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Breeding Maize (*Zea mays* L.) for for Heat Stress Tolerance

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Abstract: Maize is staple food crop for hundreds of millions farming families in Africa and heat stress is one of the environmental factors that significantly affect the production and productivity of the crop. Heat stress is one of the most important abiotic factors negatively affecting the phenology, growth, grain yield and biomass of the maize worldwide. The problem will become even more serious in future climate change scenarios due to the frequent occurrence of high temperatures and water scarcity. The development of high-yielding maize genotypes that are resistant to heat stress is one of the top priorities of maize breeders. The fundamental step in breeding for heat stress tolerance is to identify the appropriate donor plant for its effective use in the development of new cultivars. Understanding the genetic basis and the mode of inheritance of traits related to heat tolerance is paramount to successful breeding strategies. There are several approaches to improving heat tolerance including: convection breeding, transgenetics and genome editing. Heat stress can be alleviated by breeding plant varieties with improved heat tolerance using a variety of conventional or advanced genetic methods. Compared to molecular breeding, especially marker-assisted selection, which is extremely efficient and precise, conventional breeding is tedious, time-consuming and dependent on the environment. Many studies have shown that the negative effects of heat stress can be minimized by the application of osmoregulatory compounds, salicylic acid (SA), proline, ascorbic acid (AA) and silicon and by changing certain management practices that would support plants' stress tolerance at planting time, Optimization of fertilizer and irrigation management. Therefore, the present review has attempted to highlight breeding approaches for heat tolerance and management strategies that might be useful for mitigating heat stress in maize.

Key words: Stress Breeding · Global Warming · Maize · Heat Avoidance Heat Stress Tolerance

INTRODUCTION

Maize is staple food crop for more than 300 million farming families in Africa [1]. Maize cultivation in sub-Saharan Africa (SSA), is dominated by smallholder farmers and the production area of the crop is increased by about 66% from 2007 to 2020 [2]. Together with wheat and rice offers at least 30% of the food calories to more than 4.5 billion people in 94 developing countries [3]. Maize accounts for 45% of total calories and 43% of total protein from grains in East and Southern Africa [4].

Despite its importance in the region, maize yields in SSA are still the lowest compared to other regions of the world. As reported by Chaudhary *et al*. [5] 2050, the demand for maize in the developing world will be almost double to the current demand. Farmers are growing the crop under diverse constraints and climate induced maize production constraints are the major causes of yield reduction in sub-Saharan African countries. Drought is one of the most severe abiotic stresses in many regions of the world and it is one of the most urgent issues in the current climate scenario [6]. Higher temperature at reproductive stage decreased the seed setting percentage and kernel development that ultimately has negative effect on grain yield and quality as well [7, 8]. Every plant species, more precisely every genotype, has an optimal temperature range for growth and development. If the temperature rises above this critical limit, thermal stress occurs, have negatively impacts on the yield performance of the genotype. As higher temperatures and low humidity wither the open grains of silk and pollen and condense pollen germination [9]. It also negatively affects

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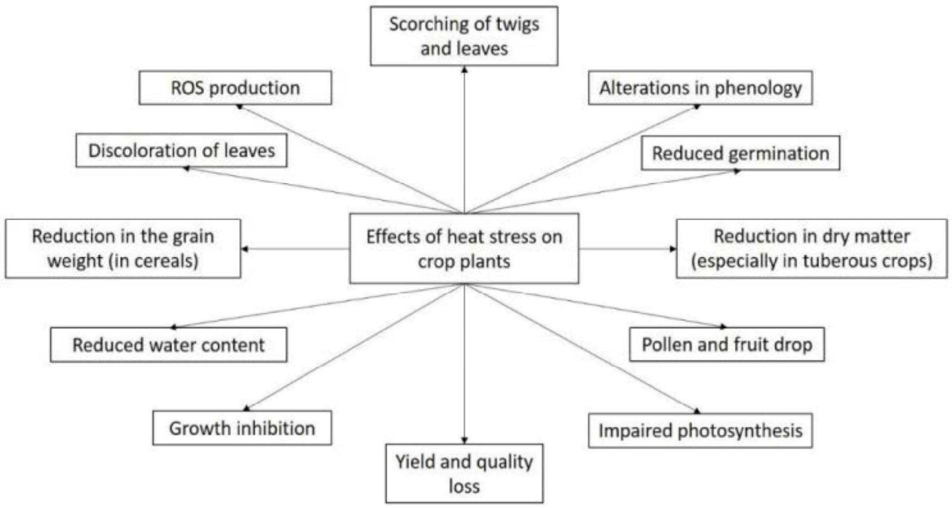

Fig. 1: General effects of heat stress on crop plants [27].

many physiological aspects of the plant, such as: B. Variations in protein metabolism, reducing the process of photosynthesis [10, 11]. Improving the productivity and maintaining the stability of maize yield under favorable as well as stressful conditions are necessary to cater to the demand of growing worldwide population. Hence, the current review has attempted to highlight breeding approaches for heat tolerance and management strategies that might be useful for mitigating heat stress in maize.

