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REVIEW PAPER

Jacks of all trades: reactive oxygen species in plant responses to stress combinations and priming-induced stress tolerance

Sophie Hendrix[1,*], Isabeau Vanbuel[1], Jasmine Colemont[1], Laura Bos Calderó[1], Mohamed Amine Hamzaoui[1,2], Kris Kunnen[1], Michiel Huybrechts[1], and Ann Cuypers[1]

[1] Environmental Biology, Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium
[2] Laboratory of Plant Physiology, Department of Biology, Universidad Autónoma de Madrid, Madrid, Spain

* Correspondence: sophie.hendrix@uhasselt.be

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Abstract

Climate change is expected to increase the frequency of heat waves, drought periods, and flooding events, thereby posing a serious risk to crop productivity and global food security. In order to develop strategies to improve plant growth under adverse environmental conditions, in-depth molecular knowledge on plant stress responses is required. In this context, particular attention should be paid to the involvement of reactive oxygen species (ROS), molecules known for causing oxidative damage, but also indispensable for intra- and intercellular signal transduction required for plant acclimation to a wide variety of stress conditions. As plants often encounter multiple stressors simultaneously and their responses to these conditions can generally not be predicted based on the effects of the individual stress factors, we first focus on the involvement of ROS and cellular redox homeostasis in plant responses to combined and multifactorial stress conditions. Then we provide an overview of the role of ROS in priming strategies aimed at improving plant tolerance to climate change-related stress conditions. Finally, approaches to advance our understanding of redox dynamics in plant responses to combined stress and priming are discussed.

Keywords: Abiotic stress, biostimulants, climate change, combined stress, drought, heat stress, flooding, phytohormones, priming, reactive oxygen species.

Introduction

As a consequence of the rapid rise in greenhouse gas emissions, atmospheric CO_2 concentrations have increased more than 50% above average pre-industrial levels (Friedlingstein *et al.*, 2022). This has led to a temperature increase of approximately 1.1 °C between the periods 1850–1900 and 2011–2020. Further global warming is expected with predicted temperature increases ranging from 1.5 °C to over 4 °C by 2100 (IPCC, 2023). This is associated with the more frequent occurrence of temperature extremes, drought periods, and flooding due to heavy precipitation, which pose major threats to crop production and global food security (Leisner *et al.*, 2023). The most severe climate change scenario is predicted to cause crop yield losses ranging from 7% to 23% (Rezaei *et al.*, 2023). Hence, it is essential to characterize plant responses to climate

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Abbreviations: ABA, abscisic acid; APX, ascorbate peroxidase; AsA, ascorbic acid;CAT, catalase; GA, gibberellic acid; GR, glutathione reductase; GSH, glutathione; JA, jasmonic acid; MDA, malondialdehyde; POD, peroxidase; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase change-related stress conditions and their combinations in order to aid the development of strategies to mitigate climate impact on crop yield and quality.

The cellular redox balance under non-stressed and stressed conditions

In general, abiotic stress conditions like heat, drought, and flooding interfere with plant physiological processes such as photosynthesis, respiration and uptake of water and nutrients. At the molecular level, plant stress responses are often characterized by increased levels of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$). These molecules are continuously generated in plant cells as by-products of energy metabolism. Whereas chloroplasts and peroxisomes are the most important ROS sources under light conditions, mitochondria contribute most to the cellular ROS burden in the dark (Das and Roychoudhury, 2014). In chloroplasts, energy transfer from excited chlorophyll to oxygen causes the formation of $^1O_2$ at photosystem II and electron leakage to oxygen yields $O_2^{\bullet-}$ at photosystem I. In peroxisomes, H_2O_2 is generated upon conversion of glycolate into glyoxylate during photorespiration. Furthermore, β-oxidation of fatty acids also significantly contributes to peroxisomal H_2O_2 production. In mitochondria, electron leakage from complexes I and III of the electron transport chain to oxygen constitutes the major source of ROS (Phua *et al.*, 2021). Besides energy-producing organelles, also the endoplasmic reticulum contributes to cellular ROS levels, as H_2O_2 is formed as a by-product of oxidative protein folding (Ozgur *et al.*, 2018). Furthermore, ROS are produced in the apoplast by the action of NADPH oxidases (also known as respiratory burst oxidase homologues; RBOHs), which generate $O_2^{\bullet-}$, and peroxidases, polyamine oxidases, and copper-containing amine oxidases, which produce H_2O_2 that induces cross-linking of cell wall components (Schmidt *et al.*, 2016).

Whereas ROS production through these mechanisms takes place under physiological conditions, it is significantly enhanced by various abiotic stress conditions, including those related to climate change. Heat stress, for example, increases membrane fluidity, thereby disrupting the integrity of electron transport chains in chloroplasts and mitochondria, leading to electron leakage to oxygen and subsequent $O_2^{\bullet-}$ production in these compartments. Furthermore, increased temperatures trigger an influx of calcium (Ca^{2+}) into the cytosol, which subsequently increases NADPH oxidase activity (Fortunato *et al.*, 2023; Hendrix *et al.*, 2023). Upon drought stress, stomatal closure is induced to prevent water loss through transpiration, thereby limiting CO_2 availability and directing electron transfer towards oxygen, generating $O_2^{\bullet-}$ at chloroplast photosystem I through the Mehler reaction. Furthermore, these conditions increase photorespiration and the associated H_2O_2 production in peroxisomes (Cruz de Carvalho, 2008). Mitochondrial respiration is also enhanced upon drought stress, thereby further contributing to increases in cellular ROS levels (Miller *et al.*, 2010). Heavy precipitation can cause flooding, which is characterized by limited oxygen availability to plants, resulting in hypoxic or even anoxic conditions. Although counterintuitive, such oxygen-limited conditions can also enhance ROS production in plants. Flooding can limit the amount of light available for photosynthesis, which can increase ROS formation, especially upon subsequent reoxygenation (Patel *et al.*, 2019; Pucciariello and Perata, 2021). Besides chloroplasts, also mitochondria are put forward as important sources of ROS in responses to flooding, likely through impaired respiration (Jethva *et al.*, 2022). Furthermore, NADPH oxidases have been proposed to play key roles in ROS formation during the early stages of hypoxia and reoxygenation (Hong *et al.*, 2020; Yu *et al.*, 2024).

Reactive oxygen species: damage versus signalling

Stress-induced increases in ROS levels can evoke damage to cellular macromolecules, but also play key roles in intra- and intercellular communication. ROS are key players in anterograde and retrograde signalling pathways, transducing signals between various subcellular compartments and the nucleus. Furthermore, ROS are involved in cell-to-cell signalling and communication between different plant organs (Peláez-Vico *et al.*, 2022; Sevilla *et al.*, 2023). In these processes, ROS are strongly interconnected with other signalling molecules such as Ca^{2+}. Indeed, activation of ROS-producing NADPH oxidases depends on Ca^{2+} binding to their EF hand motifs as well as phosphorylation by Ca^{2+}-dependent protein kinases. Vice versa, ROS can trigger cellular Ca^{2+} influx through activation of ROS-activated Ca^{2+} channels (Ravi *et al.*, 2023). Similarly, ROS signalling is also tightly intertwined with phytohormone signalling, forming an integrated redox–hormone network (Bartoli *et al.*, 2013; Xia *et al.*, 2015; Choudhury *et al.*, 2017). In addition, crosstalk with reactive nitrogen species and reactive sulfur species should also be considered. The involvement of these molecules in plant stress responses is well known and their reaction with ROS causes the generation of mixed reactive species (Martí-Guillén *et al.*, 2022).

In the context of ROS signalling, especially H_2O_2 plays an important role due to its relatively long half-life and ability to cross cellular membranes through aquaporins (Smirnoff and Arnaud, 2019). ROS signalling is primarily mediated via oxidative post-translational modifications of cysteine residues in proteins. Cysteine oxidation results in the formation of sulfenic acid (-SOH) as well as inter- or intramolecular disulfide bonds (P. Wang *et al.*, 2024). Besides ROS, also reactive nitrogen species (e.g. nitric oxide) and reactive sulfur species (e.g. hydrogen sulfide) induce post-translational modifications of cysteine residues, further strengthening their interconnection with the cellular redox network (Cejudo *et al.*, 2021; Martí-Guillén *et al.*, 2022). These oxidative modifications generally alter the conformation of proteins, thereby affecting their activity, subcellular localization, and/or interaction with other proteins. The ability of ROS to oxidize a wide range of proteins (e.g. transcription factors, kinases, phosphatases, channels) in various subcellular compartments enables these molecules to coordinate dynamic signal transduction pathways with strong spatiotemporal control, ultimately contributing to stress acclimation (P. Wang *et al*., 2024). Although ROS are able to directly oxidize proteins, ROS-mediated protein oxidation for signal transduction purposes is generally facilitated by thiol peroxidases such as glutathione peroxidase-like proteins and peroxiredoxins (Vogelsang and Dietz, 2022; Chae *et al*., 2023). While protein sulfenylation is reversible, further oxidation of sulfenic acid to sulfinic acid (-SO_2H) and subsequently sulfonic acid (-SO_3H) is considered irreversible and disturbs protein function (H. Zhou *et al*., 2023). In addition, the presence of ROS at high levels can oxidatively damage other cellular macromolecules such as DNA and membrane lipids.

