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REVIEW

Mechanisms of heat sensing and responses in plants. It is not all about Ca^{2+} ions

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Abstract

The climate shift has resulted in frequent heat waves, which cause damaging effects on plant growth and development at different life stages. All cellular processes in plants are highly sensitive to a high temperature. The plasma membrane heat receptors usually sense temperature variations directly or *via* a change in membrane fluidity. The accumulation of damaged proteins and reactive oxygen species also aid in heat perception. Calcium ions and heat sensors transfer signals to transcription factors through a series of signaling cascades. The heat stress transcription factors (HSFs) effectively regulate expression of heat induced genes. The members of the heat shock transcription factor A1 (HsfA1s) family are master regulators of a heat stress response. Different HSFs interact with each other at different levels and simultaneously operate heat induced gene expression. Interaction of HSFs with each other on multiple levels provides chances for manipulation to improve plant heat stress tolerance.

Additional key words: calcium, heat sensors, heat stress transcription factors, membrane receptors, reactive oxygen species.

Introduction

Climate change and global warming lead to frequent heat waves that damage plants not only by a change in diurnal temperature but also by occurrence of episodes of heat-stress, which impairs normal homeostasis. A high temperature interferes with all chemical processes and results in cell damage ultimately decreasing plant growth and yield (Li *et al.* 2017). To survive under a high temperature, plants have to sense ambient temperature accurately and respond to its changes.

A temperature above an optimum alters the fluidity of plasma membrane and activates membrane heat receptors. Heat stress (HS) also results in accumulation of unfolded proteins and reactive oxygen species (ROS) inside the cell. These alterations stimulate many signal transduction pathways and activate transcription factors (TFs), which operate expression of downstream genes and prepare plants for stress tolerance (Mittler *et al*. 2012, Bokszczanin *et al.* 2013, Yan *et al.* 2017). The TFs are

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Abbreviations: ABA - abscisic Acid; ACBP - acyl CoA binding-protein; AREB/ABFs - ABA responsive-element binding-factors/ABRE binding-factors; ARP6 - actin related protein 6; BIP3 - binding immunoglobulin protein; bZIP - basic leucine zipper; CaM3 - calmodulin 3; CBKS - calcium/calmodulin binding protein-kinases; CDKA1 - cyclin dependent-kinase A1; CDPK - calcium dependent protein-kinase; CNGCS - cyclic nucleotide-gated channels; DPB3 - DNA polymerase II subunit B3; DREB2A - dehydration responsive element-binding protein-2A; ERFs - ethylene responsive factors; GRF7 - growth regulation-factor 7; GRI - Grim Reaper; H2A.Z - histone cluster 1 family member Z; H2A - histone cluster 1 family member A; HS - heat stress; HSF - heat stress transcription factors; Hsfa1 - heat shock transcription factor A1; JUB1 - Jungbrunnen 1; MAPK6 - mitogen activated protein-kinase 6; MBF1C - multiprotein bridging factor 1C; MED25 - MEDIATOR 25; NAC019 - NAC domain containing Protein 19; NF-YA2/NF-YB3 - nuclear factor Y, subunit A2 or B3; NPR1 - natriuretic peptide receptor 1; NRD - negative regulatory domain; PA - phosphatidic acid; PEPC - phospho*enol*pyruvate carboxylase; PM - plasma membrane; PP7 - protein-phosphatase 7; PPI - protein-protein interaction; RCD1 - radical induced cell-death 1; RCF2 - C-repeat binding factor gene expression 2; RIM - RCD1 interacting motif; ROF1 - rotamase FKBP 1; ROF2 - rotamase FKBP 2; ROS - reactive oxygen species; STIM - stromal-interaction molecules; SWR1 - Swi2/Snf2-related ATPase; SYTA - synaptotagmin A; TDR - temperature dependent-repression; TFIIB - transcription factor IIB; TFs - transcription factors; TRP-V - transient receptor-potential cation channel subfamily V; UPR - unfolded protein response.
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critical for normal homeostasis and efficiently regulate gene expression under normal and harsh environmental conditions (Röth *et al.* 2017, Yang *et. al.* 2017). The heat stress transcription factors (HSF) are key regulators of heat stress response and enable plants to survive and adapt under temperature extremes. Plant response to HS is very complex and comprises several interconnected signal transduction pathways and processes. Plasma membrane bound kinases and HSFs play an important role in HS response.

Plants have several conserved stress gene-families (about 300 genes), which help to protect vital cellular activities from an HS-induced damage (Rasmussen *et al.* 2013). It is urgent need to reveal a molecular mechanism underlying the HS response and to develop heat stress tolerant plant cultivars. The whole genome expression and improvements in molecular methods are very helpful for plant scientists to solve these tasks. In our review, we have discussed how plants sense changes in temperature. Plasma membrane bound receptors, a mass of unfolded protein, and ROS convey a message to cellular transcriptional machinery to initiate HS response. Several membrane receptors have been identified in animals whereas their presence in plants needs experimental verifications (Yao *et al.* 2011).

