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The role of ROS and phytohormones in crops under environmental stress

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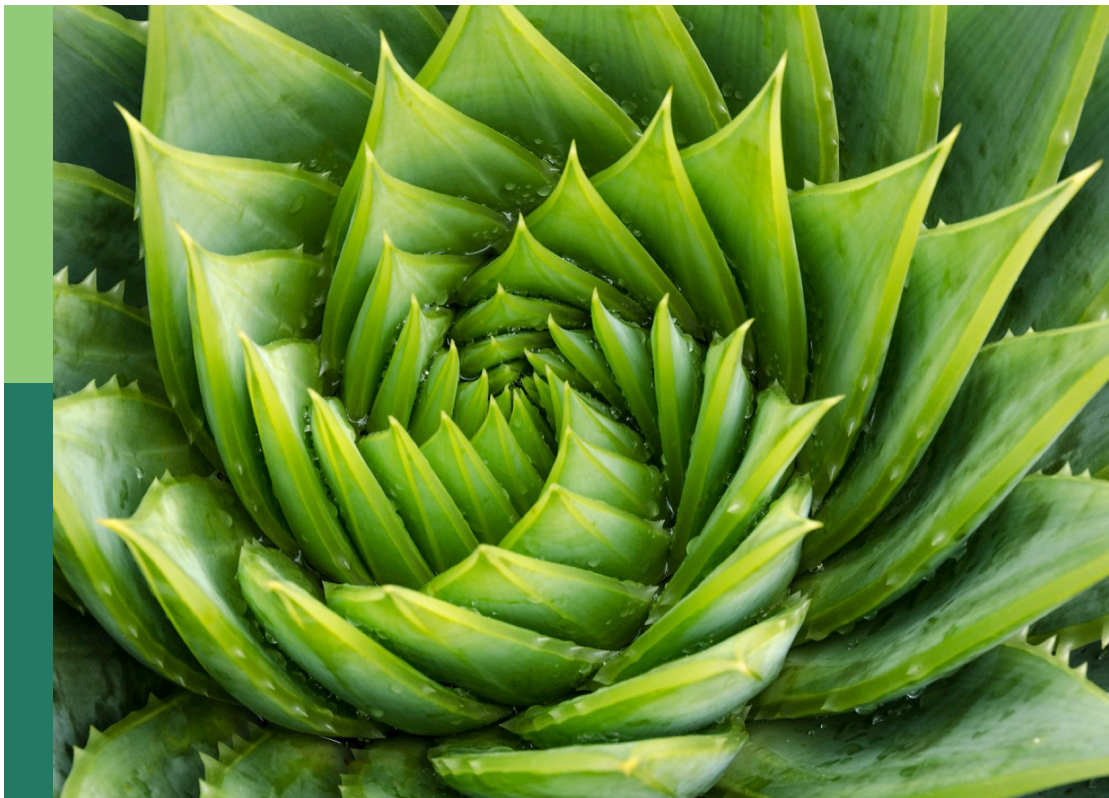

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The role of ROS and phytohormones in crops under environmental stress

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Heavy metal toxicity in plants and the potential NO-releasing novel techniques as the impending mitigation alternatives

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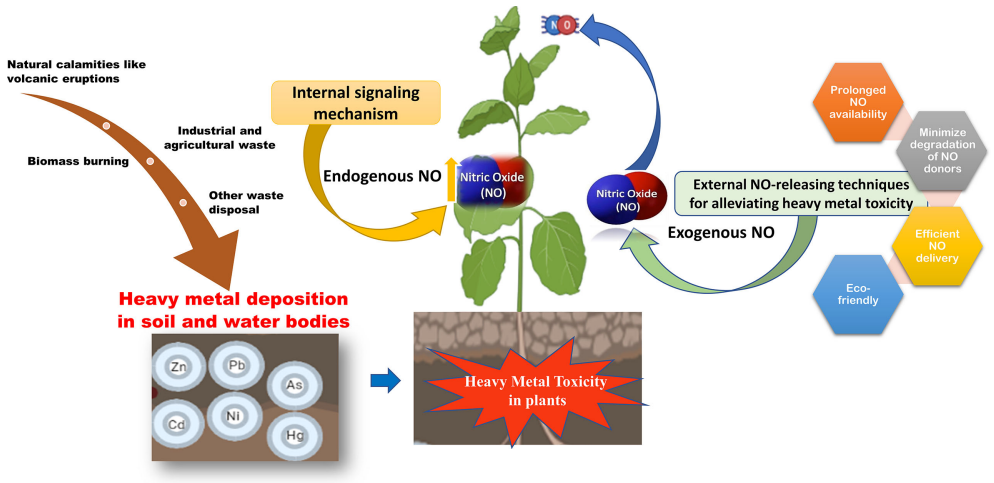
Environmental pollutants like heavy metals are toxic, persistent, and bioaccumulative in nature. Contamination of agricultural fields with heavy metals not only hampers the quality and yield of crops but also poses a serious threat to human health by entering the food chain. Plants generally cope with heavy metal stress by regulating their redox machinery. In this context, nitric oxide (NO) plays a potent role in combating heavy metal toxicity in plants. Studies have shown that the exogenous application of NO donors protects plants against the deleterious effects of heavy metals by enhancing their antioxidative defense system. Most of the studies have used sodium nitroprusside (SNP) as a NO donor for combating heavy metal stress despite the associated concerns related to cyanide release. Recently, NO-releasing nanoparticles have been tested for their efficacy in a few plants and other biomedical research applications suggesting their use as an alternative to chemical NO donors with the advantage of safe, slow and prolonged release of NO. This suggests that they may also serve as potential candidates in mitigating heavy metal stress in plants. Therefore, this review presents the role of NO, the application of chemical NO donors, potential advantages of NO-releasing nanoparticles, and other NO-release strategies in biomedical research that may be useful in mitigating heavy metal stress in plants.

KEYWORDS

heavy metal toxicity, nitric oxide, NO donors, NO-release, nanoparticles, encapsulation, agriculture



GRAPHICAL ABSTRACT
Heavy metal toxicity reduces plant growth and productivity. Since traditionally used NO donors do not provide a sustained NO-release, therefore heavy metal toxicity can be mitigated by novel NO-delivery techniques.

Introduction

Industrialization and the increasing human population have led to the exploitation of natural resources for anthropogenic activities leading to ecological imbalance. Heavy metal contamination in soil and water is one of the major examples of such human-centric activities that pose a serious threat to the environment (Chen et al., 2022b). However, natural phenomenon like volcanic eruptions and weathering of rocks also contributes to the contamination of soil and water bodies with heavy metals. Contamination of heavy metals in agricultural soil leads to decreased growth and productivity of crops and their bioaccumulation in crops poses serious health hazards as they enter the food chain (Emurotu and Onianwa, 2017). Due to their persistent, bioaccumulative, and toxic nature, these are known as major environmental pollutants (Tchounwou et al., 2012).

Recently, agricultural lands contaminated with heavy metals have gained much attention because of their detrimental effect on the agro-ecosystem. Any adverse effect on the agro-ecosystem directly affects various active and dynamic physical, chemical, and biological activities involved in plant growth and productivity. A principal consequence of heavy metal toxicity in plants is the overproduction of reactive oxygen species (ROS) leading to oxidative stress (Romero-Puertas et al., 2019). However, certain heavy metals like Cadmium (Cd) may not induce ROS production in plants but act as pro-oxidants and suppress the availability of antioxidants (Singh and Shah, 2015; Loix et al., 2017). Thus, heavy metals disturb the equilibrium between the production and scavenging of ROS resulting in oxidative stress (Demecsová and Tamás, 2019). While heavy metals induce the production of ROS, nitric oxide plays an important role in stimulating the antioxidant signaling response in plants, thus alleviating the toxic effects of ROS (Terrón-Camero et al., 2019; Sharma et al., 2020).

Nitric oxide (NO) is a versatile and key molecule known for its role in enhancing plant tolerance to abiotic stresses like drought, salinity, heavy metals, and extreme temperatures (Simontacchi et al., 2015; Begara-Morales et al., 2019; Nabi et al., 2019). Additionally, it plays an important role in various growth and developmental processes in plants such as germination, root development, photomorphogenesis (Corpas and Palma, 2020). Nitric oxide not only activates the antioxidative machinery but also activates the synthesis of phytochelatins which helps plants to cope with the deleterious effects of ROS (Groß et al., 2013). It is also documented that both endogenous and exogenous NO contribute to stress tolerance in plants (Wei et al., 2020). Moreover, the exogenous application of NO (in the form of NO donor) is highly dose-dependent and varies from plant to plant. A detailed account on the dose-dependent application of different NO donors in various plants has been reviewed by Terrón-Camero et al. (2019).

In the last decade, several studies described the use of different NO donors to understand the effects and the mechanism of NO under heavy metal stress in plants (Gill et al., 2013; Singh and Shah, 2014; Imran et al., 2016; Hashem et al., 2018; Li et al., 2019; Piacentini et al., 2020b; Ahmad et al., 2021b). These studies support the notion that the exogenous application of NO donors counterbalances the toxic and detrimental effects of heavy metals on overall plant physiology.

Despite the vast application of exogenous chemical NO donors in plants, these are generally unstable and prone to decomposition by high light or temperatures leading to rapid and uncontrolled release of NO which reduces their efficacy (Seabra and Durán, 2010; Seabra et al., 2015). Furthermore, all the NO donors have different molecular weights, different rates of absorption by the plants, and different rates of NO release *in planta*. In addition, some of these NO donors are known to release toxic byproducts along with NO. For example, Sodium Nitroprusside (SNP) is known to release cyanide once absorbed by the plants (Norris and Hume, 1987). Therefore, efforts have been made in developing biomaterials that can release NO in a controlled, efficient, and bio-safe manner. In this context, nanotechnology offers major advantages like easy and efficient encapsulation for better storage and controlled release of such chemicals to the targeted sites.

Recently, the application of nano-NO donors as potential alternatives to chemical NO donors has gained much attention. Studies have reported that the application of nanomaterials enhances the level of endogenous NO, promotes growth, and mitigates environmental stress in plants (Oliveira et al., 2016). Evaluation of the potential of nanoparticles as NO donors have recently begun for agricultural and biomedical purposes. This review aims at discussing the potential advantages of NO-releasing nanomaterials in plants and their usefulness in mitigating heavy metal stress in plants. The review also offers thoughtful insights on the prospects of applying other NO delivery platforms that are so far used in biomedical research but may be useful in plant science as well.

Heavy metal toxicity and the role of nitric oxide in mitigating heavy metal stress in plants

Heavy metals are serious environmental pollutants due to their acute and chronic toxic effects and widespread occurrence. The most hazardous heavy metals and metalloids in the environment include chromium (Cr), Nickel (Ni), Copper (Cu), Zinc (Zn), cadmium (Cd), Lead (Pb), Mercury (Hg), and Arsenic (As). The toxicity caused by these heavy metals on living organisms depends on the dose and duration of exposure (Chen et al., 2022a). However, certain heavy metals like cd, Pb, and Hg may be toxic even at very low concentrations. Heavy metal toxicity in plants leads to several physiological and morphological changes, responsible for the decline in growth (Chen et al., 2022a). For instance, plants exposed to cadmium showed reduced water and nutrient uptake and a decline in the rate of photosynthesis along with other morphological symptoms like chlorosis, inhibition of growth and browning of root tips that ultimately lead to cell death (Wojcik and Tukiendorf, 2004; Mohanpuria et al., 2007). It has been reported that heavy metals can lead to oxidative deterioration of biological molecules causing DNA fragmentation, lipid peroxidation, and protein oxidation. They can alter the content of antioxidants and may change the antioxidative enzyme activity (Sharma and Dietz, 2009).

The role of nitric oxide in mitigating the toxicity induced by heavy metals is well known and thoroughly studied. Nitric oxide (NO) is a key molecule involved in several physiological and biochemical processes in plants. NO is involved in root hair development (Lombardo et al., 2006), enabling plant-microbe interaction during nitrogen fixation (Pande et al., 2021), regulating a balance between auxin and reactive oxygen intermediates (Yu et al., 2014), and for maintaining iron homeostasis (Graziano and Lamattina, 2007). NO also plays a vital role in enhancing the immune response (Tada et al., 2008; Wang et al., 2009) and hypersensitive cell death response (Romero-Puertas et al., 2007; Yun et al., 2011). As a signaling molecule, NO plays a protective role in alleviating abiotic stress conditions (Zhang et al., 2007; Cantrel et al., 2011) including mitigation of heavy metal toxicity in plants (Singh et al., 2016; Nabi et al., 2019; Wei et al., 2020) as shown in Figure 1. Most of the studies on heavy metal toxicity in plants indicate that NO reduces the ROS levels by enhancing the levels of antioxidative enzymes (Wang et al., 2010b; Nabi et al., 2019; Terrón-Camero et al., 2019). During heavy metal stress, NO regulates the excessive production of ROS by forming less stable peroxynitrite from the superoxide radical ($O_2^{\cdot-}$) (Groß et al., 2013). Moreover, NO also regulates the antioxidant enzyme activity in the cell to control the ROS levels during heavy metal stress (Begara-Morales et al., 2019; Khator et al., 2021). Accumulation of NO also leads to the reduction of heavy metal uptake by metal transporters in the roots (Zhao et al., 2013; Singh et al., 2016).

Other studies on heavy metal toxicity suggested the role of nitric oxide in controlling the stomatal aperture (Nabi et al., 2019), in modifying proteins through S-nitrosylation or tyrosine nitration (Saxena and Shekhawat, 2013), and also in minimizing the mobility of heavy metals by enhancing the expression of phytochelatins in plants and thus reducing the heavy metal toxicity in plants (Groß et al., 2013).

Nitric oxide interacts with different biomolecules like phytohormones in response to heavy metal stress in plants. Nitric oxide regulates phytohormonal levels under heavy metal stress conditions. NO is suggested to reduce AsIII toxicity by regulating Jasmonic acid biosynthesis (Singh et al., 2017). It also increases the levels of indole acetic acid (IAA), cytokinins and gibberellic acid while decreasing the levels of ABA in order to lower lead (Pb) uptake and transport (Sadeghipour, 2017).

The interaction between NO and phytohormones is mainly influenced by NO-mediated post-translational modifications (PTMs) under basal as well as induced conditions (Terrile et al., 2012). Protein S-nitrosylation is the most prominent and widely studied PTM among others. It is the selective but

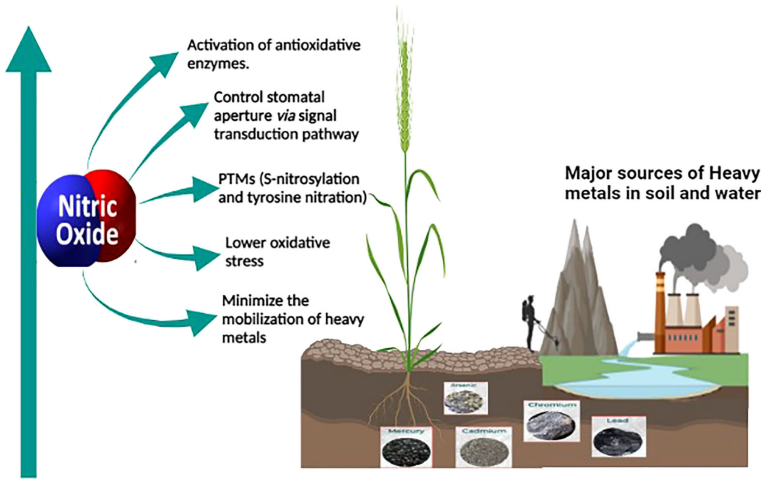


FIGURE 1
Sources of heavy metal contamination in agricultural land and the role of NO in mitigating heavy metal toxicity in plants. Anthropogenic activities such as industrialization and mining leads to heavy metal contamination in agricultural soil. The oxidative stress caused by the heavy metal toxicity is alleviated by endogenous or exogenously supplied nitric oxide which alleviates it. Nitric oxide is a versatile signaling molecule activating the antioxidative enzymes, controlling stomatal aperture or modifying important proteins through post-translational modification, minimizing mobilization of heavy metals through enhancing the phytochelatins, and thus reducing the toxicity caused by heavy metals.

reversible redox-based covalent addition of a NO moiety to the sulfhydryl group of cysteine (Cys) molecule(s) on a target protein to form S-nitrosothiols. Our group has recently reviewed a detailed account of phytohormonal regulation through S-nitrosylation under stress (Pande et al., 2022). However, in case of heavy metal stress this is still a potential and important line of inquiry in future.

NO donors used for alleviating heavy metal toxicity in plants

Exogenous application of NO is most commonly done by supplementing NO donors. Direct application of exogenous nitric oxide to plants is difficult due to its gaseous nature and requires specific equipment (Rodríguez-Ruiz et al., 2017). Moreover, a short half-life (<6 s) of NO makes it difficult to be supplied constantly at the tissue level (Seabra et al., 2015). Therefore, NO is mainly delivered through donor molecules (Wang et al., 2005; Barraud et al., 2009). The commonly used NO donors include SNP, diethylenetriamine NONOate (DETA NONOate), S-nitroso N-acetyl-DL-penicillamine (SNAP), diethylenetriamine/nitric oxide (DETANO), S-nitrosothiols (RSNO), S-nitrosocysteine (CysNO) and S-nitrosoglutathione (GSNO). Recently, a study reported the synthesis and application of N-nitrosomelatonin (NOMela) as a more efficient NO donor than GSNO in Arabidopsis seedlings (Singh et al., 2021a). However, specifically for heavy metal stress tolerance the most commonly used NO donor is SNP (Bothof et al., 2020), treated alone or in combination with other stress ameliorating agents as shown in Table 1.

However, due to the relatively unstable nature and susceptibility to decomposition by heat or light, the release of NO is uncontrolled, resulting in unpredictable and random signaling and other physiological effects (Seabra et al., 2015). This problem may be overcome by encapsulating the NO donor molecules of slow and consistent release. Therefore, NO-releasing nanoparticles may be considered as potential alternatives to unstable chemical NO donors.

Nanoparticles used for alleviating heavy metal toxicity in plants

Nanotechnological interventions in the field of agriculture have paved a way for attaining the long-term goal of sustainable agriculture by improving plant health and productivity under varying environmental conditions (Pande and Arora, 2019). The application and use of nanomaterials not only enhance plant growth and productivity but also help in mitigating biotic and abiotic stress in plants (Arora et al., 2012; Nayan et al., 2016; Bhatt et al., 2020; Zhou et al., 2021). Nanoparticles offer various advantages as compared to their macro counterparts, these include higher surface activity (more surface area available for reaction), enhanced catalytic efficiency, and unique optical and magnetic properties (Wang et al., 2019). Such unique properties add specialized functions to the nanoparticles making them effective in repairing the damage by soil remediation (Liu

TABLE 1 Studies using NO donors for mitigating heavy metal stress in plants.

NO donor	Plants	Outcome (stress alleviation)	Reference
SNP	*Glycine max*	Mitigation of mercury (Hg) stress.	(Ahmad et al., 2021b)
	Brassica juncea	Detoxification of Cd stress.	(Khator et al., 2021)
	Musa acuminata	Tolerance against osmotic stress.	(Amnan et al., 2021)
	Isatis cappadocica	Improved tolerance to As stress.	(Souri and Karimi, 2021)
	Vicia faba	Improved tolerance to As stress.	(Ahmad et al., 2020)
	Arachis hypogaea	Inhibition of programmed cell death by aluminum (Al)	(He et al., 2019)
	Oryza sativa	Modulation of As toxicity.	(Praveen and Gupta, 2018)
	Oryza sativa	Improvement in Ni tolerance.	(Rizwan et al., 2018)
	Solanum lycopersicum	Growth promotion under Cd stress.	(Ahmad et al., 2018)
	Spirodela intermedia	Alleviation of As stress.	(Da-Silva et al., 2018)
	Triticum aestivum	Amelioration of Pb toxicity.	(Kaur et al., 2015)
	Lolium perenne	Promotes growth under Pb toxicity.	(Bai et al., 2015)
	Pogonatherum crinitum	Controlled Pb uptake.	(Yu et al., 2012)
	Triticum aestivum	Mitigation of oxidative stress by enhancing the antioxidative defense response.	(Hasanuzzaman and Fujita, 2013)
	Arabidopsis thaliana	Prevention of Lead toxicity in seedlings but no effect on the accumulation	(Phang et al., 2011)
	Cucumis sativus	Alleviation of the adverse effects caused by Cd.	(Yu et al., 2013)
	Lupinus perennis L.	Mitigation of inhibitory effect of Ni.	(Hassanein et al., 2020)
	Brassica napus	Ameliorating Pb toxicity.	(Hamidi et al., 2020)
	Nasturtium officinale	Reduction in the adverse effects caused by As.	(Namdjoyan and Kermanian, 2013)
	Lactuca sativa var. *capitata*	Reduction in the adverse effects of Co.	(Samet, 2020)
	Lupinus luteus	Stimulation of germination and mitigation of inhibitory effects of Cd and Pb stress.	(Kopyra and Gwóźdź, 2003)
	Triticum aestivum	Enhancement of root growth under Ni stress.	(Wang et al., 2010b)
	Capsicum annum	Reduction in oxidative stress induced by Cd and Pb (applied alone or in combination).	(Kaya et al., 2019)
	Cicer arietinum	Reduction in accumulation, toxicity, and oxidative stress induced by Cd.	(Kumari et al., 2010)
	Typha angustifolia	Mitigation of Cd stress.	(Zhao et al., 2016)
	Oryza sativa	Decreased accumulation of Cd in roots.	(Xiong et al., 2009)
	Helianthus annuus	Protection of leaves against Cd-induced oxidative stress.	(Laspina et al., 2005)
	Lolium perenne	Mitigation of oxidative stress induced by Cd.	(Chen et al., 2018)
	Cassia tora L.	Significant reduction in Al-induced oxidative stress.	(Wang and Yang, 2005)
	Phaseolus Vulgaris	Tolerance to Al.	(Wang et al., 2010a)
	Oryza sativa	Reduced Cu toxicity and Cu-induced NH_4^+ accumulation and Cu toxicity.	(Yu et al., 2005)
	Solanum lycopersicum	Alleviation of Cu toxic effects.	(Cui et al., 2010)
	Triticum aestivum and *Phaseolus vulgaris*	Maintenance of Zn homeostasis.	(Abdel-Kader, 2007)
	Hibiscus moscheutos	Alleviation of inhibitory effects of Al on root elongation.	(Tian et al., 2007)
	Triticum aestivum	Alleviation of Cd-induced toxicity and alterations in biochemical factors in roots.	(Singh et al., 2008)
SNP + H_2O_2	*Glycine max*	amelioration of As toxicity.	(Singh et al., 2021b)
SNP+ Si	*Brassica juncea*	Mitigation of As stress.	(Ahmad et al., 2021a)
SNP+ Salicylic acid	*Eleusine coracana*	Protection from Ni stress.	(Kotapati et al., 2017)
SNP+ GSH	*Oryza sativa*	Mitigation of the adverse effects of Cu.	(Mostofa et al., 2015)

(Continued)

TABLE 1 Continued

NO donor	Plants	Outcome (stress alleviation)	Reference
SNP+ GSH	*Oryza sativa*	Decrease in oxidative stress induced by Cu by enhancing the antioxidative levels.	(Mostofa et al., 2014)
SNP+Auxin	*Oryza sativa*	Mitigation of the adverse effect of Cd stress.	(Piacentini et al., 2020a)
SNP+SA	*Carthamus tinctorius*	Decrease in adverse effects of Zn.	(Namdjoyan et al., 2018)
SNP+TiO_2 nanoparticles	*Triticum aestivum*	Alleviation of the adverse effects caused by Cd stress.	(Faraji et al., 2018)
SNP+Melatonin	*Catharanthus roseus*	Mitigation of Cd stress.	(Nabaei and Amooaghaie, 2019)
SNP+SA	*Zea mays*	Reduction in negative effects caused by Se.	(Naseem et al., 2020)
SNP ASC + $NaNO_2$ N-*tert*-butyl-α-phenylnitrone, 3-morpholinosydonimine (all are NO donors)	*Oryza sativa*	Reduction in $CdCl_2$ induced toxicity by reducing oxidative stress	(Hsu and Kao, 2004)

et al., 2021). The metal adsorption property of magnetite nanoparticles has been found to lower the accumulation of Cd and Na in rice plants (Sebastian et al., 2019). Nanoparticles also influence the formation of apoplastic barriers which suppresses the accumulation of heavy metals in the soil (Rossi et al., 2017). Nanoparticles are useful in mitigating heavy metals in various ways. For instance, they prevent the translocation of heavy metals by forming complexes with them which leads to their immobilization at inactive sites like vacuoles (Wang et al., 2021). These complexes also get adsorbed on the cell surfaces, restricting their movement and biological activity (Cui et al., 2017; Wang et al., 2021). Activation of enzymatic (superoxide dismutase (SOD), catalase (Cantrel et al., 2011), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX), and peroxidase (POD), and non-enzymatic (such as vitamin C, vitamin E, and polyphenols) anti-oxidative defense system is another strategy to cope with the toxicity caused by heavy metals (Zhou et al., 2021). However, a more effective strategy would be to combine the properties of nanoparticles with NO donors as this will have a more profound effect in combating heavy metal stress. In this context, the characteristics properties of nanoparticles like high permeability, film-forming ability, prolonged contact with the active ingredient, and high diffusion would add to the characteristic properties of NO donors for double protection against heavy metal stress.

Nitric-oxide releasing nanoparticles as potential alternatives to chemical NO donors in alleviating heavy metal toxicity in plants

The limitations associated with NO donors have led to the development of new biomaterials for the controlled and prolonged release of NO into biological systems including plants (Kim et al., 2014; Seabra et al., 2014). Evaluation of NO-releasing nanoparticles has recently begun as an alternative to chemical NO donors for various biomedical and agricultural purposes (Lopes-Oliveira et al., 2019; Ma et al., 2019; Liang et al., 2020; Pieretti and Seabra, 2020; Pelegrino et al., 2020; Pieroni, 2020; Ahmad et al., 2021a). Some of the NO-releasing nanoparticles used in biomedical research have been listed in Table 2 with their various applications in different plants.

A recent study reported the effect of free and nanoencapsulated nitric oxide donor, S-nitroso-mercaptosuccinic acid on neotropical tree seedlings, under field conditions. In this study, the donor molecule was coated with chitosan nanoparticles which protected the molecule from thermal and photochemical degradation. Their study suggested that depending on the tree species, seedling acclimation in the nursery was improved using these nanoencapsulated NO donors (Lopes-Oliveira et al., 2019). Besides, delivering NO is considered a promising approach in biomedical research and applications. The biomedical applications of NO-releasing nanoparticles suggest their importance and also provides insights into their adequacy in plant system as well. Therefore, based on the scientific knowledge we report that the application of NO-releasing nanoparticles is a useful approach to alleviate the detrimental effects of heavy metal stress on plants. The combined effects of nanoparticles and the stress mitigating properties of NO may provide an advantageous approach for combating heavy metal stress in plants. Figure 2, demonstrates the advantages of NO-releasing nanoparticles over chemical NO donors in alleviating heavy metal toxicity in plants.

Recent studies have synthesized NO-releasing nanoparticles for studying their effect in plants. NO-releasing nanoparticles are formulated by the addition of NO donor molecules in chitosan nanoparticles which encapsulates the NO donor for slow and prolonged release (Oliveira et al., 2016; Pelegrino et al., 2017a; Pelegrino et al., 2017b; Cabral et al., 2019). These have

TABLE 2 Recent examples of the advances in the applications of NO-releasing nanoparticles in agriculture and biomedical research.

S.NO.	NO-releasing Nanoparticle	Applied on	Outcome	Reference
1	Alginate/Chitosan (ratio 0.75) encapsulated with GSH	*Zea mays* *Glycine sp*	Sustained and controlled release of NO over several hours. Potentially useful as controlled release systems.	(Pereira et al., 2015)
2	Chitosan nanoparticle encapsulated with S-nitroso-mercaptosuccinic acid	*Zea mays*	Alleviation of salt stress	(Oliveira et al., 2016)
3	GSNO-loaded mineralized $CaCO_3$ nanoparticles	Human breast cancer cells, MCF-7	Improvement in therapeutic activity of doxorubicin.	(Lee et al., 2016)
4	Chitosan nanoparticle encapsulated with S-nitroso-mercaptosuccinic acid	*Heliocarpus popayanensis* *Cariniana estrellensis*	Improvement of seedling acclimation and protection of NO donor from thermal and photochemical degradation.	(Lopes-Oliveira et al., 2019)
5	Tetramethoxysilane derived hydrogel-based NO-releasing nanoparticles	Male, Balb/c mice	Reduction in the inflammatory response.	(Williams et al., 2020)
6	NO-releasing S-Nitrosoglutathione-Conjugated Poly (Lactic-Co-Glycolic Acid) Nanoparticles	Mice	Treatment of MRSA (methicillin-resistant staphylococcus aureus) infected cutaneous wounds.	(Lee et al., 2020)
7	Copper-based metal-organic framework as a controlled NO-releasing vehicle	Mice	Therapy for diabetic wounds	(Zhang et al., 2020)
8	Superparamagnetic iron oxide nanoparticles (SPIONS) based NO-releasing nanoparticles	Rat L2 epithelial cells	Reduction in the inflammatory response.	(Shurbaji et al., 2021)
9	NO-releasing chitosan nanoparticles	BALB/c mice	Treatment of cutaneous Leishmaniasis caused by *Leishmania amazonensis*	(Cabral et al., 2021)

also been tested for their efficacy in mitigating environmental stress in different plants (Oliveira et al., 2016; Lopes-Oliveira et al., 2019).

Other NO-releasing techniques used in biomedical research as alternate potential strategies for efficient NO-release in plants

NO is an important signaling molecule that has a significant role in biomedical research. In mammalian tissues, the kinetics and exposure time of NO are key determinants in its biological applications. However, its therapeutic applications are limited due to its extremely short half-life, aimless diffusion into the vasculature, and limited accumulation in the target tissues (Wang et al., 2020).

Traditionally, NO donors were used for NO-release in biomedical sciences which included various types of nitrates, N-diazeniumdiolates, Nitrosothiols, Furoxans, Metal nitrosyl compounds, and Nitrobenzenes (Yang et al., 2021). Nanotechnology has been recently employed for the delivery of NO. In such cases, the nanomaterial is usually degraded once absorbed into the system thereby releasing NO gas through NO donor. Though several different nanotech-based NO delivery platforms have been developed, some interesting studies carry significant potential for application in plant sciences. For example, Lee et al. (2016) described the pH-sensitive release of NO by $CaCO_3$ mineralized nanoparticles. This is specifically important in plant sciences as plant growth under basal conditions and soil-related stress conditions such as heavy metal stress, and salinity is significantly related to the pH of the soil. Therefore, such nanoparticle carriers can be engineered to release NO or NO donors at a specific soil pH or under a range of pH conditions. Jia et al. (2018) developed a redox-active nanosilicon-NO donor system that released NO only in response to over-accumulated GSH in tumor tissues. The same concept can be employed in plant sciences by developing nanomaterial-NO donor systems that release NO only in response to over-accumulated chemicals such as salts, heavy metal ions, phytohormones, secondary metabolites or other chemicals/ions in plant tissues. In addition, biomedical researchers developed nanomaterial-NO donors triggered by external cues such as light, heat, X-rays and ultrasound (Fan et al., 2015; Guo et al., 2017; Jin et al., 2017; Hotta et al., 2020; Zhou et al., 2020). Although their clinical applications are limited in mammals, we believe that these may prove to be highly useful in plant sciences. Sunlight is mandatory for photosynthesis. However, intense light for a longer duration and/or certain wavelengths of light are harmful to plant growth. Similarly, intense heat also limits plant growth and development. Nanomaterial-NO delivery systems that release NO under specific light and temperature conditions can be designed for target NO delivery. An alternate biomedical study also suggests the use of nanostructured CuO/SiO_2 catalysts for releasing NO by the catalytic decomposition of NO-releasing metabolites like GSNO (Kulyk et al., 2020). However, these nanoparticles are suggested to be useful in medical applications and their possibilities and applications need to be explored for agriculture purposes.

