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

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**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 drawn global attention due to its detrimental effects on crop development and productivity. In maize, high temperature stress during the reproductive stage causes poor seed germination and cellular alterations that increase the amount of reactive oxygen species (ROS), which damages lipids, proteins, and nucleic acids, ultimately causing cell death. Different strategies, such as adaptation, avoidance and acclimatization, are available for plants to use in response to heat stress conditions. Heat stress tolerance is a complicated quantitative feature and for effective breeding programme mode of inheritance of the traits related with heat stress is very much important. 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 (HSPs), 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. Maize is known as the "queen of cereals" due to its superior yield potential compared to other 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.

In terms of nutrition, both human and animals is greatly influenced by maize. The nature of maize demand is also evolving. Although maize is a significant food crop, the demand for maize as animal feed has grown drastically over the past 10 years. It is used as a raw material in a number of different sectors to make products including starch, ethanol, acetic acid, glucose, synthetic rubber, colours, and resin. There are several maize byproducts, including fructose syrup, maltodextrin, germ oil, fibre, and gluten products, that are used in the textile, paper, pharmaceutical, organic chemical, cosmetics, and edible oil sectors.

Food production for the expanding human population is seriously threatened by a variety of environmental problems. Temperature changes are one of the several abiotic stresses that often affect plant growth as well as development. During the plant's reproductive stage high temperature stress is major threat since it influencing crop production and productivity globally. Heat stress during the spring maize crop's reproductive stage causes an extended anthesis silking interval and poor seed germination. For maize plants, optimal temperature 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. By 2100, temperatures are expected to be 1.8 – 4.0 °C higher than present temperature due to a projected increase in the global air temperature is 0.2 °C per decade. (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 potential of maize germplasm to withstand several biotic and abiotic challenges will steadily enhance production and ensure sufficient food supply for the increasing world population. Therefore, understanding the genetic basis of heat stress tolerance is crucial for the creation of tolerant synthetics and hybrids for sustainable agriculture. New strategies are being developed to modify the expression of functionally related genes by modifying abiotic stress signalling pathways that control the expression of several genes that may improve stress tolerance.

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

 Heat stress, also known as high temperature stress refers to the negative effects on plants caused by temperature higher than the ideal temperature. Heat stress would affect (1) survival, (2) growth and development and (3) physiological processes.

Effect of heat stress on plants vary with the nature of crop, degree and duration of temperature. Due to high temperature, cellular damage or cell death may occur within minutes, that results in the breakdown of cellular organisation (Ahuja *et al*., 2010). High temperature affects all the important stages in plant like germination, growth, development, reproduction, yield, etc. Additionally, it influences the efficacy of enzymatic processes as well as the stability of numerous proteins, RNA, and cytoskeleton structures. (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:** Photosynthesis is one of the physiological processes in plants that is most heat-sensitive. High temperature causes reduce amount of photosynthetic pigment. Closure of stomata lead to reduced CO2 uptake. High temperature affects function of chlorophyll by altering the structural organization of thylakoids.
* **Reduction in plant growth:** High temperatures decreases cell’s water content which eventually leads to decrease in cell size and cell growth. Additionally, a decline in the net assimilation rate (NAR) also results in a lower relative growth rate (RGR).
* **Improper development:** Reproductive tissues are more sensitive. High temperature causes pollen and spikelet sterility and reduced pollen viability.
* **Yield reduction:** Even a little (1.5 °C) increase in temperature has a big detrimental impact on crop production since high temperatures are so unpleasant. The grain production of cereals declined by 4.1% to 10.0 % with a 1 °C rise in the seasonal average temperature. The major cause of reduced production during heat stress is a fall in assimilatory capacity, which is brought on by a decrease in photosynthesis caused by altered membrane stability, an increase in maintenance respiration costs, and a fall in radiation usage 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:** Under heat stress, plant water relations are more negatively impacted. Seedlings are affected by high temperatures because they require more evapotranspiration and suffering more tissue damage.
* **Inhibition of seed germination:** Temperature is one of the fundamental conditions for seed germination, hence it has a significant impact on this process. In maize crop optimum range of temperatures for seed germination is 25-30 °C.
* **Oxidative stress:** The production of ROS (reactive oxygen species), such as O2, H2O2, and other compounds that cause oxidative stress, is accelerated by temperature stress. which could severely damage a cell's structure.
1. **PLANT ADAPTATION TO HEAT STRESS**

