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Review

Beat the heat: plant- and microbe-mediated strategies for crop thermotolerance

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Heat stress (HS) affects plant growth and development, and reduces crop yield. To combat HS, plants have evolved several sophisticated strategies. The primary HS response in plants involves the activation of heat-shock transcription factors and heat-shock proteins (HSPs). Plants also deploy more advanced epigenetic mechanisms in response to recurring HS conditions. In addition, beneficial microbes can reprogram the plant epitranscriptome to induce thermotolerance, and have the potential to improve crop yield productivity by mitigating HS-induced inhibition of growth and development. We summarize the latest advances in plant epigenetic regulation and highlight microbe-mediated thermotolerance in plants.

Highlights

In nature plants encounter multiple stresses such as heat waves that negatively influence their growth and reduce productivity. Often, however, mild heat can induce tolerance to later subsequent stresses. In such circumstances, plants memorize the previous stress exposure and develop a memory termed thermopriming or thermomemory.

Epigenetic modifications play a crucial role in maintaining plant HS memory, and can be essential for adaptation to intermittent HS events. In recent years epigenetic modifications such as histone methylation and small RNAs have been shown to actively participate in thermomemory in plants.

Plant epigenetic modifications are not only shaped by mild stress but also by beneficial endophytic microorganisms. The use of these microbes as a tool to ensure robust responses under HS holds great potential to improve crop productivity in a sustainable manner.

Role of heat stress in plant growth and agriculture

Generally, HS occurs when the ambient temperature rises above a threshold level for a period that is sufficient to damage plant growth and development. Different plant species have specific temperature requirements for healthy growth [1]. For instance, wheat (*Triticum aestivum*) has an optimum temperature of 22°C for vegetative growth. Although a rise of 5–10°C can accelerate growth, temperatures above that range impair cellular processes, with adverse effects on development and reproductive success [2]. Each degree Celsius increase in global mean temperature is estimated to reduce the global yield of wheat by 6.0%, rice (*Oryza sativum*) by 3.2%, maize (*Zea mays*) by 7.4%, and soybean (*Glycine max*) by 3.1%. As a consequence of climate change, modified weather conditions cause heat waves in many parts of the world, resulting in significant crop yield losses. For example, Germany was listed as one of the most affected countries by a heat wave in 2018, with damage exceeding 3.5 billion USD in the agricultural sector. Therefore, it is imperative to produce high-yielding crop plants adapted to adverse HS conditions [3,4].

Plants have evolved thermotolerance mechanisms to combat HS, such as the production of HSPs and antioxidants. The production of HSPs is orchestrated by heat-shock factors (HSFs) and other transcription factors (TFs) [5–9]. However, depending on the magnitude, episode, and duration of HS, plants can deploy advanced mechanisms such as epigenetic modifications of chromatin to enhance HS tolerance. One such mechanism, termed thermomemory, is induced upon exposure of plants to mild non-lethal HS conditions. Plants then are capable of retaining a memory of the previous exposure to HS, thereby improving their survival upon reoccurring severe stress regimes. The duration of such stress memory can last for multiple days or may even be inherited to the next generation [10–13]. This type of mechanism can be very useful for the survival of plants under extreme conditions such recurring transient heat waves.

Microbes have helped plants to colonize this planet for millions of years, making mutual beneficial plant–microbe interactions an important part of natural ecosystems. The potential of specific microbes to enhance plant growth by decreasing the need for fertilizers has been recognized

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and applied in agriculture for a long time. The utilization of plant growth-promoting bacteria (PGPB) and plant growth-promoting fungi (PGPF) is considered as one of the ecological strategies to improve plant growth, leading to decreased pollution of agricultural soils and water in current agriculture [14–17]. The use specific microbes to improve crop performance under various abiotic stress conditions including HS has also received considerable attention. and microbes have been shown to modulate numerous cellular, biochemical, and molecular processes [18–20]. Such multifaceted action of microorganisms or their communities makes them strong and viable options for abiotic stress-mitigation strategies in crop plants, especially under the ongoing pressure of increasing climate change. We review here current knowledge regarding plant HS mechanisms and microbe-mediated thermotolerance in plants.

Primary HS responses in plants

HS negatively affects plant growth, physiology, and metabolism. HS arrests the photosynthetic process by reducing the enzymatic activity of chloroplast protein complexes [21,22]. For example, ribulose 1,5-bisphosphate carboxylase (Rubisco) and Rubisco activase are highly temperature-sensitive enzymes [22–25]. HS-mediated disruption of photosynthesis results in the accumulation of reactive oxygen species (ROS), causing protein, lipid, carbohydrate, and DNA oxidation which ultimately amalgamate to produce plant cell death [7,26]. At the developmental level, high temperatures stimulate early flowering, inhibit male and female gametophyte growth, impair anther opening, inhibit pollen germination, and can lead to abortion of early embryos as a result of disturbances in pollen tube guidance and fertilization [2,26–29]. Plants induce a primary heat stress response (HSR) via Ca^{2+}-dependent calmodulin (CaM3) and H_2O_2-induced mitogen-activated protein kinases (MAPKs) (Figure 1) [30–34]. Plants inherit a form of HS tolerance known as basal thermotolerance. In addition, plants can also acquire thermotolerance via prior acclimatization to high non-lethal temperatures over a short period of time, thereby enabling plants to survive exposure to subsequent high lethal temperatures.

