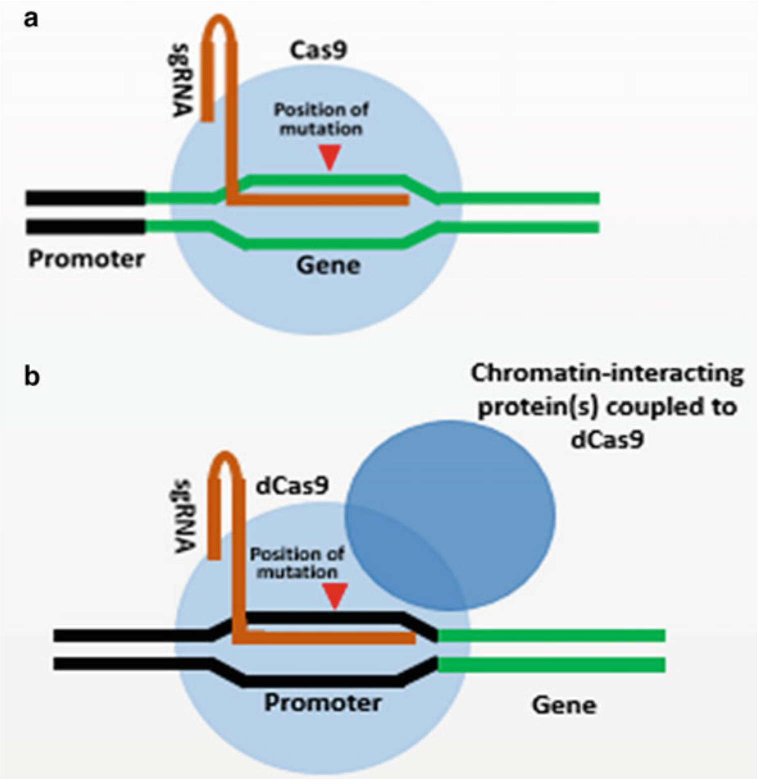

Fig. 13.2 Genome editing technologies for precise gene engineering. **a** CRISPR/Cas9 system for introducing mutations in a specific DNA sequence **b** dCas9 system for introducing epigenetic marks on a specific DNA sequence. The nature of marks created depend on the coupled proteins, which interacts with chromatin to being about desired changes

expression level in maize is low and non-uniform across tissues. Shi et al. (2015) showed that transgenic maize expressing high levels of ARGOS8 is drought tolerant. In this case they used CRISPR/Cas 9 system to generate allelic variation in a key gene ARGOS8. Shi et al. (2015) inserted a GOS2 promoter upstream of the ARGOS8 gene. GOS2 is a maize promoter that imparts a moderate level of constitutive expression. The presence of a GOS2 promoter upstream of ARGOS8 gene resulted in higher and uniform gene expression. This study demonstrates the use of single, native genes for modulating complex traits like drought tolerance.

Now-a-days, technology is available for reversibly altering the expression as well. This field, referred to as epigenome engineering, has opened new avenues in genetic manipulation of plants. This utilizes a particular type of a Cas9 endonuclease, which is capable of recognizing a nucleic acid sequence, but is unable of cleaving it (Pulecio et al., 2017). Due to lack of endonuclease activity, it is also referred to as Cas9 Endonuclease Dead (dCas9). dCas9 can be utilized in various designs to bring about epigenetic changes in the cell (Fig. 13.2b). Through histone modifications, it can be used to increase or decrease expression of particular genes.

In maize gene editing successfully utilized to alter five genes liguleless1 (LIG1), male fertility (Ms26 and Ms45), and acetolactate synthase (ALS) genes (ALS1 and ALS2) through targeted mutagenesis, and site-specific gene insertion using Cas9 and guide RNA (Svitashev et al., 2015). Using integrated multiplexed CRISPR/Cas9-based approaches Liu et al. (2020) successfully targeted 743 genes.

Thus, with the advent of epigenome engineering, the epigenetic events can now be targeted and the benefit of their reversible nature can be beneficially utilized to obtain the dual benefits of stress resistance and high yield. Hence, advanced technologies like genome editing can be used developing maize with abiotic stress resistance. Recent studies have demonstrated this by targeting specific genes.

13.12 Conclusion

There are various conventional as well as molecular and genomic techniques available through which the identified genetic variations can be dissect and explore for identification of suitable recombinants, gene(s)/QTLs and candidates' gene(s) for drought and water logging stress tolerance. Among the conventional breeding, simple selection and classical breeding methods such as pedigree and backcrossing breeding procedures etc. are the important ones which have contributed significantly for the development of stress resilient genotypes since long back. Precise phenotyping at critical crop growth stages under target environments are the keys of success for stress resilient breeding. Generally, seedling, flowering and grain filling stages are the more sensitive stages for drought, heat, cold and water logging stress in maize. Amongst all these stages, the seedling stage for water logging and flowering for drought heat and cold stress are the most sensitive one. Among the molecular and genomic approaches; MAS, MARS, genomic selection, functional genomics, genetic engineering and genome editing are the important ones which can complement the conventional breeding methodologies and therefore can improve the efficiency as well as effectiveness of abiotic stress breeding programme. Developing suitable stress resilient maize genotypes can help to increases the national as well as world average maize productivity.

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