The antioxidative defence network

To allow for oxidative signalling, while avoiding oxidative damage, tight control of cellular ROS levels is required. To this end, plant cells are equipped with an extensive antioxidative defence system consisting of enzymatic as well as non-enzymatic components. Major enzymatic antioxidants are superoxide dismutase (SOD), which converts $O_2^{\bullet-}$ to H_2O_2, and catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase-like proteins, peroxiredoxins and glutathione *S*-transferases, responsible for H_2O_2 detoxification. Non-enzymatic antioxidants can be further subdivided into water-soluble compounds such as ascorbic acid (AsA), glutathione (GSH) and various polyphenols (e.g. flavonoids), and lipid-soluble compounds such as tocopherols and carotenoids (P. Wang *et al*., 2024). Interestingly, lipoic acid is soluble in water as well as in lipids, thereby connecting the activities of antioxidants in membranes with those in the cytosol (Navari-Izzo *et al*., 2002). It should be noted that enzymatic and non-enzymatic antioxidants are tightly intertwined. An excellent example is the AsA–GSH cycle, contributing to H_2O_2 detoxification in various subcellular compartments. In this cycle, AsA serves as the electron donor for APX, which reduces H_2O_2 to water, and GSH delivers electrons required for recycling AsA to its reduced form (Foyer and Kunert, 2024). In response to developmental and environmental cues, the abundance and activity of these antioxidants is altered to enable regulation of cellular ROS levels (P. Wang *et al*., 2024).

In this review, we provide an overview of the involvement of ROS in plant responses to stress combinations including heat, drought, and/or flooding. In addition, we discuss the importance of the cellular redox balance in priming-induced plant tolerance to climate change-related stress conditions. In this framework, also the interplay between ROS and other signalling molecules is considered.

Reactive oxygen species in plant tolerance to combined stress conditions

The vast majority of studies investigating plant stress responses focus on single stress conditions (Renziehausen *et al*., 2024). Nevertheless, in the environment, plants are generally exposed to combinations of different stresses. These can occur either simultaneously or sequentially and cause a significant threat to crop yield (Suzuki *et al*., 2014; Pandey *et al*., 2015). Stress combinations can consist of biotic stress conditions like pathogen infections or herbivore attack as well as abiotic stresses. The latter comprise stresses directly linked to climate change such as heat, drought, and flooding, but also other environmental challenges such as environmental pollution with heavy metals, microplastics, and antibiotics should be considered (Zandalinas *et al*., 2021a). Whereas the term combined stress generally refers to a combination of two stress conditions, simultaneous or sequential exposure to three or more stresses is referred to as a multifactorial stress combination (Zandalinas *et al*., 2024). Recently, Zandalinas *et al*. (2021b) showed that survival rates of Arabidopsis seedlings significantly decreased with simultaneous exposure to an increasing number of stress conditions, even though each of these stresses had little to no effect on plant survival when applied individually. As the occurrence of stress conditions, and hence their combinations, is expected to increase significantly in future years as a consequence of climate change and anthropogenic activities, there is an urgent need to develop crops with an increased tolerance to combined stress conditions to safeguard food security for the continuously growing world population. To do so, it is essential to gain in-depth knowledge on the molecular mechanisms underlying plant responses to stress combinations (Sato *et al*., 2024). The fact that plant responses to combined stress conditions are often unique and cannot simply be predicted from responses to individual stressors further emphasizes the need to study plants under dual or even multifactorial stress conditions (Pandey *et al*., 2015; Zhang and Sonnewald, 2017; Desaint *et al*., 2021). For a detailed description of phenotypic, physiological and molecular plant responses to specific stress combinations, readers are referred to literature reviews by Desaint *et al*. (2021) (heat stress and pathogens), Tahjib-Ul-Arif *et al*. (2023) (waterlogging and salinity), Renziehausen *et al*. (2024) (combinations involving flooding stress), and Sato *et al*. (2024) (drought and heat stress).

The complexity of plant responses to stress combinations

The effects of combined stress conditions on phenotypic, physiological, or molecular parameters can be additive, synergistic, or antagonistic in which the effect of the stress combination is equal to, greater than, or smaller than the sum of the effects of the individual stresses, respectively (Renziehausen *et al*., 2024). This can be explained by the fact that plant responses to different stress conditions involve other stress sensors, signalling pathways, and regulatory networks. Hence, plant exposure to multiple stresses simultaneously can result in the clashing of different acclimation or defence strategies, thereby either improving or decreasing stress tolerance (Pandey *et al.*, 2015; Zandalinas *et al.*, 2021a; Renziehausen *et al.*, 2024). Improved stress tolerance is, for example, observed in tomato plants subjected to a combination of heat stress and salinity compared with plants treated with salt stress alone. Accumulation of glycine betaine and trehalose in response to the stress combination is linked to a better maintenance of cell water status and photosynthesis, resulting in a less severe impact on plant growth (Rivero *et al.*, 2014). In contrast, plant exposure to combined heat stress and drought often has detrimental consequences. When plants are subjected to heat stress alone, stomatal conductance is generally increased to enable leaf cooling via transpiration. In contrast, drought typically causes abscisic acid (ABA) accumulation in guard cells, thereby inducing stomatal closure to prevent water loss. When combined, effects of drought dominate over those induced by heat, and stomata remain closed and transpiration is repressed, resulting in a higher leaf temperature in plants exposed to a combination of both stresses compared with plants exposed to heat alone, leading to dramatic effects on crop yield (Mittler, 2006).

However, it should be noted that plant responses to stress combinations depend on several parameters including the plant species, developmental stage and the plant organ studied. For example, Sinha *et al.* (2022) recently demonstrated that leaf stomata were closed in soybean plants exposed to a combination of water deficit and heat stress, whereas flower stomata were open, thereby favouring transpiration through flowers over transpiration through leaves. This strategy is termed ‘differential transpiration’ and serves to protect reproductive processes from the effects of combined drought and heat stress by lowering the internal temperature of the flower (Sinha *et al.*, 2022). Prediction of plant responses to stress combinations is further complicated by the fact that these depend on multiple aspects of the stress conditions (stress severity, duration of exposure, and sequence of exposure) as well as on environmental conditions (nutrition, light quality, and time of day) (Anwar *et al.*, 2021; Zandalinas *et al.*, 2021a). Moreover, it is important to take into account that combined stress conditions also affect soil parameters. Indeed, Rillig *et al.* (2019) demonstrated that soil microbiome diversity significantly decreased with an increasing number of stresses in a combined stress situation. As the soil microbiome is an important determinant of plant health, this puts an additional risk on plant growth and productivity.

Stress combinations affect cellular reactive oxygen species levels

Despite the complexity of plant responses to stress combinations, signalling pathways triggered by most biotic and abiotic stresses share several common features including the involvement of Ca^{2+}, phytohormones, mitogen-activated protein kinases and ROS (Pandey *et al.*, 2015). As alterations in ROS levels and changes in the cellular redox balance are key features of plant responses to virtually all stress conditions, it is highly plausible that ROS/redox signals serve as important mediators of combined stress responses (Renziehausen *et al.*, 2024). As summarized in Table 1, combined stress conditions often induce more pronounced increases in ROS levels compared with individual stresses in the same experimental set-up (Fig. 1). For example, simultaneous exposure of sweet sorghum seedlings to drought stress and salinity caused stronger increases in leaf $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$ concentrations compared with the single exposures. The strength of the increase in $O_2^{\bullet-}$ levels seemed equal to the sum of the effects of the individual stressors, suggesting an additive effect. In contrast, elevations in H_2O_2 and $^{\bullet}OH$ induced by simultaneous drought and salt stress were more pronounced than what would simply be predicted based on the effects of the individual stressors, pointing towards a synergistic effect. These increased ROS levels coincided with a stronger rise in malondialdehyde (MDA) levels, suggesting a greater extent of lipid peroxidation. Interestingly, these differences were accompanied by a unique transcriptional footprint, as combined exposure to drought and salinity caused up-regulation of over 2500 genes that were not transcriptionally induced by either of the single stresses (Wang and Wei, 2022). The effects of combined salinity and waterlogging on leaf H_2O_2 concentrations slightly varied between different maize cultivars. In general, salinity caused stronger increases in H_2O_2 levels compared with waterlogging. Whereas a more pronounced increase in H_2O_2 levels under combined compared with single stress conditions was observed in some cultivars, the response to combined exposure did not significantly differ from that induced by salinity alone in other cultivars. These effects were observed during the stress exposure as well as during the recovery phase (Mahmood *et al.*, 2021). In cotton, leaf H_2O_2 levels were increased by drought but generally not affected by heat. Markedly, combined exposure to both stressors led to stronger increases in H_2O_2 levels in leaves of stress-sensitive genotypes, pointing towards a synergistic effect of both stressors on cellular ROS levels. However, in genotypes that were identified as either drought- or heat-tolerant, increases in H_2O_2 concentrations induced by the stress combination were similar to those induced by heat alone, suggesting an additive effect. These data again emphasize that combined stress responses can differ between cultivars of the same species (Zafar *et al.*, 2023). In cotton anthers, $O_2^{\bullet-}$ levels were not affected by drought treatment, but showed a significant increase in response to high temperature. Similarly, elevations in H_2O_2 were more pronounced in anthers of heat-treated plants compared with drought-stressed plants. In general, ROS levels in cotton anthers showed similar responses to combined heat and drought treatment as compared with those observed upon heat stress alone, indicating that combined stress does not always

Table 1. Summary of studies on the influence of combined abiotic stress conditions on reactive oxygen species (ROS) levels in plants