Generally, plants adapt to HS via HSF- and HSP-mediated HSR. Plants have multiple HSFs, and there are 21 HSFs in arabidopsis (*Arabidopsis thaliana*) which are divided into three classes: HSFA, HSFB, and HSFC [5–9,35]. In the primary heat response, HSFA1a, HSFA1b, HSFA1d, HSFA1e, HSFA2, and HSFA3 play a pivotal role [9,36–38]. HSFA1s function as master regulators of HS-regulated gene expression in plants because they further bind to other TFs [39,40]. For instance, the transcription factor DREB2A (dehydration-responsive element-binding 2A) is a direct target of HSFA1 and further activates HSFA3 to sustain the HSR for longer periods (Figure 1) [9,41,42]. HSFA2 directly interacts with HSFA1s and acts as a key regulator of plant basal and acquired thermotolerance by activating the expression of HSR genes [43,44]. The expression of *HSFA1*s is regulated by heat shock cognate 70-1 (Hsc70-1). Under non-HS conditions, Hsc70-1 forms a ternary complex with HSFA1D and HSFA1E; this complex is disrupted upon HS and induces downstream signaling mechanisms to activate HSR (Figure 1) [45]. More recently, studies have shown a novel role of ethylene in HS. In addition to HSFA1s, ethylene response factors (ERF95 and ERF97), which are downstream targets of the ethylene signaling factors EIN2 and EIN3, also regulate the expression of *HSFA2* under HS conditions [46]. Furthermore, the circadian clock transcription factors REVEILLE4 (RVE4) and RVE8 mediate early HSR gene expression independently of HSFs and instead operate via ERFs. RVE4/8 regulates the expression of the downstream transcription factor *ERF53* and *ERF54* genes to further increase thermotolerance in plants in the afternoon [47]. These studies suggest an important role of ethylene in regulating HSR genes; however, the crosstalk between different TFs and different hormones requires further investigation.