Mechanism of heat sensing

Heat stress interferes with every cell process and results in dysfunction of macromolecules, *e.g.*, in protein denaturation and partial melting DNA and RNA strands. Therefore, all macromolecules can serve as HS sensors and because of this, there is a need to identify specific molecules that act as primary HS sensors, which can accurately sense and specifically react to temperature variations. The HS sensors have ability to sense heat directly (due to a change in conformation) or indirectly. They trigger signaling pathways that ultimately result in expression of heat stress responsive genes. Several cascades of kinases and heat stress transcription factors (HSF) play roles in pathways leading from HS perception to heat induced gene expression. Some new findings in pattern of heat sensing in plants are discussed in the following sections:

Plasma membrane heat sensors: Components of the plasma membrane (PM) act as a primary heat sensors and initiate HS signal transduction pathways. The PM senses small changes in temperature and activates specific calcium channels, which cause inward flow of calcium (Fig. 1). Inward Ca^{2+} flow is the first step in signal transduction pathways to initiate HS response (Wang and Huang 2017). Use of calcium channel blockers or chelators has shown that calcium channels also behave as primary heat sensors in plants (Mittler *et al.* 2012). Calcium channels do not sense a change in

Table 1. An overview of possible heat sensing mechanisms/pathways. For abbreviations see the list.

Primary heat sensor	Possible heat sensing mechanisms	Active members of pathways	Functions
Plasma membrane	specific calcium channels	CNGCs, TRP-V, STIM	allow inward flow of Ca^{2+} ions
	synaptotagmin A mechanism	SYTA or SYTA1	perform plasma membrane repairing control expression of calmodulin 3 regulate expression of heat stress transcription factors and HSPs
	lipid signaling pathways	accumulation of phosphatidic acid	regulate CDPK, PEPC, and ABI1 control membrane Ca^{2+} channels
A mass of unfolded proteins in endoplasmic reticulum and cytosol	membrane associated transcription factors (MTFs)	bZIP28 and bZIP60	regulate expression of RbohD control expression of heat inducible genes interact with COMPASS like components
	HSF mechanism	HsfA2	regulate HS inducible genes
Accumulation of reactive oxygen species in the cell	NADPH oxidase	respiratory burst oxidase homolog D (RbohD)	control expression of *HSFs* and HSPs
	redox-potential sensitive pathways	change in redox potential on plasma membrane	interferes with ROS sensor proteins and regulate NPR1 activity
	GRIM REAPER protein	GRI derived peptides	induce cell death pathways
Histone modifications	nucleosome having H2A.Z	SWR1 complex	regulate gene expression under HS

temperature directly, but variations in PM fluidity due to HS or due to fluidity inducing chemicals trigger their opening. Research on animals has shown that certain ion channels, such as cyclic nucleotide-gated channels (CNGCs), the transient receptor-potential cation channel subfamily V (TRP-V) and stromal-interaction molecules (STIM) (Table 1) also behave as temperature sensors, but their similarity with plants has not yet been elucidated (Yao *et al.* 2011).

Synaptotagmin A (SYTA, also known as SYTA1), is a transmembrane protein that functions to maintain PM integrity with the help of Ca^{2+} ions. The SYTA has two Ca^{2+} ion binding regions (C2A and C2B) present towards the N-terminus. Inward flow of calcium *via* Ca^{2+}-channels or from a membrane rupture site (due to HS) activates SYTA, which, in the next step, repairs the damaged site (Fig. 1). The transmembrane region of SYTA, also known as C2A, is responsible for binding to phospholipids *via* Ca^{2+} (Yan *et al.* 2017). However, activation of SYTA by Ca^{2+} in the absence of a membrane damage needs experimental verification. The SYTA, activated by Ca^{2+}, also regulates the expression of calmodulin 3 (CaM3), which, in the next step, activates mitogen activated protein-kinase 6 (MAPK6). The MAPK6 on one hand controls trans-membrane Ca^{2+} flow, and on the other hand controls root elongation affected by H_2O_2. The SYTA over-expression also regulates membrane lipid peroxidation (Table 1). We can say that SYTA up-regulates expression of MAPK6 *via* a Ca-CaM3 complex, and it also regulates the expression of heat shock transcription factors *HsfA1a*, *HsfA1b*, *HsfA5*, *HsfB1*, and some *HSPs* because expressions of these genes are down-regulated in *syta* mutant plants (Yan *et al.* 2017). In conclusion, over-expression of *SYTA* increases plant thermotolerance by operating HS response and decreasing membrane lipid peroxidation, but how SYTA regulates heat stress induced genes is not yet clear.

A change in membrane fluidity due to HS initiates a lipid signaling pathway and results in accumulation of phosphatidic acid (PA) inside the cell (Table 1). The PA, in the next step, interacts with calcium dependent protein-kinase (CDPK) and phospho*enol*pyruvate carboxylase

