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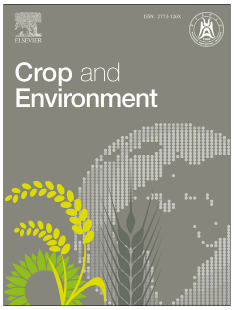

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Review Article

ROS signaling and its involvement in abiotic stress with emphasis on heat stress-driven anther sterility in plants

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Abstract

With global warming, crop plants are challenged by heat stress during reproductive growth, leading to male sterility and yield reduction. The balance between reactive oxygen species (ROS) generation and scavenging is disrupted by stress, resulting in oxidative stress, which is harmful to crop growth. However, recent advances have shown that ROS signaling via proteins that sense the redox state is positive for plant performance under stress. ROS signaling is also involved in regulating anther development, such as timely tapetum degeneration. We summarize recent progress in uncovering the mechanism of heat stress effects on ROS homeostasis and discuss the relationship between oxidative stress and metabolic disorder, which is often observed during anther development under heat stress. We propose experimental and breeding strategies to improve field crop tolerance to heat stress.

Keywords: Anther development, Crop plants, Heat stress, Heat tolerance strategies, Reactive oxygen species

1. Introduction

It has been reported that global average surface temperatures have increased by 0.95-1.20°C from 1850-1900 to 2011-2020, with a faster increase rate over land where humans cultivate crop plants than over the ocean (Gulev et al., 2023). Moreover, there are many regions where air temperatures have increased by more than 2°C during the same period (Cox et al., 2020). Elevated temperature and frequent extreme high temperature events lead to decreased yields in most crops due to vulnerable reproductive processes, such as microspore development, anther dehiscence, and grain filling (Khan et al., 2022; Muller and Rieu, 2016; Xiong et al., 2017). To meet the demand of a growing population, it is important to elucidate the mechanism by which heat stress affects anther development and to explore strategies to improve crop heat tolerance.

Reactive oxygen species (ROS), including superoxide radicals, singlet oxygen, hydroxyl radicals, and hydrogen peroxide, are derived from aerobic metabolism. Inside cells, electrons leak from the electron transport chain (ETC) of mitochondria and chloroplasts, resulting in superoxide radical production; in the extracellular space, oxygen receives electrons from NADPH under the catalysis of respiratory burst oxidase homologs (RBOHs). Because ROS can cause damage to membranes, DNA, and proteins, their concentrations must be tightly controlled. In plant cells, both enzymatic and non-enzymatic ROS scavenging pathways prevent ROS damage. Apart from their cytotoxic effects, ROS are also known to serve as signaling molecules involved in regulating plant growth and development, integrating stress signals, and establishing systemic acquired acclimation (Czarnocka and Karpiński, 2018; Mittler, 2017; Mittler et al., 2022).

During anther development, ROS levels reach a peak during meiosis and act as key signals that initiate the timely degradation of the tapetum, which is essential for the provision of nutrients needed for pollen development (Hu et al., 2011). However, heat stress often disrupts the redox homeostasis of anthers, resulting in pollen sterility and yield loss (Chaturvedi et al., 2021). In addition to abnormal ROS accumulation, heat stress also results in disordered sugar and lipid metabolism, which is usually observed in infertile anthers (Liu et al., 2021b; Min et al., 2014; Narayanan et al., 2018; Rezaul et al., 2019; Zoong Lwe et al., 2020). ROS can change metabolic profiles by affecting metabolic enzyme activity directly or regulating metabolism-related gene expression through ROS signaling. However, how ROS are involved in the heat response of anthers by reprogramming metabolic systems has rarely been discussed. We also summarize new emerging strategies, such as priming and plant growth regulation, to improve heat stress tolerance.

2. ROS homeostasis and signaling

Multiple pathways are involved in the generation and scavenging of ROS, depending on the type of stress and specific subcellular structures of the plants (Czarnocka and Karpiński, 2018). Different pathways involved in maintaining ROS homeostasis work synergistically to integrate stress signals and initiate adaptive mechanisms (Mittler et al., 2022). In this section, we discuss the mechanisms of ROS production and scavenging under stress and summarize the genetic and physiological evidence linking ROS signaling to stress tolerance.

2.1. The mechanism of ROS production

The ETC is one of the principal sites for ROS generation. When protein complexes that make up the ETC, such as complex I and complex III, are in a reduced state, they can directly transfer electrons to oxygen to produce superoxide radicals. The rate of superoxide production from the ETC is related to the concentration of potential electron donors and the local concentration of O_2 (Møller, 2001; Murphy, 2009). In chloroplasts, when the photosystem captures light that exceeds the demand for the Calvin cycle, the photosynthetic ETC becomes excessively reduced, leading to the accumulation of ROS within the chloroplasts (Ugalde et al., 2021). Similarly, in mitochondria, ROS accumulate when the NADH/NAD$^+$ ratio is high and the cellular demand for ATP is insufficient (Murphy, 2009). Oxidative stress can also occur when electron transfer from complex IV to oxygen is inhibited, such as when cytochrome C is released from the mitochondria inner membrane or when cells undergo aerobic respiration in a hypoxic environment (Blokhina and Fagerstedt, 2010; Kim et al., 2006; Pucciariello and Perata, 2017; Wagner et al., 2019). Alternative oxidase bypasses complex II without driving proton translocation, effectively preventing over-reduction of the ETC and reducing electron leakage (Selinski et al., 2018).

The RBOH protein family, located on the cell membrane, is the only protein family in plants that possesses a gp91^{phox} homologous subunit. They have NADPH oxidase activity and utilize intracellular NADPH as an electron donor to directly transfer electrons to O_2, generating superoxide radicals (Marino et al., 2012; Wang et al., 2018; Wong et al., 2007). The NADPH oxidase catalytic activity of RBOHs is strictly regulated at the protein level. The N-terminus of RBOH is located on the inner side of the cell membrane and contains two EF-hands capable of binding Ca^{2+} and serine residues that can undergo phosphorylation modifications (Hu et al., 2020a). External stimuli can promote the influx of Ca^{2+} and activate RBOH. In addition to binding the EF-hand domain of

RBOHs, Ca^{2+} can also interact with calcium-dependent protein kinases (CDPKs) to participate in the phosphorylation modification of RBOHs (Kobayashi et al., 2007). It has been shown that the Ca^{2+} transporter OsANNEXIN1 can interact with OsCDPK24 to promote the phosphorylation of RBOHs (Qiao et al., 2015). Calcium sensor calcineurin B-like (CBL) proteins can interact with CBL-interacting protein kinase (CIPKs) to promote CIPK-mediated phosphorylation of RBOHs (Drerup et al., 2013). Upon binding of Ca^{2+} to the EF-hand domain of RBOHs, the conformation of the N-terminal domain is altered, enhancing the efficiency of CIPK-mediated phosphorylation and promoting ROS production. These studies suggest that the Ca^{2+} binding site and phosphorylation modification site cooperatively regulate the catalytic activity of RBOHs (Han et al., 2019). Additionally, the phosphatase ABI1 in plants can remove the phosphorylation modification of RBOHs, thereby shutting down their catalytic activity (Han et al., 2019).

2.2. The mechanism of ROS scavenging

There are enzymatic and non-enzymatic pathways for the scavenging of ROS in plant cells, which not only play a role in ROS detoxification but also participate in ROS signaling (Czarnocka and Karpiński, 2018). The enzymatic antioxidant system consists of a series of enzymes, including superoxide dismutases, catalases, ascorbate peroxidases, glutathione reductases, monodehydroascorbate reductases, glutathione peroxidases, dehydroascorbate reductases, and glutathione transferases (Czarnocka and Karpiński, 2018). Superoxide dismutases catalyze the dismutation of superoxide radicals to produce hydrogen peroxide, which is then broken down into water and oxygen through catalases, the ascorbate-glutathione cycle, glutathione peroxidases, and other enzymatic pathways. Catalases are mainly localized in peroxisomes and catalyze the dismutation of hydrogen peroxide without requiring an electron donor, whereas other pathways rely on NADPH as an electron donor. Catalase activity is tightly regulated by phosphorylation and dephosphorylation. Under standard conditions, CatC is dephosphorylated by PHOSPHATASE OF CATALASE 1 (PC1) and disassociates to monomers. Under salt stress, PC1 is inhibited and leaves phosphorylated CatC to assemble its tetramer with catalase activity; salt stress thereby switches on catalase activity to cope with oxidative stress via inhibiting PC1 (Liu et al., 2023). Another two kinases, SALT TOLERANCE RECEPTOR-LIKE CYTOPLASMIC KINASE 1 and CDPK8, activate catalases under salt or drought stress (Zhou et al., 2018; Zou et al., 2015). Compared to catalases, glutathione peroxidases have a higher affinity for hydrogen peroxide and are more widely distributed in the cell. Recent studies suggest that glutathione peroxidases can finely regulate intracellular ROS levels and serve as sensors of the intracellular redox state, while catalases primarily clear excessive ROS under stress (Czarnocka and Karpiński, 2018; Farooq et al., 2019; Mittler et al., 2022).

The non-enzymatic antioxidant system consists of a group of small molecules with antioxidant activity, including carotenoids, ascorbic acid, glutathione, tocopherols, and flavonoids (Chapman et al., 2019). Carotenoids are primarily synthesized and accumulated in chloroplasts, where they play a crucial role in protecting the photosynthetic apparatus through non-photochemical quenching mechanisms (Caliandro et al., 2013; Kirilovsky and Kerfeld, 2016). Additionally, their oxidation products can serve as signaling molecules to transmit oxidative stress signals to the nucleus under stress conditions (Felemban et al., 2019; Hou et al., 2016). Ascorbic acid acts as an electron donor to directly scavenge superoxide radicals and hydroxyl radicals and is regenerated in the ascorbate-glutathione cycle (Gill and Tuteja, 2010). Glutathione, a tripeptide synthesized from glutamate, cysteine, and glycine, is another component of the ascorbate-glutathione cycle. The oxidized form of glutathione is reduced by glutathione reductase using NADPH as an electron donor. Flavonoids are a class of plant secondary metabolites that exhibit stronger electron-donating capacity than ascorbic acid (Hernández et al., 2008). In tomato, knockout *F3H* which involves in flavonoid synthesis leads to reduced accumulation of flavonols in pollen grains. Those pollen grains with reduced levels of flavonols show more ROS accumulation under heat stress, which results in decreased pollen viability (Muhlemann et al., 2018).

