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REVIEW PAPER

Redox-mediated responses to high temperature in plants

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Abstract

As sessile organisms, plants are particularly affected by climate change and will face more frequent and extreme temperature variations in the future. Plants have developed a diverse range of mechanisms allowing them to perceive and respond to these environmental constraints, which requires sophisticated signalling mechanisms. Reactive oxygen species (ROS) are generated in plants exposed to various stress conditions including high temperatures and are presumed to be involved in stress response reactions. The diversity of ROS-generating pathways and the ability of ROS to propagate from cell to cell and to diffuse through cellular compartments and even across membranes between subcellular compartments put them at the centre of signalling pathways. In addition, their capacity to modify the cellular redox status and to modulate functions of target proteins, notably through cysteine oxidation, show their involvement in major stress response transduction pathways. ROS scavenging and thiol reductase systems also participate in the transmission of oxidation-dependent stress signals. In this review, we summarize current knowledge on the functions of ROS and oxidoreductase systems in integrating high temperature signals, towards the activation of stress responses and developmental acclimation mechanisms.

Keywords: Antioxidants, Arabidopsis, heat stress, high temperature, redox, ROS, signalling, stress, thiol reductases.

Introduction

Climate change induced by human activities impacts on all levels of the ecological pyramid. Plants, as sessile organisms, are particularly prone to the multifactorial environmental stress induced by the increase in global temperature. Stresses induced by increases in temperature include systemic water deficit, increased ravaging by pests, and increased salt concentrations in regions susceptible to flooding. Moreover, climate change induces more frequent and intense heat waves, which negatively affect plant survival (Liu *et al.*, 2016; Bailey-Serres *et al.*, 2019; IPCC, 2021). In plants, heat stress influences most development stages including seed germination, radicle emergence, seedling growth, and development of flowers and reproductive organs. Inhibition of photosynthesis and further effects of heat stress on metabolic processes negatively affect

cell division and growth (Wang *et al.*, 2018). Plant acclimation to high temperature is highly dependent on the amplitude and the timing of temperature variations. While plants are normally adapted to seasonal and daily temperature fluctuations, they can suffer from extreme summer heat waves, particularly when combined with other stresses such as drought or high light. Reactive oxygen species (ROS) are by-products of many plant metabolic processes such as chloroplastic and mitochondrial electron transport, but their production is increased under conditions of stress such as high temperatures (Choudhury *et al.*, 2017; Noctor *et al.*, 2018). The reactivity of ROS means that their concentrations must be controlled, but also that they are excellent signalling molecules that underpin plant development and responses to high temperature (Considine and Foyer, 2014; Dietz *et al.*, 2016; Mhamdi and Van Breusegem, 2018; Waszczak *et al.*, 2018; Huang *et al.*, 2019). In particular, ROS play important roles in heat signalling cascades by regulating the expression and accumulation of heat stress response proteins (Driedonks *et al.*, 2015). Plants are equipped with an exceptional network of ROS detoxification and oxidoreductase enzymes, which also participate in high temperature signalling and response systems (Noctor *et al.*, 2018). In this review, our aim is to summarize current knowledge on the role of ROS, redox regulation, and oxidoreductases in plant responses to high temperatures, and how these players shape plant development and resistance for acclimation to changing environments.

ROS metabolism and signalling under high temperature

Plant responses to high temperatures involve complex pathways that allow them to perceive this environmental constraint and transmit the respective information. These transmission pathways also depend on the nature of the high temperature regime. This section aims to detail the major components of these pathways with respect to the nature of the high temperature regime, particularly focusing on ROS and redox signalling.

Different types of high temperature regimes induce different pathways

To study the mechanisms of plant acclimation to high temperatures under controlled conditions, Yeh *et al.* (2012) proposed to classify thermotolerance assays into three types: basal thermotolerance (BT), short- and long-term acquired thermotolerance (SAT/LAT), and thermotolerance to moderately high temperature (TMHT) (Fig. 1). Of note, this classification is based on the response of Arabidopsis (*Arabidopsis thaliana*) and might be different for other plant species. Arabidopsis is commonly grown at ~20–22 °C under controlled conditions. A short heat shock (HS) of a few hours above 40 °C leads to plant death (Yeh *et al.*, 2012). However, previous exposure to moderately high temperature, of ~37 °C, can increase the survival chances of the plant (Larkindale and Vierling, 2008; Sedaghatmehr *et al.*, 2016). The BT assay is based on an abrupt increase of the ambient temperature (20–22 °C) to 40–45 °C during a short time period of 0.5–1 h (Fig. 1A). The SAT and LAT assays (Fig. 1B) refer to experiments including a pre-treatment at a moderately high temperature (generally 1 h at 37 °C) called priming, and are used to study genes involved in thermomemory (recently reviewed by Balazadeh, 2022). The time interval between priming and a more severe heat treatment (generally 1–2 h at 45 °C) ranges from 2 h for SAT to >2 d for LAT assays. TMHT assays consist of a 33–38 °C treatment for several days (5–8 d) (Fig. 1C). Because BT, SAT, and LAT assays involve short treatments, they are generally performed using a pre-heated water bath for a rapid increase in temperature. In contrast, TMHT assays are performed in thermostatic growth chambers because they require an increase in temperature without changing light or other growth conditions. All these thermotolerance assays are typically followed by a recovery phase of several days during which plants are placed at 20 °C and thermotolerance is determined based on the survival rate of a large number of plants. The respective treatments are used to mimic a short variation in temperature observed during the day (for BT, SAT, or LAT) or longer heat waves occurring in temperate climate regions during summer (for TMHT). All these severe heat stress treatments are known to induce profound reprogramming of gene expression including massive transcriptional induction of heat shock proteins (HSPs) or ROS-scavenging enzymes, enabling plant survival (see the following sections). In contrast, a mild increase in growth temperatures, up to 30 °C (referred to as high ambient temperature in this review), causes changes in plant morphology and affects transition in developmental stages such as germination and flowering without affecting plant survival (Quint *et al.*, 2016; Casal and Balasubramanian, 2019). This treatment does not induce an HS response, but rather induces a developmental acclimation with physiological modifications such as enhanced growth of hypocotyls and petioles, early flowering, reduction of the stomatal index, and longer and thinner primary roots. This so-called thermomorphogenesis is associated with a very different reprogramming of gene expression compared with HS responses and is associated with chromatin remodelling and epigenetic modifications (see the following sections) (Kumar and Wigge, 2010; Casal and Balasubramanian, 2019). Thermomorphogenesis assays are conducted by growing the plants at 4–7 °C above their optimal growth temperature, generally for >5 d, and measuring hypocotyl or petiole elongation (Fig. 1D).

ROS production under high temperature

Like most abiotic and biotic stress conditions, high temperatures lead to the accumulation of ROS in different subcellular compartments, as summarized in Fig. 2. Temperature, as a physical factor, directly alters the fluidity of biomembranes and causes the remodelling of membrane microdomains (Niu and Xiang, 2018). High temperatures not only change the membrane composition

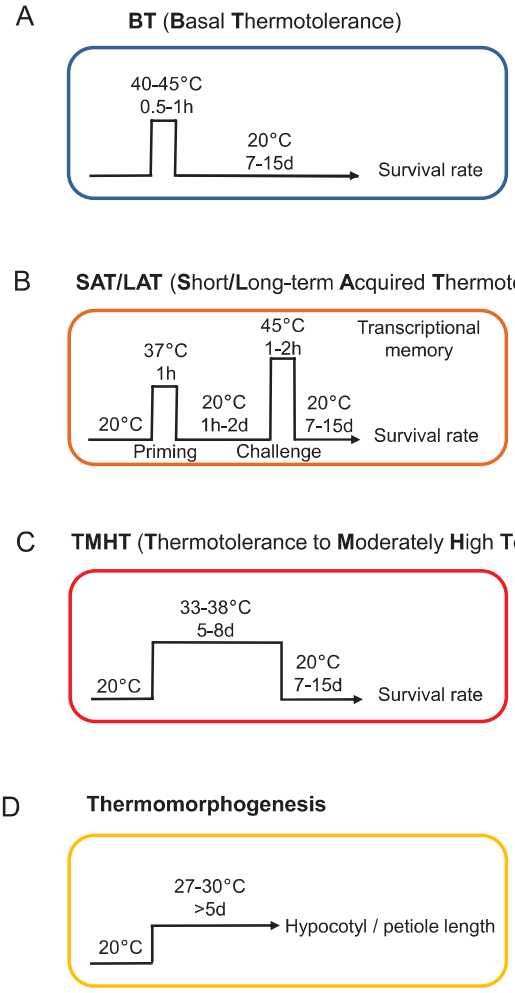

Fig. 1. Different high temperature regimes to study heat stress responses in plants. (A) Basal thermotolerance (BT). (B) Short-/long-term acquired thermotolerance (SAT/LAT). (C) Thermotolerance to moderately high temperature (TMHT). (D) Thermomorphogenesis. See text for more details on the conditions of the respective treatments.

