**High Temperature Tolerance in Maize (*Zea mays* L.)**

**Ravi D. Patel1\*, Yogesh Purohit2**

1\*Ph.D. Scholar, Department of Genetics and Plant Breeding, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar - 385506, Gujarat.

2Ph.D. Scholar, Department of Genetics and Plant Breeding, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar - 385506, Gujarat.

\*Email id: rdpatel26898@gmail.com , Mobile number: +91 9426281500

**Abstract**

Globally maize is one of the most important cultivated crop and plays a significant role in human and livestock nutrition. In India, maize has traditionally been cultivated as a kharif crop, but with new improved hybrids and irrigation facilities, it can grow effectively during rabi in many regions of the country. Among the different abiotic stresses, High temperature has become a world’s concern because it affects severely the growth and production of crops. In maize, high temperature stress during the reproductive stage causes poor seed germination and cellular alterations that increase the production of reactive oxygen species (ROS), which damages lipids, proteins, and nucleic acids, ultimately causing cell death. Plants can respond to heat stress conditions by using a variety of mechanisms like adaptation, avoidance, or acclimatisation. Heat stress tolerance is a complicated quantitative feature and mode of inheritance of traits related with heat stress, is important for effective breeding programme. New approaches are being developed to modify the expression of functionally related genes by modifying abiotic stress signalling pathways that control the expression of many genes that may contribute to stress tolerance. Different breeding approaches like conventional and non-conventional approaches to improve heat stress tolerance in maize are described in this chapter.

**Key words:** High temperature, Heat stress, Heat shock protein, Maize (*Zea mays* L.)

1. **INTRODUCTION**

 One of the most important grains cultivated worldwide, after wheat and rice, is maize (*Zea mays* L.). Maize uses water and carbon dioxide more effectively due to its C4 photosynthesis process. Due to its superior yield potential compared to other cereals, maize is referred to as the "queen of cereals". It has a pistillate and staminate flowering pattern and is a naturally monoecious plant. In India, maize has traditionally been cultivated largely as a kharif crop, but with the evolution of new, improved hybrids and the assurance that irrigation facilities will be available, it can now be grown effectively during rabi in many regions of the country.

Maize plays a significant role in human and livestock nutrition. The nature of the demand for maize is also changing. Maize is an important food crop but over the past decade, its demand as livestock feed has grown tremendously. It is utilized as raw materials for various industries for manufacturing starch, ethanol, acetic acid, glucose, synthetic rubber, dyes and resin, etc. Many by-products of maize like fructose syrup, maltodextrin, germ oil, germ meal fiber and gluten products which have applications in industries such as alcohol, textile, paper, pharmaceuticals, organic chemicals, cosmetics and edible oil.

Food production for the expanding human population is seriously threatened by a variety of environmental problems. Among the different abiotic stresses, temperature fluctuations are common during plant growth and development. During the reproductive phase of plant high temperature stress is major threat since it affecting crop production and productivity worldwide. High temperature stress during the spring maize crop's reproductive stage causes an extended anthesis silking interval and poor seed germination. The ideal temperature for maize plants is between 22 °C to 32 °C during the day and 16.7 °C to 23.3 °C at night, respectively. Plant growth is accelerated at these temperatures because the photosynthetic rate becomes faster than respiration. When the temperature drops below 5 °C or increases over 32 °C, plant growth is negatively impacted. The global air temperature is predicted to rise by 0.2 °C per decade, which will lead to temperatures 1.8 – 4.0 °C higher than the current level by 2100 (IPCC 2007). High temperature stress causes cellular alterations that increase the production of reactive oxygen species (ROS), which damages lipids, proteins, and nucleic acids, ultimately causing cell death.

The ability of maize germplasm to withstand various biotic and abiotic challenges will steadily enhance yield and ensure food supply for the increasing world population. Therefore, understanding the genetic basis of high temperature tolerance is crucial for the creation of tolerant synthetics and hybrids for sustainable agriculture. New approaches are being developed to modify the expression of functionally related classes of genes by modifying abiotic stress signalling pathways and characterising and cloning transcription factors that control the expression of many genes that may contribute to stress tolerance

1. **HEAT STRESS - ITS EFFECT ON PLANT AND PLANT RESPONSE TO HEAT STRESS**

 The adverse effects on plants due to higher temperature than the optimal temperature is considered asheat stress or high temperature stress. Heat stress would affect (1) survival, (2) growth and development and (3) physiological processes.