Plant species	Organ	Stress (i)	Stress (ii)	Combined stress	ROS detection method	Redox-related parameters	Reference
(i) Drought, (ii) heat							
Gossypium hirsutum	Leaves	$H_2O_2\uparrow$	$H_2O_2=$	$H_2O_2\uparrow\uparrow$	Spectrophotometry	AO-Enz, AO-Met	Zafar *et al.* (2023)
Xanthoceras sorbifolium	Leaves	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	Histochemical staining	AO-Enz, AO-Met	Li *et al.* (2021)
Triticum aestivum	Leaves	$O_2^{\bullet-}\uparrow$	$O_2^{\bullet-}\uparrow\uparrow$	$O_2^{\bullet}-\uparrow\uparrow\uparrow$	Spectrophotometry	AO-Enz, AO-Met	Ru *et al.* (2023)
Gossypium hirsutum	Anthers	$H_2O_2\uparrow$; $O_2^{\bullet-}=$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	Spectrophotometry	AO-Enz, AO-Met	J. Zhang *et al.* (2023)
Valeriana jatamansi; Hedychium spicatum	Leaves	$H_2O_2\uparrow\uparrow$	$H_2O_2\uparrow$	$H_2O_2\uparrow\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Pandey *et al.* (2021)
Solanum melongena	Leaves	$H_2O_2\uparrow$	$H_2O_2\uparrow$	$H_2O_2\uparrow\uparrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Hannachi *et al.* (2022)
Zea mays	Roots	$O_2^{\bullet-}\uparrow\uparrow$	$O_2^{\bullet-}\uparrow$	$O_2^{\bullet-}\uparrow\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Ru *et al.* (2022)
Solanum lycopersicum	Leaves	$H_2O_2\uparrow$	$H_2O_2=$	$H_2O_2\uparrow\uparrow$	Spectrophotometry	AO-Met, LPO	Francesca *et al.* (2022)
Lycopersicon esculentum	Leaves	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	Histochemical staining	AO-Enz, LPO	Annadurai *et al.* (2023)
Prunus cerasifera	Leaves	$H_2O_2\uparrow\uparrow$	$H_2O_2\uparrow$	$H_2O_2\uparrow\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Bolat *et al.* (2024)
Chenopodium album	Leaves	$H_2O_2\uparrow$	$H_2O_2\uparrow$	$H_2O_2\uparrow\uparrow$	Chemiluminescence	AO-Enz, AO-Met, LPO	Semwal and Khanna-Chopra (2020)
Hordeum vulgare	Shoots	$H_2O_2=$; $O_2^{\bullet-}\uparrow$	$H_2O_2=$; $O_2^{\bullet-}=$	$H_2O_2=$; $O_2^{\bullet-}=$	Spectrophotometry + histochemical staining	AO-Enz, LPO	Zhanassova *et al.* (2021)
	Roots	$H_2O_2=$	$H_2O_2\uparrow/=$	$H_2O_2\uparrow$			
Psychotria brachyceras	Leaves	$H_2O_2\uparrow$	$H_2O_2=$	$H_2O_2\uparrow$	Spectrophotometry	AO-Enz, LPO	De Palma *et al.* (2023)
(i) Drought, (ii) salinity							
Sorghum bicolor	Leaves	$H_2O_2=$; $O_2^{\bullet-}=$; $^{\bullet}OH=$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$; $^{\bullet}OH\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow$; $^{\bullet}OH\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Wang and Wei (2022)
Oryza sativa	Leaves	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Huanhe *et al.* (2024)
Solanum melongena	Leaves	$H_2O_2\uparrow\uparrow$	$H_2O_2\uparrow$	$H_2O_2\uparrow\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Kıran and Baysal Furtana (2023)
Populus euphratica, *P. pruinosa*	Leaves	$O_2^{\bullet-}\uparrow$	$O_2^{\bullet-}\uparrow$	$O_2^{\bullet-}\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Yu *et al.* (2020)
Cyperus esculentus	Leaves	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	Spectrophotometry	AO-Enz, LPO	N. Wang *et al.* (2024)
(i) Drought, (ii) cadmium							
Vigna angularis	Roots	$H_2O_2=$; $O_2^{\bullet-}\uparrow$	$H2O2\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\downarrow$	Spectrophotometry + histochemical staining	AO-Enz	Ma *et al.* (2024)
Phaseolus vulgaris	Leaves	$H_2O_2\uparrow$	$H_2O_2\uparrow\uparrow$	$H_2O_2\uparrow\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Yildirim *et al.* (2023)
(i) Drought, (ii) mercury							
Zea mays	Leaves	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	Spectrophotometry + histochemical staining	AO-Enz, LPO	Tang *et al.* (2023)
(i) Drought, (ii) cold							
Hordeum vulgare	Shoots	$H2O2=$; $O_2^{\bullet-}\uparrow$	$H_2O_2=$; $O_2^{\bullet-}\downarrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\downarrow\downarrow$	Spectrophotometry + histochemical staining	AO-Enz, LPO	Zhanassova *et al.* (2021)
	Roots	$H_2O_2=$	$H_2O_2=$	$H_2O_2=$			
(i) Heat, (ii) salinity							
Oryza sativa	Leaves	$H_2O_2=$	$H_2O_2\uparrow\uparrow$	$H_2O_2\uparrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Mendes *et al.* (2024)
Solanum lycopersicum	Shoots	$H_2O_2\downarrow$; $O_2^{\bullet-}=$	$H_2O_2\downarrow$; $O_2^{\bullet-}\downarrow$	$H_2O_2\downarrow$; $O_2^{\bullet-}\downarrow\downarrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Sousa *et al.* (2022)
	Roots	$H_2O_2\uparrow$; $O_2^{\bullet-}=$	$H_2O_2=$; $O_2^{\bullet-}=$	$H_2O_2\uparrow$; $O_2^{\bullet-}=$			
(i) Waterlogging, (ii) salinity							
Zea mays	Leaves	$H_2O_2\uparrow/=$	$H_2O_2=/\uparrow$	$H_2O_2\uparrow\uparrow$	Spectrophotometry	AO-Enz	Mahmood *et al.* (2021)
(i) Hypoxia, (ii) iron							
Soybean	Leaves	$H_2O_2\uparrow$	$H_2O_2=$	$H_2O_2\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Delias *et al.* (2022)
(i) Waterlogging, (ii) cadmium							
Solanum lycopersicum	Leaves	$H_2O_2=$; $O_2^{\bullet-}=$	$H_2O_2\downarrow$; $O_2^{\bullet-}=$	$H_2O_2=$; $O_2^{\bullet-}\uparrow$	Spectrophotometry	AO-Enz, LPO	R. Zhou *et al.* (2023)

This table compiles research from the past 5 years investigating plant responses to combinations of two abiotic stress conditions. Columns include the stress combination, plant species, organ studied, observed trends in ROS levels in response to exposure to each of the individual stress conditions and the stress combination in comparison with control conditions, the method used for ROS detection, and additional redox-related parameters studied. AO-Enz, antioxidant enzymes; AO-Met, antioxidant metabolites; LPO, lipid peroxidation.

induce a stronger increase in cellular ROS levels (J. Zhang *et al.*, 2023). As for other parameters, differences in ROS signatures induced by combined stress conditions could be related to various factors including the intensities of the individual stresses and the plant species and organ analysed. As such, it is clear that changes in ROS levels in plants exposed to stress combinations cannot simply be predicted based on the effects of each of the individual stressors. Nevertheless, the available data clearly point towards a role for ROS in plant responses to combined stress conditions. Although increased ROS levels resulting from additive or synergistic effects of multiple stressors frequently lead to cellular damage (e.g. lipid peroxidation evidenced by increased MDA concentrations), it should be considered that the ROS signatures induced by specific stress combinations also serve crucial roles in signal transduction and plant acclimation to the combined exposure.

Antioxidative defence in plant responses to combined stress conditions

Recently, two meta-analyses also highlighted the impact of combined stress conditions on ROS levels in plants. Angon *et al.* (2022) combined the results of 30 research articles reporting on the effects of combined drought stress and salinity. They found that this stress combination caused more pronounced effects on plant growth, photosynthesis, and ionic balance compared with either of the single stresses and resulted in more severe increases in H_2O_2 and MDA levels. The stronger increase in ROS levels was likely related to the fact that the antioxidative defence system was only activated to a similar extent in plants exposed to the stress combination compared with those exposed to the single stresses (Angon *et al.*, 2022). Similarly, a meta-analysis performed by Cao *et al.* (2024) including 36 research papers revealed that combined exposure to drought and salinity resulted in stronger increases in H_2O_2 and MDA levels compared with the individual stresses, whereas increases in AsA and GSH concentrations and activities of SOD, peroxidase (POD), CAT, APX, glutathione peroxidase (GPX), and glutathione reductase (GR) were similar or even less pronounced under combined compared with single stress conditions. In contrast, several other studies reported higher antioxidative enzyme activities in plants exposed to combined stress conditions in comparison with those subjected to the individual stresses. For example, Kıran and Baysal Furtana (2023) showed that a combination of drought and salinity caused stronger increases in H_2O_2 concentrations as well as SOD, CAT, and GR activities in eggplant seedlings compared with drought or salinity separately. Interestingly, Ru *et al.* (2023) demonstrated that the time of sampling during the stress exposure and recovery phase strongly affects the observed responses on the antioxidative system. In leaves of winter wheat, $O_2^{\bullet-}$ concentrations were more strongly increased by combined heat stress and drought compared with the individual stresses at all time points assessed during the stress exposure as well as during the recovery period. Nevertheless, activities of antioxidative enzymes (SOD, POD, CAT, and GR) were enhanced to a greater extent by the combined stress compared with the single stresses during the initial days of the stress treatment, whereas the opposite was observed at later time points. Interestingly, this did not coincide with increased MDA levels at the later time points, suggesting that the lack of enhanced antioxidative defence compared with the single stress conditions did not cause a greater extent of oxidative damage. Antioxidative enzyme activities determined during the recovery phase strongly depended on the duration of the prior stress exposure (Ru *et al.*, 2023). In general, increased antioxidative enzyme activities in response to stress combinations probably serve to counteract enhanced cellular ROS concentrations as a consequence of the high stress severity. However, it is unclear whether the lack of stronger activation of the antioxidative defence system in response to combined compared with single stress conditions as observed in other studies is merely a negative consequence of the stress severity (e.g. due to damage to antioxidative proteins) or whether it serves to increase cellular ROS levels, thereby triggering signalling pathways required for acclimation to the stress combination.