Besides, NO donor-conjugated chemical drugs were also designed with more sophisticated NO linkage, release position, selectivity, and amount of NO release. Chen et al. (2008) designed

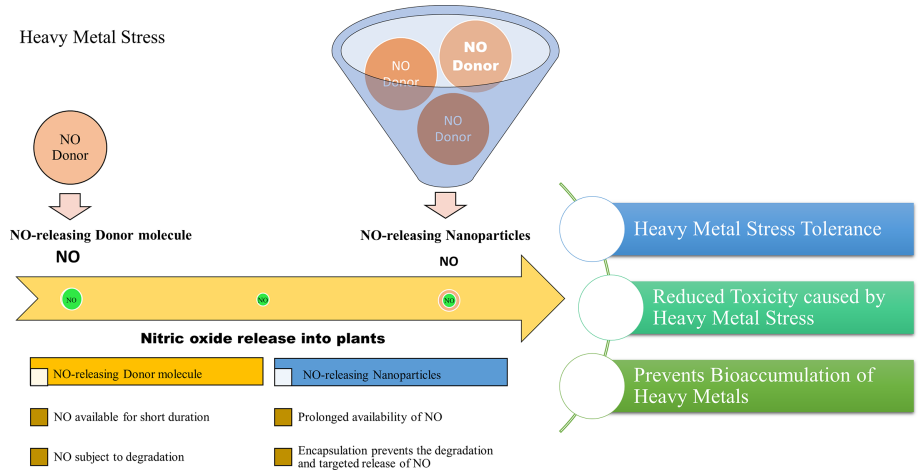


FIGURE 2
Projected advantages of NO-releasing nanoparticles in alleviating heavy metal toxicity in plants.

and synthesized multiple NO-releasing derivatives of oleanolic acid (NO-OA) with anti-hepatocellular carcinoma activity. To our knowledge, such types of conjugated NO-donors have not been tested in plants. Moreover, NO release strategies are mostly limited to a few NO donors only (as mentioned in the previous sections) therefore these can be utilized and tested in crop research as well.

The development of targeted prodrugs for gases like NO has been a special challenge in biomedical sciences that carries great prospects. Prodrugs are medications that, after administration, are metabolized and converted into a pharmacologically active drug within the body. Specific enzymes can activate NO prodrugs and release NO gas at specific sites, greatly reducing the side effects. Such types of targeted NO prodrugs developed so far are activated by glycosidases (Wu et al., 2001; Cai et al., 2004), cytochrome enzymes (Saavedra et al., 1997), oxidoreductases (Sharma et al., 2013a), esterases (Saavedra et al., 2000) and reductase enzymes (Sharma et al., 2013b) that trigger the release of NO. Interestingly, plants express a plethora of all these different enzymes in various types of tissues offering the possibility of using NO prodrugs in plant sciences.

Similarly, drug delivery systems based on monoclonal antibodies also offer high target specificity in mammals. Antibody/peptides conjugated NO donors have been widely used in cancer treatment (Sievers and Senter, 2013; Chari et al., 2014; Sun et al., 2019) offering significantly higher specificity and release of NO following the detection of target cells only (such as cancer cells) by the monoclonal antibodies. NO donors conjugated to monoclonal antibodies can be engineered for the targeted, specific, and safe release of NO in plant systems under various circumstances. Such monoclonal antibodies can be tailored to recognize specific fungal, bacterial, and viral peptides (during infection), receptor proteins for various phytohormones (for regulating plant development and responses to various abiotic stresses), and several other peptides with spatial and temporal expression profiles; for the targeted delivery of NO in plant systems. Figure 3 summarize the suggested NO-releasing techniques for potential application in plants.

Conclusions and future prospects

Heavy metals occur naturally in the earth's crust. They are often needed in very small amounts to carry out essential role in the metabolic systems of living organisms. However, natural calamities like weathering of rocks and volcanic eruptions, and other anthropogenic activities like mining and industrialization have largely overwhelmed their natural geochemical cycles (Nriagu and Pacyna, 1988). As a result, their concentration has increased in agricultural lands which negatively affects the growth and productivity of crops. To make things worse the application of chemical fertilizers containing heavy metals has further deteriorated the soil profile of agriculturally useful lands (Curtis and Smith, 2002).

Exposure of plant roots to heavy metals like cadmium (Cd), arsenic (As), lead (Pb), and copper (Cu), enhances endogenous levels of NO. NO is involved in various physiological and biochemical processes in plants that ensure optimal growth and development of plants exposed to various environmental stress conditions. Exogenous application of NO in the form of NO donors has been reported to lower oxidative stress by enhancing the activity of antioxidative enzymes under various environmental stress conditions. However, the use of NO donors is disadvantageous due to the short half-life of NO and its degradation by heat and light. A potential strategy is to use nanoparticles as encapsulating agents to effectively release NO in the plants for their optimal growth in heavy metal contaminated soils. Furthermore, to alleviate the toxicity caused by heavy

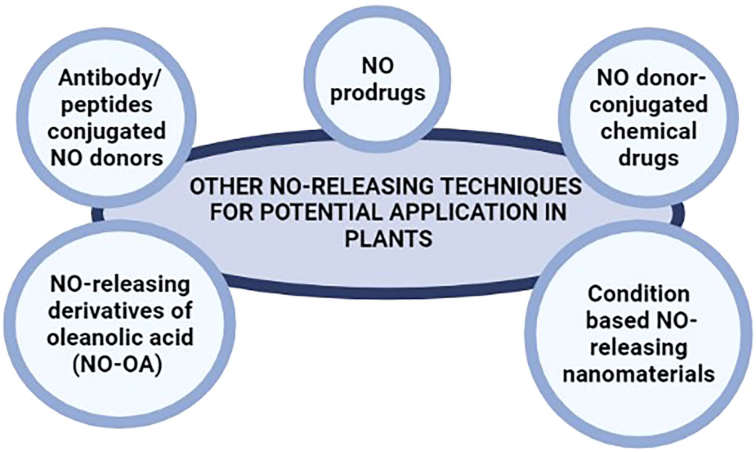


FIGURE 3
NO-releasing techniques used in biomedical research that may be potential alternatives to NO donors used in plant.

metals in plants, NO needs to be delivered efficiently and for a prolonged duration. Therefore, conjugating them with the right nanoparticle is an important consideration. In this context, chitosan nanoparticles are suggested to be the most suitable candidates for this purpose owing to their unique properties such as biodegradable and biocompatible nature. Therefore, these nanoparticles need to be tested for their role in mitigating heavy metal stress in plants to sustain agricultural productivity. In conclusion, any NO-releasing technique that promises prolonged and efficient delivery of NO in an eco-friendly manner has the potential of alleviating heavy metal toxicity in plants.

Author contributions

AP: conceptualization, visualization, writing - original draft. B-GM: project administration. NM: resources. WR: investigation. D-SL: visualization. GML resources. JH: writing - review and editing. AH: writing - review and editing. GL: final review and editing. B-WY: supervision, funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abdel-Kader, D. Z. E.-A. (2007). Role of nitric oxide, glutathione and sulfhydryl groups in zinc homeostasis in plants. *Am. J. Plant Physiol.* 2, 59–75. doi: 10.3923/ajpp.2007.59.75

Ahmad, P., Ahanger, M. A., Alyemeni, M. N., Wijaya, L., and Alam, P. (2018). Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. *Protoplasma.* 255, 79–93. doi: 10.1007/s00709-017-1132-x

Ahmad, P., Alam, P., Balawi, T. H., Altalayan, F. H., Ahanger, M. A., and Ashraf, M. (2020). Sodium nitroprusside (SNP) improves tolerance to arsenic (As) toxicity in vicia faba through the modifications of biochemical attributes, antioxidants, ascorbate-glutathione cycle and glyoxalase cycle. *Chemosphere.* 244, 125480. doi: 10.1016/j.chemosphere.2019.125480

Ahmad, P., Alyemeni, M. N., Wijaya, L., Ahanger, M. A., Ashraf, M., Alam, P., et al. (2021b). Nitric oxide donor, sodium nitroprusside, mitigates mercury toxicity in different cultivars of soybean. *J. Hazardous Materials.* 408, 124852. doi: 10.1016/j.jhazmat.2020.124852

Ahmad, A., Khan, W. U., Ali Shah, A., Yasin, N. A., Naz, S., Ali, A., et al. (2021a). Synergistic effects of nitric oxide and silicon on promoting plant growth, oxidative stress tolerance and reduction of arsenic uptake in brassica juncea. *Chemosphere.* 262, 128384. doi: 10.1016/j.chemosphere.2020.128384

Amnan, M. A. M., Pua, T.-L., Lau, S.-E., Tan, B. C., Yamaguchi, H., Hitachi, K., et al. (2021). Osmotic stress in banana is relieved by exogenous nitric oxide. *PeerJ.* 9, e10879. doi: 10.7717/peerj.10879

Arora, S., Sharma, P., Kumar, S., Nayan, R., Khanna, P., and Zaidi, M. (2012). Gold-nanoparticle induced enhancement in growth and seed yield of brassica juncea. *Plant Growth Regul.* 66, 303–310. doi: 10.1007/s10725-011-9649-z

Bai, X. Y., Dong, Y. J., Wang, Q. H., Xu, L. L., Kong, J., and Liu, S. (2015). Effects of lead and nitric oxide on photosynthesis, antioxidative ability, and mineral element content of perennial ryegrass. *Biol. Plantarum.* 59, 163–170. doi: 10.1007/s10535-014-0476-8

Barraud, N., Storey, M. V., Moore, Z. P., Webb, J. S., Rice, S. A., and Kjelleberg, S. (2009). Nitric oxide-mediated dispersal in single-and multi-species biofilms of clinically and industrially relevant microorganisms. *Microbial Biotechnol.* 2, 370–378. doi: 10.1111/j.1751-7915.2009.00098.x

Begara-Morales, J. C., Chaki, M., Valderrama, R., Mata-Pérez, C., Padilla, M. N., and Barroso, J. B. (2019). The function of s-nitrosothiols during abiotic stress in plants. *J. Exp. Bot.* 70, 4429–4439. doi: 10.1093/jxb/erz197

Bhatt, D., Bhatt, M. D., Nath, M., Dudhat, R., Sharma, M., and Bisht, D. S. (2020). Application of nanoparticles in overcoming different environmental stresses. *Protective Chem. Agents Amelioration Plant Abiotic Stress: Biochem. Mol. Perspect.*, 635–654. doi: 10.1002/9781119552154.ch32

Bothof, G., Van Rhee, K. P., Koomen, E., and Veldhoen, E. S. (2020). Change in national dosing advice of nitroprusside after potentially fatal cyanide intoxication. *SN Compr. Clin. Med.* 2, 522–525. doi: 10.1007/s42399-020-00261-9

Cabral, F. V., Pelegrino, M. T., Sauter, I. P., Seabra, A. B., Cortez, M., and Ribeiro, M. S. (2019). Nitric oxide-loaded chitosan nanoparticles as an innovative antileishmanial platform. *Nitric. Oxide.* 93, 25–33. doi: 10.1016/j.niox.2019.09.007

Cabral, F. V., Pelegrino, M. T., Seabra, A. B., and Ribeiro, M. S. (2021). Nitric-oxide releasing chitosan nanoparticles towards effective treatment of cutaneous leishmaniasis. *Nitric. Oxide.* 113, 31–38. doi: 10.1016/j.niox.2021.04.008

Cai, T. B., Lu, D., Landerholm, M., and Wang, P. G. (2004). Sialated diazeniumdiolate: A new sialidase-activated nitric oxide donor. *Organic Lett.* 6, 4203–4205. doi: 10.1021/ol048397p

Cantrel, C., Vazquez, T., Puyaubert, J., Rezé, N., Lesch, M., Kaiser, W. M., et al. (2011). Nitric oxide participates in cold-responsive phosphosphingolipid formation and gene expression in arabidopsis thaliana. *N. Phytol.* 189, 415–427. doi: 10.1111/j.1469-8137.2010.03500.x

Chari, R. V. J., Miller, M. L., and Widdison, W. C. (2014). Antibody–drug conjugates: An emerging concept in cancer therapy. *Angewandte Chemie In. Ed.* 53, 3796–3827. doi: 10.1002/anie.201307628

Chen, L., Beiyuan, J., Hu, W., Zhang, Z., Duan, C., Cui, Q., et al. (2022a). Phytoremediation of potentially toxic elements (PTEs) contaminated soils using alfalfa (Medicago sativa l.): A comprehensive review. *Chemosphere.* 293, 133577. doi: 10.1016/j.chemosphere.2022.133577

Chen, W., Dong, Y., Hu, G., and Bai, X. (2018). Effects of exogenous nitric oxide on cadmium toxicity and antioxidative system in perennial ryegrass. *J. Soil Sci. Plant Nutr.* 18, 129–143. doi: 10.4067/S0718-95162018005000601

Chen, L., Zhang, Y., Kong, X., Lan, E., Huang, Z., Peng, S., et al. (2008). Design, synthesis, and antihepatocellular carcinoma activity of nitric oxide releasing derivatives of oleanolic acid. *J. Medicinal Chem.* 51, 4834–4838. doi: 10.1021/jm800167u

Chen, L., Zhou, M., Wang, J., Zhiqin, Z., Chengjiao, D., Xiangxiang, W., et al. (2022b). A global meta-analysis of heavy metal (loid) s pollution in soils near copper mines: Evaluation of pollution level and probabilistic health risks. *Sci. Total Environ.* 835, 155441. doi: 10.1016/j.scitotenv.2022.155441

Corpas, F. J., and Palma, J. M. (2020). Assessing nitric oxide (NO) in higher plants: an outline. *Nitrogen.* 1, 12–20. doi: 10.3390/nitrogen1010003

Cui, J., Liu, T., Li, F., Yi, J., Liu, C., and Yu, H. (2017). Silica nanoparticles alleviate cadmium toxicity in rice cells: Mechanisms and size effects. *Environ. Pollut.* 228, 363–369. doi: 10.1016/j.envpol.2017.05.014

Cui, X., Zhang, Y., Wu, X., and Liu, C. (2010). The investigation of the alleviated effect of copper toxicity by exogenous nitric oxide in tomato plants. *Plant Soil Environ.* 56, 274–281. doi: 10.17221/98/2009-PSE

Curtis, L. R., and Smith, B. W. (2002). *Heavy metal in fertilizers: Considerations for setting regulations in Oregon*. Oregon: Department of Environmental and Molecular Toxicology Oregon State University Corvallis.

Da-Silva, C. J., Canatto, R. A., Cardoso, A. A., Ribeiro, C., and De Oliveira, J. A. (2018). Oxidative stress triggered by arsenic in a tropical macrophyte is alleviated by endogenous and exogenous nitric oxide. *Braz. J. Bot.* 41, 21–28. doi: 10.1007/s40415-017-0431-y

Demecsová, L., and Tamás, L. (2019). Reactive oxygen species, auxin and nitric oxide in metal-stressed roots: Toxicity or defence. *BioMetals.* 32, 717–744. doi: 10.1007/s10534-019-00214-3

Emurotu, J., and Onianwa, P. (2017). Bioaccumulation of heavy metals in soil and selected food crops cultivated in Kogi State, North Central Nigeria. *Environ. Syst. Res.* 6, 1–9. doi: 10.1186/s40068-017-0098-1

Fan, W., Bu, W., Zhang, Z., Shen, B., Zhang, H., He, Q., et al. (2015). X-Ray radiation-controlled NO-release for on-demand depth-independent hypoxic radiosensitization. *Angewandte Chemie In. Ed.* 54, 14026–14030. doi: 10.1002/anie.201504536

Faraji, J., Sepehri, A., and Salcedo-Reyes, J. C. (2018). Titanium dioxide nanoparticles and sodium nitroprusside alleviate the adverse effects of cadmium stress on germination and seedling growth of wheat (Triticum aestivum l.). *Universitas Scientiarum.* 23, 61–87. doi: 10.11144/Javeriana.SC23-1.tdna

Gill, S. S., Hasanuzzaman, M., Nahar, K., Macovei, A., and Tuteja, N. (2013). Importance of nitric oxide in cadmium stress tolerance in crop plants. *Plant Physiol. Biochem.* 63, 254–261. doi: 10.1016/j.plaphy.2012.12.001

Graziano, M., and Lamattina, L. (2007). Nitric oxide accumulation is required for molecular and physiological responses to iron deficiency in tomato roots. *Plant J.* 52, 949–960. doi: 10.1111/j.1365-313X.2007.03283.x

Groß, F., Durner, J., and Gaupels, F. (2013). Nitric oxide, antioxidants and prooxidants in plant defence responses. *Front. Plant Sci.* 4, 419. doi: 10.3389/fpls.2013.00419

Guo, R., Tian, Y., Wang, Y., and Yang, W. (2017). Near-infrared laser-triggered nitric oxide nanogenerators for the reversal of multidrug resistance in cance. *Advanced Functional Mat.* 27, 1606398. doi: 10.1002/adfm.201606398

Hamidi, H., Masoudian, N., Ebadi, M., and Roudi, B. (2020). Relationship of sodium nitroprusside with growth and antioxidant enzymes of canola under lead stress. *J. Chem. Health Risks.* 10, 57–65. doi: 10.22034/jchr.2020.1863574.1020

Hasanuzzaman, M., and Fujita, M. (2013). Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (Triticum aestivum l.) Seedlings by enhancing antioxidant defense and glyoxalase system. *Ecotoxicology.* 22, 584–596. doi: 10.1007/s10646-013-1050-4

Hashem, H. A., Hassanein, A. A., and Esmail, N. Y. (2018). Nitric oxide enhances the adaptive responses of lupine plants against heavy-metal stress. *Aust. J. Crop Sci.* 12, 1962. doi: 10.21475/ajcs.18.12.12.p1379

Hassanein, A., Esmail, N., and Hashem, H. (2020). Sodium nitroprusside mitigates the inhibitory effect of salt and heavy metal stress on lupine yield and downregulates antioxidant enzyme activities. *Acta Agrobotanica.* 73, 7336–7351. doi: 10.5586/aa.7336

He, H., Oo, T. L., Huang, W., He, L.-F., and Gu, M. (2019). Nitric oxide acts as an antioxidant and inhibits programmed cell death induced by aluminum in the root tips of peanut (Arachis hypogaea l.). *Sci. Rep.* 9, 9516. doi: 10.1038/s41598-019-46036-8

Hotta, Y., Kataoka, T., Mori, T., and Kimura, K. (2020). Review of a potential novel approach for erectile dysfunction: Light-controllable nitric oxide donors and nanoformulations. *Sex Med. Rev.* 8, 297–302. doi: 10.1016/j.sxmr.2019.05.006

Hsu, Y. T., and Kao, C. H. (2004). Cadmium toxicity is reduced by nitric oxide in rice leaves. *Plant Growth Regul.* 42, 227–238. doi: 10.1023/B:GROW.0000026514.98385.5c

Imran, Q. M., Falak, N., Hussain, A., Mun, B.-G., Sharma, A., Lee, S.-U., et al. (2016). Nitric oxide responsive heavy metal-associated gene AtHMAD1 contributes to development and disease resistance in arabidopsis thaliana. *Front. Plant Sci.* 7, 1712. doi: 10.3389/fpls.2016.01712

Jia, X., Zhang, Y., Zou, Y., Wang, Y., Niu, D., He, Q., et al. (2018). Dual intratumoral Redox/Enzyme-responsive NO-releasing nanomedicine for the specific, high-efficacy, and low-toxic cancer therapy. *Advanced Mats.* 30, 1704490. doi: 10.1002/adma.201704490

Jin, Z., Wen, Y., Hu, Y., Chen, W., Zheng, X., Guo, W., et al. (2017). MRI-Guided and ultrasound-triggered release of NO by advanced nanomedicine. *Nanoscale.* 9, 3637–3645. doi: 10.1039/C7NR00231A

Kaur, G., Singh, H. P., Batish, D. R., Mahajan, P., Kohli, R. K., and Rishi, V. (2015). Exogenous nitric oxide (NO) interferes with lead (Pb)-induced toxicity by detoxifying reactive oxygen species in hydroponically grown wheat (Triticum aestivum) roots. *PloS One.* 10, e0138713. doi: 10.1371/journal.pone.0138713

Kaya, C., Akram, N. A., Sürücü, A., and Ashraf, M. (2019). Alleviating effect of nitric oxide on oxidative stress and antioxidant defence system in pepper (Capsicum annuum l.) plants exposed to cadmium and lead toxicity applied separately or in combination. *Scientia Hortic.* 255, 52–60. doi: 10.1016/j.scienta.2019.05.029

Khator, K., Saxena, I., and Shekhawat, G. S. (2021). Nitric oxide induced cd tolerance and phytoremediation potential of b. juncea by the modulation of antioxidant defense system and ROS detoxification. *Biometals.* 34, 15–32. doi: 10.1007/s10534-020-00259-9

Kim, J., Saravanakumar, G., Choi, H. W., Park, D., and Kim, W. J. (2014). A platform for nitric oxide delivery. *J. Materials Chem. B.* 2, 341–356. doi: 10.1039/C3TB21259A

Kopyra, M., and Gwóźdź, E. A. (2003). Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of lupinus luteus. *Plant Physiol. Biochem.* 41, 1011–1017. doi: 10.1016/j.plaphy.2003.09.003

Kotapati, K. V., Palaka, B. K., and Ampasala, D. R. (2017). Alleviation of nickel toxicity in finger millet (Eleusine coracana l.) germinating seedlings by exogenous application of salicylic acid and nitric oxide. *Crop J.* 5, 240–250. doi: 10.1016/j.cj.2016.09.002

Kulyk, K., Azizova, L., Cunningham, J. M., Mikhalovska, L., Borysenko, M., and Mikhalovsky, S. (2020). Nanosized copper(ii) oxide/silica for catalytic generation of nitric oxide from s-. *nitrosothiols. J. Materials Chem. B* 8, 4267–4277. doi: 10.1039/D0TB00137F

Kumari, A., Sheokand, S., and Swaraj, K. (2010). Nitric oxide induced alleviation of toxic effects of short term and long term cd stress on growth, oxidative metabolism and cd accumulation in chickpea. *Braz. J. Plant Physiol.* 22, 271–284. doi: 10.1590/S1677-04202010000400007

Laspina, N., Groppa, M., Tomaro, M., and Benavides, M. (2005). Nitric oxide protects sunflower leaves against cd-induced oxidative stress. *Plant Sci.* 169, 323–330. doi: 10.1016/j.plantsci.2005.02.007

Lee, H. J., Kim Da, E., Park, D. J., Choi, G. H., Yang, D. N., Heo, J. S., et al. (2016). pH-responsive mineralized nanoparticles as stable nanocarriers for intracellular nitric oxide delivery. *Colloids Surf B Biointerfaces.* 146, 1–8. doi: 10.1016/j.colsurfb.2016.05.039

Lee, J., Kwak, D., Kim, H., Kim, J., Hlaing, S. P., Hasan, N., et al. (2020). Nitric oxide-releasing s-Nitrosoglutathione-Conjugated Poly(Lactic-Co-Glycolic acid) nanoparticles for the treatment of MRSA-infected cutaneous wounds. *Pharmaceutics.* 12, 618–631. doi: 10.3390/pharmaceutics12070618

Liang, H., Li, Z., Ren, Z., Jia, Q., Guo, L., Li, S., et al. (2020). Light-triggered NO-releasing nanoparticles for treating mice with liver fibrosis. *Nano Res.* 13, 2197–2202. doi: 10.1007/s12274-020-2833-6

Li, S.-W., Li, Y., Leng, Y., Zeng, X.-Y., and Ma, Y.-H. (2019). Nitric oxide donor improves adventitious rooting in mung bean hypocotyl cuttings exposed to cadmium and osmotic stresses. *Environ. Exp. Bot.* 164, 114–123. doi: 10.1016/j.envexpbot.2019.05.004

Liu, Y., Wu, T., White, J. C., and Lin, D. (2021). A new strategy using nanoscale zero-valent iron to simultaneously promote remediation and safe crop production in contaminated soil. *Nat. Nanotechnology.* 16, 197–205. doi: 10.1038/s41565-020-00803-1

Loix, C., Huybrechts, M., Vangronsveld, J., Gielen, M., Keunen, E., and Cuypers, A. (2017). Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. *Front. Plant Sci.* 8, 1867. doi: 10.3389/fpls.2017.01867

Lombardo, M. C., Graziano, M., Polacco, J. C., and Lamattina, L. (2006). Nitric oxide functions as a positive regulator of root hair development. *Plant Signaling Behav.* 1, 28–33. doi: 10.4161/psb.1.1.2398

Lopes-Oliveira, P. J., Gomes, D. G., Pelegrino, M. T., Bianchini, E., Pimenta, J. A., Stolf-Moreira, R., et al. (2019). Effects of nitric oxide-releasing nanoparticles on neotropical tree seedlings submitted to acclimation under full sun in the nursery. *Sci. Rep.* 9, 17371. doi: 10.1038/s41598-019-54030-3

Ma, Y., Fu, L., Hussain, Z., Huang, D., and Zhu, S. (2019). Enhancement of storability and antioxidant systems of sweet cherry fruit by nitric oxide-releasing chitosan nanoparticles (GSNO-CS NPs). *Food Chem.* 285, 10–21. doi: 10.1016/j.foodchem.2019.01.156

Mohanpuria, P., Rana, N. K., and Yadav, S. K. (2007). Cadmium induced oxidative stress influence on glutathione metabolic genes of camellia sinensis (L.) o. kuntze. *Environ. Toxicology: Int. J.* 22, 368–374. doi: 10.1002/tox.20273

Mostofa, M. G., Seraj, Z. I., and Fujita, M. (2014). Exogenous sodium nitroprusside and glutathione alleviate copper toxicity by reducing copper uptake and oxidative damage in rice (Oryza sativa l.) seedlings. *Protoplasma.* 251, 1373–1386. doi: 10.1007/s00709-014-0639-7

Mostofa, M. G., Seraj, Z. I., and Fujita, M. (2015). Interactive effects of nitric oxide and glutathione in mitigating copper toxicity of rice (Oryza sativa l.) seedlings. *Plant Signaling Behav.* 10, e991570. doi: 10.4161/15592324.2014.991570

Nabaei, M., and Amooaghaie, R. (2019). Interactive effect of melatonin and sodium nitroprusside on seed germination and seedling growth of catharanthus roseus under cadmium stress. *Russian J. Plant Physiol.* 66, 128–139. doi: 10.1134/S1021443719010126

Nabi, R. B. S., Tayade, R., Hussain, A., Kulkarni, K. P., Imran, Q. M., Mun, B.-G., et al. (2019). Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. *Environ. Exp. Bot.* 161, 120–133. doi: 10.1016/j.envexpbot.2019.02.003

Namdjoyan, S., and Kermanian, H. (2013). Exogenous nitric oxide (as sodium nitroprusside) ameliorates arsenic-induced oxidative stress in watercress (Nasturtium officinale r. br.) plants. *Scientia Hortic.* 161, 350–356. doi: 10.1016/j.scienta.2013.07.035

Namdjoyan, S., Kermanian, H., Soorki, A. A., Tabatabaei, S. M., and Elyasi, N. (2018). Effects of exogenous salicylic acid and sodium nitroprusside on α-tocopherol and phytochelatin biosynthesis in zinc-stressed safflower plants. *Turkish J. Bot.* 42, 271–279. doi: 10.3906/bot-1707-48

Naseem, M., Anwar-Ul-Haq, M., Akhtar, J., and Jaskani, M. J. (2020). Effect of exogenous application of salicylic acid and sodium nitroprusside on maize under selenium stress. *Pakistan J. Agric. Sci.* 57, 150–158. doi: 10.21162/PAKJAS/20.8562

Nayan, R., Rawat, M., Negi, B., Pande, A., and Arora, S. (2016). Zinc sulfide nanoparticle mediated alterations in growth and anti-oxidant status of brassica juncea. *Biologia.* 71, 896–902. doi: 10.1515/biolog-2016-0107

Norris, J., and Hume, A. (1987). *In vivo* release of cyanide from sodium nitroprusside. *Br. J. anaesthesia.* 59, 236–239. doi: 10.1093/bja/59.2.236

Nriagu, J. O., and Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *nature.* 333, 134–139. doi: 10.1038/333134a0

Oliveira, H. C., Gomes, B. C. R., Pelegrino, M. T., and Seabra, A. B. (2016). Nitric oxide-releasing chitosan nanoparticles alleviate the effects of salt stress in maize plants. *Nitric. Oxide.* 61, 10–19. doi: 10.1016/j.niox.2016.09.010

Pande, A., and Arora, S. (2019). "Nanotechnological interventions for improving plant health and productivity," in *Plant biotechnology: Progress in genomic era* (Singapore: Springer).

Pande, A., Mun, B.-G., Lee, D.-S., Khan, M., Lee, G.-M., Hussain, A., et al. (2021). NO network for plant–microbe communication underground: A review. *Front. Plant Sci.* 12, 431. doi: 10.3389/fpls.2021.658679

Pande, A., Mun, B. G., Rahim, W., Khan, M., Lee, D. S., Lee, G. M., et al. (2022). Phytohormonal regulation through protein s-nitrosylation under stress. *Front. Plant Sci.* 13, 865542–865542. doi: 10.3389/fpls.2022.865542

Pelegrino, M. T., Kohatsu, M. Y., Seabra, A. B., Monteiro, L. R., Gomes, D. G., Oliveira, H. C., et al. (2020). Effects of copper oxide nanoparticles on growth of lettuce (Lactuca sativa l.) seedlings and possible implications of nitric oxide in their antioxidative defense. *Environ. Monit. Assess.* 192, 1–14. doi: 10.1007/s10661-020-8188-3

Pelegrino, M. T., Silva, L. C., Watashi, C. M., Haddad, P. S., Rodrigues, T., and Seabra, A. B. (2017b). Nitric oxide-releasing nanoparticles: Synthesis, characterization, and cytotoxicity to tumorigenic cells. *J. Nanoparticle Res.* 19, 57. doi: 10.1007/s11051-017-3747-4

Pelegrino, M., Weller, R., Chen, X., Bernardes, J., and Seabra, A. B. (2017a). Chitosan nanoparticles for nitric oxide delivery in human skin. *MedChemComm.* 8, 713–719. doi: 10.1039/C6MD00502K

Pereira, A. E. S., Narciso, A. M., Seabra, A. B., and Fraceto, L. F. (2015). Evaluation of the effects of nitric oxide-releasing nanoparticles on plants. *J. Physics: Conf. Ser.* 617, 012025. doi: 10.1088/1742-6596/617/1/012025

Phang, C., Leung, D. W., Taylor, H. H., and Burritt, D. J. (2011). The protective effect of sodium nitroprusside (SNP) treatment on arabidopsis thaliana seedlings exposed to toxic level of Pb is not linked to avoidance of Pb uptake. *Ecotoxicology Environ. Saf.* 74, 1310–1315. doi: 10.1016/j.ecoenv.2011.02.006

Piacentini, D., Della Rovere, F., Sofo, A., Fattorini, L., Falasca, G., and Altamura, M. M. (2020a). Nitric oxide cooperates with auxin to mitigate the alterations in the

root system caused by cadmium and arsenic. *Front. Plant Sci.* 11, 1182. doi: 10.3389/fpls.2020.01182

Piacentini, D., Ronzan, M., Fattorini, L., Della Rovere, F., Massimi, L., Altamura, M., et al. (2020b). Nitric oxide alleviates cadmium-but not arsenic-induced damages in rice roots. *Plant Physiol. Biochem.* 151, 729–742. doi: 10.1016/j.plaphy.2020.04.004

Pieretti, J. C., and Seabra, A. B. (2020). "Nitric oxide-releasing nanomaterials and skin infections," in *Nanotechnology in skin, soft tissue, and bone infections* (Cham: Springer).

