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REVIEW

Mechanisms of heat tolerance in crop plants

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Abstract

Due to possible climate changes, heat stress has obtained a serious concern all over the world. Tolerance to this stress *via* knowledge of metabolic pathways will help us in engineering heat tolerant plants. A group of proteins called heat shock proteins are synthesized following stress and their synthesis is regulated by transcription factors. Under high temperature (HT), reactive oxygen species (ROS) are often induced and can cause damage to lipids, proteins, and nucleic acids. To scavenge the ROS and maintain cell membrane stability, synthesis of antioxidants, osmolytes, and heat shock proteins is of a vital importance. In view of above mentioned, this review highlights the detailed mechanism of pathways involving crucial steps that change during HT stress.

Additional key words: antioxidant defence system, cell membrane stability, heat shock proteins, osmolytes, reactive oxygen species.

Introduction

High temperature (HT) adversely affects plant growth and yield (Kurek *et al*. 2007, Ahmad and Prasad 2012). By the end of the 21st century, the Earth's climate is predicted to warm by an average of 2 - 4 °C (Pachauri and Reisinger 2007) due to both anthropogenic and natural factors (Eitzinger *et al*. 2010). Emission of greenhouse gases, such as carbon dioxide, methane, and nitrous oxide, from agricultural systems is one of the major concerns contributing to this global increase of temperature (Smith and Olesen 2010). Plant responses and adaptation to elevated temperatures, and the mechanisms underlying the development of heat tolerance need to be better understood for important agricultural crops. Under HT conditions, plants accumulate different metabolites, such as antioxidants, osmoprotectants, and heat shock proteins (Hsps), using different pathways (Bokszczanin and Fragkostefanakis 2013). Membrane lipid bilayer fluidity gets significantly enhanced, which can cause electrolyte leakage, reactive oxygen species (ROS) production, and oxidative damage. A ROS production in chloroplasts and mitochondria is of a great importance for signalling as well, and for production of protective enzymes and antioxidants (Bohnert *et al*. 2006), which results in less oxidative damage. The Hsps play roles in stress signal transduction, protecting and repairing damaged proteins and membranes, as well as regulating a cellular redox state. Some other major tolerance responders include ion transporters, osmoprotectants, free radical scavengers, abundant stress responsive proteins, and factors involved in signalling cascades and transcriptional control which are essential to counteract the effects of heat stress (Wang *et al*. 2004).

Mechanism of heat tolerance has been linked to increased tolerance of the photosynthetic apparatus (Hemantaranjan *et al*. 2014). It is well established that HT stress causes malfunction of photosystem (PS) II, reduces the efficiency of electron transport, and increases production of the ROS. Plants under HT stress usually accumulate more ROS in both chloroplasts and mitochondria, which can severely damage DNA and cause cell membrane lipid peroxidation. A number of studies have demonstrated that ROS detoxification mechanisms play important roles in protecting plants

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Abbreviations: AA - ascorbic acid; APX - ascorbate peroxidase; AsA - ascorbate; CAT - catalase; DHAR - dehydroascorbate reductase; GPX - guaiacol peroxidase; GR - glutathione reductase; GSH - reduced glutathione; GSSG - oxidized glutathione; GST - glutathione-S-transferase; Hsfs - heat stress transcription factors; Hsps - heat shock proteins; HT - high temperature; LMM - low molecular mass; LPO - lipid peroxidation; MDA - malondialdehyde; MDHAR - monodehydroascorbate reductase; MTS - membrane thermal stability; O_2^- - superoxide anion; $^{\bullet}OH$ - hydroxyl radical; PS - photosystem; ROS - reactive oxygen species; SOD - superoxide dismutase.

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against HT stress (Larkindale and Knight 2002, Suzuki and Mittler 2006). Thus, plant tolerance to this stress is closely correlated to an increased capacity of scavenging and detoxifying the ROS. Induction of thermo-tolerance may be ascribed to maintenance of a better membrane thermo-stability and low ROS accumulation (Xu *et al*. 2006, Hameed *et al*. 2012) due to an improved antioxidant capacity (Chakraborty and Pradhan 2011). However, limited information is available on ROS generation and dissipation in different cell organelles. Responses of plants to HT are mediated by an inherent ability to survive and to acquire thermotolerance to a lethal temperature. Reports in literature reveal that a genetic variability among crops is due to expression of different stress responsive genes (Kumar *et al*. 2007), and acquisition of thermotolerance and synthesis and accumulation of Hsps have been correlated with the antioxidant defence system (Maestri *et al*. 2002).