2.3. ROS signaling under stress conditions

Under heat stress, RBOHs are activated by calcium and phosphorylation signaling. It has been shown that the activation of RBOHs relies on Ca^{2+} influx mediated by cyclic nucleotide-gated calcium channels (Paradiso et al., 2020; Wang et al., 2021), and the opening of cyclic nucleotide-gated calcium channels is associated with an increase in intracellular cAMP levels at high temperature (Gao et al., 2012). Annexins can bind to Ca^{2+} and then bind to the cell membrane to promote Ca^{2+} influx, suggesting that cyclic nucleotide-gated calcium may be cascade-regulated by annexins to modulate calcium signaling in response to heat stress (Qiao et al., 2015; Wang et al., 2015a). The activation of RBOH also depends on the phosphorylation of serine residues in the N-terminal. Under heat stress, ZmCDPK7 dissociates from the membrane to cytosol and phosphorylates RBOHB, triggering ROS signaling in maize (Zhao et al., 2021). In barley, TaCDPK13 activates NADPH OXIDASE 7 to enhance drought tolerance (Hu et al., 2020b).

Numerous studies have demonstrated that ROS signaling mediated by RBOH is beneficial for plants in resisting stress stimuli (Table 1). This includes promoting the accumulation of proline in plants, helping maintain the Na^{+}/K^{+} balance, enhancing ROS scavenging capacity, and improving salt stress resistance (Liu et al., 2021a; Ma et al., 2012; Rejeb et al., 2015). Knockdown of *OsRBOHA* reduces drought tolerance in rice (Wang et al., 2016). In *Arabidopsis*, under stress conditions, ETHYLENE RESPONSE FACTOR 74 translocates into the nucleus to promote *RBOHD* expression, rapidly increasing intracellular ROS levels and inducing the expression of stress-responsive genes during the early stages of stress, thereby enhancing plant resistance to various stress conditions (Yao et al., 2017). Brassinosteroids (BRs) stimulate RBOH to produce low levels of ROS as primary signal and promote the expression of *ZmMAP65*. The interaction between ZmMAP65 and ZmMPK5 further amplifies the ROS signal and enhances antioxidant system activity (Zhang et al., 2010; Zhu et al., 2013). Calcium influx or phosphorylation of RBOH also strengthens ROS signaling. The transcription factor MYB30 suppresses the expression of *AtANNEXIN1* and *AtANNEXIN4*. Knockout of *MYB30* leads to increased intracellular Ca^{2+} levels and improved heat tolerance (Liao et al., 2017). Overexpression of *ZmCDPK7* increases the expression of *APX1*, *CAT1*, *sHSP17.4*, and *HSP70* under heat stress (Zhao et al., 2021). Overexpression of *StCDPK23* enhances potato resistance to mechanical injury (Ma et al., 2022). Furthermore, studies have shown that RBOH can mediate long-distance transport of ROS signals in plants, helping establish systemic acquired resistance (Czarnocka and Karpiński, 2018; Dubiella et al., 2013; Xiong et al., 2021; Zandalinas and Mittler, 2021). The above research finding indicates that ROS signaling is involved in the stress response and is helpful for improving stress resistance.

Unlike traditional signaling molecules such as plant hormones, there are no known specific receptors downstream of ROS. Instead, the conformation of downstream proteins can be altered through post-translational pathways to modulate their functions. Approximately 400 proteins were identified that can be oxidatively modified in *Arabidopsis* (Jacques et al., 2015). These proteins include not only metabolic enzymes but also signaling proteins involved in intracellular signal transduction, such as kinases, phosphatases, and transcription factors (Mittler et al., 2022). The post-translational modification sites on proteins involved in ROS signaling are generally conserved cysteine residues. HYDROGEN-PEROXIDE-INDUCED Ca^{2+} INCREASES 1 (an LRR receptor kinase) is identified as an extracellular H_2O_2 sensor which possesses two extra pairs of cysteine residues in its extracellular domain (Wu et al., 2020; Fig. 1A). *Arabidopsis* phospholipase D δ is another ROS sensor involved in regulating the dynamic organization of microtubules and stomatal movement (Song et al., 2020). The transcription factor TCP15 can bind to the promoters of key genes involved in anthocyanin synthesis and repress their expression. Under high light stress, the cysteine residue on TCP15 becomes oxidized, preventing its binding to DNA and thus opening up the anthocyanin synthesis pathway (Viola et al., 2016; Fig. 1B). Under low-temperature stress, thioredoxin h2 enters the nucleus to reduce COLD-RESPONSIVE C-REPEAT-BINDING TRANSCRIPTION FACTOR 1 (CBF1). Once CBF1 is reduced, it can activate downstream gene expression and enhance plant cold tolerance (Lee et al., 2021). Similarly, the heat stress transcription factor HSFA8 forms intramolecular disulfide bonds under oxidative conditions, leading to conformational changes that drive gene expression in the nucleus (Giesguth et al., 2015; Fig. 1C). HSFA4A also forms dimers under oxidative conditions to participate in stress responses (Andrási et al., 2019). The above research indicates that redox signals can directly affect cellular metabolism and regulate gene expression through signal transduction in response to stress stimuli. In addition to protein modifications, ROS may also participate in DNA methylation modifications to regulate gene expression through epigenetic mechanisms (Babbar et al., 2021).

3. ROS are involved in anther development and the heat stress response

The development of anthers is a highly complex and ordered process, which determines the success of sexual reproduction by forming fertile pollen within a dehiscent anther wall. Protein, sugar, and lipid metabolism-related genes and organelles are activated precisely at different stages, meeting the substrate and energy demand during microspore development. ROS now are widely accepted as a key signal involved in anther development, such as the initiation of tapetum programmed cell death (PCD). However, under abiotic stress, excessive ROS are produced, which is toxic and harmful to reproductive processes (Masoomi-Aladizgeh et al., 2020; Min et al., 2013). Under heat stress, sugar and lipid metabolism often becomes disrupted and is accompanied by ROS accumulation, leading to reduced pollen viability and inhibition of anther dehiscence (Begcy et al., 2019; Khan et al., 2020; Lin et al., 2023a; Min et al., 2014).

3.1. ROS involved in tapetum PCD

The anther wall is composed of four layers of cells: from the outermost to the innermost, epidermal cells, endothecium, middle cell layer, and tapetum. During anther development, the anther wall undergoes specialization to form structures that facilitate anther dehiscence (Xiao et al., 2019; Yang et al., 2017), while also provides nutrients for the development of the microspores (Zhang et al., 2011). Numerous studies have shown that sterile anthers can develop normally before and during meiosis, but abnormalities often occur after tetrad formation. Successful development of microspores depends on the timely degradation of the anther wall, especially the tapetum (Li et al., 2006; Niu et al., 2013). Premature or delayed degradation of tapetal cells is associated with increased or decreased levels of ROS. In standard conditions, the ROS level in anthers is low before meiosis, followed by a peak during meiosis, and then a decrease to normal levels (Yi et al., 2016; Yu et al., 2017; Zhao et al., 2023a). Studies in mutants have shown that the ROS levels and PCD in the anther are finely regulated genetically (Table 2).

During meiosis, the anther activates RBOH and inhibits ROS scavenging pathways, leading to a rapid increase in ROS levels and timely initiation of tapetum degradation. Knockout of the *LeRBOHE* gene in potato or blocking the upstream signaling pathway that activates RBOH expression also leads to male sterility (Dai et al., 2022). In rice anther, ABA-ACTIVATED PROTEIN KINASE 2 induces a ROS peak during the tetrad stage. Loss of function of ABA-ACTIVATED PROTEIN KINASE 2 results in impaired tapetal layer degeneration and pollen sterility (Zhao et al., 2023a). The BRs signaling regulator BRASSINAZOLE RESISTANT 1 binds to the promoter of *RBOH1* and promotes its expression, which is essential for proper tapetum degeneration (Yan et al., 2020). In rice, metallothioneins (MTs) possess ROS scavenging activity, and during meiosis, the transcription factor DEFECTIVE TAPETUM CELL DEATH 1 inhibits the expression of *OsMT2b* to maintain high ROS levels in the anther (Yi et al., 2016). These studies indicate that RBOHs and ROS signaling are necessary for normal anther development (Dai et al., 2022; Xie et al., 2014).

Excessive ROS levels before meiosis lead to premature tapetum degradation and disrupt microspore development. *ENDOTHECIUM 1*, which is expressed in the anther walls of peas and encodes a protein with four heme domains, plays a role in ROS scavenging. Mutations in this gene result in premature tapetum degradation (Gómez et al., 2004; Hamza et al., 2021). Abnormal ROS production in mitochondria also causes premature tapetum degradation and pollen sterility (Luo et al., 2013; Zheng et al., 2019). The membrane lipid oxidation product malondialdehyde can be oxidized into the corresponding acids by aldehyde dehydrogenases. Malondialdehyde accumulation in the anther of *Osaldh2b* mutant lines results in premature tapetum degradation (Xie et al., 2020). Heat stress induced ROS production result in the activation of mitogen-activated protein kinase 6 via Ca^{2+} signaling, which trigger vacuolar processing enzymes (VPEs) to initiate PCD in *Arabidopsis* leaves (Li et al., 2013). βVPE is also involved in tapetal cell degradation (Cheng et al., 2020), but it remains unclear whether the activation of βVPE in anther requires the involvement of ROS signaling. After meiosis, microspo res are released from the tetrad and form vacuoles, and the ROS levels subsequently decrease to avoid sustained oxidative stress. Mutations in the rice transcription factor gene *MADS3* result in reduced expression of *MT-1-4b*, which encodes a protein possess superoxide radical scavenging activity, leading to elevated levels of ROS in anthers at stage 10. Mutants showed abnormal tapetum degradation and impaired pollen development (Hu et al., 2011).

3.2. Heat stress disrupts ROS homeostasis and leads to male sterility

It was shown that heat stress-induced pollen sterility is closely associated with disrupted anther ROS homeostasis (Min et al., 2013; Zhao et al., 2018a, 2023a; Fig 2). In rice anthers, the disulfide isomerase like proteins 1-1 (PDIL1-1) interacts with RBOHb and inhibits its activity. Heat stress suppresses the expression of O*sPDIL1-1* and stimulates Ca^{2+} influx, accelerating ROS accumulation (Zhao et al., 2023b). High temperatures during meiosis result in ABA accumulation in the rice anther, promoting ROS production by RBOH and causing premature tapetum degradation (Zhao et al., 2023a). Jasmonic acid (JA) signaling plays a role in maintaining a higher activity of the antioxidant system, while heat stress blocks JA synthesis and its signaling, leading to over-accumulation of ROS in cotton anthers (Abouelsaad and Renault, 2018; Khan et al., 2023). However, in *Arabidopsis* leaves, JA can induce the production of ROS by RBOH (Maruta et al., 2011), indicating that JA may participate in regulating ROS levels through different signaling pathways in different species.

Nevertheless, our understanding of the specific molecular mechanisms by which JA activates ROS production or scavenging pathways is still limited.