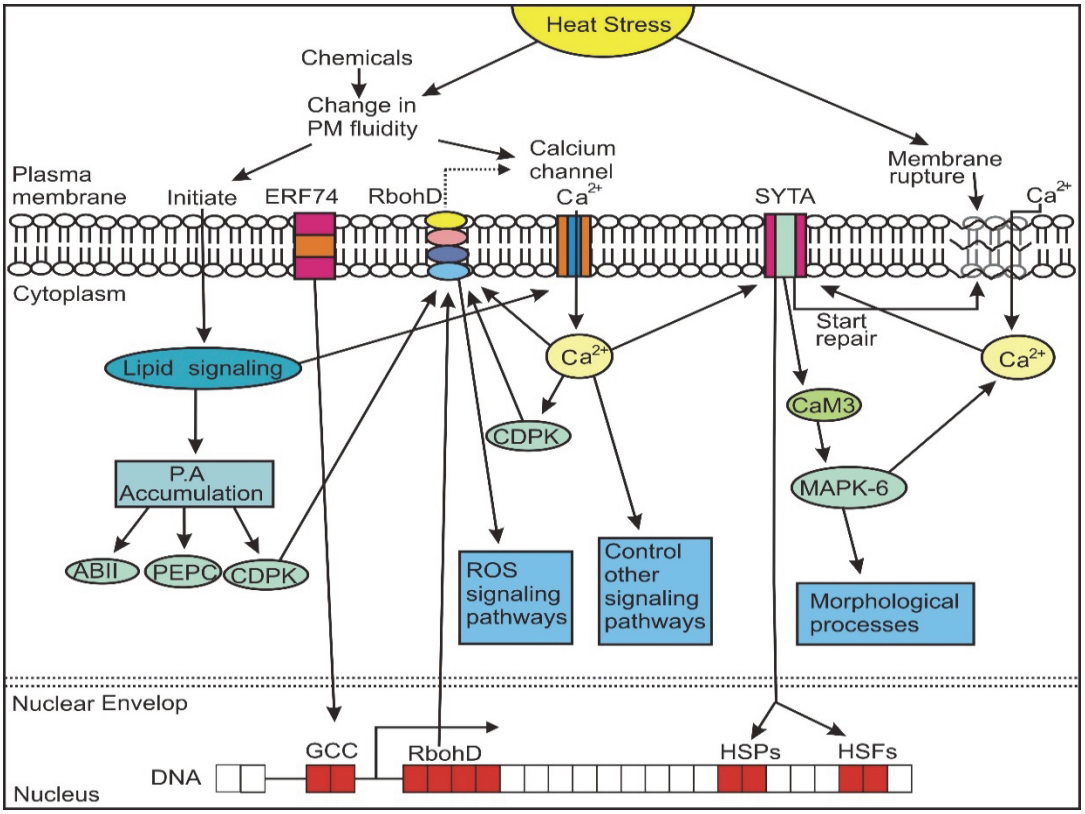

Fig. 1. Mechanism of heat sensing by plasma membrane sensors. Heat stress changes fluidity of plasma membrane and results in membrane damage. Changes in membrane fluidity initiate lipid signaling pathways and activate calcium channels. Calcium ions move in the cell through damaged sites or by channels and initiate calcium signaling pathways. A calcium dependent protein-kinase (CDPK), activated by phosphatidic acid under lipid signaling pathways or by calcium ions, stimulates a respiratory burst oxidase homolog D (RbohD) to produce reactive oxygen species, which, in turn, intensify calcium flow and operate downstream processes. Expression and plasma membrane translocation of RbohD is regulated by ethylene responsive factor 74 (ERF74), which directly senses heat stress at the plasma membrane. Reactive oxygen species and Ca^{2+} operate many downstream signaling pathways. Calcium ions also activate synaptotagmin A (SYTA), which repairs the damaged site and initiate expression of heat shock proteins (HSPs) and heat stress transcription factors (HSFs).

(PEPC) (Mishkind *et al.* 2009). In the next step, CDPK regulates accumulation of ROS (Fig. 1). Accumulation of lipid signaling molecules also controls opening Ca^{2+}-channels and regulates their flow (Mittler *et al.* 2012). A relationship between HS or lipid signaling molecule induced plasma membrane (PM) channel opening is not yet clear and understanding the number and order of steps in PM heat sensing and response is an active area of research.

The role of unfolded proteins in endoplasmic reticulum and cytosol: Protein misfolding in cytosol and endoplasmic reticulum (ER) under HS initiates unfolded protein response (UPR; Moreno and Orellana 2011). The UPR is mediated by membrane associated transcription factors (MTFs). They are mainly members of the basic leucine zipper (bZIP) family. In PM or ER, bZIP is present in a nonactive form under normal conditions (Liu and Howell 2016), but HS or misfolded proteins activate bZIP in the ER *via* Golgi resident-proteases (S1P and S2P). In a study on *Arabidopsis*, Zhang *et al*. (2017) has reported that bZIP28 and bZIP60 play important roles in UPR. Under HS, bZIP28 is translocated to the nucleus and interacts with 2-kb upstream promoter elements of heat induced genes (directly regulated by bZIP). Most of these genes are involved in protein folding under ER-UPR (Zhang *et al.* 2017). The *bZIP28* and *bZIP60* genes both regulate the expression of a UPR marker gene, binding immunoglobulin protein (BIP3), and play an important role in plant heat stress tolerance (Table 1). These results suggest that HS dependent UPR is directed by bZIP28 (Zhang *et al.* 2017). It was also speculated that other TFs can also possibly operate on *UPR* target genes, which are under bZIP28 regulation. Along this, bZIP also interacts with COMPASS like components of *Arabidopsis* and adds H3K4 trimethylated histone marks to target genes to intensify gene expression (Song *et al.* 2015). However, *bZIP* downstream genes are not well characterized until now. Similarly, unfolded proteins in the cytosol activate cytosolic UPR (Mittler *et al.* 2012). Main players of the cytosolic pathway are HSFs, specifically HSFA2, which interact with promoter elements of HS inducible genes and regulate their expression (Table 1). Sensitivity of UPR may be lower than PM calcium channels because a mild change in temperature results in masses of unfolded proteins in the cell. It is assumed that initiation of UPR needs Ca^{2+} signals from PM heat sensing pathways, but how Ca^{2+} ions initiate this pathway and its role as a primary heat sensor need experimental validation.