Role of membranes in heat tolerance

Peroxidation of lipids is considered as the most damaging process known to occur in every living organism. Heat stress results in enhanced lipid peroxidation (LPO) due to free radical damage of the cell membrane. It has been recognized that LPO products are formed from poly-unsaturated precursors that include small hydrocarbon fragments, such as ketones, malondialdehyde (MDA), and compounds related to them (Garg and Manchanda 2009). Malondialdehyde is a highly reactive three carbon dialdehyde produced as byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. Therefore, measurement of MDA is widely used as indicator of lipid peroxidation (Wagner *et al*. 2004). Some of these compounds react with thiobarbituric acid to form coloured products called thiobarbituric acid reactive substances (Hameed *et al*. 2012). The LPO in both cellular and organelle membranes takes place when above-threshold ROS levels are reached, thereby affecting normal cellular functioning (Montillet *et al*. 2005). Resulting alkoxyl and peroxyl radicals are able to induce new radical chains by interacting with additional lipid molecules. Resulting lipid hydroperoxide can easily decompose into several reactive species including lipid alkoxyl radicals, aldehydes, alkanes, lipid epoxides, and alcohols (Smirnoff 1995). A single initiation event thus has a potential to generate multiple peroxide molecules by a chain reaction. The overall effect of LPO is decreased membrane fluidity. This makes easier to exchange phospholipids between the two halves of the bilayer and to increase the leakiness of the membrane to substances that do not normally cross it, to damage membrane proteins, receptors, enzymes, and ion channels.

The integrity and functions of biological membranes are sensitive to HT as heat stress alters the tertiary and quaternary structures of membrane proteins. Membranes are moving mosaics of proteins and lipids. The lipids stagger between monolayers, diffuse within the plane of a monolayer and rotate about their own axes, with their acyl chains also rotating around carbon-carbon bonds (Hemantaranjan *et al*. 2014). Much heat sensing occurred through protein unfolding. Since protein conformation changes with temperature, both a decrease and an increase of temperature can lead to protein unfolding (Pastore *et al*. 2007). Membrane fluidity is largely dictated by a degree of membrane lipid saturation. A temperature induced change in membrane fluidity is one consequence of HT stress leading to injury (Stone and Nicolas 1994). Membrane fluidity in temperature tolerance has been delineated by mutation analysis and transgenic and physiological studies. For example, a soybean mutant deficient in fatty acid unsaturation shows a strong tolerance to an HT (Pastore *et al*. 2007). Also, the thylakoid membranes of two *Arabidopsis* mutants deficient in fatty acid unsaturation (fad5 and fad6) show an increased stability to an HT (Yamada *et al*. 2007), and an increased lipid saturation in tobacco caused by silencing an *ω-3 desaturase* gene also rendered plants more tolerant to an HT (Von Koskull-Doring *et al*. 2007).

Membrane thermal stability (MTS) can be a significant selection criterion for heat stress tolerance and is determined by measuring the electrical conductivity. Membrane stability parameters usually decrease during later developmental stages of plant growth. A genetic variation among genotypes for the MTS can be utilized in wheat breeding for heat-stressed environments. Wheat lines with a high MTS tend to yield better than lines of a low MTS when grain filling occurs under hot conditions (Gupta *et al*. 2013). Exposure of plant cells to an HT causes cellular membrane disruptions that are apparently related to temperature-specific phase changes (gel to liquid form or *vice versa*) in the membrane lipid bilayer. Heat stress was shown to cause impairments in mitochondrial membrane functions and results in the induction of oxidative damage (Sairam *et al*. 1998, Larkindale and Knight 2002, Suzuki and Mittler 2006). The photosynthetic apparatus in plants is highly thermolabile and is damaged before visible symptoms of HT are manifested. An HT affects thylakoid membrane reactions by changing the amount of the absorbed light energy and its utilization, and in turn, altering the pattern of chlorophyll fluorescence. Therefore, determining a mechanism associated with heat tolerance and identifying screening methods are vital for improvement of heat tolerance in plants.