High activity of the ROS scavenging system can help enhance heat tolerance. Heat-tolerant varieties often maintain higher ROS scavenging activity under high temperatures. For example, the heat-tolerant rice variety Huanghuazhan exhibits higher activities of ROS scavenging enzymes than heat-sensitive varieties (Al-Zahrani et al., 2022). Exogenous application of BRs can enhance antioxidant system activity and heat tolerance (Guo et al., 2022; Zhang et al., 2019, 2023). Generally, under heat stress, ROS levels increase, tapetum degradation is triggered, and pollen sterility occurs. However, in cotton, casein kinase I has been found to inhibit *RBOHD* expression and promote the expression of antioxidant system genes under heat stress, which leads to decreased hydrogen peroxide levels and delayed tapetum degradation (Min et al., 2013). The spatiotemporal characteristics of ROS in anthers are determined by both gene expression and environmental factors. The molecular regulatory mechanisms of ROS production and scavenging pathways in response to heat stress at different stages of anther development still require further research.

3.3. The relationship between ROS and energy metabolism in anther under heat stress

Anther development consumes energy, and the process relies on a strictly regulated metabolism. However, under heat stress, anther ROS homeostasis is disturbed, carbon and lipid metabolism are disrupted, and anatomical structure becomes abnormal, similar to the observed phenotype in various male-sterile mutants (Begcy et al., 2019; Hu et al., 2011; Zhao et al., 2018b; Zheng et al., 2019). It is interesting to determine how homeostasis is disturbed by heat stress. Is there any correlation between oxidative stress and metabolic disorders?

Under heat stress, the metabolic network is reprogrammed to improve heat tolerance, such as by enhancing antioxidant capacity. The mitochondrion, involved in energy metabolism, is also an active ROS production site under stress (Van Aken, 2021). Heat stress enhances the respiration rate and results in increased NADH content and a burst of ROS in mitochondria (Hou et al., 2021). Studies have shown that alternative oxidase respiration can reduce electron leakage from the ETC to oxygen (Cvetkovska and Vanlerberghe, 2012; Maxwell et al., 1999). Under heat stress, the alternative oxidase pathway helps maintain the NADH/NAD$^+$ ratio and suppresses $O_2{\cdot}^-$ production (Borovik and Grabelnych, 2018). Metabolic enzymes involved in the TCA cycle sense the redox state through proteins such as thioredoxins and glutathione, thereby regulating their own catalytic activities (Baxter et al., 2007; Daloso et al., 2015; McLain et al., 2011). For example, α-ketoglutarate dehydrogenase is the rate-limiting enzyme of the TCA cycle, which can form a disulfide bond with glutathione to repress its catalytic activity under oxidative stress (McLain et al., 2011). Heat stress-induced ROS accumulation may slow down the NADH production rate by suppressing the TCA cycle (Baxter et al., 2007; Dumont and Rivoal, 2019). The TCA cycle is central to sugar and lipid metabolism and is easily disrupted by heat stress; therefore, further research is needed to uncover the relationship between central metabolic changes and anther development under heat stress. NADPH is the byproduct of the oxidative pentose phosphate pathway and acts as an electron donor for several antioxidant pathways, such as the ascorbate-glutathione cycle and glutathione peroxidases. Under heat stress, higher glucose-6-phosphate dehydrogenase activity results in more carbon flowing into the oxidative pentose phosphate pathway and enhances the antioxidant capacity of *Phaseolus vulgaris* anthers (Santiago et al., 2021). This indicates that the ROS scavenging pathway will consume sugar under heat stress, with less energy being allocated to support anther development.

Studies from mutants show that disrupted metabolism through gene function loss also results in oxidative stress. *EDT1* encodes a subunit of ATP-citrate lyase, which is involved in acetyl-CoA synthesis. Decreased ATP levels, energy charge, and fatty acid content were observed in mutant *edt1* anthers compared with wild type, which indicates that energy and lipid metabolism were suppressed. The *edt1* mutant anther suffers oxidative stress after meiosis and shows precocious tapetum degeneration (Bai et al., 2019). OsHSP60 interacts with FLOURY ENDOSPERM6 located in the plastid and is involved in anther starch synthesis. Moreover*,* *oshsp60* mutant anthers exhibit defects in starch accumulation and suffer oxidative stress under heat stress (Lin et al., 2023a). *OsHXK1* encodes hexokinase, which is a rate-limiting enzyme in glycolysis, and OsAGO2 regulates its expression via DNA methylation. Knockdown of *OsAGO2* decreases methylation in the *OsHXK1* promoter, resulting in its over-expression. ROS accumulation in anther was observed in *Osago2* mutants and *OsHXK1* over-expression lines (Zheng et al., 2019). Most studies suggest that the reason for ROS accumulation under heat stress is insufficient antioxidant capacity, but the relationship between abundant ROS production and heat stress-induced changes in metabolism also needs consideration.

4. Strategies to enhance fertility under heat stress during the reproductive stage

4.1. Breeding heat-tolerant cultivars

Exploring heat tolerance genes and breeding heat-tolerant cultivars are beneficial approaches for reducing yield loss under heat stress. The heat tolerance of crop anthers is a quantitative trait controlled by multiple genes. Chen et al. (2021) successfully identified a novel heat tolerance QTL locus on chromosome 8 in rice using the bulked segregant analysis sequencing method. Li et al. (2015) localized a QTL on chromosome 3 in rice that is involved in regulating protein homeostasis. Ma et al. (2021) identified three heat tolerance QTL loci in cotton using genome-wide association and transcriptome-wide association study analyses and discovered a novel negative regulator of heat tolerance in anthers, heat related receptor kinase *GhHRK1*. By using the rice super pan-genome, *THERMO-TOLERANCE AND GRAIN LENGTH 1* was identified recently as a negative regulator of heat stress and grain size (Lin et al., 2023b). The identification of heat stress-related genes and the elucidation of heat tolerance molecular mechanisms provides a resource for the genetic improvement of crop heat stress tolerance.

As we previously discussed, ROS signaling plays a role in abiotic stress tolerance. We hypothesized that the difference in heat stress tolerance between cultivars is related to the natural variation in the functional genes involved in ROS signaling. It has been observed that the methylation patterns of the promoter regions of RBOH genes differ between sensitive and tolerant cultivars in response to heat stress (Ma et al., 2018). Furthermore, based on our analysis of natural population data, we identified a single-nucleotide mutation within the coding sequence of a RBOH gene, resulting in an average 25.7% increase in pollen viability under heat stress (unpublished data). In addition to RBOHs, other genes involved in ROS signaling, such as those involved in ROS perception, also influence heat stress tolerance. Identifying and aggregating advantageous alleles in the ROS signaling pathway may help enhance the heat tolerance of crops.

4.2. Priming

Plants can enhance their tolerance to heat stress after pre-adaptation to moderate stress (Begcy et al., 2019; Conrath et al., 2015; Hilker et al., 2016). For instance, in maize, after being exposed to high temperatures for 48 h during the tetrad stage, a significant upregulation of heat shock protein gene expression is observed in the pollen even after 15 d (Begcy et al., 2019). It has been shown that drought priming during the vegetative growth period can improve heat tolerance during the grain filling stage in wheat (Wang et al., 2015b). In *Arabidopsis*, after experiencing drought stress for 3-7 d, the phosphorylation of RNA polymerase and the methylation of histone H3K4 were still maintained, leading to increased transcription efficiency of drought stress-responsive genes during subsequent drought stress. This suggests that plants can preserve stress information through epigenetic inheritance (Ding et al., 2012). In tall fescue (*Festuca arundinacea*), drought training can increase the phosphorylation levels of proteins involved in RNA splicing, transcription regulation, stress response, and stress signaling transduction, indicating that post-translational pathways can enhance plant stress resistance through stress pre-adaptation (Zhang et al., 2020). These studies indicate that plants can retain stress information, either in the short term or long term, to enhance their stress resistance.

In addition to the abovementioned mechanisms, ROS also play a role in the process of priming. It was reported that H_2O_2 treatment could stimulate superoxide dismutase activity under heat stress (Qiao et al., 2015). Moderate heat treatment can improve the expression of heat stress-responsive genes and antioxidant enzyme activity in tomato seedlings through increasing the level of RBOHs-dependent H_2O_2 (Sun et al., 2019). ABA is a plant hormone induced by various stresses. In the promoter region of RBOHs genes, many *cis*-elements that responded to ABA was identified, and ABA could trigger ROS burst in rice anthers (Wang et al., 2020; Zhao et al., 2023a). RBOH-dependent ROS were identified as organ-organ communication signal, which is essential for plant systemic acquired acclimation (Suzuki et al., 2013). Such ABA-triggered ROS systemic signaling may explain how drought priming enhances heat stress tolerance. Stress preadaptation will be an effective strategy to prevent heat stress during the reproductive growth stage. However, further research is needed to determine the optimal methods, timing, and intensity of priming.

4.3. Exogenous application of plant growth regulators

Exogenous application of plant growth regulators has been demonstrated to mitigate abnormal microspore development caused by heat-induced ROS over-accumulation. Exogenous application of BRs or SA under heat stress has been shown to increase antioxidant enzyme activity and improve grain yield in rice (Zhang et al., 2023; Zhao et al., 2018a). In cotton, the inhibition of JA synthesis under heat stress leads to the excessive accumulation of ROS and reduced fertility. Conversely, the exogenous application of JA has been shown to enhance pollen fertility and promote anther dehiscence (Khan et al., 2023; Li et al., 2023). ABA has been reported to induce an increase in H_2O_2 levels via RBOHs. Studies have demonstrated that the exogenous application of ABA in maize seedlings triggers H_2O_2 accumulation, leading to enhanced heat shock protein accumulation and up-regulation of antioxidant enzyme activities (Hu et al., 2009). Moreover, ABA sprayed on rice has induced higher expression of heat shock proteins, contributing to heat tolerance (Rezaul et al., 2019). However, the mechanism of ABA-triggered ROS involved in heat stress response is still largely unknown. Nanomaterials such as CeO_2 nanoparticles, MoS_2 nanosheets, and C_{60} have been found to possess ROS scavenging activity and can enhance the salt stress resistance of crops (Fu et al., 2023; Wu and Li, 2022; Zhao et al., 2020). Exogenous application of these nanomaterials may compensate for the impaired antioxidant system activity in anthers under high temperature, thereby improving their heat tolerance.

5. Conclusions

A large number of studies have shown that the levels of ROS are tightly controlled by various mechanisms. We have summarized key genes that play crucial roles in responding to abiotic stresses and in the regulation of anther development via ROS signaling. ROS over-accumulation is often observed in crop anthers under heat stress, and whether ROS signaling plays a role in maintaining anther redox balance under heat stress requires further investigation. Moreover, ROS signaling exhibits cross-talk with the metabolic system. However, further research is needed to reveal how ROS signaling is involved in metabolic regulation and how metabolic changes influence redox homeostasis in developing anthers under stress.

Abbreviations

BRs	Brassinosteroids
CDPK	Calcium-dependent protein kinase
ETC	Electron transport chain
JA	Jasmonic acid
PCD	Programmed cell death
RBOHs	Respiratory burst oxidase homologs
ROS	Reactive oxygen species
TCA	Tricarboxylic acid cycle
VPE	Vacuolar processing enzymes

Authors' contributions

W.X. and Y. M. wrote the main manuscript; L.M., X.Z., J.K., and K. L. provided comments and revised the manuscript. All the authors reviewed the manuscript.