but also affect specific interactions between lipids and proteins such as fatty acid desaturases, ion channels, kinases, or proteins in the organellar electron transport chains (ETCs). For example, the ROS-generating respiratory burst oxidase homologue D (RBOHD) localized in plasma membrane microdomains is activated by a heat-induced cytosolic calcium (Ca^{2+}) increase to produce superoxide ($O_2^{\cdot-}$) in the apoplast, which is chemically and possibly also enzymatically dismutated to hydrogen peroxide (H_2O_2) (Lherminier *et al.*, 2009; Suzuki *et al.*, 2012; Chen *et al.*, 2022). The major positive role of extracellular ROS generation under high temperature is emphasized by the high sensitivity of *rbohd* null mutants to basal and acquired thermotolerance regimes (Larkindale *et al.*, 2005). A rapid accumulation of RBOH-dependent H_2O_2 occurs in suspension cells subjected to 38 °C (Königshofer *et al.*, 2008), which is also observed in Arabidopsis plants subjected to a BT regime (Babbar *et al.*, 2021). Apoplastic H_2O_2 can enter cells through aquaporins in the plasma membrane and covalently modify cytoplasmic proteins to trigger signalling processes (Rodrigues *et al.*, 2017). Apoplastic H_2O_2 oxidizes cysteine residues in the plasma membrane-localized phospholipase D (PLD), resulting in microtubule depolarization, induction of mitogen-activated protein kinase (MAPK) activity, and possibly induction of *HSP70* expression (Song *et al.*, 2020). Upon stress, Ca^{2+} is also rapidly released into the cytosol through the activation of Ca^{2+} channels, which contribute to the induction of signalling cascades (McAinsh and Pittman, 2009).

Heat stress signalling by apoplastic H_2O_2 could also be mediated by the leucine rich-repeat receptor kinase HPCA1 (hydrogen peroxide-induced Ca^{2+} increases 1) in the plasma membrane. HPCA1 is activated by H_2O_2 via the formation of two disulfide bridges between four extracellular cysteine residues, leading to the autophosphorylation of HPCA1 at its cytosolic domain. This ultimately leads to activation of Ca^{2+} channels in guard cells, which is required for stomatal closure (Wu *et al.*, 2020) and the propagation of cell to cell ROS signals following local high light stress (Fichman *et al.*, 2022). Yet, the contribution of HPCA1 to heat stress signalling has not been explored.

Plant organelles are also hotspots of ROS production under high temperature. ROS are continuously formed in photosynthetic and respiratory ETCs in chloroplasts and mitochondria. Heat stress can disrupt the integrity of thylakoid membranes by impacting protein–lipid and lipid–lipid interactions, leading to membrane leakiness and a loss of the proton gradient (Schrader *et al.*, 2007; Zhang and Sharkey, 2009). Hence, heat stress-induced destabilization of the ETC results in the formation of ROS and concomitant membrane lipid peroxidation, further contributing to the loss of membrane integrity (Hüve *et al.*, 2011; Copolovici *et al.*, 2012). While electron transfer in PSII is altered by heat stress, Rubisco activity also decreases because Rubisco activase activity is redox controlled and depends on photosynthetic electron flow (Salvucci and Crafts-Brandner, 2004). An alternative source of ROS can be provided by the over-reduction of the photosynthetic ETC during stress, due to a slowdown of ATP- and NADPH-consuming enzymes (Foyer *et al.*, 2012). On the other hand, H_2O_2 produced by imbalances in chloroplast energetics activates cyclic electron flow around PSI, which can contribute to heat stress acclimation (Soursa, 2015; Strand *et al.*, 2015).

Temperature regimes such as those used to assess TMHT cause aggregation of components of the mitochondrial ETC complexes, resulting in excess ROS production (Maziak *et al.*, 2021). In addition, photorespiratory processes also generate ROS in peroxisomes (Waszczak *et al.*, 2018; Mittler *et al.*, 2022).

Cytosolic and nuclear compartments under high temperature conditions

The cytosol is not a major ROS-generating compartment under HS conditions. However, it constitutes a crucial

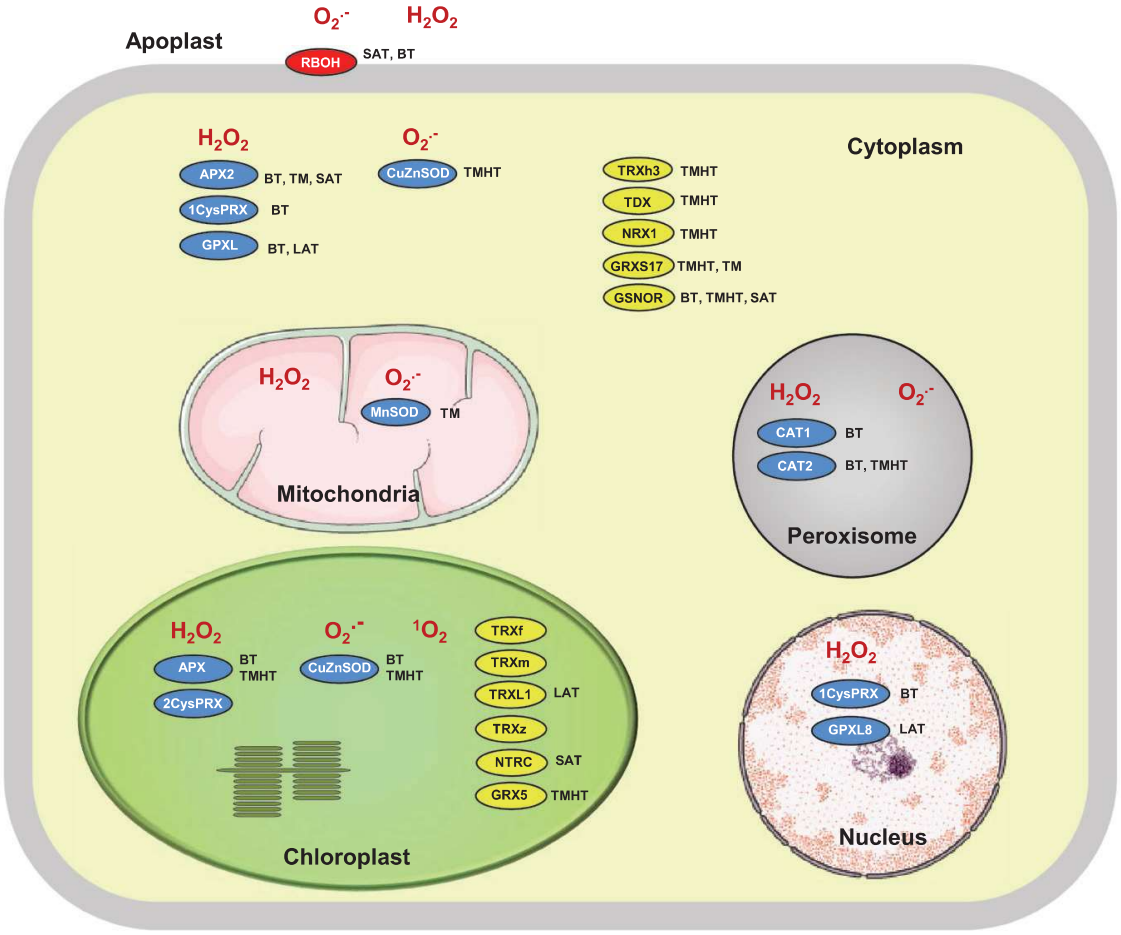

Fig. 2. ROS and plant antioxidative enzymes involved in high temperature responses. The figure summarizes the available information on the subcellular localization of ROS (H_2O_2, hydrogen peroxide; $O_2^{\cdot-}$, superoxide; $^1O_2$, singlet oxygen), enzymes involved in ROS reduction (blue ellipses), ROS production (red ellipse), and redox modification of protein thiols (yellow ellipses) in high temperature responses in Arabidopsis and other plants. The information is not comprehensive, and other ROS and proteins may be involved in heat stress responses. When available, the type of high temperature regime used to show the role of the respective protein is indicated: BT, basal thermotolerance; SAT, short-term acquired thermotolerance; LAT, long-term acquired thermotolerance; TMHT, thermotolerance to moderately high temperature; TM, thermomorphogenesis. APX, ascorbate peroxidase; CAT, catalase; RBOH, respiratory burst oxidase homologue; GPXL, glutathione peroxidase-like; GRX, glutaredoxin; NTRC, NADPH-thioredoxin reductase C; TRX, thioredoxin; NRX, nucleoredoxin; TDX, tetratricoredoxin; GSNOR, nitrosoglutathione reductase; PRX, peroxiredoxin; SOD, superoxide dismutase.