When it comes to how plants respond to and tolerate extreme temperatures, plant species vary greatly. Plants may be divided into three categories based on their ideal growth temperature. (Figure 1). There are (a) Psychrophiles, (b) Mesophyles and (c) Thermophyles. (Zrobek-Sokolnik *et al.,* 2012; Larcher *et al*., 1995)

1. Psychrophiles : Psychrophiles are plants that can survive low temperatures between 0 and 10 °C
2. Mesophyles : Mesophyles are plants that survive temperatures between 10 and 30 °C.
3. Thermophyles : Plants, which prefer high temperature ranges of 30 °C to 65 °C or even more for optimal growth are considered as thermophyles.

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 based on heat tolerance.

Plant uses different adaptation mechanisms for its survival in hot, dry environments to high temperature (Fitter *et al.,* 2002). Avoidance and tolerance mechanisms are part of the plant's adaptation to high temperatures. (Figure 2).

* **Avoidance Mechanisms**
* During heat stress conditions, early maturation is directly correlated with lower yield losses.
* Presence of small hairs (tomentose), cuticles, waxy covering, etc. helps in avoidance of heat stress by decreasing the absorption of solar radiation
* Additionally, plants with tiny leaves are more capable to resist heat stress.
* In these plants, leaf blades frequently arrange themselves parallel to the sun's rays and away from the light (paraheliotropism).
* Intensive transpiration keeps the leaf temperature below the surrounding temperature and protects leaves from heat stress.

**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.** Various adaptive mechanisms of plants to high temperature stress condition.

* **Tolerance Mechanisms**
* High temperature tolerance is the ability of plant to survive, grow and produce significant yield under heat stress condition.
* Major techniques for regulating and balancing the heat stress situation in crops include ion transporters, late embryogenesis abundant proteins (LEA), osmoprotectants, and antioxidant defense.
* In situations of extreme heat stress, short-term responses including leaf orientation, transpirational cooling, and alterations in membrane lipid composition are crucial to survival.
1. **MECHANISM OF SIGNAL TRANSDUCTION & DEVELOPMENT OF HEAT TOLERANCE**

Numerous genes have been discovered to be upregulated to aid the plant's resistance to stress, which results in 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. Depending on the kind of plant and the type of stress, different signal transduction molecules are involved in the activation of genes that respond to stress. These include NO, sucrose (as a signalling molecule), Ca-dependent protein kinases (CDPKs), mitogen-activated protein kinases (MAPK/MPKs), and phytohormones (Ahmad *et al.,* 2012). These molecules, together with transcriptional factors, activate genes that respond to stress.

**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**

**Figure 3.** Diagrammatic illustration of heat-induced signal transduction pathway and development of heat tolerance in plants.

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

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

Under high temperature stress conditions, Akbar *et al*., (2008) revealed that non-additive gene action is responsible for yield and yield-contributing traits in maize. Dominance gene action has been found to be predominant for traits such as seed vigour, viability, germination percentage, relative water content, plant growth rate, anthesis silking interval, silk receptivity, pollen size, rolling of leaves, number of ears per plant, shelling percentage, leaf senescence, plant maturity days, and economic yield. (Tassawar *et al*., 2012). The non-additive genes effect regulates the percentage of cell membrane thermostability, stomata conductance, transpiration rate, turgor potential, growing degree days to 50% tasseling, and grain yield per plant, while the additive genes effect governs growing degree days to 50% maturity at high and normal temperature conditions (Akbar *et al*., 2009). In high temperature stress conditions, traits had dominant or non-additive gene action that may be improved by hybrid breeding method to increase heat stress resistance in maize.  Populations improvement programs can also increase the frequency of genes that make individuals more resistant to heat in populations of maize.

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. Plants have a variety of physiological mechanisms for survival, growth, and development, including photosynthesis, assimilate partitioning, water use efficiency, nutrient use efficiency, and membrane stability. Under conditions of heat stress, heat shock proteins were used to control and improve these phenomena (Camejo *et al*., 2005; Ahn and Zimmerman 2006; Momcilovic and Ristic 2007). Genetic engineering and molecular breeding are being used in addition to stress tolerance breeding to increase crop yields under heat stress conditions.

