



HEAT STRESS: PLANT RESPONSES AND MITIGATION STRATEGIES

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ABSTRACT

Rising temperatures across the globe poses an urgent challenge for the food security. “Business as usual” crop development will not be sufficient to handle this situation. Plants respond at morphological, physiological and biochemical level to the heat stress. However, the impact of HT depends on the duration, intensity and developmental stage of the crop. It is very important to understand the factors involved in HT stress for better management. To acclimatise themselves to the elevated temperature, plants upregulate the synthesis of heat shock proteins. This review provides the response of plants to heat stress, molecular mechanism of acclimation to HT stress and the mitigation strategies of Heat stress. The feasible option to mitigate the HT stress is the development of thermotolerance in plants. Genetic approaches of identifying QTL’s conferring thermotolerance will smooth the progress of breeding for heat tolerance and focuses on the illustration of various factors which could be of practical use to develop HT tolerant transgenics.

KEY WORDS: Heat Stress, Acclimation, Themotolerance.

INTRODUCTION

Heat condition is the climatic variable of greatest weight in agricultural zoning for the various cultivated species. By the end of 21st century, it is conjectured that global climate will witness the increase of 2–4°C temperature and by the mid 21st century it would rise by 1-3°C (IPCC 2012). Average global combined temperature of land and ocean surface has increased by 0.85°C between 1880 and 2012 (IPCC 2014). Developing countries population would be seriously affected and it is predicted that the worldwide food production needs to be increased by 70% in order to feed the ever-growing population (Bita and Gerats, 2013). Therefore there is an urgent need to incorporate the heat tolerance in the cultivated crops to combat against climate change. Plants being static poikilotherms are much exposed to extremities of climate such as high temperatures or drought, which often occur simultaneously. These stresses usually lead to a disruption of cellular homeostasis and in acute conditions may even lead to death (Mittler, 2006). Heat stress may be defined as the increase in temperature above the critical value for a period enough to cause an irreversible damage to the growth, development and physiological processes of the plant. Excessive heat may lead to a catastrophic decrease in crop production and may lead to a widespread famine (Bita and Gerats, 2013). Protein denaturation and aggregation and increased fluidity of membrane lipids are the immediate effects of high temperatures while the secondary effects include inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, protein degradation and loss of membrane integrity (Howarth *et al.*, 2005). However, effect of heat stress is influenced by the intensity, duration, temperature

increase rate and the sensitive stage of the plant. The heat threshold level value varies with the different stages of development (Hemantaranjan *et al.*, 2014). Excessive heat, in addition to inducing quantitative and qualitative losses in crop production, shortens the duration of the cycle, reduces flower fertility percentage, accelerates the swelling period, decreases the mean grain weight, and increases fruit fall. In foods consumed fresh, for example fruits and vegetables, heat stress causes morphological defects that in the eyes of the consumer, mean quality loss (M. A. de Souza *et al.*, 2012). To surmount the effects of heat stress, plants show adjustments at the physiological and biochemical level. The process of developing thermo tolerant crop varieties is always of the foremost preference. For this reason, a meticulous understanding of physiological responses of plants to high temperature, mechanisms of heat tolerance and possible strategies for improving crop thermotolerance is crucial (Hemantaranjan *et al.*, 2014). Conventional breeding approaches have contributed meagrely to the development of thermotolerant varieties. There is the need to pick up the pace for developing the heat tolerant varieties by the molecular approaches. This review focuses on the basic plant responses to heat stress, morphological, physiological and biochemical responses to heat stress as well as on the breeding approaches both conventional and molecular for developing thermotolerant varieties.

How do plants respond to heat stress?

Elevated air temperature leads to faster plant development and shorter crop duration and consequently a reduction in cumulative light perception and assimilation over the plant’s life cycle (Driedonks *et al.*, 2016). The primary effects of heat stress is depicted in the figure 1. Besides

this, disturbance of fundamental processes such as carbon assimilation, respiration and transpiration may reduce overall metabolic efficiency and result in vegetative developmental defects such as fewer, malformed and/or smaller organs (Takeoka *et al.* 1991, Maestri *et al.* 2002, Stone 2001). Negative impact on sexual reproduction and consequently fruit and seed yield has been observed (Peet *et al.* 1997, Erickson and Markhart 2002, Zinn *et al.* 2010). On roots high soil temperature leads to heat

necrosis as well as reduced germination capability and plant emergence (Stevenson *et al.* 2001). Warm-season annuals usually cope better with high temperatures than cool-season annuals (Driedonks *et al.* 2016). The effects of HT stress are more pronounced on reproductive development than on vegetative growth and thereby leading to the catastrophic loss of yield (Young *et al.*, 2004, Zinn *et al.*, 2010). The loss in crop yield of major crops in some regions is showed in table 1.