The role of reactive oxygen species: ROS, a class of reactive forms of molecular oxygen, play a dual role in the cell: signaling molecules and toxic by-products. The ROS are involved in multiple abiotic stress signaling pathways. Plants use ROS because they are small molecules and can be easily synthesized. The concentration of ROS inside the cell must be precisely controlled (Mittler *et al.* 2011). Accumulation of ROS in the cell plays a part in the early phase of HS response and effectively controls expressions of *HSFs* and accumulation of heat stress proteins (HSPs) as reported for rice and *Arabidopsis* by Wang *et al.* (2009). Accumulation of ROS due to biotic and abiotic stresses can be abscisic acid (ABA) dependent or independent (Yao *et al.* 2017).

Two different enzymes control production of ROS: a membrane bound NADPH oxidase (RbohD) and a cell-wall bound type-III peroxidase (Daudi *et al.* 2012). Ten respiratory burst oxidase homologs (Rboh) have been reported for *Arabidopsis*, and RbohD is most active and unique (Li *et al.* 2015). Accumulation of ROS in the cell is crucial and for this purpose, regulation of RbohD activity is indispensable (Table 1). Plants can also regulate RbohD by supervising activity of ethylene responsive factors (ERFs). In a study on *Arabidopsis*, Jiao *et al.* (2013) has reported that ERF74 and ERF75, two important MTFs, play roles in temperature sensing and HS response. Normally, EFR74 is inactive and fixed in the PM with the help of an acyl CoA binding-protein (ACBP) (Licausi *et al.* 2011). Upon HS, ERF74 is released, moved to the nucleus, binds with promoter elements of *RbohD* (GCC-sequence) present 1 709 kb upstream from the transcription initiation and induces its expression, which ultimately results in ROS production (Fig. 1). Importance of GCC in *RbohD* expression has been analyzed through the use of GCC-mutant promoters, and down-regulation of expression has been observed (Blomberg *et al.* 2012). This is an important mechanism for a quick response against a sudden change in temperature.

The role of ERF75 is less significant as compared to ERF74. In short, ERF74 and ERF75 work as on-off switches and control *RbohD* expression and build-up of ROS in the cell under variable environmental conditions. When the conditions return to normal, content of ERF74 decreases *via* N-end rule-pathway, and RbohD dependent mechanism is switched-off (Yao *et al.* 2017). Although the mechanism of controling ERF74 with the N-end rule has still not been elucidated, *ERF74* and *ERF75* knockout mutants have shown a reduced ROS accumulation and a high sensitivity to HS (Yao *et al.* 2017). It indicates that ERF plays an important role in ROS dependent stress response. A report has also shown that coronatine-insensitve-1 (COI-1) also regulates the formation of ROS through RbohD in the jasmonic acid pathway (Maruta *et al.* 2011).

Binding Ca^{2+} ions to specific RbohD domains or phosphorylation by CDPKs also control its function (Suzuki *et al.* 2011). By controling RbohD function, Ca^{2+} also plays a role in ROS accumulation (Fig. 1). Activation of RbohD and accumulation of ROS interact with calcium ion signaling pathways by a positive feedback mechanism and results in opening additional

PM calcium channels and, finally, intensify HS response (Mittler *et al.* 2012). Accumulation of ROS can also be regulated by the use of PM fluidity inducing chemicals or by inhibitors that block ROS producing enzymes. It has also been suggested that pathways of programmed cell death under HS are also activated by ROS accumulation in the plant cell (Königshofer *et al.* 2008).

A mechanism of ROS sensing at the PM may also involve redox-potential sensitive pathways. A high redox potential interferes with ROS sensor proteins directly by changing a redox potential or indirectly by oxidizing cell wall or PM components. One evidence in this regard is regulation of natriuretic peptide receptor 1 (NPR1) by an intracellular redox potential, which results in its monomerization and translocation to the nucleus where it up-regulates defense responsive genes (Rao and Chaitanya 2016). A grim reaper (GRI) protein is another strong candidate for ROS sensing (Wrzaczek *et al.* 2011). Peptides derived by GRI may induce cell death in response to the presence of superoxide radicals, but experimental evidence is required to support this mechanism.

Heat stress induced histone modifications: Experiments with heat sensing mutant *Arabidopsis thaliana* lines result in identification of an actin related protein 6 (*ARP6*) gene which plays a role in HS response. A subunit of a Swi2/Snf2-related ATPase (SWR1) complex, encoded by *ARP6,* operates in histone octamer development and adds histone cluster 1 family member Z (H2A.Z) in place of histone cluster 1 family member A (H2A) in the nucleosome. Content of H2A.Z in chromosomes is reduced in *arp6* mutant plants (Table 1). Nucleosomes containing H2A.Z can sense HS and regulate gene expression, but this sensing is completely temperature dependent because wild type plants grown at a low temperature show a similar transcriptome for the *ARP6* gene as compared with mutants (Röth *et al.* 2017). A high temperature displaces H2A.Z from the nucleosomes located at the promotor region of HS induced genes and possibly upregulates their expression. However, a mechanism and role of this pathway in development of a basal thermotolerance is not clear and it needs further justifications. Evidence from *arp6* mutants supports this argument because a decreased H2A.Z