Role of reactive oxygen species detoxification in heat tolerance

Reactive oxygen species including superoxide anion (O_2^-), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) are natural products of cell metabolism (for details see Fig. 1). Insufficient energy dissipation during photosynthesis can lead to formation of the chlorophyll triplet state. The chlorophyll triplet state can react with triplet oxygen to give up a very reactive singlet oxygen which has a damaging effect on PS I and II as well as on the whole photosynthetic machinery. HT stress enhances rapid production and accumulation of the ROS (Sairam *et al*. 2000, Mittler 2002, Almeselmani *et al*. 2006). Overproduction of the ROS above a constitutive level is potentially harmful to all cellular compounds and negatively influence cell metabolism (Breusegem *et al*. 2001, Esfandiari *et al*. 2007). Detoxification of these ROS is of a prime importance (Suzuki *et al*. 2011) and the plant defence system involves enzymatic and non-enzymatic antioxidants (Fig. 1). The major enzymatic antioxidants are catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and glutathione-S-transferase (GST) (Noctor and Foyer 1998). The SOD converts O_2^- to H_2O_2 whereas CAT and peroxidases dismutate H_2O_2. The CAT eliminates H_2O_2 by breaking it down to H_2O and O_2 but peroxidases require reducing equivalents to scavenge H_2O_2. The GPX requires a phenolic compound guaiacol as electron donor to decompose H_2O_2 whereas APX uses a reduced form of ascorbate (AsA) to protect cells against damaging effects of H_2O_2 (Tripathy and Oelmüller 2012). The oxidized form of AsA produced by the action of APX is regenerated *via* ascorbate-glutathione cycle or Halliwell-Asada pathway involving monodehydroascorbate reductase (MDHAR) and DHAR, and finally the oxidized glutathione (GSSG) is reduced by GR using the reducing power of NADPH (Fig. 1).

Glutathione-S-transferases are a collection of multifunctional proteins that are found essentially in all organisms. Apart from participation in metabolism of natural secondary compounds (Dixon *et al*. 2001) and

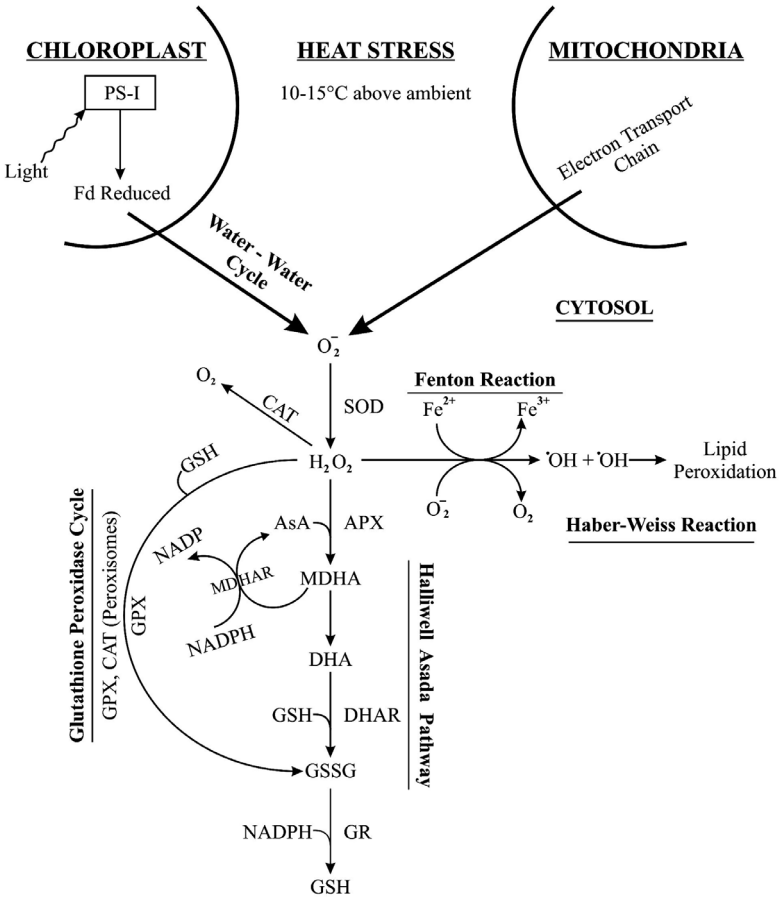

Fig. 1. The mechanism of reactive oxygen species production and utilization under high temperature stress conditions. APX - ascorbate peroxidase, AsA - ascorbate, CAT - catalase, DHA - dehydroascorbate, DHAR - dehydroascorbate reductase, Fd - ferredoxin, GPX - guaiacol peroxidase, GR - glutathione reductase, GSH - reduced glutathione, GSSG - oxidized glutathione, $^{\bullet}OH$ - hydroxyl radical, MDHA - monodehydroascorbate, MDHAR - monodehydroascorbate reductase, O_2^- - superoxide anion, PS I - photosystem I, SOD - superoxide dismutase. Adapted partially from Wahid *et al*. (2007).