Availability of data and materials

Not applicable.

Declaration of competing interest

The authors declare that they have no conflicts of interest related to this paper.

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References

Abouelsaad, I., Renault, S. 2018. Enhanced oxidative stress in the jasmonic acid-deficient tomato mutant def-1 exposed to NaCl stress. J. Plant Physiol. 226, 136-144.

Al-Zahrani, H.S., Alharby, H.F., Fahad, S. 2022. Antioxidative defense system, hormones, and metabolite accumulation in different plant parts of two contrasting rice cultivars as influenced by plant growth regulators under heat stress. Front. Plant Sci. 13, 911846.

Andrási, N., Rigó, G., Zsigmond, L., Pérez-Salamó, I., Papdi, C., Klement, E., Pettkó-Szandtner, A., Baba, A.I., Ayaydin, F., Dasari, R., Cséplő, Á., Szabados, L. 2019. The mitogen-activated protein kinase 4-phosphorylated heat shock factor A4A regulates responses to combined salt and heat stresses. J. Exp. Bot. 70, 4903-4918.

Babbar, R., Karpinska, B., Grover, A., Foyer, C.H. 2021. Heat-induced oxidation of the nuclei and cytosol. Front. Plant Sci. 11, 617779.

Bai, W., Wang, P., Hong, J., Kong, W., Xiao, Y., Yu, X., Zheng, H., You, S., Lu, J., Lei, D., Wang, C., Wang, Q., Liu, S., Liu, X., Tian, Y., Chen, L., Jiang, L., Zhao, Z., Wu, C., Wan, J. 2019. Earlier Degraded Tapetum1 (EDT1) encodes an ATP-Citrate Lyase required for tapetum programmed cell death. Plant Physiol. 181, 1223-1238.

Baxter, C.J., Redestig, H., Schauer, N., Repsilber, D., Patil, K.R., Nielsen, J., Selbig, J., Liu, J., Fernie, A.R., Sweetlove, L.J. 2007. The metabolic response of heterotrophic *Arabidopsis* cells to oxidative stress. Plant Physiol. 143, 312-325.

Begcy, K., Nosenko, T., Zhou, L.Z., Fragner, L., Weckwerth, W., Dresselhaus, T. 2019. Male sterility in maize after transient heat stress during the tetrad stage of pollen development. Plant Physiol. 181, 683-700.

Blokhina, O., Fagerstedt, K.V. 2010. Oxidative metabolism, ROS and NO under oxygen deprivation. Plant Physiol. Biochem. 48, 359-373.

Borovik, O.A., Grabelnych, O.I. 2018. Mitochondrial alternative cyanide-resistant oxidase is involved in an increase of heat stress tolerance in spring wheat. J. Plant Physiol. 231, 310-317.

Caliandro, R., Nagel, K.A., Kastenholz, B., Bassi, R., Li, Z., Niyogi, K.K., Pogson, B.J., Schurr, U., Matsubara, S. 2013. Effects of altered α- and β-branch carotenoid biosynthesis on photoprotection and whole-plant acclimation of *Arabidopsis* to photo-oxidative stress. Plant Cell Environ. 36, 438-453.

Chapman, J.M., Muhlemann, J.K., Gayomba, S.R., Muday, G.K. 2019. RBOH-dependent ROS synthesis and ROS scavenging by plant specialized metabolites to modulate plant development and stress responses. Chem. Res. Toxicol. 32, 370-396.

Chaturvedi, P., Wiese, A.J., Ghatak, A., Zaveska Drabkova, L., Weckwerth, W., Honys, D. 2021. Heat stress response mechanisms in pollen development. New Phytol. 231, 571-585.

Chen, Z.S., Liu, X.F., Wang, D.H., Chen, R., Zhang, X.L., Xu, Z.H., Bai, S.N. 2018. Transcription factor OsTGA10 is a target of the MADS protein OsMADS8 and is required for tapetum development. Plant Physiol. 176, 819-835.

Cheng, Z., Guo, X., Zhang, J., Liu, Y., Wang, B., Li, H., Lu, H. 2020. βVPE is involved in tapetal degradation and pollen development by activating proprotease maturation in *Arabidopsis thaliana*. J. Exp. Bot. 71, 1943-1955.

Chen, L., Wang, Q., Tang, M., Zhang, X., Pan, Y., Yang, X., Gao, G., Lv, R., Tao, W., Jiang, L., Liang, T. 2021. QTL mapping and identification of candidate genes for heat tolerance at the flowering stage in rice. Front. Genet. 11, 621871.

Conrath, U., Beckers, G.J., Langenbach, C.J., Jaskiewicz, M.R. 2015. Priming for enhanced defense. Annu. Rev. Phytopathol. 53, 97-119.

Cox, D.T.C., Maclean, I.M.D., Gardner, A.S., Gaston, K.J. 2020. Global variation in diurnal asymmetry in temperature, cloud cover, specific humidity and precipitation and its association with leaf area index. Glob. Chang. Biol. 26, 7099-7111.

Cvetkovska, M., Vanlerberghe, G.C. 2012. Alternative oxidase modulates leaf mitochondrial concentrations of superoxide and nitric oxide. New Phytol. 195, 32-39.

Czarnocka, W., Karpiński, S. 2018. Friend or foe? Reactive oxygen species production, scavenging and signaling in plant response to environmental stresses. Free Radic. Biol. Med. 122, 4-20.

Dai, X., Han, H., Huang, W., Zhao, L., Song, M., Cao, X., Liu, C., Niu, X., Lang, Z., Ma, C., Xie, H. 2022. Generating novel male sterile tomatoes by editing respiratory burst oxidase homolog genes. Front. Plant Sci. 12, 817101.

Daloso, D.M., Muller, K., Obata, T., Florian, A., Tohge, T., Bottcher, A., Riondet, C., Bariat, L., Carrari, F., Nunes-Nesi, A., Buchanan, B.B., Reichheld, J.P., Araujo, W.L., Fernie, A.R. 2015. Thioredoxin, a master regulator of the tricarboxylic acid cycle in plant mitochondria. Proc. Natl. Acad. Sci. U.S.A. 112, 1392-1400.

Ding, Y., Fromm, M., Avramova, Z. 2012. Multiple exposures to drought 'train' transcriptional responses in *Arabidopsis*. Nat. Commun. 3, 740.

Drerup, M.M., Schlucking, K., Hashimoto, K., Manishankar, P., Steinhorst, L., Kuchitsu, K., Kudla, J. 2013. The Calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the *Arabidopsis* NADPH oxidase RBOHF. Mol. Plant 6, 559-569.

Dubiella, U., Seybold, H., Durian, G., Komander, E., Lassig, R., Witte, C.P., Schulze, W.X., Romeis, T. 2013. Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. Proc. Natl. Acad. Sci. U.S.A. 110, 8744-8749.

Dumont, S., Rivoal, J. 2019. Consequences of oxidative stress on plant glycolytic and respiratory metabolism. Front. Plant Sci. 10, 166.

Farooq, M.A., Niazi, A.K., Akhtar, J., Saifullah, Farooq, M., Souri, Z., Karimi, N., Rengel, Z. 2019. Acquiring control: The evolution of ROS-Induced oxidative stress and redox signaling pathways in plant stress responses. Plant Physiol. Biochem. 141, 353-369.

Felemban, A., Braguy, J., Zurbriggen, M. D., Al-Babili, S. 2019. Apocarotenoids involved in plant development and stress response. Front. Plant Sci. 10, 1168.

Fu, C., Khan, M.N., Yan, J., Hong, X., Zhao, F., Chen, L., Ma, H., Li, Y., Li, J., Wu, H. 2023. Mechanisms of nanomaterials for improving plant salt tolerance. Crop Environ. 2, 92-99.

Gao, F., Han, X., Wu, J., Zheng, S., Shang, Z., Sun, D., Zhou, R., Li, B. 2012. A heat-activated calcium-permeable channel - *Arabidopsis* cyclic nucleotide-gated ion channel 6 - is involved in heat shock responses. Plant J. 70, 1056-1069.

Giesguth, M., Sahm, A., Simon, S., Dietz, K.J. 2015. Redox-dependent translocation of the heat shock transcription factor AtHSFA8 from the cytosol to the nucleus in *Arabidopsis thaliana*. FEBS Lett. 589, 718-725.

Gill, S.S., Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909-930.

Gómez, M.D., Beltrán, J.P., Cañas, L.A. 2004. The pea END1 promoter drives anther-specific gene expression in different plant species. Planta 219, 967-981.

Gulev, S.K., Thorne, P.W., Ahn, J., Dentener, F.J., Domingues, C.M., Gerland, S., Gong, D., Kaufman, D.S., Nnamchi, H.C., Quaas, J., Rivera, J.A., Sathyendranath, S., Smith, S.L., Trewin, B., von Schuckmann, K., Vose, R.S. 2021. Changing state of the climate system.. In: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L. Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, O., Yu, R., Zhou, B. (Eds.), Climate change 2021: the physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, UK and New York, USA, pp. 287-422.

Guo, C., Chen, Y., Wang, M., Du, Y., Wu, D., Chu, J., Yao, X. 2022. Exogenous brassinolide improves the antioxidant capacity of *Pinellia ternata* by enhancing the enzymatic and nonenzymatic defense systems under non-stress conditions. Front. Plant Sci. 13, 917301.

Hamza, R., Roque, E., Gomez-Mena, C., Madueno, F., Beltran, J.P., Canas, L.A. 2021. PsEND1 is a key player in pea pollen development through the modulation of redox homeostasis. Front. Plant Sci. 12, 765277.

Han, J.P., Koster, P., Drerup, M.M., Scholz, M., Li, S., Edel, K.H., Hashimoto, K., Kuchitsu, K., Hippler, M., Kudla, J. 2019. Fine-tuning of RBOHF activity is achieved by differential phosphorylation and Ca^{2+} binding. New Phytol. 221, 1935-1949.

Hernández, I., Alegre, L., Van Breusegem, F., Munné-Bosch, S. 2008. How relevant are flavonoids as antioxidants in plants? Trends Plant Sci. 14, 125-132.

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Baurle, I., Geiselhardt, S., Hincha, D.K., Kunze, R., Mueller-Roeber, B., Rillig, M.C., Rolff, J., Romeis, T., Schmulling, T., Steppuhn, A., van Dongen, J., Whitcomb, S.J., Wurst, S., Zuther, E., Kopka, J. 2016. Priming and memory of stress responses in organisms lacking a nervous system. Biol. Rev. 91, 1118-1133.

Hou, L., Zhao, M., Huang, C., He, Q., Zhang, L., Zhang, J. 2021. Alternative oxidase gene induced by nitric oxide is involved in the regulation of ROS and enhances the resistance of *Pleurotus ostreatus* to heat stress. Microb. Cell Factories 20, 137.

Hou, X., Rivers, J., Leon, P., McQuinn, R.P., Pogson, B.J. 2016. Synthesis and function of apocarotenoid signals in plants. Trends Plant Sci. 21, 792-803.