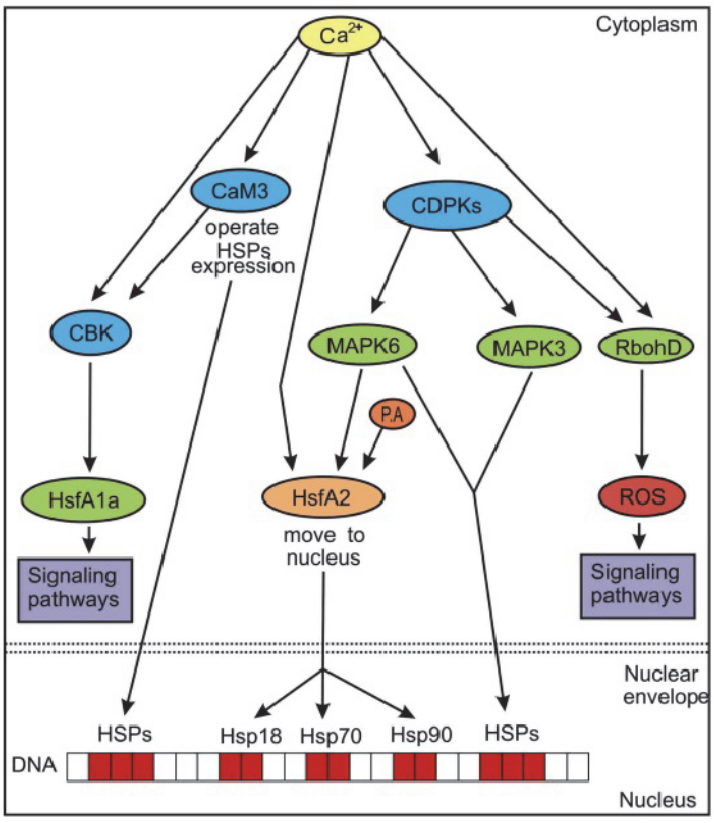

Fig. 2. A calcium signaling pathway. Inside the cell, Ca^{2+} interacts with multiple signaling molecules to initiate heat stress response. The Ca^{2+} directly interacts with heat stress transcription factors (HSFs) HsfA2 and HsfA1a *via* calmodulin 3 (CaM3) and a calcium/calmodulin binding protein-kinase (CBK) to operate heat shock protein (HSP) expressions. The Ca^{2+} also controls reactive oxygen species (ROS) accumulation by directly interacting with a respiratory burst oxidase homolog D (RbohD) or calcium dependent protein-kinases (CDPKs). Calcium also initiates membrane repair *via* a synaptotagmin A (SYTA). By regulating activity of master HSFs and ROS accumulation, calcium initiates expressions of other *HSFs* and *HSPs* and regulates enzyme activities and so prepare the plant to tolerate heat stress.

content in nucleosomes of *HSP* promoter regions interferes with binding certain TFs as reviewed by Mittler *et al*. (2012). However, the heat induced displacement of H2A.Z from promoter regions of *HSP* genes and regulation of their expressions in the absence of calcium ion signaling pathways still need experimental evidence.

Calcium signaling pathways

Inside the cell, Ca^{2+} interacts with and directs many signaling pathways (Fig. 2). First, inward calcium flux activates CaM3 and CDPKs. A multiprotein bridging factor 1C (MBF1C) plays its role as a co-activator of CDPK to operate expression of antioxidant enzymes (Qu *et al.* 2013). In the next step, CDPK activates MAPKs and RbohD (Suzuki *et al.* 2011). The MAPKs (MAPK3 and MAPK6) in the next step, regulate expressions of *HSPs* and play their roles in HS response (Wang and Huang 2017). The MAPK6 also phosphorylates HsfA2 and this phosphorylation on T249 position results in its nuclear localization (Evrard *et al.* 2013). It is purposed that all these genes are downstream to the Ca^{2+} signaling pathways, and calcium regulates expressions of *HSFs* and *HSPs* under HS.

Cellular Ca^{2+} content also regulates expression of *HsfA2c*, and a differential expression pattern has been observed under different treatments with calcium as noted by Wang and Huang (2017). The *HsfA2c*, up-regulated by Ca^{2+} or phosphatidic acid (PA), operates expressions of *HSP* genes, and a significant up-regulation of *Hsp18*, *Hsp70*, and *Hsp90* has been observed (Fig. 2). Repression in DNA binding ability of some *HSFs* due to Ca^{2+} chelators supports this argument. Calmodulin activated by Ca^{2+} is also involved in HS signal transduction in wheat and operates expressions of some *HSPs* (Wang and Huang 2017). The Ca^{2+} also up-regulates expression of *HSP70/90* which, in the next step, controls regulation of HsfA1a by protein-protein interaction (PPI). Crosstalk between different signal transduction molecules to induce expressions of HSF and HSPs is very complex and interconnected.

Regulation of heat stress response in plants

Key functional proteins expressed under HS are HSPs and ROS detoxifying enzymes. A number of proteins has been identified and classified into different HSP classes and they function as molecular chaperons to protect and reactivate proteins damaged by HS (Qu *et al.* 2013). The importance of HSPs in heat as well as in other abiotic stresses has been reported in literature but their exact function and specificity in target recognition is not yet clear. Production of ROS significantly increases under HS, and their imperative role in stress response has been already discussed. Enzymes detoxifying ROS are important HS inducible proteins to control buildup of ROS in the cell. Genetic mutants of ROS detoxifying enzymes have shown sensitivity to HS.

Heat sensors deliver signals through a series of reactions to HSF-regulatory proteins and precisely operate their activation and function at transcriptional and translational levels. Recent evidence has shown that many factors regulate HSF expression under stress conditions (Morimoto *et al.* 2013, Sato *et al.* 2014, Ohama *et al.* 2016, Ahanger *et. al.* 2017) (Fig. 3). Eukaryotic HSF shows a certain level of homology, forms trimers, and binds to the *cis*-elements of heat shock elements (Scharf *et al.* 2012). Plants have many TF families having dozens of members and showing unique properties compared with animals. The plant HSFs are broadly classified into three classes: A, B, and C. This classification depends upon the presence of a DNA-binding domain and an oligomerization domain (Li *et al.* 2017). All HSFs share heat shock elements in their promoter regions, which indicates that they are either auto-regulated or other HSFs operate their expressions.