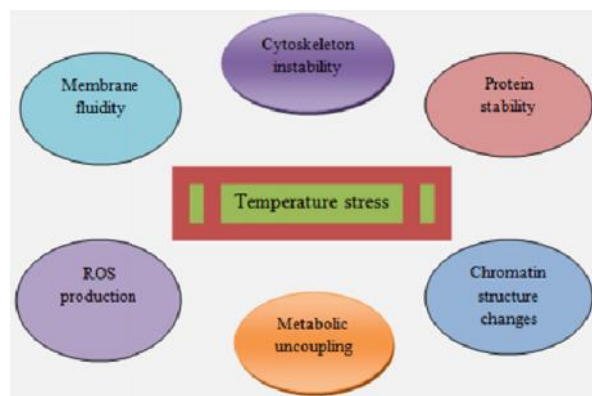


FIGURE 1: Primary effects of temperature stress on plants

Plants respond to heat stress by promptly activating unfolded protein response (UPR) mechanisms which in turn induce several processes ranging from the recruitment of heat shock proteins (HSPs) to the induction of

antioxidant defense systems and enzymes such as catalases and superoxide dismutases and the synthesis of osmoprotectants like proline and sugar alcohols (Liu and Huang, 2000, Moreno and Orellana, 2011).

TABLE 1: Loss of crop yield due to heat stress

Crop	Loss in yield due to heat	Region	References
Rice	4-14%	South east Asia	Lobell 2007
wheat	30%	Russia`	USDA report 2010
wheat	10%	China	You <i>et al.</i> 2009
Wheat	5.5%	Globally	Lobell <i>et al.</i> 2011
Maize	3.8%	Globally	Lobell <i>et al.</i> 2011
Maize	1.7%	Africa	Lobell <i>et al.</i> 2011

Effect of Heat stress on plant morphology and physiology

Heat stress has an independent mode of action on the morphology, physiology and metabolism of plant cells (Bita and Gerats, 2013). High temperatures adversely affect the germination percentage and thereby reduce the crop stand. Temperature above 40°C restricts the seedling emergence in rice (Akman, 2009). In wheat, seed germination and seedling emergence are adversely affected (Tewolde *et al.*, 2006). Visual symptoms of heat stress include sunburn, leaf senescence, scorching of twigs, growth inhibition and discoloration of leaves and fruits (Fahad *et al.*, 2017). Elevated air temperatures are generally involved in regulation of leaf appearance rates and leaf elongation rates along with decreasing leaf-elongation duration (Bos *et al.*, 2000). In addition, there is a significant increase in number of leaves, particularly during the arrested reproductive development stage and without any decrease in leaf photosynthetic rate (Porter and Gawith, 1999, Iqbal *et al.*, 2017). Heat stress causes the limitation to plant growth by affecting the shoot net assimilation rates and thereby the dry weight of the plant (Wahid *et al.*, 2007). Flower development and pollen

viability are impaired in some crop species. The difference in the flower development and pollen viability of HT stress sensitive and tolerant genotypes of tomato are depicted in the figure 2. On roots the effects of HT ranges from decrease in number of roots, root length and root diameter (Iqbal *et al.*, 2017). Heat stress induces changes in the physiological processes such as respiration and photosynthesis which eventually lead to the reduction in the plant productivity. C₃ plants are more susceptible to high temperature stress than C₄ plants. High temperature causes injury to chloroplast by altering structural organization of thylakoid, swelling of grana stacks and impairing grana stackability (Fahad *et al.*, 2017). These structural changes are accompanied by ion-leakage from leaf cells exposed to heat (Wahid and Shabbir, 2005). Increased fluidity of thylakoid at high temperatures leads to dislodging of photosystem II (PSII) light harvesting complexes from thylakoid membrane (Iqbal *et al.*, 2017). Production of the reactive oxygen species (ROS's) are known to be associated in the detrimental effect of heat on chlorophyll and photosynthetic apparatus (Camejo *et al.*, 2006, Guo *et al.*, 2007). Chloroplast, mitochondria and peroxisomes are the main sites of ROS synthetic pathway.

Hence they exhibit intense rate of electron flow in cells. These organelle membranes constitute PUFA and there is heavy oxidising reaction and lipid peroxidation takes place. As a result plant cells face oxidation stress under heat stress oxidations. In addition oxidative stress also damages protein by aminoacid modifications, peptide chain breakages, changes in the electrical potential of the cells and makes the cell vulnerable to proteolysis. Therefore ROS has the ability to damage cellular macromolecules. Under severe circumstances death of plant may occur. Reduction in the photosynthetic activity due to these structural changes creates a situation of plant starvation. Rubisco is the first to respond to HT stress due to the presence of the two substrates, Carbon dioxide and oxygen. Rubisco inactivation occurs at faster rates at elevated temperatures (Iqbal *et al.*, 2017).