Pieroni, M. (2020). Nitric oxide-releasing cyclodextrins as biodegradable antibacterial scaffolds: A patent evaluation of US2019343869 (A1). *Expert Opin. Ther. Patents.* 30, 1–5. doi: 10.1080/13543776.2020.1822328

Praveen, A., and Gupta, M. (2018). Nitric oxide confronts arsenic stimulated oxidative stress and root architecture through distinct gene expression of auxin transporters, nutrient related genes and modulates biochemical responses in oryza sativa l. *Environ. Pollut.* 240, 950–962. doi: 10.1016/j.envpol.2018.04.096

Rizwan, M., Mostofa, M. G., Ahmad, M. Z., Imtiaz, M., Mehmood, S., Adeel, M., et al. (2018). Nitric oxide induces rice tolerance to excessive nickel by regulating nickel uptake, reactive oxygen species detoxification and defense-related gene expression. *Chemosphere.* 191, 23–35. doi: 10.1016/j.chemosphere.2017.09.068

Rodríguez-Ruiz, M., Mateos, R. M., Codesido, V., Corpas, F. J., and Palma, J. M. (2017). Characterization of the galactono-1,4-lactone dehydrogenase from pepper fruits and its modulation in the ascorbate biosynthesis. Role of nitric oxide. *Redox Biol.* 12, 171–181. doi: 10.1016/j.redox.2017.02.009

Romero-Puertas, M. C., Laxa, M., Matte, A., Zaninotto, F., Finkemeier, I., Jones, A. M., et al. (2007). S-nitrosylation of peroxiredoxin II e promotes peroxynitrite-mediated tyrosine nitration. *Plant Cell.* 19, 4120–4130. doi: 10.1105/tpc.107.055061

Romero-Puertas, M. C., Terrón-Camero, L. C., Peláez-Vico, M. Á., Olmedilla, A., and Sandalio, L. M. (2019). Reactive oxygen and nitrogen species as key indicators of plant responses to cd stress. *Environ. Exp. Bot.* 161, 107–119. doi: 10.1016/j.envexpbot.2018.10.012

Rossi, L., Zhang, W., Schwab, A. P., and Ma, X. (2017). Uptake, accumulation, and in planta distribution of coexisting cerium oxide nanoparticles and cadmium in glycine max (L.) merr . *Environ. Sci. Technol.* 51, 12815–12824. doi: 10.1021/acs.est.7b03363

Saavedra, J. E., Billiar, T. R., Williams, D. L., Kim, Y.-M., Watkins, S. C., and Keefer, L. K. (1997). Targeting nitric oxide (NO) delivery *in vivo*. Design of a liver-selective NO donor prodrug that blocks tumor necrosis factor-α-Induced apoptosis and toxicity in the liver. *J. Medicinal Chem.* 40, 1947–1954. doi: 10.1021/jm9701031

Saavedra, J. E., Shami, P. J., Wang, L. Y., Davies, K. M., Booth, M. N., Citro, M. L., et al. (2000). Esterase-sensitive nitric oxide donors of the diazeniumdiolate family: *In vitro* antileukemic activity. *J. Medicinal Chem.* 43, 261–269. doi: 10.1021/jm9903850

Sadeghipour, O. (2017). Nitric oxide increases Pb tolerance by lowering Pb uptake and translocation as well as phytohormonal changes in cowpea (Vigna unguiculata (L.) walp.). *Sains Malaysiana.* 46, 189–195. doi: 10.17576/jsm-2017-4602-02

Samet, H. (2020). Alleviation of cobalt stress by exogenous sodium nitroprusside in iceberg lettuce. *Chilean J. Agric. Res.* 80, 161–170. doi: 10.4067/S0718-58392020000200161

Saxena, I., and Shekhawat, G. (2013). Nitric oxide (NO) in alleviation of heavy metal induced phytotoxicity and its role in protein nitration. *Nitric. Oxide.* 32, 13–20. doi: 10.1016/j.niox.2013.03.004

Seabra, A. B., and Durán, N. (2010). Nitric oxide-releasing vehicles for biomedical applications. *J. Of Materials Chem.* 20, 1624–1637. doi: 10.1039/B912493B

Seabra, A. B., Justo, G. Z., and Haddad, P. S. (2015). State of the art, challenges and perspectives in the design of nitric oxide-releasing polymeric nanomaterials for biomedical applications. *Biotechnol. Adv.* 33, 1370–1379. doi: 10.1016/j.biotechadv.2015.01.005

Seabra, A. B., Rai, M., and Durán, N. (2014). Nano carriers for nitric oxide delivery and its potential applications in plant physiological process: A mini review. *J. Plant Biochem. Biotechnol.* 23, 1–10. doi: 10.1007/s13562-013-0204-z

Sebastian, A., Nangia, A., and Prasad, M. N. V. (2019). Cadmium and sodium adsorption properties of magnetite nanoparticles synthesized from hevea brasiliensis muell. arg. bark: Relevance in amelioration of metal stress in rice. *J. Hazardous Materials.* 371, 261–272. doi: 10.1016/j.jhazmat.2019.03.021

Sharma, S. S., and Dietz, K.-J. (2009). The relationship between metal toxicity and cellular redox imbalance. *Trends Plant Sci.* 14, 43–50. doi: 10.1016/j.tplants.2008.10.007

Sharma, K., Iyer, A., Sengupta, K., and Chakrapani, H. (2013a). INDQ/NO, a bioreductively activated nitric oxide prodrug. *Organic Lett.* 15, 2636–2639. doi: 10.1021/ol400884v

Sharma, K., Sengupta, K., and Chakrapani, H. (2013b). Nitroreductase-activated nitric oxide (NO) prodrugs. *Bioorganic Medicinal Chem. Lett.* 23, 5964–5967. doi: 10.1016/j.bmcl.2013.08.066

Sharma, A., Soares, C., Sousa, B., Martins, M., Kumar, V., Shahzad, B., et al. (2020). Nitric oxide-mediated regulation of oxidative stress in plants under metal stress: a review on molecular and biochemical aspects. *Physiologia plantarum* 168, 318–344. doi: 10.1111/ppl.13004

Shurbaji, S., El-Sherbiny, I. M., Alser, M., Ali, I. H., Kordi, H., Al-Sadi, A., et al. (2021). Nitric oxide releasing hydrogel nanoparticles decreases epithelial cell injuries associated with airway reopening. *Front. bioengineering Biotechnol.* 8, 579788–579788. doi: 10.3389/fbioe.2020.579788

Sievers, E. L., and Senter, P. D. (2013). Antibody-drug conjugates in cancer therapy. *Annual Rev. Med.* 64, 15–29. doi: 10.1146/annurev-med-050311-201823

Simontacchi, M., Galatro, A., Ramos-Artuso, F., and Santa-María, G. E. (2015). Plant survival in a changing environment: the role of nitric oxide in plant responses to abiotic stress. *Front. Plant Sci.* 6, 977. doi: 10.3389/fpls.2015.00977

Singh, H. P., Batish, D. R., Kaur, G., Arora, K., and Kohli, R. K. (2008). Nitric oxide (as sodium nitroprusside) supplementation ameliorates cd toxicity in hydroponically grown wheat roots. *Environ. Exp. Bot.* 63, 158–167. doi: 10.1016/j.envexpbot.2007.12.005

Singh, A. P., Dixit, G., Kumar, A., Mishra, S., Singh, P. K., Dwivedi, S., et al. (2016). Nitric oxide alleviated arsenic toxicity by modulation of antioxidants and thiol metabolism in rice (Oryza sativa l.). *Front. Plant Sci.* 6, 1272. doi: 10.3389/fpls.2015.01272

Singh, S., Husain, T., Kushwaha, B. K., Suhel, M., Fatima, A., Mishra, V., et al. (2021b). Regulation of ascorbate-glutathione cycle by exogenous nitric oxide and hydrogen peroxide in soybean roots under arsenate stress. *J. Hazardous Materials.* 409, 123686. doi: 10.1016/j.jhazmat.2020.123686

Singh, P. K., Indoliya, Y., Chauhan, A. S., Singh, S. P., Singh, A. P., Dwivedi, S., et al. (2017). Nitric oxide mediated transcriptional modulation enhances plant adaptive responses to arsenic stress. *Sci. Rep.* 7, 1–13. doi: 10.1038/s41598-017-03923-2

Singh, N., Jain, P., Gupta, S., Khurana, J. M., and Bhatla, S. C. (2021a). N-nitrosomelatonin, an efficient nitric oxide donor and transporter in arabidopsis seedlings. *Nitric. Oxide.* 113-114, 50–56. doi: 10.1016/j.niox.2021.05.001

Singh, P., and Shah, K. (2014). Evidences for reduced metal-uptake and membrane injury upon application of nitric oxide donor in cadmium stressed rice seedlings. *Plant Physiol. Biochem.* 83, 180–184. doi: 10.1016/j.plaphy.2014.07.018

Singh, I., and Shah, K. (2015). Evidences for suppression of cadmium induced oxidative stress in presence of sulphosalicylic acid in rice seedlings. *Plant Growth Regul.* 76, 99–110. doi: 10.1007/s10725-015-0023-4

Souri, Z., and Karimi, N. (2021). The effect of arsenic and sodium nitroprusside on the physiological responses and antioxidant enzymes activity of isatis cappadocica. *jispp* 10, 187–208. doi: 20.1001.1.23222727.1400.10.41.3.3

Sun, F., Wang, Y., Luo, X., Ma, Z., Xu, Y., Zhang, X., et al. (2019). Anti-CD24 antibody–nitric oxide conjugate selectively and potently suppresses hepatic carcinoma. *Cancer Res.* 79, 3395–3405. doi: 10.1158/0008-5472.CAN-18-2839

Tada, Y., Spoel, S. H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., et al. (2008). Plant immunity requires conformational charges of NPR1 *via* s-nitrosylation and thioredoxins. *Science.* 321, 952–956. doi: 10.1126/science.1156970

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., and Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Molecular Clin. Environ. Toxicol.* 101, 133–164. doi: 10.1007/978-3-7643-8340-4_6

Terrile, M. C., París, R., Calderón-Villalobos, L. I., Iglesias, M. J., Lamattina, L., Estelle, M., et al. (2012). Nitric oxide influences auxin signaling through s-nitrosylation of the arabidopsis TRANSPORT INHIBITOR RESPONSE 1 auxin receptor. *Plant J.* 70, 492–500. doi: 10.1111/j.1365-313X.2011.04885.x

Terrón-Camero, L. C., Peláez-Vico, M. Á., Del-Val, C., Sandalio, L. M., and Romero-Puertas, M. C. (2019). Role of nitric oxide in plant responses to heavy metal stress: Exogenous application versus endogenous production. *J. Exp. Bot.* 70, 4477–4488. doi: 10.1093/jxb/erz184

Tian, Q. Y., Sun, D. H., Zhao, M. G., and Zhang, W. H. (2007). Inhibition of nitric oxide synthase (NOS) underlies aluminum-induced inhibition of root elongation in hibiscus moscheutos. *N. Phytol.* 174, 322–331. doi: 10.1111/j.1469-8137.2007.02005.x

Wang, P. G., Cai, T. B., and Taniguchi, N. (2005). *Nitric oxide donors: For pharmaceutical and biological applications* (John Wiley & Sons).

Wang, Y.-Q., Feechan, A., Yun, B.-W., Shafiei, R., Hofmann, A., Taylor, P., et al. (2009). S-nitrosylation of AtSABP3 antagonizes the expression of plant immunity. *J. Biol. Chem.* 284, 2131–2137. doi: 10.1074/jbc.M806782200

Wang, H.-H., Huang, J.-J., and Bi, Y.-R. (2010a). Nitrate reductase-dependent nitric oxide production is involved in aluminum tolerance in red kidney bean roots. *Plant Sci.* 179, 281–288. doi: 10.1016/j.plantsci.2010.05.014

Wang, Y., Jiang, F., Ma, C., Rui, Y., Tsang, D. C. W., and Xing, B. (2019). Effect of metal oxide nanoparticles on amino acids in wheat grains (Triticum aestivum) in a life cycle study. *J. Environ. Manage.* 241, 319–327. doi: 10.1016/j.jenvman.2019.04.041

Wang, K., Wang, Y., Wan, Y., Mi, Z., Wang, Q., Wang, Q., et al. (2021). The fate of arsenic in rice plants (Oryza sativa l.): Influence of different forms of selenium. *Chemosphere.* 264, 128417. doi: 10.1016/j.chemosphere.2020.128417

Wang, Y.-S., and Yang, Z.-M. (2005). Nitric oxide reduces aluminum toxicity by preventing oxidative stress in the roots of cassia tora l. *Plant Cell Physiol.* 46, 1915–1923. doi: 10.1093/pcp/pci202

Wang, Y., Yang, T., and He, Q. (2020). Strategies for engineering advanced nanomedicines for gas therapy of cancer. *Natl. Sci. Rev.* 7, 1485–1512. doi: 10.1093/nsr/nwaa034

Wang, S. H., Zhang, H., Jiang, S. J., Zhang, L., He, Q. Y., and He, H. Q. (2010b). Effects of the nitric oxide donor sodium nitroprusside on antioxidant enzymes in wheat seedling roots under nickel stress. *Russian J. Plant Physiol.* 57, 833–839. doi: 10.1134/S1021443710060129

Wei, L., Zhang, M., Wei, S., Zhang, J., Wang, C., and Liao, W. (2020). Roles of nitric oxide in heavy metal stress in plants: Cross-talk with phytohormones and protein s-nitrosylation. *Environ. Pollut.* 259, 113943. doi: 10.1016/j.envpol.2020.113943

Williams, A. T., Muller, C. R., Govender, K., Navati, M. S., Friedman, A. J., Friedman, J. M., et al. (2020). Control of systemic inflammation through early nitric oxide supplementation with nitric oxide releasing nanoparticles. *Free Radical Biol. Med.* 161, 15–22. doi: 10.1016/j.freeradbiomed.2020.09.025

Wojcik, M., and Tukiendorf, A. (2004). Phytochelatin synthesis and cadmium localization in wild type of arabidopsis thaliana. *Plant Growth Regul.* 44, 71–80. doi: 10.1007/s10725-004-1592-9

Wu, X., Tang, X., Xian, M., and Wang, P. G. (2001). Glycosylated diazeniumdiolates: A novel class of enzyme-activated nitric oxide donors. *Tetrahedron Lett.* 42, 3779–3782. doi: 10.1016/S0040-4039(01)00614-1

Xiong, J., An, L., Lu, H., and Zhu, C. (2009). Exogenous nitric oxide enhances cadmium tolerance of rice by increasing pectin and hemicellulose contents in root cell wall. *Planta.* 230, 755–765. doi: 10.1007/s00425-009-0984-5

Yang, Y., Huang, Z., and Li, L.-L. (2021). Advanced nitric oxide donors: Chemical structure of NO drugs, NO nanomedicines and biomedical applications. *Nanoscale.* 13, 444–459. doi: 10.1039/D0NR07484E

Yu, L., Gao, R., Shi, Q., Wang, X., Wei, M., and Yang, F. (2013). Exogenous application of sodium nitroprusside alleviated cadmium induced chlorosis, photosynthesis inhibition and oxidative stress in cucumber. *Pak J. Bot.* 45, 813–819.

Yu, C. C., Hung, K. T., and Kao, C. H. (2005). Nitric oxide reduces Cu toxicity and Cu-induced NH4+ accumulation in rice leaves. *J. Plant Physiol.* 162, 1319–1330. doi: 10.1016/j.jplph.2005.02.003

Yu, M., Lamattina, L., Spoel, S. H., and Loake, G. J. (2014). Nitric oxide function in plant biology: A redox cue in deconvolution. *N. Phytol.* 202, 1142–1156. doi: 10.1111/nph.12739

Yun, B.-W., Feechan, A., Yin, M., Saidi, N. B., Le Bihan, T., Yu, M., et al. (2011). S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. *Nature.* 478, 264–268. doi: 10.1038/nature10427

Yu, Q., Sun, L., Jin, H., Chen, Q., Chen, Z., and Xu, M. (2012). Lead-induced nitric oxide generation plays a critical role in lead uptake by pogonatherum crinitum root cells. *Plant Cell Physiol.* 53, 1728–1736. doi: 10.1093/pcp/pcs116

Zhang, A., Jiang, M., Zhang, J., Ding, H., Xu, S., Hu, X., et al. (2007). Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. *N. Phytol.* 175, 36–50. doi: 10.1111/j.1469-8137.2007.02071.x

Zhang, P., Li, Y., Tang, Y., Shen, H., Li, J., Yi, Z., et al. (2020). Copper-based metal–organic framework as a controllable nitric oxide-releasing vehicle for enhanced diabetic wound healing. *ACS Appl. Materials Interfaces.* 12, 18319–18331. doi: 10.1021/acsami.0c01792

Zhao, H., Jin, Q., Wang, Y., Chu, L., Li, X., and Xu, Y. (2016). Effects of nitric oxide on alleviating cadmium stress in typha angustifolia. *Plant Growth Regul.* 78, 243–251. doi: 10.1007/s10725-015-0089-z

Zhao, X.-F., Lin, C., Rehmani, M. I., Wang, Q.-S., Wang, S.-H., Hou, P.-F., et al. (2013). Effect of nitric oxide on alleviating cadmium toxicity in rice (Oryza sativa l.). *J. Integr. Agric.* 12, 1540–1550. doi: 10.1016/S2095-3119(13)60417-7

Zhou, P., Adeel, M., Shakoor, N., Guo, M., Hao, Y., Azeem, I., et al. (2021). Application of nanoparticles alleviates heavy metals stress and promotes plant growth: An overview. *Nanomaterials.* 11, 26. doi: 10.3390/nano11010026

Zhou, X., Meng, Z., She, J., Zhang, Y., Yi, X., Zhou, H., et al. (2020). Near-infrared light-responsive nitric oxide delivery platform for enhanced radioimmunotherapy. *Nano-Micro Lett.* 12, 100. doi: 10.1007/s40820-020-00431-3

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Sulfur reduces the root-to-shoot translocation of arsenic and cadmium by regulating their vacuolar sequestration in wheat (*Triticum aestivum* L.)

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Accumulation of arsenic (As) and cadmium (Cd) in wheat grain is a serious threat to human health. Sulfur (S) can simultaneously decrease wheat grain As and Cd concentrations by decreasing their translocation in wheat; however, the mechanisms are unclear. We conducted hydroponic experiments to explore the mechanisms by which S modulates As and Cd translocation and their toxicity in wheat. Wheat seedlings were grown in deficient sulfate (2.5 μM) or sufficient sulfate (1.0 mM) nutrient solutions for 6 days and then exposed to zero (control), low As+Cd (1 μM As plus 0.5 μM Cd), or high As+Cd (50 μM As plus 30 μM Cd) for another 6 days. Compared with the control, plant growth was not affected by low As+Cd, but was significantly inhibited by high As+Cd. In the low As+Cd treatment, S supply had no significant effect on plant growth or root-to-shoot As and Cd translocation. In the high As+Cd treatment, sufficient S supply significantly alleviated As and Cd toxicity and their translocation by increasing phytochelatin (PC) synthesis and the subsequent vacuolar sequestration of As and Cd in roots, compared with deficient S supply. The use of L-buthionine sulfoximine (a specific inhibitor of γ-glutamylcysteine synthetase) confirmed that the alleviation of As and Cd translocation and toxicity in wheat by S is mediated by increased PC production. Also, *TaHMA3* gene expression in wheat root was not affected by the As+Cd and S treatments, but the expression of *TaABCC1* was upregulated by the high As+Cd treatment and further increased by sufficient S supply and high As+Cd treatment. These results indicate that S-induced As and Cd subcellular changes affect As and Cd translocation mainly by regulating thiol metabolism and *ABCC1* expression in wheat under As and Cd stress.

KEYWORDS

cadmium, wheat, arsenic, subcellular distribution, phytochelatin, TaABCC1

Introduction

Heavy metal/metalloid pollution in soil-crop systems is a potential threat to human health. Arsenic (As) and cadmium (Cd) are ubiquitously distributed, highly toxic, and readily transferred from contaminated soil to the food chain (Zhao and Wang, 2020). Long-term exposure to these two toxic elements can cause lung disease, skin disease, kidney disease, and Itai-Itai disease (Godt et al., 2006; Balali-Mood et al., 2021). Wheat (*Triticum aestivum* L.) is one of the most important staple foods and ranks first in terms of global consumption, accounting for about 28% of cereal consumption annually (Food and Agriculture Organization, 2021). Compared with other grain crops such as barley and maize, wheat grain has greater ability to accumulate Cd (Yang et al., 2014; Rizwan et al., 2016). In alkaline soil, wheat can still accumulate considerable amounts of Cd in its grain at concentrations even higher than those seen in rice (Yang et al., 2020). As a result, wheat and wheat-based products are major dietary sources of Cd (Greger and Löfstedt, 2004; Punshon and Jackson, 2018). Although wheat grain has lesser As accumulation than rice (Williams et al., 2007; Bhattacharya et al., 2010; Saeed et al., 2021), high As concentration was commonly detected in the grain of wheat grown in As-contaminated soils (Duncan et al., 2017; Suman et al., 2020). Arsenic is typically present in wheat grain in an inorganic form (Zhao et al., 2010; Shi et al., 2013; Rasheed et al., 2018), which is more toxic to humans than the organic form (Styblo et al., 2000; Sun et al., 2008). Risks to human health as a result of inorganic As exposure through consumption of wheat have been reported in India (Suman et al., 2020), Pakistan (Rasheed et al., 2018), and Argentina (Sigrist et al., 2016). Therefore, there is a need for strategies to decrease As and Cd accumulation in the grain of wheat grown in As and Cd co-contaminated soils.

Most previous studies focused on the uptake and translocation of As and Cd individually in soil-crop systems, and many mitigation strategies have been employed to reduce As or Cd accumulation in food crops. However, farmland contaminated with both As and Cd has been reported worldwide (Wan et al., 2020; El-Naggar et al., 2021). Simultaneous decreases in As and Cd accumulation in crops are a challenge because of their opposite behaviors in soil (Honma et al., 2016; Qiao et al., 2018). For example, liming of acidic soils with lime or biochar is effective in decreasing Cd accumulation in crops, but not for As (Zhu et al., 2016; Chen et al., 2018). Water and phosphorous fertilizer management exert opposite effects on As and Cd bioavailability to plants (Lee et al., 2016; Wan et al., 2019; Khan et al., 2020). Likewise, the effect of sulfate on As and Cd bioavailability in wheat rhizosphere soil is inconsistent; interestingly, sulfur (S) supply decreased As and Cd accumulation in wheat grain by decreasing their translocation in wheat plants grown in As and Cd co-contaminated soil (Shi et al., 2020). However, the mechanism underlying S-mediated regulation of As and Cd translocation in wheat is largely unknown.

The subcellular distribution of As and Cd significantly affects the concentration of free As and Cd ions in plant cells, modulating As and Cd translocation and toxicity to plants (Srivastava et al., 2016; Cao et al., 2018; Huang et al., 2021). In rice, S supply alters the Cd subcellular distribution by regulating *OsHMA3* (OsHMA3, a transporter for Cd sequestration into root vacuoles) expression (Cao et al., 2018). However, the TaHMA3 transporter may not be able to transport Cd in wheat (Zhang et al., 2020). Therefore, whether S can alter Cd subcellular distribution in wheat by another pathway warrants further investigation. The ATP-binding cassette transporters ABCC1 and ABCC2 are reportedly involved in the vacuolar sequestration of Cd in *Arabidopsis thaliana* (Park et al., 2012). In contrast to HMA3, which transports the ionic form of Cd, the ABCC transporters transport Cd-phytochelatin (PC) conjugates (Zhang et al., 2018). PCs also have a high affinity for As binding to their thiol groups. AtABCC1 and AtABCC2 transport both Cd-PC and As-PC complexes (Park et al., 2012; Song et al., 2014a). Therefore, the magnitudes of PC synthesis and *ABCC* expression in plants may determine As and Cd mobility in roots. In a recent study, 18 wheat ABCC proteins were identified; a phylogenetic analysis showed that only TaABCC1 is an ortholog of AtABCC1 and AtABCC2 (Bhati et al., 2015). However, the relationship between *TaABCC1* expression and As and Cd translocation in wheat is unclear.

Sulfur is a vital macronutrient for plant growth and responses to abiotic and biotic stresses (Tao et al., 2018). Numerous crucial S-containing compounds are derived directly or indirectly from this element, including cysteine (Cys), glutathione (GSH), PCs, and nicotinamide (Na and Salt, 2011). We hypothesized that S supply alters the subcellular distribution of As and Cd by upregulating PC synthesis and *TaABCC1* expression, thus reducing the root-to-shoot

translocation of As and Cd in wheat plants. Accordingly, in this study, we exposed wheat seedlings to both As and Cd under various levels of S supply in solution culture to investigate the influence of S on As and Cd uptake, translocation, and subcellular distribution, as well as plant growth. Furthermore, the effect of S supply on GSH and PC synthesis, *TaABCC1* and *TaHMA3* expression, and their relationships with As and Cd translocation in wheat seedlings were analyzed. The findings provide theoretical guidance for bioremediation of As and Cd in wheat plants to reduce environmental and health hazards.

Materials and methods

Plant culture and treatment

Wheat (*Triticum aestivum* L. cv. Jinmai 85) seeds were surface-sterilized in 10% (w/w) hydrogen peroxide for 10 min, washed thoroughly with clean water and soaked in distilled water for 12 h, and germinated in wet quartz sand for 3 days. After germination, uniform seedlings were selected and transplanted into a black plastic beaker (twelve seedlings per beaker) with 1.1 L of 0.5 mmol L^{-1} $CaCl_2$ solution for 3 days, and then the seedlings with residual endosperm removed were transferred into nutrient solution with deficient sulfur (S1: 2.5 μM sulfate, the concentration of sulfate in micronutrients) or sufficient sulfur (S2: 1.0 mM sulfate, the concentration of sulfate in full nutrient solution). The sufficient S nutrient solution (full nutrient solution) contained 1.0 mM $Ca(NO_3)_2$, 1.5 mM KCl, 1 mM $MgSO_4$, 0.2 mM KH_2PO_4, 1.5 mM $CaCl_2$, 1 μM H_3BO_3, 0.05 μM $(NH_4)_6Mo_7O_{24}$, 1 μM ZnSO4, 1 μM MnSO4, 0.5 μM $CuSO_4$, and 40 μM $FeNa_2$-EDTA (Ren et al., 2012). For deficient S supply, $MgCl_2$ was instead of $MgSO_4$. Nutrient solution was refreshed every 3 days, and solution pH was adjusted to 6.0 using HCl or NaOH. Beakers were placed in a growth cabinet with a 12/12-h light/dark photoperiod and 25/20°C day/night temperatures.

After growing the wheat seedlings in deficient- or sufficient-S nutrient solution for 6 days, they were exposed to zero (0 μM As plus 0 μM Cd; control), low (1 μM As plus 0.5 μM Cd; low As +Cd), or high As and Cd (50 μM As plus 30 μM Cd; high As+Cd) for another 6 days maintained in deficient- or sufficient-S nutrient solution under the above-mentioned growth conditions. The concentrations of As and Cd selected were based on the results of preliminary toxicity assays. Arsenic at 1–10 μM or Cd at 0.5–5 μM has no visual negative effect on the growth of wheat seedlings after 6 days; and As at 50 μM or Cd at 30 μM both caused about 40% inhibition in root elongation (Figure S1). Furthermore, to substantiate the inhibition by S of As and Cd translocation in wheat plants by increasing GSH and PC production under As and Cd stress, a GSH synthetase inhibitor, $_L$-buthionine sulfoximine (BSO, 0.25 mM) was added to high As+Cd + deficient S supply and high As+Cd + sufficient S supply. The concentration of BSO used in this study was set according to previous studies (Tang et al., 2016; Shi et al., 2017). The effects of the two treatments were compared with that of high As+Cd + deficient S-supply plants. The treatments were arranged in a completely randomized block design with three replicates and twelve plants per beaker. The sources of As and Cd were Na_2HAsO_4 and $CdCl_2$, respectively.

Determination of plant growth and concentrations of As and Cd

The lengths of roots (the average value of the two longest roots of each plant) and shoots were measured using a ruler before and after 6 days of As+Cd treatment. After measurement of root and shoot length, wheat plants were harvested. The roots were rinsed in deionized water and immersed in an ice-cold desorption solution containing 1 mM K_2HPO_4, 0.5 mM $Ca(NO_3)_2$, and 5 mM MES (pH 6.0) for 10 min to remove root surface-adsorbed As and Cd (Xu et al., 2022). Roots and shoots were separated, washed with distilled water, blotted dry, and weighed. Root and shoot samples were divided into two sets. One set for biochemical analysis was immediately frozen in liquid nitrogen and stored at –80°C, and the other set was dried to a constant weight at 60°C for total As and Cd analysis. The dried samples were weighed and digested with HNO_3–H_2O_2 (2:1, v/v) at 125°C (Shi et al., 2020). Arsenic and Cd concentrations were determined by inductively coupled plasma mass spectroscopy (ICP-MS, Perkin Elmer NexION 2000, Waltham, MA). For quality control, reagent blanks and a certified reference plant material (Orange Leaves GBW10020, National Research Center for Standards, China) were included in the analysis. The recovery rates of the standard reference materials for As and Cd were 92–98% and 95–101%, respectively.

Determination of As and Cd subcellular distribution

Frozen root and shoot tissues were powdered in liquid nitrogen and extracted with 10 mL of extraction solution containing 50 mM Tris-HCl buffer (pH 7.5), 0.25 mM sucrose, and 1.0 mM DL-dithioerythritol. The homogenate was centrifuged at 1500 × *g* for 15 min at 4°C; the residue was considered the cell-wall fraction. The supernatant was centrifuged at 20,000 × *g* for 30 min at 4°C (Su et al., 2014; Xiao et al., 2020). The supernatant and the residue were referred to as the soluble and organelle fractions, respectively. These fractions were dried to constant weight at 60°C and digested with HNO_3–H_2O_2. Arsenic and Cd concentrations were determined by ICP-MS (Shi et al., 2020).

Analysis of non-protein thiol compounds

Non-protein thiol compounds in wheat samples were extracted and analyzed as described previously (Wu et al., 2013; Shi et al., 2017). In short, frozen plant samples were ground into powder in liquid nitrogen and extracted in 2 mL of trifluoroacetic acid (TFA, 0.1%) containing 6.3 mM diethylenetriaminepentaacetic acid (DTPA) and 1 mL of 1 mM tris(2-carboxyethyl)phosphate hydrochloride (TCEP) prepared in 200 mM HEPES buffer (pH 8.2) and 6.3 mM DTPA. The homogenate was centrifuged at 12,000 × *g* for 20 min at 4°C. The supernatant was passed through a 0.22-μm membrane filter, and a 250-μL aliquot of the supernatant was mixed with 20 μL of 1 mM TCEP and immediately derivatized with 10 μL of 25 mM monobrombimane, together with 420 μL of 200 mM HEPES buffer containing 6.3 mmol L^{-1} DTPA. Derivatization was conducted in the dark for 30 min at 45°C and terminated by adding 300 μL of 1 M methansulphonic acid (MSA). The thiol derivatives were separated and analyzed by reverse-phase ultra-performance liquid chromatography. The details of the procedure are described elsewhere (Shi et al., 2017).

Quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from flash-frozen fresh roots using the Promega SV Total RNA Isolation System (Promega) based on the manufacturer's instructions, and RNA quality was examined by agarose gel electrophoresis and a NanoDrop 2000 spectrophotometer. First-strand cDNA was synthesized from total RNA using M-MLV Reverse Transcriptase (Fermentas). Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was carried out on a Bio-Rad CFX96 Real-Time PCR system with SYBR Green detection in accordance with the manufacturer's instructions. The primers used for *TaABCC1* and *TaABCC2* were as reported previously (Bhati et al., 2015), and those for *TaHMA3* were designed according to the sequence KF683296.1 (https://www.ncbi.nlm.nih.gov/). The *tubulin* gene was used as an internal standard control gene (He et al., 2018). The primer sequences are listed in Supplementary Table S1. The qRT-PCR reaction conditions comprised pre-denaturation at 95℃ for 1 min followed by 36 cycles of denaturation at 95℃ for 10 s, annealing at 58℃ for 15 s, and extension at 72℃ for 20 s with a final incubation at 72℃ for 5 min. Three technical replications were performed for each sample. The $2^{-\Delta\Delta Ct}$ comparative method was employed to calculate relative gene expression levels (Livak and Schmittgen, 2001).