 Plant responses to heat stress vary with the degree of temperature, duration of temperature and nature of crop. Due to extreme temperature, cellular damage or cell death may occur within minutes, which causes collapse of cellular organization (Ahuja *et al*., 2010). High temperature affects all the important stages in plant like germination, growth, development, reproduction, yield, etc. and it also affects the stability of various protein, RNA, cytoskeleton structures, and efficiency of enzymatic reactions (Mittler and Blumward 2010). If leaf temperature is rise greater than 30 °C it causes inactivates the Rubisco and if temperature is rise greater than 38 °C it causes thermal inactivation of enzymes, which efficiently reduces photosynthesis (Steven *et al*., 2002). In maize its affecting pollination due to silk desiccation and pollen abortion. Apart from these effects, other effects caused by high temperature were shown below.

* **Alteration in photosynthesis:** In plants photosynthesis is one of the most heat sensitive physiological processes. High temperature causes reduce amount of photosynthetic pigment. Closure of stomata lead to reduced CO2 uptake. High temperature affects function of chlorophyll by altered structural organization of thylakoids.
* **Reduction in plant growth:** High temperature causes loss of cell water content for which the cell size and ultimately the growth is reduced. Reduction in net assimilation rate (NAR) is also another reason for reduced relative growth rate (RGR).
* **Improper development:** Reproductive tissues are more sensitive. High temperature causes pollen and spikelet sterility and reduced pollen viability.
* **Yield reduction:** High temperature is so terrible that even a small (1.5 °C) increase in temperature have significant negative effects on crop yields. Increase of the seasonal average temperature 1 °C decreased the grain yield of cereals by 4.1% to 10.0%. Loss of productivity in heat stress is chiefly related to decreased assimilatory capacity which is due to reduced photosynthesis by altered membrane stability and enhanced maintenance respiration cost, reduction in radiation use efficiency.
* **Alteration in phenology:** The crop duration was reduced under elevated temperature.
* **Alteration in dry matter partitioning:** Heat may reduce grain growth, which in turn reduces photosynthate translocation to the grain (due to a reduced sink size).
* **Water loss:** Plant water relation is more affected under the heat stress. High temperatures affect seedlings, by increasing evapotranspiration demand and tissue damage.
* **Inhibition of seed germination:** Seed germination is highly dependent on temperature as temperature is one of the basic requisites of this process. In maize crop optimum range of temperatures for seed germination is 25-30 °C.
* **Oxidative stress:** Temperature stress accelerates the generation of ROS including O2, H2O2, etc. induced oxidative stress. Which can result in significant damage to cell structure.
1. **PLANT ADAPTATION TO HEAT STRESS**

Plant species shows great variation in terms of their response and tolerance to high temperature. Plants can be classified into three groups, on the basis of preferred temperature of growth (Figure 1). There are (a) Psychrophiles, (b) Mesophyles and (c) Thermophyles. (Zrobek-Sokolnik *et al.,* 2012; Larcher *et al*., 1995)

1. Psychrophiles : it include plants, which grow well at low temperature ranges between 0 °C to 10 °C.
2. Mesophyles : Plants which favor moderate temperature and grow well between 10 °C to 30 °C.
3. Thermophyles : which grow optimally at high temperature ranges between 30 °C to 65 °C or even higher.

Heat sensitive species

**Plants**

Psychrophiles (Temp. – 0 to 10 °C)

Mesophyles (Temp.– 10 to 30 °C)

Thermophyles (Temp.– 30 to 65 °C)

Relatively heat resistant species

Heat tolerant species

**Figure 1.** Classification of plants on the basis of their heat tolerance.

Plant uses different adaptation mechanisms for its survival in hot, dry environments to high temperature (Fitter *et al.,* 2002). Plant adaptation to high temperature stress includes avoidance and tolerance mechanisms (Figure 2).

* **Avoidance Mechanisms**
* Early maturation is closely correlated with smaller yield losses under high temperature.
* Presence of small hairs (tomentose), cuticles, waxy covering, etc. helps in avoidance of heat stress by reducing the absorption of solar radiation
* Plants with small leaves are also more likely to avoid heat stress
* In such plants, leaf blades often turn away from light and orient themselves parallel to sun rays (paraheliotropism).
* Intensive transpiration prevents leaves from heat stress, and leaf temperature may be 6 °C or even 10–15 °C lower than ambient temperature.