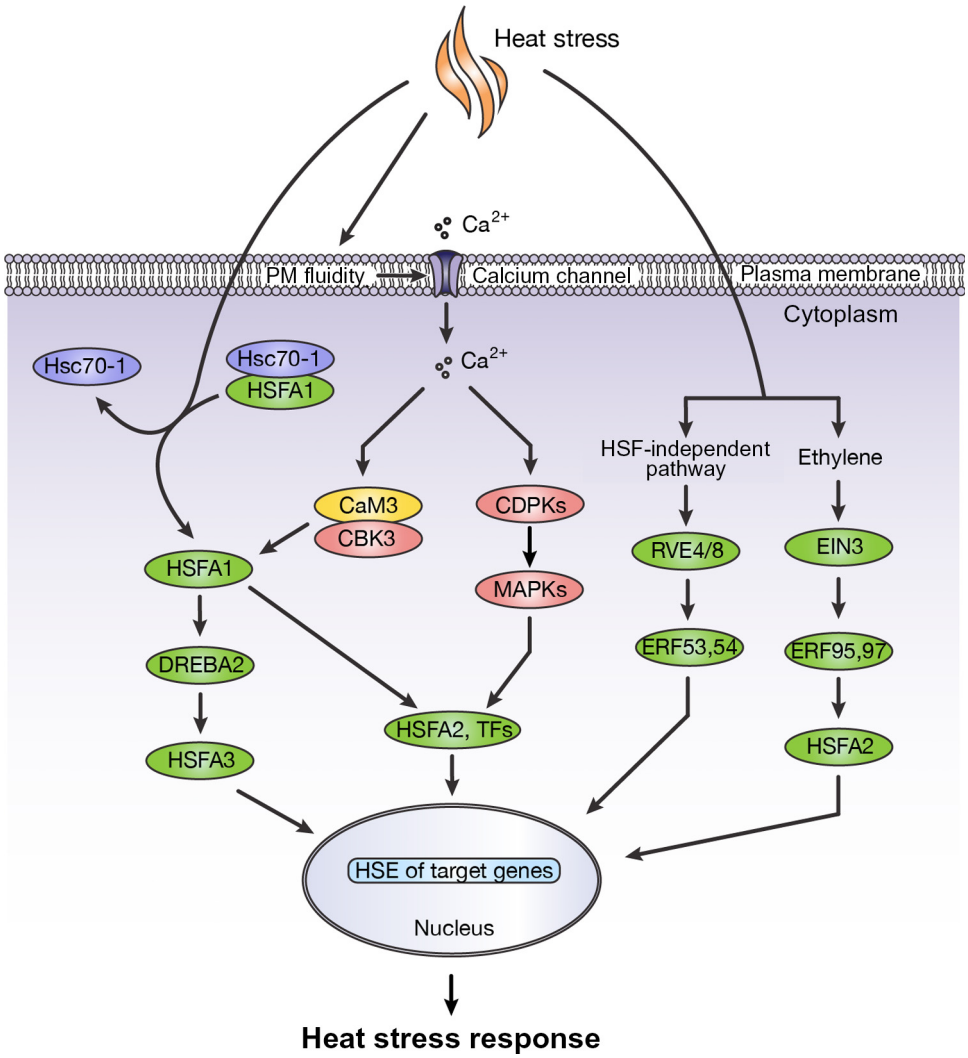

Figure 1. Diagrammatic representation of heat stress (HS) signaling and the primary heat stress response (HSR) in plants. Under normal conditions, heat shock cognate 70-1 (HSC70-1) represses the HSR by forming a complex with HSFA1, which dissociates upon HS. Free HSFA1 activates *HSFA2* and dehydration-responsive element-binding protein (*DREBA2*) to initiate the expression of further genes such as *HSFA3* in the HSR. HS changes plasma membrane fluidity resulting in calcium influx. Ca^{2+} influx activates calcium-dependent protein kinases (CDPKs), further activating MAPKs (mitogen-activated protein kinases) which phosphorylate HSFA2 and other transcription factors. Ca^{2+} also binds to calmodulin 3 (CaM3) and activates calmodulin-binding kinase CBK3 to activate HSFA1 by phosphorylation. HSFs directly bind to heat stress elements (HSEs) in the promoter regions of *HSP* genes to activate their transcription and contribute to the HSR. An HSF-independent pathway regulated by the circadian clock transcription factors REVEILLE4 and 8 (RVE4/8) also mediates early HS-induced gene expression. RVE4/8 regulates the expression of downstream ethylene response factor *ERF53* and *ERF54* genes to provide thermotolerance, especially during the afternoon. HS also triggers ethylene signaling by activating the transcription factor EIN3 to bind to the promoters of *ERF95* and *ERF97*, and these in turn regulate *HSFA2* expression and a large number of downstream targets including *HSP* genes to regulate the HRS. Arrows indicate positive regulation (heat stress transcription factor 1). Abbreviation: PM, plasma membrane.

Epigenetic regulation of HS

In recent years, epigenetic mechanisms have been shown to play an essential role in the adaptation of plants to different stresses. The chromatin structure is known to be altered by post-translational modification of histones including acetylation, mono/di/trimethylation, phosphorylation, and

sumoylation. These histone modifications can activate or repress transcription by generating either 'open' or 'closed' chromatin configurations, thereby regulating the accessibility of chromatin to transcriptional regulators. Characterization of the HSR revealed that epigenetic modifications play a role in acquired thermotolerance by enhancing the expression of a set of HSR genes during subsequent HS conditions [48–51].

Histone methylation as an epigenetic mark to provide thermotolerance

In arabidopsis, histone methylation mainly occurs on histone H3 at Lys4 (K4), Lys9 (K9), Lys27 (K27), Lys36 (K36), and on histone H4 at Arg3 (R3) and Arg17 (R17). These different methylation sites have different roles. Histone H3 methylated on K4 (H3K4me) and H3K36me mainly generate an open chromatin configuration to activate transcription, whereas H3K9me and H3K27me are responsible for closed chromatin states that result in transcriptional repression [51–53]. Plants remember the earlier exposure to heat via these histone methylation modifications. After a mild primary HS, plants show enrichment of di- and trimethylated histone H3 (H3K4me2 and H3K4me3) at loci of HS memory genes such as *APX2*, *HSP18.2*, and *HSP22*, thereby providing higher and longer expression of these HS memory genes which is responsible for the maintenance of acquired thermotolerance (Figure 2) [19,38,44]. The accumulation of H3K4me2 and H3K4me3 is HSFA2- and HSFA3-dependent. After priming, HSFA3 binds to HSFA2 and forms heteromeric complexes with other HSFs, which results in enhanced HS memory.