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 population in a hot target environment and identifying genotypes 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.
* **Conventional breeding protocols to develop high temperature tolerant plants**
* Identifying genetic material with traits associated to heat tolerance
* It is important to distinguish between heat tolerance and growth potential when testing various genotypes, especially wild accessions, for growth under high temperatures. Often, irrespective of the growing environment the performance of plants with higher growth potential performs better.
* When breeding for stress tolerance, it is often crucial for the developed cultivars to perform well in both stressful and non-stressful environments.
* **Non-conventional approaches**
1. Heat-Shock Proteins (HSPs)
2. Genetic Engineering and Transgenic Approaches
3. Omics Approach
4. **Heat Shock Proteins (HSPs)**
* Heat inducible genes, often known as "heat shock genes (HSGs)", which encode "heat shock protein (HSPs)" which are generally stimulated in response to heat stress.
* Heat shock proteins are also called as master player for heat stress tolerance.
* The majority of these proteins are expressed at high temperatures, which works as chaperones to inhibit the degradation of intracellular proteins and maintain their function and stability.
* HSPs is only expressed during specific plant developmental phases, such as microsporogenesis, embryogenesis, seed germination and fruit maturity.
* Heat-shock proteins (HSPs), also known as stress-induced proteins, are a class of proteins that are induced when a plant experiences heat stress.
* Heat tolerance role of heat shock proteins was first assumed by Vierling in 1991
* **Classification of Heat Shock Protein (HSP)**

Based on their molecular weight, heat shock proteins are divided into five main groups:

* HSP100 (100-114 kDa)
* HSP90 (80-94 kDa)
* HSP70 (69-71 kDa)
* HSP60 (57-60 kDa)
* Small heat-shock proteins (15-30 kDa)

**Table 1.** Major heat shock proteins (HSPs) and their response during heat stress

|  |  |  |
| --- | --- | --- |
| **Heat Shock Proteins** | **Found in crop** | **Response / Functions** |
| HSP100 | Wheat, Rice, Tomato | It facilitates in reactivating proteins that have been heat-damaged. |
| HSP90 | Maize, Wheat, Tomato | It controls protein folding and functions as a co-regulator of heat stress-related signal transduction complexes. (ATP is needed). It Coupled with HSP70 functions as a multi-chaperone machine. |
| HSP70 | Soybean, Tomato, Wheat | Primary stabilization of newly synthesized proteins, ATP-dependent binding and release |
| HSP60 | Wheat, Rye, Barley, Maize | It promotes folding and aggregation of many proteins. HSP60 having specialized folding machinery (ATP-dependent). |
| Small HSP (sHSP) | Barley, Maize, Rice, Tomato, Sorghum, Pearl millet, Wheat,  | In order to prevent irreversible unfolding or improper protein aggregation, it binds to partly folded or denatured substrate proteins. |

1. **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

|  |  |  |  |
| --- | --- | --- | --- |
| **Transgenic plant** | **Transgenes** | **Function of transgenes** | **Reference** |
| *Zea mays* L. | Hsp100, Hsp101 from *Ar*abidopsis*. thaliana* L. | Synthesis of heat shock protein for high temperature tolerance  | Queitsch *et al.* (2000) |

**Table 2.** Transgenic plant, transgenes (linked for heat stress) and their role for enhancing plants towards heat stress tolerance.

1. **Omics Approaches:**

The identification of transcriptional, translational, and post-translational pathways which regulate the plant response to heat stress has become more feasible and anticipated due to the development of omics technology.

All molecular evidence for plant’s ability to withstand heat stress begins with DNA, which also contains several genes that are sensitive to high temperature stress (genomics). Several genes with possible roles in heat stress responses have been found through genetic screening and genome-wide expression studies. (Yeh *et al*., 2012). 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) (Hasanuzzaman *et al*., 2013). In plants, numerous high temperature sensitive miRNAs have been discovered and it plays a significant functional role in high temperature stress tolerance. miRNAs accumulate their target gene mRNAs, that could help to improve the tolerance to high temperatures. Moreover, proteomes participate in a number of biochemical activities and generate a number of metabolic products. (metabolome).