**Adaptive mechanism to heat stress**

**Tolerance**

**Avoidance**

 Changing leaf orientation Osmoprotectants

 Transpirational cooling Antioxidant defense

 Leaf rolling Expression of stress protein

Signaling cascades and transcriptional control

 Early maturity

 Alteration of membrane

 lipid composition

**Figure 2.** Different adaptation mechanisms of plants to high temperature.

* **Tolerance Mechanisms**
* Ability of plant to grow and produces economic yield under heat stress condition is known as heat tolerance.
* Ion transporters, late embryogenesis abundant proteins (LEA), osmoprotectants, antioxidant defense are major mechanisms as well as factors responsible for signaling cascades and transcriptional control are important for control and balance the heat stress condition in crop.
* In case of sudden heat stress, short term response, i.e., leaf orientation, transpirational cooling and changes in membrane lipid composition are more important for survival.
1. **MECHANISM OF SIGNAL TRANSDUCTION AND DEVELOPMENT OF HEAT TOLERANCE**

Upregulation of many genes has been reported to help the plant to withstand the stress conditions which leads to plant adaptation (Tuteja *et* al., 2009). During stress, plants interpret internal and external signals through multiple independent or connected pathways that are employed to control diverse responses for the development of their tolerance (kaur *et al*., 2005; Figure 3). Multiple pathways are involved in the numerous interconnected systems which make up a plant's reaction to stress. Cofactors and signalling molecules must interact for a response to be produced in certain cellular compartments or tissues in response to a given stimulus. Genes which respond to stress are activated by signalling molecules. There are various signal transduction molecules related to stress responsive gene activation depending upon plant type, types of stresses. Some broad group of those are the Ca-dependent protein kinases (CDPKs), mitogen-activated protein kinase (MAPK/MPKs), NO, sugar (as signaling molecule), phytohormones (Ahmad *et al*., 2012). These molecules together with transcriptional factors activate stress responsive genes.

**Figure 3.** Schematic illustration of heat induced signal transduction mechanism and development of heat tolerance in plants.

**High temperature**

Signal sensing and transduction

**Oxidative stress**

**Disruption of membrane properties, proteins/enzymes, and cellular homeostasis**

Signal sensing and transduction

 Transcriptional factor

**Activation of stress responsive gene**

**Activation of antioxidant enzymes, free radical scavengers, signaling molecules, osmoprotectants**

**ROS detoxification reactivation of protein and enzymes, re-establishment of cellular homeostasis**

**Development of heat tolerance**

1. **GENETICS OF HEAT TOLERANCE AND GENE EXPRESSION**

 Heat stress tolerance is a complicated quantitative feature and mode of inheritance of traits related with heat stress, is critical for effective breeding programme. Heat tolerance is also quantitatively inherited in maize, and successful heat tolerance selection involves evaluating genotypes in replicated experiments in different environments (Bai 2003).

 Akbar *et al.* (2008) also reported non-additive gene action is responsible for yield and yield contributing characters under high temperature stress condition in maize. Dominance gene action has been found to be predominant for seed vigor, germination percentage, relative water content, growth rate of plant, rolling of leaf, pollen size, anthesis silking interval, viability, shelling percentage, silk receptivity, number of ear per plant, leaf senescence, plant maturity days and economic yield (Tassawar *et al*., 2012). Cell membrane thermo stability percentage, stomata conductance, turgor potential, transpiration rate, growing degree days to 50% tasseling and grain yield per plant are governed by the non-additive genes effect and for growing degree-days to 50% maturity governed by additive genes effect at high and normal temperature condition (Akbar *et al*., 2009). Traits had dominant or non-additive gene action in high temperature stress condition that could be improve by hybrid breeding strategy to improve heat stress tolerance in maize. Population improvement programs can also boost the frequency of favorable heat tolerant genes in maize populations.

 When a plant is exposed to heat stress, changes in signal interpretation occur that are responsible for gene expression and the production of transcripts that are responsible for the formation of stress-related proteins (Iba 2002). One of the most significant tactics in this respect is the production of heat shock proteins (Feder and Hoffman 1999). During high temperatures, heat shock proteins act as chaperones and perform signal transduction functions. Plant has different physiological mechanism for survival and growth and development such as photosynthesis, assimilate partitioning, water use efficiency, nutrient use efficiency, membrane stability and these phenomena were control and improved by heat shock protein under heat stress condition for development of thermo tolerance plant (Camejo *et al*., 2005; Ahn and Zimmerman 2006; Momcilovic and Ristic 2007). Now, molecular breeding as well as genetic engineering as an additional tool for stress tolerance breeding to improved crops yield under heat stress condition.