Data analysis

Data are expressed as means ± standard deviation of three independent replicates and statistically analyzed by analysis of variance (ANOVA) using SPSS software (v. 25.0). Duncan's multiple comparison test was used to assess differences among treatments, and a p-value ≤ 0.05 was considered indicative of statistical significance. Figures and tables were generated using Excel 2016 and SigmaPlot 12.5 software. The translocation factor (TF), an indicator of the ability of plants to translocate As or Cd from roots to shoots, was calculated as the ratio of As or Cd concentration in shoots to roots (He et al., 2015; Huang et al., 2021).

Results

Influence of S on the growth of As- and Cd-treated plants

We evaluated the impact of S supply and As+Cd treatment on the growth of wheat seedlings in terms of root and shoot elongation and dry weight (Figure 1). Compared with the control, the low As+Cd treatment did not significantly alter any of the parameters. However, the high As+Cd treatment caused significant ($p \leq 0.05$) inhibition of plant growth, leading to 58.2%, 44.7%, 13.3%, and 15.2% decreases in root elongation, shoot elongation, root dry weight, and shoot dry weight, respectively, under deficient S supply. By contrast, sufficient S supply significantly alleviated the plant growth inhibition induced by high As+Cd, because the four growth parameters were increased by sufficient compared to deficient S supply. The root elongation, shoot elongation, root dry weight, and shoot dry weight in sufficient S supply were 24.7%, 17.8%, 12.7%, and 6.9% higher than those in deficient S supply, respectively. In the zero (control) and low As+Cd treatments, the differences in the four growth parameters between the two S supplies were not significant.

Influence of S on As and Cd accumulation and translocation in wheat plants

The concentrations of As and Cd in wheat roots and shoots increased significantly with increasing As and Cd concentrations in nutrient solution (Figure 2). Arsenic and Cd accumulated mainly in wheat roots irrespective of S supply. In deficient S- and sufficient S-treated plants, the As and Cd concentrations in wheat roots were 17.9- and 11.7-fold higher than those in shoots in the low As+Cd treatment, and were further increased to 54.8- and 27.0-fold by the high As+Cd treatment, respectively (Figures 2A–D). In the low As+Cd treatment, S supply had no significant effect on As and Cd accumulation in wheat plants (Figures 2A–D). In the high As+Cd treatment, sufficient S supply increased the root As and Cd concentrations by 13.5% and 19.5%, respectively, in comparison with deficient S supply (Figures 2A, B). By contrast, sufficient S supply significantly decreased the shoot As concentration by 15% but

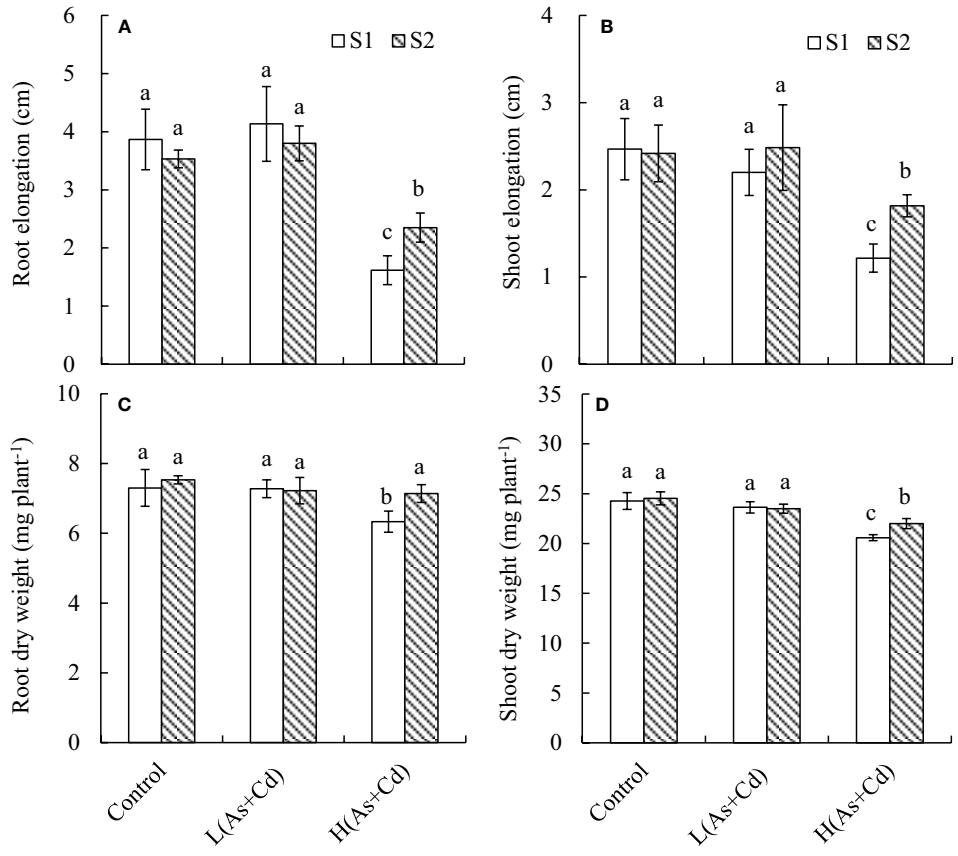

FIGURE 1
Root **(A)** and shoot **(B)** elongation, and root **(C)** and shoot **(D)** dry weight of wheat plants grown in low S or high S nutrient solution after 6 days of As + Cd exposure. S1, deficient sulfate; S2, sufficient sulfate; L(As+Cd), low As plus Cd; H(As+Cd), high As plus Cd. Data are means ± SD (n = 3). Different letters in the same column indicate significant differences among treatments at $p \leq 0.05$ according to Duncan's multiple comparison test.

had no significant effect on the shoot Cd concentration (Figures 2C, D). Sufficient S supply significantly decreased As and Cd translocation from root to shoot in plants treated with high As and Cd concentrations but had no significant effect on As and Cd translocation in wheat plants treated with low As and Cd concentrations (Figures 2E, F).

Influence of S on the subcellular distribution of As and Cd in wheat roots

As shown in Table 1, in both low As+Cd- and high As+Cd-treated plants, most As and Cd in root cells was distributed in the cell wall and soluble fractions, and little was in the organelle fraction. In the low As+Cd treatment, S supply had no significant effect on the concentration and proportion of As and Cd in the various subcellular fractions. However, the subcellular distributions of As and Cd varied according to S supply in the high As+Cd treatment. In the high As+Cd treatment, sufficient S supply significantly increased As and Cd concentrations in the soluble fraction by 80% and 108%, respectively, and decreased the As concentration in the organelle fraction by 19%, when compared with deficient S supply. No significant differences were found in the concentrations of As and Cd in the cell wall and the concentration of Cd in the organelle fraction between deficient and sufficient S supply. Therefore, sufficient S supply significantly increased the As and Cd proportions in the soluble fraction compared to deficient S supply. Also, sufficient S supply significantly decreased the proportion of As and Cd in the cell wall and organelle fractions.

Influence of S on thiol synthesis in wheat roots

Table 2 shows the concentrations of Cys, GSH, and PCs in roots of wheat plants exposed to various As and Cd concentrations under deficient or sufficient S supply. The concentrations of Cys and GSH in wheat roots were significantly higher in the high As+Cd

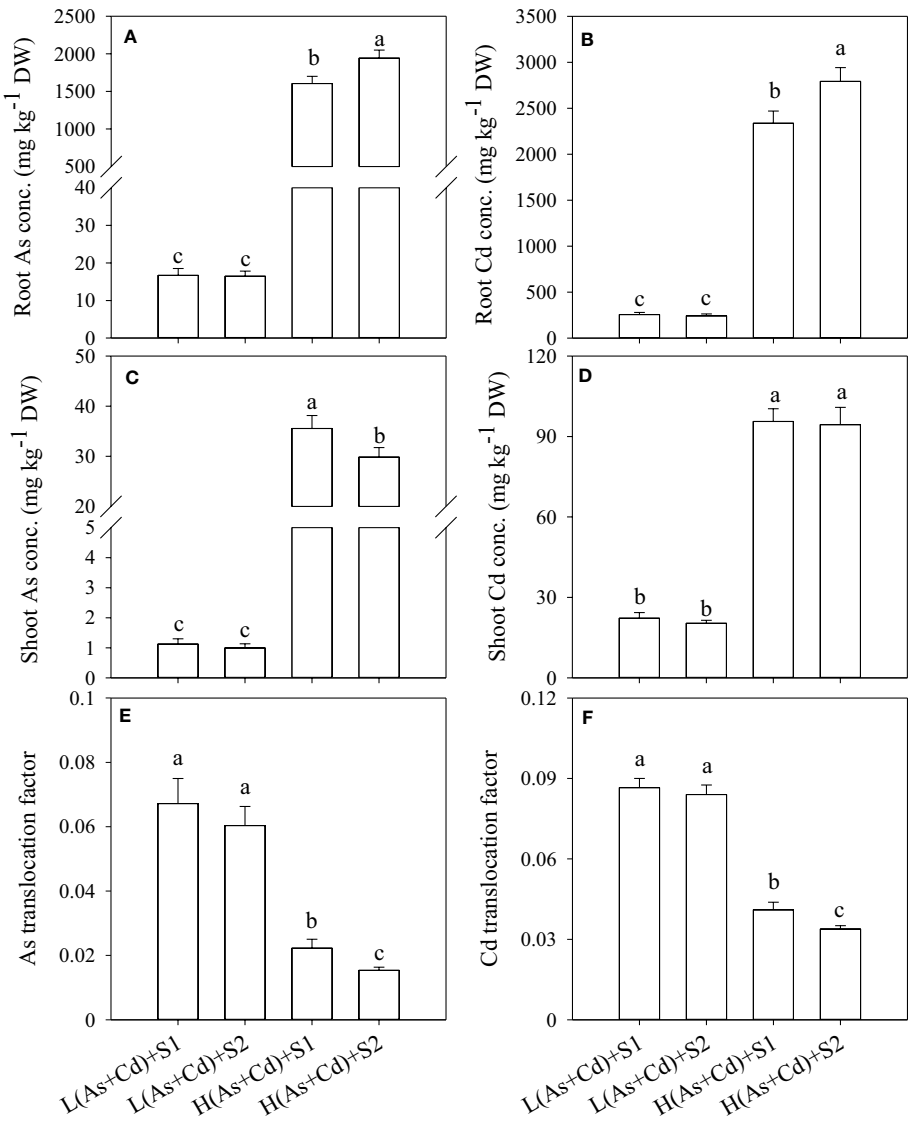

FIGURE 2
Arsenic and Cd accumulation and translocation in wheat plants grown in low S or high S nutrient solution after 6 days of As plus Cd exposure. S1, deficient sulfate; S2, sufficient sulfate; L(As+Cd), low As plus Cd; H(As+Cd), high As plus Cd. Data are means ± SD (n = 3). Different letters in the same column indicate significant differences among treatments at $p \leq 0.05$ according to Duncan's multiple comparison test.

treatment as compared to the controls, whereas no significant differences were observed between the low and zero As+Cd treatments. High As+Cd exposure induced PC synthesis in wheat roots, and PC_4 was only detected in high As+Cd-treated plants. The average (of two S treatments) concentrations of PC_2 and PC_3 in wheat roots in the high As+Cd treatment were 38.2- and 22.2-fold higher than those in the low As+Cd treatment, and 88.9- and 37.9-fold higher than those in the zero As+Cd treatment, respectively (Table 2). Compared to deficient S supply, sufficient S supply had no significant effect on Cys, GSH, and PC concentrations in wheat roots in the zero and low As+Cd treatments, but significantly increased the Cys, GSH, PC_2, PC_3, and PC_4 concentrations in roots by 27%, 51%, 81%, 95%, and 57%, respectively, in the high As+Cd treatment.

Influence of S on the expression of TaABCC1, TaABBCC2, and TaHMA3 in wheat roots

Compared to the control, *TaABCC1* expression in wheat roots was not affected by low As+Cd exposure but was significantly increased by the high As+Cd treatment (Figure 3A). The average (of two S treatments) expression

TABLE 1 Subcellular distribution of As and Cd in the roots of wheat plants grown in deficient S or sufficient S nutrient solution after 6 days of As plus Cd exposure.

Subcellular fraction	Treatment	Concentration (mg kg^{-1} FW)		Proportion (%)	
		As	Cd	As	Cd
Cell wall	L(As+Cd) +S1	1.06 ± 0.03 b	8.45 ± 0.59 b	58.1 ± 1.5 a	67.8 ± 5.2 a
	L(As+Cd) + S2	0.94 ± 0.19 b	7.89 ± 1.16 b	55.5 ± 3.7 a	66.5 ± 5.4 ab
	H(As+Cd) + S1	63.26 ± 7.60 a	73.20 ± 12.29 a	56.9 ± 7.0 a	74.8 ± 2.3 a
	H(As+Cd) + S2	57.26 ± 9.10 a	81.32 ± 8.13 a	40.7 ± 0.6 b	62.4 ± 0.8 b
Soluble fraction	L(As+Cd) +S1	0.73 ± 0.08 c	3.86 ± 0.78 c	39.7 ± 1.9 b	30.8 ± 5.0 ab
	L(As+Cd) + S2	0.69 ± 0.05 c	3.79 ± 1.03 c	41.8 ± 4.3 b	31.6 ± 5.4 ab
	H(As+Cd) + S1	45.05 ± 8.16 b	22.60 ± 1.12 b	40.5 ± 7.2 b	23.3 ± 2.1 b
	H(As+Cd) + S2	81.12 ± 15.27 a	46.93 ± 6.31 a	57.8 ± 0.8 a	36.0 ± 0.8 a
Organelle fraction	L(As+Cd) +S1	0.04 ± 0.01 c	0.16 ± 0.03 b	2.1 ± 0.4 ab	1.3 ± 0.2 b
	L(As+Cd) + S2	0.05 ± 0.01 c	0.22 ± 0.07 b	2.7 ± 0.5 a	1.8 ± 0.3 ab
	H(As+Cd) + S1	2.91 ± 0.25 a	1.85 ± 0.07 a	2.6 ± 0.2 a	1.9 ± 0.2 a
	H(As+Cd) + S2	2.37 ± 0.17 b	1.96 ± 0.21 a	1.7 ± 0.2 b	1.5 ± 0.0 b

S1, deficient sulfate; S2, sufficient sulfate; L(As+Cd), low As plus Cd; H(As+Cd), high As plus Cd. Data are means ± SD (n = 3). Different letters in each column indicate significant differences among treatments at $p \leq 0.05$ according to Duncan's multiple comparison test.

TABLE 2 Concentrations of thiol (-SH) compounds in the roots of wheat plants grown in deficient S or sufficient S nutrient solution after 6 days of As plus Cd exposure (nmol SH g^{-1} FW).

Treatment	Cys	GSH	PC_2	PC_3	PC_4
Control (S1)	38.84 ± 3.28 c	3.51 ± 0.07 c	3.11 ± 0.62 d	2.64 ± 0.25 e	ND
Control (S2)	47.57 ± 7.75 c	4.16 ± 0.49 c	2.85 ± 0.57 d	2.84 ± 0.44 de	ND
L(As+Cd) + S1	45.25 ± 6.97 c	3.06 ± 0.29 c	6.53 ± 1.43 c	4.04 ± 0.59 cd	ND
L(As+Cd) + S2	47.73 ± 9.04 c	3.18 ± 0.23 c	7.36 ± 1.42 c	5.33 ± 1.47 c	ND
H(As+Cd) + S1	119.62 ± 3.69 b	19.20 ± 1.21 b	188.83 ± 34.40 b	70.63 ± 14.47 b	11.65 ± 1.26 b
H(As+Cd) + S2	151.95 ± 11.19 a	29.06 ± 5.71 a	340.97 ± 65.19 a	137.45 ± 28.04 a	18.33 ± 3.32 a

S1, deficient sulfate; S2, sufficient sulfate; L(As+Cd), low As plus Cd; H(As+Cd), high As plus Cd; ND, not detected. Data are means ± SD (n = 3). Different letters in the same column indicate significant differences among treatments at $p \leq 0.05$ according to Duncan's multiple comparison test.

level of *TaABCC1* in roots of high As+Cd-treated wheat plants was 4.8-fold higher than that of low As+Cd-treated plants, and 3.9-fold higher than that of zero As+Cd-treated plants (Figure 3A). Although sufficient S supply had no significant effect on *TaABCC1* expression in wheat roots in the low and zero As+Cd treatments, it significantly increased *TaABCC1* expression in wheat roots by 83% in the high As+Cd treatment compared with deficient S treatment. *TaABBCC2* and *TaHMA3* expression levels were not significantly affected by S supply or the As+Cd treatments (Figures 3B, C).

Effect of BSO on S-mediated influence on growth and As and Cd translocation in wheat plants

To confirm that S alleviates As and Cd toxicity and root-to-shoot translocation in wheat plants by increasing GSH and PC production under As and Cd stress, BSO was used to manipulate the synthesis of GSH and PCs in high As+Cd-treated plants. As shown in Table 3, in the high As+Cd + deficient S treatment, addition of BSO decreased the GSH, PC_2, PC_3, and PC_4 concentrations in wheat roots by 69%, 75%, 84%, and 83%, respectively. In the presence of BSO, the differences in root GSH and PC concentrations between deficient and sufficient S supply were not significant in the high As+Cd treatment. The effects of BSO on plant growth, As and Cd accumulation, the subcellular distribution of As and Cd in roots, and the root-to-shoot translocation of As and Cd in wheat plants exposed to high As +Cd are shown in Table 4. Under high As+Cd with deficient S treatment, BSO supply significantly aggravated the toxicity of As and Cd to wheat plants and decreased root elongation, shoot elongation, root dry weight, and shoot dry weight by 59%, 55%, 18%, and 13%, respectively. BSO treatment also decreased As and Cd concentrations in the soluble fraction by 71% and 66%, respectively, and increased the As concentration in the organelle

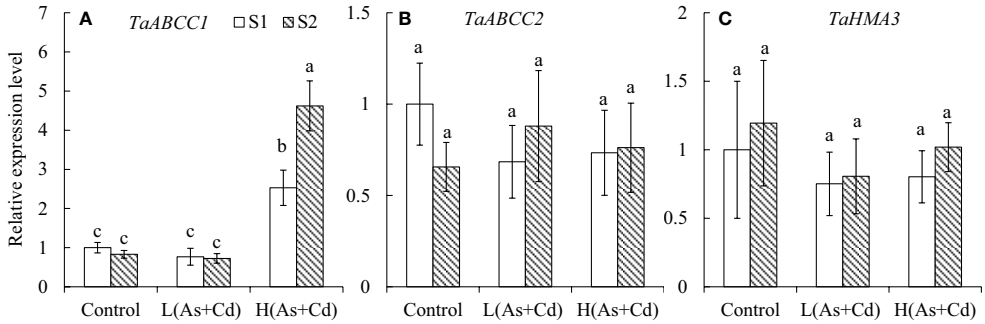

FIGURE 3
Expression levels of *TaABCC1* **(A)**, *TaABCC2* **(B)**, and *TaHMA3* **(C)** in the roots of wheat plants grown in low S or high S nutrient solution after 6 days of As plus Cd exposure. S1, deficient sulfate; S2, sufficient sulfate; L(As+Cd), low As plus Cd; H(As+Cd), high As plus Cd. Data are means ± SD (n = 3). Different letters in the same column indicate significant differences among treatments at $p \leq 0.05$ according to Duncan's multiple comparison test.

TABLE 3 Concentrations of thiol (-SH) compounds in the roots of wheat plants grown in deficient S or sufficient S nutrient solution after 6 days of exposure to high As plus Cd with or without BSO (nmol SH g^{-1} FW).

Treatment	Cys	GSH	PC_2	PC_3	PC_4
H(As+Cd) + S1	128.16 ± 14.50 a	19.55 ± 2.04 a	175.70 ± 17.72 a	79.44 ± 12.46 a	10.17 ± 1.80 a
H(As+Cd) + S1 + BSO	111.12 ± 17.54 a	6.11 ± 0.49 b	44.44 ± 4.89 b	12.98 ± 3.45 b	1.72 ± 0.29 b
H(As+Cd) + S2 + BSO	114.39 ± 17.2 a	6.78 ± 0.72 b	47.45 ± 6.50 b	16.74 ± 2.99 b	2.01 ± 0.29 b

S1, deficient sulfate; S2, sufficient sulfate; H(As+Cd), high As plus Cd; BSO, L-buthionine sulfoximine. Different letters in each column indicate significant differences among treatments at p ≤ 0.05 according to Duncan's multiple comparison test.

fraction by 31%. BSO treatment decreased the root As concentration by 59%, root Cd concentration by 46%, and shoot Cd concentration by 64%, but had no significant effect on shoot As concentration. Consequently, As and Cd TFs from roots to shoots increased by 2.6- and 1.4-fold, respectively, in response to BSO. Furthermore, in the presence of BSO, the differences in the four plant growth parameters, As and Cd accumulation, the subcellular distribution of As and Cd in roots, and root-to-shoot translocation of As and Cd in wheat plants between deficient S and sufficient S supply were not significant under the high As+Cd treatment.

Discussion

It's well known that the translocation of As and Cd from roots to shoots in plants is species specific and depends on the external metal/metalloid concentration and the intensity of metal/metalloid phytotoxicity (Siemianowski et al., 2011). Therefore, to systematically study the physiological and molecular mechanisms governing the effects of S on As and Cd translocation in wheat, we exposed wheat seedlings to low As +Cd (1 μM As plus 0.5 μM Cd; no significant effect on wheat growth) or high As+Cd (50 μM As plus 30 μM Cd; significant inhibition of plant growth) under deficient S or sufficient S supply. In this study, 6 days of high As+Cd stress resulted in significant reductions in root and shoot elongation and root and shoot dry weight under deficient S supply. Notably, the magnitudes of the reductions in the four growth parameters were less in the high As +Cd treatments with sufficient S supply, suggesting that S enhances the growth of wheat plant under high As+Cd stress. This can be attributed to the alleviating effect of S on As and Cd toxicity in wheat because the growth of plants was not affected by S supply under the zero or low As+Cd treatment (Figure 1). These results are consistent with prior reports that S supply improves rice plant growth under As or Cd stress (Srivastava et al., 2016; Cao et al., 2018), but not under control conditions for 14 days (Srivastava et al., 2016). Consistent with other studies on rice (Srivastava et al., 2016) and barley (Reid et al., 2013), this study also showed that 12 days of S deficiency did not have a significant impact on wheat growth under the control conditions. This is not surprising considering the high capacity of plants to buffer changes in shoot and root biomass production in response to S limitation (Elberse et al., 2003). Plants achieve this buffering effect by altering the expression of S metabolism-related hormones and genes (Reid et al., 2013; Srivastava et al., 2016).

TABLE 4 Plant growth, As and Cd accumulation, the subcellular distribution of As and Cd in roots, and As and Cd translocation from roots to shoots of wheat plants grown in deficient S or sufficient S nutrient solution after 6 days of exposure to high As plus Cd with or without BSO.

Parameter	H(As+Cd) + S1	H(As+Cd) + S1 + BSO	H(As+Cd) + S2 + BSO
Root elongation (cm)	2.03 ± 0.25 a	0.83 ± 0.15 b	0.93 ± 0.21 b
Shoot elongation (cm)	1.26 ± 0.15 a	0.57 ± 0.06 b	0.63 ± 0.06 b
Root dry weight (mg plant^{-1})	6.56 ± 0.29 a	5.39 ± 0.29 b	5.44 ± 0.35 b
Shoot dry weight (mg plant^{-1})	20.72 ± 0.46 a	18.08 ± 1.26 b	18.97 ± 0.42 b
Root As conc. (mg kg^{-1} DW)	1461.8 ± 176.8 a	601.5 ± 58.7 b	647.7 ± 45.4 b
Shoot As conc. (mg kg^{-1} DW)	32.3 ± 3.4 a	34.7 ± 2.5 a	31.5 ± 3.2 a
Root Cd conc. (mg kg^{-1} DW)	2108.7 ± 241.9 a	974.4 ± 83.1 b	1006.5 ± 102.6 b
Shoot Cd conc. (mg kg^{-1} DW)	91.6 ± 8.2 a	58.2 ± 3.3 b	60.8 ± 5.1 b
As in cell wall (mg kg^{-1} FW)	57.7 ± 6.7 a	34.2 ± 6.3 b	36.6 ± 4.7 b
As in soluble fraction (mg kg^{-1} FW)	39.8 ± 6.8 a	11.6 ± 2.9 b	14.8 ± 1.9 b
As in organelle fraction (mg kg^{-1} FW)	2.99 ± 0.4 b	3.9 ± 0.4 a	3.9 ± 0.3 a
Cd in cell wall (mg kg^{-1} FW)	77.7 ± 6.7 a	47.1 ± 4.6 b	48.4± 4.0 b
Cd in soluble fraction (mg kg^{-1} FW)	29.5 ± 4.1 a	10.0 ± 1.8 b	11.0 ± 1.5 b
Cd in organelle fraction (mg kg^{-1} FW)	1.7 ± 0.2 a	1.8 ± 0.2 a	1.8 ± 0.3 a
As translocation factor	0.022 ± 0.001 b	0.058 ± 0.008 a	0.049 ± 0.004 a
Cd translocation factor	0.044 ± 0.007 b	0.060 ± 0.007 a	0.061 ± 0.007 a

S1, deficient sulfate; S2, sufficient sulfate; H(As+Cd), high As plus Cd; BSO, L-buthionine sulfoximine. Data are means ± SD (n = 3). Different letters in the same row indicate significant differences among treatments at $p \leq 0.05$ according to Duncan's multiple comparison test.

The vacuole is the largest plant organelle, and is crucial for detoxification (Martinoia et al., 2012). For normal cell development, plants attempt to sequestrate As and Cd into vacuoles (Song et al., 2014a; Cao et al., 2018). Our results showed that sufficient S supply increased the proportion of As and Cd in the soluble fraction (mainly in vacuoles) of wheat plants grown under the high As+Cd treatment (Table 1). This suggests that, under high As+Cd stress, S supply increased vacuolar sequestration of As and Cd. Sequestration of As and Cd into the vacuole decreased their activity in the cytosol (Table 1), thereby alleviating As and Cd toxicity (Song et al., 2014a; Cao et al., 2018). This explains why S supply has different effects on plant growth at different As and Cd concentrations (Figure 1). Previous study demonstrated that S supply can increase Cd vacuolar sequestration in rice roots by two pathways: 1) transporting Cd^{2+} into vacuoles *via* the OsHMA3 transporter and 2) transporting Cd-PC complexes into vacuoles *via* a PC-dependent pathway (Cao et al., 2018). However, the HMA3 transporter in wheat plants may not transport Cd (Zhang et al., 2020). In this study, *TaHMA3* expression was not affected by the S and As+Cd treatments (Figures 3B, C).

PCs play an important role in As and Cd tolerance and translocation in plants (Batista et al., 2014; Zanella et al., 2016; Das et al., 2021). PCs are high-affinity metal/metalloid chelators that can be induced by As and Cd stress in many plants (Shi et al., 2017; Das et al., 2021; González et al., 2021). Similarly, in this study, high As+Cd treatment increased the synthesis of GSH and PCs in wheat roots in comparison with zero and low As+Cd treatment (Table 2). Supply of S further increased the synthesis of GSH and PCs under high As+Cd stress (Table 2), possibly enhancing As and Cd tolerance and reducing As and Cd translocation in wheat plants (Figures 1, 2). By contrast, S supply in the zero and low As+Cd treatments had no significant impact on the Cys, GSH, and PC concentrations in wheat roots (Table 2), and thus likely had no significant effect on As and Cd accumulation and translocation in wheat (Figure 2). In addition, the suppression of GSH and PC synthesis by BSO hypersensitized wheat plants to As and Cd toxicity, and increased root-to-shoot As and Cd translocation (Tables 3, 4). The differences in plant growth, the subcellular distribution of As and Cd in roots, and the root-to-shoot translocation of As and Cd between deficient S and sufficient S supply were not significant in the presence of BSO (Table 4), although they differed significantly in its absence (Figures 1, 2). Taken together, these results indicate that S decreased As and Cd translocation and their toxicity to wheat plants mainly by increasing the synthesis of GSH and PCs, which then chelated more As and Cd and promoted As and Cd transfer into vacuoles (Table 1).

After chelating with PCs, the complexes of As-PCs and Cd-PCs should be sequestered into plant vacuoles for final detoxification (Song et al., 2010; Park et al., 2012). However, due to the large size and polyploid complexity of the wheat genome, the transporters involved in the vacuolar sequestration of As-PCs and Cd-PC in wheat plants are unknown. In Arabidopsis, two transporters (AtABCC1 and AtABCC2) of the ATP-binding cassette transporter family transport As-PCs and Cd-PCs into the vacuole (Song et al., 2010; Park et al., 2012). Similarly, OsABCC1 is the main transporter for the vacuolar sequestration of As-PCs complexes in rice (Song et al., 2014b). In wheat, only one gene, *TaABCC1*, shows high similarity to

AtABCC1 and *AtABCC2* (Bhati et al., 2015). In this study, *TaABCC1* expression in wheat roots was significantly induced by the high As+Cd treatment, which was further enhanced by sufficient S supply. Furthermore, the changes in *TaABCC1* expression under various As+Cd and S conditions were consistent with the changes in GSH and PCconcentrations in wheat roots, which were correlated with As and Cd translocation and plant tolerance to As and Cd. Therefore, S supply increases As and Cd vacuolar sequestration in wheat roots by increasing PC synthesis and *TaABCC1* expression, suggesting a mechanism by which S regulates As and Cd translocation and toxicity in wheat.

Conclusion

Sufficient S supply simultaneously decreased As and Cd translocation and their toxicity in wheat plants under As and Cd stress. This could be attributed to induction of the synthesis of PCs and the expression of *TaABCC1*, increasing As and Cd chelation and transfer into vacuoles. Furthermore, the HMA3 transporter in wheat roots is not involved in S-mediated regulation of Cd vacuolar sequestration, even through this transporter is crucial for S-induced Cd vacuolar sequestration in rice (Cao et al., 2018). This study sheds light on the physiological and molecular mechanisms by which S modulates As and Cd translocation in wheat and will facilitate the development of strategies to simultaneously decrease the accumulation of As and Cd in wheat grain.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

GS, LL, and YG conceived and designed this study. HL, HZ, GF, and WC conducted the experiment. HL, HZ, and JL analyzed the data and prepared the tables and figures. GS and LL wrote the first draft of the manuscript. DZ and YG reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1032681/full#supplementary-material

References

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., and Sadeghi, M. (2021). Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Front. Pharmacol.* 12. doi: 10.3389/fphar.2021.643972

Batista, B. L., Nigar, M., Mestrot, A., Alves Rocha, B., Barbosa Junior, F., Price, A. H., et al. (2014). Identification and quantification of phytochelatins in roots of rice to long-term exposure: evidence of individual role on arsenic accumulation and translocation. *J. Exp. Bot.* 65, 1467–1479. doi: 10.1093/jxb/eru018

Bhati, K. K., Sharma, S., Aggarwal, S., Kaur, M., Shukla, V., Kaur, J., et al. (2015). Genome-wide identification and expression characterization of ABCC-MRP transporters in hexaploid wheat. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00488

Bhattacharya, P., Samal, A. C., Majumdar, J., and Santra, S. C. (2010). Accumulation of arsenic and its distribution in rice plant (*Oryza sativa* l.) in gangetic West Bengal, India. *Paddy. Water Environ.* 8, 63–70. doi: 10.1007/s10333-009-0180-z

Cao, Z. Z., Qin, M. L., Lin, X. Y., Zhu, Z. W., and Chen, M. X. (2018). Sulfur supply reduces cadmium uptake and translocation in rice grains (*Oryza sativa* l.) by enhancing iron plaque formation, cadmium chelation and vacuolar sequestration. *Environ. pollut.* 238, 76–84. doi: 10.1016/j.envpol.2018.02.083

Chen, H., Zhang, W., Yang, X., Wang, P., McGrath, S. P., and Zhao, F. J. (2018). Effective methods to reduce cadmium accumulation in rice grain. *Chemosphere* 207, 699–707. doi: 10.1016/j.chemosphere.2018.05.143

Das, U., Rahman, M. A., Ela, E. J., Lee, K. W., and Kabir, A. H. (2021). Sulfur triggers glutathione and phytochelatin accumulation causing excess cd bound to the cell wall of roots in alleviating cd-toxicity in alfalfa. *Chemosphere* 262, 128361. doi: 10.1016/j.chemosphere.2020.128361

Duncan, E. G., Maher, W. A., Foster, S. D., Krikowa, F., O'Sullivan, C. A., and Roper, M. M. (2017). Dimethylarsenate (DMA) exposure influences germination rates, arsenic uptake and arsenic species formation in wheat. *Chemosphere* 181, 44–54. doi: 10.1016/j.chemosphere.2017.04.043

Elberse, I. A. M., Van Damme, J. M. M., and Van Tienderen, P. H. (2003). Plasticity of growth characteristics in wild barley (*Hordeum spontaneum*) in response to nutrient limitation. *J. Ecol.* 91, 371–382. doi: 10.1046/j.1365-2745.2003.00776.x

El-Naggar, A., Chang, S. X., Cai, Y., Lee, Y. H., Wang, J., Wang, S. L., et al. (2021). Mechanistic insights into the (im)mobilization of arsenic, cadmium, lead, and zinc in a multi-contaminated soil treated with different biochars. *Environ. Int.* 156, 106638. doi: 10.1016/j.envint.2021.106638

Food and Agriculture Organization. (2021) *FAO cereal supply and demand brief*. Available at: http://www.fao.org/worldfoodsituation/csdb/en/ (Accessed March 10, 2022).

Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., et al. (2006). The toxicity of cadmium and resulting hazards for human health. *J. Occup. Med. Toxicol.* 1, 22. doi: 10.1186/1745-6673-1-22

González, A., Laporte, D., and Moenne, A. (2021). Cadmium accumulation involves synthesis of glutathione and phytochelatins, and activation of CDPK, CaMK, CBLPK, and MAPK signaling pathways in *Ulva compressa*. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.669096

Greger, M., and Löfstedt, M. (2004). Comparison of uptake and distribution of cadmium in different cultivars of bread and durum wheat. *Crop Sci.* 44, 501–507. doi: 10.2135/cropsci2004.5010

He, J., Li, H., Ma, C., Zhang, Y., Polle, A., Rennenberg, H., et al. (2015). Overexpression of bacterial γ-glutamylcysteine synthetase mediates changes in cadmium influx, allocation and detoxification in poplar. *New Phytol.* 205, 240–254. doi: 10.1111/nph.13013

He, Y., Zhang, X., Zhang, Y., Ahmad, D., Wu, L., Jiang, P., et al. (2018). Molecular characterization and expression of PFT, an FHB resistance gene at the Fhb1 QTL in wheat. *Phytopathology* 108, 730–736. doi: 10.1094/PHYTO-11-17-0383-R

Honma, T., Ohba, H., Kaneko-Kadokura, A., Makino, T., Nakamura, K., and Katou, H. (2016). Optimal soil eh, pH, and water management for simultaneously minimizing arsenic and cadmium concentrations in rice grains. *Environ. Sci. Technol.* 50, 4178–4185. doi: 10.1021/acs.est.5b05424

Huang, Y., Chen, J., Zhang, D., Fang, B., YangJin, T., Zou, J., et al. (2021). Enhanced vacuole compartmentalization of cadmium in root cells contributes to glutathione-induced reduction of cadmium translocation from roots to shoots in pakchoi (*Brassica chinensis* l.). *Ecotox. Environ. Saf.* 208, 111616. doi: 10.1016/j.ecoenv.2020.111616

Khan, Z. S., Rizwan, M., Hafeez, M., Ali, S., Adrees, M., Qayyum, M. F., et al. (2020). Effects of silicon nanoparticles on growth and physiology of wheat in cadmium contaminated soil under different soil moisture levels. *Environ. Sci. pollut. Res.* 27, 4958–4968. doi: 10.1007/s11356-019-06673-y

Lee, C. H., Wu, C. H., Syu, C. H., Jiang, P. Y., Huang, C. C., and Lee, D. Y. (2016). Effects of phosphorous application on arsenic toxicity to and uptake by rice seedlings in as-contaminated paddy soils. *Geoderma* 270, 60–67. doi: 10.1016/j.geoderma.2016.01.003

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_{T}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Martinoia, E., Meyer, S., De Angeli, A., and Nagy, R. (2012). Vacuolar transporters in their physiological context. *Annu. Rev. Plant Biol.* 63, 183–213. doi: 10.1146/annurev-arplant-042811-105608

Na, G., and Salt, D. E. (2011). The role of sulfur assimilation and sulfur-containing compounds in trace element homeostasis in plants. *Environ. Exp. Bot.* 72, 18–25. doi: 10.1016/j.envexpbot.2010.04.004

Park, J., Song, W. Y., Ko, D., Eom, Y., Hansen, T. H., Schiller, M., et al. (2012). The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. *Plant J.* 69, 278–288. doi: 10.1111/j.1365-313X.2011.04789.x

Punshon, T., and Jackson, B. P. (2018). Essential micronutrient and toxic trace element concentrations in gluten containing and gluten-free foods. *Food Chem.* 252, 258–264. doi: 10.1016/j.foodchem.2018.01.120

Qiao, J. T., Liu, T. X., Wang, X. Q., Li, F. B., Lv, Y. H., Cui, J. H., et al. (2018). Simultaneous alleviation of cadmium and arsenic accumulation in rice by applying zero-valent iron and biochar to contaminated paddy soils. *Chemosphere* 195, 260–271. doi: 10.1016/j.chemosphere.2017.12.081

Rasheed, H., Kay, P., Slack, R., and Gong, Y. Y. (2018). Arsenic species in wheat, raw and cooked rice: Exposure and associated health implications. *Sci. Total Environ.* 634, 366–373. doi: 10.1016/j.scitotenv.2018.03.339

Reid, R., Gridley, K., Kawamata, Y., and Zhu, Y. (2013). Arsenite elicits anomalous sulfur starvation responses in barley. *Plant Physiol.* 162, 401–409. doi: 10.1104/pp.113.216937

Ren, Y., He, X., Liu, D., Li, J., Zhao, X., Li, B., et al. (2012). Major quantitative trait loci for seminal root morphology of wheat seedlings. *Mol. Breed.* 30, 139–148. doi: 10.1007/s11032-011-9605-7

Rizwan, M., Ali, S., Abbas, T., Zia-ur-Rehman, M., Hannan, F., Keller, C., et al. (2016). Cadmium minimization in wheat: a critical review. *Ecotox. Environ. Saf.* 130, 43–53. doi: 10.1016/j.ecoenv.2016.04.001

Saeed, M., Quraishi, U. M., and Malik, R. N. (2021). Arsenic uptake and toxicity in wheat (*Triticum aestivum* l.): A review of multi-omics approaches to identify tolerance mechanisms. *Food Chem.* 355, 129607. doi: 10.1016/j.foodchem.2021.129607

Shi, G. L., Lou, L. Q., Li, D. J., Hu, Z. B., and Cai, Q. S. (2017). Phytochelatins play key roles for the difference in root arsenic accumulation of different *Triticum aestivum* cultivars in comparison with arsenate uptake kinetics and reduction. *Chemosphere* 175, 192–199. doi: 10.1016/j.chemosphere.2017.02.017

Shi, G. L., Lou, L. Q., Zhang, S., Xia, X. W., and Cai, Q. S. (2013). Arsenic, copper, and zinc contamination in soil and wheat during coal mining, with assessment of health risks for the inhabitants of huaibei, China. *Environ. Sci. pollut. Res.* 20, 8435–8445. doi: 10.1007/s11356-013-1842-3

Shi, G., Lu, H., Liu, H., Lou, L., Zhang, P., Song, G., et al. (2020). Sulfate application decreases translocation of arsenic and cadmium within wheat (*Triticum aestivum* l.) plant. *Sci. Total Environ.* 713, 136665. doi: 10.1016/j.scitotenv.2020.136665

Siemianowski, O., Mills, R. F., Williams, L. E., and Antosiewicz, D. M. (2011). Expression of the P_{1B}-type ATPase *AtHMA4* in tobacco modifies zn and cd root to shoot partitioning and metal tolerance. *Plant Biotechnol. J.* 9, 64–74. doi: 10.1111/j.1467-7652.2010.00531.x

Sigrist, M., Hilbe, N., Brusa, L., Campagnoli, D., and Beldoménico, H. (2016). Total arsenic in selected food samples from Argentina: Estimation of their contribution to inorganic arsenic dietary intake. *Food Chem.* 210, 96–101. doi: 10.1016/j.foodchem.2016.04.072

Song, W. Y., Mendoza-Cózatl, D. G., Lee, Y., Schroeder, J. I., Ahn, S. N., Lee, H. S., et al. (2014a). Phytochelatin–metal (loid) transport into vacuoles shows different substrate preferences in barley and *Arabidopsis*. *Plant Cell Environ.* 37, 1192–1201. doi: 10.1111/pce.12227

Song, W., Park, J., Mendoza-Cózatl, D. G., Suter-Grotemeyer, M., Shim, D., and Hörtensteiner, S. (2010). Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin transporters. *Proc. Natl. Acad. Sci.* 107, 21187–21192. doi: 10.1073/pnas.101396410

Song, W. Y., Yamaki, T., Yamaji, N., Ko, D., Jung, K. H., Fujii-Kashino, M., et al. (2014b). A rice ABC transporter, OsABCC1, reduces arsenic accumulation in the grain. *Proc. Natl. Acad. Sci.* 111, 15699–15704. doi: 10.1073/pnas.1414968111

Srivastava, S., Akkarakaran, J. J., Sounderajan, S., Shrivastava, M., and Suprasanna, P. (2016). Arsenic toxicity in rice (*Oryza sativa* l.) is influenced by sulfur supply: impact on the expression of transporters and thiol metabolism. *Geoderma* 270, 33–42. doi: 10.1016/j.geoderma.2015.11.006

Styblo, M., Del Razo, L. M., Vega, L., Germolec, D. R., LeCluyse, E. L., Hamilton, G. A., et al. (2000). Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. *Arch. Toxicol.* 74, 289–299. doi: 10.1007/s002040000134

Su, Y., Liu, J., Lu, Z., Wang, X., Zhang, Z., and Shi, G. (2014). Effects of iron deficiency on subcellular distribution and chemical forms of cadmium in peanut roots in relation to its translocation. *Environ. Exp. Bot.* 97, 40–48. doi: 10.1016/j.envexpbot.2013.10.001

Suman, S., Sharma, P. K., Siddique, A. B., Rahman, M. A., Kumar, R., Rahman, M. M., et al. (2020). Wheat is an emerging exposure route for arsenic in bihar, India. *Sci. Total Environ.* 703, 134774. doi: 10.1016/j.scitotenv.2019.134774

Sun, G. X., Williams, P. N., Carey, A. M., Zhu, Y. G., Deacon, C., Raab, A., et al. (2008). Inorganic arsenic in rice bran and its products are an order of magnitude higher than in bulk grain. *Environ. Sci. Technol.* 42, 7542–7546. doi: 10.1021/es801238p

Tang, Z., Kang, Y., Wang, P., and Zhao, F. J. (2016). Phytotoxicity and detoxification mechanism differ among inorganic and methylated arsenic species

in. *Arabidopsis. thaliana. Plant Soil* 401, 243–257. doi: 10.1016/j.envpol.2020.114157

Tao, Z., Chang, X., Wang, D., Wang, Y., Ma, S., Yang, Y., et al. (2018). Effects of sulfur fertilization and short-term high temperature on wheat grain production and wheat flour proteins. *Crop J.* 6, 413–425. doi: 10.1016/j.cj.2018.01.007

Wan, X., and Parikh, S. J. (2020). Simultaneous removal of arsenic, cadmium, and lead from soil by iron-modified magnetic biochar. *Environ. Pollut.* 261, 114157. doi: 10.1016/j.envpol.2020.114157

Wan, Y., Huang, Q., Camara, A. Y., Wang, Q., and Li, H. (2019). Water management impacts on the solubility of cd, Pb, as, and cr and their uptake by rice in two contaminated paddy soils. *Chemosphere* 228, 360–369. doi: 10.1016/j.chemosphere.2019.04.133

Williams, P. N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A. J., et al. (2007). Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. *Environ. Sci. Technol.* 41, 6854–6859. doi: 10.1021/es070627i

Wu, Z., Zhang, C., Yan, J., and Ge, Y. (2013). Separation and quantification of cysteine, glutathione and phytochelatins in rice (*Oryza sativa* l.) upon cadmium exposure using reverse phase ultra performance liquid chromatography (RP-UPLC) with fluorescence detection. *Anal. Methods* 5, 6147–6152. doi: 10.1039/c3ay40849c

Xiao, Y., Wu, X., Liu, D., Yao, J., Liang, G., Song, H., et al. (2020). Cell wall polysaccharide-mediated cadmium tolerance between two *Arabidopsis thaliana* ecotypes. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.00473

Xu, M., Lin, Y., da Silva, E. B., Cui, Q., Gao, P., Wu, J., et al. (2022). Effects of copper and arsenic on their uptake and distribution in as-hyperaccumulator *Pteris vittata*. *Environ. pollut.* 300, 118982. doi: 10.1016/j.envpol.2022.118982

Yang, J. L., Cang, L., Wang, X., Xu, H. T., and Zhou, D. M. (2020). Field survey study on the difference in cd accumulation capacity of rice and wheat in rice-wheat rotation area. *J. Soil. Sediment.* 20, 2082–2092. doi: 10.1007/s11368-020-02587-2

Yang, Y., Nan, Z., and Zhao, Z. (2014). Bioaccumulation and translocation of cadmium in wheat (*Triticum aestivum* l.) and maize (*Zea mays* l.) from the polluted oasis soil of northwestern China. *Chem. Spec. Bioavailab.* 26, 43–51. doi: 10.3184/095422914X13888342841789

Zanella, L., Fattorini, L., Brunetti, P., Roccotiello, E., Cornara, L., D'Angeli, S., et al. (2016). Overexpression of AtPCS1 in tobacco increases arsenic and arsenic plus cadmium accumulation and detoxification. *Planta* 243, 605–622. doi: 10.1007/s00425-015-2428-8

Zhang, L., Gao, C., Chen, C., Zhang, W., Huang, X. Y., and Zhao, F. J. (2020). Overexpression of rice *OsHMA3* in wheat greatly decreases cadmium accumulation in wheat grains. *Environ. Sci. Technol.* 54, 10100–10108. doi: 10.1021/acs.est.0c02877

Zhang, J., Martinoia, E., and Lee, Y. (2018). Vacuolar transporters for cadmium and arsenic in plants and their applications in phytoremediation and crop development. *Plant Cell Physiol.* 59, 1317–1325. doi: 10.1093/pcp/pcy006

Zhao, F. J., Stroud, J. L., Eagling, T., Dunham, S. J., McGrath, S. P., and Shewry, P. R. (2010). Accumulation, distribution, and speciation of arsenic in wheat grain. *Environ. Sci. Technol.* 44, 5464–5468. doi: 10.1021/es100765g

Zhao, F. J., and Wang, P. (2020). Arsenic and cadmium accumulation in rice and mitigation strategies. *Plant Soil* 446, 1–21. doi: 10.1007/s11104-019-04374-6

Zhu, H. H., Chen, C., Zhu, Q. H., and Huang, D. Y. (2016). Effects of soil acidification and liming on the phytoavailability of cadmium in paddy soils of central subtropical China. *Environ. pollut.* 219, 99–106. doi: 10.1016/j.envpol.2016.10.043

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Supplementation of nitric oxide and spermidine alleviates the nickel stress-induced damage to growth, chlorophyll metabolism, and photosynthesis by upregulating ascorbate–glutathione and glyoxalase cycle functioning in tomato

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Experiments were conducted to evaluate the role of exogenously applied nitric oxide (NO; 50 μM) and spermidine (Spd; 100 μM) in alleviating the damaging effects of Ni (1 mM $NiSO_4 6H_2O$) toxicity on the growth, chlorophyll metabolism, photosynthesis, and mineral content in tomato. Ni treatment significantly reduced the plant height, dry mass, and the contents of glutamate 1-semialdehyde, δ-amino levulinic acid, prototoporphyrin IX, Mg–prototoporphyrin IX, total chlorophyll, and carotenoids; however, the application of NO and Spd alleviated the decline considerably. Supplementation of NO and Spd mitigated the Ni-induced decline in photosynthesis, gas exchange, and chlorophyll fluorescence parameters. Ni caused oxidative damage, while the application of NO, Spd, and NO+Spd significantly reduced the oxidative stress parameters under normal and Ni toxicity. The application of NO and Spd enhanced the function of the antioxidant system and upregulated the activity of glyoxalase enzymes, reflecting significant reduction of the oxidative effects and methylglyoxal accumulation. Tolerance against Ni was further strengthened by the accumulation of proline and glycine betaine due to NO and Spd application. The decrease in the uptake of essential mineral elements such as N, P, K, and Mg was alleviated by NO and Spd. Hence, individual and combined supplementation of NO and Spd effectively alleviates the damaging effects of Ni on tomato.

KEYWORDS

antioxidants, glyoxalase, oxidative stress, nickel, nitric oxide, spermidine

Introduction

In recent years, rapid industrial progress has raised many serious issues including heavy metal pollution, thereby imposing serious threats to livelihood. Among the key contributors of heavy metal pollution are the processes of smelting, mining, electroplating, burning of fossils, and phosphate fertilization (Salt and Kramer, 2000). Heavy metals are continuously disposed and added to the environment, rendering the surroundings unsafe for humans and crops. Excess accumulation of heavy metals seriously affects soil health and renders it less fertile, thereby affecting crop growth and productivity (Ahmad and Ashraf, 2011; Hassan et al., 2019). Nickel (Ni) is one of the metal pollutants with serious influence on the ecosystem and on human and plant health (Duda-Chodak and Baszczyk, 2008). Ni is a natural component of water and soil, but occurs at very low concentrations (McIlveen and Negusantil, 1994; McGrath, 1995). Excess concentrations of Ni can prove toxic and result in damaging alterations in the metabolism and growth of plants. Reduced plant growth due to Ni toxicity has been attributed to the significant reduction in photosynthesis, enzyme activity, membrane stability, and mineral nutrition (Kazemi et al., 2010; Khan and Khan, 2014). Metal stress-induced growth retardation is mainly attributed to the oxidative damage triggered by the excess accumulation of toxic reactive oxygen species (ROS) and methylglyoxal (MG), which severely affects the redox homeostasis, membrane function, and protein stability, among others (Shahid et al., 2014; Berni et al., 2019; Ahmad et al., 2020). In addition, the stress-induced decline in chlorophyll metabolism significantly contributes to overall growth alterations (Dalal and Tripathy, 2012; Qin et al., 2020). Plants upregulate their tolerance mechanisms to alleviate the damaging effects of ROS, and included in these key tolerance mechanisms are the antioxidant system, glyoxalase system, and osmolyte accumulation (Ahmad et al., 2020). The enzymatic and non-enzymatic components of the antioxidant system work in close coordination to maintain the cellular ROS concentrations, and they also contribute to redox homeostasis, maintenance of enzyme activity, and photosynthesis (Begum et al., 2020; Qin et al., 2021). The glyoxalase system consists of two key enzymes, i.e., glyoxalase I (Gly I) and glyoxalase II (Gly II), that act on MG to prevent its cytotoxic effects (Ahmad et al., 2020).

Nitric oxide (NO) is an endogenous signaling molecule that has been proven to be a ubiquitous molecule involved in regulating an array of physiological, biochemical, and molecular processes from germination to stress signalling and tolerance (Ahmad et al., 2018; Ahmad et al., 2020). The impact of NO produced endogenously or applied exogenously depends on its concentration and the site of production determining its beneficial or deleterious effect (Fatma et al., 2016; Asgher et al., 2017). At optimal concentrations, NO forms a key component of the signaling network, leading to the modulation of key physiological and biochemical pathways for better stress tolerance (Fatma et al., 2016; Soliman et al., 2019; Shang et al., 2022). Due to its unique chemical properties and biological action, NO has been considered as either a stress-inducing (Gould et al., 2003) or a protective agent (Hsu and Kao, 2004; Bai et al., 2015). NO-derived molecules, also known as reactive nitrogen species (RNS), play key roles in maintaining the intracellular redox homeostasis and signaling for the activation of antioxidant mechanisms. It has been reported that the application of NO in plants under stressful conditions helps protect the growth, photosynthesis, and enzyme activity by upregulating the tolerance mechanisms (Fatma et al., 2016; Nabi et al., 2019; Singhal et al., 2021). Recently, in *Hordeum vulgare* L., the application of NO has been reported to alleviate the damaging effects of copper on plant growth and photosynthesis by upregulating the antioxidant function and maintaining the redox homeostasis (Massoud et al., 2022).

Spermidine (Spd) is one of the polyamines and has been reported to actively participate in the growth and cellular function regulation in plants under normal and adverse conditions (Chen et al., 2019; Rakesh et al., 2021). Polyamines are low-molecular-weight nitrogen-containing molecules that are produced during metabolism in almost all types of cells (Chen et al., 2019). They have strong capacity to bind DNA, RNA, and proteins, and the accumulation of polyamines, including spermine, Spd, and putrescine, has been reported to occur under stress conditions, which is associated with the upregulation of their biosynthetic pathway (Hu et al., 2016; Bano et al., 2020). Polyamines play key roles in the alleviation of the oxidative damage to plants through their active role in upregulating the tolerance mechanisms aimed to neutralize toxic ROS (Minocha et al., 2014). Treatment with polyamines has been reported to alleviate the deleterious effects of water (Hassan et al., 2018) and heat stress-induced (Jing et al., 2020) oxidative damage by improving the activity of antioxidant enzymes and the osmolyte accumulation. However, reports on the interactive effects of NO and polyamines are rare.

Tomato (*Solanum lycopersicum* L.) is an important crop grown worldwide. It has rich antioxidative and anticancer activities ascribed to the presence of key metabolites including lycopene and carotene, among others. A considerable increase in soil Ni concentrations can adversely influence the growth and productivity of tomato, and the accumulation of Ni in edible fruit can affect humans. In this backdrop, it was hypothesized that foliar treatment with NO and Spd (individual and combined) can alleviate the Ni stress-induced alterations in the growth, chlorophyll metabolism, and mineral uptake by upregulating the ascorbate–glutathione cycle, glyoxalase cycle, and osmolyte accumulation. The growth, chlorophyll metabolism, photosynthesis, oxidative stress parameters, and tolerance mechanisms were calculated.

Material and methods

Seeds of tomato (*S. lycopersicum* L. cultivar Dongfeng-199) were sterilized with 0.001% $HgCl_2$ for 5 min, followed by thorough washing with distilled water. The sterilized seeds were sown in trays filled with a nutrient-rich peat-based substrate (Pindstrup, Ryomgård, Denmark). Five days after germination, the seedlings were transplanted into pots (diameter, 20 cm) filled with acid-washed sand and were regularly irrigated with full-strength Hoagland nutrient solution (200 ml per pot) on alternate days. Fifteen days after successful seedling establishment, the pots were divided into two groups: one group irrigated with normal Hoagland solution and another group irrigated with modified Hoagland solution containing 1 mM Ni ($NiSO_46H_2O$). However, treatments with NO [in the form of 50 μM sodium nitroprusside (SNP)] and Spd (100 μM Spd trihydrochloride; Sigma-Aldrich, St. Louis, MO, USA) were given foliarly (10 ml per pot) with a hand sprayer using teepol as a surfactant. Detailed experimental treatments are as follows: a) control; b) Ni; c) 50 μM NO; d) 100 μM Spd; e) NO+Spd; f) Ni+NO; g) Ni+Spd; and h) Ni+NO+Spd. Treatments with Ni, NO, and Spd were given for another 15 days; therefore, analysis of the different parameters was done on 30-day-old plants. Pots were arranged in a completely randomized block design with four replicates for each treatment in a greenhouse with day/night temperatures of 30°C/25°C and relative humidity of 70 ± 5%. The different physiological and biochemical parameters determined included photosynthesis, oxidative stress markers, and the antioxidant and glyoxalase systems.

Estimation of pigments, photosynthetic gas exchange parameters, and PSII function

The contents of total chlorophyll and carotenoids were estimated in accordance with the method of Arnon (1949). After homogenizing 100 mg fresh leaf in 80% acetone using a pestle and mortar, the extract was centrifuged and the optical density of the supernatant determined at 480, 645, and 663 nm. Gas exchange parameters such as the net photosynthesis (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were recorded using the LI-6400 photosynthesis system (LI-COR, Lincoln, NE, USA) between 0900 and 1200 hours. The modulated chlorophyll fluorometer PAM-2500 (Heinz Walz, Effeltrich, Germany) was used to calculate the chlorophyll fluorescence parameters including photosystem II (PSII) activity (F_v/F_m), photochemical quenching (qP), non-photochemical quenching (NPQ), and electron transport rate (ETR) after dark adapting the leaves for 25 min.

Estimation of glutamate-1-semialdehyde, δ-amino levulinic acid, prototoporphyrin IX, Mg-prototoporphyrin IX, and protochlorophyllide

The glutamate-1-semialdehyde (GSA) content was measured as described by Kannangara and Schouboe (1985), and absorbance was taken at 620 nm after incubating the tissue with gabaculine. The content of δ-amino levulinic acid (δ-ALA) was estimated according to Harel and Klein (1972), and optical density was read at 555 nm after incubating the samples for 4 h with levulinic acid. For estimation of the contents of prototoporphyrin IX (Proto IX), Mg-prototoporphyrin IX (Mg-Proto IX), and protochlorophyllide (Pchlide), the method of Hodgins and Huystee (1986) was followed. Briefly, 300 mg of fresh leaf samples was extracted in 5 ml alkaline acetone and the optical density read at 575, 590, and 628 nm.

Estimation of proline and glycine betaine

The proline content was determined by homogenizing the dry powdered sample in 3% sulfosalicylic acid using a pestle and mortar. The homogenate was centrifuged at 3,000 × *g* for 20 min and 2 ml supernatant mixed with glacial acetic acid and ninhydrin reagent. The resultant mixture was incubated at 100°C for 1 h. Thereafter, the samples were cooled in an ice bath, proline was separated using toluene, and the optical density was read at 520 nm (Bates et al., 1973). For the estimation of glycine betaine (GB), the method of Grieve and Grattan (1983) was followed. Dry samples were extracted in distilled water, and the extract was filtered and diluted by the addition of 2 N H_2SO_4. An appropriate aliquot of the diluted extract was mixed with cold KI–I_2 reagent, followed by centrifugation at 10,000 × *g* for 15 min. The periodide crystals formed were dissolved in 1,2-dichloroethane and the optical density taken at 365 nm. The standard curve of GB was used for calculation.

Measurement of lipid peroxidation, hydrogen peroxide, and activity of lipoxygenase

Lipid peroxidation was measured following the method of Heath and Packer (1968). Of the fresh leaf tissue, 100 mg was macerated in 1% trichloroacetic acid (TCA) and the extract centrifuged at 10,000 × *g*. The supernatant (1.0 ml) was reacted with 4 ml thiobarbituric acid for half an hour at 95°C. After cooling the samples in an ice bath, centrifugation was done at 5,000 × *g* for 5 min and the absorbance measured at 532 and 600 nm (Heath and Packer, 1968). Lipid peroxidation was expressed as the amount of malondialdehyde (MDA) formed. The

hydrogen peroxide content was estimated by homogenizing fresh tissue in 0.1% TCA. After centrifugation at 12,000 × *g*, 0.5 ml of the supernatant was mixed with potassium phosphate buffer (pH 7.0) and potassium iodide. Absorbance was then taken at 390 nm, and the standard curve of H_2O_2 was used for calculation (Velikova et al., 2000). The method described by Doderer et al. (1992) was used to assay the activity of lipoxygenase (LOX; EC 1.13.11.12), and absorbance was taken at 234 nm. Linoleic acid was used as the substrate, and the extinction coefficient of 25 mM^{-1} cm^{-1} was used for calculation.

Activity of glyoxalase I and glyoxalase II and content of methylglyoxal

Fresh tissue was extracted in cold 50 mM potassium phosphate buffer (pH 7.0) containing 10 mM KCl, 1 mM ascorbate, 1 mM β-mercaptoethanol, and 10% glycerol. The homogenate was centrifuged at 11,500 x g for 15 minutes at 4°C and the supernatant used as the enzyme source for assaying the activities of Gly I (EC 4.4.1.5) and Gly II (EC 3.1.2.6). The method described by Hasanuzzaman et al. (2011) was employed to estimate the activity of Gly I. A change in the optical density was noticed at 240 nm. For calculation, an extinction coefficient of 3.37 mM^{-1} cm^{-1} was used. For assay of the Gly II activity, the optical density was recorded at 412 nm and an extinction coefficient of 13.6 mM^{-1} cm^{-1} was used for calculation, in accordance with Principato et al. (1987). The content of MG was estimated according to Wild et al. (2012). After extracting leaf tissue in perchloric acid and centrifuging the homogenate for 10 min at 11,000 × *g*, the supernatant was reacted with sodium dihydrogen phosphate and *N*-acetyl-L-cysteine. After 10 min, the *N*-α-acetyl-*S*-(1-hydroxy-2-oxo-prop-1-yl)cysteine formed was read at 288 nm.

Assay of the antioxidant enzymes and contents of ascorbate and reduced glutathione

Antioxidant enzymes were extracted by homogenizing 1.0 g fresh leaf tissue in 100 mM cold phosphate buffer (pH 7.8) containing 1% polyvinylpyrrolidone (PVP), 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl (PMSF) using a pre-chilled pestle and mortar. After centrifuging the homogenate at 12,000 × *g* for 15 min at 4°C, the supernatant was used as the enzyme source. The activity of superoxide dismutase (SOD; EC 1.15.1.1) was assayed following Bayer and Fridovich (1987), and absorbance was taken at 560 nm after incubating the samples for 15 min. The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured according to Nakano and Asada (1981) and the absorbance taken at 290 nm for 3 min; for glutathione reductase (GR; EC 1.6.4.2), the method of Foyer and Halliwell (1976) was used and absorbance was taken at 340 nm for 2 min. To measure the activity of dehydroascorbate reductase (DHAR; EC 1.8.5.1), the method of Nakano and Asada (1981) was followed and the absorbance taken at 265 nm for 2 min. The method described by Hossain et al. (1984) was used to assay the activity of monodehydroascorbate reductase (MDHAR; EC 1.6.5.4) and the absorbance taken for 2 min at 340 nm. On the other hand, the method of Mukherjee and Choudhuri (1983) was used for the estimation of the ascorbate (AsA) content, while Ellman (1959) method was followed for the estimation of the content of reduced glutathione (GSH). Standard curves of AsA and GSH were used for their calculation.

Estimation of ions

The concentrations of Mg, K, and Ni were determined using an atomic absorption spectrophotometer (AA-6300; Shimadzu, Kyoto, Japan) (Sagner et al., 1998). The nitrogen content was estimated in dry plant tissue according to the micro-Kjeldahl method (Jackson, 1973), while phosphorus was estimated using the spectrophotometric method (Olsen et al., 1954).

Statistical analysis

The values presented are the mean ± SE of four replicates. Duncan's multiple range test was performed using one-way ANOVA to determine the least significant difference (LSD) among the mean values at $p < 0.05$.