Reactive oxygen species–phytohormone interplay in plant responses to combined stresses

When considering the involvement of ROS in plant responses to combined stress conditions, also their interplay with other signalling molecules such as Ca^{2+} and phytohormones should be taken into account (Fig. 1). Although studies focusing on the impact of stress combinations on both ROS and Ca^{2+} are currently scarce, several recent publications have reported combined stress-induced changes in both ROS and phytohormone concentrations. Hannachi *et al.* (2022), for example, demonstrated that combined exposure to drought and heat stress caused a stronger increase in leaf H_2O_2 levels of different eggplant cultivars compared with exposure to both individual stresses. Whereas individual heat or drought treatment did not affect phytohormone concentrations in some cultivars, ABA, salicylic acid (SA), and jasmonic acid (JA) levels were slightly increased by heat treatment and showed a stronger response to drought in others. In all cultivars, combined exposure to both stressors caused a similar effect on levels of these phytohormones to drought treatment alone (Hannachi *et al.*, 2022). In leaves of *Prunus cerasifera*, combined exposure to heat and drought caused a stronger increase in H_2O_2 concentrations than exposure to the individual stressors, both during the stress and upon recovery. However, the effect was less pronounced than would be predicted based on addition of the individual effects. Both drought and heat caused significant increases in ABA concentrations and significant decreases in indole-3-acetic acid, gibberellic acid (GA), and cytokinin levels. In general, these effects were more pronounced in plants simultaneously exposed to both stress conditions (Bolat *et al.*, 2024). Zaman *et al.* (2024) investigated ROS levels and transcriptional responses in dragon

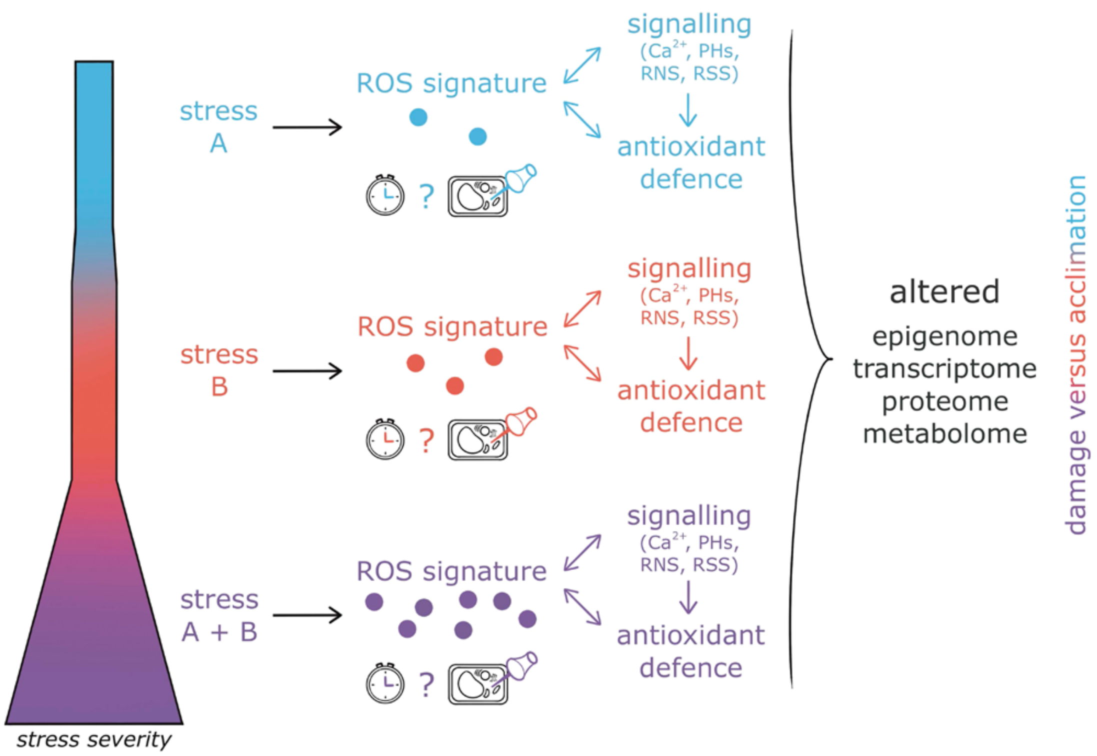

Fig. 1. Schematic overview of the involvement of unique reactive oxygen species (ROS) signatures in plant acclimation to combined stress conditions. Exposure to a combination of two or more abiotic stresses generally leads to a higher overall stress severity and stronger increases in cellular ROS levels compared with exposure to each of the stresses individually. Although ROS can cause oxidative damage, they are also key players in cellular signal transduction. Cellular ROS levels are controlled by an extensive antioxidative defence system and are tightly interconnected with other signalling molecules such as calcium (Ca^{2+}), phytohormones (PHs), reactive nitrogen species (RNS), and reactive sulfur species (RSS). Together, the signal transduction pathways activated by specific stress conditions trigger alterations at various biological organization levels (epigenome, transcriptome, proteome, metabolome), ultimately aimed at stress acclimation. Whereas it is clear that specific stress conditions trigger unique redox signatures, detailed information regarding the dynamics and subcellular origin of ROS production is generally lacking.

fruit seedlings exposed to drought, heat, vanadium, and their combinations. In cladodes, H_2O_2 concentrations were not affected by any of the individual stresses, but showed a significant increase to combined drought and vanadium treatment, suggesting a synergistic effect of both stressors on cellular ROS levels. Interestingly, addition of heat to the stress combination did not further increase H_2O_2 concentrations. Although the authors did not assess phytohormone concentrations, they performed an RNA-sequencing analysis, which revealed differential expression of a large number of genes related to ABA, GA, JA, brassinosteroid, ethylene, cytokinin, SA and auxin signalling pathways under combined stress conditions in comparison with single stress exposure (Zaman *et al.*, 2024).

Reactive oxygen species: key drivers of plant acclimation to stress combinations?

The coordinated action of phytohormones, ROS, and other key signalling molecules such as Ca^{2+} likely triggers unique transcriptional and/or metabolic responses required for plant survival and acclimation to specific single, combined, or multifactorial stress scenarios. However, despite the strong increase in the number of studies investigating plant responses to combined stress conditions over recent years, the importance of ROS in plant acclimation to stress combinations remains largely unknown. It could be speculated that stronger increases in ROS levels under combined stress compared with single stress conditions are merely a consequence of the higher stress intensity and are detrimental to plant function. Nevertheless, higher ROS levels in plants exposed to combined stress conditions could also serve as an important signal to trigger unique responses at various biological organization levels (transcriptome, proteome, metabolome, etc.) required for plant survival (Fig. 1). This hypothesis is supported by findings of Zandalinas *et al.* (2021b), showing that ROS levels increased in wild-type Arabidopsis plants with an increasing number of stressors in a multifactorial stress combination. Interestingly, both *apx1* and *rbohd* mutants displayed lower survival rates compared with wild-type plants upon exposure to stress combinations consisting of four or more stressors. These data suggest that not only ROS detoxification but also ROS generation plays important roles in plant survival upon stress combination.

Reactive oxygen species: key players in priming-induced stress tolerance

Stress priming: sequential stress exposure as a strategy towards improved stress tolerance

Whereas two or more stresses can occur simultaneously, stress events can also take place sequentially. When plants are first exposed to a mild, sublethal stress event, followed by a recovery period under optimal growth conditions, they often show an increased tolerance to subsequent stresses (Fig. 2). This phenomenon is termed stress priming and involves the development of a so-called stress memory, which allows plants to respond in a faster and more efficient manner to subsequent stress exposures. This process is also referred to as acquired stress tolerance, while the ability of plants to withstand stress conditions without prior exposure is known as basal stress tolerance (Nair *et al.*, 2022). The increased stress resilience is generally characterized by a smaller impact of the stress treatment on various parameters such as plant growth rate, yield and photosynthetic capacity in primed compared with non-primed plants and is linked to alterations at multiple biological organization levels including the transcriptome, proteome, and metabolome (H. Liu *et al.*, 2022). Furthermore, epigenetic effects such as changes in DNA methylation, chromatin remodelling, and histone modifications play key roles in stress memory (Nair *et al.*, 2022) (Fig. 2). Stress memory duration can vary from hours to weeks, depending on the stress type, intensity, duration, and recurrence of the priming stress, as well as on the plant species and its genetic background (H. Liu *et al.*, 2022; Nair *et al.*, 2022). When the memory effect is retained in the current generation only, it is referred to as somatic stress memory. However, stress memory can also be transferred from one generation to the next. Whereas the term intergenerational memory refers to the situation when a memory effect is only detected in the first non-stressed generation, the term transgenerational memory indicates the occurrence of memory effects in at least two stress-free generations (Lämke and Bäurle, 2017).

Stress priming can also be subdivided into *cis*-priming and *trans*-priming. These terms indicate situations in which exposure to a specific stress factor increases plant tolerance to a subsequent exposure to the same or a different stressor, respectively (Khan *et al.*, 2022; Nair *et al.*, 2022). A well-known example of *cis*-priming is the observation that survival of various plant species upon exposure to high temperature is significantly increased by prior treatment with a milder temperature stress (Jagadish *et al.*, 2010; Sedaghatmehr *et al.*, 2016; Zhang *et al.*, 2016; Wang *et al.*, 2020; B. Liu *et al.*, 2021; Olas *et al.*, 2021; Samantaray *et al.*, 2023; John *et al.*, 2024). This phenomenon is known as acquired thermotolerance and involves various mechanisms including increases in the expression and abundance of heat shock proteins and heat shock factors as well as enhanced activity of various antioxidative enzymes (Khan *et al.*, 2022). Interestingly, high temperature treatment also functions in *trans*-priming, as it was shown to enhance plant tolerance to a variety of other abiotic stress conditions including metal exposure (Hsu and Kao, 2007; Chou *et al.*, 2012; W. Liu *et al.*, 2021; Amin *et al.*, 2024), salinity (Gong *et al.*, 2001; Hossain *et al.*, 2013; Faralli *et al.*, 2015; Körner *et al.*, 2024), drought (Gong *et al.*, 2001; Hossain *et al.*, 2013), anoxia (Banti *et al.*, 2008), and chilling stress (Gong *et al.*, 2001; Zhang *et al.*, 2005, Zhang et al., 2013). Vice versa, other abiotic stress conditions can also improve plant thermotolerance (Zhang *et al.*, 2020; De Pascali *et al.*, 2022; Feijó *et al.*, 2023; Bryant *et al.*, 2024; Lamelas *et al.*, 2024; H. Wang *et al.*, 2024). For a detailed overview of this topic, readers are referred to Khan *et al.* (2022). The occurrence of *trans*-priming is likely based on the fact that plant responses to different stress conditions rely on overlapping sets of signalling molecules such as ROS and phytohormones (H. Liu *et al.*, 2022).

