



# Ethylene involvement in the regulation of heat stress tolerance in plants

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## Abstract

Because of the rise in global temperature, heat stress has become a major concern for crop production. Heat stress deteriorates plant productivity and alters phenological and physiological responses that aid in precise monitoring and sensing of mild-to-severe transient heat stress. Plants have evolved several sophisticated mechanisms including hormone-signaling pathways to sense heat stimuli and acquire heat stress tolerance. In response to heat stress, ethylene, a gaseous hormone, is produced which is indispensable for plant growth and development and tolerance to various abiotic stresses including heat stress. The manipulation of ethylene in developing heat stress tolerance targeting ethylene biosynthesis and signaling pathways has brought promising outcomes. Conversely increased ethylene biosynthesis and signaling seem to exhibit inhibitory effects in plant growth responses from primitive to maturity stages. This review mainly focuses on the recent studies of ethylene involvement in plant responses to heat stress and its functional regulation, and molecular mechanism underlying the plant responses in the mitigation of heat-induced damages. Furthermore, this review also describes the crosstalk between ethylene and other signaling molecules under heat stress and approaches to improve heat stress tolerance in plants.

**Keywords** Ethylene · Heat stress · Phytohormones crosstalk · Plant tolerance

## Abbreviations

ACC	1-Aminocyclopropane-1-carboxylic acid	DRM	Domains rearranged methyltransferase
ACO	1-Aminocyclopropane-1-carboxylic acid oxidase	EBP	Ethylene-responsive element-binding protein
ACS	1-Aminocyclopropane-1-carboxylic acid synthase	EIL	Ethylene insensitive-like
AVG	Aminoethoxy vinyl glycine	EIN	Ethylene insensitive
CEF	Cyclic electron flow	EREB	Ethylene-responsive element-binding protein
CMT	Chromomethylase	ERF	Ethylene response factor
CTR	Constitutive triple response	ERS	Ethylene response sensor
		ETR	Ethylene response
		FPKM	Fragments Per Kb per Million fragments
		GEO	Gene Expression Omnibus
		Hsf	Heat shock factor
		HSP	Heat shock protein
		LHC	Light-harvesting complexes
		MCP	1-Methylcyclopropene
		NADPH oxidase	Nicotinamide adenine dinucleotide phosphate oxidase
		NO	Nitric oxide
		Nr	Never ripe
		OEC	Oxygen-evolving complex
		PCD	Programmed cell death
		PS	Photosystem

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RAP	The Rice Annotation Project
RNS	Reactive nitrogen species
SNP	Sodium nitroprusside
TAIR	The Arabidopsis Information Resource
UBP	Oligouridylate-binding protein

## Introduction

A rise in temperature above the threshold level for a certain period is sufficient to cause damage to crop plants and limit global plant productivity. However, the threshold level of temperature varies from species to species and among different compartments within the cell (Hasanuzzaman et al. 2013; Asseng et al. 2015). Heat stress hampers plant growth by affecting a multitude of biological functions that ultimately leads to reduction in plant growth and yield loss (Savada et al. 2017; Jegadeesan et al. 2018a, b). In response to such environmental cues, there is an intricate network of plant responses that together mediate plant growth and survivability under unfavorable conditions. Heat stress affects many cellular, physiological, and molecular functions leading to membrane dysfunction, protein denaturation, nucleotide damage, and accumulation of reactive oxygen species (ROS) which subsequently results in oxidative stress and may cause programmed cell death (PCD) in plants (Kuk et al. 2003; Santisree et al. 2017; Narayanan 2018). Prolonged exposure to heat stress disrupts membrane integrity and thus affects cellular functions (Xu et al. 2006; Asthir 2015). Moreover, heat stress also results in loss of photosynthetic pigments and efficiency, abnormal organization of thylakoids, disruption of the electron transport chains in mitochondria and chloroplasts, decreased production of photo-assimilates, and depletion of carbohydrate reserves (Khan et al. 2013; Tan et al. 2011; Cortleven et al. 2019; Paul et al. 2020). Furthermore, inhibition of carbohydrate metabolism induced by heat stress results in impairment of plant reproductive events such as stamen and pollen development, ovule fertilization, fruit set, seed development, and loss of grain yield in a wide variety of crops (Kotak et al. 2007; Wahid et al. 2007; Schauburger et al. 2017; Ozga et al. 2017; Liu et al. 2019).

Plants have evolved different mechanisms to cope up with the heat stress which include intrinsic heat stress tolerance in which plants have the integral ability to resist heat stress and acquired tolerance which enables the plants to survive under lethally high temperature following acclimatization to such temperatures over a short period (Liu and Charnig 2012; Song et al. 2012). Furthermore, plants develop adaptation strategies to protect themselves from heat-induced oxidative stress by physical changes within the plant body and frequently by raising signals for changing metabolism to restore the cellular redox balance and homeostasis (Park

and Seo 2015). The unfavorable effects of heat stress can be mitigated by developing dynamic approaches for improving thermotolerance to crop plants is crucial.

The involvement of phytohormones is not only in plant growth and in development, but could also be implicated in the plant response to heat stress to improve plant growth and yield (Dobra et al. 2015; Torres et al. 2017). The plant hormone ethylene (ET) is an important regulator of abiotic stress responses, ET accumulation at various range of concentration is associated with the plant responses to abiotic stresses that influence growth and development (Khan et al. 2014, 2020; Thao et al. 2015). Moreover, increased production of ET in the plant during heat stress results in a decrease of grain yield (Hays et al. 2007; Kaur et al. 2020), suggesting the concentration-dependent function of ET in plants. It is reported that enhanced production of ET leads to yield penalty/yield loss mainly by reducing reproductive attributes such as spikelet fertility and grain weight by accelerating senescence (Huberman et al. 2013; Savada et al. 2017). Heat stress regulates ET production majorly in the reproductive tissues such as floral, pedicel, and fruit tissues, and optimizes the dynamics of resource allocation (Savada et al. 2017). Exogenous treatment of ET (such as ethephon) plays an important role in thermotolerance by activating several stress-related proteins that are involved in maintaining functional integrity and stability of the plant cells (Jegadeesan et al. 2018b). ET signaling is also involved in the mitigation of heat stress-induced adversities in plants and enhancement of thermotolerance by reducing oxidative stress and maintaining chlorophyll content (Wu and Yang 2019). Moreover, heat-shock proteins (HSPs) and genes related to ET signaling are involved in the intricate network of signal transduction for thermotolerance (Wu and Yang 2019). Broadly, ET has crucial involvement in plant responses to heat stress and mediates thermotolerance.

Thus, it can be hypothesized that heat stress-induced ET production and its response could play a crucial role in plant defense and can mediate the significant improvement of thermotolerance in plants through modulating various mechanisms. Therefore, in this review, we briefly highlight the impact of heat stress on ET-mediated mechanisms and traits associated with thermotolerance which is involved in management strategies for ROS and reactive nitrogen species (RNS)-signaling and defense system in plants. Moreover, to increase our understanding of thermotolerance to plants, the crosstalk of ET with phytohormones and other signaling molecules are briefly discussed.

## Ethylene biosynthesis and signaling during heat stress

Research in the past few years has documented the role of ET in the regulation of heat stress responses in plants (Cheng et al. 2013; Takács et al. 2018; Pistelli et al. 2019; Wu and Yang 2019). ET, being a gaseous plant hormone, exhibit time and dose-dependent effects on the plants during heat stress conditions. While a low concentration of ET facilitates the activation of defense signaling in plants, its high concentration seems to be inhibitory in the cucumber and wheat during post-harvest storage (Chang et al. 2010; Khan et al. 2013; Riyazuddin et al. 2020). ET production during heat stress has been linked to the onset of leaf senescence and reduction in pollen germination, pollen tube growth, fruit set, spike fertility, and grain weight, which, however, seems to be a survival strategy to maintain the optimal progeny load under heat stress conditions (Valluru et al. 2017). High temperature, particularly in the range of 30–38 °C, induce a rise in the ET production in plants including *Phaseolus vulgaris* (Sauter et al. 1990), *Solanum lycopersicum* (Pan et al. 2019), *Mangifera indica* (Ketsa et al. 1999), *Actinidia deliciosa* (Antunes and Sfakiotakis 2000), and *Triticum aestivum* (Khan et al. 2013); however, at 40 °C, inhibition of ET production has been observed also in *Lycopersicon esculentum* (Biggs et al. 1988). In addition to these, some plants such as creeping bentgrass did not show any change in the ET concentration during heat stress at 35 °C, suggesting that HEAT-tolerant heat stress responses vary in different plant species (Larkindale and Huang 2005).

### Heat stress-induced ethylene production in vegetative tissues

The heat-induced ET production is positively linked to the adaptation to heat stress in plants. At first, it was shown that short-term heat stress causes transient inhibition of wounding induced ET production in etiolated pea stems; however, a threefold increase in ET production was observed after 80 min of wounding that was 1.36-fold higher after heat stress (Steed and Harrison 1993). Subsequent analysis of ACC content suggested that the drop in the ET concentration after short-term heat stress was because of the inhibition of ACC oxidation (Steed and Harrison 1993). Larkindale and Knight (2002) reported that pre-treatment of 100 µM ACC to Arabidopsis seedlings significantly enhanced heat stress tolerance. The survival rate of the Arabidopsis seedlings was increased by approximately threefold when the seedlings were pre-treated with ACC, while the lipid peroxidation was reduced by 50%.

Moreover, ET-insensitive mutant *etr-1* showed enhanced susceptibility to heat stress. *etr-1* mutants showed threefold increased lipid peroxidation and 50% reduced survival as compared to the WT (Larkindale and Knight 2002). Similarly, pre-treatment of a cool-season or creeping bentgrass (*Agrostis stolonifera* var. *palustris*) with ET precursor ACC enhanced the heat tolerance of the plants as evident by the turf quality and increased leaf photosynthetic rates (Larkindale and Huang 2004). ACC treatment also resulted in the enhanced activities of antioxidant enzymes including ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX), and reduced the lipid peroxidation by approximately twofold as compared to control. Although no significant increase in the endogenous ET concentration was observed in the heat stress-exposed cool-season grass in a follow-up study, the concentration of ET increased significantly upon recovery of the heat stress treated cool-season grass (Larkindale and Huang 2005). Recently, it was shown that the exogenous application of ACC improved the heat stress tolerance of rice seedlings (Wu and Yang 2019). ACC-treated seedlings showed reduced lipid peroxidation and relative electrolyte leakage during heat stress as compared to control. Besides, ACC treatment recovered the activities of antioxidant enzymes including CAT, APX, and POX which otherwise reduced during heat stress. Moreover, enhanced expression of various heat-shock factors, ET biosynthesis including *ACC oxidase 1* and *ACC oxidase 3*, and ET-signaling genes including *EIN-2*, *EIL-1*, and *EIL-2* were observed in the rice seedlings treated with ACC as compared to the untreated ones under heat stress conditions. ACC-treated rice seedlings showed higher chlorophyll content and less reduction in the fresh weight under heat stress as compared to the untreated seedlings. Moreover, ACC-treated seedlings showed reduced cell damage and lesser accumulation of H<sub>2</sub>O<sub>2</sub> as compared to the untreated seedlings under heat stress (Wu and Yang 2019). Taken together, these results commonly suggest that ET improves thermotolerance in plants by various mechanisms including reduction of oxidative damage and maintenance of chlorophyll content and overall leaf quality during heat stress.