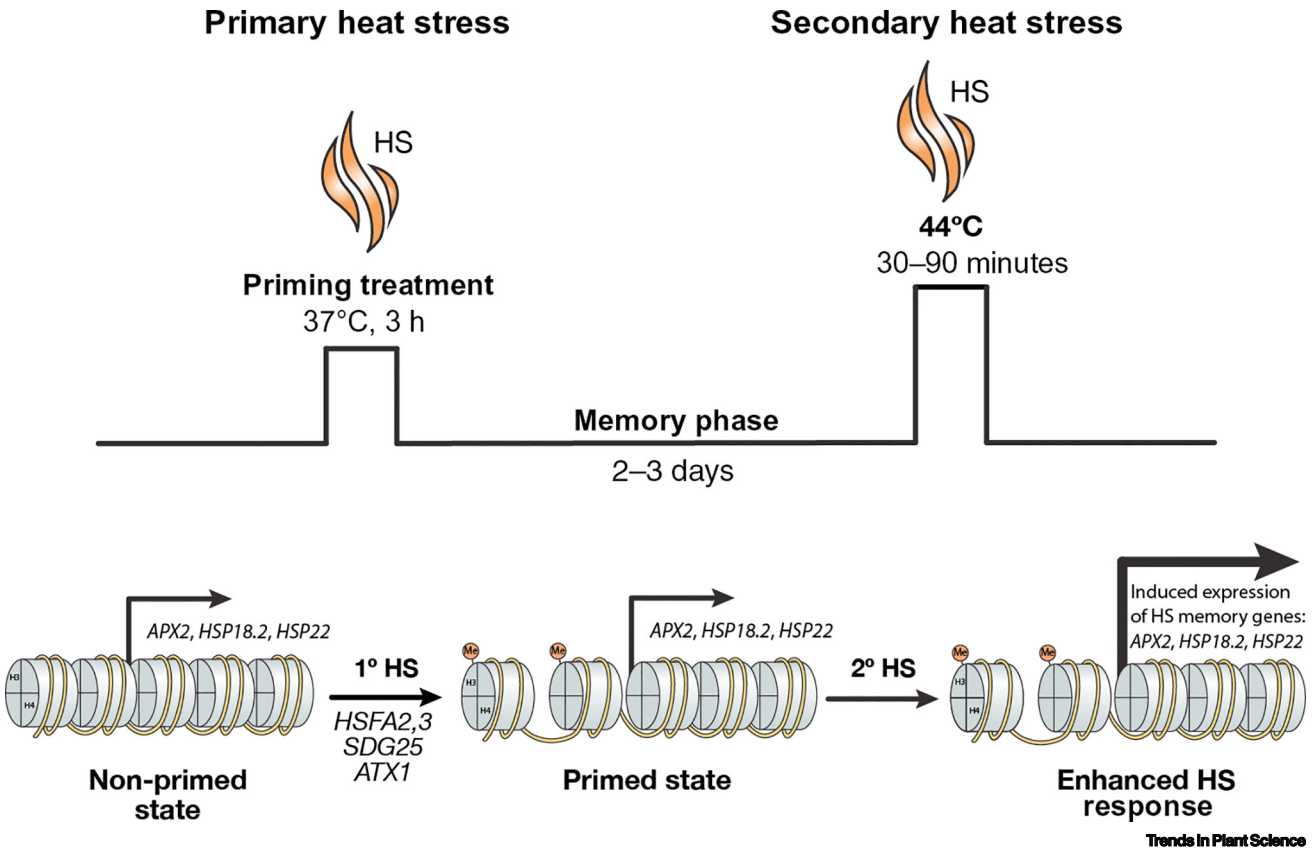

Figure 2. Schematic representation of epigenetic modifications involved in priming. Upon heat stress (HS), SDG25 and ATX1 prime the promoters of HS memory genes by histone H3 lysine 4 (H3K4) methylation via a HSFA2/3-dependent pathway. H3K4 methylation results in chromatin decondensation of HS memory genes, allowing faster onset of transcription mediated by HSFA2 and HSFA3 upon secondary HS.

These epigenetic modifications can be inherited in the next generation as transgenerational thermomemory mediated via HSFA2. A recent study by Song *et al.* [54] showed that histone H3K4 methyltransferases SDG25 and ATX1 induce histone H3K4me3 levels in arabidopsis (Figure 2). *Sdg25 atx1* double mutants show disruption of epigenetic memory and abrogate the longevity response to HS genes, suggesting that SDG25 and ATX1 play a role in maintaining stress-responsive gene expression during stress recovery. Gene regulation during HS is modulated by both positive and negative histone marks. After primary exposure to HS, the expression of HS memory genes declines gradually while H3K4me3 levels remain high. In this situation, the proper maintenance of repressive histone marks should also play an important role in the down-regulation of *HSP* genes. In this context, JUMONJI (JMJ) proteins, which are demethylases involved in regulating H3K27me3, maintain repressive histone marks on memory genes, indicative of JMJ-mediated control of HS memory [53,54].

How exactly the deposition of H3K4me occurs is not well understood, but the majority of H3K4me appears to be deposited by the COMPASS-like complex which is known to regulate different aspects of plant development [55]. Under abiotic stress conditions, unfolded proteins accumulate, and the COMPASS-like complex is recruited by the TFs bZIP28/60 which are required for the deposition of H3K4me3 at a subset of genes [56]. Under HS, it is assumed that the same mechanism might trigger the deposition of this mark at some HS memory genes. The deposition of the marks might occur by recruitment of the COMPASS-like complex by stress-specific TFs to particular genes. Several stress-induced TFs have been identified as key components of transcriptional memory and could account for this specificity in recruiting specific chromatin-regulatory proteins to target loci. For instance, upon HS, H3K4me deposition is dependent on HSFA2 and HSFA3. However, why HSFA2 and HSFA3 mediate this sustained chromatin modification remains to be further investigated.

Histone acetylation as an epigenetic mark for thermotolerance

Histone acetylation is mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation of histone weakens the interaction of DNA with histone and promotes chromatin decondensation resulting in enhanced transcriptional activity. The arabidopsis genome contains 12 HAT genes, among which *GCN5* (general control nonderepressible 5) encodes a key HAT that is required for gene expression changes in numerous plant developmental pathways and responses to environmental conditions, including HS [44,57,58]. GCN5 facilitates enhanced levels of histone H3 acetylation (H3K9/14ac) at the promoter regions of the *HSFA3* and *UV-HYPERSENSITIVE 6* (*UVH6)* genes which leads to higher transcription of *HSFA2*, *HSFA3*, and *UVH6* under HS. Therefore, loss of *GCN5* results in severe defects in thermotolerance [57]. H3K9ac enrichment in the promoter and coding regions of *HSP18.2* and *APX2* is associated with increased transcription and better thermotolerance after secondary HS [44]. Deacetylation also plays an important role in chromatin modification-mediated thermotolerance. In arabidopsis, 18 genes encode HDACs. Among the 18 HDACs, HD2C acts as a transcriptional repressor. The *hd2c* mutant plants show an increase in the global levels of H3K9K14ac and H3K4me2, and decreased levels of H3K9me2. Transcriptome analysis of heat-treated *hd2c* revealed upregulation of 25 *HSP* and four *HSF* genes, demonstrating that HD2C represses heat-activated genes by removing lysine acetylation at their chromatin loci [59,60].