The cellular redox balance in stress priming

Whereas the beneficial effects of stress priming are well established, the underlying mechanisms are currently not fully understood. What is clear, however, is that ROS are important players in the process (González-Bosch, 2018). In many cases, stress priming results in an increase in cellular ROS levels prior to exposure to a second stress condition. While exposure to a second stress episode still induces an increase in ROS concentrations compared with the control, this effect is generally less pronounced in primed compared with non-primed plants (Table 2; Fig. 2). In roots and leaves of *Alhagi sparsifolia*, for example, drought priming resulted in significant elevations in H_2O_2 and $O_2^{\bullet -}$ levels. After a recovery period of 15 d followed by another drought episode, H_2O_2 concentrations were lower in roots and leaves of primed compared with non-primed plants. The same was observed for $O_2^{\bullet -}$ concentrations in leaves, whereas no differences were observed in roots. In general, the drought-primed plants showed a higher tolerance to a subsequent drought exposure, as evidenced by a larger root length and a higher root dry weight, leaf relative water content, and total plant biomass, as well as a lower extent of lipid peroxidation. As such, it can be speculated that the priming-induced increase in ROS levels serves a key role in the induction of a somatic stress memory. This is likely related to the significant increases in SOD, POD, CAT, GPX, and APX activities induced by drought priming. Interestingly, activities of GR and monodehydroascorbate reductase, which are involved in the reduction of antioxidative metabolites rather than in direct ROS detoxification, showed the opposite pattern. Furthermore, the interplay between ROS and phytohormones could also play an essential role in the establishment of drought stress memory, as concentrations of many phytohormones were significantly affected upon drought priming (Ullah *et al.*, 2024b). Highly similar results were reported in *Calligonum mongolicum* plants in a comparable experimental set-up (Ullah *et al.*, 2024a). Similarly, Ru *et al.* (2022) demonstrated that two consequent episodes of

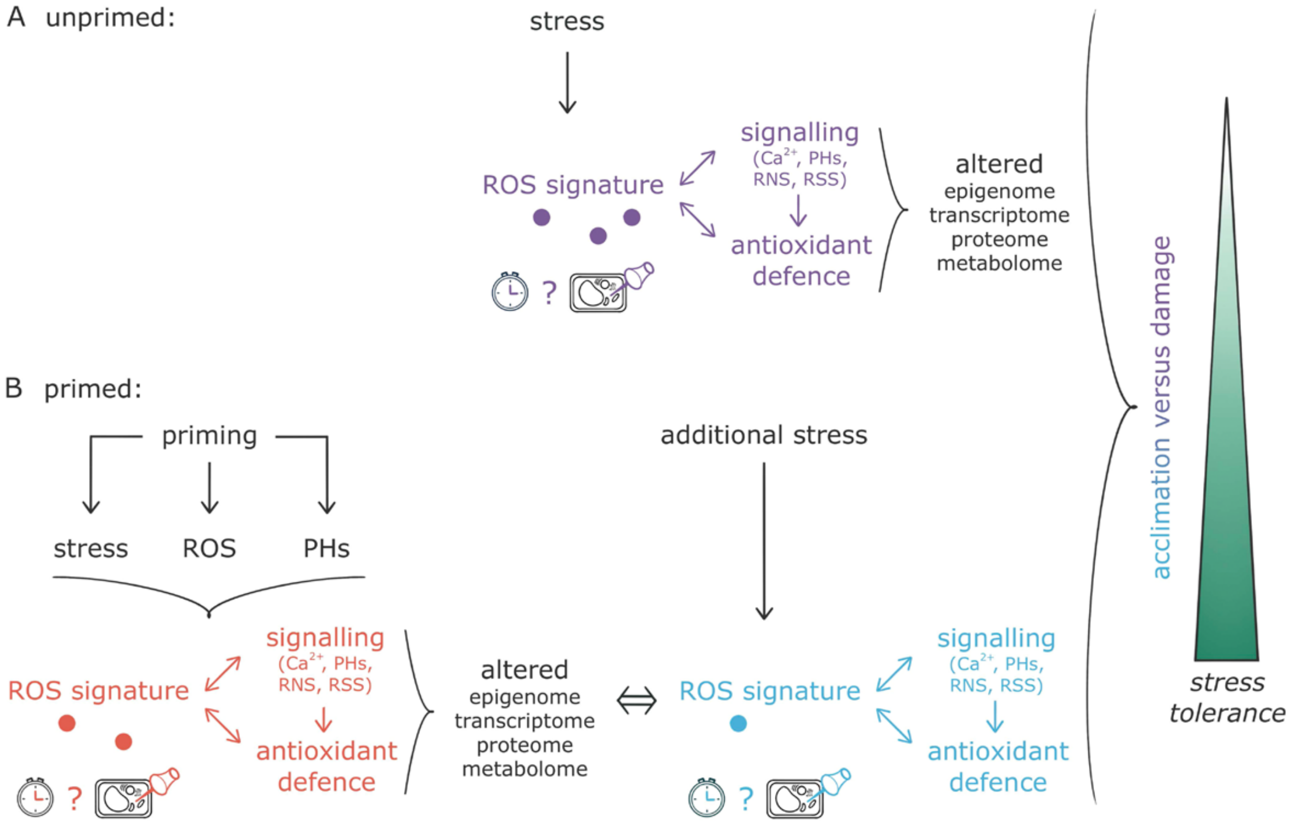

Fig. 2. Schematic overview of the involvement of reactive oxygen species (ROS) in priming-mediated increases in plant stress tolerance. Priming through a mild stress exposure or through treatment with ROS or phytohormone (PH)-related compounds induces a so-called stress memory, which is associated with alterations at various biological organization levels (epigenome, transcriptome, proteome, metabolome). Together with other signalling molecules such as calcium (Ca^{2+}), phytohormones, reactive nitrogen species (RNS), and reactive sulfur species (RSS), ROS are key players in establishing stress memory. This enhances plant tolerance to subsequent stress exposures and is generally associated with less pronounced stress-induced increases in cellular ROS levels in primed (B) compared with non-primed (A) plants. Whereas it is clear that ROS are important mediators of priming-dependent improvement of plant stress tolerance, detailed information regarding the dynamics and subcellular origin of ROS production is generally lacking.

priming with heat, drought, or a combination of both stresses for 2 d increased the tolerance of maize roots to a subsequent prolonged exposure to the same stressor(s) for 6 d. This was evidenced by the fact that root morphology of primed plants was less affected by subsequent stress exposure in comparison with that of non-primed plants. Interestingly, priming with the respective stress conditions resulted in higher root $O_2^{\bullet-}$ concentrations immediately prior to subsequent stress exposure, but less pronounced $O_2^{\bullet-}$ elevations at two time points during the subsequent stress treatment. Again, this observation is likely related to priming-induced increases in antioxidative enzyme activities, as SOD, CAT, and POD activities were higher in roots of primed plants both prior to and during the final stress episode (Ru *et al.*, 2022). These data suggest that priming strategies are not only useful to improve plant tolerance to individual stress conditions, but that they can also be employed to enhance plant resilience to combined or even multifactorial stress scenarios.

It should be noted that the intensity of the priming stress can affect to what extent plant tolerance to subsequent stress exposure is improved. Indeed, Khanzada *et al.* (2022) demonstrated that the temperature used for thermopriming influences subsequent heat stress tolerance of winter wheat. In this study, plants were primed with two episodes of either low (22/16 °C) or moderate heat stress (30/24 °C) at the four-leaf stage and the six-leaf stage and were subsequently exposed to high temperature (34/30 °C) during the booting or flowering stage. Low heat priming did not affect H_2O_2 and $O_2^{\bullet-}$ in flag leaves, while these parameters were significantly increased in response to moderate heat priming. Upon subsequent high temperature exposure, elevations in $O_2^{\bullet-}$ levels were similar between non-primed and low heat-primed plants. Increases in H_2O_2 concentrations induced by exposure to high temperature during the flowering stage were less pronounced in low heat-primed plants compared with non-primed plants, but such a difference was not observed when plants were subjected to high temperature stress during the booting stage. In contrast, moderate heat priming resulted in smaller stress-induced increases in flag leaf ROS levels compared with non-primed plants irrespective of the developmental stage during which the plants

Table 2. Summary of studies on abiotic stress-induced priming and reactive oxygen species (ROS) production under subsequent stress in plants