Changes in the plant growth regulators due to the HT stress also affect the plants. For example, Studies on maize have suggested that heat stress disrupts cellular and nuclear integrity, particularly in the cells present in the

periphery of the endosperm due to mediation of cytokinins (Jones and Settler, 2000). Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation in wheat (Iqbal M R Khan *et al.*, 2013).

HT modifies the carbon metabolism enzymes, starch accumulation and sucrose synthesis by down regulating specific genes in carbohydrate metabolism (Fahad *et al.*, 2017, Ruan *et al.*, 2010). Heat stress also affects cell cycle and cell division through changing the microtubules organization, elongation of phragmoplast microtubules, and formation of microtubule asters in mitotic cells (Smertenko *et al.*, 1997). All these injuries together ultimately cause starvation, growth inhibition, decreased ion flux, accumulation of toxic compounds and reactive oxygen species (ROS) (Howarth *et al.*, 2005). ROS interferes in the cell functioning by damaging the lipids and proteins.

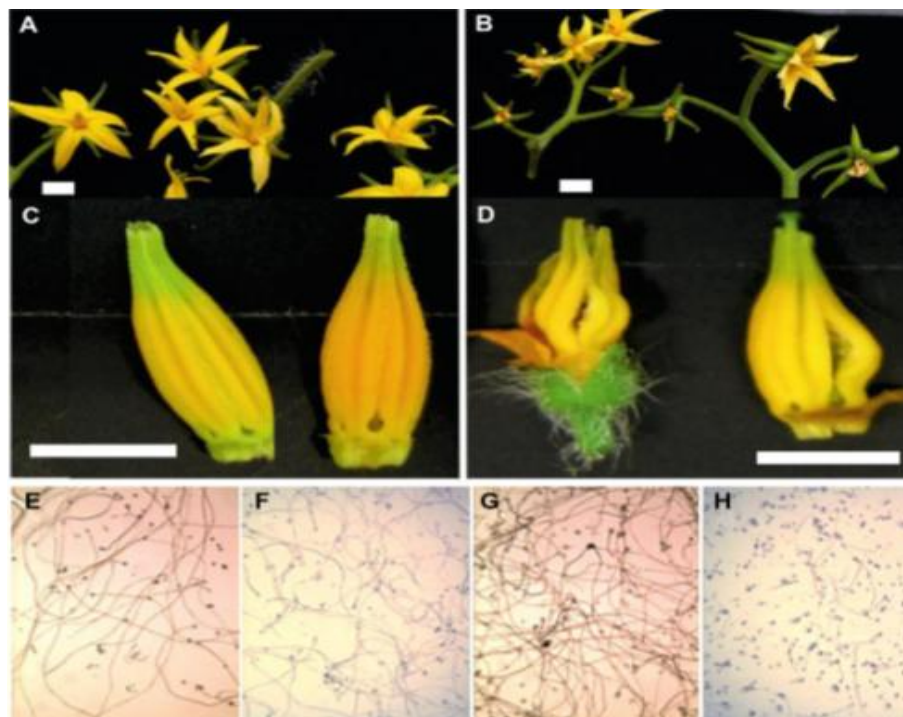


FIGURE 2: Flower development and pollen viability of tolerant (left) and sensitive tomato genotypes (right). (Source: Bitra and Gerats 2013) The top shows inflorescences of a heat-tolerant genotype on the left (A) and a sensitive genotype on the right (B). In the second panel, the morphology of the anther cones is shown for both genotypes (C) and (D). Below (E–H) in vitro pollen germination under standard conditions (E, heat-tolerant genotype, G, heat-sensitive) and high temperature for both genotypes (F, heat-tolerant genotype, H, heat-sensitive).

Effect of HT Stress on Yield:

Rise in the global temperature presents an alarming threat to the stable crop yields. The annual temperature might increase by 2.5°C to 4.3°C in important crop-growing regions of the world by 2080 to 2099, according to the Intergovernmental Panel on Climate Change (IPCC) A1B scenario (Christensen *et al.*, 2007). Reduction in the fertility is the problem associated with the heat stress when crop plants are subjected to heat during meiosis and fertilization *e.g.* tomato, rice, cowpea, barley (Giorno *et al.*, 2013, Jagadish *et al.*, 2014, Ehlers and Hall, 1998,