detoxification of xenobiotics (Dixon *et al*. 1998), plant GSTs can catalyze reduction of H_2O_2 to a less harmful alcohol (Roxas *et al*. 2000). By doubling the GST/GPX activity in transgenic tobacco, the seedlings and plants show significantly faster growth than wild-type and thus provide a better protection against oxidative stress (Roxas *et al*. 2000).

Activities of different antioxidant enzymes are temperature sensitive and activation occurs at different temperature ranges. Chakraborty and Pradhan (2011) observed in *Lens culinaris* that CAT, APX, and SOD activities initially increase before decline at 50 °C whereas POX and GR activities decline at all temperatures ranging from 20 to 50 °C. In addition, a maximum antioxidant activity is at 35 - 40 °C in tolerant cultivars and at 30 °C in susceptible ones (Kaushal *et al*. 2011). Their activities also differ depending upon tolerance or susceptibility of different crop cultivars, their growth stages, and a growing season (Almeselmani *et al*. 2006). Activities of GST, APX, and CAT are more enhanced in wheat cultivars that show a better tolerance to heat stress (Goyal and Asthir 2010, Ahmad and Prasad 2012); thus, the tolerance of the wheat cultivars appears to be correlated with their antioxidant capacities.

Ascorbate, GSH, and α-tocopherol are major non-enzymatic antioxidants that play crucial roles in ROS detoxification (Hameed *et al*. 2012). Ascorbate is distributed in almost all plant parts and is synthesized in mitochondria and transported to other parts of a plant. Ascorbate is used as substrate by APX to reduce H_2O_2 to H_2O in the ascorbate-glutathione cycle and generate monodehydroascorbate which further dissociate to AsA and dehydroascorbate (Fig. 1).

The α-tocopherol (the major vitamin E compound found in leaf chloroplasts especially in the thylakoid membranes and plastoglobuli) deactivates photosynthesis -derived ROS (mainly singlet oxygen and $OH^{\cdot}$) and along with other antioxidants, scavenges lipid peroxy-radicals. It acts as lipophilic antioxidant and interacts with polyunsaturated acyl groups of lipids (Tripathy and Oelmüller 2012) and so stabilizes the membrane; it also acts as substance that modulates signal transduction. The amount of α-tocopherol changes in response to environmental constraints, the magnitude of stress, and species sensitivity. Changes in α-tocopherol result from altered expression of genes related to its biosynthesis pathway (Sandorf and Hollander-Czytko 2002). Compensatory mechanisms exist to afford an adequate protection to the photosynthetic apparatus in the absence of α-tocopherol (Kanwischer *et al*. 2005) and to provide further evidence that it is the whole set of antioxidant defences (AsA, GSH, carotenoids, tocopherols and other isoprenoids, flavonoids, and enzymatic antioxidants), rather than a single antioxidant, which helps plants to withstand environmental stress (Bosch 2005).

Glutathione is a non-protein thiol that has a key role in H_2O_2 detoxification. It has been reported that the conversion ratio of GSH to its oxidized form GSSG during the detoxification of H_2O_2 is the indicator of cellular redox balance (Goyal and Asthir 2010). These events were widely reported in plants under various abiotic stresses. Glutathione and AsA are now considered as important components of redox signalling in plants (Suzuki *et al*. 2012).

Role of heat shock proteins in heat tolerance

Heat stress triggers expression of defence genes that are not expressed under "normal" conditions (Morimoto 1993). All stresses induce gene expression and synthesis of stress-induced proteins (Lindquist and Crig 1988, Morimoto *et al*. 1994, Gupta *et al*. 2010) in cells that are subjected to stress. However, stressing agents firstly lead to an immediate block of important metabolic processes, including DNA replication, transcription, mRNA export, and translation, until cells start to recover (Biamonti and Caceres 2009).