Small non-coding RNA-mediated thermotolerance in plants

Non-coding RNAs such as *trans*-acting small interfering RNAs (ta-siRNAs), microRNAs (miRNAs), and long non-coding circular RNAs (lncRNAs) play an indispensable role in regulating HSR [51,61–63]. ta-siRNAs are involved in thermotolerance via post-transcriptional gene silencing. Arabidopsis has eight families of non-coding precursor genes that generate ta-siRNAs – *trans*-

acting siRNA precursor 1 (TAS1a-c), TAS2, TAS3a-c, and TAS4. Among these families, TAS1-derived siRNAs negatively regulate the HEAT-INDUCED TAS1 TARGET genes *HTT1* and *HTT2* under normal conditions [48,64]. However, exposure to HS decreases the abundance of TAS1-derived siRNAs, causing higher expression of *HTT* genes, which act as cofactors of HEAT SHOCK PROTEIN 70-14 (HSP70-14) to mediate thermotolerance. Plants overexpressing *TAS1* show reduced *HTT* gene expression levels and thermotolerance, whereas overexpression of *HTT* genes upregulates several *HSF* genes, resulting in greater thermotolerance. siRNAs are also linked with a Copia-type retrotransposon named ONSEN which becomes transcriptionally induced in arabidopsis seedlings upon HS. Arabidopsis seedlings impaired in the biogenesis of siRNAs showed ONSEN accumulation upon HS, indicating that siRNAs mediate silencing of ONSEN. The transcriptional regulation of ONSEN is attributed to HSFA2 because it binds to heat-response element (HRE) in the long terminal repeat (LTR) of ONSEN (Figure 3) [50,65]. Other microRNAs are also involved in thermotolerance. miR398 is a positive regulator of plant thermotolerance because it