compartment for the integration of multiple stress signalling pathways. Together with the endoplasmic reticulum (ER), the cytosol signals heat stress-induced protein and RNA damage by inducing the unfolded protein response (UPR) pathway (C.-Y. Yu *et al.*, 2022). It is also the site of synthesis of proteins involved in heat stress responses, such as protein chaperones and antioxidant enzymes. ROS and Ca^{2+} generated or released during HS are transmitted to the cytosol through aquaporins and Ca^{2+} channels. Cytosolic Ca^{2+} accumulation induces several downstream phosphorylation cascades involving calcium-dependent protein kinases (CDPKs), calmodulin CAM3, and MAPKs, activating several HS-responsive transcription factors (TFs) such as heat shock factors (HSFs), DREBs, WRKYs, and the transcriptional co-activator multiprotein bridging factor 1 (MBF1) (Liu *et al.*, 2008; Zhang *et al.*, 2009; Suzuki *et al.*, 2011; Mittler *et al.*, 2012). For example, HSFA4a might be a sensor of H_2O_2 signalling, while HSFA5 serves as a repressor of this pathway (Miller and Mittler, 2006; Baniwal *et al.*, 2007). MAPKs can affect the expression and activity of catalase (CAT) to control ROS levels while conversely regulating the expression of *RBOHD* in various species (Ortiz-Masia *et al.*, 2008; Pitzschke and Hirt, 2009; Liu and He, 2017).

The dynamics and the pattern of ROS production, as well as their impact on the cytosolic redox state, have been poorly investigated under different high temperature regimes. The development of genetically encoded ROS and redox sensors allows these questions to be addressed. For example, the use of Grx1–roGFP2 (reduction–oxidation-sensitive green fluorescent protein 2) for monitoring the redox state of glutathione has shown glutathione oxidation in the cytosol and

the nucleoplasm during a 1 h BT assay at 42 °C (Babbar *et al.*, 2021). In other studies, in contrast, severe heat stress at 55 °C for 30 min caused oxidation of cytosolic and mitochondrial roGFP while less severe heat treatments (<45 °C, 30 min) did not affect the roGFP redox state (Schwarzländer *et al.*, 2009). This emphasizes the high plasticity of the cellular redox state under contrasting high temperature treatments. Moreover, other environmental constraints (e.g. light or CO_2) also influence the cellular redox state under high temperatures (see the following sections).

Targeting genetically encoded sensors for H_2O_2 and the glutathione redox potential (E_{GSH}) along with Ca^{2+}, pH, and NADPH biosensors to different subcellular compartments allows the dynamics of these parameters under high temperature regimes to be deciphered at the subcellular scale, as has been approached under other abiotic and biotic stresses (Knight *et al.*, 1991; Miesenböck *et al.*, 1998; Schwarzländer *et al.*, 2009; Exposito-Rodriguez *et al.*, 2017; Lim *et al.*, 2020; Hipsch *et al.*, 2021; Müller-Schüssele *et al.*, 2021; Ugalde *et al.*, 2021; Moreau *et al.*, 2022). Such integrated approaches might help to understand how ROS signatures and redox modifications triggered by specific stress conditions are transmitted to other cell compartments, such as the nucleus. Nuclear ROS generation is not documented and the glutathione pool in the nucleus has a highly reduced redox state. However, rapid oxidation of the nucleoplasm occurs upon heat stress, which is probably relayed through the passive diffusion of ROS from the cytosol (Babbar *et al.*, 2021). Moreover, the transfer of H_2O_2 from chloroplasts to the nucleus has also been suggested under high light conditions, which has been proposed to occur through physical contacts between chloroplastic stromules and nuclei (Estavillo *et al.*, 2011; Maurel *et al.*, 2015; Exposito-Rodriguez *et al.*, 2017). Nevertheless, the regulation of ROS and redox levels in the nucleus is poorly understood, particularly under stress conditions.

High temperature influence on antioxidative systems

To prevent oxidative damage and to regulate the thiol oxidation state, plant cells rely on a wide range of enzymatic and non-enzymatic antioxidants, including thiol reductases (Mittler *et al.*, 2022). This section of the review provides an overview of HS-induced effects on specific antioxidants and summarizes how these contribute to thermotolerance (Fig. 2).

Superoxide dismutase

Superoxide dismutases (SODs) catalyse the dismutation of $O_2^{\cdot-}$ into H_2O_2 and molecular oxygen (O_2). Based on their metal cofactor, SODs are classified into different groups: copper/zinc SOD (Cu/Zn-SOD), iron SOD (Fe-SOD), and manganese SOD (Mn-SOD). They can be found in virtually all subcellular compartments including chloroplasts, mitochondria, peroxisomes, the cytosol, the nucleus, and possibly the apoplast (Alscher *et al.*, 2002; Gill *et al.*, 2015; Chen *et al.*, 2022).

Information on the influence of high temperatures on SODs and their involvement in thermotolerance is relatively scarce. However, it has been shown that HS affects SOD protein levels and/or activity in a wide variety of plant species including *Rehmannia glutinosa* (Chung *et al.*, 2006), *Solanum lycopersicum* (Camejo *et al.*, 2007), *Lotus japonicus* (Sainz *et al.*, 2010), and *Oryza sativa* (Shah and Nahakpam, 2012; Zhao *et al.*, 2018). Furthermore, the importance of SOD in high temperature responses is emphasized by the altered thermotolerance of plants with altered SOD levels. For example, decreases in photosynthetic activity upon treatment with a temperature of 42 °C for 20 h were less pronounced in potato plants expressing Cu/Zn-SOD and ascorbate peroxidase (APX) in the chloroplast under an oxidative stress-inducible *SWPA2* promoter (Tang *et al.*, 2006). Similarly, rice plants overexpressing a plastid/Golgi-localized Mn-SOD (*MSD1*) had a higher percentage of perfect grains when grown at increased day/night temperatures of 33 °C/28 °C during the grain-filling stage, whereas the opposite was observed for an *MSD1* knockdown line (Shiraya *et al.*, 2015).

Catalase

CATs mediate the conversion of H_2O_2 into H_2O and O_2, and do not require an additional reductant, in contrast to peroxidases, which are also involved in H_2O_2 neutralization (discussed below). CATs are especially important for the maintenance of redox homeostasis in peroxisomes (Mhamdi *et al.*, 2012; Anjum *et al.*, 2016).

Recently, a broad screen of 41 rice mutants to identify heat-tolerant genotypes revealed that morpho-physiological as well as biochemical traits could explain variations in grain yield upon HS. Among these, CAT seemed of particular importance as many heat-sensitive genotypes were characterized by reduced CAT activity and enhanced H_2O_2 levels after 12 h exposure to 45 °C followed by a 3 d recovery period. Based on their results, the authors proposed that CAT activity could be used as a biomarker for heat tolerance of rice plants at early growth stages (Zafar *et al.*, 2020). The role of CAT in thermotolerance was also supported by the observation that Arabidopsis *cat2* mutants had a lower TMHT. Interestingly, their BT was like that of WT plants, indicating that the importance of specific antioxidants during HS responses strongly depends on the imposed stress regime. Furthermore, thermotolerance was unaltered in *cat1* and *cat3* mutants, suggesting a specific role for CAT2 in TMHT (Ono *et al.*, 2021). This finding is in line with the observation that CAT2 accounts for ~90% of CAT activity in Arabidopsis leaves (Mhamdi *et al.*, 2010). The importance of CAT2 for H_2O_2 detoxification is also emphasized by

the inability of *cat2* null mutants to detoxify externally applied H_2O_2 efficiently (Ugalde *et al.*, 2021). In maize, on the other hand, *cat2* knockout lines displayed an enhanced germination rate at 40 °C, implying a negative effect of CAT2 on these severe high temperature conditions (Scandalios *et al.*, 2000). Hence, it is likely that the involvement of specific antioxidants depends on the developmental stage during which plants are subjected to HS as well as the plant species. The latter is further supported by the fact that overexpression of the native *CAT2* gene did not enhance thermotolerance of Arabidopsis (Ono *et al.*, 2021), whereas transgenic expression of *Brassica oleracea BoCAT1* and *BoCAT2* limited heat-induced increases in H_2O_2 levels in Arabidopsis (Chiang *et al.*, 2014).

Ascorbate–glutathione cycle

Besides CAT, APX also contributes to H_2O_2 detoxification in plant cells. This enzyme functions in the ascorbate–glutathione (AsA–GSH) cycle, which can be found in most subcellular compartments. As its name implies, APX relies on AsA as an electron donor for the reduction of H_2O_2 to H_2O. This reaction yields two molecules of monodehydroascorbate (MDHA), which is recycled to AsA by the action of monodehydroascorbate reductase (MDHAR) or in a non-enzymatic disproportionation reaction resulting in one molecule of AsA and one molecule of dehydroascorbate (DHA). The latter is reduced back to AsA in a reaction catalysed by dehydroascorbate reductase (DHAR), using electrons derived from reduced glutathione (GSH). As a consequence, glutathione disulfide (GSSG) is formed and needs to be recycled back to GSH by glutathione reductase (GR), which relies on NADPH as a reductant (Gill and Tuteja, 2010; Foyer and Noctor, 2011).