Sakata and Higashitani, 2008). In wheat, the reduction in seed set has been noticed when the plants were exposed to high-temperature stress (>30 °C) from early meiosis to pollen maturity (Saini & Aspinall, 1982). This reduction in seed set was due to the lack of pollen viability. The number of grains per spike was reduced under temperatures 35°C in cv. Bohemia and 38°C in cv. Tobak which resulted in a similar response of spike productivity (Hlavacova *et al.*, 2017). The exposure of spring wheat during mid anthesis period to the elevated temperature decreased the grain number per ear at

maturity in spring wheat (Ferris *et al.*, 1998), indicating the heat sensitivity of fertilization and grain setting. The heat sensitivity of the pollen might be explained by its inability to synthesize all the HSPs (Mascarenhas & Crone, 1996). Embryo sac malformation has been reported in wheat at 30°C and rapeseed at 32°C when subjected to heat stress (Hedhly, 2011). Global wheat production was simulated to decline by 6% for each degree Celsius rise in temperature (Asseng *et al.*, 2015). In rice, stress due to the elevated temperature at flowering stage reduces spikelet fertility in rice (*Oryza sativa* L.). Sterility is caused by poor anther dehiscence (caused by the tight closure of the locules) and low pollen production, and hence low number of germinating pollen grains on the stigma (Matsui, Omasa

& Horie 2001, Matsui & Omasa 2002, Prasad *et al.* 2006, Barnabas *et al.* 2007). In maize, at temperatures higher than 38 °C the reduction in pollen germination ability and pollen tube elongation has been recorded which eventually led to the reduction in seed set (Dupuis & Dumas 1990, Stone 2001). A remarkable decrease in fruit set has been recorded in tomato in response to heat stress, especially when applied during microsporogenesis (Zhang and Yang, 2014). During this stage, a short period at 40 °C or extended exposure just a few degrees above optimal temperature (32 °C rather than 26 °C during the day) results in male sterility (Sato *et al.*, 2006, Giorno *et al.*, 2013).

TABLE 2. Effects of high temperature stress in different crop species (Source: Hasanuzzaman *et al.*, 2013).

Crops	Heat treatment	Growth stage	Major effects	References
Chili pepper (<i>Capsicum annuum</i>)	38/30 °C (day/night)	Reproductive, maturity and harvesting stage	Reduced fruit width and fruit weight, increased the proportion of abnormal seeds per fruit.	Pagamas and Nawata, 2008
Rice (<i>Oryza sativa</i>)	Above 33 °C, 10 days	Heading stage	Reduced the rates of pollen and spikelet fertility.	Rahaman <i>et al.</i> , 2009
Wheat (<i>Triticum aestivum</i>)	38 °C, 24 and 48 h	Seedling stage	Decreased chl and relative water content (RWC), diminished antioxidative capacity.	Zhang <i>et al.</i> , 2013
Wheat (<i>Triticum aestivum</i>)	32/24 °C (day/ night), 24 h	At the end of spikelet initiation stage	Spikelet sterility, reduced grain yield.	Zhang <i>et al.</i> , 2013
Wheat (<i>Triticum aestivum</i>)	37/28 °C (day/ night), 20 days	Grain filling and maturity stage	Shortened duration of grain filling and maturity, decreases in kernel weight and yield.	Rahaman <i>et al.</i> , 2009
Wheat (<i>Triticum aestivum</i>)	30/25 °C day/night	From 60 DAS to maturity stage	Reduced leaf size, shortened period for days to booting, heading, anthesis, and maturity, drastic reduction of number of grains/spike and smaller grain size and reduced yield.	Rahaman <i>et al.</i> , 2009
Rice (<i>Oryza sativa</i>)	32 °C (night temperature)	Reproductive stage	Decreased yield, increased spikelet sterility, decreased grain length, width and weight.	Mohammed and Tarpley, 2010
Maize (<i>Zea mays</i>)	35/27 °C (day/night), 14 days	Reproductive stage	Reduced ear expansion, particularly suppression of cob extensibility by impairing hemicellulose and cellulose synthesis through reduction of photosynthate supply.	Suwa <i>et al.</i> , 2010
Maize (<i>Zea mays</i>)	33–40 °C, 15 days	During Pre-anthesis and silking onwards	Severe effect on plant and ear growth rates.	Suwa <i>et al.</i> , 2010
Rice (<i>Oryza sativa</i>)	25–42.5 °C	Vegetative growth stage	Decrease in the CO ₂ assimilation rate.	Rahaman <i>et al.</i> , 2009
Soybean (<i>Glycine max</i>)	38/28 °C (day/night), 14 days	Flowering stage	Decreased the leaf Pn and stomatal conductance (<i>gs</i>), increased thicknesses of the palisade and spongy layers, damaged plasma membrane, chloroplast membrane, and thylakoid membranes, distorted mitochondrial membranes, cristae and matrix.	Djanaguiraman <i>et al.</i> , 2011
Tobacco (<i>Nicotiana tabacum</i>)	43 °C, 2 h	Early growth stage	Decrease in net photosynthetic rate (Pn), stomatal conductance as well as the apparent quantum yield (AQY) and carboxylation efficiency (CE) of photosynthesis. Reduced the activities of antioxidant enzymes.	Tan <i>et al.</i> , 2011
Okra (<i>Abelmoschus esculentus</i>)	32 and 34 °C	Throughout the growing period	Reduced yield, damages in pod quality parameters such as fibre content and break down of the Ca-pectate.	Gunawardhana and de silva, 2011

Mechanisms of heat stress and its associated characteristics

Mechanisms to mitigate heat stress are avoidance and tolerance.