Heat shock proteins (Hsps) are grouped into five different families based on their molecular masses: *1*) Hsp100, *2*) Hsp90, *3*) Hsp70, *4*) Hsp60, and *5*) small Hsps (sHsps). Plants in general have the highest diversity of sHsps. The expressions of genes for these sHsps are limited to certain developmental stages of a plant like seed germination, embryogenesis, development, or fruit ripening (Sun *et al*. 2002). Major Hsps have some kind of related roles in solving the problem of misfolding and aggregation as well as acting as chaperones. The diversification and abundance of Hsps reflect an adaptation of a plant to heat stress. Transcriptions of *Hsp* genes are mainly controlled by regulatory proteins called heat stress transcription factors (Hsfs) located in the cytoplasm in an inactive state (Nover and Baniwal 2006). Each factor has one carboxylic terminal (C-terminal) and three amino terminals (N-terminals) and includes the amino acid leucine (Schuetz *et al*. 1991). Plants show at least 21 Hsfs each having its role in regulation. However, they cooperate in all phases of heat stress responses during triggering, maintenance, and recovery. Therefore, these factors are considered as transcriptional activators for heat shock response (Baniwal *et al*. 2004, Hu *et al*. 2009).

In a tomato system, HsfA1a is the master transcriptional regulator that is responsible for a stress-induced gene expression including the synthesis of both HsfB1 and HsfA2 (Fig. 2). These factors have been named into three classes according to the structural differences in their aggregation in triplets such as oligomerisation domain *viz.* HsfA (A1 and A2), HsfB (B1) and HsfC (Tripp *et al*. 2009). All these factors are necessary for plant acquisition of heat tolerance (Baniwal *et al*. 2004). Hence, there is an acquired thermotolerance phenomenon which is supported by a study on *A. thaliana* that indicates the participation of HsfA2

(Charng *et al*. 2007). Furthermore, HsfA2 is finely regulated with Hsp17-CII during anther development of a heat tolerant tomato genotype and is further induced under both short and prolonged heat stress (Giorno *et al*. 2010). Expression of heat-inducible genes to synthesize Hsps is composed of mechanism of sensing and transmitting signal of HT stress to Hsfs (regulatory protein) (Schöffl *et al*. 1998, Larkindale *et al*. 2005). Heat shock factors binds to HSE in the promoter region that consists of alternating units of pentameric nucleotides (5'-nGAAn-3'). Efficient binding of HSF requires at least three alternating units (Morimoto 1998, Schöffl *et al*. 1998). In the absence of stress, Hsfs are present in the cytoplasm as single free monomeric units without any binding activity with DNA. Upon sensing stress, these factors aggregate in a triplet and accumulate in the nucleus (Sorger and Nelson 1989). The binding of Hsfs to DNA in tomato seedlings is promoted by salicylic acid, however, it does not promote the transcription of Hsp70 mRNA or the expressions of HsfA2 and HsfB1. This can indicate that salicylic acid has a role in modulating the Hsf for binding (Snyman and Cronje 2008).