Regulation of heat stress response at the transcriptional level

A heat shock transcription factor A1 (HsfA1), known as a 'master regulator of HS', plays a key role in HS response. Knockout mutants *HsfA1* of *Arabidopsis* have shown sensitivity to HS due to a decreased expression of heat stress responsive (*HSR*) genes as reported by Yoshida *et al.* (2011) and Liu *et al.* (2011). The HsfA1s directly regulate expression of several HS responsive TFs such as HsfBs, HsfA2, HsfA7a, a multiprotein bridging factor 1C (MBF1C), and a dehydration responsive element-binding protein-2A (DREB2A) (Yoshida *et al.* 2011). Many other HSFs have been identified in model plants. Here, we review recent findings about some important HSFs.

HsfA1: The HsfA1 is considered as a principal and fundamental regulator of initiation of HS response in plants. Although HsfA1 regulates gene expression under HS, its role is rather less significant compared with other HsfA family members, which indicates that activity of

HsfA1 is tightly regulated (Ohama *et al.* 2017). Post-translational modifications, PPI, and HSP70/90 play imperative roles in HsfA1 activation and regulation.

The HsfA1 is the main regulator of HS response in *Chlamydomonas*, and phosphorylation plays an important role in its activation as it is highly phosphorylated upon HS. An experiment designed to inhibit kinase activity has shown a decreased expression of *HSPs* under HS (Schmollinger *et al.* 2013). Calcium ions or AtCaM3 are imperative for phosphorylation of HSFA1a under HS. First, AtCaM3 activates calcium/calmodulin binding protein-kinases (CBKs), which, in turn, performs phosphorylation. Protein-phosphatase 7 (PP7) dephosphorylates HSFA1a, but this needs further verification (Mittler *et al.* 2012). The DNA binding ability of HsfA1 is regulated by protein kinases, *i.e.*, a cyclin dependent-kinase A1 (CDKA1) and a calmodulin binding-protein kinase-3 (CBK3) in *Arabidopsis*, and it plays an important role in this regard (Fig. 3). Studies have shown that *pp7* and *cbk3* mutants exhibit a variable thermotolerance as reviewed by Ohama *et al.* (2017). It is difficult to predict the importance of phosphorylation for activation of HsfA1 because a range and level along with timing and sites of phosphorylation are not yet elucidated.

Under certain conditions, PPI negatively regulates activity of HsfA1. The HSP70 and HSP90 (HSP70/90) interact with HsfA1 and decrease its transactivation and change nuclear localization (Fig. 3). The presence of a specific domain entitled as a temperature dependent-repression temperature dependent repression (TDR) domain responsible for HS activation has been reported for HsfA1 (Ohama *et al.* 2016). Under normal conditions, HSP70/90 interacts with TDR to press-down HsfA1, whereas HS release and activate it. Depression of HsfA1 by HSP70/90 dynamics is conserved across the kingdoms although mechanism of interaction in animals is different due to an absence of a TDR domain. It has been reported that HSP90 along with its co-chaperones rotamase FKBP 1 (ROF1) and ROF2 regulate expression of HsfA2, which is another example of PPI (Meiri *et al.* 2010).

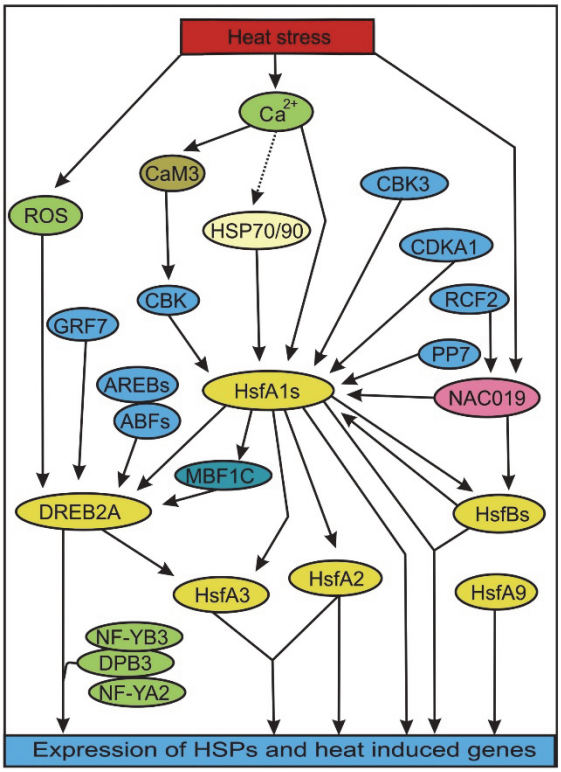

Fig. 3. Cross-talk between different heat stress transcription factors. Under heat stress (HS), heat stress transcription factors HsfA1s act as a master regulator to operate HS response. Under normal conditions, heat shock proteins HSP70 and 90 repress HsfA1s activity, but upon HS, calcium binding protein kinases (CBKs), a cyclin dependent kinase A1 (CDKA1), and calmodulin binding protein kinases 3 (CBK3) perform phosphorylation and activate HsfA1s which, in turn, regulate expression of a dehydration responsive element binding protein 2a (DREB2A), a multiprotein binding factor 1C (MBF1C), HsfA2, and HsfBs. The DREB2A is also activated by reactive oxygen species (ROS) and by abscisic acid responsive element binding factors/ABRE binding factors (AREB/ABFs). The HS also initiates expression of DNA polymerase ii subunit B3 (DPB3), nuclear factor Y, and subunits A2 and A3 (NF-YA2 and NF-YA3), which bind with DREB2A and enhance its activity to induce HS response. The HsfA2, activated by HsfA1s, in collaboration with HsfA3 also operates HS response. The HsfBs, activated by HsfA1s or by HS *via* NAC domain containing protein 19 (NAC019), also operate HS response and intensify HsfA1s activity. Some HsfA family members, such as HsfA9, independently regulate HS response.