Avoidance mechanisms

Avoidance mechanisms involve all those changes that are associated with the escape of plants from being exposed to heat stress. Long term changes and short term changes are

exhibited by the plants in the process of avoidance. These include changing leaf orientation, transpirational cooling, or alteration of membrane lipid compositions. The avoidance mechanisms in plants are depicted in the figure 3. Agronomical practices to avoid the heat stress include selection of proper sowing method, choice of sowing dates, method of irrigation and seed priming *etc.* (Hasanuzzaman *et al.*, 2013).

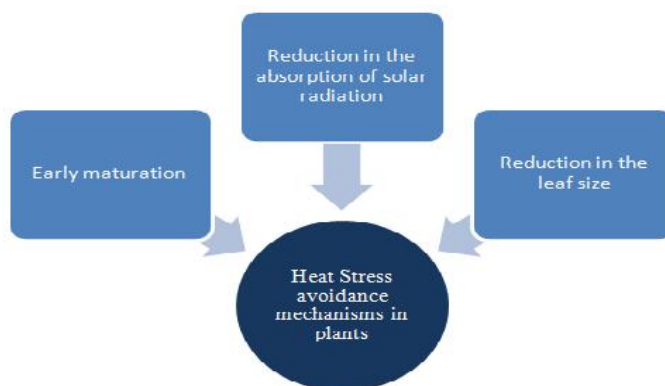


FIGURE 3: Heat stress avoidance mechanisms in plants

Acclimation is the process in which the plants adjust to the changed environmental conditions and maintain its performance in a wide range of environmental conditions. These molecular changes for heat stress acclimation are shown in the figure 4 containing the flow chart. Changes in the temperature are sensed by the sensors in the plants. When the plant receives the stimuli, generation of an integrated signalling cascade is the prior step that takes place. This signalling cascade is triggered by the receptors present in the plasma membrane of the cell (Vigh *et al.*, 1998, Sangwan *et al.*, 2002, Los and Murata, 2004.). The increased fluidity of the lipid membrane leads to the increased Ca^{2+} flux (Bita and Gerats, 2013). This activates

the mitogen activated protein (MAP) kinases followed by the activation of transcription factors related to the synthesis of Heat Shock Protein (HSP) genes. For example, the CNGC2 gene of Arabidopsis encodes a component of the membrane cyclic nucleotide gated Ca^{2+} channels that acts as the primary thermo-sensors of land plant cells. These channels in the plasma membrane respond to increments in the ambient temperature by triggering an optimal heat shock response (Saidi *et al.*, 2009, Bita and Gerats, 2013). Recent researches have revealed that in vegetative and reproductive tissues, there exists variation in the various signalling pathways of tissue specific activation (Mittler *et al.*, 2011).

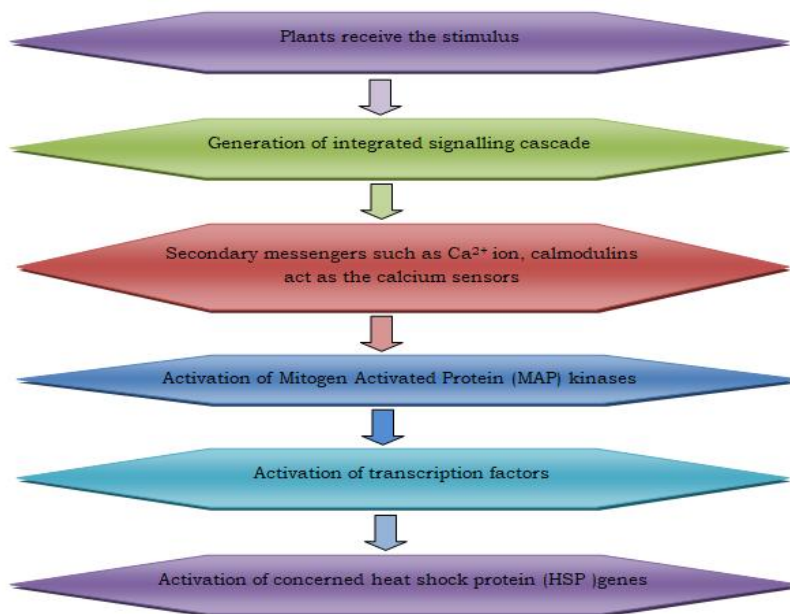


FIGURE 4 : Molecular mechanism involved in the acclimation of crop plants for heat stress