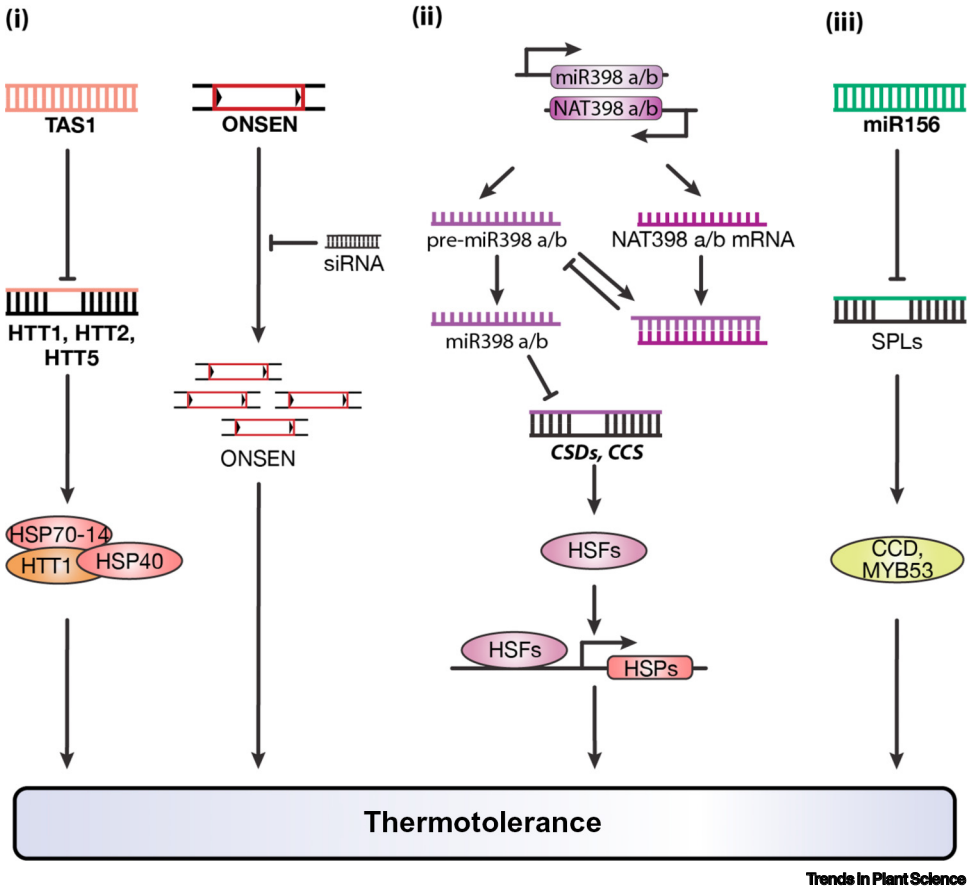

Figure 3. Schematic representation of small non-coding RNA-mediated thermotolerance in plants. *Trans*-acting siRNA precursor 1 (TAS1) negatively regulates heat-induced tas1 target (*HTT1*). HTT proteins bind to heat stress (HS) proteins HSP70 and HSP40 to enhance thermotolerance (i). The Ty1/Copia-type retrotransposon ONSEN is activated by HS and contributes to thermotolerance. The accumulation of ONSEN is mediated by small interfering RNAs (siRNAs) (i). miR398 represses copper/zinc superoxide dismutases (*CSD*) genes and copper chaperone for SOD1 (*CCS*), triggering higher expression of heat-shock factors (HSFs, and subsequently of heat-shock proteins (HSPs). miR398 is regulated by natural antisense transcripts (NATs). In *Arabidopsis*, *cis*-NATs of *MIR398* genes repress the processing of their pri-miRNAs (ii). In microRNA-mediated thermotolerance, miR156 represses Squamosa promoter binding-like transcription factors (SPLs). Downregulation of SPLs is essential to sustain higher expression of carotenoid cleavage dioxygenase (*CCD*) and transcription factor *MYB53* genes after HS (iii). Pointed and blunt arrows indicate positive and negative regulation, respectively.

suppresses the expression of its targets *CCS*, *CSD1*, and *CSD2* (copper/zinc superoxide dismutase), which are negative regulators of the HSR [66,67]. HSFA1B and HSFA7B can bind directly to the promoter regions of *MIR398* to induce its expression. miR398 is also regulated by natural antisense transcripts (NATs) because *cis*-NATs of *MIR398* genes repress the processing of pri-miRNAs, and deletion of *NAT398b* and *NAT398c* promotes miR398 processing and thermotolerance. These results suggest that *NAT398b/c* repress miR398 biogenesis and thereby weaken plant thermotolerance (Figure 3) [67]. Another miRNA, miR156, is highly expressed under HS conditions and its expression can persist for several days to promote the expression of HSR genes to maintain thermotolerance. The action of miRNA156 is mediated by members of SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE (SPL) transcription factors (Figure 3). Some members of the SPL family, such as SPL2 and SPL11, act as repressors of HSR genes such as *APX2*, *HSA32*, *HSFA2*, *HSP17.6A*, and *HSP22.0*. The function of miRNA156 is attributed to the repression of SPL2 and SPL11 expression through cleavage or translational repression, thereby activating HSR [68–71]. Lately, another category of small RNAs called circRNA was reported to be involved in HS. Pan *et al.* [62] reported 1583 heat-specific circRNAs. The study showed that many more circRNAs are expressed upon HS than under control conditions. A greater number of circRNAs were detected in the nucleolus compared to plastids and mitochondria, suggesting that they might play a role in gene regulation. We predict that circRNAs may interact with other regulators to control the expression of the HSR genes. However, no studies have addressed how circRNAs regulate HS [72].

Beneficial microbe-driven plant thermotolerance

HS is a major abiotic stress that limits the growth and productivity of plants worldwide. In this context, harnessing the potential of plant growth-promoting microbes could be an alternative strategy to mitigate HS in plants. Bacteria belonging to the genera *Pseudomonas*, *Bacillus*, and *Enterobacter*, as well as fungi such as *Paecilomyces formosus*, can improve the performance of plants including wheat, tomato (*Solanum esculentum*), soybean, sorghum (*Sorghum bicolor*), rice, and arabidopsis under HS [17] (Table 1). Beneficial microbes can modulate plant HS regulation via direct and indirect mechanisms, but the amelioration mechanisms are intricate and

Table 1. Microbe-mediated heat stress tolerance in plants

Plant species	Beneficial microbe	Mechanism	Refs
Triticum aestivum	*Pseudomonas brassicacearum*, *Bacillus thuringiensis*, *Bacillus subtilis*	Enhanced activity of antioxidant enzymes, and increased amino acid and protein content	[73]
Lycopersicon esculentum	*Bacillus cereus*	Reduced ABA, increased SA and antioxidant enzyme activities, and increased APX, SOD, and GSH levels	[15]
Arabidopsis thaliana, *Triticum aestivum*	*Enterobacter* sp. SA187	Ethylene-mediated enhanced expression of *HSP*, *HSF*, and *EIN2*, genes. HSFA2-dependent H3K4me3 enrichment at memory genes	[19]
Sorghum bicolor	*Bacillus cereus* TCR17	Reduced oxidative stress via upregulation of *SOD*, *APX1*, *CAT*, and stress-tolerance *HSP* genes	[75]
Sorghum	*Pseudomonas* sp. strain AKM-P6	induced biosynthesis of proteins, reduced membrane injury, higher levels of chlorophyll, sugars, and amino acids	[76]
Triticum aestivum	*Bacillus amyloliquefaciens* UCMB5113 or *Azospirillum brasilense* NO40	Reduced ROS, enhanced transcripts of *HSP* and *HSF* genes	[77]
Triticum aestivum	*Pseudomonas putida*	Reduced membrane injury, increased activity of SOD, APX, and CAT	[78]
Soybean	*Bacillus cereus* SA1	Increased SA, reduced ABA, increased expression of *APX*, *SOD*, *HSP*, *LAX3*, and *AKT2* genes, elevated GSH amino acid content, and enhanced potassium gradients	[82]
Japonica rice	*Paecilomyces formosus*	Lower endogenous level of AA and jasmonic acid, and increased total protein content	[80]
Triticum aestivum	*Bacillus velezensis* 5113	Metabolic and molecular reprogramming	[74]