In contrast to the above reports, some other studies revealed a negative effect of ET on heat stress tolerance in plants. It was shown that foliar application of ET inhibitor AVG delayed heat stress-induced leaf senescence by partially recovering the activities of antioxidant enzymes including CAT and SOD and reducing free radicals and H<sub>2</sub>O<sub>2</sub> content in creeping bentgrass (Xu and Huang 2009). However, lipid peroxidation was declined in response to AVG treatment during heat stress, suggesting that the heat stress-induced lipid peroxidation is mediated by the ET (Xu and Huang 2009). Another similar study showed that exogenous treatment of AVG suppressed leaf senescence,

improved turf quality, and chlorophyll content while reduced the relative electrolyte leakage during heat stress in creeping bentgrass; *A. stolonifera* var. *pencross* (Jespersen and Huang 2015). Furthermore, metabolite profiling showed accumulation of organic acids, sugar alcohols, and sucrose during heat stress after AVG treatment, while the levels of glucose and fructose were greatly reduced (Jespersen et al. 2015), suggesting that differential accumulation of metabolites involved in osmoregulation and antioxidant metabolism imparts tolerance to heat stress in absence of ET (Jespersen et al. 2015). Since both positive and negative effects of ET in heat stress tolerance in creeping bent-grass have been observed, it can be speculated that there is a cross-talk of ET with other phytohormones during heat stress and the outcome of the interaction is temporally and spatially regulated. The hint for this interaction was observed in wheat where it was shown that the salicylic acid improved the heat stress tolerance of wheat by increasing proline accumulation and by reducing heat stress-induced ET production (Khan et al. 2013). In the absence of salicylic acid, heat stress resulted in an approximately twofold increase in the ACS activity and a 1.5-fold increase in ET production as compared to the control. However, treatment of salicylic acid reduced ET production via decreasing approximately 34.3% ACS activity as compared to control, suggesting that ET and salicylic acid function antagonistically under heat stress in wheat (Khan et al. 2013).

### Heat stress-induced ethylene production in the reproductive tissues

The effect of heat stress and subsequent ET production has been investigated in multiple reproductive tissues including pollen grains (Firon et al. 2012), anthers (Jegadeesan et al. 2018a), stigma/style, ovaries (Savada et al. 2017), flowers (Jegadeesan et al. 2018b), kernels (Cicchino et al. 2013), developing grains (Dias et al. 2010), and fruits (Alba et al. 2005; Ketsa et al. 1999). While the majority of the studies showed a positive role of ET in heat stress tolerance, a few highlighted a negative interaction between ET and heat stress tolerance. For example, exogenous application of ET biosynthesis inhibitor improved grain weight under heat stress conditions in wheat (Hays et al. 2007), while exogenous treatment of ET releasing compound ethephon showed beneficial results in terms of kernel set and grain yield during heat stress conditions in maize (Cicchino et al. 2013).

Since pollen grains are highly heat-sensitive and the onset of heat stress during pollen development can directly influence the crop yield, the effect of heat stress on pollen development of tomato was analyzed using proteomics and transcriptomics approaches (Jegadeesan et al. 2018a, b). Heat stress resulted in the production of ET in pollens, anthers,

and flowers with upregulation of several genes involved in ET biosynthesis including *SIACS3* and *SIACS11*. Moreover, the upregulation of ET receptor *SIETR3* and downstream components of ET-signaling cascade including *SICTR2* was also observed in response to heat stress in mature pollens (Jegadeesan et al. 2018a). Furthermore, ethephon treatment improved the pollen viability and pollen germination following heat stress (Jegadeesan et al. 2018b). The proteome of heat-stressed pollen grains showed marked differences as compared to the proteome of control pollen grains; however, exogenous treatment of ethephon shifted the proteome of heat-stressed pollens close to the proteome of control pollen grains, suggesting the roles of ET in negating the negative effects of heat stress on pollen development. Moreover, pre-treatment of ethephon also resulted in the increased abundance of enzymes involved in responses to oxidative stress such as glutathione-disulfide reductase, glutaredoxin, and protein disulfide isomerase (Jegadeesan et al. 2018b). Similarly, heat stress led to the upregulation of the genes encoding for ACC synthase and ACC oxidase (*PsACO*) in the pre-pollinated ovaries together with the higher production of ET in pea (Savada et al. 2017). However, ET production was inhibited in the style/stigma and petals after pollination during heat stress, suggesting a tissue-specific modulation of ET biosynthesis during heat stress. Moreover, this study also suggested that the expression of *PsACO2* is more tightly associated with the ET production as up- and downregulation of this gene resulted in increased and decreased production of ET in various reproductive tissues of pea (Savada et al. 2017). Likewise, tomato ET-insensitive mutant Never ripe (Nr), defective in an ET response sensor (ERS)-like ET receptor, showed enhanced sensitivity to heat stress (Firon et al. 2012). Nr mutants showed the development of a low number and less viable pollen grains as compared to the WT under heat stress conditions. In addition, the mature pollens showed 60% less accumulation of sucrose as compared to the mature pollens of the WT. Furthermore, exogenous treatment of ethephon resulted in the increased quality and quantity of the developed pollen grains during heat stress in WT, while pre-treatment of AVG reduced the number of germinating pollen grains by more than fivefold during heat stress in WT as compared to control (Firon et al. 2012). In the case of fruits, Nr mutation inhibited tomato fruit ripening in tomato and affected fruit carotenoid biosynthesis, ET production, and expression of approximately 37% of the total identified genes during fruit maturation (Alba et al. 2005). Mature green Nr fruits were longer, developed thicker outer pericarp, fewer locules, and fewer mature seeds as compared to the WT. Moreover, these Nr mature green fruits also showed a higher accumulation of reduced ascorbate than WT (Alba et al. 2005).

In wheat, heat stress-induced spike in ET concentration has been observed in both bread wheat and durum



wheat during grain filling stages (Dias et al. 2010). In heat stress susceptible cultivar of wheat, heat-stress resulted in a 12-fold increase in the ET concentration in the flag leaf, while embryo and kernels showed a sevenfold and sixfold increase, respectively (Hays et al. 2007). However, in case of heat stress-tolerant cultivar, no such heat-induced change in the ET concentration was observed in response to heat stress. This heat stress-induced spike in ET concentrations in susceptible cultivar resulted in kernel abortion, reduced grain set per ear, and reduced kernel weight. Pre-treatment of ET inhibitor 1-MCP did not show heat-induced kernel abortion and reduced kernel weight, further confirming the involvement of heat-induced ET in kernel abortion and reduced kernel weight (Hays et al. 2007). In case of kiwifruits, propylene-induced fruit ripening and subsequent quantification of ET production were studied at normal and heat stress conditions (Antunes and Sfakiotakis 2000). It was observed that propylene induced normal ripening of kiwifruits at 30–34 °C; however, ripening was greatly reduced at higher temperatures. At 38 °C, kiwifruits failed to ripe, while at 40 °C, ripening was completely inhibited. Subsequent analysis of ET concentration showed that heat stress of 38 °C reduced ET production, while it was completely inhibited at 40 °C. In the case of ACC, no change in its concentration was observed at 38 °C, while it was greatly reduced at 40 °C. The enzymatic analysis suggested that the decrease in the ET concentrations at higher temperatures was because of the decreased activities of ACC synthase and ACC oxidase. These results suggested that heat stress inhibits the ripening of kiwifruits by limiting the amount of ET production (Antunes and Sfakiotakis 2000). Similarly, heat stress resulted in the inhibition of ripening-associated ET production in tomato fruits because of the decline in activities of ACC synthase and ACC oxidase in the fruit pericarp (Biggs et al. 1988). However, this heat stress-induced inhibition of ET production was reversible as the transfer of tomato fruits to ambient temperatures even after prolonged heat stress showed a fourfold increase in the ET concentration as compared to the heat-stressed fruits. Interestingly, no ET production was observed in the heat stress-exposed pericarp disks following protein synthesis inhibitor cycloheximide treatment, suggesting that the recovered ET production in tomato fruits was not due to the reactivation of ACC synthase and ACC oxidase and was because of the de novo synthesis of both of these enzymes (Biggs et al. 1988). In yet another similar study, the link between the post-harvesting storage of tomato fruits at high temperatures and ET production was deciphered (Atta-Aly 1992). It was reported that post-harvest storage of tomato fruits at 20 °C showed the highest production of ET followed by a decline in the concentrations of both ET and ACC up to 30 °C. At temperatures above 30–35 °C, a further decline in ET production was observed; however, the ACC concentration

showed a sharp increase. The inhibition of ET production at higher temperatures was found to be reversible as shifting of heat stress-exposed fruits to 20 °C led to similar levels of ET production as compared to the controls. The conversion of ACC to ET was maximum at 30 °C and was inhibited at 35 °C, suggesting that inhibition of ET production at temperatures above 30 °C is probably because of the inhibition of ACC-to-ET conversion (Atta-Aly 1992).

### Ethylene signaling in plants under heat stress

An RNA-Seq-based transcriptome analysis of reproductive stage soybean leaves showed upregulation of various genes of ET-signaling cascade under heat stress conditions. Among several genes identified, an upregulation of 12 ET response factors (ERF) was observed (Xu et al. 2019) that play key roles in ET signaling (Riyazuddin et al. 2020). In particular, *ERF021* showed a marked 78.7-fold induction during heat stress conditions, suggesting the pivotal roles of ET in heat stress tolerance in soybean (Xu et al. 2019). Similarly, heat stress treatment to developing pollens led to the upregulation of multiple genes involved in ET biosynthesis and signaling. These genes included genes encoding for ACC synthase, *ER5*, *ER21*, *LeJERF1*, and *ER24* (a homolog of MBF-1) (Frank et al. 2009). In addition, a significant upregulation of genes related to ET biosynthesis and signaling including *ACO1*, *ACO4*, *EREB* (an *ERF*), and *ETR4* was observed in tomato under heat stress (Pan et al. 2019). In contrast, the treatment of 1-MCP abolished the CO<sub>2</sub>-induced heat stress responses without interfering with the endogenous ET production, suggesting that 1-MCP might interact with downstream components of ET signaling to regulate the heat stress response. Furthermore, 1-MCP treated plants showed reduced expression of *ERF1* during heat stress, suggesting the key roles of *ERF1* in regulating the CO<sub>2</sub>-induced heat stress responses in tomato. Moreover, the silencing of *ERF1* showed reduced levels of several heat-shock proteins and compromised elevated CO<sub>2</sub>-induced responses to heat stress (Pan et al. 2019). Similarly, overexpression of *ERF1* in Arabidopsis improved heat stress tolerance of transgenic lines as compared to the WT by enhancing the expression of heat tolerance genes (Cheng et al. 2013). In addition, Arabidopsis ET-signaling mutants, *ein2*, and *etr1* showed a 40% reduced basal thermotolerance than WT. However, in the case of acquired thermotolerance, only a minor difference was observed as the acquired thermotolerance of these mutants was 75–80% similar to the WT (Larkindale et al. 2005). Besides, the ET over-accumulating mutants *eto1*, 2, and 3 did not show any difference in the thermotolerance and was similar to the WT. Taken together, these results suggested the key roles of ET in heat stress tolerance in plants.