Overexpressing different native or heterologous *APX* genes could be a promising strategy to enhance plant tolerance to high temperatures. Indeed, overexpression of its native stromal APX in Arabidopsis slightly enhanced its ability to retain chlorophyll when subjected to a temperature of 33 °C. Strikingly, overexpression of the stromal APX from the red alga *Cyanidioschyzon merolae* in Arabidopsis had a much more pronounced beneficial effect on TMHT (33 °C for 7 d) (Hirooka *et al.*, 2009). Similarly, Arabidopsis plants overexpressing different *APX* isoforms from *Brassica campestris* showed enhanced thermotolerance, as evidenced by higher chlorophyll contents, better germination rates, and lower malondialdehyde (i.e. a lipid peroxidation marker) levels compared with wild-type (WT) plants after exposure to 40 °C for 5 d (Chiang *et al.*, 2015). Transgenic *B. oleracea* lines overexpressing *APX* also displayed an enhanced thermotolerance (Jiang *et al.*, 2016). It should be noted, however, that the impact of altered *APX* expression on heat sensitivity strongly depends on the high temperature regime applied, as Arabidopsis *apx2* knockout mutants showed a reduced BT but are not affected in SAT (Suzuki *et al.*, 2013b). Furthermore, the developmental stage at the time of heat treatment is crucial, since these *apx2* knockouts even showed an enhanced thermotolerance during the reproductive stage, as revealed by increased seed production. Based on these data, the authors suggested that redundant mechanisms are activated in *apx2* mutants to protect reproductive tissues from heat-induced damage (Suzuki *et al.*, 2013b). In addition, APX was also found to play an important role in floral transitioning upon prolonged elevated temperature exposure (30 °C during 14 d) in the *Oncidium* hybrid orchid, which is probably related to its role in regulating the AsA redox state (Chin *et al.*, 2014).

From these data, it is clear that APX2 is the major APX isoform involved in plant thermotolerance. As such, APX2 is subject to many layers of regulation during HS responses. First of all, the promoter of Arabidopsis *APX2* contains a heat shock element (HSE) responsible for its transcriptional up-regulation upon binding of HSFs (Wang *et al.*, 2016). Interestingly, such thermal activation of *APX2* transcription is more pronounced under light compared with dark conditions, as a consequence of phytochrome B-mediated light priming (Han *et al.*, 2019). Furthermore, APX2 is also regulated at the post-translational stage, as a study in tomato revealed that the calcium-dependent protein kinase CPK28 phosphorylates APX2 at Thr59 and Thr164, thereby improving thermotolerance (Hu *et al.*, 2021).

The importance of the AsA–GSH cycle in plant tolerance to elevated temperatures is further supported by the fact that exogenous AsA application partially mitigates the heat sensitivity of several species including *Phaseolus aureus* (Kumar *et al.*, 2011), *Triticum aestivum* (Kumar *et al.*, 2014), and *Festuca arundinacea* (K. Chen *et al.*, 2017). In addition, data from AsA-deficient and AsA-overproducing lines support the involvement of this antioxidative metabolite in plant thermotolerance (Larkindale *et al.*, 2005; Tóth *et al.*, 2011; Zhang *et al.*, 2018). In this context, AsA seems crucial for the prevention of heat-induced photoinhibition in chloroplasts (Tóth *et al.*, 2011; K. Chen *et al.*, 2017). The role of GSH in governing plant thermotolerance is supported by the fact that sulfur incorporation into GSH is increased under HS conditions in maize (Nieto-Sotelo and Ho, 1986). Furthermore, the rate of sulfur incorporation into GSH and hydroxymethylglutathione was higher in wheat genotypes with a higher TMHT (Kocsy *et al.*, 2004). Using genetically encoded biosensors, two recent studies showed that treatment with 37 °C or 42 °C caused oxidation of the cytosolic E_{GSH} in Arabidopsis (Babbar *et al.*, 2021; Dard *et al.*, 2023). Moreover, GSH biosynthesis mutants and WT plants treated with the GSH biosynthesis inhibitor buthionine sulfoximine were characterized by a decreased TMHT (Dard *et al.*, 2023). In earlier work, Larkindale *et al.* (2005) also reported that the GSH-deficient *cad2* mutants displayed a reduced BT compared with WT plants. In contrast, SAT was not greatly different from that of the WT (Larkindale *et al.*, 2005). Besides its role in H_2O_2 detoxification, GSH might also enhance thermotolerance through other mechanisms. In *Vigna radiata*, exogenous GSH application was suggested to improve thermotolerance by modulating methylglyoxal detoxification (Nahar *et al.*, 2015). In addition, GSH

was shown to modulate the expression of several HSFs via the BZIP10 and MYB21 TFs (Kumar and Chattopadhyay, 2018). Finally, *GR* expression might also be subject to thermal regulation, as promoters of all GR-encoding genes in *Brassica juncea* were shown to contain a heat-responsive *cis*-element (Verma and Singh, 2021), and enhanced GR activities were observed in soybean, Arabidopsis, and poplar in response to heat stress (Weston *et al.*, 2011).

Glutathione peroxidase-like proteins

Although their functions in plant cells have not been fully elucidated to date, it is well known that glutathione peroxidases (GPXs) are involved in detoxifying H_2O_2 as well as organic peroxides. In plants, these thiol peroxidases rely on the presence of conserved cysteines in their active site and depend on thioredoxins (TRXs) rather than GSH as an electron donor. Hence, it is more appropriate to refer to these enzymes as GPX-like (GPXL). The Arabidopsis genome encodes eight GPXLs, which are localized in the nucleus, cytosol, chloroplasts, mitochondria, the endoplasmic reticulum (ER), and at the plasma membrane (Gaber *et al.*, 2012; Attacha *et al.*, 2017).

Currently, information on the involvement of plant GPXLs in (a)biotic stress responses is scarce, probably as a consequence of a high degree of functional redundancy. Nevertheless, increased *GPXL* transcript levels have been reported in different plant species including Arabidopsis (Rodriguez Milla *et al.*, 2003), *Camellia sinensis* (Fu, 2014), *Nelumbo nucifera* (Diao *et al.*, 2014), and *Gossypium hirsutum* (M. Chen *et al.*, 2017). Furthermore, a *gpxl8* Arabidopsis mutant showed a reduced LAT, as evidenced by a stronger extent of lipid peroxidation and increased oxidized protein levels compared with WT plants (Gaber, 2014). Similarly, transient expression of a tomato *GPXL* in tobacco improved its tolerance to salt and BT-related heat stresses, and limited the appearance of apoptosis-like features in response to these conditions (Chen *et al.*, 2004). Hence, GPXLs might be useful targets to enhance plant thermotolerance, and this topic is an interesting avenue for further research.

Peroxiredoxins

Peroxiredoxins (PRXs), the second type of thiol peroxidases in plants, are also involved in detoxification of H_2O_2 and organic peroxides. Based on their biochemical characteristics, PRXs are further classified into typical 2-Cys PRXs, typical 1-Cys PRXs, and atypical 2-Cys PRXs. The latter group is further subdivided into PRXQ and type II PRXs. These subgroups differ in their interaction and regeneration mechanisms, as reviewed by Liebthal *et al.* (2018). Plant PRXs can be found in the cytosol, nucleus, chloroplasts, and mitochondria (Liebthal *et al.*, 2018).

The involvement of PRXs in HS responses has often been proposed based on the altered thermotolerance of PRX mutant or overexpressing lines. For example, transgenic expression of a *N. nucifera* 1-Cys PRX enhanced the thermotolerance of Arabidopsis seeds, as evidenced by an enhanced germination rate and a higher percentage of green cotyledons compared with the WT (Chen *et al.*, 2016). Moreover, overexpression of native or heterologous 2-Cys PRXs was shown to enhance the HS tolerance of different species including *F. arundinacea* (Kim *et al.*, 2010), *Solanum tuberosum* (Kim *et al.*, 2011), and Arabidopsis (Mishra *et al.*, 2021). Furthermore, heterologous expression of a putative chloroplastic 2-Cys PRX from rice enhanced HS tolerance in yeast (Kim *et al.*, 2013). Using RNAi in tobacco, H. Yu *et al.* (2022) revealed that 2-Cys PRX plays a key role in alleviating heat-induced oxidative damage and photoinhibition. Virus-induced gene silencing of 2-Cys PRX and PRXIIB in *Nicotiana benthamiana* revealed that both proteins are involved in AsA regeneration during HS (Vidigal *et al.*, 2015). Furthermore, virus-induced gene silencing of 2-Cys PRX in tomato led to increased transcript levels of genes involved in AsA biosynthesis and autophagy, as well as enhanced autophagosome formation during HS, hinting at the involvement of PRXs in stress-induced autophagy (Cheng *et al.*, 2016).