Tolerance mechanisms

Heat tolerance is the ability of the plant to survive and produce yield even under heat stress with the minimal loss. Tolerance mechanisms include ion transporters, osmoprotectants, free-radical scavengers, late embryogenesis abundant proteins and factors involving in the signalling cascade and transcriptional control. Figure 5 depicts the heat stress tolerance mechanisms in plants (source: Wahid *et al.*, 2007). The antioxidant mechanism is a part of heat stress adaptation and its strength is

correlated with the acquired thermotolerance (Maestri *et al.*, 2002). Additionally, physiological approach offers the benefit of maximizing the probability to harness the more relevant additive gene actions (Reynolds and Trethown, 2007). The exogenous application of protectants such as osmoprotectants, phytohormones, signalling molecules and trace elements have shown beneficial effects on plants grown under heat tolerance as these protectants has growth-promoting and antioxidant capacity (Hasanuz zaman *et al.*, 2011). The accumulation of osmolytes such

as proline, glycine betaine and trehalose is a well known adaptive mechanism in plants against abiotic stress conditions including heat tolerance (Asthir, 2015). Proline and glycine, betaine application considerably reduced the H_2O_2 production, improved the accumulation of soluble sugars, improved the K^+ and Ca^{2+} contents and protected the developing tissues from heat stress effects. Identically, exogenous applications of several phytohormones were found to be effective in mitigating heat stress in plants. However the concentration should not be lethal or toxic to

its growth seedling stage (Asthir, 2015). Tocopherol plays an important role in signal transduction pathways and in the gene expression regulation in different processes such as plant defense and export of photoassimilates (Falk & Munne-Bosch, 2010). Tocopherol plays an important role in regulating the membrane stability. The role of tocopherol in preventing LPO has been noticed in many reports. Lipid peroxyl radicals, which are involved in the propagation of LPO, are scavenged by tocopherol (Asthir, 2015).

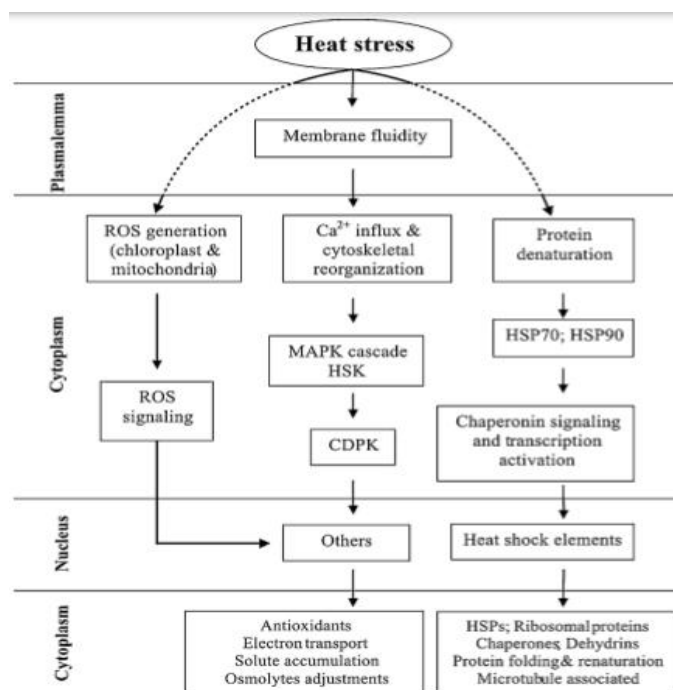


FIGURE 5: Proposed heat-stress tolerance mechanisms in plants. MAPK, mitogen activated protein kinases, ROS, reactive oxygen species, HAMK, heat shock activated MAPK, HSE, heat shock element, HSPs, heat shock proteins, CDPK, calcium dependent protein kinase, HSK, histidine kinase. Source: Wahid *et al.* 2007.

Heat Shock Proteins and their role:

For the plant to survive under heat stress, the upregulation of several heat inducible genes, commonly referred as “heat shock genes” (HSGs) which encode HSPs and these active products is the most important mechanism. Heat Shock Proteins are highly heterogeneous in nature. Synthesis of HSP’s seems to be the universal response to heat from bacteria to human (Veiriling, 1991). According to Wahid *et al.* (2007) HSP triggered thermotolerance is attributed to the observations that their induction coincides with the organism under stress, their biosynthesis is extremely fast and sensitive and they are induced in a wide variety of cells and organisms. On the basis of molecular weight, three classes of proteins account for most HSP, s, they are HSP70, HSP90 and low molecular weight proteins of 15-30 kDa. HSP 70 and HSP 90 can increase in ten-fold while the low molecular weight HSP’s can increase by 200 fold. Expression of all the HSP’s is not same for all the crop species. When maize, rye and wheat seedlings are subjected to heat shock ($42^{\circ}C$), five mitochondrial LMW-HSP’s were expressed in maize while only one HSP is expressed in wheat indicating that maize shows higher thermotolerance than wheat and rye (Korotaeva *et al.*, 2001). Over expression of plant HSFs

can increase plant’s thermo-tolerance, but gene knockouts of individual HSFs tested so far had little effect on survival at high temperature (Hemantaranjan *et al.*, 2014). A cis-acting DNA sequence, the heat-shock element (HSE), has been found to be necessary for heat-induced transcription (Nover & Baniwal, 2006). The HSE has a common consensus sequence of –GAA–TTC– and is found in multiple copies upstream of all HS genes. The induction of HS gene expression is mediated by the binding of a trans-acting transcriptional activator, the heat stress transcription factors (HSFs), to the HSE (Asthir, 2015). HSP’s help in shuttling problems from one compartment to another and also help in transporting protein to the “garbage disposals” inside the cell (Wahid *et al.*, 2007).