1. **EFFECTS OF HEAT STRESS ON THE MORPHOLOGY AND PHYSIOLOGY OF PLANTS**

Oxidative stress, which is caused by an excess of reactive oxygen species (ROS), is one of the major consequences of heat stress. Plants must constantly fight for existence because high temperatures prevent plant growth and development (Hasanuzzaman *et al*., 2013). Plants undergo physical changes and produce signals to adjust their various metabolic processes in response to high temperature stress. Due to high temperature, cells lose water content, which reduces cell size and growth (Rodriguez *et al*., 2005).

During heat stress, the relative growth rate (PGR) of maize and millet is decreased as a result of a decrease in the net assimilation rate (NAR) (Wahid *et al*., 2007). Crop life cycles are shortened by heat stress. Temperatures > 1-2°C higher or lower than normal, result in shorter grain filling times and have a detrimental impact on yield and yield contributing characteristics (Zhang *et al*., 2006). Compared to the vegetative stage, the reproductive stage of plants is more susceptible to heat stress, and small temperature rises around the time of blooming result in the complete loss of the grain cycle (Lobell *et al*., 2011). The reduction in floral bud and flower abortion during high temperature stress conditions varied significantly among plant species. (Demirevskya-kepova *et al*., 2005).

By choosing primary characters like yield and secondary characters related with improved yield potential, genetic improvement can be achieved under severe environmental conditions. Under abiotic stress conditions, secondary characteristics are more crucial for genetic improvement in the maize population (Betran *et al*., 2003).

**Anthesis silking interval**

According to Chapman *et al*., (1997), most high yielding plants have short anthesis silking interval (ASI) and more ears per plant (EPP) in drought-prone conditions. According to Cicchino *et al*., (2010a), a large difference between tasseling and anthesis in maize causes a prolonged anthesis silking interval in conditions of high temperature.

**Tassel blast**

Hussain *et al*., (2006) was reported that tassel blast in maize had a positive significant connection with leaf burning and was negatively and highly significantly associated with grain yield.

**Leaf firing**

Leaf firing decreases the photosynthetic apparatus under heat stress conditions (Chen *et al*., 2010). During heat stress, Bai (2003) found a substantial decrease in yield per plant with an increase in percent leaf firing, days to flowering, a decrease in chlorophyll fluorescence, and a decrease in the number of tassel branches.

**Silk receptivity (%)**

The quantity of pollen present at the time of silking in maize regulate the number of kernels per cob. In Maize, kernel set determine by The pattern of silk elongation and a duration of silk receptivity. Grain yield is determined by variations in senescence and silk elongation (Anderson *et al*., 2004).

**Leaf senescence (%)**

Senescence is a phenomenon that restricts grain filling and yields under high temperature stress Lobell *et al*., (2012). The relationship between leaf dead score and grain yield was not significant, but it was strongly correlated with leaf area index (LAI), demonstrating the significance of green area, which is linked to chlorophyll content and is necessary for photosynthesis and helps maintain high grain yield under stressful conditions (Kamara *et al*., 2003). Delayed senescence refers to a plant's ability to remain green under stressful conditions during late grain filling (Zaidi *et al*., 2004).

**Crop maturity days**

 Most of the time, there is no significant correlation between grain yield and the length of grain filling between the heading date and physiological maturity. According to Talbert *et al*., (2001), maturity days were linked to higher yields of grains when there was a water shortage.

**Chlorophyll content**

According to Betran *et al*., 2003 grain production was significantly associated with chlorophyll content and ear per plant during extreme stress conditions.

**Plant height**

In maize during high temperature stress conditions, growth rate of first internode of plant is decrease which determine plant height at the time of maturity (Weaich *et al*.,1996).

**Number of kernels per ear**

The production of the crop under heat stress conditions is hampered in maize by kernel number loss that is caused by abortion, pollen viability, and pollination dynamics (Cicchino *et al*., 2010b).

**Grain yield**

During high temperature stress condition, grain filling is one of the most vulnerable stages in maize (Thompson 1986). According to Khodarahmpour *et al*., (2011), inbred lines of maize reduce the grain production by up to 70% when temperatures are high.

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