Hu, C.H., Wang, P.Q., Zhang, P.P., Nie, X.M., Li, B.B., Tai, L., Liu, W.T., Li, W.Q., Chen, K.M. 2020a. NADPH Oxidases: The vital performers and center hubs during plant growth and signaling. Cells 9, 437.

Hu, C.H., Zeng, Q.D., Tai, L., Li, B.B., Zhang, P.P., Nie, X.M., Wang, P.Q., Liu, W.T., Li, W.Q., Kang, Z.S., Han, D.J., Chen, K.M. 2020b. Interaction between TaNOX7 and TaCDPK13 contributes to plant fertility and drought tolerance by regulating ROS production. J. Agric. Food Chem. 68, 7333-7347.

Hu, L., Liang, W., Yin, C., Cui, X., Zong, J., Wang, X., Hu, J., Zhang, D. 2011. Rice MADS3 regulates ROS homeostasis during late anther development. Plant Cell 23, 515-533.

Hu, X., Liu, R., Li, Y., Wang, W., Tai, F., Xue, R., Li, C. 2009. Heat shock protein 70 regulates the abscisic acid-induced antioxidant response of maize to combined drought and heat stress. Plant Growth Regul. 60, 225-235.

Jacques, S., Ghesquiere, B., De Bock, P.J., Demol, H., Wahni, K., Willems, P., Messens, J., Van Breusegem, F., Gevaert, K. 2015. Protein methionine sulfoxide dynamics in *Arabidopsis thaliana* under oxidative stress. Mol. Cell Proteomics 14, 1217-1229.

Jung, K.H., Han, M.J., Lee, Y.S., Kim, Y.W., Hwang, I., Kim, M.J., Kim, Y.K., Nahm, B.H., An, G. 2005. Rice *Undeveloped Tapetum1* is a major regulator of early tapetum development. Plant Cell 17, 2705-2722.

Khan, A.H., Ma, Y., Wu, Y., Akbar, A., Shaban, M., Ullah, A., Deng, J., Khan, A.S., Chi, H., Zhu, L., Zhang, X., Min, L. 2023. High-temperature stress suppresses allene oxide cyclase 2 and causes male sterility in cotton by disrupting jasmonic acid signaling. Crop J. 11, 33-45.

Khan, A.H., Min, L., Ma, Y., Wu, Y., Ding, Y., Li, Y., Xie, S., Ullah, A., Shaban, M., Manghwar, H., Shahid, M., Zhao, Y., Wang, C., Zhang, X. 2020. High day and night temperatures distinctively disrupt fatty acid and jasmonic acid metabolism, inducing male sterility in cotton. J. Exp. Bot. 71, 6128-6141.

Khan, A.H., Min, L., Ma, Y., Zeeshan, M., Jin, S., Zhang, X. 2022. High-temperature stress in crops: male sterility, yield loss and potential remedy approaches. Plant Biotechnol. J. 21, 680-697.

Kim, M., Lim, J. H., Ahn, C.S., Park, K., Kim, G.T., Kim, W.T., Pai, H.S. 2006. Mitochondria-associated hexokinases play a role in the control of programmed cell death in *Nicotiana benthamiana*. Plant Cell 18, 2341-2355.

Kimura, S., Hunter, K., Vaahtera, L., Tran, H. C., Citterico, M., Vaattovaara, A., Rokka, A., Stolze, S. C., Harzen, A., Meissner, L., Wilkens, M.M.T., Hamann, T., Toyota, M., Nakagami, H., Wrzaczek, M. 2020. CRK2 and C-terminal phosphorylation of NADPH oxidase RBOHD regulate reactive oxygen species production in *Arabidopsis*. Plant Cell 32, 1063-1080.

Kirilovsky, D., Kerfeld, C. A. 2016. Cyanobacterial photoprotection by the orange carotenoid protein. Nat. Plants 2, 16180.

Ko, S.S., Li, M.J., Sun-Ben Ku, M., Ho, Y.C., Lin, Y.J., Chuang, M.H., Hsing, H.X., Lien, Y.C., Yang, H.T., Chang, H.C., Chan, M.T. 2014. The bHLH142 transcription factor coordinates with TDR1 to modulate the expression of *EAT1* and regulate pollen development in rice. Plant Cell 26, 2486-2504.

Kobayashi, M., Ohura, I., Kawakita, K., Yokota, N., Fujiwara, M., Shimamoto, K., Doke, N., Yoshioka, H. 2007. Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. Plant Cell 19, 1065-1080.

Lee, E.S., Park, J.H., Wi, S.D., Kang, C.H., Chi, Y.H., Chae, H.B., Paeng, S.K., Ji, M.G., Kim, W.Y., Kim, M.G., Yun, D.J., Stacey, G., Lee, S.Y. 2021. Redox-dependent structural switch and CBF activation confer freezing tolerance in plants. Nat. Plants 7, 914-922.

Lee, S., Seo, P.J., Lee, H.J., Park, C.M. 2012. A NAC transcription factor NTL4 promotes reactive oxygen species production during drought-induced leaf senescence in *Arabidopsis*. Plant J. 70, 831-844.

Li, N., Zhang, D.S., Liu, H.S., Yin, C.S., Li, X.X., Liang, W.Q., Yuan, Z., Xu, B., Chu, H.W., Wang, J., Wen, T.Q., Huang, H., Luo, D., Ma, H., Zhang, D.B. 2006. The rice tapetum degeneration retardation gene is required for tapetum degradation and anther development. Plant Cell 18, 2999-3014.

Li, X.M., Chao, D.Y., Wu, Y., Huang, X., Chen, K., Cui, L. G., Su, L., Ye, W.W., Chen, H., Chen, H.C., Dong, N.Q., Guo, T., Shi, M., Feng, Q., Zhang, P., Han, B., Shan, J. X., Gao, J.P., Lin, H.X. 2015. Natural alleles of a proteasome alpha2 subunit gene contribute to thermotolerance and adaptation of African rice. Nat. Genet. 47, 827-833.

Li, Y., Chen, M., Khan, A.H., Ma, Y., He, X., Yang, J., Zhang, R., Ma, H., Zuo, C., Li, Y., Kong, J., Wang, M., Zhu, L., Zhang, X., Min, L. 2023. Histone H3 lysine 27 trimethylation suppresses jasmonate biosynthesis and signaling to affect male fertility under high temperature in cotton. Plant Commun. 4, 100660.

Li, Z., Yue, H., Xing, D. 2013. MAP Kinase 6-mediated activation of vacuolar processing enzyme modulates heat shock-induced programmed cell death in *Arabidopsis*. New Phytol. 198, 1291-1292.

Liao, C., Zheng, Y., Guo, Y. 2017. MYB30 transcription factor regulates oxidative and heat stress responses through ANNEXIN-mediated cytosolic calcium signaling in *Arabidopsis*. New Phytol. 216, 163-177.

Lin, S., Liu, Z., Sun, S., Xue, F., Li, H., Tursun, A., Cao, L., Zhang, L., Wilson, Z.A., Zhang, D., Liang, W. 2023a. Rice HEAT SHOCK PROTEIN60-3B maintains male fertility under high temperature by starch granule biogenesis. Plant Physiol. 192, 2301-2317.

Lin, Y., Zhu, Y., Cui, Y., Qian, H., Yuan, Q., Chen, R., Lin, Y., Chen, J., Zhou, X., Shi, C., He, H., Hu, T., Gu, C., Yu, X., Zhu, X., Wang, Y., Qian, Q., Zhang, C., Wang, F., Shang, L. 2023b. Identification of natural allelic variation in *TTL1* controlling thermotolerance and grain size by a rice super pan-genome. J. Integr. Plant Biol. 00, 1-12.

Liu, C., Lin, J.Z., Wang, Y., Tian, Y., Zheng, H.P., Zhou, Z.K., Zhou, Y.B., Tang, X.D., Zhao, X.H., Wu, T., Xu, S.L., Tang, D.Y., Zuo, Z.C., He, H., Bai, L.Y., Yang, Y.Z., Liu, X.M. 2023. The protein phosphatase PC1 dephosphorylates and deactivates CatC to negatively regulate H_2O_2 homeostasis and salt tolerance in rice. Plant Cell 9, 3604-3625.

Liu, D., Li, Y.Y., Zhou, Z.C., Xiang, X., Liu, X., Wang, J., Hu, Z.R., Xiang, S.P., Li, W., Xiao, Q.Z., Wang, Y., Hu, R.S., Zhao, Q. 2021a. Tobacco transcription factor bHLH123 improves salt tolerance by activating NADPH oxidase *NtRbohE* expression. Plant Physiol. 186, 1706-1720.

Liu, S., Li, Z., Wu, S., Wan, X. 2021b. The essential roles of sugar metabolism for pollen development and male fertility in plants. Crop J. 9, 1223-1236.

Luo, D., Xu, H., Liu, Z., Guo, J., Li, H., Chen, L., Fang, C., Zhang, Q., Bai, M., Yao, N., Wu, H., Wu, H., Ji, C., Zheng, H., Chen, Y., Ye, S., Li, X., Zhao, X., Li, R., Liu, Y. G. 2013. A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. Nat. Genet. 45, 573-577.

Ma, L., Jiang, H., Ren, Y.Y., Yang, J.W., Han, Y., Si, H.J., Prusky, D., Bi, Y., Wang, Y. 2022. Overexpression of *StCDPK23* promotes wound healing of potato tubers by regulating *StRbohs*. Plant Physiol. Biochem. 185, 279-289.

Ma, L., Zhang, H., Sun, L., Jiao, Y., Zhang, G., Miao, C., Hao, F. 2012. NADPH oxidase AtrbohD and AtrbohF function in ROS-dependent regulation of Na^+/K^+homeostasis in *Arabidopsis* under salt stress. J. Exp. Bot. 63, 305-317.

Ma, Y., Min, L., Wang, M., Wang, C., Zhao, Y., Li, Y., Fang, Q., Wu, Y., Xie, S., Ding, Y., Su, X., Hu, Q., Zhang, Q., Li, X., Zhang, X. 2018. Disrupted genome methylation in response to high temperature has distinct affects on microspore abortion and anther indehiscence. Plant Cell 30, 1387-1403.

Ma, Y., Min, L., Wang, J., Li, Y., Wu, Y., Hu, Q., Ding, Y., Wang, M., Liang, Y., Gong, Z., Xie, S., Su, X., Wang, C., Zhao, Y., Fang, Q., Li, Y., Chi, H., Chen, M., Khan, A. H., Lindsey, K., Zhu, L., Li, X., Zhang, X. 2021. A combination of genome-wide and transcriptome-wide association studies reveals genetic elements leading to male sterility during high temperature stress in cotton. New Phytol. 231, 165-181.

Marino, D., Dunand, C., Puppo, A., Pauly, N. 2012. A burst of plant NADPH oxidases. Trends Plant Sci. 17, 9-15.