The plant hormone ET is putatively a key regulator in abiotic and biotic stress conditions. ET interacts with one of its

receptor complexes to trigger the inactivation of constitutive triple response 2 kinase, which initially dephosphorylates ET-insensitive 2 (Savada et al. 2017) and afterward initiates a cleavage on the C-terminal of EIN2, and eventually translocate it to the nucleus, where it activates EIN3/EIL1. EIN3/EIL1 further promotes the transcriptional cascade through regulating ERFs (Yoshida et al. 2011) which eventually control several genes under the ET-signaling pathway (Müller and Munné-Bosch 2015). Under heat stress, *ET receptor 1* and EIN2 mutants show lower survival rates; this finding suggested the involvement of ET signaling in acquiring thermotolerance (Larkindale and Knight 2002; Larkindale et al. 2005). Many studies reported that exposure to heat stress accelerates the production and accumulation of ET in most plant species (Field and Barrowclough 1989) (Antunes and Sfakiotakis 2000) while also decrease in some like tomato (Atta-Aly 1992). A recent study suggests that ET-mediated signaling confers thermotolerance in plants and facilitates regulations of heat-shock factors (Wu and Yang 2019).

Different environmental stress conditions have different mechanisms to acclimatizes and adapt to it. ET, a plant hormone, is reportedly an important regulator involved in heat stress responses. Here, we performed the in silico analyses of ET biosynthesis and signaling genes in Arabidopsis and rice in response to heat stress. The more details have been given in Supplementary file. First, we retrieved all ET-related genes of *Arabidopsis thaliana* and *Oryza sativa* from The Arabidopsis Information Resource (TAIR) and The Rice Annotation Project (RAP) databases, respectively. We further divided these genes into three categories; genes involved in the biosynthesis of ET, genes involved in ET signaling, and genes involved in ET perception. Finally, 28 genes were obtained from each *A. thaliana* and *O. sativa* which are enlisted in Supplementary table S1 and S2.

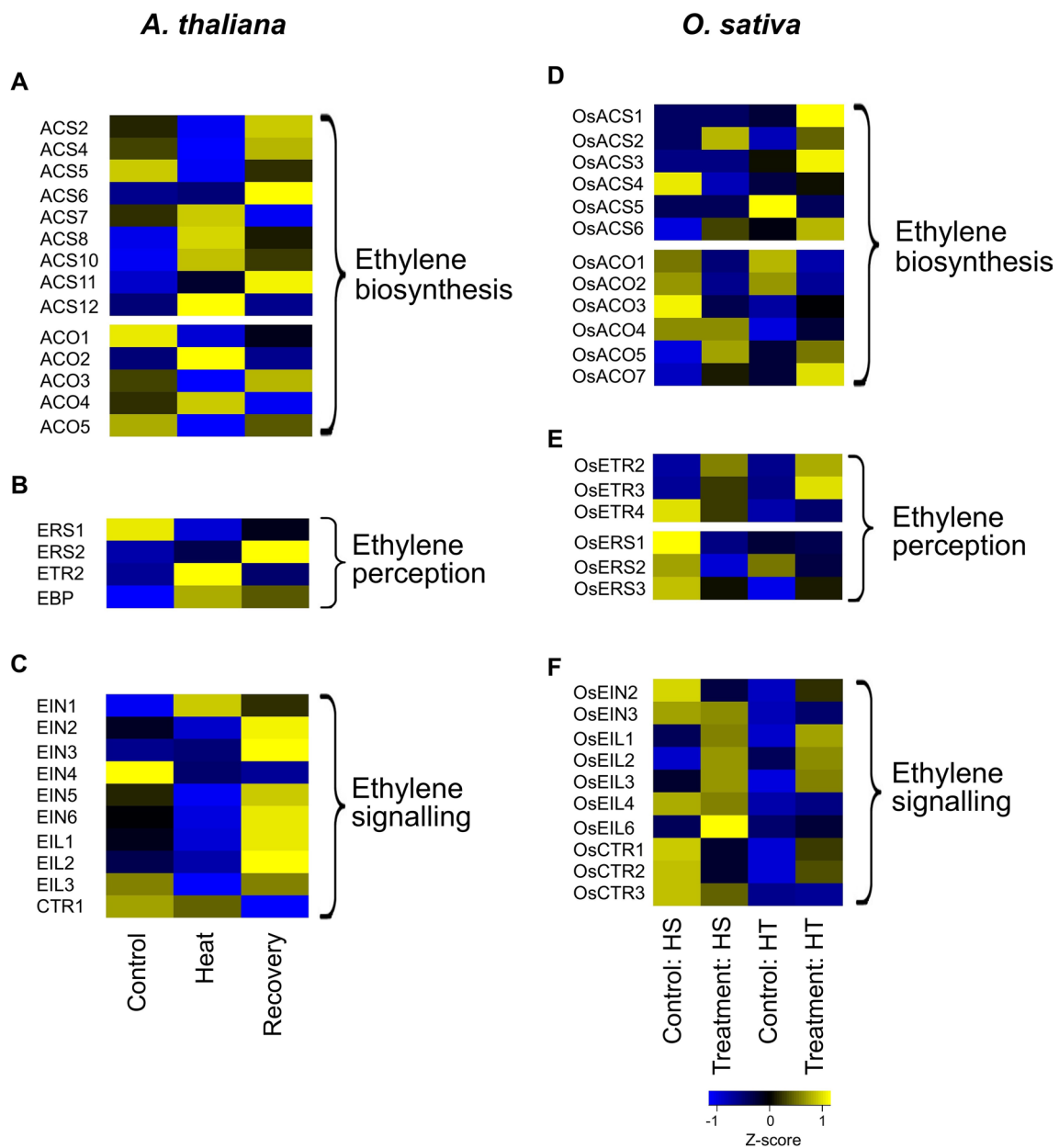
The normalized gene expression level from Arabidopsis from control, heat, and recovery conditions have been shown in sequential order in Fig. 1a, b, and c. The ET biosynthesis genes (1-AMINOCYCLOPROPANE-1-CARBOXYLATE: ACS and 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE: ACO) *ACS6*, *ACS7*, *ACS8*, *ACS10*, *ACS11*, *ACS12*, *ACO2*, and *ACO4* were strongly induced under heat stress, whereas *ACS2*, *ACS4*, *ACS5*, *ACO1*, *ACO3*, and *ACO4* were negatively regulated (Fig. 1a). All these genes retain their original expression level in the recovery condition. In rice, we used transcriptomic data from two different lines; heat-sensitive (HS) and heat-tolerant (HT). We found *OsACS2*, *OsACS6*, *OsACO5*, and *OsACO7* were upregulated in both the lines at heat stress, while, on the contrary, *OsACO1* and *OsACO2* get downregulated (Fig. 1d). ET perception genes in Arabidopsis (ENHANCER OF SHOOT REGENERATION: ESR, ETHYLENE RESPONSE: ETR, and ETHYLENE-RESPONSIVE ELEMENT BINDING PROTEIN: EBP) we found *ESR2*, *ETR2*, and *EBP* get

upregulated, while *ERS1* gets downregulated at heat stress (Fig. 1b). However, in rice, ET perception genes *OsETR2* and *OsETR3* were upregulated and *OsERS1* and *OsERS2* were downregulated in both the lines (Fig. 1e). ET-signaling genes (ETHYLENE INSENSITIVE: EIN, ETHYLENE-INSENSITIVE3-LIKE: EIL and CONSTITUTIVE TRIPLE RESPONSE: CTR) in Arabidopsis, EIN1 and EIN3 upregulated under heat stress, whereas EIN1 expression reverts, but EIN3 further upregulates. While EIN2, EIN4, EIN5, EIN6, EIL1, EIL2, EIL3, and CTR1 show reduced expression under heat stress (Fig. 1c). Most of these genes intensify their expression when placed in recovery condition except EIN4 and CTR1. While in rice, the ET-signaling genes *OsEIL1*, *OsEIL2*, *OsEIL3*, and *OsEIL6* were upregulated in both the lines, while no genes were found downregulated in both lines (Fig. 1f).

### Impact of heat stress on DNA methylation patterns of ethylene-associated genes

DNA methylation is one of the most important epigenetic mechanisms, which involve the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine. It has been shown to regulate gene expression by recruiting proteins involved in gene repression or by inhibiting the binding of transcription factor(s) to the functional region of DNA such as promoters (Zhang et al. 2018). It also plays a vital role in the growth and development of the plants (Finnegan et al. 1996, 2000; Xiao et al. 2006). In plants, along with CG methylation, DNA methylation is found to have a specific function in CHG and CHH contexts (H = A, C, or T). Previous findings show that in Arabidopsis, CG methylation is maintained by DNA METHYLTRANSFERASE 1 (MET1) (Finnegan and Dennis 1993; Vongs et al. 1993; Finnegan et al. 2000; Kankel et al. 2003), while methylation of CHG and CHH are maintained by CHROMOMETHYLASE 2 and 3 (CMT2 and CMT3) (Bartee et al. 2001; Lindroth et al. 2001; Zemach et al. 2013) and the de novo DNA methyltransferases DOMAINS REARRANGED METHYLTRANSFERASE 1 and 2 (DRM1 and DRM2) (Cao and Jacobsen 2002; Zhang and Jacobsen 2006).

Along with its essential role in the regulation of genes for growth and development, DNA methylation also regulates the genes with the stress response. NCBI Project number PRJNA587704 has BS-seq data of *A. thaliana* from control, HS 6 hr, HS 12 hr, HS 24 hr, recovery 6 hr, recovery 12 hr, and recovery 24 hr. We first perform the genome-wide DNA methylation in all three contexts: CG, CHG, and CHH, and analyses then checked the average methylation pattern in all samples for our all-28 ET-associated genes in Arabidopsis under heat stress. Globally, we found that CG methylation is overall higher in all up- and downregulated