Results

Exogenous NO and/or Spd improves growth

The impacts of Ni stress on plant height and plant dry weight with and without the application of NO and Spd are shown in Table 1. Relative to the control, Ni treatment reduced the plant height (30.39%) and plant dry weight (41.50%) significantly. However, the application of NO and Spd significantly mitigated the decline in plant height and plant dry weight, with maximal ameliorations of 42.44% and 60.58% in Ni+NO+Spd-treated plants compared to their Ni-stressed counterparts. Under normal conditions, the application of NO and Spd considerably enhanced the plant height and dry weight, attaining maximal increases of 46.30% and 25.89%, respectively, due to the combined of NO and Spd treatment (Table 1).

NO and/or Spd alleviates the decline in nutrient uptake and reduced the Ni content

Nickel toxicity resulted in a significant decline in the uptake of essential elements, including N, P, K, and Mg; however, the application of NO and Spd, individually and in combination,

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TABLE 1 Effect of nitric oxide (NO; 50 μM) and spermidine (Spd; 100 μM) supplementation on nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg), nickel (Ni), proline, glycine betaine, plant height, and plant dry weight in *Solanum lycopersicum* L. treated with nickel (Ni).

	Control	Spd	NO	Spd+NO	Ni	Ni+Spd	Ni+NO	Ni+Spd+NO
N	17.02 ± 1.31d	21.67 ± 1.61c	24.03 ± 1.82b	26.44 ± 2.3a	7.15 ± 0.37g	9.23 ± 0.52f	12.97 ± 0.62e	15.98 ± 1.01d
P	14.03 ± 0.99d	17.06 ± 1.17c	19.11 ± 1.32b	21.86 ± 2.1a	6.16 ± 0.31g	8.71 ± 0.49f	10.67 ± 0.81e	13.23 ± 0.79d
K	16.09 ± 0.83d	18.36 ± 1.36c	22.12 ± 1.62b	24.50 ± 1.82a	8.13 ± 0.62g	10.03 ± 0.88f	12.44 ± 0.99e	15.42 ± 1.17d
Mg	8.12 ± 0.45d	9.55 ± 0.63c	11.02 ± 0.77b	12.98 ± 0.83a	4.66 ± 0.23g	5.44 ± 0.25f	6.13 ± 0.28e	7.82 ± 0.33d
Ni	0.0024 ± 0.00019g	0.0017 ± 0.00016f	0.0013 ± 0.00011e	0.0009 ± 0.0001e	3.11 ± 0.21a	2.39 ± 0.18b	2.01 ± 0.13c	1.55 ± 0.011d
Proline	42.1 ± 3.4f	63.8 ± 4.5d	72.7 ± 5.4c	85.1 ± 5.70b	52.2 ± 3.8e	73.1 ± 5.6c	86.5 ± 6.1b	101.4 ± 6.4a
Glycine betaine	2.08 ± 0.11f	2.93 ± 0.16d	3.29 ± 0.19c	3.58 ± 0.19b	2.61 ± 0.21e	3.34 ± 0.23c	3.63 ± 0.25b	4.32 ± 0.28a
Plant height	35.2 ± 2.8d	39.5 ± 3.2c	44.3 ± 3.6b	51.5 ± 4.1a	24.5 ± 2.1g	27.7 ± 2.4f	30.3 ± 2.5e	34.9 ± 2.5d
Plant dry weight	4.12 ± 0.24d	4.73 ± 0.27c	5.17 ± 0.31b	5.56 ± 0.34a	2.41 ± 0.15g	2.98 ± 0.17f	3.36 ± 0.19e	3.87 ± 0.21d

Data are the mean ± SE of four replicates. Different letters designate significant difference at $p < 0.05$.

increased the uptake of these elements. Relative to the control, Ni toxicity reduced N by 57.99%, P by 56.09%, K by 49.47%, and Mg by 42.61%, but significantly increased Ni (Table 1). Treatment with NO+Spd in Ni-stressed plants maximally alleviated the decreases in N (123.49%), P (114.77%), K (89.66%), and Mg (67.81%) compared to Ni-treated plants. Under normal conditions, the uptake of N, P, K, and Mg increased maximally by 55.34%, 55.80%, 52.26%, and 59.85% due to NO+Spd treatment. The application of Spd, NO, and Spd +NO to Ni-treated plants reduced the Ni accumulation by 23.15%, 35.36%, and 50.16%, respectively, compared to the Ni-stressed plants (Table 1).

Proline and glycine betaine accumulation increased due to NO and Spd treatments

Plants treated with Ni exhibited increased accumulation of proline (23.99%) and GB (25.48%) compared to the control. The contents of proline and GB further increased due to the application of Spd and NO, attaining maximal increases of 140.85% and 107.69%, respectively, in Ni+Spd+NO-treated plants compared to the control. Under normal conditions, increases of 51.54% and 29.01% due to Spd, 72.68% and 58.17% due to NO, and 102.13% and 72.11% due to Spd+NO supplementation were observed compared to the control (Table 1).

Supplementation of NO and/or Spd improves pigment synthesis

Treatment of tomato with Ni resulted in a significant decline in the synthesis of GSA (45.02%), δ-ALA (44.72%), Proto IX (46.92%), Mg-Proto IX (51.23%), Pchlide (48.20%), total chlorophyll (48.73%), and carotenoids (34.17%). Relative to the control, treatment with Spd+NO increased GSA by 65.80%, δ-ALA by 40.26%, Proto IX by 28.82%, Mg-Proto IX by 47.64%, Pchlide by 39.00%, total chlorophyll by 49.26%, and carotenoids by 19.30%. Alleviation of the negative effects of Ni toxicity due to individual and combined Spd and NO treatments was observed. Maximal mitigation rates of 70.07% for GSA, 76.59% for δ-ALA, 81.45% for Proto IX, 94.93% for Mg-Proto IX, 81.38% for Pchlide, 92.66% for total chlorophyll, and 47.54% for carotenoids in Ni+Spd+NO-treated compared to Ni-treated plants were observed (Figures 1A–G).

NO and/or Spd alleviates the decline in gas exchange and fluorescence parameters

The effects of Ni toxicity on the photosynthetic parameters P_n, E, C_i, g_s, F_v/F_m, qP, NPQ, and ETR are shown in Figures 2 and 3. Relative to the control, Ni-treated plants exhibited decline rates of 49.18% in P_n, 30.46% in C_i, 60.00% in g_s, 39.09% in E, 25.03% in F_v/F_m, 18.69% in qP, and 36.51% in ETR; however, NPQ increased by 43.87%. However, the application of NO and Spd individually and in conjunction alleviated the Ni-induced decline to a considerable extent. Under normal conditions, the combined application of Spd and NO maximally increased the P_n, C_i, g_s, E, F_v/F_m, qP, and ETR by 58.74%, 31.44%, 40.00%, 28.18%, 16.37%, 22.82%, and 42.95%, respectively, compared to the control, but decreased NPQ by 47.98% (Figures 2 and 3).

NO and/or Spd alleviates the oxidative damage induced by Ni

Treatment with Ni resulted in the increased generation of H_2O_2 (174.25%), the activity of lipoxygenase (102.39%), and lipid peroxidation (266.55%) compared to the control. Relative to the control, the application of Spd and NO significantly reduced H_2O_2, the activity of lipoxygenase, and lipid

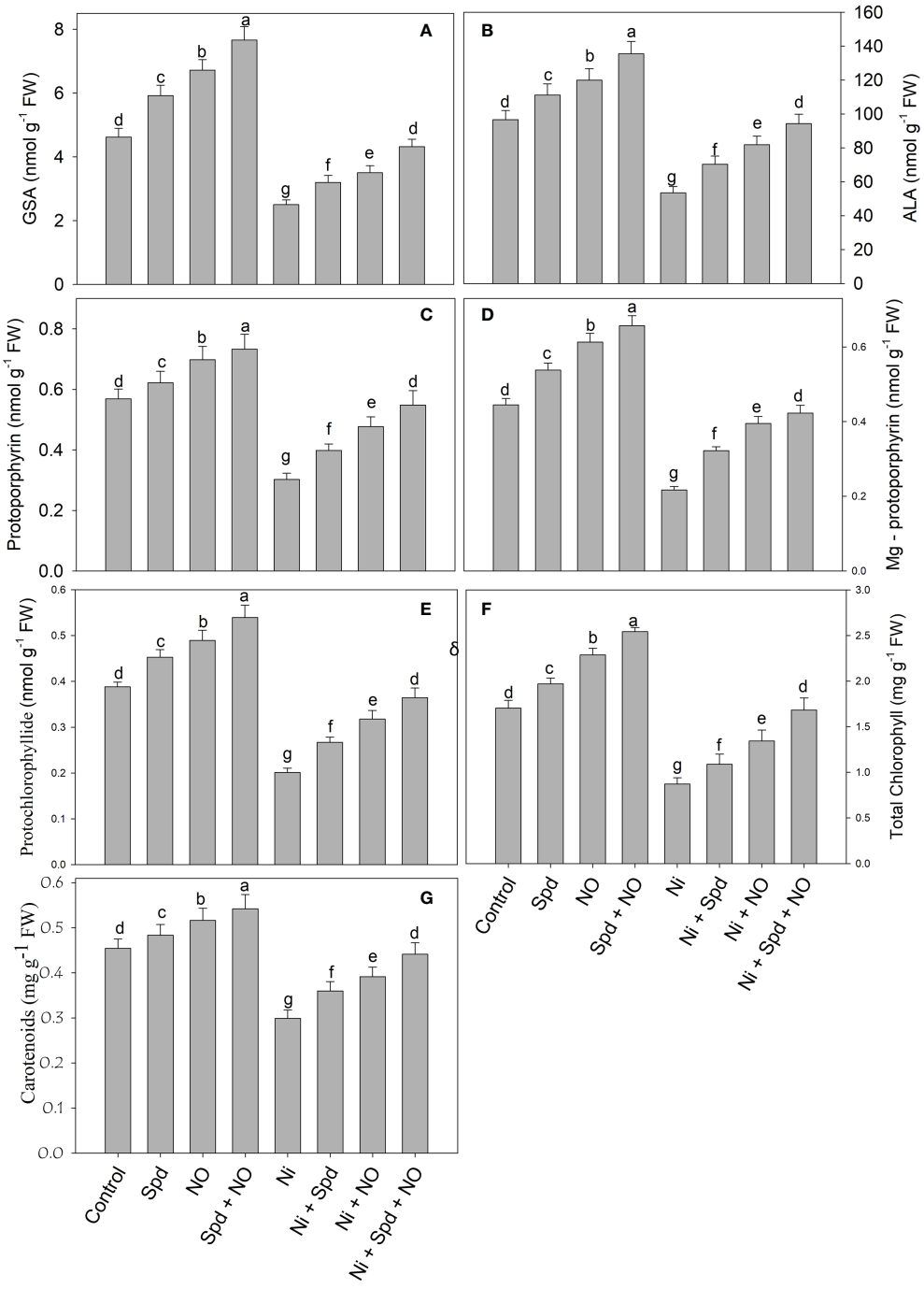

FIGURE 1
Effect of nitric oxide (50 μM) and spermidine (100 μM) supplementation on **(A)** glutamate 1-semialdehyde, **(B)** δ-amino levulinic acid, **(C)** prototoporphyrin IX, **(D)** Mg-prototoporphyrin IX, **(E)** protochlorophyllide (Pchlide), **(F)** total chlorophyll and **(G)** carotenoids content in *Solanum lycopersicum* L. subjected to nickel stress. Data is mean (±SE) of four replicates and different letters on bars denote significant difference at $P < 0.05$.

peroxidation, attaining maximal reductions of 38.13%, 48.67%, and 42.45%, respectively, in seedlings treated with Spd+NO compared to the control (Figure 4). Treatment with Spd and NO individually or in combination in Ni-treated plants decreased the generation of H_2O_2, the activity of lipoxygenase, and lipid peroxidation, with maximal reductions of 49.34%, 46.78%, and 48.98%, respectively, observed in Ni+Spd+NO-treated compared to Ni-treated plants (Figures 4A–C).

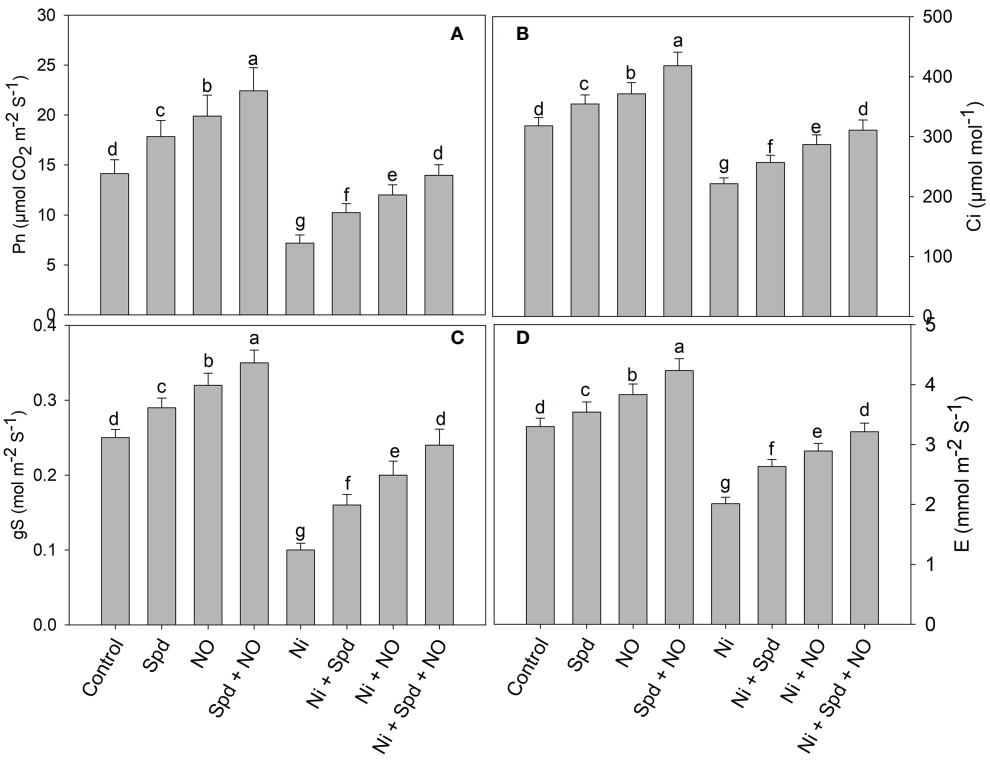

FIGURE 2
Effect of nitric oxide (50 μM) and spermidine (100 μM) supplementation on **(A)** photosynthesis, **(B)** intercellular CO2 concentration, **(C)** stomatal conductance and **(D)** transpiration rate in *Solanum lycopersicum* L. subjected to nickel stress. Data is mean (±SE) of four replicates and different letters on bars denote significant difference at $P < 0.05$

Activity of glyoxalase system enzymes is upregulated due to NO and/or Spd

Plants treated with Ni exhibited increased accumulation of MG and activities of Gly I and Gly II compared to the control (Figures 5A–C). Increases of 94.11% in the MG content, 37.40% in the activity of Gly I, and 42.42% in Gly II activity due to Ni toxicity were observed compared to the control. The application of Spd and NO to Ni-treated plants further increased the activities of Gly I and Gly II, attaining maximal increases of 65.00% and 90.57%, respectively, in Ni+Spd+NO-treated plants compared to the control. Under normal conditions, the application of Spd and NO increased the activities of Gly I and Gly II compared to the control, with increases of 30.42% and 31.41%, respectively, in plants treated with Spd+NO. The content of MG exhibited decline rates of 15.24%, 27.61%, and 44.65% due to Spd, NO, and NO+Spd treatments, respectively, compared to the control. The application of Spd and NO individually or in combination to Ni-treated plants reduced the MG content significantly compared to Ni-stressed plants, attaining a maximal decline rate of 47.18% in Ni+Spd+NO-treated plants compared to Ni-treated plants (Figure 5).

Influence of NO and/or Spd on the antioxidant system

The activities of SOD, APX, MDHAR, DHAR, and GR increased by 92.58%, 37.91%, 84.39%, 65.74%, and 49.59%, respectively, due to Ni toxicity compared to the control. The application of Spd and NO to Ni-stressed plants caused a further increase in their activities, which showed maximal enhancements of 196.00%, 140.82%, 146.47%, 132.50%, and 160.48% for SOD, APX, MDHAR, DHAR, and GR, respectively in Ni+Spd+NO-treated plants compared to the control (Table 2). Under normal growth conditions, Spd and NO treatments enhanced the activities of the antioxidant enzymes assayed; however, maximal increases of 66.86% for SOD, 29.28% for APX, 61.48% for MDHAR, 49.70% for DHAR, and 50.80% for GR were exhibited by plants that received treatment with Spd+NO compared to the control. The contents of AsA and GSH respectively increased by 11.28% and 12.69% due to Spd, by 18.61% and 20.36% due to NO, and by 30.17% and 23.75 due to Spd+NO treatments compared to the control. Relative to the control, Ni treatment reduced AsA by 27.61%, but increased GSH by 22.81%. The application of Spd

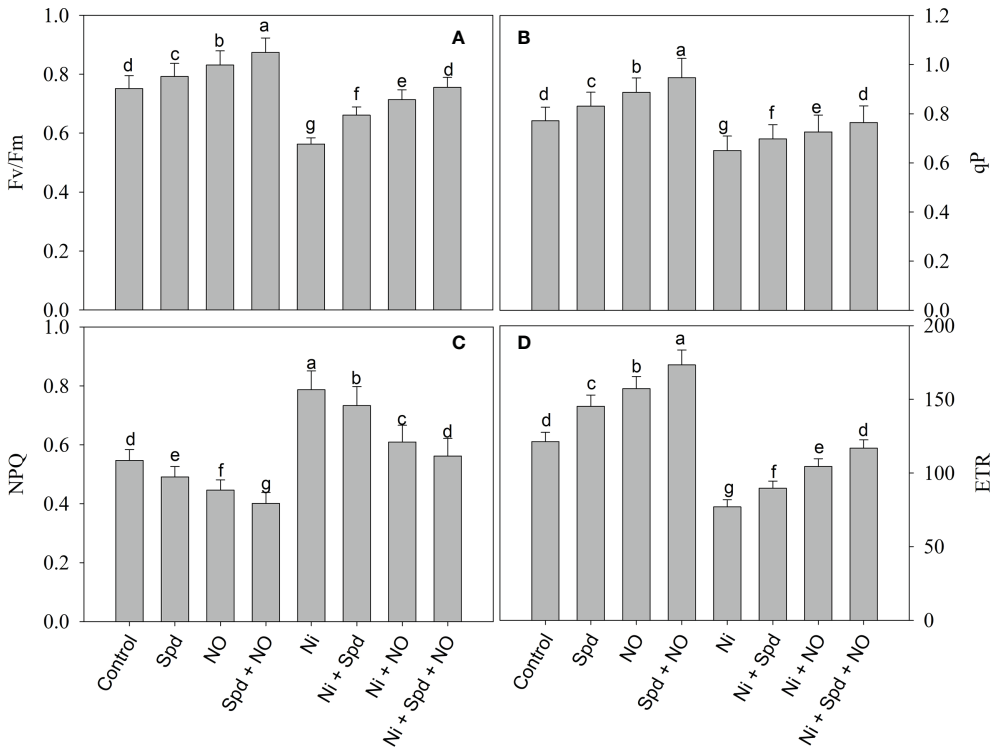

FIGURE 3
Effect of nitric oxide (50 μM) and spermidine (100 μM) supplementation on **(A)** PSII activity (Fv/Fm), **(B)** photochemical quenching (qP), **(C)** non photochemical quenching (NPQ) and **(D)** electron transport rate (ETR) in *Solanum lycopersicum* L. subjected to nickel stress. Data is mean (±SE) of four replicates and different letters on bars denote significant difference at $P < 0.05$.

and NO alleviated the decline in AsA, with a maximum alleviation of 35.42% in Ni+Spd+NO-treated plants compared to their Ni-stressed counterparts. The content of GSH further increased due to the application of Spd and NO, gaining a maximum increase of 49.42% in Ni+Spd+NO-treated plants compared to the control (Table 2).

Discussion

Metal pollution is one of the primary problems impeding sustainable crop production. Metals and metalloids hinder growth by causing alterations in root development, enzyme activity, photosynthesis, and floral development (Hameed et al., 2016; Alyemeni et al., 2018). Management techniques and biotechnological approaches have been adopted to strengthen the tolerance of plants to the toxic effects of metals so that the damage to productivity can be lessened. In the present study, an effort was made to investigate the beneficial role of the application of NO and Spd for the protection of tomato from the damaging effects of excess Ni. The application of NO and Spd was proven beneficial in improving growth in terms of plant height and dry biomass accumulation under normal conditions, which also alleviated the decline induced by Ni; however, the effect was more obvious in plants given both NO and Spd treatments. Ni-induced growth reduction has been previously reported by others (Soliman et al., 2019; Amjad et al., 2020). Treatment with Ni restricts growth by interfering with the cell division of the root and metaxylem cells, thereby restricting the cellular proliferation and tissue elongation (Demchenko et al., 2005). Treatment with NO and Spd may have regulated the cellular division and tissue proliferation (Shen et al., 2013); however, the mechanisms underlying the mitigation of Ni-induced growth retardation by NO and Spd are largely unknown. Improved growth and alleviation of Ni-induced toxic effects due to NO and Spd supplementation can be ascribed to the significant improvement in the uptake of key nutrients such as N, P, K, and Mg. Maintaining sufficient concentrations of the essential mineral nutrients triggers growth stimulation and other regulatory mechanisms through their active involvement in key functions including enzyme activity, cell division, photosynthesis, and activation of the tolerance mechanisms (Sarwar et al., 2019). For example, N (Iqbal et al., 2015), P (Begum et al., 2020), and K (Ahanger and Agarwal, 2017) have been reported to regulate growth under different stresses by upregulating the tolerance mechanisms,

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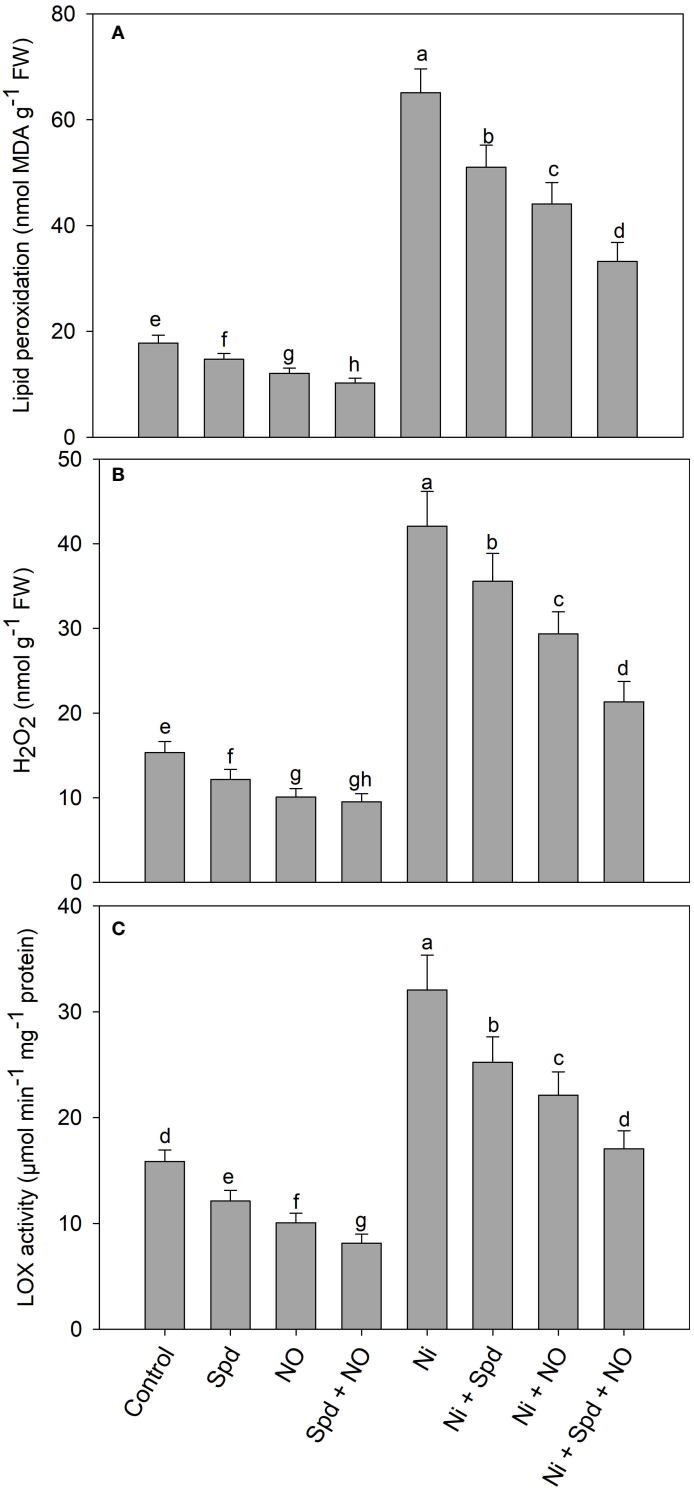

FIGURE 4
Effect of nitric oxide (50 µM) and spermidine (100 µM) supplementation on **(A)** lipid peroxidation, **(B)** hydrogen peroxide and **(C)** activity of lipoxygenase in *Solanum lycopersicum* L. subjected to nickel stress. Data is mean (±SE) of four replicates and different letters on bars denote significant difference at $P < 0.05$.

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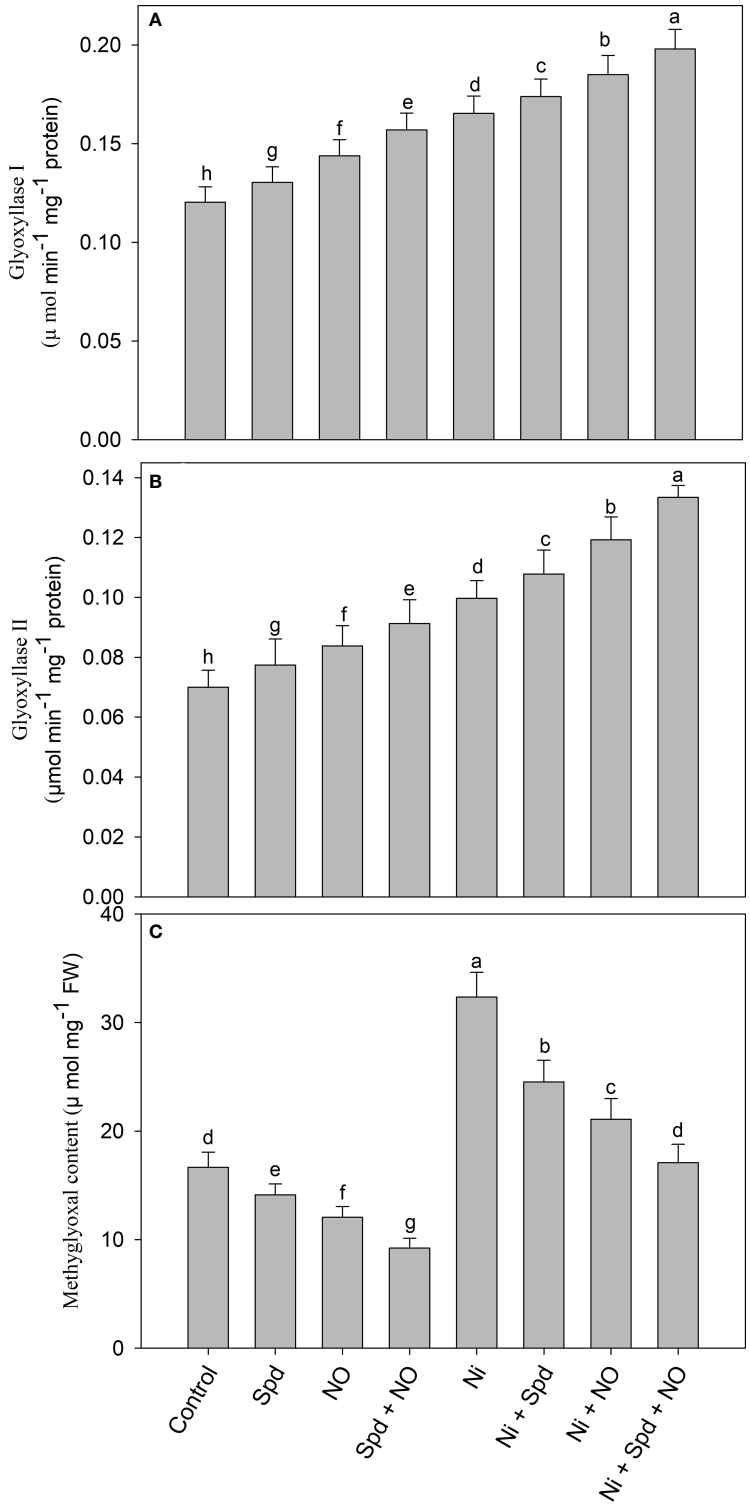

FIGURE 5
Effect of nitric oxide (50 μM) and spermidine (100 μM) supplementation on **(A)** activity of **(A)** glyoxylase I, **(B)** glyoxylase II and **(C)** methylglyoxal content in *Solanum lycopersicum* L. subjected to nickel stress. Data is mean (±SE) of four replicates and different letters on bars denote significant difference at $P < 0.05$.

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TABLE 2 Effect of nitric oxide (NO; 50 μM) and spermidine (Spd; 100 μM) supplementation on the activity of superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) and the contents of ascorbate (AsA) and reduced glutathione (GSH) in *Solanum lycopersicum* L. treated with nickel (Ni).

	Control	Spd	NO	Spd+NO	Ni	Ni+Spd	Ni+NO	Ni+Spd+NO
SOD	12.01 ± 0.917h	15.12 ± 1.27g	17.53 ± 1.58f	20.04 ± 1.91e	23.13 ± 2.32d	28.87 ± 2.52c	31.21 ± 2.88b	35.55 ± 3.24a
APX	0.823 ± 0.063h	0.907 ± 0.068g	0.961 ± 0.073f	1.064 ± 0.087e	1.135 ± 0.091d	1.491 ± 0.098c	1.709 ± 0.097b	1.982 ± 0.10a
MDHAR	32.04 ± 2.7h	37.21 ± 3.2g	43.06 ± 3.7f	51.74 ± 4.2e	59.08 ± 4.6d	65.15 ± 4.8c	70.83 ± 5.2b	78.97 ± 5.5a
DHAR	51.44 ± 3.7h	60.28 ± 4.5g	68.06 ± 4.7f	77.01 ± 4.8e	85.26 ± 5.3d	94.8 ± 5.8c	107.51 ± 6.1b	119.6 ± 6.4a
GR	1.24 ± 0.061h	1.34 ± .065g	1.51 ± 0.069f	1.87 ± 0.077e	2.46 ± 0.82d	2.73 ± 0.087c	2.96 ± 0.091b	3.23 ± 0.11a
AsA	333.1 ± 13.2d	370.7 ± 16.6c	395.1 ± 18.6b	433.6 ± 20.1a	241.1 ± 9.2g	278.7 ± 10.1f	298.8 ± 11.6e	326.5 ± 13.0d
GSH	319.1 ± 11.3h	359.6 ± 13.5g	384.1 ± 15.7f	418.5 ± 17.8e	391.9 ± 16.6d	436.3 ± 20.3c	456.8 ± 20.8b	476.8 ± 21.2a

Data are the mean ± SE of three replicates. Different letters designate significant difference at $p < 0.05$.

thereby neutralizing the damaging effects of ROS on delicate molecules and pathways. A reduction in the mineral elements caused by Ni stress has been reported by Soliman et al. (2019) and Amjad et al. (2020). Ni not only interferes with the uptake of mineral ions through the roots but also disrupts their translocation to the shoot and fruit (Pandey and Sharma, 2002). Treatment with Ni reduced the influx and translocation of essential elements, such as S, P, Mg, Ca, Zn, Fe, and Mn, in different crops (Yang et al., 2008). The exogenous application of NO and Spd resulted in a significant increase in the uptake of N, P, K, and Mg, but reduced the uptake of Ni. The application of NO to tomato plants has been reported to alleviate the decrease in the uptake of essential elements under Cd stress (Ahmad et al., 2018). In rice, supplementation of Spd alleviated the decrease in Mg, K, Ca, Fe, Mo, and Mn under Al stress, thereby preventing the growth retardation and photosynthetic decline (Jiang et al., 2021). In corroboration with our findings, Kotapati et al. (2017) have also demonstrated an alleviation of the decrease in the growth and mineral uptake of *Eleusine coracana* subjected to Ni stress with exogenous application of NO. However, there are no reports on the combined effect of NO and Spd on the alleviation of the Ni-induced decline in growth and mineral uptake.