Characteristics associated with the assessment of heat tolerance

Although there are many methodologies to assess and quantify the heat stress in the natural populations and controlled environments physiologically, the following characteristics are mostly used. These include cell membrane thermal stability, chlorophyll fluorescence, canopy temperature Depression (CTD), triphenyl tetrazolium test (M. A. de Souza *et al.*, 2012).

Cell membrane thermal stability can be used as the physiological characteristic for stress breeding in some crops (Nagarajan *et al.*, 2010, Scafaro *et al.*, 2010, M. A. de Souza *et al.*, 2012, Bitá and Gerats, 2013). Rupturing of the cell membrane cause the flow of electrolytes into the cells and their concentration can be evaluated by electric conductance. Higher electrical conductance is associated with higher ability to withstand heat stress (Saadalla *et al.*, 1990). Limitations do exist for this characteristic *i.e.* this characteristic is not found to be strongly associated with the heat stress when worked in wheat (Blum *et al.*, 2001). Membrane thermostability can be considered as an important trait for evaluating heat stress in many crops (Blum and Ebercorn, 1981). In winter and spring wheat lower heritability of the membrane thermostability suggested the use of multiple replications during selection to limit environmental effects (Ibrahim and Quick, 2001). Chlorophyll fluorescence is measured using a portable apparatus called fluorometer. Chlorophyll fluorescence quantifies the damage caused by elevated temperature in the thylakoid tissues and the related reactions with photosynthesis. It gives information about the relative efficiency of the electron transport from PSII to PSI (M. A. de Souza *et al.*, 2012). In some legumes like groundnut and soybean, when the membrane thermostability and chlorophyll fluorescence are used as parameters for screening heat stress, it was observed that membrane thermostability is negatively associated with the specific leaf weight (Srinivasan *et al.*, 1996). In the heat stress tolerance studies in common bean, this characteristic helped in classifying the genotypes into stress susceptible, intermediate and tolerant (Petkova *et al.*, 2007). For the selections in Brassica and wheat a combination of two parameters *i.e.*, membrane thermal stability and chlorophyll content are used (Ristic *et al.*, 2007, Kumar *et al.*, 2013). Canopy temperature Depression (CTD) is an easily measured manifestation of crop metabolic and physiological response to the environment. Infrared thermometry is used for the measurement of the CTD. The principle behind this is that plants with greater heat-stress tolerance can maintain their organ temperature, respiration, and transpiration activities at normal levels, even under stress conditions (M. A. de Souza *et al.*, 2012). Under heat stress conditions, CTD exhibited positive correlation with yield in wheat (Reynolds *et al.*, 1994). In spring wheat CTD is used as an integrative trait for screening spot blotch resistance and heat stress tolerance. CTD proved to be an integrative trait for both types of stresses and has promise for further application in selection of stress tolerant genotypes in tropical environments (Rosyara *et al.*, 2008). 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) cell viability test has been used in various studies to quantify the level of acquired thermal tolerance in plants (Blum, 1988). The 2, 3, 5 Triphenyl Tetrazolium chloride reduction assay measures the reduction of TTC by electrons from mitochondrial electron transport chain (Towill and Mazur, 1979). Presumably, inhibition of TTC reduction is an indicator of respiratory enzyme inactivation or mitochondrial dysfunction that, in turn, reflects the relative level of cell viability. TTC is determined by the spectrophotometry (M. A. de Souza *et al.*, 2012). One assay of heat tolerance was

which measures electrolyte leakage from leaf tissue after exposure to high temperature. Heat injury was assessed by membrane thermal stability (MTS) and by quantifying the reduction of triphenyl tetrazolium chloride (TTC) to formazan by mitochondrial dehydrogenase respiratory enzymes in heat-stressed seedlings of winter and spring wheat. Results from the two assays were highly associated ($r = 0.62$, $n = 14$, $P < 0.05$). The high heritability of TTC warrants good progress from selection in early generations (Ibrahim and Quick 2001).

Breeding for Thermotolerance

Thermotolerance means the ability of plants to cope up with excessively high temperature (Wahid *et al.*, 2007). The existence of genetic variability in heat stress is an indispensable factor for the development of more tolerant cultivars. When adopting any breeding method for a determined crop, the type of reproduction of the species should be considered, that is, whether it is self-pollinating, cross-pollinating, or asexual. The thermotolerant varieties can be bred either by conventional breeding or molecular breeding.