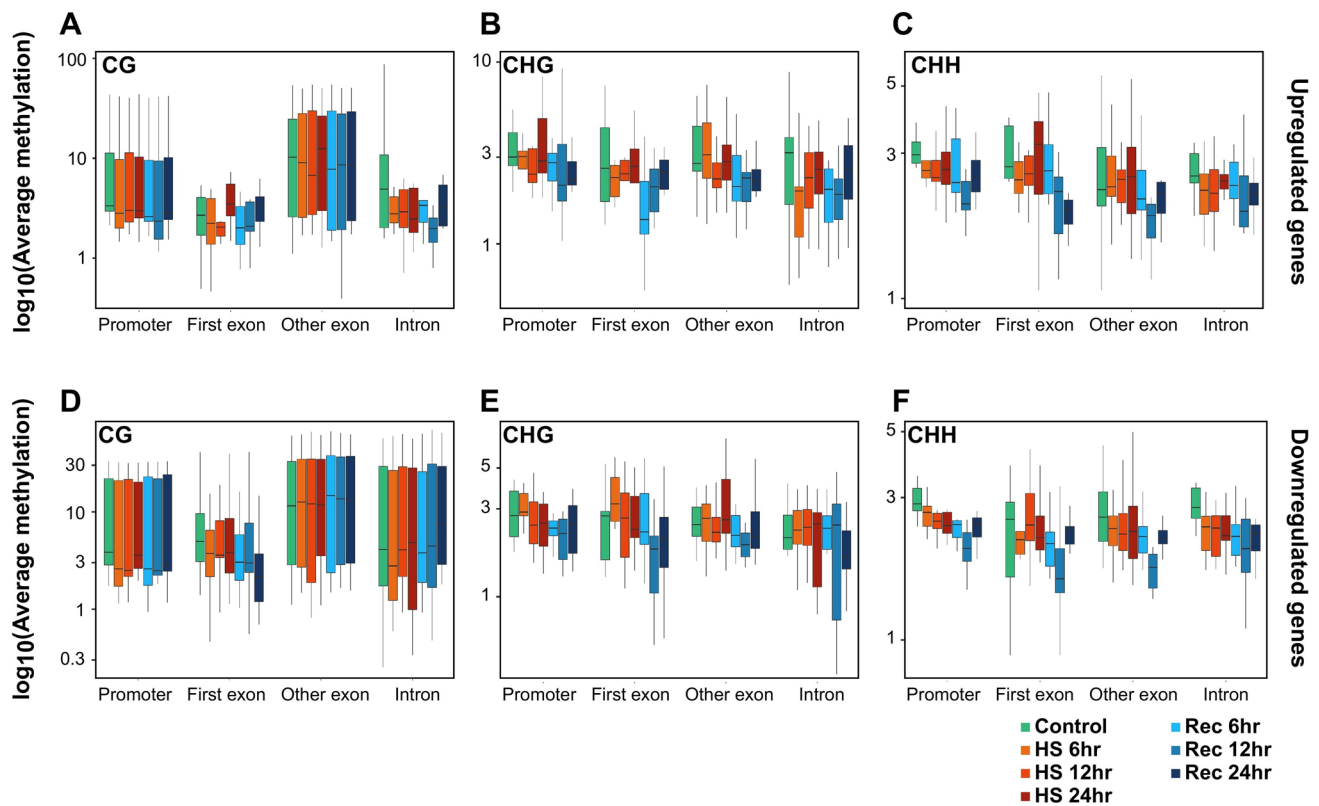


**Fig. 1** Gene expression (FPKM) profile of ethylene-associated genes from *Arabidopsis thaliana* and *Oryza sativa* at different temperature points. Gene expression of **a** 14 ethylene biosynthesis genes, **b** 4 ethylene perception genes, and **c** 10 ethylene signaling genes from *A. thaliana* at control (7 days in 22 °C), heat: HS (75 h in 37 °C) and recovery (3 days in 22 °C) conditions are shown. Similarly, gene expression from **d** 12 ethylene biosynthesis genes, **e** 6 ethylene per-

ception genes, and **f** 10 ethylene signaling genes from two variants of *O. sativa*; heat-sensitive (HS: XN0437S) and heat-tolerant (HT: XN0437T), at control (25 °C) and treatment (38 °C) conditions are shown. List of all these genes from *A. thaliana* and *O. sativa* are enlisted in Supplementary Table S1 and S2, respectively. RNA-seq raw data used for *A. thaliana* and *O. sativa* are from GSE132415 (Sun et al. 2020) and SRP050315 (Liao et al. 2015) respectively

genes in comparison to CHG and CHH (Fig. 2). This finding is consistent with the previous studies in *Arabidopsis* and *Brassica rapa*, suggesting that the relative proportion of mCs in CG context at the genome-wide level is highest compare to CHG and CHH (Wang et al. 2015; Liu et al. 2018). In upregulated genes, it was observed a significant increase in average CG and CHH methylation in the first

exons (start of the gene after TSS) under the highest stress level (HS 24 hr) and then started decreasing to retain its original position when subjected to recovery condition. A similar trend was also observed in other exons, but the extent of change is not high as in the first exon. However, CHG methylation in exons does not show such dynamics with the change in temperature (Fig. 2a, b, and c). Similar



**Fig. 2** Average DNA methylation value from *A. thaliana* categorized based on their contexts (CG, CHG, and CHH) and further sub-categorized into gene location under control heat stress (HS) and recovery conditions of differentially expressed genes.  $\log_{10}$  value of average CG (a), CHG (b) and CHH (c) methylation for upregulated genes location; promoters, first exon, other exons, and introns are shown.

Similarly, average CG (d), CHG (e), and CHH (f) methylation for downregulated gene are shown. For each plot, conditions are control, HS 6 hr, HS, 12 hr, HS 24 hr, Rec 6 hr, Rec 12 hr, and Rec 24 hr. Where HS if heat stress for 42 °C and Rec is recovery at control temperature. BS-seq raw data used for *A. thaliana* from PRJNA587704

trends were reported in *Brassica rapa* in the exons of the gene body (Liu et al. 2018). While in promoters and introns, a small decrease has been observed under HS and a pattern of increase to regain its original profile in recovery condition (Fig. 2a, b and c). On the contrary, CHG methylation shows a decrease in promoters and introns, and upon removal of HS, they try to regain its ambient profile. These observations suggest that in upregulated ET-associated genes, the expression is positively correlated with CG and CHH methylation in exons under HS, while negatively correlates when CG and CHH methylation present in promoters. In the case of downregulated genes, methylation of all three contexts decreases in promoters and first exons under HS and shows signs of recovery at 24 hr after removal HS (Fig. 2d, e, and f). This means that there is a decrease in methylation (CG, CHG, and CHH) in both gene-body and promoter regions of downregulated genes.

These findings for up- and downregulated gene expressions with DNA methylation goes in line with previous investigations which show that heat stress tends to increase the overall DNA methylation levels. This means that DNA

methylation at most of the locations has been increased, but for some locations, it also shows a decrease to some extent (Boyko et al. 2010; Gao et al. 2014). Therefore, we surmise that the modulation of methylation for differential gene expression and its change in expression under heat stress may be greatly different.

### Involvement of ethylene in ROS and RNS-signaling and defense systems under heat stress

The accumulation of ROS and RNS plays an important role in the regulation of plant responses upon heat stress (Wahid et al. 2007; Asthir 2015; Parankusam et al. 2017; Ali et al. 2020). Usually, ROS and RNS production rapidly becomes excessive in plants subjected to heat stress, but it depends on the severity and duration of heat exposure (Uchida et al. 2002; Locato et al. 2008; Pucciariello et al. 2012). Heat stress can cause deregulation and disruption of the electron transport chains in mitochondria and chloroplasts, as



well as influence the activity of NADPH oxidase in plasma membrane thus induces rapid production and accumulation of ROS in plant tissues (Wahid et al. 2007; Asthir 2015; Prerostova et al. 2020). Under heat stress, singlet oxygen ( $^1\text{O}_2$ ), hydroxyl radical ( $\cdot\text{OH}$ ), superoxide radical ( $\text{O}_2^{\cdot-}$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) can be formed rapidly, which are all strongly oxidizing compounds, and therefore, they can be harmful to cell integrity and can initiate programmed cell death (PCD) in plants (Vacca et al. 2004; Locato et al. 2008). Among them,  $\text{H}_2\text{O}_2$  is the most stable ROS, which is diffusible and can act as a toxic compound by inducing high oxidative stress or can function as a signaling molecule mediating heat stress tolerance by influencing antioxidant enzymes (Locato et al. 2008; Kocsy et al. 2013; Van Aken and Van Breusegem 2015). It is well known that the levels and activities of these non-enzymatic and enzymatic antioxidants are highly varying upon heat stress (Locato et al. 2008), which can be dependent on the action of defense phytohormones like ET or can be mediated by other signaling compounds such as the nitric oxide (NO) (Yang et al. 2011; Hasanuzzaman et al. 2012; Parankusam et al. 2017). ROS, especially  $\text{H}_2\text{O}_2$ , can act together with ET in a self-amplifying feedback loop where ET induces  $\text{H}_2\text{O}_2$  accumulation, which then enhances the production of ET, and thus, high level of  $\text{H}_2\text{O}_2$  and ET may initiate detrimental processes such as leaf senescence or chlorosis under heat stress (Wi et al. 2010; Xia et al. 2015). At the same time, ET could also regulate ROS metabolism by modulating antioxidant enzymes (Takács et al. 2018). In addition, ET-induced ERFs play a role in redox regulation and oxidative stress induces ERFs (Müller and Munné-Bosch 2015). It was found that HSPs have been upregulated in *ERF1* overexpressing Arabidopsis and less expressed in *ERF1* RNAi plants. Moreover, the overexpression of *ERF1* leads to proline accumulation and induces the expression of the key enzyme of proline biosynthesis (Cheng et al. 2013). In other cases, the overexpression of *ERF1* in tomato resulted higher proline accumulation and lower lipid peroxidation as well as increased the activity of antioxidant enzymes under salt stress (Hu et al. 2014). *ERF3* also regulated ROS metabolism in tobacco resulting in lower accumulation of ROS and enhancing various abiotic stress tolerance (Wu et al. 2008). These studies suggest the key role of *ERFs* in redox regulation which needs further investigations in the case of thermal tolerance.

Heat stress (43/38 °C) induced significantly higher ET production in winter rape (*Brassica napus*) seedlings and parallelly decreased the activity of SOD and POD, but increased lipid peroxidation based on the elevated malondialdehyde (MDA) content (Zhou and Leul 1999). In contrast to this observation, high accumulation of  $\text{H}_2\text{O}_2$  in the chloroplast, as well as increased activity of SOD, APX, and GR was measured in maize leaves exposed to heat (42 °C, 8 h). In parallel, higher levels of the ET-responsive protein were

detected which could be in relation to the induction of leaf senescence under heat stress in maize (Zhao et al. 2016). Similarly, heat stress (40 °C, 6 h) significantly increased ET production and  $\text{H}_2\text{O}_2$  content, as well as lipid peroxidation in wheat (*Triticum aestivum*) leaves after 30 days (Khan et al. 2013). The application of the ET biosynthesis inhibitor AVG also confirmed the role of ET in heat stress-induced senescence by modulating ROS levels. Authors found that AVG treatments significantly decreased  $\text{O}_2^{\cdot-}$  and  $\text{H}_2\text{O}_2$  production in heat-stressed (35/30 °C) perennial ryegrass (*Lolium perenne*) after 24 days. These changes positively correlated with the increase in SOD and CAT activity in these leaves (Zhang et al. 2019). Others also observed that heat stress (38 °C) rapidly induced the production of ROS in leaves of *Melissa officinalis*, since the peak in  $\text{H}_2\text{O}_2$  and  $\text{O}_2^{\cdot-}$ , the content was already detected after 1 h of the heat exposure (Pistelli et al. 2019). In parallel to these changes, the activities of SOD, CAT, and POD, as well as MDA content significantly increased under heat stress already from the first hour. In contrast, APX activity decreased, but the activity of MDHAR and DHAR increased in the heat-treated leaves (Pistelli et al. 2019). Interestingly, the authors measured significantly higher ET production only after 2 h which suggested that ET takes part in consecutive events to regulate heat tolerance and to protect plants against heat-induced oxidative stress (Pistelli et al. 2019). While heat shock (45 °C, 3 h) slightly elevated ACC content in leaves but decreased it in roots of *A. thaliana*. At the same time, ACC accumulation was significantly promoted by applying heat stress as pre-acclimation (1 h at 37 °C followed by 2 h at optimal temperature 20 °C) in this plant. In addition, suppression of NADPH oxidase and CAT activity was measured, but the activity of Cu/ZnSOD was elevated by heat shock (Prerostova et al. 2020). Interestingly, AsA levels increased in response to heat stress (35 °C), but it was not influenced by exogenously applied airborne ET (approximately 100 nL L<sup>-1</sup>) in holm oak (*Quercus ilex*) leaves. However, ET-treated plants showed lower levels of AsA than controls under strong heat stress (50 °C) and also above 35 °C when the heat was combined with drought stress (Munné-Bosch et al. 2004). Foliar application of ACC (100 μM) prior to exposure of plants to heat stress (35 °C) did not change the decreasing tendency in CAT and APX activity upon heat stress, but increased the activity of SOD and POD in creeping bentgrass (*Agrostis stolonifera*) shoots (Larkindale and Huang 2004). In contrast, it was found that rice (*Oryza sativa*) seedlings treated with a lower concentration of ACC (10 μM) showed a significantly higher survival rate and biomass production after heat treatment (45 °C). Moreover, ACC treatment ameliorated the heat stress-induced  $\text{H}_2\text{O}_2$  production as well as increased CAT, APX, and POX activities in heat-stressed leaves. At the same time, the application of ACC did not influence the heat-induced decrease in

SOD activity (Wu and Yang 2019). These results suggest that ET plays a significant role in ROS metabolism under heat stress. However, the effects of ET on promoting heat-induced senescence or thermal tolerance regulated by ROS are highly dependent on the plant species and organs with different sensitivity to ET and the levels of ET-ROS production, as well as other environmental conditions (e.g., light and drought).