Final stressor	Priming stressor	Plant species	Organ	Priming [a]	Unprimed stressed plants [b]	Primed stressed plants [c]	ROS detection method	Redox-related parameters	Reference
Drought	Drought	*Morus multicaulis* (Husang 32)	Leaves	$H_2O_2\downarrow$ or =; $O_2^{\bullet-}=$	$H_2O_2\downarrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow$ or =; $O_2^{\bullet-}=$	Spectrophotometry	AO-Enz, LPO	L. Liu *et al.* (2022)
			Roots	$H_2O_2=$; $O_2^{\bullet-}=$	$H_2O_2\downarrow$; $O_2^{\bullet-}\uparrow$ or =	$H_2O_2\downarrow$; $O_2^{\bullet-}=$			
		Morus multicaulis (Husang 7307)	Leaves	$H_2O_2\downarrow$ or =; $O_2^{\bullet-}=$	$H_2O_2\downarrow$ or =; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow$ or =; $O_2^{\bullet-}=$	Spectrophotometry		
			Roots	$H_2O_2\downarrow$; $O_2^{\bullet-}=$	$H_2O_2\downarrow$; $O_2^{\bullet-}\uparrow$ or =	$H_2O_2=$; $O_2^{\bullet-}=$			
		Alhagi sparsifolia	Leaves	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Ullah *et al.* (2024b)
			Roots	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow$			
		Calligonum mongolicum	Leaves	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\downarrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Ullah *et al.* (2024a)
			Roots	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow$			
		Triticum aestivum	Leaves	—	$H_2O_2\uparrow$	$H_2O_2\uparrow$	Spectrophotometry	AO-Enz, LPO	Wang *et al.* (2021a)
		Triticum aestivum (Yangmai16)	Leaves	—	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Li *et al.* (2023)
		Triticum aestivum (Xumai33)	Leaves	—	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$			
Drought	Drought	*Glycine max*	Leaves	—	$H_2O_2\uparrow\uparrow$	$H_2O_2\uparrow$	Spectrophotometry	AO-Enz, LPO	Bester *et al.* (2024)
			Roots						
Flooding	Flooding		Leaves						
			Roots						
Drought	Flooding		Leaves						
			Roots						
Flooding	Drought		Leaves						
			Roots						
Heat	Heat (low)	*Triticum aestivum*	Leaves	$H_2O_2=$; $O_2^{\bullet-}=$	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow\uparrow$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Khanzada *et al.* (2022)
	Heat (high)			$H_2O_2\uparrow$; $O_2^{\bullet-}=$	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet-}\uparrow\uparrow\uparrow$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow\uparrow$			
Heat	Heat	*Zea mays*	Roots	$O_2^{\bullet-}\uparrow$	$O_2^{\bullet-}\uparrow\uparrow$	$O_2^{\bullet-}\uparrow$	Spectrophotometry	AO-Enz, LPO	Ru *et al.* (2022)
Drought	Drought			$O_2^{\bullet-}\uparrow$	$O_2^{\bullet-}\uparrow\uparrow$	$O_2^{\bullet-}\uparrow$			
Drought & Heat	Drought and Heat			$O_2^{\bullet-}\uparrow\uparrow$	$O_2^{\bullet-}\uparrow\uparrow\uparrow$	$O_2^{\bullet-}\uparrow\uparrow$			
Heat	Waterlogging	*Gossypium hirsutum*	Leaves	$H_2O_2=$; $O_2^{\bullet-}=$	$H_2O_2\uparrow$; $O_2^{\bullet-}\uparrow$	H2O2=; $O_2^{\bullet-}=$	Spectrophotometry	AO-Enz, LPO	H. Wang *et al.* (2024)

This table compiles research from the past 5 years investigating priming of plants with various abiotic stressors (drought, flooding, heat) prior to exposing them to a final stressor to investigate the ROS production in plants. Columns include the final stressor, priming stressor, plant species, organ studied, observed trends in ROS levels, the method used for ROS detection, and additional redox-related parameters studied. AO-Enz, antioxidant enzymes; AO-Met, antioxidant metabolites; LPO, lipid peroxidation.

[a] ROS production following priming alone is considered only when it is measured during the interval after the priming treatment and prior to the application of the subsequent final stressor. These ROS levels are compared with those in control plants (no priming, no stress) to evaluate the specific oxidative response induced by priming.

[b] ROS production following exposure to the final stressor without a previous priming treatment. These ROS levels are compared with those in control plants (no priming, no stress) to evaluate the specific oxidative response upon stress exposure.

[c] ROS production following exposure to the final stressor with a previous priming treatment. These ROS levels are compared with those in control plants (no priming, no stress) to evaluate the specific oxidative response upon stress exposure after priming.

were subjected to the stress treatment. The different ability of both priming treatments to protect plants against subsequent heat stress is again likely related to the degree of activation of the antioxidative defence system, as moderate heat priming induced stronger increases in SOD, CAT, and APX activities compared with low heat priming. Whereas monodehydroascorbate reductase activity showed the same response, GR and dehydroascorbate reductase activities were lower in moderate heat-primed plants in comparison with non-primed and low heat-primed plants (Khanzada *et al.*, 2022).

Besides the intensity of the priming stress, the plant genetic background can influence the effect of stress priming on subsequent stress tolerance. L. Liu *et al.* (2022) investigated the impact of drought priming on drought tolerance of two *Morus multicaulis* cultivars. Whereas $O_2^{\bullet-}$ concentrations did not differ between primed and non-primed plants after an 18-day period of drought stress priming, H_2O_2 concentrations were either not affected or decreased after the priming event depending on the cultivar studied. Upon subsequent drought stress, $O_2^{\bullet-}$ levels were generally lower in primed compared with non-primed plants, whereas H_2O_2 showed the opposite pattern, with the extent of the observed effects again differing between the two cultivars (L. Liu *et al.*, 2022). These data suggest that stress priming does not necessarily limit ROS production upon renewed stress exposure, but that it can also affect the type of ROS that is generated.

Interestingly, ROS are likely not only involved in the mechanisms underlying *cis*-priming, but also contribute to plant cross-tolerance to different abiotic stress conditions. In a recent study, Bester *et al.* (2024) investigated the influence of *cis*-priming and *trans*-priming on water deficit and waterlogging tolerance of soybean. Plants were primed with a 5 day period of either water deficit or waterlogging during the vegetative stage and again exposed to either of the two stressors during the reproductive stage. The obtained results showed that stress-induced increases in root and leaf H_2O_2 concentrations were significantly less pronounced in primed compared with non-primed plants, irrespective of the stressor used for priming. This response generally coincided with stronger stress-induced increases in SOD, CAT, and APX activities in roots and leaves of primed plants in comparison with those observed in non-primed plants (Bester *et al.*, 2024).

Besides enhancing plant stress tolerance by activating the enzymatic antioxidative defence system, ROS can also contribute to stress memory through other mechanisms. It was shown in tomato, for example, that inhibition of NADPH oxidase-mediated apoplastic ROS production in thermo-primed plants by treatment with diphenylene iodonium resulted in a decreased expression of heat stress-responsive genes and the loss of heat stress memory. During recovery upon prolonged heat stress exposure (termed overacclimation), H_2O_2 levels in the apoplast decreased, whereas those in chloroplasts increased. This response coincided with an increased extent of programmed cell death, emphasizing the importance of the subcellular localization of ROS production in determining plant stress tolerance (Sun *et al.*, 2018).

Chemical priming: shaping reactive oxygen species dynamics in plant stress acclimation

Although the underlying mechanisms are not fully understood, it is clear that ROS are key players in stress priming-mediated increases in plant tolerance to various abiotic stress conditions, including those related to climate change. Instead of using a prior stress exposure, also chemical priming is frequently used to improve plant stress resilience (Fig. 2) (Savvides *et al.*, 2016). In the next sections of this review, we focus on the influence of priming with ROS, phytohormones, and various biostimulants on plant tolerance to climate change-related stress conditions and explore the effects of these priming strategies on the cellular redox state.

Reactive oxygen species-based priming strategies

Due to its chemical properties and important role in signalling processes, H_2O_2 is frequently used in low concentrations for priming purposes (Hossain *et al.*, 2015; Kerchev *et al.*, 2020) (Table 3). Research has demonstrated positive effects of H_2O_2 priming on plant tolerance to a wide range of stress conditions including those directly linked to climate change such as heat (Uchida *et al.*, 2002; Zheng *et al.*, 2018; Iqbal *et al.*, 2020), drought (Iqbal *et al.*, 2018; Jira-anunkul and Patanagul, 2021; Wang *et al.*, 2021b), and waterlogging (S. Wang *et al.*, 2022). Treatment of plants with H_2O_2 is often carried out during the seed stage (Savvides *et al.*, 2016). Alternatively, plants can be treated with H_2O_2 at later stages during their development, for example by foliar spraying or through surface irrigation. Recently, Iqbal and Yaning (2024) compared the effects of these different H_2O_2 priming methods on drought tolerance of *Chenopodium quinoa* plants. In a preliminary experiment, they determined that a concentration of 15 mM H_2O_2 was most suitable for foliar spraying, whereas 5 mM H_2O_2 was the optimal concentration for surface irrigation, as these were the highest concentrations tested that did not induce an obvious increase in MDA levels. Interestingly, these treatments did not affect internal H_2O_2 and $O_2^{\bullet-}$ concentrations measured in the seedlings. Comparison of both methods with seed H_2O_2 priming at a concentration of 80 mM revealed that seed priming and foliar spraying were the most effective in preventing negative effects of drought exposure on various parameters including plant height and grain yield. This was in agreement with the fact that these methods caused the strongest reduction of drought-induced H_2O_2 production. Upon drought stress, SOD and POD activities were significantly higher in primed compared with non-primed plants, irrespective of the priming method. However, SOD activity was most strongly induced in seed-primed plants, whereas POD activity was highest in plants treated with H_2O_2 through foliar spraying (Iqbal and Yaning, 2024). Besides the method of H_2O_2 treatment, the

Table 3. Summary of studies on reactive oxygen species (ROS)-induced priming and ROS production under subsequent drought stress in plants