most often are not well understood. Beneficial microbe-induced thermotolerance seems to be regulated by a complex network of signaling events occurring during the plant–microbe interaction. Experimental evidence using high-throughput techniques suggests that microbes induce HS tolerance by reprogramming the transcriptome and metabolome of plants to modify antioxidant enzyme activities, nutrient uptake, stomatal conductance, ion transport, carbohydrate metabolism, and phytohormone status [17,74,80]. In particular, a variety of ROS are generated when plants are subjected to HS. ROS can have a dual role: in low amounts, ROS work as signaling molecules to prepare the plants for stress responses. However, high levels of ROS can significantly affect plant growth and yield by directly affecting multiple processes including photosynthesis [31,81]. Under these circumstances, microbes can reduce ROS levels by enhancing the antioxidant capacity of the plant. This predominantly involves maintaining the levels of reduced glutathione (GSH) and ascorbate as well as enhanced activities of catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) that together help to protect photosynthesis [15].

Although it is clear that beneficial microbes enhance the expression of antioxidant genes, how they induce their upregulation is not well understood. Microbe-mediated stimulation of plant growth under HS may be explained by the regulation of plant phytohormone levels. Inoculation of plants with phytohormone-producing bacteria [such as indole-3-acetic acid, IAA, and bioactive gibberellic acid, GA] could mitigate the adverse effects of HS on tomato and wheat plants. Bioactive GAs and IAA are key phytohormones that play a vital role in plant growth, development, and tolerance to HS through the regulation of gene expression leading to activation of antioxidant enzymes, synthesis of osmoprotectants, and enhanced photosynthesis [82]. Under HS conditions, plants tend to produce more abscisic acid (ABA) to regulate ABA-dependent signaling. However, high ABA levels cause growth inhibition by inducing stomatal closure. Some beneficial plant bacteria reduce overshooting of ABA levels to ease the adverse effects of HS [17,82]. Salicylic acid (SA) is another plant hormone that plays an important role in plant abiotic stress management. The association of microbes with plants enriches the endogenous levels of SA in plants which in turn induces systemic resistance and ameliorates abiotic stress by inducing SA-mediated signaling. The association of beneficial microbes with plants might trigger the accumulation of low amounts of ROS, which further induces SA production, thereby activating SA-dependent signaling pathways to improve plant responses to HS [19,82]. Recently, ethylene has been linked to plant adaptation to HS. Ethylene signaling and HSR genes, including *HSP*s, form an intricate network of signal transduction for thermotolerance [46]. Ethylene is also triggered upon plant–microbe symbiotic interaction, which ultimately results in enhanced HSR gene expression and thereby increased thermotolerance. Higher expression of *HSF* and *HSP* genes enhances HS tolerance, and beneficial microbes can enhance *HSF* and *HSP* transcript levels in wheat and arabidopsis during HS [19,76]. In these studies it was shown that microbes modulate the plant transcriptome and metabolome, but how exactly microbes regulate these genes in plants is not well understood. A recent study in arabidopsis unraveled that the beneficial root endophyte *Enterobacter* sp. SA187 reprograms the plant transcriptome via ethylene-dependent regulation. SA187 produces 2-keto-4-methylthiobutyric acid (KMBA), which can be converted to ethylene *in planta*. KMBA is a sulfur-containing compound, and plant ethylene signaling is linked to the sulfur metabolism via SAM (*S*-adenosyl methionine) as a precursor of ethylene [18,83]. This suggests that microbe-produced compounds trigger ethylene signaling in arabidopsis to regulate HS tolerance via increased ERF-mediated expression of *HSFA2* and *HSP* genes. Interestingly, the presence of SA187 induces EIN2- and HSFA2-dependent H3K4me3 modification of HS memory genes such as *APX2* and *HSP18.2*, thereby generating robust constitutive plant thermotolerance (Figure 4). These results indicate that beneficial microbes prime plants to HS with *EIN3* and *HSFA2* as the main regulators of bacteria-mediated thermopriming.