Among ROS, superoxide and NO can generate the production of toxic RNS, such as peroxynitrite ( $\text{ONOO}^-$ ), which can initiate PCD at high concentrations in plants (Delledonne et al. 2001). However, NO can enhance tolerance mechanism in plants under various abiotic stress conditions including heat stress (Del Río 2015; Fancy et al. 2017). ET can cooperate not only with ROS but also with RNS during stress acclimation. At the same time, it was found that while NO generator sodium nitroprusside (SNP) stimulated significant emission of ET from Arabidopsis, exogenous ACC did not enhance the production of NO in calli (Wang et al. 2009) suggesting antagonism in ET-NO interaction (Kolbert et al. 2019). Despite the evident role of NO in maintaining thermal tolerance (Parankusam et al. 2017; Zhang et al. 2019), the interaction between ET and NO under heat stress remains poorly explored (Kolbert et al. 2019). Nevertheless, an increase NO production was observed after 2 h and an opposite trend in ET production was measured under heat stress (37 °C) in alfalfa (*Medicago sativa*) plantlets (Leshem et al. 1998). It can be concluded that further research regarding the temporal regulation of ET-NO interaction is necessary to understand the regulatory mechanism under heat stress.

### Involvement of ethylene in the regulation of photosynthetic components under heat stress

It is well known that heat stress can increase the fluidity of membranes, which can cause the disintegration of the lipid bilayer of cell membranes (Sharkey 2005). Thus, not only the heat stress-induced lipid peroxidation but also other changes in membrane lipid profile (e.g., changes in the lipid composition, decrease in the lipid unsaturation levels, and alteration in the ratio of the bilayer to non-bilayer-forming lipids) can lead to membrane damage, electrolyte leakage and cell death (Larkindale and Huang, 2004; Zheng et al. 2011; Narayanan et al. 2016). At the same time, growing evidence shows that ET can mediate significant changes in membrane lipid composition during heat stress (Zhang et al. 2020). It is known that ET contributes to decreasing in lipids levels (except phosphatidic acid), increasing in the ratios of digalactosyl diglyceride/monogalactosyl diglyceride, and phosphatidylcholine/phosphatidylethanolamine

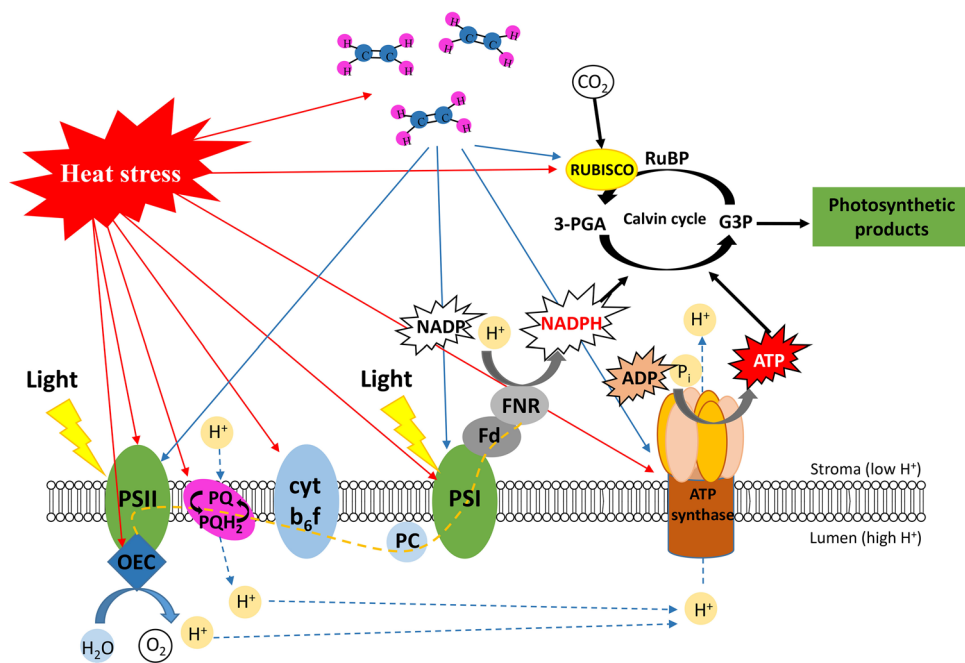
under senescence (Jia and Li 2015). Hence, membrane lipid remodeling through the regulation of lipid composition and fatty acyl group unsaturation under heat stress is a crucial step of tolerance mechanism, but eliciting the role of ET needs further research.

The photosynthetic apparatus, especially photosystem II (PSII) with its oxygen-evolving complex (OEC), as well as ATP synthesis and carbon fixation by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) are primary targets of heat stress in plants (Sharkey 2005; Murata et al. 2007; Allakhverdiev et al. 2008). At the same time, PSI, Cytochrome b559 (Cytb559), and plastoquinone (PQ) are also affected (Mathur et al. 2014; Ivanov et al. 2017; Hu et al. 2020). Thylakoid membranes are highly sensitive to heat stress and these can sense heat as a cellular thermometer inside chloroplasts (Horváth et al. 1998). Loss of membrane integrity, swelling of grana, decreased membrane stacking, and abnormal organization of thylakoids have been reported under heat stress in various plants (Downs et al. 2013; Yuan et al. 2017; Balfagón et al. 2019; Paul et al. 2020). Moreover, heat stress induces the redistribution and randomization of chlorophyll–protein complexes of both PSII and PSI (Ivanov et al. 2017; Hu et al. 2020). An increase in the fluidity of the thylakoid membranes causes dislodging of PSII light-harvesting complexes (LHC) and decreases the integrity of PSII (Mathur et al. 2014; Ivanov et al. 2017). OEC is one of the most sensitive parts of PSII and heat stress causes the loss of two out of four Mn from the Mn-containing cluster of OEC (Enami et al. 1998; Allakhverdiev et al. 2008). Besides the direct effects of heat on the photosynthetic apparatus, indirect outcomes such as the production of ROS are also significant which can destroy the components of the photosynthetic machinery and inhibit the repair of PSII and/or PSI (Murata et al. 2007; Allakhverdiev et al. 2008; Pospíšil 2016). Over-reduction of electron transport chain in chloroplasts results in enhanced ROS production. This increased amount of ROS, such as OH,  $\text{O}_2^-$ , and  $\text{H}_2\text{O}_2$ , can damage the reaction center (RC) of PSII (Asada 2006; Foyer and Noctor 2009; Suzuki et al. 2012). D1 protein, one of the key polypeptides of PSII reaction center, is highly affected by ROS, resulting in disruption of linear electron flow, reduced rates of oxygen evolution, as well as carbon fixation (Kato and Sakamoto 2009; Pospíšil 2016). Dysfunction in carbon assimilation processes is originated from the decline of the activity of Rubisco and/or Rubisco activase which are both heat-sensitive (Salvucci and Crafts-Brandner 2004; Demirevska-Kepova et al. 2005; Sharkey 2005; Perdomo et al. 2017). Thus, the photosynthetic capacity of plants, especially  $\text{C}_3$  plants rather than  $\text{C}_4$  plants, can be influenced more significantly by heat stress. The  $\text{CO}_2$  availability is also changed under heat stress as it induces rapid stomatal closure (decreased stomatal conductance,  $g_s$ ) which affects the intercellular  $\text{CO}_2$  concentration impairing the efficiency

of photosynthesis (Yang et al. 2006; Greer and Weedon 2012). In addition to the decline in Rubisco activity, ribulose-1,5-bisphosphate (RuBP) regeneration and ATP synthesis can also be caused by heat stress (Schrader et al. 2004; Yamori and von Caemmerer 2009; Ivanov et al. 2017). Thus, the limitation of photosynthesis under heat stress is associated with impaired ATP synthesis and metabolism. At the same time, the harmful effects of heat stress can be dependent not only on the damage of the photosynthetic apparatus but also on the activity of its defense and repair mechanisms (Murata et al. 2007; Ivanov et al. 2017). Moreover, not only the duration and quantity of heat stress determine the effects on photosynthesis but also the sensitivity of the tested plant genotypes or the developmental stages of the exhibited organs (Zhang et al. 2012; Ruocco et al. 2019; Obiero et al. 2020). From this aspect, the photosynthetic thermotolerance and energy-dependent defense responses of plants upon heat stress can be mediated by several phytohormones such as abscisic acid (ABA), jasmonic acid (JA) (Hu et al. 2020), or ET (Figs. 3, 4 and 5).