Priming agent and application method	**Plant species**	**Organ**	**Priming** [a]	**Unprimed stressed plants** [b]	**Primed stressed plants** [c]	**ROS detection method**	**Redox-related parameters**	**Reference**
H_2O_2								
Seed	*Chenopodium quinoa*	Leaves	—	$H_2O_2\uparrow\uparrow\uparrow$	$H_2O_2\uparrow\uparrow$	Spectrophotometry	AO-Enz, LPO	Iqbal and Yaning (2024)
Foliar			H_2O_2=; $O_2^{\bullet-}$=	$H_2O_2\uparrow\uparrow\uparrow$	$H_2O_2\uparrow$			
Irrigation			H_2O_2=; $O_2^{\bullet-}$=	$H_2O_2\uparrow\uparrow\uparrow$	$H_2O_2\uparrow\uparrow$			
Foliar	*Nicotiana tabacum*	Leaves	H_2O_2=	H_2O_2= or $\downarrow$	$H_2O_2\downarrow$ or $\downarrow\downarrow$	Spectrophotometry	AO-Enz	Villagómez-Aranda *et al.* (2024)
Seed	*Triticum aestivum* (tolerant)	Shoots	—	$H_2O_2\uparrow$	$H_2O_2\downarrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Bhardwaj *et al.* (2021)
		Roots		$H_2O_2\uparrow$	$H_2O_2\uparrow$			
Seed	*Triticum aestivum* (intermediate sensitivity)	Shoots		$H_2O_2\downarrow$	H_2O_2=			
		Roots		$H_2O_2\downarrow$	$H_2O_2\uparrow$			
Seed	*Triticum aestivum* (sensitive)	Shoots		H_2O_2=	H_2O_2=			
		Roots		H_2O_2=	$H_2O_2\uparrow$			
H_2O_2 and NO								
Seed	*Triticum aestivum* (S24—intermediate sensitivity)	Leaves	H_2O_2=	$H_2O_2\uparrow$	$H_2O_2\uparrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Habib *et al.* (2020)
Seed	*Triticum aestivum* (Fsd-2008—tolerant)	Leaves	H_2O_2=	$H_2O_2\uparrow$	$H_2O_2\uparrow$			

This table compiles research from the past 5 years investigating priming of plants with ROS (H_2O_2 and NO) prior to exposing them to drought stress to investigate the ROS production in plants. Columns include the priming agent and application method, plant species, organ studied, observed trends in ROS levels, the method used for ROS detection, and additional redox-related parameters studied. AO-Enz, antioxidant enzymes; AO-Met, antioxidant metabolites; LPO, lipid peroxidation.

[a] ROS levels following priming alone are compared with those in control plants (no priming, no stress) to evaluate the specific oxidative response induced by priming.

[b] ROS levels following exposure to the final stressor without a previous priming treatment are compared with those in control plants (no priming, no stress) to evaluate the specific oxidative response upon stress exposure.

[c] ROS levels following exposure to the final stressor with a previous priming treatment are compared with those in control plants (no priming, no stress) to evaluate the specific oxidative response upon stress exposure after priming.

plant genetic background can affect the extent to which H_2O_2 priming improves drought tolerance, as recently demonstrated in a study by Bhardwaj *et al.* (2021) using different wheat cultivars. Similar to the effects of stress priming, H_2O_2 priming can also positively impact stress tolerance in an intergenerational manner. Priming of *Nicotiana tabacum* via foliar spraying with H_2O_2 improved not only the drought tolerance of the primed generation, but also that of the offspring. This response was likely modulated by epigenetic alterations, as a large number of differentially methylated cytosines were detected in the offspring, many of which were associated with genes involved in cellular responses to environmental stimuli (Villagómez-Aranda *et al.*, 2024).

Phytohormone-based priming strategies

Furthermore, plant tolerance to various stress conditions can be increased by pre-treatment with phytohormone-related compounds (Kerchev *et al.*, 2020) (Fig. 2). Similar to ROS priming, phytohormone priming can be achieved via treatment at the seed stage as well as at later stages during plant development through foliar spray or root application. As summarized in Table 4, priming with brassinosteroids, GA, JA, ethylene, ABA, SA, and strigolactones improves tolerance of various plant species to drought and/or heat stress. In general, the phytohormone-induced increased stress tolerance coincides with lower ROS levels in primed as compared with non-primed plants after stress exposure, suggesting an influence of phytohormone priming on cellular redox homeostasis. It should be noted, however, that prior to the stress treatment, differences in ROS levels between primed and non-primed plants are variable. In some cases, phytohormone priming resulted in significant increases in H_2O_2 levels. This was, for example, observed in roots of tomato plants primed with SA (Galviz *et al.*, 2021) as well as in leaves of *Oryza sativa* treated with ABA (X. L. Liu *et al.*, 2022). In contrast, decreased H_2O_2 concentrations were reported in *Brassica napus* seedlings (M. N. Khan *et al.*, 2020) and leaves of *Vicia faba* plants primed with GA (Rady *et al.*, 2021) as well as in leaves of JA-primed *Glycine max* plants (Rahman *et al.*, 2024). Nevertheless, phytohormone priming was generally found to enhance antioxidative enzyme activities, thereby likely contributing to the lower extent of stress-induced ROS production in primed plants compared with their non-primed counterparts (Table 4).

In addition to the traditional plant hormones, also signalling molecules with hormone-like functions can be used in priming strategies. In this context, melatonin and polyamines

Table 4. Summary of studies on phytohormone-induced priming and reactive oxygen species (ROS) production under subsequent abiotic stress in plants

Priming agent and application method	Stressor	Plant species	Organ	Priming [a]	Unprimed stressed plants [b]	Primed stressed plants/unprimed stressed plants [c]	ROS detection method	Redox-related parameters	Reference
Brassinosteroid									
Seed (EBL)	Heat	*Brassica juncea*	Seedling	$H_2O_2\uparrow\uparrow\uparrow$; $O_2^{\bullet -}=$; $HO^{\bullet}\downarrow$	$H_2O_2\uparrow$; $O_2^{\bullet -}\uparrow$; $HO^{\bullet}\uparrow$	$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow$; $HO^{\bullet}\downarrow$	Spectrophotometry + histochemical staining	AO-Enz, AO-Met, LPO	Neha *et al.* (2022)
Seed (HBL)				$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow\downarrow$; $HO^{\bullet}\downarrow\downarrow$		$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow\downarrow$; $HO^{\bullet}\downarrow\downarrow$			
Gibberellic acid									
Seed	Drought	*Brassica napus*	Seedling	$H_2O_2=$	$H_2O_2\uparrow$	$H_2O_2\downarrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	K. K. Zhang *et al.* (2023)
Seed	Drought	*Brassica napus*	Seedling	$H_2O_2\downarrow$	$H_2O_2\uparrow$	$H_2O_2\downarrow\downarrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	M. N. Khan *et al.* (2020)
Seed	Drought	*Oryza sativa*	Leaves	—	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet -}\uparrow\uparrow$	$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow$	Spectrophotometry	AO-Enz, LPO	K. K. Zhang *et al.* (2024)
			Roots	—	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet -}\uparrow\uparrow$	$H_2O_2\downarrow\downarrow$; $O_2^{\bullet -}\downarrow$			
Foliar	Drought	*Vicia faba*	Leaves	$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow$	$H_2O_2\uparrow$; $O_2^{\bullet -}\uparrow$	$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow$	Spectrophotometry	AO-Enz, AO-Met, LPO	Rady *et al.* (2021)
Jasmonic acid									
Root	Heat	*Glycine max*	Leaves	$H_2O_2\downarrow$	$H_2O_2\uparrow$	$H_2O_2\downarrow$	Spectrophotometry	AO-Enz, LPO	Rahman *et al.* (2024)
	Drought				$H_2O_2\uparrow\uparrow$	$H_2O_2\downarrow$			
	Drought & Heat				$H_2O_2\uparrow\uparrow$	$H_2O_2\downarrow$			
Ethylene									
Seed (ethephon)	Drought	*Oryza sativa*	Leaves	—	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet -}\uparrow\uparrow$	$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow$	Spectrophotometry	AO-Enz, LPO	K. K. Zhang *et al.* (2024)
			Roots	—	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet -}\uparrow\uparrow$	$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow\downarrow$			
Abscisic acid									
Foliar	Heat	*Oryza sativa*	Grains	$H_2O_2=$; $O_2^{\bullet -}=$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet -}\uparrow\uparrow$	$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow$	Spectrophotometry	AO-Enz	Liu *et al.* (2023)
Root		*Oryza sativa*	Leaves	$H_2O_2\uparrow/=$; $O_2^{\bullet -}=$	$H_2O_2\uparrow\uparrow$; $O_2^{\bullet -}\uparrow\uparrow$	$H_2O_2\downarrow$; $O_2^{\bullet -}\downarrow$	Spectrophotometry	LPO	X. L. Liu *et al.* (2022)
Salicylic acid									
Root	Heat	*Gossypium hirsutum*	Leaves	$H_2O_2=$	$H_2O_2\uparrow\uparrow$	$H_2O_2\downarrow\downarrow$	Spectrophotometry	AO-Enz, LPO	Das *et al.* (2024)
Seed	Drought	*Solanum lycopersicum*	Leaves	$H_2O_2=$	$H_2O_2\uparrow\uparrow$	$H_2O_2=$	Spectrophotometry	AO-Enz, LPO	Galviz *et al.* (2021)
			Roots	$H_2O_2\uparrow$	$H_2O_2\uparrow$	$H_2O_2\uparrow\uparrow$			
Root			Leaves	$H_2O_2=$	$H_2O_2\uparrow\uparrow$	$H_2O_2\downarrow$			
			Roots	$H_2O_2\uparrow$	$H_2O_2\uparrow$	$H_2O_2\uparrow$			
Seed	Drought	*Triticum aestivum*	Leaves	$H_2O_2=$	$H_2O_2\uparrow$	$H_2O_2\downarrow$	Spectrophotometry	LPO	Maswada *et al.* (2021)
Strigolactone									
Root (GR24)	Drought	*Triticum aestivum*	Leaves	—	$H_2O_2\uparrow$	$H_2O_2\downarrow$	Spectrophotometry	MDA, AO-Enz	Song *et al.* (2023)

This table compiles research from the past 5 years investigating priming of plants with phytohormones prior to exposing them to a final stressor (heat, drought, combination) to investigate the ROS production in plants. Columns include the priming agent and application method, the stressor, plant species and organ studied, observed trends in ROS levels, the method used for ROS detection, and additional redox-related parameters studied. AO-Enz, antioxidant enzymes; AO-met, antioxidant metabolites; EBL: 4-epibrassinolide; HBL: 28-homobrassinolide; LPO, lipid peroxidation.