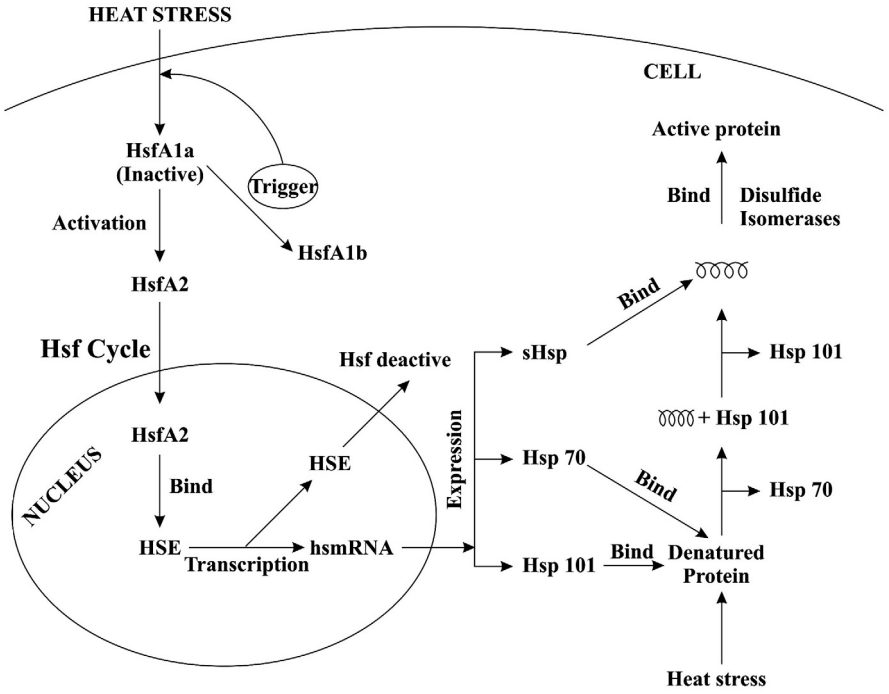

Fig. 2. The mode of action of heat shock proteins. The HsfA1a triggers a heat stress response through the induction of HsfA1b and HsfA2 which produce co-activators. Active HsfA2 homotrimers bind to a heat shock promoter element (HSE) which induces the expression of various Hsps, Hsf70, Hsf101, and sHsp that participate in repair. Adapted from Qu *et al*. (2013)**.**

The function of any protein is determined by its formation and folding into three dimensional structure (Levitt *et al*. 1997). Formation of thiol structure requires 50 % of conserved amino acid sequences. The role of Hsps in folding other proteins is important as Morimoto and Santoro (1998) indicated that Hsps protect cells from injury and facilitate recovery and survival after a return to normal growth conditions. Similarly, Timperio *et al*. (2008) specified that upon heat stress, the role of Hsps as molecular chaperones is without doubt, but their function in non-stress conditions can be different: Unfolding proteins is not the main effect, and protection from damage can occur in an alternative way apart from ensuring the maintenance of a correct protein structure.

The general role of Hsps is to act as molecular chaperones regulating folding and accumulation of proteins as well as their localization and degradation (Feder and Hofmann 1999, Schulze-Lefert 2004, Panaretou and Zhai 2008, Hu *et al*. 2009, Gupta *et al*. 2010). These proteins, as chaperones, prevent the irreversible aggregation of other proteins and participate in refolding proteins during heat stress (Levitt *et al*. 1997, Tripp *et al*. 2009). Each group of these Hsps has a unique mechanism and a defined role to play. Denaturation of proteins under HT stress leads to new synthesis of proteins (Hsps) that are assumed to be result of the pool of free chaperones present in latent form mostly found in the cytoplasm (Timperio *et al*. 2008). Stress causes activation, oligomerization, and eventually recompartmentation of HsfA1 to the nucleus where it binds to its target sequences (HSE) present in the promoter of *Hsp* genes (Fig. 2).

Overexpression of HSP101 from *Arabidopsis* in rice plants results in a significant improvement of growth performance during recovery from heat stress (Liu *et al*. 2011). *Arabidopsis* has 21 *Hsf* genes belonging to three major classes, *HsfA*, *HsfB*, and *HsfC*, based on structural differences. The HsfAs appear to be the major factors

responsible for a heat-induced activation of heat shock genes. The HsfBs apparently lack the heat-inducible transactivation function in spite of having the normal DNA binding function and might act as co-activators of transcription with *HsfAs* (Czarnecka-Varner *et al*. 2000). In spite of extensive studies on Hsfs, no immediate upstream factors to Hsf in heat signal transduction have been identified. Several *Hsf* genes are heat inducible indicating the presence of transcriptional activators for *Hsf* genes (Sung *et al*. 2003). Whether they are Hsfs themselves or other novel transcriptional factors awaits further investigation.

Developing grains contain Hsp100, and a more tolerant cultivar of wheat maintains a higher content of Hsp100 at an elevated temperature (Sumesh *et al*. 2008). The LMM heat shock proteins represent a set of homologous proteins in the range of 15 - 30 kDa (DeRocher and Vierling 1994, Hemantaranjan *et al*. 2014). Under stress conditions, LMM-Hsps may comprise up to 1 % of the cellular proteins. Plant LMM-Hsps are divided into six classes. Three classes are localized in the cytosol or in the nucleus, and the other three in the plastids, endoplasmic reticulum, and mitochondria (Agarwal *et al*. 2003). The organellar forms of LMM-Hsps appear to be unique to plants with the exception of *Drosophila* Hsp22 that is localized in mitochondria (Morrow *et al*. 2000). Significance of Hsps in thermotolerance has been mostly shown on the basis of correlation with metabolic activity in the cell (Vierling 1991, Morrow and Tanguay 2012). In most cases, specific functions of Hsps have not been demonstrated *in vivo*. It has been, however, shown that LMM-Hsps located in mitochondria and chloroplasts protect respiratory electron transport in mitochondria and PS II electron transport in chloroplasts (Sharma-Natu *et al*. 2010). A chloroplastic 22 kDa Hsp from *Chenopodium album*, which is localized in thylakoid lumen, interacts specifically with the thermolabile oxygen evolving complex of PS II and protects it from heat stress damage (Heckathorn *et al*. 1998, Sun *et al*. 2002). The LMM-Hsps have been demonstrated to act as molecular chaperones *in vitro* and *in vivo*. An 18 kDa Hsp has been shown to prevent protein aggregation and maintain them in active refolding form by other chaperones (Sun *et al*. 2002). Starch synthesis in grains takes place in amyloplasts (Preiss and Sivak 1996). In view of the reported localization of LMM-Hsps in plastids, the role of Hsp18 in providing thermotolerance for grain growth in wheat could be through providing protection to soluble starch synthase, an enzyme shown to be extremely sensitive to HT.