The key role of PRXs in thermotolerance is likely to be linked to their dual function as peroxidases and molecular chaperones. This was, for example, demonstrated for the 2-Cys PRX1 from *Brassica campestris*, which switches between both functions in response to HS and oxidative stress (Kim *et al.*, 2009). Similarly, chloroplast-localized 2-Cys PRX from Arabidopsis was also shown to function as a chaperone, inhibiting the aggregation of citrate synthase during heat treatment (Fig. 3A) (Muthuramalingam *et al.*, 2009). Interestingly, E.M. Lee *et al.* (2015) were able to enhance peroxidase as well as chaperone activities of Arabidopsis 2-Cys PRX by site-directed mutagenesis of Cys to Ser at position 150 of the α-helix, suggesting that mutagenesis of PRXs could be a promising strategy for improvement of plant tolerance to heat. In addition to a peroxidase–chaperone functional switch, PRXs are also regulated at the transcriptional level in response to HS. Indeed, the Arabidopsis 1-Cys PRX PER1 is transcriptionally regulated by the ABI5 TF, which interacts with the RNA-binding protein FCA, thereby contributing to thermotolerance (S. Lee *et al.*, 2015).

Thioredoxins

TRXs are responsible for the reduction of target proteins, including PRXs and GPXLs, via a thiol–disulfide exchange mechanism catalysed by two redox-active cysteines in the active site. Two systems exist for the subsequent reduction of oxidized TRXs: NADPH-dependent thioredoxin reductase (NTR) in the cytosol and mitochondria, and ferredoxin-dependent thioredoxin reductase (FTR) in chloroplasts, using the reductive power of NADPH and ferredoxin, respectively (König *et al.*, 2012). An additional NTR (NTRC) in plastids differs from the cytosolic and mitochondrial isoforms in that it contains its own TRX domain (Pérez-Ruiz *et al.*, 2006). Plants

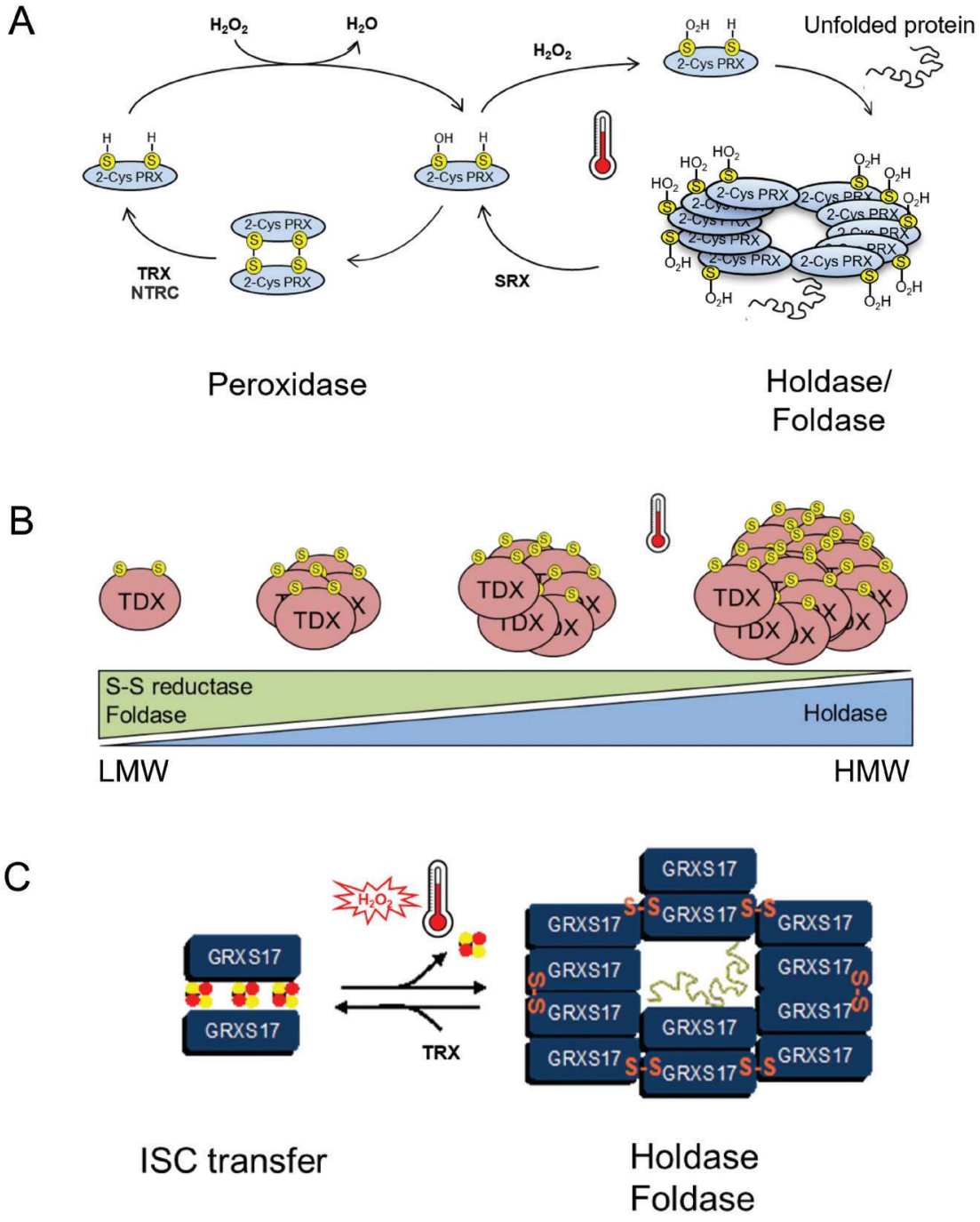

Fig. 3. Thiol reductase-based redox switches upon high temperatures. (A) Redox-dependent switch of 2-Cys peroxiredoxin (PRX) from peroxidase to chaperone activity. At 20 °C, the reduced 2-Cys PRX dimer functions as a peroxidase, scavenging H_2O_2. After initial oxidation (S-OH), 2-Cys PRXs dimerize and form two intermolecular disulfide bridges, which are reduced by thiol reductases such as thioredoxin (TRX) and NADPH-dependent thioredoxin reductase C (NTRC) (Pérez-Ruiz *et al.*, 2006). At high temperatures, overaccumulation of H_2O_2 induces sulfinylation (S-O_2H) and decamerization of 2-Cys PRX. 2-Cys PRX decamers function as holdase or foldase chaperones *in vitro* on citrate synthase (Muturamalingam et al., 2009; König *et al.*, 2013). 2-Cys PRX decamers are reduced by sulfiredoxins (SRXs) (Iglesias-Baena *et al.*, 2010). (B) Redox-dependent switch of tetratricoredoxin (TDX) from a disulfide reductase/foldase to a holdase activity. At 20 °C, TDX is mainly found in low molecular weight (LMW) complexes with disulfide reductase and foldase chaperone activity. At high temperatures, heat-generated H_2O_2 participates in TDX oligomerization and the formation of high molecular weight (HMW) complexes with holdase chaperone activity (Lee *et al.*, 2009). Substitution of the active site cysteines C304 and C307 by serines does not impair thermotolerance to moderately high temperatures (TMHT) (35 °C, 5 d) of the *tdx* null mutant (Lee *et al.*, 2009). (C) Redox-dependent switch of GRXS17 from iron–sulfur cluster transfer to chaperone activity. At 20 °C, the holo-GRXS17 is dimeric and coordinates

three iron–sulfur clusters (ISCs) that are to be transferred to cytosolic and nuclear target proteins (e.g. BOLA). At 35 °C, heat-generated H_2O_2 induces intermolecular disulfide formation and oligomerization of GRXS17 that acquires chaperone activities as a holdase and foldase. Substitution of active site cysteines C33, C179, C309, and C416 by serines impairs thermotolerance to moderately high temperatures (TMHT) (35 °C, 6 d) of the *grxS17* null mutant (Martins *et al.*, 2020).

contain at least 10 subfamilies of TRXs, together consisting of >40 members, present in almost all subcellular compartments (Sevilla *et al.*, 2015). No free TRXs have been reported for the vacuole, the secretory pathway, and the apoplast in plants.