[a] ROS levels following priming alone are compared with those in control plants (no priming, no stress) to evaluate the specific oxidative response induced by priming.

[b] ROS levels following exposure to the stressor without a previous priming treatment are compared with those in control plants (no priming, no stress) to evaluate the specific oxidative response upon stress exposure.

[c] ROS levels following exposure to the stressor with a previous priming treatment are compared with those in unprimed, stressed plants to evaluate the effect of priming on the oxidative response upon stress exposure.

are of particular interest. Melatonin is an indolic compound produced from tryptophan and is known to play important roles in a variety of plant processes, including seed germination, fruit ripening, photosynthesis, circadian rhythm, and many others. Furthermore, its role in plant responses to abiotic stress conditions and its strong connection to the cellular redox network are well established (A. Khan *et al.*, 2020). Interestingly, several recent research efforts have also demonstrated the effectivity of exogenous melatonin applications for priming purposes, as reviewed by Rajora *et al.* (2022). For example, Muhammad *et al.* (2023) reported that melatonin priming improved drought tolerance of maize. This response coincided with less pronounced drought-induced increases in H_2O_2 and $O_2^{\bullet-}$ levels and stronger inductions in SOD, POD, and CAT activities as well as in GSH and AsA concentrations (Muhammad *et al.*, 2023). Similarly, Jahan *et al.* (2019) revealed that melatonin pre-treatment significantly enhanced heat stress tolerance of tomato, which was linked to a lower extent of heat-induced ROS production and stronger induction of antioxidant enzyme activities. Exogenous melatonin also affected heat-induced alterations in endogenous nitric oxide and polyamine levels, emphasizing its cross-talk with other signalling molecules (Jahan *et al.*, 2019). Notably, exogenous melatonin treatment also enhanced the tolerance of tomato leaves to combined drought and heat conditions, likely as a consequence of increased antioxidative defence (Annadurai *et al.*, 2023). A beneficial effect of melatonin priming on antioxidative enzyme activities was also observed in *Cyperus esculentus* exposed to a combination of salt and drought (N. Wang *et al.*, 2024).

Polyamines such as putrescine, spermidine, and spermine are small polycationic molecules synthesized from amino acids (mainly ornithine, arginine, and methionine) that have been implicated in several plant developmental processes including seed germination, senescence, vascular development, and reproduction (Blázquez, 2024). Furthermore, they are important players in plant stress responses and can be exogenously applied for priming purposes (Wojtyla *et al.*, 2024). For example, Liu *et al.* (2014) reported that pre-treatment with spermidine mitigated waterlogging-induced increases in cellular ROS levels. This response coincided with increased SOD, CAT, POD, and GR activities and lower extents of lipid peroxidation and DNA damage, suggesting the interconnection of polyamines with the cellular redox network (Liu *et al.*, 2014).

Biostimulant-based priming strategies

Besides ROS and phytohormones, a broad range of biostimulants can be used for priming purposes. Biostimulants are naturally derived molecules that are non-toxic in low concentrations and are typically used to stimulate plant growth. Examples include fungi such as *Trichoderma harzianum*, non-pathogenic bacterial strains, protein hydrolysates, and plant-derived extracts (Kerchev *et al.*, 2020). Although detailed knowledge on their mode of action is often lacking, several recent studies revealed an impact of treatment with various biostimulants on stress-induced ROS production, suggesting a role for redox-related processes in their underlying mechanism. For example, prior inoculation of Arabidopsis plants with *Brevundimonas vesicularis* (S1T13) significantly minimized increases in $O_2^{\bullet-}$ and H_2O_2 concentrations induced by subsequent drought stress. This response coincided with stronger drought-induced increases in antioxidative enzyme activities and a stronger transcriptional up-regulation of antioxidative genes and drought-responsive transcription factors of the dehydration-responsive element binding family (Tran *et al.*, 2023). Similarly, priming with two biostimulants based on *Ascophyllum nodosum* extracts (i.e. Phylgreen) and animal L-α-amino acids (i.e. Delfan plus) resulted in protection of Arabidopsis against short-term heat stress. Analyses at different biological organization levels revealed that both agents were able to reduce the extent of oxidative damage in leaves, which could be explained by a priming-induced transcriptional up-regulation of heat shock proteins and antioxidants (Cocetta *et al.*, 2022). Likewise, priming with a pig blood-derived protein hydrolysate through foliar application minimized the extent of drought-induced increases in ROS production and lipid peroxidation in tomato plants. At the same time, higher SOD, CAT, POD, and APX activities were measured in roots and leaves of primed compared with non-primed plants. Whereas a concentration of $2\ g\ L^{-1}$ of the protein hydrolysate showed the most pronounced beneficial effect, both lower and higher concentrations were less potent in promoting drought tolerance (W. Wang *et al.*, 2022). These results emphasize the importance of the concentration applied in determining the effects of biostimulant-mediated priming. Taken together, the application of biostimulants is a promising approach to enhancing plant tolerance to various abiotic stress conditions, including those related to climate change. As biostimulants are naturally occurring molecules, their use in priming strategies significantly aids in enhancing agricultural sustainability. Furthermore, extraction of biostimulants from waste and by-products contributes to a circular economy (Wazeer *et al.*, 2024; X. Zhang *et al.*, 2024).

Conclusion and perspectives

Although ROS can oxidatively damage cellular macromolecules when present at high concentrations, they are also indispensable for signal transduction in plants exposed to various stress conditions. Furthermore, research suggests that they play key roles in plant responses to stress combinations as well as in priming-induced increases in plant stress tolerance. Nevertheless, the detailed molecular mechanisms underlying the involvement of ROS in these processes remain largely elusive. To further dissect the importance of ROS signals and their interplay with other signalling molecules in plant acclimation to combined stress conditions and priming-dependent increased stress tolerance, experiments should be performed in plants with disturbed redox homeostasis, either through mutations of important pro- or antioxidative genes or through pharmacological treatments. Furthermore, particular attention should be paid to the timing as well as the subcellular localization of ROS production (Figs 1, 2). Indeed, current studies generally assess ROS concentrations in entire plants or plant organs (roots, leaves, flowers, etc.) without subcellular resolution and analyses are often limited to one specific time point. Nevertheless, it is known that cellular ROS levels are highly dynamic and can rapidly change. Furthermore, most frequently used methods for ROS detection via spectrometry, histochemical staining, and staining using fluorescent dyes suffer from a lack of specificity (Ortega-Villasante *et al.*, 2018). Hence, caution should be taken when comparing results between studies, as different methodologies for ROS detection might yield different results. As it allows direct detection of reactive species, electron paramagnetic resonance provides a more accurate method for analysis of $O_2^{\bullet-}$ and $^{\bullet}OH$ levels (Steffen-Heins and Steffens, 2015). However, its use in studies investigating the involvement of ROS in plant responses to combined stress conditions and in priming-mediated plant stress tolerance is scarce. Also genetically encoded biosensors could be highly useful tools in the investigation of these processes, as they could significantly enhance the spatial and temporal resolution of our knowledge on plant responses to combined stress conditions. This approach is non-invasive and hence allows dynamic monitoring of redox-related parameters (e.g. GSH redox potential, H_2O_2, $NADP^+$/NADPH) as well as the levels of other signalling molecules such as Ca^{2+} and certain phytohormones with high specificity. Furthermore, the sensors can be targeted to specific subcellular compartments (e.g. mitochondria, plastids, peroxisomes, endoplasmic reticulum) to gain in-depth knowledge on subcellular responses to stress combinations (Walia *et al.*, 2018; Müller-Schüssele *et al.*, 2021). This method can be combined with various large-scale -omics approaches to reveal how specific ROS signatures are connected to alterations at the epigenome, transcriptome, proteome, and metabolome level (Joshi *et al.*, 2024).

It is clear from the currently available data that ROS play important roles in establishing improved plant stress tolerance through various priming strategies. Such stress memory can be highly beneficial to plants upon encountering future stress conditions. However, maintenance of the memory state can result in suboptimal growth and development under environmentally favourable conditions, as allocation of resources to sustained memory results in a lower resource availability for growth and development. As such, it is essential for plants to control the duration of the stress memory and reset the cellular state at the appropriate time after a stress episode ends. In heat-stressed plants, autophagy is known to play an important role in the resetting of thermomemory (Sedaghatmehr and Balazadeh, 2024). In addition, RNA turnover is an essential process involved in forgetting stress memories (Crisp *et al.*, 2016). In-depth investigation of the mechanisms underlying the delicate balance between maintaining and resetting stress memories and the role of ROS and redox signalling in these processes will further contribute to the development of strategies to improve plant stress tolerance.

Whereas stress-tolerant plant varieties could be obtained through conventional breeding, this is highly time-consuming and might hence not be suitable in the context of rapidly changing climatological and environmental conditions. Genetic modification could yield crop varieties with improved stress resilience in a shorter time frame, but this approach is subject to regulatory restrictions and public concern (Savvides *et al.*, 2016). As such, plant priming through prior stress exposure or treatment with ROS, phytohormones, or biostimulants is an interesting alternative approach to improve plant survival under single as well as combined stress conditions and could contribute to sustainable agriculture and a circular economy.

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Author contributions

SH and AC: conceptualization; IV, JC, LBC, MAH, KK and MH: data collection; SH and AC: writing—original draft, writing—revision and editing; IV: visualization. All authors have read and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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