Plant Response to Heat Stress: Plant responses to heat stress vary with the degree of heat stress, duration and type of the plant under stress conditions. Among the abiotic factors, heat stress is one of the most significant limiting factors that affect the productivity of the crops [12]. At extreme high temperature, cellular damage or cell death may occur within minutes, which may lead to a catastrophic collapse of cellular organization [13]. Heat stress affects all aspects of plant processes like germination, growth, development, reproduction and yield [14]. Heat stress negatively affects various physiological, biochemical and growth processes in plants. [15], also reported that heat stress causes a decrease in the production of carbohydrate, while, increase in carbohydrate starvation throughout the period of high respiration. In addition to this, heat stress also resulted in a significant reduction in the growth and net assimilation [16].

In a case of maize, the effects of heat stress vary across plant tissues and organs, depending on the vulnerability of active metabolic processes at the time of stress [17]. Heat stress affects the vegetative and reproductive growth of maize, starting from germination to grain filling [18]. Hot spells and moisture stress (or physiological drought) are common in tropical, arid and semi-arid corn-growing regions. It is predicted that heat stress alone or in combination with drought will become a major production constraint for maize in the future. High temperatures can alter cellular level metabolism and pollen dehiscence, pollen fertility, silk emergence and stigma receptivity, seed formation and grain filling, ultimately reducing grain yield [19]. Excessive heat also leads to a reduction in net photosynthesis, leaf area, decreased biomass accumulation and lower seed weight [20]. Ambient temperatures higher than optimum can hinder both vegetative and reproductive growth. However, the reproductive stage (anthesis, silk formation, grain filling and seed set) is the most sensitive stage that eventually leads to complete sterility and grain breakage in maize [21]. The temperature threshold for heat stress damage is lower in the reproductive organs than in the vegetative parts [22, 23]. The effects of heat stress on corn at different stages of plant growth, from germination and seedling formation to late grain filling, have been extensively studied [24]. There is a categorized sensitivity to heat stress in maize crop. The maximum yield loss was observed when it occurred around the reproductive stage and during flowering and the delay phase of grain filling, followed by the grain filling phase and the late vegetative stage [17, 23, 24]. The main effect of heat stress on the reproductive stage is manifested in reduced pollen viability, which in turn affects pollination, fertilization and nuclear count [25] and reduced seed set resulting from increased nuclear abortion rate [26].

Genetic Resource for Heat Stress Tolerance: The ability to survive an otherwise fatal temperature stress can be conferred by exposure to a mild nonfatal temperature stress. This induced ability to survive a normally fatal stress is called acquired thermo-tolerance [28]. A heat hardy cultivar is defined as a cultivar that exhibits higher productivity than other cultivars when grown in environments where heat stress occurs. Large differences in the heat tolerance of different crops have been found in both cultivated and related wild species. The presence of genetic variation within a species is a prerequisite for a breeding program to develop genotypes responsive to heat stress [29]. Basically, heat tolerance is not tied to a single trait; rather, it is a complex and polygenic mechanism in crops. Today's crop varieties have limited heat tolerance as previous domestication, green revolution and conventional breeding all aimed to increase yield and quality traits. However, knowledge of the genes/markers/QTL regions associated with heat tolerance is now required to improve heat tolerance. Previous studies suggested that there is still a great deal of genetic diversity in the germplasm of different crops. There are significant differences between genotypes when it comes to heat stress, giving breeders the opportunity to improve their plants through genetic improvements. Variation in maize landrace genetics is natural and helpful for future breeding advances [30]. It is a crucial requirement to enable a steady development of agricultural productivity [31]. The genetic resources of plants are the essential part of all agricultural systems, therefore the conservation, evaluation and improvement of the germplasm is essential [32]. For many years, dominant landraces were the sources for the development of new freely pollinated varieties.