Furthermore, the increased growth due to NO and Spd treatments under normal and Ni stress conditions can be attributed to the significant enhancement in the chlorophyll synthesis and photosynthesis. The contents of intermediates of the chlorophyll synthesis pathway, i.e., GSA, δ-ALA, Proto IX, Mg-Proto IX, and Pchlide, were significantly enhanced by the application of NO and Spd, thereby causing an evident increase in chlorophyll synthesis. Environmental stresses impart deleterious effects on the synthesis of chlorophyll by hindering the synthesis of intermediate compounds (Dalal and Tripathy, 2012). Qin et al. (2020) have demonstrated that salinity considerably decreases the expression of genes coding for enzymes that catalyze the key step in the chlorophyll biosynthesis pathway, thereby affecting the synthesis of chlorophyll and its intermediate compounds. Previously, a significant reduction in the chlorophyll content of *Phaseolus vulgaris* (Taibi et al., 2016) and wheat (Parlak, 2016) due to Ni toxicity has been reported; however, there are no available reports on the influence of Ni on the contents of intermediate compounds of the chlorophyll synthesis pathways. Supplementation of NO and Spd improved the uptake of Mg, a key component of the chlorophyll molecule, and, in addition, may have upregulated the activity of enzymes that mediate the synthesis of chlorophyll. Supplementation of NO (Ahmad et al., 2020) and Spd (Li et al., 2018) has been reported to alleviate the stress-induced decrease in chlorophyll synthesis, resulting in a significant enhancement in photosynthesis and gas exchange. Carotenoids act as accessory light-harvesting pigments and protect the photosynthetic apparatus from the toxic effects of radicals, act as redox intermediates in the secondary pathway of electron transfer with PSII, and bring about the stabilization of pigment–protein complexes (Frank and Brudvig, 2004; Hashimoto et al., 2016; Zulfiqar et al., 2021). Recently, Al-Mushhin (2022) has demonstrated significant alleviation in the salinity-induced decrease in δ-ALA, Proto IX, Mg-Proto IX, chlorophyll, and photosynthesis with Spd application. The combined application of NO and Spd maximally enhanced the chlorophyll and carotenoid synthesis and the stomatal and non-stomatal attributes of photosynthesis under normal conditions and Ni treatment. The enhanced chlorophyll synthesis, photosynthesis, and fluorescence parameters due to NO and Spd treatments may be due to the significant improvement in antioxidant function resulting in the quick elimination of toxic ROS, thereby protecting the major structures of the photosynthetic apparatus. Similar to our observations, Khan and Khan (2014); Soliman et al. (2019), and Khan et al. (2016) have reported significant decreases in the stomatal (P_n, C_i, g_s, and E) and non-stomatal (F_v/F_m, qP, and ETR) attributes of photosynthesis due to Ni toxicity. Both NO and Spd have been reported to impart beneficial effects on the photosynthetic efficiency by regulating the gas exchange, water uptake, and the PSII function (Li et al., 2015; Ahmad et al., 2018;

Ahmad et al., 2020). Feng et al. (2021) have demonstrated that treatment with NO induces photoprotection of PSI and PSII by initiating the D1 protein repair pathway under cold stress. Similarly, Spd-induced protection to D1 protein under salinity–alkalinity stress has been reported by Hu et al. (2016). Recently, in heat-stressed lettuce, He et al. (2022) have also observed significant alleviation in the parameters P_n, C_i, g_s, E, F_v/F_m, qP, and ETR with Spd application, which resulted in improved growth, water use efficiency, and biomass production. Improved chlorophyll synthesis and photosynthesis in NO- and Spd-treated plants can directly influence the carbon metabolism and the carbon–nitrogen balance (Du et al., 2016; Farag et al., 2017), which will otherwise be drastically affected by Ni stress (Sheoran et al., 1990). NO and Spd might have imparted synergistic effects to protect photosynthesis. Further studies are of interest.

In addition to the growth enhancement and photoprotection, the application of NO and Spd induced a significant reduction in the oxidative damage by reducing the generation of toxic radicals such as H_2O_2. Excess concentrations of ROS are toxic to normal plant metabolism and cause membrane dysfunction due to the oxidation of membrane lipids and proteins, thereby affecting the functions of key organelles such as chloroplast and the mitochondria (Shahid et al., 2014; Kapoor et al., 2019). An increased ROS production and lipid peroxidation due to Ni treatment has been reported in *Brassica juncea* (Khan et al., 2016), *Zea mays* (Amjad et al., 2020), and *Vicia faba* (Helaoui et al., 2022). Increased ROS due to stress conditions triggers the peroxidation of membrane lipids and proteins, thereby reducing their structural and functional stability, causing the leakage of essential cellular constituents to occur (Ahanger et al., 2019; Qin et al., 2021). Exogenous application of NO (Fatma et al., 2016) and Spd (Li et al., 2015) significantly reduces the generation of ROS, hence protecting the growth and photosynthetic function by maintaining the structural and functional stability of the key components. However, the combined effect of NO and Spd has not been investigated. Increased lipoxygenase activity determines the enhanced hydroperoxidation of polyunsaturated fatty acids (Babenko et al., 2017), and elevated activity of lipoxygenase reflects a surge in damage to membrane fatty acids (Nahar et al., 2016). The reduced accumulation of ROS in NO- and Spd-treated plants can be due to the significant upregulation of the antioxidant system, which reduces the peroxidation of membranes, hence protecting the function of the major cellular organelles. SOD forms the key defense against superoxide radicals, while APX, MDHAR, DHAR, and GR are important enzymatic components of the ascorbate–glutathione cycle, which also involves AsA and GSH (Ahanger et al., 2017; Kapoor et al., 2019). Although Ni toxicity triggered the activity of antioxidant enzymes, the application of NO and Spd further increased their activity, thereby strengthening ROS scavenging. Spd (Jiang et al., 2021) and NO (Fatma et al., 2016) treatment has previously been reported to upregulate the antioxidant function under aluminium and salt stress, thereby reducing the ROS accumulation and lipid peroxidation. The upregulation of the antioxidant system leads to the regulation of growth, membrane function, redox homeostasis, photosynthesis, and mitochondrial electron transport (Ahmad et al., 2018). The ascorbate–glutathione cycle eliminates excess H_2O_2 from the chloroplast and the mitochondria and maintains the optimal concentrations of the redox components, including AsA and GSH (Hasanuzzaman et al., 2019b). GSH has an important role in glyoxalase cycle as well. In addition, the upregulation of the ascorbate–glutathione cycle leads to the maintenance of optimal NADPH/NADP so that the electron transport is least affected and radical generation is reduced (Ahmad et al., 2020). In the present study, NO- and Spd-treated plants maintained an increased ETR and concentration of the redox components, which can be ascribed to the upregulated function of the ascorbate–glutathione cycle. Both ascorbate and glutathione are involved in the elimination of key components of the redox system and the ascorbate–glutathione cycle; therefore, their increased accumulation due to NO and Spd can eliminate the stress-induced damaging effects on the key cellular organelles, macromolecules, and pathways. By maintaining the redox state of α-tocopherol and zeaxanthin, glutathione protects the biological membranes and prevents the oxidative denaturation of proteins under stress conditions by protecting thiol groups. In addition, it acts as a substrate for the key antioxidant enzymes glutathione peroxidase and glutathione *S*-transferase (Hasanuzzaman et al., 2017). Ascorbate effectively scavenges ROS directly or indirectly, thereby playing a critical role in the tolerance to oxidative damage, besides acting as a cofactor for several enzymes (Xiao et al., 2021).

The accumulation of compatible osmolytes, including proline and GB, was enhanced with Ni treatment, and supplementation of NO and Spd further enhanced their accumulation, attaining maximal accumulation in plants treated with both NO and Spd. Both proline and GB accumulate in significant concentrations to alleviate the deleterious effects of stress on plant function (Hasanuzzaman et al., 2019a; Ali et al., 2020). GB treatment significantly reduces the oxidative effects of Ni by reducing ROS and the lipoxygenase activity and increasing the proline content in *Pennisetum typhoideum* (Xalxo et al., 2017). The accumulation of proline (Singh et al., 2012; Parlak, 2016) and GB (Soliman et al., 2019) due to Ni toxicity has been reported earlier. Accumulation of compatible osmolytes protects metabolism, protein structure, and function; maintains the cellular water content; and scavenges ROS (Ali et al., 2020; Ghosh et al., 2021). The increased accumulation of osmolytes such as proline and GB due to NO and Spd application has been reported by others, similarly resulting in the alleviation of the damaging effects of

different stressors on plant growth (Fatma et al., 2016; Pal et al., 2018; Ahanger et al., 2019; Wang et al., 2020). The influence of NO and Spd individually or in combination on the alleviation of Ni toxicity has been rarely reported. The accumulation of compatible osmolytes significantly affects the antioxidant potential of plants, hence the stress tolerance potential (Jday et al., 2016). Increased accumulation of osmolytes is regulated at the gene expression level to directly affect the function of the enzymes controlling their metabolism (Meena et al., 2019). Osmolytes such as proline, amino acids, sugars, and GB have been suggested to maintain redox homeostasis and mediate stress signaling to protect key plant functions including photosynthesis (Rosa et al., 2009; Kishor et al., 2015; Trovato et al., 2021; Kishor et al., 2022). Therefore, the increased accumulation of osmolytes due to NO and Spd treatment under Ni stress determines their beneficial role in preventing the damaging effects on plant growth.

The glyoxalase system is another interesting mechanism to protect plants from toxic MG, and manipulating the activity of glyoxalase enzymes helps plants counter the damaging effects efficiently (Gupta et al., 2018). The activities of both Gly I and Gly II were significantly enhanced with the supplementation of NO and Spd, and the effect was more obvious due to their combined treatment. The application of NO (Ahmad et al., 2020) and Spd (Nahar et al., 2017) has been demonstrated to improve the activities of Gly I and Gly II, which was reflected in the reduced accumulation of MG, hence preventing growth retardation under arsenic and aluminium stress. The effect of NO or Spd on the function of the glyoxalase system has not been reported under Ni stress. In cadmium-stressed mung bean, Nahar et al. (2016) reported that the combined application of NO and Spd enhanced the glyoxalase function more efficiently than did individual treatments, which was reflected in the reduced MG accumulation. MG is cytotoxic at higher concentrations (Kaur et al., 2014) and can be an important signaling molecule if maintained at low concentrations (Hoque et al., 2016). Plants able to maintain the significantly increased activity of the glyoxalase system combat the stress-induced damaging effects more efficiently (Maier et al., 2012). Crop cultivars exhibiting increased activities of the antioxidant and glyoxalase system enzymes display better stress adaptation potential and strengthening of the tolerance mechanism due to exogenous protectants, proving their beneficial role in stress mitigation and their contribution toward sustainable food production (Ahmad et al., 2021).

Conclusion

Conclusively, it can be said that exogenously supplied NO and Spd have been proven beneficial in assuaging the damaging effects of Ni on the growth, chlorophyll synthesis, and photosynthesis of tomato. The alleviatory effects of NO and Spd were evident as significant reductions in ROS and MG accumulation, lipid peroxidation, and lipoxygenase activity. Furthermore, increased osmolyte accumulation and enhanced function of the antioxidant and glyoxalase systems justify the beneficial influence of NO and Spd on Ni tolerance. The results suggest a crosstalk mechanism between NO and Spd for efficient Ni toxicity adaptation at the physiological and biochemical levels.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

CQ and MA conceived and designed the study. CQ and JS carried out the experimentation. CQ, JS, and MA compiled the literature. CQ and JS wrote the initial draft. MA crosschecked the results and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ahanger, M. A., and Agarwal, R. M. (2017). Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* l) as influenced by potassium supplementation. *Plant Physiol. Biochem.* 115, 449–460. doi: 10.1016/j.plaphy.2017.04.017

Ahanger, M. A., Qin, C., Maodong, Q., Dong, X. X., Ahmad, P., Abd_Allah, E. F., et al. (2019). Spermine application alleviates salinity induced growth and photosynthetic inhibition in *Solanum lycopersicum* by modulating osmolyte and secondary metabolite accumulation and differentially regulating antioxidant metabolism. *Plant Physiol. Biochem.* 144, 1–13. doi: 10.1016/j.plaphy.2019.09.021

Ahanger, M. A., Tomar, N. S., Tittal, M., Argal, S., and Agarwal, RM (2017) Plant growth under water/ salt stress: ROS production; antioxidants and significance of added potassium under such conditions. *Physiol. Mol. Bio. Plants.* 23 (4), 731–744. doi: 10.1007/s12298-017-0462-7

Ahmad, P., Ahanger, M. A., Alyemeni, M. N., Wijaya, L., and Alam, P. (2018). Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. *Protoplasma.* 255 (1), 79–93. doi: 10.1007/s00709-017-1132-x

Ahmad, P., Alam, P., Balawi, T. H., Altalayan, F. H., Ahanger, M. A., and Ashraf, M. (2020). Sodium nitroprusside (SNP) improves tolerance to arsenic (As) toxicity in *Vicia faba* through the modifications of biochemical attributes, antioxidants, ascorbate-glutathione cycle and glyoxalase cycle. *Chemosphere.* doi: 10.1016/j.chemosphere.2019.125480

Ahmad, P., Alyemeni, M. N., Wijaya, L., Ahanger, M. A., Ashraf, M., Alam, P., et al. (2021). Nitric oxide donor, sodium nitroprusside, mitigates mercury toxicity in different cultivars of soybean. *J. Hazardous Mat.* 408. doi: 10.1016/j.jhazmat.2020.124852

Ahmad, M. S. A., and Ashraf, M. (2011). Essential roles and hazardous effects of nickel in plants. *Rev. Environ. Contam. Toxicol.* 214, 125–167. doi: 10.1007/978-1-4614-0668-6_6

Ali, S., Abbas, Z., Seleiman, M. F., Rizwan, M., Yavas, I., Alhammad, B. A., et al. (2020). Glycine betaine accumulation, significance and interests for heavy metal tolerance in plants. *Plants (Basel).* 9 (7), 896. doi: 10.3390/plants9070896

Alyemeni, M. N., Ahanger, M. A., Wijaya, L., Alam, P., Bhardwaj, R., and Ahmad, P. (2018). Selenium mitigates cadmium-induced oxidative stress in tomato (*Solanum lycopersicum* l.) plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. *Protoplasma.* 255 (2), 459–469. doi: 10.1007/s00709-017-1162-4

Al-Mushhin, A. A. (2022). Interactive effect of potassium and spermidine protects growth, photosynthesis and chlorophyll biosynthesis in Vigna angularis from salinity induced damage by up-regulating the tolerance mechanisms. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 50 (1), 12607. doi: 10.15835/nbha50112607

Amjad, M., Raza, H., Mutaza, B., Abbas, G., Imran, M., Shahid, M., et al. (2020). Nickel toxicity induced changes in nutrient dynamics and antioxidant profiling in two maize (Zea mays L.) hybrids. *Plants (Basel).* 9 (1), 5. doi: 10.3390/plants9010005

Arnon, D. I. (1949). Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* 24, 1–15. doi: 10.1104/pp.24.1.1

Asgher, M., Per, T. S., Masood, A., Fatma, M., Freschi, L., Corpas, F. J., et al. (2017). Nitric oxide signaling and its crosstalk with other plant growth regulators in plant responses to abiotic stress. *Environ. Sci. Pollut. Res.* 24, 2273–2285. doi: 10.1007/s11356-016-7947-8

Babenko, L. M., Shcherbatiuk, M. M., Skaterna, T. D., and Kosakivska, I. V. (2017). Lipoxygenases and their metabolites in formation of plant stress tolerance. *Ukr Biochem. J.* 89 (1), 5–21. doi: 10.15407/ubj89.01.005

Bai, X. Y., Dong, Y. J., Wang, Q. H., Xu, L. L., Kong, J., and Liu, S. (2015). Effects of lead and nitric oxide on photosynthesis, antioxidative ability, and mineral element content of perennial ryegrass. *Biol. Plant* 59, 163–170. doi: 10.1007/s10535-014-0476-8

Bano, C., Amist, N., and Singh, N. B. (2020). "Role of polyamines in plants abiotic stress tolerance: Advances and future prospects," in *Plant life under changing environment- responses and management.* (Elsevier USA: Academic Press), 481–496. doi: 10.1016/B978-0-12-818204-8.00021-7

Bates, L. S., Waldre, R. P., and Teare, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant Sci.* 39, 205–207. doi: 10.1007/BF00018060

Bayer, W. F., and Fridovich, J. L. (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.* 161, 559–566. doi: 10.1016/0003-2697(87)90489-1

Begum, N., Ahanger, M. A., and Zhang, L. (2020). AMF inoculation and phosphorus supplementation alleviates drought induced growth and photosynthetic decline in *Nicotiana tabacum* by up-regulating antioxidant metabolism and osmolyte accumulation. *Environ. Exp. Bot.* doi: 10.1016/j.envexpbot.2020.104088

Berni, R., Luyckx, M., Xu, X., Legay, S., Sergeant, K., Huasman, J. F., et al. (2019). Reactive oxygen species and heavy metal stress in plants: Impact on the cell wall and secondary metabolism. *Exp. Environ. Bot.* 161, 98–106. doi: 10.1016/j.envexpbot.2018.10.017

Chen, D., Shao, Q., Yin, L., Younis, A., and Zheng, B. (2019). Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. *Front. Plant Sci* 9, 1945. doi: 10.3389/fpls.2018.01945

Dalal, V. K., and Tripathy, B. C. (2012). Modulation of chlorophyll biosynthesis by water stress in rice seedlings during chloroplast biogenesis. *Plant Cell Environ.* 35, 1685–1703. doi: 10.1111/j.1365-3040.2012.02520.x

Demchenko, N. P., Kalimova, I. B., and Demchenko, K. N. (2005). Effect of nickel on growth, proliferation, and differentiation of root cells in *Triticum aestivum* seedlings. *Russian J. Plant Physiol.* 52 (2), 220–228. doi: 10.1007/s11183-005-0034-5

Doderer, A., Kokkelink, I., van der Veen, S., Valk, B., Schram, A., and Douma, A. (1992). Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochim. Biophys. Acta* 112, 97–104. doi: 10.1016/0167-4838(92)90429-H

Duda-Chodak, A., and Baszczyk, U. (2008). The impact of nickel on human health. *J. Elementol.* 13 (4), 685–696.

Du, J., Shu, S., Shao, Q., An, Y., Zhou, H., Guo, S., et al. (2016). Mitigative effects of spermidine on photosynthesis and carbon–nitrogen balance of cucumber seedlings under $Ca(NO_3)_2$ stress. *J. Plant Res.* 129, 79–91. doi: 10.1007/s10265-015-0762-3

Ellman, G. L. (1959). Tissue sulphydryl groups. *Arch. Biochem. Biophys.* 82, 70–77. doi: 10.1016/0003-9861(59)90090-6

Farag, M., Najeeb, U., Yang, J., Hu, Z., and Fang, Z. M. (2017). Nitric oxide protects carbon assimilation process of watermelon from boron-induced oxidative injury. *Plant Physiol. Biochem.* 111, 166–173. doi: 10.1016/j.plaphy.2016.11.024

Fatma, M., Masood, A., Per, T. S., and Khan, N. A. (2016). Nitric oxide alleviates salt stress inhibited photosynthetic response by interacting with sulfur assimilation in mustard. *Front. Plant Sci.* 7, 521. doi: 10.3389/fpls.2016.00521

Feng, Y., Fu, X., Han, L., Xu, C., Liu Bi, H., and Ai, X. (2021). Nitric oxide functions as a downstream signal for melatonin-induced cold tolerance in cucumber seedlings. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.686545

Foyer, C. H., and Halliwell, B. (1976). The presence of glutathione and glutathione reductase in chloroplast: A proposed role in ascorbic acid metabolism. *Planta.* 133, 21–25. doi: 10.1007/BF00386001

Frank, H. A., and Brudvig, G. W. (2004). Redox functions of carotenoids in photosynthesis. *Biochem.* 43 (27), 8607–8615. doi: 10.1021/bi0492096

Ghosh, U. K., Islam, M. N., Siddiqui, M. N., and Khan, M. A. R. (2021). Understanding the roles of osmolytes for acclimatizing plants to changing environment: a review of potential mechanism. *Plant Sig. Beh.* 16 (8), 1913306. doi: 10.1080/15592324.2021.1913306

Gould, K. S., Lamotte, O., Klinguer, A., Pugin, A., and Wendehenne, D. (2003). Nitric oxide production in tobacco leaf cells: a generalized stress response? *Plant Cell Environ.* 26, 1851–1862. doi: 10.1046/j.1365-3040.2003.01101.x

Grieve, C. M., and Grattan, S. R. (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil.* 70, 303–307. doi: 10.1007/BF02374789

Gupta, B. K., Sahoo, K. K., Ghosh, A., Tripathi, A. K., Anwar, K., Das, P., et al. (2018). Manipulation of glyoxalase pathway confers tolerance to multiple stresses in rice. *Plant Cell Environ.* 41 (5), 1186–1200. doi: 10.1111/pce.12968

Hameed, A., Rasool, S., Azooz, M. M., Hossain, M. A., Ahanger, M. A., and Ahmad, P. (2016). "Heavy metal stress: plant responses and signaling." in: *Plant Metal Interaction Emerging Remediation Techniques.* pp.557–583. doi: 10.1016/B978-0-12-803158-2.00024-2

Harel, E., and Klein, S. (1972). Light dependent formation of 5-aminolevulinic acid in etiolated leaves of higher plants. *Biochem. Biophy. Res. Commun.* 49, 364–370. doi: 10.1016/0006-291X(72)90419-6

Hasanuzzaman, M., Anee, T. I., Bhuiyan, T. F., Nahar, K., and Fujita, M. (2019a). "Emerging role of osmolytes in enhancing abiotic stress tolerance in rice," in *Advances in rice research for abiotic stress tolerance*. (Woodhead publishing/Elsevier), 677–708. doi: 10.1016/B978-0-12-814332-2.00033-2

Hasanuzzaman, M., Bhuyan, M. M. B., Anee, T. I., Parvin, K., Nahar, K., Mahmud, J. A., et al. (2019b). Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants (Basel).* 8 (9), 384. doi: 10.3390/antiox8090384

Hasanuzzaman, M., Hossain, M. A., and Fujita, M. (2011). Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. *Plant Biotechnol. Rep.* 5, 353–365. doi: 10.1007/s11816-011-0189-9

Hasanuzzaman, M., Nahar, K., Anee, T. I., and Fujita, M. (2017). Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. *Physiol. Mol. Biol. Plants* 23 (2), 249–268. doi: 10.1007/s12298-017-0422-2

Hassan, F. A. S., Ali, E. F., and Alamer, K. H. (2018). Exogenous application of polyamines alleviates water stress-induced oxidative stress of Rosa damascena Miller var. trigintipetala Dieck. *South Afr. J Bot.* 116, 96–102. doi: 10.1016/j.sajb.2018.02.399

Hashimoto, H., Uragami, C., and Cogdell, R. J. (2016). Carotenoids and photosynthesis. *Subcell Biochem.* 79, 111–139. doi: 10.1007/978-3-319-39126-7_4

Hassan, M. U., Chattha, M. U., Khan, I., Chattha, M. B., Aamer, M., Nawaz, M., et al. (2019). Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities–a review. *Environ. Sci. Pollut. Res.* 26, 12673–12688. doi: 10.1007/s11356-019-04892-x

Heath, R. L., and Packer, L. (1968). Photoperoxidation in isolated chlorplasts. i. kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125, 189–198. doi: 10.1016/0003-9861(68)90654-1

He, X., Hao, J., Fan, S., Liu, C., and Han, Y. (2022). Role of spermidine in photosynthesis and polyamine metabolism in lettuce seedlings under high-temperature stress. *Plants (Basel).* 11 (10), 1385. doi: 10.3390/plants11101385

Helaoui, S., Hattab, S., Mkhinini, M., Boughattas, I., Majdoub, A., and Banni, M. (2022). The effect of nickel exposure on oxidative stress of *Vicia faba* plants. *Bull. Environ. Contam Toxicol.* 108, 1074–1080. doi: 10.1007/s00128-022-03535-1

Hodgins, R. R., and Huystee, R. B. V. (1986). Rapid simultaneous estimation of protoporphyrin and mg-porphyrins in higher plants. *J. Plant Physiol.* 125, 311–323. doi: 10.1016/S0176-1617(86)80153-5

Hoque, T. S., Hossain, M. A., Mostafa, M. G., Burritt, D. J., Fujita, M., and Tran, L. S. P. (2016). Methylglyoxal: an emerging signaling molecule in plant abiotic stress responses and tolerance. *Front. Plant Sci.* 7, 1341. doi: 10.3389/fpls.2016.01341

Hossain, M. A., Nakano, Y., and Asada, K. (1984). Monodehydroascorbate reductase in spinach chloroplasts and its participation in the regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiol.* 25, 385–395. doi: 10.1093/oxfordjournals.pcp.a076726

Hsu, Y. T., and Kao, C. H. (2004). Cadmium toxicity is reduced by nitric oxide in rice leaves. *Plant Growth Reg.* 41, 227–238. doi: 10.1023/B:GROW.0000026514.98385.5c

Hu, L., Xiang, L., Li, S., Zou, Z., and Hu, X. H. (2016). Beneficial role of spermidine in chlorophyll metabolism and D1 protein content in tomato seedlings under salinity–alkalinity stress. *Physiol. Plant* 156, 468–477. doi: 10.1111/ppl.12398

Iqbal, N., Umar, S., and Khan, N. A. (2015). Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). *J. Plant Physiol.* 178, 84–91. doi: 10.1016/j.jplph.2015.02.006

Jackson, M. L. (1973). *Soil chemical analysis* (New Delhi: Prentice Hall of India Pvt. Ltd).

Jday, A., Rejeb, K. B., Slama, I., Saadallah, K., Bordenave, M., Planchais, S., et al. (2016). Effects of exogenous nitric oxide on growth, proline accumulation and antioxidant capacity in *Cakile maritima* seedlings subjected to water deficit stress. *Funct. Plant Biol.* 43 (10), 939–948. doi: 10.1071/FP15363

Jiang, D., Hou, J., Gao, W., Tong, X., Li, M., Chu, X., et al. (2021). Exogenous spermidine alleviates the adverse effects of aluminum toxicity on photosystem II through improved antioxidant system and endogenous polyamine contents. *Ecotox. Environ. Saf.* 207, 111265. doi: 10.1016/j.ecoenv.2020.111265

Jing, J., Guo, S., Li, Y., and Li, W. (2020). The alleviating effect of exogenous polyamines on heat stress susceptibility of different heat resistant wheat (*Triticum aestivum* l.) varieties. *Sci. Rep.* 10, 7467. doi: 10.1038/s41598-020-64468-5

Kannangara, C. G., and Schouboe, A. (1985). Biosynthesis of d-ALA in greening barley leaves. VII. glutamate-1-semialdehyde accumulation in gabaculine treated leaves. *Carlsberg Res. Commun.* 50, 179–191. doi: 10.1007/BF02907144

Kapoor, D., Singh, S., Kumar, V., Romero, R., Prasad, R., and Singh, J. (2019). Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). *Plant Gene.* 19. doi: 10.1016/j.plgene.2019.100182

Kaur, C., Ghosh, A., Pareek, A., Sopory, S. K., and Singla-Pareek, S. L. (2014). Glyoxalases and stress tolerance in plants. *Biochem. Soc. Trans.* 42 (2), 485–490. doi: 10.1042/BST20130242

Kazemi, N., Khavari-Nejad, R. A., Fahimi, H., Saadatmand, S., and Nejad-Sattari, T. (2010). Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* l. under nickel stress. *Scientia Hortic.* 126, 402–407. doi: 10.1016/j.scienta.2010.07.037

Khan, M. I. R., and Khan, N. A. (2014). Ethylene reverses photosynthetic inhibition by nickel and zinc in mustard through changes in PS II activity, photosynthetic nitrogen use efficiency, and antioxidant metabolism. *Protoplasma.* 251, 1007–1019. doi: 10.1007/s00709-014-0610-7

Khan, M. I. R., Khan, N. A., Masood, A., Per, T. S., and Asgher, M. (2016). Hydrogen peroxide alleviates nickel-inhibited photosynthetic responses through increase in use-efficiency of nitrogen and sulfur, and glutathione production in mustard. *Front. Plant Sci.* 7, 44. doi: 10.3389/fpls.2016.00044

Kishor, P. B. K., Suravajhala, P., Rathnagiri, P., and Sreenivasulu, N. (2015). Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. *Front Plant Sci.* 6, 544. doi: 10.3389/fpls.2015.00544

Kishor, P. B. K., Suravajhala, P., Rathnagiri, P., and Sreenivasulu, N. (2022). Intriguing role of proline in redox potential conferring high temperature stress tolerance. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2022.867531

Kotapati, K. V., Palaka, B. K., and Ampasala, D. R. (2017). Alleviation of nickel toxicity in finger millet (*Eleusine coracana* l.) germinating seedlings by exogenous application of salicylic acid and nitric oxide. *Crop J.* 5, 240–250. doi: 10.1016/j.cj.2016.09.002

Li, L., Gu, W., Li, J., Li, C., Xie, T., Qu, D., et al. (2018). Exogenously applied spermidine alleviates photosynthetic inhibition under drought stress in maize (*Zea mays* l.) seedlings associated with changes in endogenous polyamines and phytohormones. *Plant Physiol. Biochem.* 129, 35–55. doi: 10.1016/j.plaphy.2018.05.017

Li, J., Hu, L., Zhang, L., Pan, X., and Hu, X. (2015). Exogenous spermidine is enhancing tomato tolerance to salinity–alkalinity stress by regulating chloroplast antioxidant system and chlorophyll metabolism. *BMC Plant Biol.* 15, 303. doi: 10.1186/s12870-015-0699-7

Maier, A., Fahnenstich, H., von Caemmerer, S., Engqvist, M. K. M., Weber, A. P. M., Flugge, U. I., et al. (2012). Transgenic introduction of a glycolate oxidative cycle into *A. thaliana* chloroplasts leads to growth improvement. *Front. Plant Sci.* 3. doi: 10.3389/fpls.2012.00038

Massoud, M. B., Kharbech, O., Mahjoubi, Y., Chaoui, A., and Wingler, A. (2022). Effect of exogenous treatment with nitric oxide (no) on redox homeostasis in barley seedlings (*Hordeum vulgare* l.) under copper stress. *J. Soil Sci. Plant Nutr.* 22, 1604–1617. doi: 10.1007/s42729-021-00757-w

McGrath, S. P. (1995). "Chromium and nickel," in *In heavy metals in soils*. Ed. B. J. Alloway (London: Blackie Academic and Professional), 152–174.