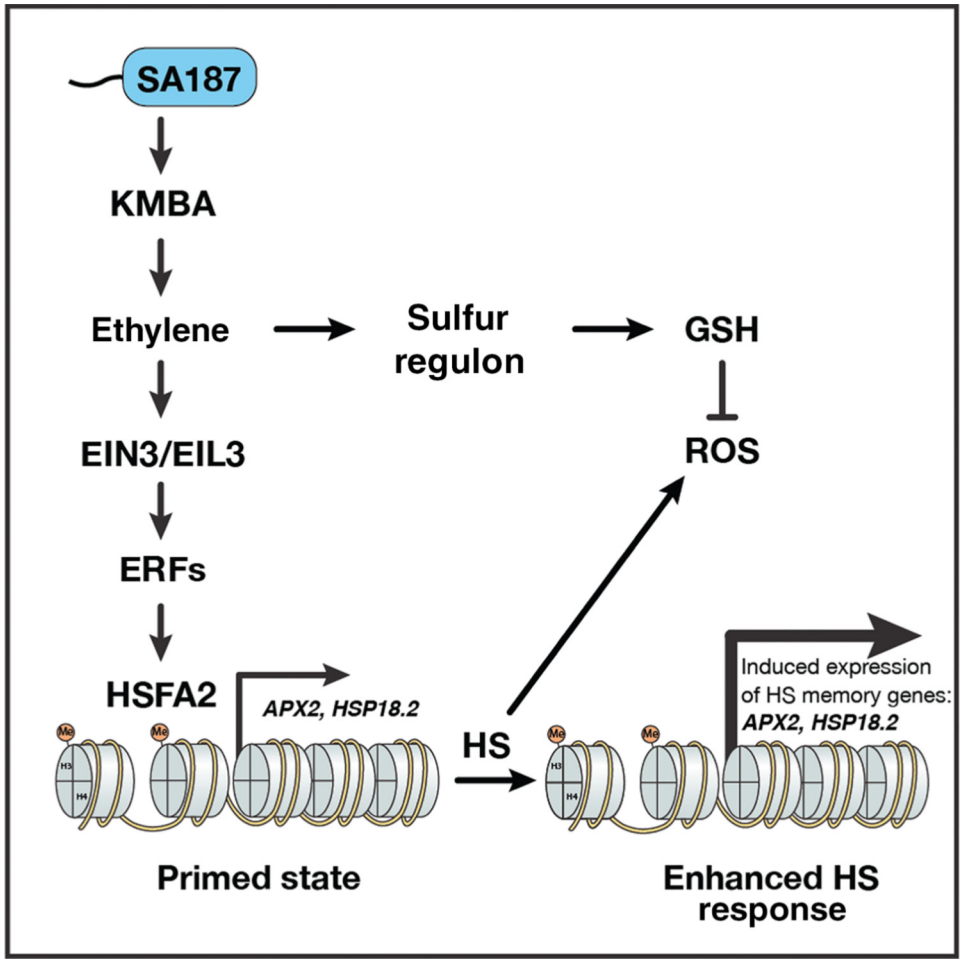

Figure 4. Schematic representation of plant thermotolerance mediated by the root endophyte *Enterobacter sp.* SA187. This bacterium produces 2-keto-4-methylthiobutyric acid (KMBA) which can be converted to ethylene in plants. Ethylene is linked to the sulfur pathway, and this pathway further regulates glutathione production. Glutathione (GSH) can block heat stress (HS)-induced reactive oxygen species (ROS) production by plants. SA187 also primes HS memory genes via ethylene signaling by HSFA2-dependent trimethylation of histone H3 (H3K4me3) at the promoters of memory genes such as *APX2* and *HSP18.2*.

Interestingly, whereas priming in acquired thermotolerance requires prior exposure of plants to high non-lethal temperatures, SA187 constitutively primes memory genes without any heat treatment. Moreover, in contrast to many transgenic approaches using HSF genes, SA187 did not show any negative effects on plants under ambient conditions. This mechanism has two potential applications in agriculture: (i) it offers the possibility to make plants thermotolerant in an ecologically sustainable manner, and (ii) it indicates which types of targets are likely to be of importance in molecular breeding of stress-tolerant crops.

Although our knowledge of the complex relationship between plants and microbes in HS is advancing, most of our understanding comes from non-crop plants in controlled experiments. We propose that forthcoming research efforts on the potential of beneficial microbes should explicitly focus on food crops.

Concluding remarks and future perspectives

Major progress has been made in the characterization of the HS regulatory networks in plants. However, despite this progress, many questions remain unanswered regarding the coordination between different TFs and their roles at different temperatures (see Outstanding questions). It is clear that plants use fine-tuned regulation of HSR, such as changes in chromatin structure for storing the information of previous environmental conditions, hence allowing plants to save energy by responding faster and/or more strongly to recurrent stresses. Recent evidence indicates that various beneficial microbes have the potential to improve crop yield under HS conditions [15,73–81], and one study showed that beneficial microbes use epigenetic mechanisms to improve plant thermotolerance [19]. There is a clear need for more studies into the complex interactions between plants and microbes and their role during HS, and these approaches promise to generate novel possibilities in agriculture to enhance plant performance and yield as a means to combat the effects of climate change and overpopulation on this planet that we all share.

Acknowledgments

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Declaration of interests

The authors declare no conflicts of interest.

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Outstanding questions

What defines the role of different TFs in different stresses? For instance, DREB2A, ERFs, and HSFA2 are also known to be involved in drought, salt, and high light stress, respectively.

Why does histone methylation enrichment in response to HS take place at particular gene loci and not at others?

A large number of circRNAs are induced upon HS, and a key question will be to identify the target genes that are epigenetically regulated by circRNA.

Beneficial microbes prime plants via H3K4me3 chromatin modification. However, few studies have shown similar or different chromatin modifications by beneficial microbes, and further investigation will be necessary to determine how general this mechanism is for beneficial microbes.

Normally, temperatures surpass thresholds for only a few hours in the afternoon, whereas flowers typically open in the morning. The effect of the potential mismatch between the time of flowering and that of the daily maximum temperature maximum remains unknown.

Given the challenges of climate change, one of the most important questions concerns how crop plants can be made more heat tolerant.

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