Similar to PRXs, TRXs play major roles in plant thermotolerance because of their ability to switch to a chaperone function. Indeed, the transition of the TRX-like protein TDX from Arabidopsis from low molecular weight (LMW) to high molecular weight (HMW) complexes in response to an HS coincided with a shift in its function from a disulfide reductase and foldase chaperone to a holdase chaperone (Fig. 3B). Furthermore, plants overexpressing the *TDX* showed increased TMHT (Lee *et al.*, 2009). A similar heat-induced, oligomerization state-related switch from disulfide reductase to chaperone activities was observed for Arabidopsis TRXh3. Whereas *TRXh3*-overexpressing plants were more tolerant to heat stress, the opposite was observed for a *trxh3* knockout mutant (Park *et al.*, 2009). Furthermore, tobacco TRXf and TRXm and maize TRXh also showed holdase chaperone activity, as evidenced by their ability to prevent heat-induced aggregation of malate dehydrogenase *in vitro* (Sanz-Barrio *et al.*, 2012; Liu *et al.*, 2017). Besides their role as molecular chaperones, plant TRXs might also mediate thermotolerance via additional mechanisms. For example, it was shown that the rice TRXz interacts with plastid multiple organellar RNA-editing factors (MORFs) and regulates their redox state, thereby modulating chloroplast RNA editing at high temperature (Wang *et al.*, 2020). Furthermore, rice TRXz also interacts with white leaf panicle 2 (WLP2) and fructose kinase-like 2 (FLN2), which both modulate the activity of the plastid-encoded RNA polymerase (PEP). As such, this FLN–TRX module regulates the expression of PEP-encoded genes, but it also contributes to the maintenance of chloroplast redox homeostasis in response to heat stress (Lv *et al.*, 2017). Recently, chloroplastic thioredoxin-like 1 (TRXL1) was also shown to play a role in thermotolerance in Arabidopsis. Whereas a *trxl1* mutant showed a reduced LAT, the opposite was observed for *TRXL1*-overexpressing lines. The positive effect of TRXL1 on thermotolerance could be linked to the fact that it stimulates the activity of malate dehydrogenase. Through the conversion of OAA to malate, this enzyme generates $NADP^+$, which can prevent $O_2^{\cdot-}$ overproduction by quenching electrons from PSII. At the post-translational level, TRXL1 is regulated by caseinolytic protease 1-mediated degradation, which is antagonized by chaperonin 60A (Pant *et al.*, 2020). In tomato, the TRX homologue nucleoredoxin 1 (SlNRX1) has been shown to positively regulate TMHT by enhancing the transcription of antioxidant and HSP genes (Cha *et al.*, 2022).

Interestingly, the TRX-reducing NTRs also play a role in plant thermotolerance. Chae *et al.* (2013) reported that plants overexpressing an Arabidopsis NTRC showed an enhanced SAT, whereas the opposite was observed for an *ntrc* knockout mutant. Like TRXs, the role of NTRC in HS tolerance is also associated with a switch from its disulfide reductase and foldase functions to a holdase function, related to its oligomerization state. Mutation of the two active site cysteines disrupted the former functions, but the effect on holdase activity was only minor. Although plants overexpressing this mutated *NTRC* version had a similar thermotolerance to plants overexpressing WT *NTRC*, they showed a higher sensitivity upon prolonged HS, suggesting that disulfide reductase and foldase functions reinforce stress resistance. Furthermore, NTRC holdase activity was enhanced by the addition of NADPH, emphasizing the importance of the redox state in determining the holdase function (Chae *et al.*, 2013).

Glutaredoxins

In addition to TRXs, the closely related glutaredoxins (GRXs) also function as reducing systems in plant cells. They generally rely on GSH as an electron donor, but some GRXs are also recycled by TRX reductases (Rouhier, 2010). Whereas TRXs mainly reduce intra- and intermolecular disulfide bonds in target proteins, GRXs are mostly involved in reducing glutathionylated proteins. Furthermore, certain GRXs function in iron–sulfur (Fe–S) cluster transfer to target proteins or Fe sensors rather than thiol reductases (Rouhier *et al.*, 2007; Bandyopadhyay *et al.*, 2008; Couturier *et al.*, 2011; Riondet *et al.*, 2012; Knuesting *et al.*, 2015; Trnka *et al.*, 2020). Based on their active site motif, GRXs are further subdivided into different classes (Cxx[C/S] for Class I, CGFS for Class II, and CCxC/S for Class III) (Couturier *et al.*, 2010; Rouhier, 2010). Class IV GRXs are atypical, large proteins containing an N-terminal GRX domain and two domains of unknown function (Couturier *et al.*, 2010).

The involvement of GRXs in plant responses to HS is supported by the observation that transgenic expression of *Pteris vittata GRX5* in Arabidopsis enhanced its TMHT and reduced oxidative protein and membrane damage (Sundaram and Rathinasabapathi, 2010). Furthermore, heterologous expression of Arabidopsis GRXS17, a class II monothiol GRX, was shown to enhance the thermotolerance of the ornamental plant *Chrysanthemum morifolium* (Kang *et al.*, 2019), tomato (Wu *et al.*, 2012), and maize (Sprague *et al.*, 2022). In tomato, mutation of CGFS-type GRXs via CRISPR/Cas9 also showed their involvement in TMHT (Kakeshpour *et al.*,

2021). Moreover, GRXS17 was found to be involved in high temperature- and photoperiod-dependent regulation of meristem development, possibly through interaction with the TF NF-YC11/NC2a (Knuesting *et al.*, 2015). Recently, Rao *et al.* (2023) reported that an Arabidopsis *grxs17* mutant was more sensitive to an increased temperature of 28 °C and showed an altered transcriptional response of many genes related to HSFs, auxin responses, cellular communication, and abiotic stress in comparison with WT plants. Hence, the authors suggested that GRXS17 plays a crucial role in gene expression regulation upon HS exposure (Rao *et al.*, 2023).

In addition, the involvement of GRXS17 in thermotolerance is also related to its ability to function as a holdase chaperone. Under physiological conditions, this protein is an Fe–S cluster-charged holoenzyme consisting of three GRX and one TRX domain that probably contributes to the maturation of cytosolic and nuclear Fe–S cluster proteins. In response to a combination of oxidative stress and HS, GRXS17 loses its Fe–S clusters, resulting in disulfide bridge formation yielding HMW complexes associated with its holdase activity (Fig. 3C). Furthermore, it associates with a different set of proteins upon HS. Hence, GRXS17 can be considered a redox-dependent molecular chaperone. It should be noted that the *grxs17* mutant displays a decreased TMHT, but SAT and LAT are unaltered in this genotype (Martins *et al.*, 2020). These data again emphasize that antioxidants can play specific roles in plant response to specific HS regimes.

Gene expression and plant acclimation to high temperature

A major output of the plant response to high temperatures is a reprogramming of gene expression, which participates in reprogramming plant metabolism to allow acclimation to challenging environmental conditions. This section aims to introduce some known redox functions in the regulation of gene expression upon high temperature conditions.

Redox-mediated transcriptional responses to high temperature

Exposure to high temperature induces profound gene expression reprogramming, which is regulated at both transcriptional and post-transcriptional levels (Merret *et al.*, 2013; Jacob *et al.*, 2017; Ohama *et al.*, 2017; Zandalinas *et al.*, 2020; Babbar *et al.*, 2021; Dard *et al.*, 2023). The transcriptional control of high temperature responses involves complex networks of TFs, some of which are redox regulated (Driedonks *et al.*, 2015; Mittler *et al.*, 2022). Among these factors, the HSFA family members are key players in the heat protection response, directly regulating the expression level of genes encoding important HS response TFs, but also triggering the depletion of repressive H2A.Z-nucleosomes on genes and transposable elements (Kumar and Wigge, 2010; Liu *et al.*, 2011; Cortijo *et al.*, 2017; Jacob *et al.*, 2017). Emphasizing the close connections between ROS and high temperature responses, large sets of genes encoding ROS metabolism and antioxidant enzymes are induced upon HS (Zandalinas *et al.*, 2020; Babbar *et al.*, 2021). For example, the *APX2* gene, encoding a major H_2O_2 detoxification enzyme, is among the main genes induced upon BT and TMHT regimes (Babbar *et al.*, 2021; Dard *et al.*, 2023). The expression of *APX2* is regulated by HSFA2 (Schramm *et al.*, 2006). Another HSFA isoform, HSFA4, induces the expression of *APX1*, possibly via its activation by the H_2O_2-dependent TF ZAT12 (Davletova *et al.*, 2005). Direct transcriptional activation by HSF is also suggested by the presence of HSEs in the promoters of several antioxidant-related genes, including *ZAT12*, *APX1*, and *APX2* (Reddy *et al.*, 2009; Liu *et al.*, 2011; Wu *et al.*, 2022). Recently, another layer of complexity has been revealed in *APX2* gene activation during the SAT regime. A first HS (priming) induces nuclear accumulation of BRI1 EMS-SUPPRESSOR 1 (BES1) and HSFA1. Both proteins interact and bind to HSEs and induce the transcription of thermotolerance genes (Albertos *et al.*, 2022). In parallel, the repressive histone mark H3K27me3 is removed from the surrounding chromatin in a BES1-dependent manner (Yao *et al.*, 2022) and replaced with the positive H3K4me3 mark (Oberkofler and Bäurle, 2022). During a second HS (challenge), *APX2* and *HSFA3* containing a 'primed chromatin' (positive histone mark H3k4me3 instead of the repressive H3K27me3) show stronger expression leading to enhanced thermotolerance. HS is also known to rapidly induce *miR398*, which in turn lowers the transcript abundance of its target genes encoding the SODs CSD1 and CSD2, in a regulatory loop that is critical for TMHT in Arabidopsis (Guan *et al.*, 2013).