1. **Breeding Approaches**
* **Conventional Approaches:** different conventional breeding approaches are
* Introduction
* Selection
* Pedigree Method
* Heterosis breeding
* Back cross method
* The majority of abiotic stress tolerance features are complicated, multi-gene-controlled, and strongly influenced by environmental variation.
* Direct selection in field condition is typically challenging due to unpredictable environmental influences.
* Growing breeding materials in a hot target production environment and identifying lines with higher yield potential has shown to be one of the best ways of selecting plants for heat-stress resistance.
* It has been suggested that one strategy is to find selection criteria in the early phases of plant growth that may be associated to heat tolerance in the reproductive stages.
* **Traditional breeding protocols to develop heat-tolerant crop plants**
* Identification of genetic resources with heat tolerance attributes.
* When screening different genotypes (in particular wild accessions) for growth under high temperatures, distinction must be made between heat tolerance and growth potential. Often plants with higher growth potential perform better regardless of the growing conditions.
* When breeding for stress tolerance, often it is necessary that the derived lines/cultivars be able to perform well under both stress and non-stress conditions.
* **Non-conventional Approaches**
1. Heat-Shock Proteins (HSPs)
2. Genetic Engineering and Transgenic Approaches
3. Omics Approach
4. **Heat-Shock Proteins (HSPs): (Master players for heat stress tolerance)**
* In general, Heat stress is responsible for the up-regulation of heat inducible genes, commonly referred as “heat shock genes” (HSGs) which encode HSPs.
* The majority of these proteins are expressed at high temperatures, which works as chaperones to prevent denaturation of intracellular proteins and maintain their stability and function.
* HSPs is only expressed during specific plant developmental phases, such as seed germination, embryogenesis, microsporogenesis, and fruit maturity.
* A group of proteins which are induced during plant under heat stress are called heat-shock proteins (**HSPs**) or stress induced proteins.
* **Classification of Heat Shock Protein (HSP)**

There are five major classes of HSP:

* HSP100 (100-114 kDa)
* HSP90 (80-94 kDa)
* HSP70 (69-71 kDa)
* HSP60 (57-60 kDa)
* Small heat-shock proteins (15-30 kDa)
* **Major classes of heat shock protein and its function**
1. HSP100: ATP-dependent dissociation and degradation of aggregate protein.
2. HSP90: Co-regulator of heat stress linked signal transduction complexes and manages protein folding. It requires ATP for its function.
3. HSP70: Primary stabilization of newly formed proteins, ATP-dependent binding and release
4. HSP60: ATP-dependent specialized folding machinery.
5. Small HSP (sHSP) :- Formation of high molecular weight oligomeric complexes which serve as cellular matrix for stabilization of unfolded proteins. HSP100, HSP70 and HSP40 are needed for its release.
6. **Transgenic Approach:**

 This approach includes following procedure for develop transgenic plant.

* Selection of gene of interest and its cloning
* Agrobacterium mediated gene transformation
* Molecular analysis of transgenic plant
* Physiological performance of the plants in greenhouse and chamber condition
* Molecular responses in transgenic plants
* Field trial

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| **Transgenic plants** | **Transgenes** | **Function of transgenes** | **References** |
| *Z. mays* L. | Hsp100, Hsp101 from *A. thaliana* L. | HSP synthesis for HT tolerance  | Queitsch *et al.* (2000) |

**Table 1.** Transgenic plants, heat stress linked transgenes and their responsible role for enhancing plants towards stress tolerance.

1. **Omics Approaches:**

Omics technologies have provided new opportunities and hopes for the identification of transcriptional, translational and post-translational mechanisms that regulate the plant response to high temperature stress.