Maruta, T., Inoue, T., Tamoi, M., Yabuta, Y., Yoshimura, K., Ishikawa, T., Shigeoka, S. 2011. *Arabidopsis* NADPH oxidases, AtrbohD and AtrbohF, are essential for jasmonic acid-induced expression of genes regulated by MYC2 transcription factor. Plant Sci. 180, 655-660.

Masoomi-Aladizgeh, F., Najeeb, U., Hamzelou, S., Pascovici, D., Amirkhani, A., Tan, D. K. Y., Mirzaei, M., Haynes, P. A., Atwell, B. J. 2020. Pollen development in cotton (*Gossypium hirsutum*) is highly sensitive to heat exposure during the tetrad stage. Plant Cell Environ. 44, 2150-2166.

Maxwell, D. P., Wang, Y., McIntosh, L. 1999. The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc. Natl. Acad. Sci. U.S.A. 96, 8271–8276.

McLain, A.L., Szweda, P.A., Szweda, L.I. 2011. α-Ketoglutarate dehydrogenase: a mitochondrial redox sensor. Free Radic. Res. 45, 29-36.

Min, L., Li, Y., Hu, Q., Zhu, L., Gao, W., Wu, Y., Ding, Y., Liu, S., Yang, X., Zhang, X. 2014. Sugar and auxin signaling pathways respond to high-temperature stress during anther development as revealed by transcript profiling analysis in cotton. Plant Physiol. 164, 1293-1308.

Min, L., Zhu, L., Tu, L., Deng, F., Yuan, D., Zhang, X. 2013. Cotton *GhCKI* disrupts normal male reproduction by delaying tapetum programmed cell death via inactivating starch synthase. Plant J. 75, 823-835.

Mittler, R. 2017. ROS are good. Trends Plant Sci. 22, 11-19.

Mittler, R., Zandalinas, S.I., Fichman, Y., Van Breusegem, F. 2022. Reactive oxygen species signalling in plant stress responses. Nat. Rev. Mol. Cell Biol. 23, 663-679.

Møller, I.M. 2001. Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 561-591.

Muhlemann, J.K., Younts, T.L.B., Muday, G.K. 2018. Flavonols control pollen tube growth and integrity by regulating ROS homeostasis during high-temperature stress. Proc. Natl. Acad. Sci. U.S.A. 115, 11188-11197.

Muller, F., Rieu, I. 2016. Acclimation to high temperature during pollen development. Plant Reprod. 29, 107-118.

Murphy, M. P. 2009. How mitochondria produce reactive oxygen species. Biochem. J. 417, 1-13.

Narayanan, S., Prasad, P. V. V., Welti, R. 2018. Alterations in wheat pollen lipidome during high day and night temperature stress. Plant Cell Environ. 41, 1749-1761.

Niu, N., Liang, W., Yang, X., Jin, W., Wilson, Z. A., Hu, J., Zhang, D. 2013. EAT1 promotes tapetal cell death by regulating aspartic proteases during male reproductive development in rice. Nat. Commun. 4, 1445.

Paradiso, A., Domingo, G., Blanco, E., Buscaglia, A., Fortunato, S., Marsoni, M., Scarcia, P., Caretto, S., Vannini, C., de Pinto, M. C. 2020. Cyclic AMP mediates heat stress response by the control of redox homeostasis and ubiquitin-proteasome system. Plant Cell Environ. 43, 2727-2742.

Pucciariello, C., Perata, P. 2017. New insights into reactive oxygen species and nitric oxide signalling under low oxygen in plants. Plant Cell Environ. 40, 473-482.

Qiao, B., Zhang, Q., Liu, D., Wang, H., Yin, J., Wang, R., He, M., Cui, M., Shang, Z., Wang, D., Zhu, Z. 2015. A calcium-binding protein, rice annexin OsANN1, enhances heat stress tolerance by modulating the production of H_2O_2. J. Exp. Bot. 66, 5853-5866.

Rejeb, K.B., Lefebvre-De Vos, D., Le Disquet, I., Leprince, A.S., Bordenave, M., Maldiney, R., Jdey, A., Abdelly, C., Savoure, A. 2015. Hydrogen peroxide produced by NADPH oxidases increases proline accumulation during salt or mannitol stress in *Arabidopsis thaliana*. New Phytol. 208, 1138-1148.

Rezaul, I.M., Baohua, F., Tingting, C., Weimeng, F., Caixia, Z., Longxing, T., Guanfu, F. 2019. Abscisic acid prevents pollen abortion under high-temperature stress by mediating sugar metabolism in rice spikelets. Physiol. Plant 165, 644-663.

Santiago, J.P., Soltani, A., Bresson, M.M., Preiser, A.L., Lowry, D.B., Sharkey, T.D. 2021. Contrasting anther glucose-6-phosphate dehydrogenase activities between two bean varieties suggest an important role in reproductive heat tolerance. Plant Cell Environ. 44, 2185-2199.

Selinski, J., Scheibe, R., Day, D.A., Whelan, J. 2018. Alternative oxidase is positive for plant performance. Trends Plant Sci. 23, 588-597.

Sirichandra, C., Gu, D., Hu, H.C., Davanture, M., Lee, S., Djaoui, M., Valot, B., Zivy, M., Leung, J., Merlot, S., Kwak, J.M. 2009. Phosphorylation of the *Arabidopsis* AtrbohF NADPH oxidase by OST1 protein kinase. FEBS Lett. 583, 2982-2986.

Song, P., Jia, Q., Chen, L., Jin, X., Xiao, X., Li, L., Chen, H., Qu, Y., Su, Y., Zhang, W., Zhang, Q., Vissenberg, K. 2020. Involvement of *Arabidopsis* phospholipase D δ in regulation of ROS-mediated microtubule organization and stomatal movement upon heat shock. J. Exp. Bot. 71, 6555-6570.

Sun, M., Jiang, F., Zhou, R., Wen, J., Cui, S., Wang, W., Wu, Z. 2019. Respiratory burst oxidase homologue-dependent H_2O_2 is essential during heat stress memory in heat sensitive tomato. Sci. Hortic. 258, 108777.

Suzuki, N., Miller, G., Salazar, C., Mondal, H. A., Shulaev, E., Cortes, D. F., Shuman, J. L., Luo, X., Shah, J., Schlauch, K., Shulaev, V., Mittler, R. 2013. Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants. Plant Cell, 25, 3553-3569.

Szarka, A., Horemans, N., Kovács, Z., Gróf, P., Mayer, M., Bánhegyi, G. 2007. Dehydroascorbate reduction in plant mitochondria is coupled to the respiratory electron transfer chain. Physiol. Plant 129, 225-232.

Ugalde, J.M., Fuchs, P., Nietzel, T., Cutolo, E.A., Homagk, M., Vothknecht, U.C., Holuigue, L., Schwarzlander, M., Muller-Schussele, S.J., Meyer, A.J. 2021. Chloroplast-derived photo-oxidative stress causes changes in H_2O_2 and EGSH in other subcellular compartments. Plant Physiol. 186, 125-141.

Van Aken, O. 2021. Mitochondrial redox systems as central hubs in plant metabolism and signaling. Plant Physiol. 186, 36-52.

Viola, I.L., Camoirano, A., Gonzalez, D.H. 2016. Redox-dependent modulation of anthocyanin biosynthesis by the TCP transcription factor TCP15 during exposure to high light intensity conditions in *Arabidopsis*. Plant Physiol. 170, 74-85.

Wagner, S., Steinbeck, J., Fuchs, P., Lichtenauer, S., Elsasser, M., Schippers, J.H.M., Nietzel, T., Ruberti, C., Van Aken, O., Meyer, A.J., Van Dongen, J.T., Schmidt, R.R., Schwarzlander, M. 2019. Multiparametric real-time sensing of cytosolic physiology links hypoxia responses to mitochondrial electron transport. New Phytol. 224, 1668-1684.

Wang, W., Chen, D., Zhang, X., Liu, D., Cheng, Y., Shen, F. 2018. Role of plant respiratory burst oxidase homologs in stress responses. Free Radic. Res. 52, 826-839.

Wang, W., Zhang, J., Ai, L., Wu, D., Li, B., Zhang, L., Zhao, L. 2021. Cyclic nucleotide-gated ion channel 6 mediates thermotolerance in *Arabidopsis* seedlings by regulating hydrogen peroxide production via cytosolic calcium ions. Front. Plant Sci. 12, 708672.

Wang, X., Ma, X., Wang, H., Li, B., Clark, G., Guo, Y., Roux, S., Sun, D., Tang, W. 2015a. Proteomic study of microsomal proteins reveals a key role for *Arabidopsis* annexin 1 in mediating heat stress-induced increase in intracellular calcium levels. Mol. Cell Proteomics 14, 686-694.

Wang, X., Vignjevic, M., Liu, F., Jacobsen, S., Jiang, D., Wollenweber, B. 2015b. Drought priming at vegetative growth stages improves tolerance to drought and heat stresses occurring during grain filling in spring wheat. Plant Growth Regul. 75, 677-687.

Wang, X., Zhang, M.M., Wang, Y.J., Gao, Y.T., Li, R., Wang, G.F., Li, W.Q., Liu, W.T., Chen, K.M. 2016. The plasma membrane NADPH oxidase OsRbohA plays a crucial role in developmental regulation and drought-stress response in rice. Physiol. Plant 156, 421-443.

Wang, W., Chen, D., Liu, D., Cheng, Y., Zhang, X., Song, L., Hu, M., Dong, J., Shen, F. 2020. Comprehensive analysis of the *Gossypium hirsutum* L. respiratory burst oxidase homolog (*Ghrboh*) gene family. BMC Genomics 21, 91.

Wong, H.L., Pinontoan, R., Hayashi, K., Tabata, R., Yaeno, T., Hasegawa, K., Kojima, C., Yoshioka, H., Iba, K., Kawasaki, T., Shimamoto, K. 2007. Regulation of rice NADPH oxidase by binding of Rac GTPase to its N-terminal extension. Plant Cell 19, 4022-4034.

Wu, F., Chi, Y., Jiang, Z., Xu, Y., Xie, L., Huang, F., Wan, D., Ni, J., Yuan, F., Wu, X., Zhang, Y., Wang, L., Ye, R., Byeon, B., Wang, W., Zhang, S., Sima, M., Chen, S., Zhu, M., Pei, J., Johnson, D. M., Zhu, S., Cao, X., Pei, C., Zai, Z., Liu, Y., Liu, T., Swift, G. B., Zhang, W., Yu, M., Hu, Z., Siedow, J. N., Chen, X., Pei, Z. M. 2020. Hydrogen peroxide sensor HPCA1 is an LRR receptor kinase in *Arabidopsis*. Nature 578, 577-581.

Wu, H., Li, Z. 2022. Recent advances in nano-enabled agriculture for improving plant performance. Crop J. 10, 1-12.

Xiao, Y., You, S., Kong, W., Tang, Q., Bai, W., Cai, Y., Zheng, H., Wang, C., Jiang, L., Wang, C., Zhao, Z., Wan, J. 2019. A GARP transcription factor *anther dehiscence defected 1* (*OsADD1*) regulates rice anther dehiscence. Plant Mol. Biol. 101, 403-414.