Heat Tolerance and Inheritance of Heat Stress Tolerance: Heat tolerance is when one genotype is more productive than another genotype in environments where heat stress occurs [33]. Heat tolerance can also be defined as the relative performance of a plant or plant process under heat compared to its performance at optimum temperature. In plant breeding, prospective heat stress tolerance is a complex quantitative trait and the mode of inheritance of traits associated with heat stress is of paramount importance for successful breeding strategies. Understanding the genetic basis of heat tolerance-related traits is critical to the development of heat-tolerant genotypes. Greater heat tolerance is defined as a specific plant process that is less damaged by high tissue temperatures and may have constitutive effects or require acclimation [33]. According to Paulsen [34], numerous traits associated with the resistance of plants to high temperatures indicate that thermo-tolerance is highly complex. Heat stress tolerance involves complex traits that depend on many attributes. The ability to survive an otherwise fatal temperature stress can be conferred by exposure to a mild nonfatal temperature stress. This induced ability to survive a normally fatal stress is called acquired thermo-tolerance [28]. A heat hardy cultivar is defined as a cultivar that exhibits higher productivity than other cultivars when grown in environments where heat stress occurs.

Heat tolerance is quantitative in nature and is controlled by a number of genes/QTL (quantitative trait loci) [35, 36]. Research on heat stress in maize is not as exhaustive as research on drought [37]. In recent years there has been increasing interest in using functional genomics tools such as transcriptomics, proteomics and metabolomics to identify and understand the components of heat stress tolerance and the underlying mechanisms at the molecular level [38].

Breeding for Heat-stress Tolerance in Maize: Historically, breeding maize for heat stress tolerance has not been given as high a priority in tropical corn breeding programs as other abiotic stresses such as drought, waterlogging and low soil nitrogen. However, over the past years, heat stress tolerance has emerged as one of the key traits for the CIMMYT corn breeding program, particularly in the South Asian tropics P.H. Zaidi, T. 2020. Development of heat tolerance in maize inbred lines by breeding can maintain optimal plant growth and productivity under heat stress [40]. The use of conventional plant breeding methods and skills has proven very useful in selecting stress tolerance in corn. The international and national research institutes in SSA have made significant progress in developing corn varieties with improved DS and/or HS tolerance [41]. For example, the CIMMYT and ITA maize breeding programs in collaboration with National Agricultural Research Institutes (NARS) in West Africa have successfully developed drought-tolerant inbred lines, hybrids and free-pollinated varieties.

Conventional breeding is almost always based on phenotypic variation of the crops, which is affected by environments and crop improvement cycle takes long time. This approaches based mainly on phenotype selection and it is tedious, time-consuming and dependent on the environment as compared to molecular breeding, particularly marker-assisted selection, which is highly efficient and precise. Heat stress can be alleviated by breeding plant varieties with improved thermo-tolerance using a variety of conventional or advanced genetic methods [42]. Development of heat-tolerant genotypes through conventional breeding methods reduces reported yield loss due to heat stress.

Marker assisted selection techniques for breeding are extremely useful. Recently, 41 polymorphic SSR markers between the heat-tolerant rice cultivar N22 and the heat-sensitive, high-yielding cultivar Uma were identified for the development of new high-yielding, heat-tolerant rice cultivars. Functional genomic studies on the effects of heat stress have been performed in many crops, including maize, under controlled laboratory conditions [40]. However, reports of genetic mapping of traits associated with heat stress tolerance in maize, particularly under field conditions, are rare. Some studies have investigated the molecular and genetic basis of seedling stage heat stress tolerance in maize [43, 44].

To cope with heat stress, plants utilize several mechanisms including maintenance of membrane stability, scavenging of reactive oxygen species (ROS), production of antioxidants, accumulation and adaptation of compatible solutes, induction of mitogen-activated protein kinase (MAPK) and CDPK- Cascades and especially chaperone signaling and transcriptional activation. All of these mechanisms, regulated at the molecular level, allow plants to thrive under heat stress. Based on the understanding of these mechanisms, possible genetic strategies to improve heat stress tolerance of plants include traditional and modern molecular breeding protocols and transgenic approaches [45]. With the advent of DNA marker technology in the 1980s and early 1990s, many of the limitations associated with isozymes and morphological markers were overcome and genetic mapping entered a new and exciting era, which allowed a significant increase in the Efficiency promised plant breeding and genetic study. As suggested by Hall [46] the genetic engineering is a useful tool for ideotype breeding because a single gene can be inserted without altering the background of the cultivars and there are numerous success stories in the literature of the development of isogenic lines in short Time. In isogenic lines, the differential expression of an inserted gene in a range of environments can be assessed very accurately. Isogenic lines developed by traditional backcrossing methods take several years to develop generations compared to marker-assisted selection (MAS). It is an efficient approach to improving a stress-tolerant crop. This approach requires the availability of genetic markers linked to stress-tolerant genes. Genetic markers associated with various environmental stresses such as drought, heat and salinity have been identified in different regions.