DREB2A: Following induction of expression by HS, or by MBF1C, DREB2A along with its co-activators, a nuclear factor Y, subunit A2 or B3 (NF-YA2 and NF-YB3), regulate expression of *HsfA3* (Yoshida *et al.* 2011) (Fig. 3). Proteins DREB2 require post-transcriptional modifications for operation, and they directly interact with DRE1 and DRE2 motifs present in the promoter region of *HsfA3* and operate its expression. In the next step, *Hsf3A* controls expressions of *HSPs* as reported by Li *et al.* (2017). A DREB2A homolog DREB2C also induces expression of the *HsfA3* gene even under normal conditions, which indicates a potential role of DREB2C for induction of *HsfA3* under stress conditions (Chen *et al.* 2010). Studies done on mutants proved that DREB2s control activity of *HsfA3* in the heat regulation pathways. The DREB2A is also activated by ABA responsive-element binding-factors/ABRE binding-factors (AREB/ABFs: AREB1 & 2, ABF1 & 3), and a growth regulation-factor 7 (GRF7) presses down it under normal conditions (Fig. 3). The relation of ABA with DREBA2A under HS is not yet elucidated. It was found that *aba1* and *abi1* mutants have shown sensitivity to HS (Larkindale *et al.* 2005). A negative regulatory domain (NRD) rich in Ser- and Thr-motifs regulate DREB2A protein stability, and it acts as a degradation signal-sequence in eukaryotes (Phukan *et al*. 2017). Under non-stress conditions, the 26S proteasome pathway degrades the DREB2A protein. However, experiments planned to inhibit the 26S proteasome pathway have divulged that DREB2A protein can accumulate in the cell under normal conditions, but it is not operational (Ohama *et al*. 2017). It shows that post translational modifications, not the proteasome inhibition, is required for its stability (Anckar and Sistonen 2011). In his review, Ohama *et al*. (2017) have stated that the stability of a wild-type DREB2A is decreased under normal conditions. A constitutively active DREB2A (DREB2A-CA) that lacks NRD accumulates under non-stress conditions, and it also up-regulates many HS inducible genes (Ohama *et al*. 2017). Degradation of DREB2A is also organized by a radical induced cell-death 1 (RCD1) protein. A role of RCD1 to cut-down DREB2A has been justified with a protein isoform encoded by a DREB2A splicing variant, which lacks an RCD1 interacting motif (RIM) (Vainonen *et al*. 2012). The RCD1 links with RIM present at the C-terminal region of DREB2A to degrade it, however, the mechanism of its action is not yet elucidated.

It has been purposed that Ser and Thr residues present in the NRD may be target-sites for kinases, and these kinase dependent modifications activate DREB2A under stress (Agarwal *et al*. 2007). This argument has also been supported by the presence of Ser and Thr residues in all DREB2A homologs present across the species (Mizoi *et al*. 2013). Therefore, future research efforts should focus on discovery of novel DREB2A interacting factors that control and regulate it under normal and heat stress conditions.

Changes in the form of DREB2A due to post transcriptional regulations also play an important role in target selectivity during drought or HS elicited gene expression (Kim *et al*. 2011). A DNA polymerase II subunit B3-1 (DPB3-1) and NF-Y factors interact with DREB2A to regulate its activity. Expressions of *DPB3-1*, *NF-YA2*, and *NF-YB3* genes are induced under HS (Sato *et al*. 2014, Singh and Laxmi, 2015). Inside the nucleus, DPB3-1 and NF-YB3 form a dimer, which binds with the NF-YA2 to form a trimer and ultimately enhance activity of DREB2A to induce HS response (Sato *et al*. 2016). Another DREB2A interacting protein is MEDIATOR 25 (MED25). Enhancement of stress tolerance of *med25* mutant plants suggested that MED25 negatively regulates DREB2A function. Under stress conditions, MED25 emancipates DREB2A, which then operates HS response. Studies have revealed that DRE sequences restrain binding MED25 with DREB2A (Blomberg *et al*. 2012). However, the mechanism of MED25 interaction with DREB2A under HS is largely unknown and further research is required to uncover the detailed molecular functions of MED25.