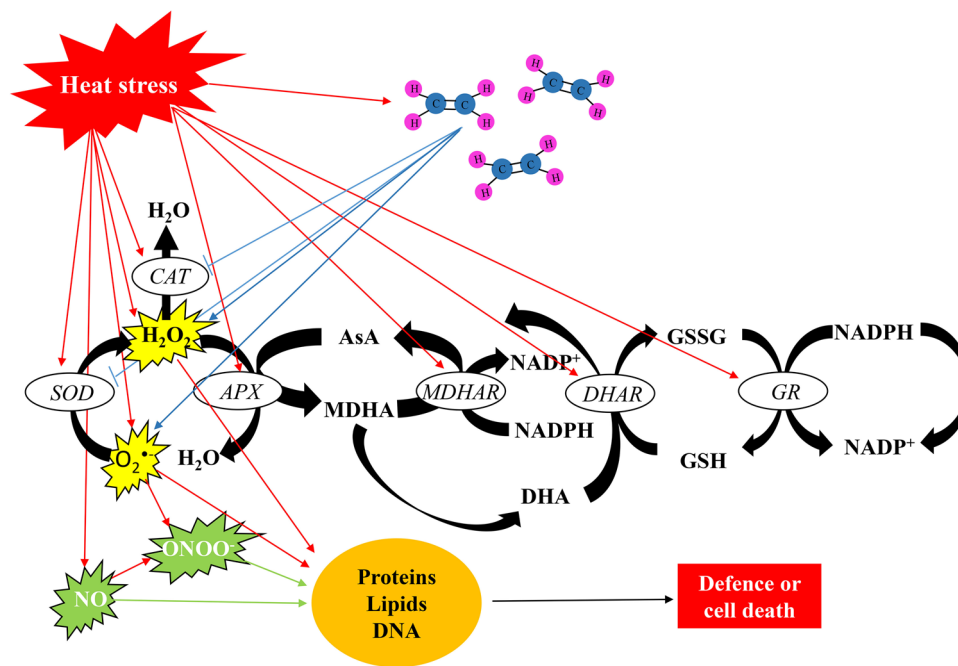
Protective responses of chloroplasts to heat stress cover multiple molecular, biochemical, biophysical, and physiological steps. These processes can be mediated by phytohormones such as ET. Interestingly, the effect of ET itself on plant photosynthesis is highly dependent on the duration

and concentration of its application (Borbély et al. 2019), it can inhibit (Taylor and Gunderson 1986) or stimulate photosynthetic activity (Khan 2004) differently by plant species and leaf ages (Ceusters and Poel 2018). At the same time, the direct effects of ET on photosynthesis are less investigated under heat tolerance. First of all, Munné-Bosch and his co-worker (2004) investigated the role of ET under heat stress. Airborne ET (approximately  $100 \text{ nL L}^{-1}$ ) did not influence significantly leaf hydration, net  $\text{CO}_2$  assimilation, stomatal conductance, the content of chlorophyll  $a + b$  and total carotenoids, and the maximal quantum yield of PSII (Fv/Fm) in holm oak exposed to heat stress (35 and 45 °C) under irrigated conditions. However, An and  $g_s$  significantly decreased upon ET under 50/52 °C. Moreover, ET also decreased the de-epoxidation state of the xanthophyll cycle (DPS) and  $\alpha$ -tocopherol ( $\alpha$ -Toc) concentrations in leaves exposed to 45 °C (Munné-Bosch et al. 2004). Heat stress (40 °C) significantly elevated ET formation by ACC synthase (ACS) in wheat which was effectively decreased by the application of salicylic acid (SA), and thus, photosynthetic  $\text{CO}_2$  assimilation and stomatal conductance were elevated (Khan et al. 2013). The exogenous application of ET inhibitor, AVG suppressed heat-induced senescence and improved heat tolerance in cool-season grass species creeping bentgrass (*Agrostis stolonifera*). Foliar application



**Fig. 3** A schematic illustration of the effects of heat stress and ethylene (ET) on the photosynthesis in plants. Heat stress can alter or damage the function or activity of photosystem II (PSII) with its oxygen-evolving complex (OEC), PSI, ATP synthesis by ATP synthase, and carbon fixation by ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO). ET also by concentration- and time-dependent manner influence activity of PSII, PSI, ATP synthase, and RUBISCO.

(*Cytb6f* Cytochrome  $b_6f$  complex, *Fd* Ferredoxin, *FNR* Ferredoxin-NADP<sup>+</sup> reductase, *G3P* Glycerol 3-phosphate, *OEC* oxygen-evolving complex, *PSII* and *PSI* photosystem II and I, *3-PGA* 3-Phosphoglyceric acid, *PQ* plastoquinone, *PQH<sub>2</sub>* plastoquinol, *RUBISCO* ribulose-1,5-bisphosphate carboxylase/oxygenase, *RuBP* ribulose-1,5-bisphosphate. Figure was prepared based on Taiz and Zeiger: Plant Physiology, 2010)



**Fig. 4** Effects of heat stress and ethylene on the homeostasis of reactive nitrogen species (RNS) and reactive oxygen species (ROS) regulated by non-enzymatic and enzymatic antioxidants. Heat stress induces oxidative and nitrosative stress in plant cells elevating intracellular levels of  $O_2^{\cdot-}$ ,  $H_2O_2$ , NO, and  $ONOO^-$ . In parallel with the involvement of ethylene, antioxidant enzymes are activated by different ways diminishing the harmful effects of the heat stress. APX

ascorbate peroxidase, AsA ascorbate, ET ethylene, CAT catalase, DHA dehydroascorbate, DHAR DHA reductase, GR glutathione reductase, GSH glutathione, GSSG glutathione disulphide,  $H_2O_2$  hydrogen peroxide, MDAH monodehydroascorbate, MDAHR MDHA reductase, NO nitric oxide,  $O_2^{\cdot-}$  superoxide,  $ONOO^-$  peroxynitrite, SOD superoxide dismutase

of AVG significantly elevated chlorophyll contents and decreased electrolyte leakage from heat-stressed leaves (Jespersen and Huang 2015). Moreover, AVG treatments increased the abundance of photosynthesis-related Rubisco, Rubisco activase, and chlorophyll *alb*-binding proteins under heat stress decreasing the senescence-inducing effects of ET (Jespersen and Huang 2015). In contrast, AVG treatments did not influence significantly the net photosynthetic rate, and yield of PSII in heat-stressed cotton (*Gossypium hirsutum*) compared to the only heat-exposed plants (Najeeb et al. 2017). Interestingly, the application of ET precursor ACC also did not influence more seriously these parameters compared to the effects of different degrees (36 and 45 °C) of heat exposure (Najeeb et al. 2017). Chlorophylls are the main photosynthetic pigments, which harvest light energy and drive electron transfer during photosynthesis. A serious reduction in chlorophyll level is also induced by heat stress as a part of protective responses of plants by increased activity of chlorophyllase and chlorophyll-degrading peroxidase, which can contribute to reducing the harmful effects of excessive light energy on photosynthetic apparatus (Wang et al. 2018; Hu et al. 2020). It is well known that ET promotes the transcription and activity of these enzymes in various plant species (Jacob-Wilk et al. 1999; Costa et al. 2005;

Shemer et al. 2008; Zhang et al. 2011), but the role of ET under heat stress remained uninvestigated. The balance of chlorophyll breakdown and biosynthesis is crucial to maintain the stability and optimal function of the photosynthetic apparatus, but the role of ET in these processes under heat stress is less known.

Heat stress (42 °C) for 2 days significantly elevated ET emission from the leaves of tomato plants and decreased the parameter of Fv/Fm (Pan et al. 2019). The linear electron transport is decreased by heat, because heat stress destroys OEC and induces dissociation of LHCII complexes from PSII photochemistry. These changes in the components of the electron transport chain can increase the efficiency of P700 photo-oxidation and the formation of PSI-LHCII-Cyt b6/f super complex, resulting in upregulation of PSI-dependent cyclic electron flow (CEF) which can help to alleviate the over-reduction of chloroplast stroma, increase in the trans-thylakoid proton motive force providing ATP, and also plays role in the photo-protection of both PSII and PSI (Zhang and Sharkey 2009; Agrawal et al. 2016; Ivanov et al. 2017; Sun et al. 2017; Tan et al. 2020). However, the role of ET on CEF has been not investigated under heat stress. At the same time, a recent investigation suggested that ACC



in concentration-dependent manner transiently increased CEF in tomato leaves (Borbély et al. 2019). Authors found that the highest concentration of ACC decreased CEF in a time-dependent manner, which is correlated well with the accumulation ROS, suggesting that molecular oxygen may function as an electron acceptor when natural acceptors of the linear electron transport were over-reduced (Borbély et al. 2019). Consequently, the role of ET in photochemistry needs further investigation under heat stress.

Among the components of PSII, the D1 protein is one of the most vulnerable under stress conditions. The repair of the damaged PSII proteins (primarily the D1 protein) is a multistep process called the ‘PSII repair cycle’ (Aro et al. 2005; Takahashi and Badger 2011). Interestingly, heat-treated leaves exhibited only trace amounts of Deg2 in the thylakoid membranes, which plays role in the primary cleavage of the photo-damaged D1 protein (Haußühl et al. 2001). Another protease, FtsH is also involved in D1 turnover upon heat stress (Yoshioka et al. 2006). It was observed that of the 12 known FtsHs, FtsH11 plays role in thermotolerance in Arabidopsis protecting the photosynthetic apparatus from heat stress at all stages of plant development (Chen et al. 2006). At the same time, there are no relevant data about the role of ET on proteases in chloroplast neither under control conditions nor under heat stress. Investigations of other components (e.g., tocopherol, carotenoids, and SOD) that alleviate the ROS causing damage in photosynthetic machinery are also required for understanding the role of ET in the heat-stress acclimation process.

Heat stress also greatly affects Rubisco levels and activity (Salvucci and Crafts-Brandner 2004; Demirevska-Kepova et al. 2005; Sharkey 2005; Perdomo et al. 2017) which can also be mediated by ET (Jespersen and Huang 2015). ET plays a crucial role in progressing senescence and a decrease of Rubisco content (Young et al. 2004; Martínez et al. 2008; Liu et al. 2019; Zheng et al. 2020). This was confirmed by the application of ET inhibitor AVG which elevated Rubisco and Rubisco activase protein levels under heat stress (35/30 °C) in bentgrass suggesting the role of ET in promoting heat-induced senescence (Jespersen and Huang 2015). However, transcript levels of Rubisco (*RBCS*) and CO<sub>2</sub> assimilation were significantly lower in ET-insensitive tobacco (*Nicotiana tabacum*) (Tholen et al. 2007). Moreover, exogenous ethephon treatments not only elevated CO<sub>2</sub> assimilation but also Rubisco activity depending on the plant cultivars of mustard (*Brassica juncea*) plants (Iqbal et al. 2012). These results show that the effects of the ET on Rubisco can be diverse depending on plant species, leaf developmental stages, and/or stress conditions that need further investigation in case of heat stress.

## Involvement of ethylene in the regulation of heat-shock proteins under heat stress

Heat stress induces protein mis-folding, and in parallel, the accumulation of HSPs play important role in cell protection against high temperature and other stresses (Wahid et al. 2007). Under heat stress, HSPs play a vital role in diminishing the effect of heat stress by stabilizing other proteins and assisting their refolding (Park and Seo 2015), prevent protein denaturation and aggregation, as well as play a role as membrane stabilizers or as site-specific antioxidants (Mathur et al. 2014). HSPs can be classified into five different families such as HSP60, HSP70, HSP90, HSP100, and the small heat-shock proteins (sHsp) based on molecular weight (Gupta et al. 2010). In vitro and in vivo experiments demonstrated that sHsp could associate with thylakoids and protect O<sub>2</sub> evolution and the OEC proteins of PSII under heat stress (Heckathorn et al. 1998, 2002; Barua et al. 2003). Among chloroplast sHsp, the tomato HSP21, which is induced by heat treatment, takes a crucial part in the establishment of PSII thermotolerance by protecting PSII from oxidative stress (Neta-Sharir et al. 2005). Thus, the rapid synthesis of HSPs could be essential in the thermal protection of chloroplast which can be mediated by various defense hormones such as ET. Heat stress (42 °C) significantly increased ET production in tomato, which was associated with elevated HSP70 expression at both transcript and protein levels (Pan et al. 2019). The proteins of HSP70 family vary in size from 68 to 75 kDa and are one of the most highly conserved classes of HSPs (Gupta et al. 2010). At the same time, HSP70, as well as HSP90, not only play a role as a chaperone in control of folding but their co-chaperones also are linked to signaling, protein targeting, and degradation (Jacob et al. 2017). Early observation has already demonstrated that ET-induced the accumulation of HSP70 (Rickey and Belknap 1991) and HSP90 (Salman et al. 2009) within several hours. Application of inhibitor of ET responses, 1-methylcyclopropene (1-MCP) significantly attenuated the heat-induced expression of HSP70 and HSP90 (Pan et al. 2019) which play role in mitigating stress impacts (Jacob et al. 2017). These results support the role of ET in the induction of HSPs under heat stress. However, the interaction between ET and HSP production and the role of HSPs in chloroplasts needs to be elucidated.