Many HS responses inherently require ROS accumulation through the activation of TFs. For example, the expression of HS response genes such as *HSF* and *HSP* is highly responsive to elevated ROS conditions (Willems *et al.*, 2016). A rapid oxidative burst during the first 15 min of the HS stimulates HSF–DNA binding and is essential for the induction of heat-responsive gene expression, for example of HSPs and APX2 (Volkov *et al.*, 2006). Under elevated levels of ROS, HSFA4a is activated through trimerization and translocation into the nucleus to induce HS target genes (Fig. 4A). The oligomerization mechanism was suggested to depend on conformational modifications induced by cysteine oxidation (Pérez-Salamó *et al.*, 2014). A similar mechanism was proposed for HSFA1a trimerization, although the cysteines involved in stress-inducible HSFA1a–DNA binding could not be identified (Liu *et al.*, 2013). Interestingly, alteration of the redox status of the chloroplast was also shown to affect the binding of HSFA1a to its target genes independent of an HS. This study further links the light-dependent regulation of the Calvin–Benson cycle to signalling of diurnal temperature variations (Dickinson *et al.*, 2018). Nuclear translocation of HSFA8 was also proposed to be regulated through cysteine oxidation (Fig. 4A) (Giesguth *et al.*, 2015).

Elevated ROS also cause translocation of the HS-responsive TF MBF1C from the cytosol to the nucleus to regulate the plant systemic acclimation to high light and HS (Suzuki *et al.*, 2013a). Redox regulation of bZIP68 plays a role in balancing HS tolerance and growth. At ambient temperature, bZIP68 is present in the nucleus and represses transcription of HS response genes such as DREB2F, while activating expression of growth-related genes. Under heat stress, H_2O_2 accumulation induces an unknown redox modification on C320 of bZIP68 that releases repression of HS response gene transcription (Fig. 4B) (Li *et al.*, 2019).

High temperature-induced developmental signals are also relayed by redox signalling. For example, the opposite effects of light and high ambient temperature (27–30 °C in Arabidopsis) on seed germination are tightly regulated by *S*-nitrosylation of the HFR1 transcriptional repressor of *PIF1* (Ying *et al.*, 2022). High temperature stimulates the *S*-nitrosylation of HFR1 at a single cysteine residue (C164), inducing its degradation, and thereby releasing PIF1 to induce the expression of the seed dormancy *SOMNUS* (*SOM*) gene and altering gibberellin and abscisic acid metabolism (Fig. 4C). In contrast, light activates the photoreceptor phytochrome B (PhyB) and antagonizes high temperature-induced *S*-nitrosylation and degradation of HFR1 by increasing *S*-nitrosoglutathione reductase (GSNOR) activity (Ying *et al.*, 2022). Interestingly, the PhyB/HFR1/PIF module regulates several developmental adaptations to light and high temperature, such as photo- and thermomorphogenesis, suggesting that these pathways might be under redox control (Casal and Balasubramanian, 2019; Zhao and Bao, 2021). Indeed, mutants affected in glutathione synthesis (*cad2* and *pad2*) or inactivated in several GRXs (*grxS17*, *grxC3*, and *grxC4*) are affected in hypocotyl elongation induced by high ambient temperatures, suggesting the involvement of thiol reduction systems in thermomorphogenesis regulation (Dard *et al.*, 2023; Rao *et al.*, 2023). Very recently, Fichman *et al.* (2023) have reported the role of PhyB and RBOHs as part of a regulatory module that controls ROS production, transcript expression, and plant acclimation to excess light, heat stress, wounding, cold, and bacterial infection (Fig. 4D).

Epigenetic mechanisms

Epigenetic regulation of heat responses has been extensively described and includes DNA methylation, histone modifications, histone variants, ATP-dependent chromatin remodelling, histone chaperones, small RNAs, long non-coding RNAs, and other undefined epigenetic mechanisms [for reviews, see J. Liu *et al.* (2015) and Perrella *et al.* (2022)]. However, evidence for the involvement of redox regulation in these pathways is still scarce. In mammals, the redox regulation of writer and eraser enzymes of histone marks is the best-documented example. Oxidative stress alters global histone modification, histone acetylation, and DNA methylation, affecting chromatin conformation and transcription (Ito *et al.*, 2004; Chen *et al.*, 2006; Ago *et al.*, 2008; Nott *et al.*, 2008; Zhou *et al.*, 2008; Doyle and Fitzpatrick, 2010; Niu *et al.*, 2015). *S*-Nitrosylation at conserved cysteines of HDAC2 in neurons results in changes in histone modification and gene expression (Nott *et al.*, 2008). In other class II histone deacetylases (HDACs), a ROS/TRX-dependent redox switch in key cysteine residues affects nuclear trafficking and subsequent gene expression (Ago *et al.*, 2008). In plants, members of the class I RPD-3 like HDACs were identified as redox-sensitive proteins during early salicylate and flg22 responses (P. Liu *et al.*, 2015). Nitric oxide (NO) has also been shown to modulate histone acetylation of stress-responsive genes by inhibiting HDAC activities and facilitating stress-induced gene transcription (Mengel *et al.*, 2017). Changes in NO content induced by light intensity modify the global histone acetylation (H3, H3K9, and H3K9/K14) in Arabidopsis, in relation to GSNOR and histone deacetylase 6 (HDA6) (Ageeva-Kieferle *et al.*, 2021).

Other key metabolic enzymes involved in DNA methylation are candidates for redox regulation. These include enzymes of the *S*-adenosylmethionine cycle, which provide precursors for DNA and histone methylation (Shen *et al.*, 2016). The DNA demethylases Repressor of Silencing1 (ROS1) and Demeter-like (DME, DML2, and DML3) enzymes remove methylated bases from the DNA backbone (Zhu, 2009). These enzymes contain an Fe–S cluster, which might be susceptible to oxidation by ROS. Moreover, different members of the cytosolic Fe–S cluster assembly machinery (i.e. MET18 and AE7) are involved in DNA methylation, probably because they affect provision of the nuclear DNA demethylases with their cofactors (Duan *et al.*, 2015; J. Liu *et al.*, 2015).

Small RNAs (siRNA and miRNA) are other important regulators of gene expression and are involved in various developmental and stress response processes in eukaryotic cells (D'Ario *et al.*, 2017; Leisegang *et al.*, 2017). For example, miRNAs targeting antioxidant genes are altered by HS (Guan *et al*, 2013). The biogenesis of small RNAs is coordinated by Dicer-like (DCL) and RNase III-like (RTL) endonucleases, which process almost all classes of dsRNA precursors. In Arabidopsis, members of the DCL and RTL families were shown to be redox regulated through disulfide bond formation or *S*-glutathionylation of conserved cysteines in their RNA-binding domains, thereby affecting their RNase III activity. Indeed, RTL1 activity can be restored by GRXs, indicating that a redox switch controls small RNA biogenesis (Charbonnel *et al.*, 2017; Seta *et al.*, 2017). Therefore, these examples show an emerging link between redox regulation and epigenetic regulation. However, whether such regulation is also involved in high temperature signalling has not been explored yet.

Redox regulation under combined stress conditions

Increasing evidence suggests a central role for redox regulation as an integrator of multiple stress signalling (Balfagón