Role of other protectants in heat tolerance

In recent decades, exogenous application of osmo-protectants, phytohormones, signalling molecules, trace elements, *etc*., has shown beneficial effects on plants grown under HT stress as these protectants usually have growth promoting and antioxidant capacities (Hasanuzzaman *et al*. 2011). Accumulation of osmolytes, such as proline, glycine betaine, and trehalose is a well-known adaptive mechanism in plants against abiotic stresses including heat. Since heat sensitive plants apparently lack the ability to accumulate these substances, heat tolerance in such plants can be improved by exogenous application of osmoprotectants (Sakamoto and Murata 2002, Rasheed *et al*. 2011). Proline or glycine betaine application considerably reduces H_2O_2 production, improves accumulation of soluble sugars and protects developing tissues from heat stress effects. However, proline is usually more effective than glycine betaine. Exogenous proline and glycine betaine also improve K^+ and Ca^{2+} content, and increase content of endogenous proline or glycine betaine and soluble sugars which render plants more tolerant to heat stress (Rasheed *et al*. 2011). Cytosolic Ca^{2+} also seems to be linked to acquisition of tolerance to temperature stresses. A mild heat treatment that elicits development of acquired thermotolerance fortifies cytosolic Ca^{2+} rise after heat stress (Larkindale and Knight 2002).

Identically, several phytohormones are effective in mitigating heat stress in plants. Chhabra *et al*. (2009) studied phytohormone induced amelioration of heat tolerance stress in *Brassica juncea* and found that soaking seeds in 100 μM indole acetic acid or gibberrelic acid, 50 and 100 μM kinetin, and 0.5 and 1 μM abscisic acid are effective for mitigating the effect of heat stress. It is interesting that both growth promoting and growth retarding hormones can mitigate heat stress effects. The role of growth promoting hormone in the mitigation of heat stress is at a concentration which is otherwise toxic to growth at the seedling stage (Hasanuzzaman *et al*. 2011). Similarly, phytohormones might play a significant role in imparting thermotolerance to cereal grains. Chandrasekar *et al*. (2000) have reported a higher accumulation of abscisic acid in heat tolerant wheat cv. C 306 than in susceptible cv. Hira in response to water stress.

Some major tolerance mechanisms involving ion transporters, late embryogenesis abundant proteins, osmoprotectants, antioxidant defence and factors involved in signalling cascades and transcriptional control are essentially significant to counteract stress effects (Wang *et al*. 2004, Rodriguez *et al*. 2005). The downstream stress responsive mechanisms help to re-establish homeostasis and to protect and repair damaged proteins and membranes (Vinocur and Altman 2005).

Future pioneering studies in model plants can pave the way to identify key regulators as target for gene manipulation of stress tolerance in crop plants. It has also been envisaged that metabolic fingerprinting can be used as breeding tool for development of plants with the best potential to tolerate abiotic stresses.

Conclusion

A complete understanding of the nature of the heat shock signalling cascades as well as the specific genes expressed in response to HT will be valuable for developing stress tolerant plants. However, detailed mechanisms of thermotolerance remain indefinable and needs an appropriate research. A successful improvement of crop tolerance to heat by altering sensing, signalling, or regulatory pathways will depend on identifying targets for modification that does not disrupt other vital processes. Though significant advances have been achieved to understand the roles of ROS in plants, it is still far from clarity what roles ROS plays in stress regulation and metabolism. Metabolic engineering plants to synthesize compatible compounds may be an alternative way of developing thermotolerance in important crop plants.

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