Genetic Engineering: Several heat-responsive genes and proteins have been described in maize. Work on maize heat stress tolerance at CIMMYT is based on exploiting native genetic tolerance, but genetic modification through genetic engineering and gene editing are also powerful strategies. Biotechnology has made significant contributions to understanding and improving the heat stress tolerance of crops. Genetic modification through biotechnology and other new breeding techniques is a powerful strategy that offers new opportunities to improve crop adaptability. However, general public concerns and complex legislation limit the use of new breeding techniques. In maize, transcriptome and metabolome studies have identified hundreds of genes that respond to heat and induce heat tolerance. Heat shock transcription factors (HSFs), which regulate the production of heat shock proteins (HSPs) are studied in detail for their utility in the development of heat tolerance in crop plants. Crops such as wheat, corn, tomato and rice have been genetically modified to improve thermo-tolerance and mainly targeted HSPs and HSFs [40, 47-49]. The availability of genomic sequences for multiple crops along with genome editing techniques has opened up new breeding opportunities for almost any desired trait [50]. Gene editing tools that can affect multiple gene knockouts are of huge importance to accelerate and achieve efficient plant breeding [51].

For the first time, multiplex genome editing was demonstrated in maize by [52] using a tRNA-RNA processing system. An intensive study by [53] confirmed that multiple regulators of important traits in a single strain can be modified by CRISPR/Cas9, facilitating the dissection of complex gene regulatory networks in the same genome. Currently, attempts to use genome editing to increase heat tolerance are targeting several genes mainly involved in the ethylene response and TFs, with the ultimate goal of increasing yield under abiotic stress, including HS [10, 53].

Recently, transgenic and genetic engineering strategies for quantitatively inherited complex traits such as heat stress tolerance/resistance are actively explored to accelerate the designing of heat stress tolerant maize varieties. The study conducted by [54, 55] on RNA-guided genome editing in plants using CRISPR demonstrated an RNA-guided approach to genome editing by developing two vectors suitable for genome editing in rice, pRGE3 and pRGE6. To improve editing frequency, researchers have improved the utility of programmable endonucleases that create DNA double-strand breaks (DSBs) at target sites. The development of various GE technologies such as transcription activator-like effector nucleases (TALEN) and zinc finger nuclease (ZFN) have been previously used in the intended modification of animal and plant cell genomes [56-58]. Overall, the availability of genome sequences for multiple crops and advances in genome editing approaches has opened up opportunities to improve almost any desired trait.

CONCLUSION

Heat stress has become a major and wide problem for crop production because it affects the development, growth and productivity of crop plants and it may affect any plant species at any stage. However, the degree of occurrence of heat stress varies greatly among climatic zones and depends on the period and probability of high temperature and on the timing of diurnal plants during high temperature. Plants have evolved several adaptive mechanisms to cope with such environmental stresses. These strategies may involve several metabolic adjustments and morpho-physiological alterations. Most recently, the development of more heat-tolerant maize has been a primary focus for breeders and geneticists and researchers examining heat-stress-related effects with chemical ameliorants. The very basic and first approach in breeding for heat stress tolerance is to identify the suitable donor parents for their effective utilization in tailoring of new cultivars. Heat stress tolerance is a complex quantitative traits and mode of inheritance of traits associated with heat stress prime importance for successful breeding strategies. Understanding the genetic basis of traits related to heat tolerance is essential for the development of heat-tolerant genotypes. Over the past three decades efforts have been made to elucidate the genetic basis of heat tolerance. Development of maize lines by conventional breeding methods reduces the loss of yield reported due to heat stress. But, conventional breeding is tedious, time-consuming and dependent on the environment as compared to molecular breeding, particularly marker-assisted selection, which is highly efficient and precise. In recent years, there has been increasing interest in using functional genomics tools, such as transcriptomics, proteomics and metabolomics, to identify and understand the components of heat stress tolerance and underlying mechanisms at the molecular level. Biotechnology also has contributed significantly in understanding and improving for heat stress tolerance in maize plants. A combined approach using several techniques for improving complex traits like heat tolerance would be a more appropriate. Research results also revealed that combination of heat and drought stress adversely affect the physiological processes including growth and development compared to single stress. So maize breeding programs need to focus on selection under both heat and drought stresses instead of focusing single stresses individually.

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