Other HsfA family members: An HsfA2 group is another key player to operate HS response (Scharf *et al*. 2012). The HsfA2 operates downstream to HsfA1, but they have a strong interaction with each other to regulate HS response pathways although all genes involved in this pathway are not known yet. It has been reported that expression of HsfA2 is regulated by HsfA1d and HsfA1e, whereas HsfA1a/b has no effect on it (Nishizawa-Yokoi *et al*. 2011). On the other hand, Li *et al*. (2010) has shown that HsfA1a and HsfA1b interact with HsfA2 at the protein level as determined by bimolecular fluorescence complementation. This interaction indicates that activity of HsfA2 is also regulated by many factors (Fig. 3). Enhanced expression of HsfA3 in *hsfa2* mutant plants shows that it directly interacts with HsfA2 but they can also work independently (Li *et al.* 2017). This interaction has also been justified by treatment with β-galactosidase, nutritional deficiencies, and in a yeast two hybrid test system (Aumond *et al*. 2017, Li *et al.* 2017). Genetic and expression analyses have indicated that HsfA3 is present downstream to the HsfA2. They function in the same regulatory pathways, but the role of former is less significant (Li *et al.* 2017). They have a direct interaction at the protein level (Fig. 3). Similar screening tests have also proved a strong interaction of HsfA3 with HsfA1a and HsfA1b (Li *et al*. 2017). The HsfA2 and HsfA3 regulate expressions of HSP18 and HSP25 because *hsfA2* and *hsfA3* mutant plants fail to express them. The *HSFA2* and *HSFA3* play roles in prolonged heat stress, whereas *HsfA1s* plays a role in an early phase of HS response (Brestic *et al*. 2014). The *HsfA9* has shown a seed specific expression, and it induces expression of HSP genes when over-expressed under stress conditions as reviewed by Ohama *et al*.

(2017) (Fig. 3). Oxidative stress and HS induce expression of *HsfA4a*, which then regulate expression of *APX1*. Activity of HsfA4a under normal conditions is regulated by HsfA4 and HsfA5, they form a hetero-oligomer and destroy DNA binding ability of HsfA4a (Yan *et al*. 2017).

Identification of a novel HSR TF known as NAC domain containing protein 19 (NAC019) has been reported recently (Xie *et. al.* 2010, Fragkostefanakis *et al*. 2015). It binds to the promoters of some TFs, such as HsfB1 and HsfA1b, to regulate their expressions under HS, as an *nac019* mutant has shown an enhanced sensitivity to HS. The regulator of the C-repeat binding factor gene expression 2 (RCF2) activates NAC019 under HS as reported by Guan *et al*. (2014). Another NAC family member, jungbrunnen 1 (JUB1) has been reported to regulate expression of DREB2A (Wu *et al*. 2012), but how HS and oxidative stress induce expression of JUB1 is not yet clear. Members of HsfAs interact with each other to regulate their expressions. For example, HsfA5 represses the functions of HsfA4a and HsfA4c. It means that HsfA expression is not only repressed by HsfBs but also by its own members. The HsfB1, an important player of HS response, works in synergism with HsfA1a and operates expressions of HSP70 and HSP101. Synergism in HsfA1s and HsfA2 activities is also reported to regulate HS response (Yan *et al*. 2017).

HsfBs: Members of the class of HsfBs generally act as transcriptional repressors. The presence of a repressor domain, a short peptide sequence (R/KLFGV) nearby the C-terminal region, is responsible for this repression (Scharf *et al*. 2012). A distant role of tomato HsfB1 (SIHsfB1) has been reported (Hahn *et al*. 2011). The presence of a histone GRGK sequence motif helps SIHsfB1 to interact directly with a histone acetyltransferase of the CBP family 1. The HsfA2 and HsfB1 form a complex with HsfA1a to up-regulate and control its activity. Contact of HSP90 with HsfB1 has a dual effect. It not only aids in DNA-binding but also promotes HsfB1 degradation by a 26S proteasome, but this mechanism of degradation needs further exploration. Interaction of soybean HsfB1 with transcription factor IIB (TFIIB) by the C-terminal region gives an alternative function to it. Interaction of TFIIB decreases if the GRGK motif is absent, but such HsfB1 works as a repressor as reported for *Arabidopsis* HsfB1 (Ikeda *et al*. 2011). In his study on tobacco, Zhu *et al*. (2012) has reported that an enhanced activity of *Arabidopsis* HsfB1 leads to cell death. Reports indicate that a precise control is required to drive HSF expression for cell survival under normal or stress conditions (Zhu 2016). It not only aids in DNA-binding but also promotes HsfB1 degradation by a 26S proteasome.

Outlook and perspectives

Heat, one of the most severe abiotic stresses, negatively affects plant growth and yield. High temperature affects activities of all enzymes and disturbs normal metabolism. Research in the last few years has resulted in better understanding HS perception and response, but still some gaps exist, which need to be fulfilled. Plasma membrane sensors effectively pick up a change in temperature, but the mechanism is not completely understood, and a number of questions remains to be answered like how heat sensors recognized abrupt and gradual change in temperature, how stress signals are transferred to respective genetic units for early and late heat stress responses, and which sensor exactly sense temperature change at first and operate initiations of other sensors. It is speculated that different heat sensors simultaneously detect heat stress and initiate response. It is also envisaged that inward flow of Ca^{2+} ions through calcium channels, through damaged plasma membrane site, and transmembrane Ca^{2+} flow is also regulated, but controlling factors are not completely characterized. Development in genome editing techniques specifically in *CRISPR/Cas9* platform make it easy to develop genetic mutant and because of this reason, characterization of genetic units involved in heat perception and response will boost up (Hassan *et al*. 2017, Sajid *et al.* 2017). Control of gene expression by MTFs under abiotic stress is a novel method of gene regulation and many studies have confirmed their role (Seo 2014). Heat stress signal transduction cascades are connected at different levels to activate all signaling pathways. Some signaling cascades may not be beneficial so they must be turned off to ensure cell survival. Interdependence of signaling pathways provides multiple sites for manipulation. Development of heat tolerant cultivars is need under present conditions of climate change and population explosion so that world food demand can be fulfilled and for this, a better understanding the molecular mechanism involved in heat perception and response is imperative.

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