## Involvement of ethylene in osmoregulation under heat stress

Products of photosynthesis affected by heat stress conditions and sugars for instance glucose, fructose, and sucrose interact with phytohormones to maintain the overall cell



structure (e.g., protecting cell membranes), in growth and development processes, and in regulating stress responses in plants (Couée et al. 2006; Rosa et al. 2009; Wind et al. 2010; Sharma et al. 2019). However, heat stress can disturb sugar (carbohydrate) metabolism, resulting in inhibited plant growth and development, pollen sterility, failure of fertilization, and loss of grain yield in a wide variety of crops. Accordingly, these metabolites can affect heat tolerance in plants (Rizhsky et al. 2004; Frank et al. 2009; Ruan et al. 2010; Snider et al. 2011; Kaushal et al. 2013; Li et al. 2015; Janda et al. 2020). Effects of ET on sugar metabolism are well discussed especially in the case of senescence or fruit ripening (León and Sheen 2003; Sami et al. 2016; Chen et al. 2018; Durán-Soria et al. 2020), but less-investigated under heat stress. Interestingly, the application of 1-MCP resulted in the reduction of soluble sugar content under heat stress in cotton plants (Yuan et al. 2015). These results confirmed that ET plays role in sugar accumulation under heat stress conditions in which high sugar levels can promote leaf senescence (Wingler et al. 2006, 2009). Application of AVG also resulted in decreased monosaccharide levels such as glucose or fructose under heat stress in bentgrass plants. However, sucrose content increased under the same condition (Jespersen et al. 2015). It will be also important to examine carefully the role of ET in sugar homeostasis under heat stress.

Glycinebetaine (GB) is a compatible osmolyte, which also takes part in the stabilization of PSII (Allakhverdiev et al. 2008; Chen and Murata 2008). In vitro investigations confirmed that GB protects D1/D2/Cytb559 complex from heat stress (35 °C) (Allakhverdiev et al. 2003). Barley seeds (*Hordeum vulgare*) treated with 20 mM GB had greater biomass associated with higher Pn under heat stress (Wahid and Shabbir 2005). It was also reported that foliar application of GB (100 mM) improved the heat tolerance of wheat by maintaining chlorophyll content, Fv/Fm, and stomatal conductance under heat stress (39 ± 2 °C) (Wang et al. 2014). In addition, GB accumulated in transgenic tobacco plants enhanced the thermotolerance of PSII (Fv/Fm,  $\Phi$ PSII, and qP) and protected OEC under heat stress (Yang et al. 2007). Moreover, over-accumulation of GB in wheat resulted in higher chlorophyll content, gas exchange, and decreased superoxide and H<sub>2</sub>O<sub>2</sub> levels, as well as electrolyte leakage under heat stress (40 °C), compared to wild-type leaves (Wang et al. 2010). Earlier various compounds were analyzed in GB synthesis such as SA or ABA (Jagendorf and Takabe 2001), but the potential role of ET in this process remained less investigated.

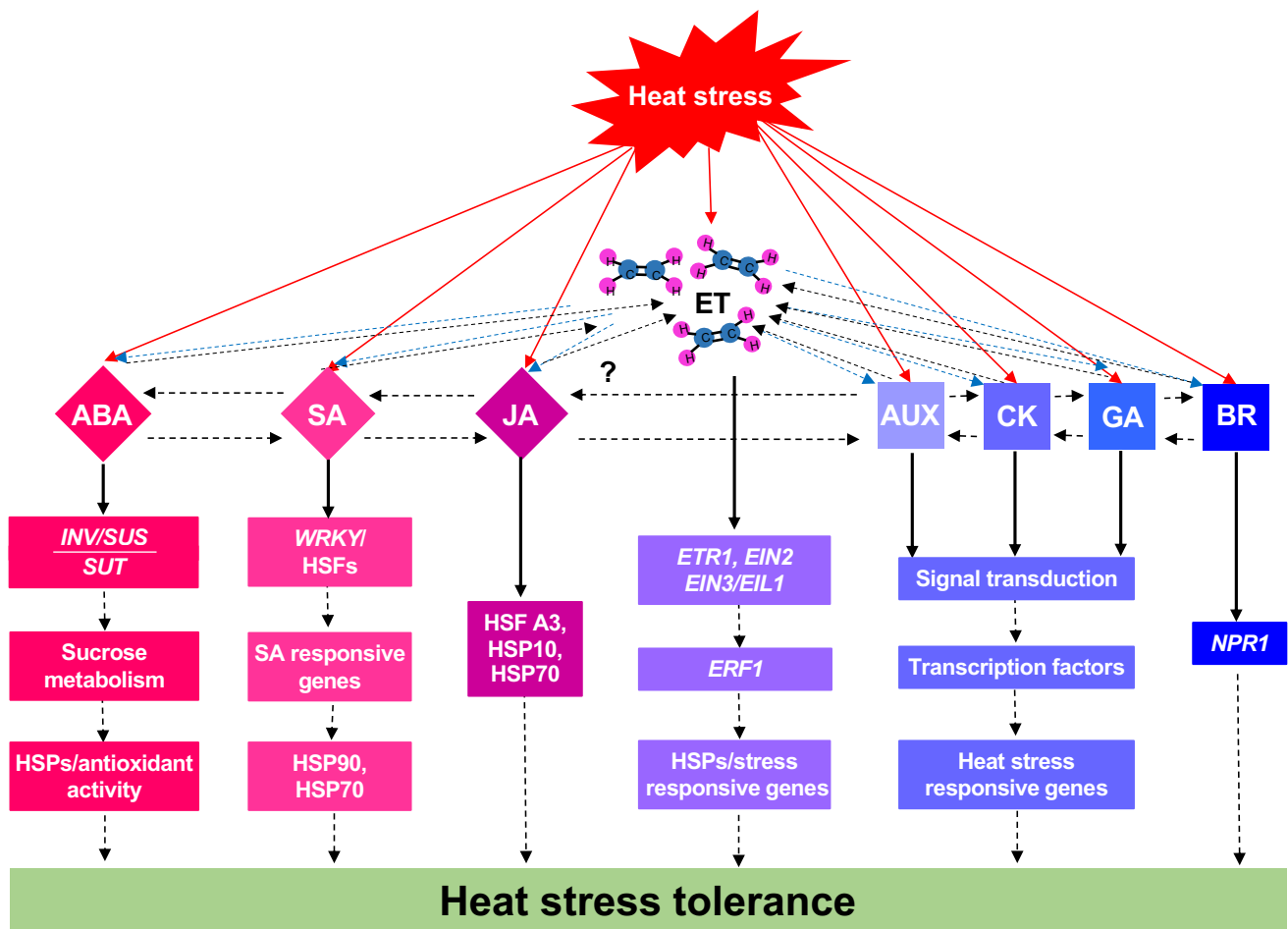
Proline acts as a compatible osmolyte and stabilizer of subcellular structures, but it also plays role in stress tolerance mechanism as ROS scavenger, enzyme protectant, and cytosolic pH buffer (Iqbal et al. 2014). Although, stress-induced proline accumulation has been well documented

and its positive role in enhancing plant tolerance to various abiotic stressors, such as salt, cold, UV radiation, and heavy metal stress, is well known, but its adverse effects in case of heat stress were also found (Lv et al. 2011). Using transgenic Arabidopsis, proline accumulation accelerated ROS production, increase in MDA levels, and ion leakage under heat stress. Moreover, ET biosynthesis was partially inhibited by proline accumulation under heat stress; thus, low levels of ET could contribute to proline-induced heat sensitivity in these plants (Lv et al. 2011). Interestingly, proline content was increased in leaves of Arabidopsis (Hsieh et al. 2013) and in wheat under heat stress (Khan et al. 2013). However, the interaction between osmolytes and ET under heat stress needs further researches.

### Ethylene and its crosstalk with phytohormones and other signaling molecules under heat stress

Analysis of phytohormones crosstalk in plants under heat stress is crucial to describe and understand the outcome of stress impacts. The activation and regulation of a large number of genes and transcription factors are dependent on various defense hormones. Besides ET, ABA, JA, and SA are also involved locally and systemically in the rapid regulation of cellular homeostasis and protection responses of plants. Auxins, cytokinins (CKs), gibberellic acids (GAs), and brassinosteroids (BRs) regulate plant growth and development under normal and stress conditions such as in the case of heat stress tolerance (Song et al. 2012; Ozga et al. 2017; Tiwari et al. 2017; Sharma et al. 2019). A summary of different plant growth regulators in regulation of heat stress is summarized in Table 1.

Based on the earliest investigations, all of the key defense-related hormones play role in the protection against heat stress-induced oxidative damage (40 °C, 3d) in Arabidopsis (Larkindale and Knight 2002). It was found that exogenous application of ACC, SA, and ABA significantly decreased the heat-induced lipid peroxidation and contributed to survival. Besides, heat stress-induced mortality (37 °C, 1 h) was significantly higher in the ET-insensitive mutant *etr-1*, the ABA-insensitive mutant *abi-1*, and a transgenic line expressing *nahG* (consequently inhibited in SA production) suggesting their role in the protection mechanism in Arabidopsis (Larkindale and Knight 2002). Later, it was also found that ABA signaling (*abi1* and *abi2*), ABA biosynthesis mutants (*aba1*, *aba2*, and *aba3*), and *NahG* transgenic lines showed defects in acquired thermotolerance (heated for 7 d to 38 °C for 90 min, cooled to room temperature for 120 min, and then heated to 45 °C for 180 min) based on the seedlings survival. At the same time, ET-signaling mutants (*ein2* and *etr1*) were more defective in basal- (heated to 45 °C for



**Fig. 5** Phytohormones crosstalk in the regulation of heat stress tolerance in plants, as discussed in the manuscript. *ABA* abscisic acid, *AUX* auxins, *BR* brassinosteroids, *CK* cytokinin, *ET* ethylene, *GA* gibberellins, *JA* jasmonic acid, *SA* salicylic acid, *ETR1* ethylene response1, *EIN2* ethylene insensitive2, *EIN3* ethylene insensitive3,

*EIL1* ethylene insensitive-like1, *ERFs* ethylene response factors, *HSFs* heat-shock factors, *INV* invertase, *NPR1* nonexpressor of pathogenesis-related genes 1, *SUS* sucrose synthase, *SUT* sucrose transporter

60 min) than acquired thermotolerance which was dependent on the light intensity (Larkindale et al. 2005). Others also confirmed using *abi1* mutants that ABA is required for heat stress acclimatization (Suzuki et al. 2016). Analysis of young developing seeds and pericarps of pea (*Pisum sativum*) revealed that heat stress elevated the content of ET, ABA, and auxins in developing seeds, but decreased GA levels and induced GA catabolism (Kaur et al. 2020). Earlier, it was shown that heat stress (43/38 °C, 3 d) increased both ABA and ET, but decreased GA, indole-3-acetic acid (IAA), and zeatin contents in winter rape (*Brassica napus*) seedlings (Zhou and Leul 1999). Further investigation based on pre-treatment with exogenous ACC, SA, and ABA in creeping bentgrass (*Agrostis stolonifera*) confirmed the role of these phytohormones in improving heat stress tolerance (35 °C), as evaluated by measurements of photosynthesis and lipid peroxidation (Larkindale and Huang 2004). Others observed similarly that exogenous spraying with SA,

6-benzylaminopurine (6-BA; a CK), and ET biosynthesis inhibitor AVG resulted in suppression of heat stress-induced increase in electrolyte leakage and chlorophyll loss in perennial ryegrass (*Lolium perenne*) (Zhang et al. 2019).