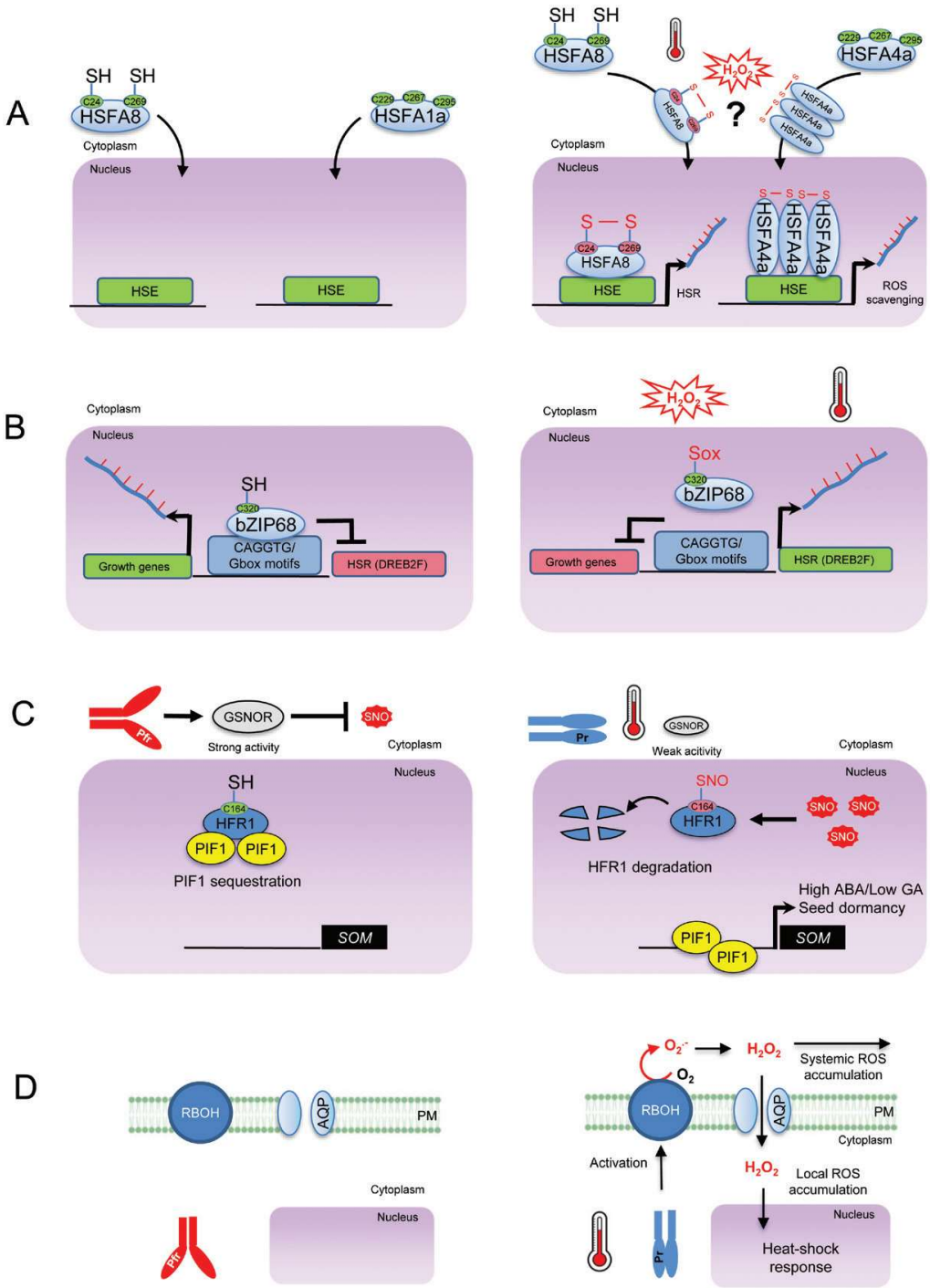

Fig. 4. Transcriptional activation mechanisms upon high temperature. (A) Redox-dependent relocalization and activation of heat shock factors (HSFs). Under ambient temperatures, HSFA8 and HSFA4A are present in the cytosol in their reduced state. Heat-generated H_2O_2 could induce a disulfide bridge between the redox-sensitive cysteines C24 and C269 of HSFA8, leading to nuclear translocation and activation of heat shock response elements (HSEs) (Giesguth *et al*, 2015). On the other hand, homotrimerization of HSFA4a during salt stress and H_2O_2 treatment is dependent on disulfide bridge formation involving C229/C267/C295 (Pérez-Salamó *et al.*, 2014). Finally, reducing conditions can favour HSFA4A monomerization and inactivation (Andrási *et al.*, 2019). (B) Redox regulation of bZIP68 plays a role in balancing heat stress tolerance and growth. At 20 °C, bZIP68 is present in the nucleus and represses transcription of HS response gene such as DREB2F and activates growth gene expression. Upon heat stress, accumulation of H_2O_2 induces an unknown redox modification of C320 on bZIP68 that releases repression of DREB2F (Li *et al.*, 2019). (C) *S*-Nitrosylation of HFR1 regulates seed germination under heat stress. At 20 °C, active PhyB (Pfr) enhances *S*-nitrosoglutathione reductase (GSNOR) activity, maintaining a low

S-nitrosothiol (SNO) concentration, and HFR1 sequesters PIF1 in the nucleus. At 37 °C, thermoinhibition of PhyB (Pr) decreases GSNOR activity leading to SNO accumulation. High SNO concentrations induce *S*-nitrosylation of HFR1 at C164 and subsequent HFR1 degradation (Ying *et al.*, 2022). HFR1 degradation releases PIF1 to induce *SOMNUS* (*SOM*) expression required for induction of seed dormancy. (D) PhyB-dependent local and systemic redox signalling during heat stress. At 20 °C, PhyB is mainly present in its active (Pfr) state. Upon local heat treatment (32 °C heat exposure of one leaf), local (in the treated leaf) and systemic (in distal leaves) accumulation of ROS is observed. This accumulation is dependent on the presence of PhyB in the cytosol and RBOHD/F activation. PhyB is likely to be necessary for RBOH-mediated ROS accumulation for local and systemic signalling (Fichman *et al.*, 2023). Given that high temperatures induce thermoinhibition of PhyB, it is therefore represented in its inactive (Pr) state in the right panel. PhyB, phytochrome B; RBOH, respiratory burst oxidase homologue; AQP, aquaporin.

et al., 2020; Zandalinas *et al.*, 2021; Mittler *et al.*, 2022). This is of particular significance *in natura*, where plants face multiple stress conditions simultaneously. For example, transcriptomic analyses combining high temperature with other abiotic stress conditions (e.g. drought, salt, or high light) have shown large overlaps in their transcriptomic signatures, including many genes involved in antioxidant processes (Suzuki *et al.*, 2016; Ashoub *et al.*, 2018; Wang *et al.*, 2018; Balfagón *et al.*, 2019). Consistently, mutants deficient in APX1 or RBOHD are more sensitive to a combination of heat stress and other abiotic stresses (Koussevitzky *et al.*, 2008; Zandalinas *et al.*, 2021). On the other hand, the accumulation of protective proteins, such as chaperones or ROS-scavenging enzymes, is a common response involved in the tolerance of plants to different combinations of stresses (Zandalinas *et al.*, 2016, 2018; Balfagón *et al*, 2018). Consistently, proteomic analyses performed in citrus showed that accumulation of APX, HSP101, and HSP17.6 correlates with tolerance to combined heat and drought stress (Balfagón *et al.*, 2018). Therefore, ROS signalling appears to play an important role in integrating different signals generated by multiple stress combinations.

Concluding remarks and future prospects

Global warming is likely to have an increasing impact on crop yield. It is therefore important to elucidate how plants respond to high temperatures. It is clear that redox regulation, which is mediated by ROS and oxidoreduction systems, plays a central role in this response. Some players involved in the perception and signalling of the response have been identified. Among them, the identification of ROS receptors (e.g. HPCA1), ROS docking components (e.g. aquaporins), redox-regulated TFs (e.g. HSFs), and ROS-producing and thiol-dependent reduction enzymes (e.g. RBOH, APX, TRX, and GRX) are probably involved in these pathways. However, many questions remain to be answered. How are different high temperature regimes perceived by plants to induce specific responses such as thermomorphogenesis, basal, moderate, or acquired thermotolerance responses? Are these responses mediated by specific ROS signatures or compartmentations? How are specific redox signals transmitted to the nucleus to reprogram gene expression? How is redox regulation involved in the complex network of transcriptional, post-transcriptional, and epigenetic gene regulatory mechanisms? What is the mechanism behind the variable susceptibility of different plant species to high temperature conditions? Plants growing *in natura* are facing multiple stress conditions, which will very probably become more intense in the future (e.g. high temperatures associated with drought, high CO_2, degraded soils, or pathogens). Understanding the redox mechanisms involved in responses to these different abiotic or biotic stress conditions will be key to identify suitable targets to enhance multistress resistance.

The development of live imaging tools to monitor ROS and redox metabolism at a subcellular scale is a highly useful approach to answer these questions. Redox proteomic techniques that allow the identification of specific thiol modifications in proteins under different environmental stress conditions are also useful developments. Furthermore, next-generation RNA-sequencing or ChIP sequencing (ChIP-seq) are also important tools to identify gene regulatory networks and new (redox) players regulating plant responses to high temperatures.

Progress in understanding the role of redox signalling in developmental and stress responses has been greatly accelerated in model plants such as Arabidopsis, notably through genome sequencing programmes and publicly available mutant collections. Nevertheless, the development of new techniques that allow sequencing or editing of the genome of hundreds of plant species/varieties will be key to transfer this knowledge to crop plants. Validation of the studied responses in candidate plants in the field upon high temperature conditions, alone or combined with other environmental stress conditions, will be a further challenge for the improvement of crop plants to withstand a changing environment.

Author contributions

SH, AD, AJM, and JPR: writing, review, and editing; all authors have read and agree with the present version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Data availability

All data supporting the findings of this study are available within the paper.

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