Xie, H.T., Wan, Z.Y., Li, S., Zhang, Y. 2014. Spatiotemporal production of reactive oxygen species by NADPH oxidase is critical for tapetal programmed cell death and pollen development in *Arabidopsis*. Plant Cell 26, 2007-2023.

Xie, X., Zhang, Z., Zhao, Z., Xie, Y., Li, H., Ma, X., Liu, Y.G., Chen, L. 2020. The mitochondrial aldehyde dehydrogenase OsALDH2b negatively regulates tapetum degeneration in rice. J. Exp. Bot. 71, 2551-2560.

Xiong, D., Ling, X., Huang, J., Peng, S. 2017. Meta-analysis and dose-response analysis of high temperature effects on rice yield and quality. Environ. Exp. Bot. 141, 1-9.

Xiong, H., Hua, L., Reyna-Llorens, I., Shi, Y., Chen, K.M., Smirnoff, N., Kromdijk, J., Hibberd, J.M. 2021. Photosynthesis-independent production of reactive oxygen species in the rice bundle sheath during high light is mediated by NADPH oxidase. Proc. Natl. Acad. Sci. U.S.A. 118, 2022702118.

Yan, MY., Xie, D.L., Cao, J.J., Xia, X.J., Shi, K., Zhou, Y.H., Zhou, J., Foyer, C.H., Yu, J.Q. 2020. Brassinosteroid-mediated reactive oxygen species are essential for tapetum degradation and pollen fertility in tomato. Plant J. 102, 931-947.

Yang, C., Song, J., Ferguson, A.C., Klisch, D., Simpson, K., Mo, R., Taylor, B., Mitsuda, N., Wilson, Z.A. 2017. Transcription factor MYB26 is key to spatial specificity in anther secondary thickening formation. Plant Physiol. 175, 333-350.

Yang, Z., Sun, L., Zhang, P., Zhang, Y., Yu, P., Liu, L., Abbas, A., Xiang, X., Wu, W., Zhan, X., Cao, L., Cheng, S. 2019. *TDR INTERACTING PROTEIN 3*, encoding a PHD-finger transcription factor, regulates Ubisch bodies and pollen wall formation in rice. Plant J. 99, 844-861.

Yao, Y., He, R.J., Xie, Q.L., Zhao, X.H., Deng, X.M., He, J.B., Song, L., He, J., Marchant, A., Chen, X.Y., Wu, A.M. 2017. *ETHYLENE RESPONSE FACTOR 74* (*ERF74*) plays an essential role in controlling a respiratory burst oxidase homolog D (RbohD)-dependent mechanism in response to different stresses in *Arabidopsis*. New Phytol. 213, 1667-1681.

Yi, J., Moon, S., Lee, Y.S., Zhu, L., Liang, W., Zhang, D., Jung, K.H., An, G. 2016. Defective Tapetum Cell Death 1 (DTC1) regulates ROS levels by binding to metallothionein during tapetum degeneration. Plant Physiol. 170, 1611-1623.

Yu, S.X., Feng, Q.N., Xie, H.T., Li, S., Zhang, Y. 2017. Reactive oxygen species mediate tapetal programmed cell death in tobacco and tomato. BMC Plant Biol. 17, 76.

Zandalinas, S.I., Mittler, R. 2021. Vascular and nonvascular transmission of systemic reactive oxygen signals during wounding and heat stress. Plant Physiol. 186, 1721-1733.

Zhang, A., Zhang, J., Ye, N., Cao, J., Tan, M., Zhang, J., Jiang, M. 2010. ZmMPK5 is required for the NADPH oxidase-mediated self-propagation of apoplastic H_2O_2 in brassinosteroid-induced antioxidant defence in leaves of maize. J. Exp. Bot. 61, 4399-4411.

Zhang, D., Luo, X., Zhu, L. 2011. Cytological analysis and genetic control of rice anther development. J. Genet. Genomics 38, 379-390.

Zhang, M., Li, Z., Feng, K., Ji, Y., Xu, Y., Tu, D., Teng, B., Liu, Q., Liu, J., Zhou, Y., Wu, W. 2023. Strategies for indica rice adapted to high-temperature stress in the middle and lower reaches of the Yangtze River. Front. Plant Sci. 13, 1081807-1081807.

Zhang, W., Sheng, J., Xu, Y., Xiong, F., Wu, Y., Wang, W., Wang, Z., Yang, J., Zhang, J. 2019. Role of brassinosteroids in rice spikelet differentiation and degeneration under soil-drying during panicle development. BMC Plant Biol. 19:409.

Zhang, X., Zhuang, L., Liu, Y., Yang, Z., Huang, B. 2020. Protein phosphorylation associated with drought priming-enhanced heat tolerance in a temperate grass species. Hort. Res. 7:207.

Zhao, L., Lu, L., Wang, A., Zhang, H., Huang, M., Wu, H., Xing, B., Wang, Z., Ji, R. 2020. Nano-biotechnology in agriculture: use of nanomaterials to promote plant growth and stress tolerance. J. Agric. Food Chem. 68, 1935-1947.

Zhao, Q., Guan, X., Zhou, L., Asad, M.-A.-U., Xu, Y., Pan, G., Cheng, F. 2023a. ABA-triggered ROS burst in rice developing anthers is critical for tapetal programmed cell death induction and heat stress-induced pollen abortion. Plant Cell Environ. 46:1453–1471.

Zhao, Q., Guan, X., Zhou, L., Xu, Y., Asad, M.-A.-U., Pan, G., Cheng, F. 2023b. *OsPDIL1-1* controls ROS generation by modulating NADPH oxidase in developing anthers to alter the susceptibility of floret fertility to heat for rice. Environ. Exp. Bot. 205:105103.

Zhao, Q., Zhou, L., Liu, J., Cao, Z., Du, X., Huang, F., Pan, G., Cheng, F. 2018a. Involvement of CAT in the detoxification of HT-induced ROS burst in rice anther and its relation to pollen fertility. Plant Cell Rep. 37, 741-757.

Zhao, Q., Zhou, L., Liu, J., Du, X., Asad, M. A., Huang, F., Pan, G., Cheng, F. 2018b. Relationship of ROS accumulation and superoxide dismutase isozymes in developing anther with floret fertility of rice under heat stress. Plant Physiol. Biochem. 122, 90-101.

Zhao, Y., Du, H., Wang, Y., Wang, H., Yang, S., Li, C., Chen, N., Yang, H., Zhang, Y., Zhu, Y., Yang, L., Hu, X. 2021. The calcium-dependent protein kinase ZmCDPK7 functions in heat-stress tolerance in maize. J. Integr. Plant Biol. 63, 510-527.

Zheng, S., Li, J., Ma, L., Wang, H., Zhou, H., Ni, E., Jiang, D., Liu, Z., Zhuang, C. 2019. *OsAGO2* controls ROS production and the initiation of tapetal PCD by epigenetically regulating *OsHXK1* expression in rice anthers. Proc. Natl. Acad. Sci. U.S.A. 116, 7549-7558.

Zhou, Y.B., Liu, C., Tang, D.Y., Yan, L., Wang, D., Yang, Y.Z., Gui, J.S., Zhao, X.Y., Li, L.G., Tang, X.D., Yu, F., Li, J.L., Liu, L.L., Zhu, Y.H., Lin, J.Z., Liu, X.M. 2018. The receptor-like cytoplasmic kinase STRK1 phosphorylates and activates CatC, thereby regulating H_2O_2 homeostasis and improving salt tolerance in rice. Plant Cell 30, 1100-1118.

Zhu, Y., Zuo, M., Liang, Y., Jiang, M., Zhang, J., Scheller, H. V., Tan, M., Zhang, A. 2013. MAP65-1a positively regulates H_2O_2 amplification and enhances brassinosteroid-induced antioxidant defence in maize. J. Exp. Bot. 64, 3787-3802.

Zoong Lwe, Z.S., Welti, R., Anco, D., Naveed, S., Rustgi, S., Narayanan, S. 2020. Heat stress elicits remodeling in the anther lipidome of peanut. Sci. Rep. 10, 22163.

Zou, J.J., Li, X.D., Ratnasekera, D., Wang, C., Liu, W.X., Song, L.F., Zhang, W.Z., Wu, W.H. *Arabidopsis* CALCIUM-DEPENDENT PROTEIN KINASE8 and CATALASE3 function in abscisic acid-mediated signaling and H_2O_2 homeostasis in stomatal guard cells under drought stress. Plant Cell 27, 1445-1460.

Table 1 Stress response proteins involved in ROS signaling

Species	Stress response proteins	inducer	Downstream protein or gene	Molecular and physiology function	Reference
Arabidopsis	CPK5	pathogen	AtRBOHD	Phosphorylating RBOHD and enhances pathogen resistance	(Dubiella et al., 2013)
Arabidopsis	CRK2	pathogen	AtRBOHD	Phosphorylating C-terminal region of RBOHD	(Kimura et al., 2020)
Arabidopsis	OST1/SnRK2E	ABA	AtRBOHF	Phosphorylating N-terminal region of RBOHF	(Sirichandra et al., 2009)
Arabidopsis	CIPK11 CIPK26	Ca^{2+}	AtRBOHE	Bind with Ca^{2+} sensor and phosphorylate RBOHE	(Han et al., 2019)
Arabidopsis	AtNTL4	Heat or drought stress	*AtRBOHC*, *AtRBOHE*	Promote expression of *AtRBOHC* and *AtRBOHhE*, improve drought tolerance	(Lee et al., 2012)
Maize	ZmCDPK	Heat or drought stress	ZmRBOHB	Phosphorylate RBOHB	(Zhao et al., 2021)
Tobacco	NtbHLH123	Salt stress	*NtRBOHE*	Directly bind to the promoter of *NtRBOHE* and promote its expression，enhance ROS scavenging activity	(Liu et al., 2021a)
Arabidopsis	ERF74/75	Heat, drought stress and ABA	*AtRBOHD*	Directly binds to the promoter of *AtRBOHD* and activates its expression, enhance stress tolerance	(Yao et al., 2017)

Abbreviations: CPK/CDPK, calcium-dependent protein kinase; CIPK, CBL-interacting protein kinase; OST1, open stomata 1; NTL4, NAC transcription factor 4; ERF74/75, ethylene response factor 74/75.