Conventional Breeding for developing heat tolerant varieties:

Germplasm having genetic variability for the heat tolerance has been introduced in the traditional cultivating areas. An example of successful use of this strategy was reported by Giordano *et al.* (2005), who assessed tomato lines in the region of Brasília derived from two introduced populations from Roraima, North Brazil. Heat-stress-tolerant lines were identified in this study with good yield and fruit quality (M. A. de Souza *et al.*, 2012). Some of the new heat-tolerant varieties have been developed unintentionally by conventional breeding (Driedonks *et al.*, 2016). For example, a variety of broccoli has an improved head quality thanks to early maturation, because this trait prevents hot days later in season to affect the heat-sensitive flower initiation developmental stage (Farnham and Bjorkman, 2011). In wheat, recurrent selection has been used with satisfactory results, as reported by Maich *et al.* (2000), who observed 15% genetic progress in grain yield after two selection cycles. Genetic progress of 8.4% was obtained in the third recurrent selection cycle for heat-stress tolerance in wheat, in research developed by the Wheat Breeding Program at the Federal University of Viçosa, in 2011. In potato breeding a genetic gain was obtained after three cycles of recurrent selection for heat tolerance leading to strong increase in yield up to 37.8% (Benites and Pinto, 2011). Screening for heat tolerance in the field presents a challenge due to interactions with other environmental factors but a wide variety of screenable traits is available that allows successful selection in the field (Hall, 2011, Bitá and Gerats, 2013). Tolerant genotypes may also be selected in controlled environments.

Molecular Approaches for breeding heat tolerant varieties

As the conventional breeding approaches for thermotolerance have not proved their reliability, molecular approaches have gained importance. Yield potential is hard to separate from heat-stress tolerance when assessing wild accessions. Linkage drag of heat stress tolerance with undesirable characteristics is one of

the serious limitations. Greater success can be obtained by integrating classic breeding and molecular biology. Using biotechnological tools allows the identification of candidate genes that once their function and association to determine molecular marker are proven, can be easily incorporated by assisted selection. Furthermore, introgression of genes that are known to be involved in the stress response, via genetic engineering, can be a faster breeding strategy to develop tolerant cultivars. However, this would be the only option when the genes of interest were derived from other sexually incompatible species, such as distant relatives, or from non-plant sources (M. A. de Souza *et al.*, 2012).

The common plant biotechnological processes to develop thermotolerant varieties are the Marker Assisted Selection (MAS) and transgenic approach. The use of MAS requires the use of molecular markers associated with the QTL's of interest. Use of RFLP revealed mapping of 11 QTL's for pollen germination and pollen tube growth in maize (Frova and Sari-Gorla, 1994). In Arabidopsis, four genomic loci (QTLs) deterring its capacity to acquire thermotolerance were identified. Multiple loci for heat tolerance have been identified in wheat (Paliwal *et al.*, 2012) and maize (Bai, 2011). In tobacco, a transgenic plant showing a better photosynthetic activity under heat stress has been produced by alteration of the chloroplast membranes (Murakami *et al.*, 2000). An enhanced tolerance against high temperature was reported in tobacco by the transfer of a gene Dnak1 (Ono *et al.*, 2001). The transgenic plants having better production of glycine betaine due to transfer a gene (BADH) showed more tolerance to heat stress (Yang *et al.*, 2005). The improved tolerance against heat stress can also be achieved by over expression of the HSPs through genetic manipulations. A transgenic tobacco plant was produced by the transfer of MT-sHSP from tomato for better thermo-tolerance (Sanmiya *et al.*, 2004). Similarly, HSFs and DREB2A genes have been identified to engineer heat tolerant transgenic plants (Ohama *et al.*, 2017, Fahad *et al.*, 2017).

CONCLUSION

Heat stress or high temperature, is prominent among the cardinal ecological factors that determine crop growth and productivity in some regions (Ashram and Hafeez, 2004). To figure out the response of plants to heat stress, it must be considered that they are subjected to adverse conditions. Preliminary consideration is essential to understand the performance of plants under stress and also to identify strategies to improve stress tolerance (Rosa M. Pérez-Clemente *et al.*, 2010). Nevertheless, foster understanding of physiological, biochemical, molecular and genetic basis of thermotolerance needs to be made. This would help the scientific community to excel far beyond the available natural variation for stress tolerance through genetic engineering and other molecular tools and techniques. It is a prerequisite to identify true QTLs responsible for thermotolerance so that the biotechnologists can plan for cloning and successful incorporation of such true QTLs in crop plants. An additional imperative improvement needed is the development of effective inducible expression systems such as stress inducible promoters to increase the

efficiency of various physiological parameters for the reduction of crop losses due to HT stress.

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