DNA is the starting point of all molecular evidences related to heat stress tolerance in plants and contains several heat stress responsive genes in their genome (genomics). A large number of genes with potential roles in heat stress responses have been identified using genetic screens and genome wide expression studies. Transcriptory products (mRNAs), from such genes in the genome, have made their transcriptome (transcriptomics) and then proteome (proteomics) when they translate into the functional proteins (responsible for stress tolerance). Micro RNA plays an important functional role and several heat stress responsive miRNAs have been identified in plants. miRNAs may result in accumulation of their target gene mRNAs, which may positively regulate the heat stress tolerance. Moreover, proteomes are interlinked in various biochemical processes and will produce several metabolic products (metabolome).

1. **PLANT MORPHOLOGY AND PHYSIOLOGY UNDER HEAT STRESS**

One of the principal consequences of heat stress is oxidative stress caused by the generation of excess reactive oxygen species (ROS). Because high temperatures inhibit plant growth and development, so plant need to continuously struggle for survival (Hasanuzzaman *et al*., 2013). During heat stress condition plants do physical changes and creating signals for alter the different metabolism to cope high temperature. Heat stress responsible for loss of cell water content due to that cell size and growth is decreases (Rodriguez *et al*., 2005).

In maize and millet relative growth rate (PGR) reduced due to reduction in net assimilation rate (NAR) under heat stress (Wahid *et al*., 2007). Heat stress causes shorter life cycle of crop. Temperature > 1-2 ℃ than the normal temperature leads to reduction in grain filling duration and negatively affect yield and yield attributing traits (Zhang *et al*. 2006). In plants reproductive stage is more susceptible to heat stress as compare to vegetative stages and few degrees increases in temperature at the time of flowering causes entire loss of grain cycle (Lobell *et al*., 2011). In Heat stress conditions plant species showed significant variation in reduction in floral bud and flower abortion (Demirevskya-kepova et al. 2005).

In stressful environmental condition, genetic improvement can be achieved by selection of primary traits such as yield and secondary trait related to improved yield potential. Secondary traits more important for genetic improvement for maize population under abiotic stress condition (Betran *et al*., 2003).

**Anthesis – silking interval (ASI)**

Chapman *et al*., (1997) reported that in drought environments most of high yielding plants had short ASI and higher ear per plant (EPP). Cicchino *et al*., 2010a reported high difference between anthesis and tasseling in maize leads to responsible for longer anthesis silking interval under high temperature condition.

**Tassel blast**

Hussain *et al*., 2006 was found that tassel blast was negatively and highly significantly correlated with grain yield and positive significant association between leaf firing in maize.

**Leaf firing**

Under high temperature stress condition leaf firing reduces photosynthetic apparatus (Chen *et al*., 2010). Bai (2003) observed significant reduction in yield per plant with increase in percent leaf firing and days to flowering and reduction in chlorophyll fluorescence and number of tassel branches in high temperature stress.

**Silk receptivity (%)**

In maize kernel number per cob was control by number of pollen available at time of silking. Maize kernel set determine by silk elongation pattern and duration of silk receptivity. Silk elongation and senescence variation lead to determine grain yield (Anderson *et al*., 2004).

**Leaf senescence (%)**

Senescence is a limiting factor for grain filling and grain yields under high temperature stress Lobell *et al*., (2012). Leaf dead score did not significantly correlated with grain yield but it was highly correlated with LAI which indicating the importance of green area which is related to chlorophyll content and responsible for photosynthesis and help in maintains of high grain yield under stress condition Kamara *et al*., (2003). Delayed senescence means stay green nature of plant is important character which shows relatively high leaf chlorophyll during late grain filling in stress (Zaidi *et al*., 2004).

**Crop maturity days**

 In most of the cases, grain filling duration time between heading date to physiological maturity has no significant association with grain yield. Talbert *et al*., 2001 reported that in the water deficient condition maturity days were associated with increases yield in cereals.

**Chlorophyll content**

Betran *et al*., 2003 reported that grain yield was significantly correlated with chlorophyll content and Ear Per Plant under severe stress condition.

**Plant height**

Decrease in the rate of growth of first internode of plan under the high temperature stress condition which initial step of plant height development in maize and it determine plant height in maturity (Weaich *et al*.,1996).

**Number of kernel per ear**

In maize, kernel number loss due to kernel abortion, pollen viability and pollination dynamics which limit the yield of the crop under heat stress condition (Cicchino et al. 2010b).

**Grain yield**

Grain filling as one of most sensitive stage in maize under heat stress condition (Thompson 1986). Inbred lines of maize, reduced grain yield up to 70% in high temperature condition (Khodarahmpour *et al*., 2011).

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