Table 2 Functional genes involved in regulating ROS levels in anthers and tapetum degeneration

Species	Functional gene	High expression stage	Histological localization	Protein function	Phenotype of mutant	reference
Rice	*DTC1*	S7-S10	Tapetum cell	Bind to OsMT2b and inhibit its ROS scavenging activity, help maintain high ROS level during stage9.	Tapetum degeneration delayed due to low ROS level; substrate exchange was disrupted.	(Yi et al., 2016)
Rice	*MADS3*	S9-S11	Tapetum cell	Promote expression of protein MT-1-4b, which scavenging ROS during later anther development stage.	ROS overaccumulate in anther during S10-S11; microspores subject to oxidative stress.	(Hu et al., 2011)
Rice	*UDT1*	Tapetum development stage	Anther	Regulate tapetum development relate gene expression.	Meiocytes degenerate at tetrads.	(Jung et al., 2005)
Rice	*OsAGO2*	S4-S11	Tapetum cell	Methylate promoter of *OsHXK1* and suppress its expression.	ROS overaccumulate in anther, leading premature initiation of tapetum PCD and pollen abortion.	(Zheng et al., 2019)
Rice	*TDR1*	Around meiosis stage	Tapetum cell	Promote the expression of *OsCP1* which involved in PCD.	Tapetum PCD delayed and resulting in pollen abortion.	(Li et al., 2006)
Rice	*EAT1*	S7-S11	Tapetum cell	Promote the expression of *OsAP25* and *OsAP37* who encodes aspartic protease.	Tapetum PCD delayed and resulting in pollen abortion.	(Niu et al., 2013)
Rice	*bHLH142*	Meiosis stage and microspore stage	Tapetum and meiocytes	Interact with TDR1 and promote the expression of *OsEAT1*.	Mutant did not enter meiosis and tapetum did not degeneration.	(Ko et al., 2014)
Rice	*EDT1*	S8-S10	Tapetum cell	Involved in lipid metabolism by catalyze cleavage of citric acid to acetyl-CoA.	Lipid and energy metabolism was disrupted; ROS overaccumulated; tapetum PCD premature and pollen abortion.	(Bai et al., 2019)
Rice	*OsTGA10*	S4-S6	Tapetum and microspore cell	Regulate tapetum development relate gene expression.	Endothecium thickening and tapetum degeneration was defective.	(Chen et al., 2018)
Rice	*TIP3*	S8-S10	Tapetum and microspore cell	Interact with TDR and promote the expression of downstream genes.	Tapetal degeneration delayed; no pollen wall formation; abnormal ubisch body morphology.	(Yang et al., 2019)
Arabidopsis	*RBOHE*	S6-T11	Tapetum cell	Oxidate NADPH and produce Superoxide.	Functional loss of RBOHE resulted in delayed tapetal degeneration	(Xie et al., 2014)
Tomato	*BZR1*			Involved in BR signaling and promote the expression of *RBOH1*.	Tapetum PCD delayed and resulting in pollen abortion	(Yan et al., 2020)
Tomato	*LeRBOHE*	S9-S10	Anther	Oxidate NADPH and produce Superoxide.	Abnormal tapetal degeneration result in pollen abortion.	(Dai et al., 2022)
Pea	*END1*	Before flowering	Anther wall expect tapetum	Encode ROS scavenging protein containing four hemopexin domains.	Tapetum PCD premature and resulting in pollen abortion	(Hamza et al., 2021) (Gómez et al., 2004)

Abbreviations: *DTC1*, *Defective Tapetum Cell Death 1*; *END1*, *ENDOTHECIUM 1*; *UDT1*, *Undeveloped Tapetum 1*; *TDR1*, *Tapetum Degeneration Retardation*; *EAT1*, *Eternal Tapetum 1*; *EDT1*, *Earlier Degraded Tapetum 1*; *TIP3*, *TDR Interactiving Protein 3; BZR1*, *Brassinazole Resistant 1;* MDA, malondialdehyde; PCD, Programmed Cell death.

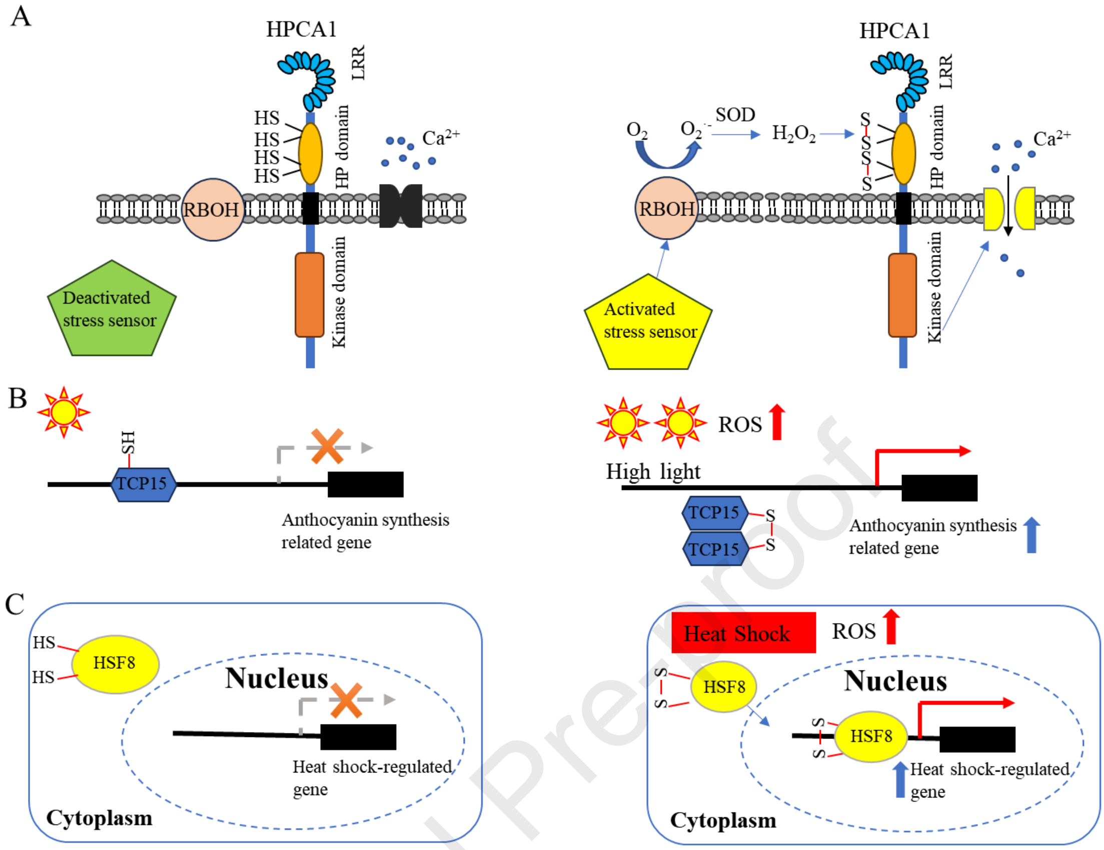

Fig. 1. Models of redox sensors involved in ROS signaling. (A) Extracellular ROS activate Ca^{2+} channels through oxidization of HPCA1. Under standard conditions, the extracellular ROS concentration is low, and HPCA1 is in a reduced state. Under stress conditions, RBOH is activated, resulting in the accumulation of extracellular ROS. Thus, HPCA1 undergoes autophosphorylation due to covalent modification of extracellular cysteine residues. Activated HPCA1 phosphorylates downstream proteins such as Ca^{2+} channels to trigger physiological responses. (B) ROS signaling plays a role in high light-induced anthocyanin accumulation. TCP15 inhibits the expression of anthocyanin synthesis-related genes under normal conditions High light stress induces TCP15 dimerization via the formation of disulfide bridges between two cysteine residues due to ROS accumulation. TCP15 dimers cannot bind to DNA and switch on anthocyanin synthesis. (C) Cytoplasmic ROS accumulation induces heat shock-regulated genes. Under normal temperatures, HSFA8 is in a reduced state and located in the cytoplasm. Heat shock-induced ROS accumulation results in HSFA8 oxidization and its translocation to the nucleus to drive heat shock-regulated gene expression. HPCA1, HYDROGEN PEROXIDE-INDUCED Ca^{2+} INCREASES 1; SOD, superoxide dismutase.

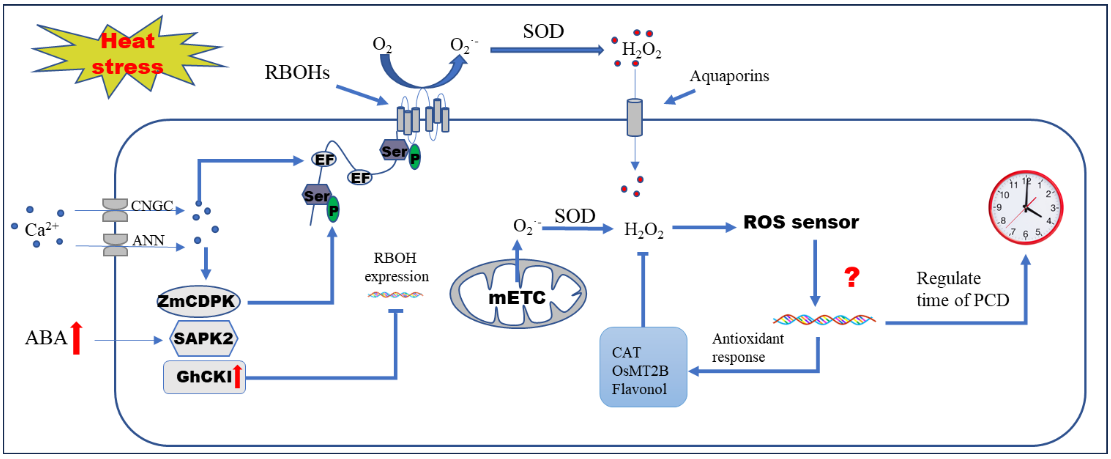

Fig. 2: The molecular mechanism by which heat stress disrupts anther ROS homeostasis and tapetum PCD. Ca^{2+} channels such as CNGC and ANN open and kinases upstream of RBOH are activated under heat stress. Membrane-located RBOH is activated by Ca^{2+} binding to its EF-hands, and kinases such as ZmCDPK and SAPK2 transfer phosphate to its Ser residues. Heat stress induces ABA accumulation and increased SAPK2 activity, leading to oxidative stress. In cotton, heat stress promotes *GhCKI* expression, which inhibits *RBOH* expression. $O2^{\cdot-}$ is relevated to the extracellular space by RBOH and can be transferred to the cytoplasm via aquaporins. The mitochondrial ETC is another site that produces $O2^{\cdot-}$ under heat stress. $O2^{\cdot-}$ is quickly converted to H_2O_2 via SOD, which is more stable under physiological conditions. To avoid oxidative stress, redox sensors that involve ROS signaling promote the expression of antioxidant-related genes. In anthers, CAT, OsMT2b and flavonols reportedly play roles in ROS detoxification. Tapetum degeneration is tightly controlled by ROS levels and downstream signaling. ABA, abscisic acid; ANN, annexin; CAT, catalase; CNGC, cyclic nucleotide-gated calcium channel; mETC, mitochondrial electron transport; PCD, programmed cell death; ROS, reactive oxygen species; SAPK2, ABA-activated protein kinase 2; SOD, superoxide dismutase.

Conflict of Interest

The authors declare that they have no conflicts of interest related to this paper.

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