Later, the dynamics in phytohormone synthesis were also investigated in the first 6 h of heat stress (35 °C). Rapid production of H<sub>2</sub>O<sub>2</sub> was measured within minutes and levels of SA increased after the first hour of the heat treatment. Interestingly, ET and ABA levels did not change significantly during this time (Larkindale and Huang 2005). Subsequent investigations have highlighted the differences in the dynamics of ET/ABA synthesis in heat-tolerant rough bentgrass (*Agrostis scabra*) and heat-sensitive creeping bentgrass (*Agrostis stolonifera*). ET and ABA content increased earlier in *Agrostis stolonifera* as compared to *Agrostis scabra*. ABA content was higher in this plant species, but its peak declined in both species after prolonged heat stress. Levels of CKs (transzeatin/zeatin riboside and isopentenyl adenosine) also

**Table 1** Some representative studies of plant growth regulators effects (PGRs) in the regulation of heat stress tolerance in plants

Plants	Temperature	PGRs	Concentration	Response	References
<i>Oryza sativa</i>	45 °C	ACC	10 µM	Enhanced antioxidant enzymes activities, reduced lipid peroxidation and ion leakage	Wu and Yang (2019)
<i>Lolium perenne</i>	35 °C	BA, SA and SNP	25, 250 and 100 µM respectively	Suppression of leaf senescence and oxidative damage, and down-regulation of Chl catabolic genes (CCGs)	Zhang et al. (2019)
<i>Solanum lycopersicum</i>	50 °C	Ethephon	1 µL L <sup>-1</sup>	Increased pollen viability and pollen germination, upregulation of several stress-related proteins	Jegadeesan et al. (2018b)
<i>Capsicum annuum</i>	39 °C	ABA, ET and MeJa	100 µM	Modulation of <i>CaW-RKY6</i> , a transcription factor that regulates thermotolerance	Cai et al. (2015)
<i>Agrostis stolonifera</i>	35 °C	Zeatin (CK)	25 µM	Improved membrane stability, osmoregulation, antioxidant, carbon and N metabolism	Jespersen et al. (2015)
<i>Zea mays</i>	35 and 48 °C	Ethephon	250 g ha <sup>-1</sup>	Improved phenological events and crop biomass	Cicchino et al. (2013)
<i>Triticum aestivum</i>	40 °C	SA	0.5 mM	Improved, proline metabolism, N assimilation and photosynthesis, promoted osmotic and water potential while restricted ET production	Khan et al. (2013)
<i>Solanum lycopersicum</i>	50 °C	Ethephon	1 µL L <sup>-1</sup>	Improved pollen viability and quality	Firon et al. (2012)
<i>Triticum aestivum</i>	38 °C	SNP	0.25 mM	Increased Chl, GSH content, and GSH/GSSG ratio and upregulation of antioxidant defense and methylglyoxal detoxification system	Hasanuzzaman et al. (2012)
<i>Chrysanthemum morifolium</i>	45 °C	SNP	200 µM	Decreased non-photochemical quenching and MDA content, maintained higher antioxidant enzymatic activities	Yang et al. (2011)
<i>Arabidopsis thaliana</i>	38 °C	MeJa	5 µM	Protects membrane from heat stress, reduced electrolyte leakage and upregulation of JA-inducible genes	Clarke et al. (2009)
<i>Agrostis stolonifera</i>	35 °C	Zeatin (CK)	25 µM	Suppressed leaf senescence, lipid peroxidation and increased photosynthetic pigments and antioxidant metabolism	Xu and Huang (2009)
<i>Agrostis stolonifera</i>	35 °C	ABA, ACC and SA	100, 5 and 10 µM respectively	Decreased oxidative damage and membrane leakage	Larkindale and Huang (2005)

**Table 1** (continued)

Plants	Temperature	PGRs	Concentration	Response	References
<i>Agrostis stolonifera</i>	35 °C	ABA, ACC and SA	5, 100 and 10 $\mu\text{M L}^{-1}$ respectively	Reduced oxidative damage by activating ROS scavenging enzymes and increased photosynthesis	Larkindale and Huang (2004)
<i>Arabidopsis</i>	40 °C	ABA, ACC and SA	100 and 10 $\mu\text{M}$ respectively	Reduced oxidative damage and increased survival rate	Larkindale and Knight (2002)

ABA Abscisic acid, ACC 1-aminocyclopropane-1-carboxylic acid, 6-BA 6-benzylaminopurine, SA salicylic acid, SNP sodium nitroprusside, MeJa methyl jasmonate

decreased, but it was delayed and it was less pronounced in *Agrostis scabra* suggesting their role in delaying senescence and thermotolerance (Xu and Huang 2007). The dynamics of the synthesis of defense hormones were analyzed for the first time by Clarke et al. (2009). Heat stress (38 °C) rapidly induced the ET production which was modulated by in JA mutant (*opr3*; mutated in OPDA reductase3) and SA transgenic (*NahG*) Arabidopsis plants. It was also revealed that ET mutant *ein2-1* conferred greater heat stress tolerance based on the measurements of electrolyte leakage. These data confirmed that JA and SA act together conferring basal thermotolerance, while ET may promote cell death under heat stress in Arabidopsis (Clarke et al. 2009). Later, Pistelli and her co-worker (Pistelli et al. 2019) found that heat stress (38 °C, 5 h) rapidly induced the synthesis of ABA and SA in the first hour of the heat exposure. In contrast, levels of both ET and JA increased later from the second hour of the heat treatment. Interestingly, ET emission remained constantly high during the investigated 5 h but ABA, SA, and JA contents decreased in leaves of *Melissa officinalis* (Pistelli et al. 2019). At the same time, it was found that SA can regulate ET levels, thus promoting thermotolerance in wheat (*Triticum aestivum*), because exogenous SA treatments decreased heat stress-induced high ET production and ACS activity in this species (Khan et al. 2013). Recently, significant differences in phytohormone content were found in non-vernalized and vernalized root chicory (*Cichorium intybus*) seedlings upon heat stress which could influence the flowering of this plant species. ET concentration was increased in both treated groups, but ABA and JA contents were higher while ACC was lower in the non-vernalized root chicory plants (Mathieu et al. 2020). Pre-treatment with heat (1 h at 37 °C followed by 2 h at optimal temperature 20 °C) also influenced the thermal acclimation process in the different organs of Arabidopsis (Prerostova et al. 2020). ACC, ABA, and JA levels were significantly higher in the leaves, while the IAA and the active CK decreased in plants pre-acclimated with heat stress as compared to the only heat-stressed ones after 24 h (Prerostova et al. 2020).

These results suggest that environmental impacts influence phytohormone-regulated heat stress tolerance. BRs have been found to intervene heat stress tolerance by inducing the expression of several hormone (ABA, ET, SA, and JA) responsive genes in Arabidopsis (Divi et al. 2010). EBR treatment increased survival rates of WT, *ein2* (*ET-insensitive*), and *eto1-1* (*ET-overproducer*) seedlings. The levels of *PR-1*, *WRKY70*, and *WAK1* transcripts were elevated by EBR in WT and SA-related genotypes, including *npr1-1*, albeit. EBR-treated *aba1-1* seedlings accumulated approximately 3- and 2.5-fold higher levels of Hsp90 at 3 and 4 h of HS (Divi et al. 2010). ABA and proline induce the overexpression of *ERF1* resulting acclimation to stresses including heat stress in Arabidopsis (Cheng et al. 2013). Moreover, treatment of SA induced the expression of ET transcription factor genes including *GmERF3* (Zhang et al. 2009), *MsERF8* (Chen et al. 2012), *AtERF6* (Sewelam et al. 2013), and *CarERF116* (Deokar et al. 2015), while the expression of *CsERF* in citrus was neither induced nor reduced by auxin, GA and BR (Ma et al. 2014).

The phytohormone-regulated signaling elements in relation to ET were also investigated by some authors under heat stress. Dong et al. (2011) suggested in their work that increased heat stress tolerance (43 °C 3 h) of ET biosynthesis mutant (*acs7*) might result from activated ABA synthesis or signaling pathways in these plants suggesting an antagonistic relationship between the two hormones. Interestingly, it was demonstrated that *ETHYLENE RESPONSE FACTOR 53* (*AtERF53*) conferred heat-stress tolerance and was induced by ABA (Hsieh et al. 2013). Moreover, there are important transcription factors, which positively regulate thermotolerance modulated by ET and ABA such as *CaWRKY6* in pepper (Cai et al. 2015). Another protein, the oligouridylate-binding protein 1b (UBP1b) plays also an important role in plant heat stress tolerance by protecting mRNAs from degradation. The RT-qPCR analysis showed that expression of ABA signaling-related genes and genes of ET biosynthesis (*ACS6*, *ACS7*, *ACS8*, and *ACS11*) were higher in UBP1b-overexpressing Arabidopsis plants

(Nguyen et al. 2017). It can be concluded that ET, ABA, JA, and SA are rapidly accumulated by heat stress but with different kinetics depending on the plant species, the strength of heat, or other environmental factors such as light intensity. At the same time, levels of auxins, CKs, and GAs were reduced in a different way depending on the listed factors and plant organs. Description of hormonal crosstalk under heat stress needs further investigations. In particular, analysis of genes encoding transcription factors provides further data to this complex relation.

## Conclusion and future prospects

In conclusion, heat stress significantly impacts the restriction of plant growth and development influencing damaged photosynthetic machinery, membrane permeability, protein dysfunction, ROS production, and oxidative stress. Research studies have shown that phytohormones play a crucial role in imparting heat stress tolerance and in sustaining crop production. Here, we provided considerable evidence for the ET-mediated heat stress tolerance in plants through various mechanisms to maintain functional integrity of cells, photosynthesis, and reduced oxidative damage during heat stress. ET regulates the ROS and RNS metabolism by modulating osmoprotectants and antioxidant defense system and provide plant tolerance against heat stress. Multiple ET biosynthetic and signaling genes included *ACS3* and *ACS11*, *FRF1*, *ER5*, *ER21*, *ER24*, *ACO1*, *ACO4*, *EREB*, and *ETR4* were induced by heat stress. Moreover, ET-mediated signaling regulates the transcript level of *HSPs*, involved in the intricate network of signal transduction that confers heat stress tolerance in plants. Although considerable progress has been achieved in understanding the mechanism and signaling pathways involved in the development of thermotolerance in plants, however, there is a need to further elucidate the role of ET in the regulation of molecular and biochemical processes for crop improvement against heat stress. To recognize this, identification and characterization of key genes would be supportive in phytohormones including ET-mediated stimulation of thermotolerance will give a new approach to establish heat-tolerant crop varieties. There are still few gaps in our knowledge regarding hormonal crosstalk to increase our understanding of stress tolerance. Description of hormonal crosstalk under heat stress needs further investigations. In particular, analyses of genes encoding transcription factors provide further data to this complex relation.

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## Compliance with ethical standards

**Conflict of interest** Authors declare that they have no conflict of interest.

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