McIlveen, W. D., and Negusantil, J. J. (1994). Nickel in the terrestrial environment. *Sci. Total Environ.* 148, 109–138. doi: 10.1016/0048-9697(94)90390-5

Meena, M., Divyanshu, K., Kumar., S., Swapnil, P., Zehra, A., Shukla, V., et al. (2019). Regulation of l-proline biosynthesis, signal transduction, transport, accumulation and its vital role in plants during variable environmental conditions. *Heliyon.* 5 (12), e02952. doi: 10.1016/j.heliyon.2019.e02952

Minocha, R., Majumdar, R., and Minocha, S. C. (2014). Polyamines and abiotic stress in plants: a complex relationship. *Front. Plant Sci.* 5. doi: 10.3389/fpls.2014.00175

Mukherjee, S. P., and Choudhuri, M. A. (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. *Physiol. Plant* 58, 166–170. doi: 10.1111/j.1399-3054.1983.tb04162.x

Nabi, R. B. S., Tayade, R., Hussain, A., Kulkarni, K. P., Imran, Q. M., Mun, B. G., and Yun, B.W (2019). Nitric oxide regulates plant responses to drought, salinity, and heavy metal stress. *Environ. Exp. Bot.* 161, 120–133. doi: 10.1016/j.envexpbot.2019.02.003

Nahar, K., Hasanuzzaman, M., Alam, M. M., Rahman, A., Suzuki, T., and Fujita, M. (2016). Polyamine and nitric oxide crosstalk: Antagonistic effects on cadmium toxicity in mungbean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems. *Ecotox. Environ. Saf.* 126, 245–255. doi: 10.1016/j.ecoenv.2015.12.026

Nahar, K., Hasanuzzaman, M., Suzuki, T., and Fujita, M. (2017). Polyamines-induced aluminum tolerance in mung bean: A study on antioxidant defense and methylglyoxal detoxification systems. *Ecotoxicology.* 26 (1), 58–73. doi: 10.1007/s10646-016-1740-9

Nakano, Y., and Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts. *Plant Cell Physiol.* 22, 867–880. doi: 10.1093/oxfordjournals.pcp.a076232

Olsen, S. R., Cole, C. V., Watandbe, F., and Dean, L. (1954). Estimation of available phosphorus in soil by extraction with sodium bi-carbonate. *J. Chem. Inf. Model.* 53 (9), 1689–1699.

Pal, M., Tajti, J., Szalai, G., Peeva, V., Vegh, B., and Janda, T. (2018). Interaction of polyamines, abscisic acid and proline under osmotic stress in the leaves of wheat plants. *Sci. Rep.* 8, 12839. doi: 10.1038/s41598-018-31297-6

Pandey, N., and Sharma, C. P. (2002). Effect of heavy metals Co^{2+}, Ni^{2+} and Cd^{2+} on growth and metabolism of cabbage. *Plant Sci.* 163, 753–758. doi: 10.1016/S0168-9452(02)00210-8

Parlak, K. U. (2016). Effect of nickel on growth and biochemical characteristics of wheat (*Triticum aestivum* l.) seedlings. *NJAS - Wageningen J. Life Sci.* 76, 1–5. doi: 10.1016/j.njas.2012.07.001

Principato, G. B., Rosi, G., Talesa, V., Govannini, E., and Uolila, L. (1987). Purification and characterization of two forms of glyoxalase II from rat liver and brain of wistar rats. *Biochem. Biophys. Acta* 911, 349–355. doi: 10.1016/0005-2744(80)90052-2

Qin, C., Ahanger, M. A., Zhou, J., Ahmed, N., Wei, C., Yuan, S., et al. (2020). Beneficial role of acetylcholine in chlorophyll metabolism and photosynthetic gas exchange in *Nicotiana benthamiana* seedlings under salinity stress. *Plant Biol.* 22 (3), 357–365. doi: 10.1111/plb.13079

Qin, C., Ahanger, M. A., Lin, B., Huang, Z., Zhou, J., Ahmed, N., et al. (2021). Comparative transcriptomic analysis reveals the regulatory effects of acetylcholine on salt tolerance of *Nicotiana benthamiana*. *Phytochemistry* 181, 112582. doi: 10.1016/j.phytochem.2020.112582

Rakesh, B., Sudheer, W. N., and Nagella, P. (2021). Role of polyamines in plant tissue culture: An overview. *Plant Cell. Tiss. Organ. Cult.* 145, 487–506. doi: 10.1007/s11240-021-02029-y

Rosa, M., Prado, C., Podazza, G., Interdonato, R., Gonzalez, J. A., Hilal, M., et al. (2009). Soluble sugars–metabolism, sensing and abiotic stress a complex network in the life of plants. *Plant Signal Behav.* 4 (5), 388–393. doi: 10.4161/psb.4.5.8294

Sagner, S., Kneer, R., Wanner, G., Cosson, J. P., Deus-Neumann, B., and Zenk, M. H. (1998). Hyperaccumulation, complexation and distribution of nickel in *Sebertia acuminata*. *Phytochemistry* 47, 339–347. doi: 10.1016/S0031-9422(97)00593-1

Salt, D. E., and Kramer, U. (2000). "Mechanisms of metal hyperaccumulation in plants," in *Phytoremediation of toxic metals: using plants to clean-up the environment*. Eds. B. D. Ensley and I. Raskin (New York: Wiley), 231–246.

Sarwar, M., Saleem, M. F., Ullah, N., Ali, S., Rizwan, M., Shahid, M. R., et al. (2019). Role of mineral nutrition in alleviation of heat stress in cotton plants grown in glasshouse and field conditions. *Sci. Rep.* 9, 13022. doi: 10.1038/s41598-019-49404-6

Shahid, M., Pourrut, B., Dumat, C., Nadeem, M., Aslam, M., and Pinelli, E. (2014). Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. *Rev. Environ. Contam Toxicol.* 232, 1–44. doi: 10.1007/978-3-319-06746-9_1

Shang, J. X., Li, X., Li, C., and Zhao, L. (2022). The role of nitric oxide in plant responses to salt stress. *Int. J. Mol. Sci.* 236167. doi: 10.3390/ijms23116167

Shen, Q., Wang, Y. T., Tian, H., and Guo, F. Q. (2013). Nitric oxide mediates cytokinin functions in cell proliferation and meristem maintenance in. *Molecular Plant.* 6 (4), 1214–1225. doi: 10.1093/mp/sss148

Sheoran, I. S., Singal, H. R., and Singh, R. (1990). Effect of cadmium and nickel on photosynthesis and the enzymes of the photosynthetic carbon reduction cycle in pigeon pea (*Cajanus cajan* l.). *Photosynth Res.* 23, 345–351. doi: 10.1007/BF00034865

Singh, G., Agnihotri, R. K., Reshma, R. S., and Ahmad, M. (2012). Effect of lead and nickel toxicity on chlorophyll and proline content of urd (*Vigna mungo* l.) seedlings. *Int. J. Plant Physiol. Biochem.* 4 (6), 136–141. doi: 10.5897/IJPPB12.005

Singhal, R. K., Jatav, H. S., Aftab, T., Pandey, S., Mishra, U. N., Chauhan, J., et al. (2021). Roles of nitric oxide in conferring multiple abiotic stress tolerance in plants and crosstalk with other plant growth regulators. *J. Plant Growth Reg.* 40, 2303–2328. doi: 10.1007/s00344-021-10446-8

Soliman, M., Alhaithloul, H. A., Hakeem, K. R., Alharbi, B. M., El-Esawi, M., and Elkelish, M. (2019). Exogenous nitric oxide mitigates nickel-induced oxidative damage in eggplant by upregulating antioxidants, osmolyte metabolism, and glyoxalase systems. *Plants.* 8, 562. doi: 10.3390/plants8120562

Taibi, K., Taibi, F., Abderrahim, L. A., Ennajah, A., Belkhodja, M., and Mulet, J. M. (2016). Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in Phaseolus vulgaris L. *South Afr. J. Bot.* 105, 306–312. doi: 10.1016/j.sajb.2016.03.011

Trovato, M., Funck, D., Forlani, G., Okumoto, S., and Amir, R. (2021). Amino acids in plants: Regulation and functions in development and stress defense. *Front. Plant Sci.* 12, 772810. doi: 10.3389/fpls.2021.772810

Velikova, V., Yordanov, I., and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain treated bean plants, protective role of exogenous polyamines. *Plant Sci.* 151, 59–66. doi: 10.1016/S0168-9452(99)00197-1

Wang, Y., Xiong, F., Nong, S., Liao, J., Xing, A., Shen, Q., et al. (2020). Effects of nitric oxide on the GABA, polyamines, and proline in tea (*Camellia sinensis*) roots under cold stress. *Sci. Rep.* 10, 12240. doi: 10.1038/s41598-020-69253-y

Wild, R., Ooi, L., Srikanth, V., and Münch, G. (2012). A quick, convenient and economical method for the reliable determination of methylglyoxal in milimolar concentrations: the n-acetyl- l-cysteine assay. *Anal. Bioanal. Chem.* 403, 2577–2581. doi: 10.1007/s00216-012-6086-4

Xalxo, R., Yadu, B., Chakraborty, P., Chandrakar, V., and Keshavkant, S. (2017). Modulation of nickel toxicity by glycine betaine and aspirin in *Pennisetum typhoideum*. *Acta Biologica Szegediensis.* 61 (2), 163–171.

Xiao, M., Li, Z., Zhu, L., Wang, J., Zhang, B., Zheng, F., et al. (2021). The multiple roles of ascorbate in the abiotic stress response of plants: antioxidant, cofactor, and regulator. *Front. Plant Sci.* 12, 598173. doi: 10.3389/fpls.2021.598173

Yang, X., Baligar, V. C., Martens, D. C., and Clark, R. B. (2008). Plant tolerance to nickel toxicity: II nickel effects on influx and transport of mineral nutrients in four plant species. *J. Plant Nutt.* 19 (2), 265–279. doi: 10.1080/01904169609365121

Zulfiqar, S., Sharif, S., Saeed, M., and Tahir, A. (2021). "Role of carotenoids in photosynthesis," in *Carotenoids: Structure and function in the human body*. Eds. M. Zia-Ul-Haq, S. Dewanjee and M. Riaz (Cham: Springer). doi: 10.1007/978-3-030-46459-2_5

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The interaction of ABA and ROS in plant growth and stress resistances

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The plant hormone ABA (abscisic acid) plays an extremely important role in plant growth and adaptive stress, including but are not limited to seed germination, stomatal closure, pathogen infection, drought and cold stresses. Reactive oxygen species (ROS) are response molecules widely produced by plant cells under biotic and abiotic stress conditions. The production of apoplast ROS is induced and regulated by ABA, and participates in the ABA signaling pathway and its regulated plant immune system. In this review, we summarize ABA and ROS in apoplast ROS production, plant response to biotic and abiotic stresses, plant growth regulation, ABA signal transduction, and the regulatory relationship between ABA and other plant hormones. In addition, we also discuss the effects of protein post-translational modifications on ABA and ROS related factors.

KEYWORDS

ABA, ROS, plant growth, resistances, signal regulation

Introduction

In nature, plants must adapt to their living environment to survive, which achieve the goal of individual growth and population reproduction, especially when plants are under stress conditions, including abiotic and biotic stresses. With the deepening of research, it has been found that ABA, as an important plant hormone. The main source of ABA is a *de novo* synthesis pathway that relies on carotenoids in higher plants (Nambara and Marion-Poll, 2005). ABA signal transduction begins with the ABA receptor perceiving the ABA signal, and then transmits the ABA signal downwards through a series of regulatory factors in the cell, forming a complex intracellular signal transduction network, and finally transformed into a visible physiological effect (Hauser et al.,

2011). The regulatory network downstream of the ABA signal has protein phosphatase Type-2C Protein Phosphatase (PP2C) as the main node in the cell. In plants, PP2C is a serine/threonine protein phosphatase as a negative regulator in the ABA signaling pathway (Santiago et al., 2009; Umezawa et al., 2009). Studies have reported that PP2C functionally acquired mutants *abi1-1* and *abi2-1* are highly insensitive to ABA in seed germination and seedling growth (Gosti et al., 1999). In addition, the important regulatory factors downstream of ABA signal also include a large number of transcription factors that transmit and realize the gene functions regulated by ABA signal, such as ABI3, ABI4, ABI5, etc. these are all important transcription factors in ABA affecting seed germination and seedling morphology (Finkelstein et al., 2002).

In plants, ROS are stress-responsive substance, such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radical (OH^-). In the process of aerobic metabolism, such as respiration and photosynthesis could inevitably lead to the production of various ROS in mitochondria, chloroplast and peroxisome, and a large amount of cellular ROS will also be caused in various biotic and abiotic stresses, and then leading to programmed cell death (PCD). A common feature of these different sources and types of ROS is that they cause oxidative damage to living substances such as protein, DNA, and lipid. Therefore, the balance of ROS in plants will also be strictly regulated. Plants have two ROS scavenging systems, namely the enzymatic scavenging system and the non-enzymatic scavenging system (Mittler, 2002; Mittler, 2017). The enzyme scavenging system is mainly through the reduction of enzymatic peroxides such as superoxide dismutase (SOD) and peroxidase (POD) to eliminate ROS, and the non-enzymatic scavenging system uses the ascorbate-glutathione cycle to eliminate ROS (Mittler, 2002). Interestingly, some studies found that there are many different mechanisms in plants that link ABA signaling with redox balance. Firstly, ABA induces the transcription of genes related to the ascorbate-glutathione cycle in the ROS scavenging system (Ghassemian et al., 2008). Secondly, the oxidation-decyclization from xanthine to zeaxanthin requires ascorbate oxidize to dehydroascorbate in ABA biosynthesis (Nambara and Marion-Poll, 2005; Hartung, 2010), which indirectly affects the level of ascorbate and the content of ROS (Ye et al., 2012), and also explains why ABA deficient mutants have lower ascorbate content and higher ROS enrichment (Ton et al., 2009).

ABA mediates the response of plants to a variety of environmental stresses, including abiotic stresses such as drought, salt, osmotic and cold stresses (Yoshida et al., 2014; Arif et al., 2018; Huang et al., 2018; Silva et al., 2018). Simultaneously, the degree of stress hormones response is also related to the adaptive capacity of plants to these adversity conditions (Nambara and Marion-Poll, 2005). Meanwhile, the role of ROS in plants which subjected to adversity stresses has been widely reported. Some studies believe that the accumulation of ROS is an adaptive response of plants under stress conditions (Suzuki et al., 2011), and it is not simply toxicity of metabolism. As a by-product, more and more evidences show that ROS also plays a role as a signaling molecule to regulate plant development and the expression of stress response genes, including encoding antioxidant enzyme genes, in order to regulate the production of H_2O_2 (Neill, 2002; Gechev et al., 2003; Choudhury et al., 2017; Mittler, 2017; Xue et al., 2020).

In addition, the function of ABA in plant-pathogen interactions has also been intensively studied (Ton et al., 2009; Cao et al., 2011). When facing multiple stresses, plants will initiate responses in the order of stresses or severity. In many cases though, ABA could promote the process of abiotic stress response to accelerate the initiation of defense responses to biotic stresses, such as pathogenic fungi (Robert-Seilaniantz et al., 2007; AbuQamar et al., 2017; Darma et al., 2019). In the early view, ROS would damage plant cell and accelerate PCD, as a signal molecule involved in the normal disease resistance process of plants, considered a universal early response substance to the infection of pathogenic microorganisms (Wong et al., 2008; Chi et al., 2009).

ABA not only promoted the shedding of plant organs, but also participates in the other plant growth and development processes, such as seed maturation, dormancy and germination, root growth and flower development (Leung and Giraudat, 1998; White et al., 2000; Finkelstein et al., 2002; Yoshida et al., 2006; Fujii et al., 2007). With the continuous progress of research, it has been found that under certain stress conditions, plants could generate a large amount of ROS to inhibit plant growth and development (Mittler, 2002; Agurla et al., 2018; Muhlemann et al., 2018), affect plant cell differentiation, root growth and stomata closure, etc. (Dietz et al., 2016; Simmons and Bergmann, 2016; Xu et al., 2017a).

At present, most researches concentrate on the role of ABA as a signal molecule in the response of plants to multiple environmental factors, and focus on the crosstalk between ABA and the response to biotic or abiotic stress in plants (Ma et al., 2009; Lee and Luan, 2012; Skubacz et al., 2016). ROS not only strengthens the cell wall through protein cross-linking to achieve antibacterial effect, but also could be used as a signal molecule to induce plant hypersensitivity (HY) and systemic acquired resistance (SAR) (Apel and Hirt, 2004; Xu et al., 2017b). In turn, plant pathogen associated molecular patterns, bacterial effector, signal transcription cascade, ion movement and protein kinase activation, etc., these biological processes will further promote the production of ROS. Meanwhile, another molecular mechanism has also been proposed to explain the inhibitory effect of ABA on ROS enrichment (Grant and Jones, 2009), that ABA may affect the stability of DELLA protein and induce the expression of genes which encoding ROS scavenging system related enzymes to inhibit the accumulation of ROS, among them, DELLA protein is one of the key regulators that integrate the plant hormones and environmental signals

(Achard et al., 2008; Yang et al., 2012), indicating that there is a complex regulatory relationship between ABA and ROS.

The roles of ABA and ROS in plant resistances

In adversities, such as low temperature, high light intensity, osmotic stress, etc., the content of plant endogenous hormones will change significantly and affect the physiological state of plants. Research reported that plant endogenous hormones have undergone great changes under low temperature stress, such as the decrease of gibberellin content and the sharp increase of ABA content (Shu et al., 2018). In the research on the changes of plant hormones under low temperature stress, which had more attention to ABA, showing that ABA is closely related to the cold resistance of plants (Huang et al., 2017; Agurla et al., 2018; Lv et al., 2018). It is generally believed that the ABA content of plant varieties with strong cold resistance is higher than that of varieties with weak cold resistance, and the ABA content is positively correlated with the cold resistance of plants (Huang et al., 2017; Rubio et al., 2019). Meanwhile, the rate of calvin cycle metabolism decreases under low temperature stress, which will lead to excessive consumption of photoreaction and accumulation of ROS in photosynthesis (Ensminger et al., 2006). Studies had suggested that ABA could improve oxidase activity and induce stomatal closure to reduce CO2 fixation, thereby inhibit the accumulation of ROS (Lim et al., 2015). However, a recent study has shown that OPEN STOMATA 1 (OST1), as an important kinase in the ABA signaling pathway, can phosphorylate the photosynthetic oxygen-producing protein PPD5 of chloroplasts in the photosynthesis to reduce the accumulation of ROS, promote stomatal opening, and lead to weaken the photo-protective mechanism of plants, that reflects the negative regulation effect of ABA in regulating stomata opening and drought resistance (Hong et al., 2020). In plants, proline is considered to be a molecular substance with multiple functions, many studies have shown that the enrichment of proline can promote the response of plants to different abiotic stresses (Haudecoeur et al., 2009; Szabados and Savouré, 2010; Kavi Kishor and Sreenivasulu, 2014). As a substance related to osmotic regulation, proline has been recognized as a protective molecule that protects plant cells from osmotic stress, and proline has the ability to increase the activity of antioxidant enzymes, implying its positive regulation of ROS scavenging activity function (Matysik et al., 2002). In addition, with the increase of ROS levels during different metabolic changes caused by abiotic stresses (Foyer and Noctor, 2005), ABA signaling and ABA-dependent proline enrichment have been shown as an important part of the tolerance signal network while the plants resist different stresses (Pastori and Foyer, 2002). Under osmotic and salt stresses, the activities of proline biosynthesis related enzymes pyrroline-5-carboxylate synthetase (P5CS) and P5C reductase (P5CR) (Hu et al., 1992) are activated by ABA (Verslues et al., 2007; Szabados and Savouré, 2010). Research finding that the reduction of ROS enrichment is accompanied by the increase of proline content in the *P5CS* over-expression transgenic tobacco (Hong et al., 2000; Siripornadulsil et al., 2002). Correspondingly, the decrease of proline level promotes the ROS enrichment and leads to oxidative damage of plant cells in the *p5cs1* insertion mutant (Szekely et al., 2008). Previous reports have also shown that salt stress-induced proline enrichment is dependent on the presence of ABA, which is consistent with the regulation of proline on redox, indicating that proline may act as an antioxidant in plants (Frank and James, 2006; Hoque et al., 2008). In addition, ABA also participates in ROS clearance process induced by salt stress in rice, however, ABA not only induces the expression of *OsP5CR* to generate proline through OsMADS25, but also induces the expression of *OsGST4* to generate glutathione S–transferase (GST), thereby promotes ROS clearance and enhances the salt tolerance of rice, simultaneously, this study also shows that auxin may play a very important role in the ABA regulation of ROS clearance (Xu et al., 2018) (Figure 1).

In the process of plant disease resistance and defense response, ABA exhibits dual characteristics in the process of pathogen infection and reproduction. For example, when plants are infected by the bacterial pathogen *Pseudomonas syringae pv. tomato* strain DC3000 (DC3000), ABA inhibits callose deposition and the expression of related genes induced by pathogen-associated molecular pattern (PAMP), that plays a negative regulatory role (de Torres Zabala et al., 2009). Meanwhile, when plant leaves are infected by bacterial pathogens, ABA will promote the closure of stomata to prevent the pathogens from spreading further in the plant (Melotto et al., 2006). In addition, ABA can promote the formation of callosum to block pathogenic bacteria continue to invade plants (Oide et al., 2013), that plays a positive regulatory role (Figure 1). The two-way regulation function is a hotspot on the interaction between plants and pathogens, and the kinetics of pathogen infection in the current research. When plants are infected by pathogens, that will induce the production of ROS and act as a signal molecule to activate plant hypersensitivity, which further starts systemic acquired resistance (SAR) (Apel and Hirt, 2004), or triggers off PCD to prevent further spread of the pathogens (Suzuki and Katano, 2018) (Figure 1). Interestingly, ABA can become the member of the infection strategy of pathogens in plants. For example, DC3000 can induce the enrichment of ABA and the expression of ABA signaling elements in *Arabidopsis thaliana*, this result is beneficial to the propagation of bacteria and the acceleration of the infection process (de Torres-Zabala et al.,

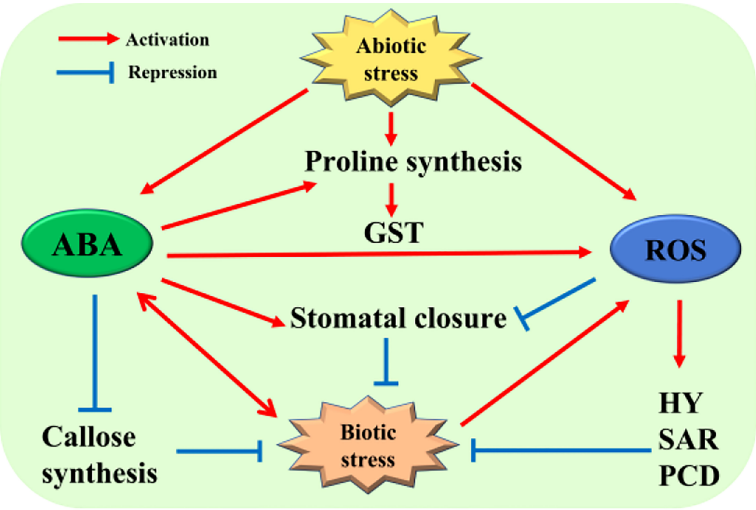

FIGURE 1
Regulatory network of the plant response to stress between ABA and ROS. Both biotic and abiotic stresses can cause the accumulation of ABA and ROS in plants, and ABA and ROS can also inhibit or adapt to external stress by regulating the content of some substances in plants or regulating the defense response of plants to maintain normal growth and development. For detailed explanation, please see text.

2007). In addition, some pathogenic fungi, such as *Botrytis cinerea* and *Magnaporthe grisea*, directly promote the production of ABA to further accelerate the infection process and efficiency of pathogens (Audenaert et al., 2002; Cao et al., 2011).

The relationship between ABA and ROS in plant growth and development

In plants, the root development is attributed to the elongation and growth of root cells, as well as the continuous division and differentiation of cells (Ramirez-Parra et al., 2017), which is affected by the homeostasis of ROS in plants (Tsukagoshi et al., 2010). As an important signal molecule, ROS affects many aspects of plant growth and development, such as cell cycle, PCD, hormone signaling, and plant response to environmental stress (Xie et al., 2014; You et al., 2014; Leng et al., 2017; Tognetti et al., 2017), if the balance of ROS is disturbed, the growth and development of plants will be affected (Zhang et al., 2014). As an important hormone that regulates plant metabolism, ABA also plays an important role in the growth and development of plant roots. In *Arabidopsis*, the ABA hypersensitive mutant *abo6* accumulates a high concentration of ROS in the root tip, which slows down the growth rate of the root tip and reduced the activity of the root meristem (He et al., 2012; Yang et al., 2014). However, the exogenous application of GSH can partially restore the phenotype of root growth in *abo6* mutant, it indicates that ROS generated in root tissues have a negative regulatory effect on root growth and development (He et al., 2012; Yang et al., 2014). Other reports also show the same regulatory relationship, exogenous application of GSH promotes the growth of *Arabidopsis* roots, while reducing the content of related antioxidants will inhibit the development of roots (Sanchez-Fernandez et al., 1997; Vernoux et al., 2000). Study has found that the domesticated mung bean seedling is detected the high content of ABA and the hypersensitivity to ABA, and showing a faster stomatal closure response, enhancing extracellular ROS production, and elevating antioxidant enzyme activity to adapt to environmental stresses, such as the damage caused by the burst of oxidation generated by osmotic stress, that is beneficial to the growth and development of mung bean seedlings (Sahu and Kar, 2018). Although the molecular interaction mechanism of ABA, ROS and antioxidant in the process of plant domestication is not clear, it still shows that there is a possible cross network which regulates plant stress tolerance and growing development.

Plant roots are often exposed to a variety of abiotic stresses, among them, high salt stress is the most common adversity condition, which strongly restricts the growth of plant roots and acts as a stress signal to promote the production of root tissue ROS. In plants, overmuch concentration of ROS will be toxic to plant cells. Therefore, the level of ROS must be strictly controlled (Qi et al., 2017). In order to reduce the oxidative damage of ROS to cells and maintain the intracellular redox balance, plants have evolved many defense systems, including ROS enzyme scavenging systems, such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and glutathione

peroxidase (GPX) (Foyer and Noctor, 2005). A large number of studies have shown that the ROS enzyme scavenging system is related to the tolerance and growth status of plants under abiotic stress (Dong et al., 2013; Huda et al., 2013; You et al., 2014). Constitutive overexpression of *APX2* in the gain-of-function mutants can significantly enhance plant drought tolerance, improve plant water utilization efficiency, and maintain H_2O_2 homeostasis in plant cells, play a role in chloroplast protection and plant growing development (Rossel et al., 2006; Wu et al., 2018). Overexpression of *APX* also shows high tolerance to salt stress in transgenic plants (Lu et al., 2007; Yan et al., 2016). Similarly, constitutive overexpression of *OsGSTU4* (glutathione S-transferase) in *Arabidopsis* can increase plant tolerance to salt and oxidative stresses, thereby promoting plant growth and development under adversity conditions (Sharma et al., 2014).

Under abiotic stresses, plants will cause premature senility of leaf through the changes of endogenous factors, which are important limiting factors for plant growth and crop yields. In the processes of these endogenous factors regulate leaf premature senility, ABA plays an important role in the connection between the oxidative damage of cell and the signal molecule response to abiotic stresses. ABA signaling mediates the expression of *NYC*, *bZIP*, *WRKY* and other transcription factors on transcription level, and indirectly affects the premature senility of leaf. In addition, Ca^{2+} signaling, ROS generation, and protein degradation also lead to leaf premature senility (Asad et al., 2019). Both exogenous environmental stimulating factors and endogenous senility factors can induce the activation of plant antioxidant systems and promote NADPH oxidase to catalyze the generation of ROS (Jiang and Zhang, 2002; Foreman et al., 2003; Ma et al., 2012). At the same time, ABA-mediated ROS production by NADPH oxidase acts as a second messenger in the ABA signal transduction pathway, negatively regulating the speed of ABA signal transmission, and then inducing leaf senescence (Kwak, 2003). Reports have shown that exogenous ABA can activate the NOX activity of genes encoding *OsNox2*, *OsNox5*, *OsNox6*, *OsNox7*, etc. in rice, and then promote the generation of ROS in guard cells. (Jiang and Zhang, 2002; Zhang et al., 2009). Thereinto, the expression of *OsNox5* and *OsNox7* are dependent on low and high concentrations of ABA respectively, indicating that OsNox5 and OsNox7 have a significant correlation with the level of ABA in plant tissues (Li et al., 2018).

In the process of plant photosynthesis, excessive light damage will lead to the degradation of the photosynthetic reaction center binding protein D1 by increasing the level of ROS and reducing the activity of PSII. In the light-dependent leaf senescence process, ABA can promote the degradation of D1 protein to trigger light damage (Gan and Amasino, 1997), however, partial shielding of light will cause the accumulation of low concentration ABA in local leaves and delay the senescence of leaves (Wang et al., 2016), which indicate that low horizontal ROS accumulation can cause lower photoinhibition and oxidative damage, and induce the degradation of D1 protein. On the contrary, low concentration ABA can not only inhibit the degradation of D1 protein, but also accelerate the biosynthesis of D1 protein. Study has found that ABA can induce the expression of the D1 protein-encoding gene *PsbA* during leaf senescence, thereby increase the content of D1 protein (Asad et al., 2019), indicating that low concentration ABA and ROS have opposite effects on the regulation of leaf senescence. In the darkness, the accumulation of D1 protein in rice leaves can reduce the level of ABA and further promote the photodamage repair of the PSII system.

Studies have reported that the transcription factor OsMADS25 regulates the elongation of main root and the number of lateral roots in rice through mediating the ABA signal pathway and the ROS scavenging system. Meanwhile, OsMADS25 can specifically activate the expression of *OsP5CR*, a key element of proline synthesis, in addition, OsMADS25 can activate the expression of *OsYUC4* to promote auxin signaling, which in turn regulates root growth (Xu et al., 2018). In the process of plant root development, in addition to auxin and glucose, ROS also acts as a key signal molecule to regulate the activity of plant meristems. In particular, ROS is continuously generated in the root tip. The level of ROS controls the direction and scope of root tip growth (Liszkay et al., 2004). As a byproduct of plant cell metabolism, low level of ROS play a key role as a second messenger, regulating many important growth and development processes, including the division and differentiation of root tip meristem cells (Dunand et al., 2007; del Pozo, 2016). However, excessive ROS accumulation can cause oxidative damage to root tip cells. In mutants with altered ROS production or disrupted redox balance, the growth statuses of the taproots show significant differences (Dunand et al., 2007; Tsukagoshi et al., 2010; Yu et al., 2016; Zeng et al., 2017). The latest research showed that the increase of Ca^{2+} signal level in the cytoplasm caused by the external environment or growing development can indirectly inhibits the ABI4-mediated ABA signal pathway, thereby promoting the germination of *Arabidopsis* seeds (Kong et al., 2015). ROS also can promote the increase of Ca^{2+} content in the cytoplasm (Schroeder and Hagiwara, 1990; Pei et al., 2000; Kwak, 2003). Meanwhile, ABI4 can promote the generation of ROS through inhibiting the expression of *VTC2* (Yu et al., 2019), and ABA-induced H_2O_2 production to activate Ca^{2+} channels in the

plasma membrane (Pei et al., 2000). Therefore, whether ROS becomes an intermediate between Ca^{2+} signal and ABA signal through feedback effect in this regulatory network remains to be studied.

The roles of ABA and ROS in plant signaling

In plant cells, ABA is recognized and bound by ABA receptor RCAR/PYR1/PYL (Ma et al., 2009; Park et al., 2009), which interacts with downstream type 2C protein phosphatases (PP2Cs) to activate SNF1 (Sucrose-Non-Fermenting Kinase 1)-related protein kinase OPEN STOMATA1 (OST1)/SnRK2, which is inhibited by PP2C that promotes the dephosphorylation of Ser/Thr residues of OST1/SnRK2 to inactivate their activity (Umezawa et al., 2009; Mittler and Blumwald, 2015). Once activated, OST1 could be directly or indirectly combined, phosphorylate anion channel protein SLOW ANION CHANNEL-ASSOCIATED1, mediate ion release, cause guard cell movement, and promote stomatal closure (Geiger et al., 2009; Lee et al., 2009; Geiger et al, 2010; Brandt et al., 2012). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is known as respiratory burst oxidase homologs (RBOHs), which is the main enzyme that promotes the generation of ROS in terrestrial plants (Umezawa et al., 2010; Mittler et al., 2011), and that is a homologue with mammalian NADPH oxidase in structure and function. RBOHs cross the plasma membrane to transport electrons from the inside to outside of the cell to form $O^{2\cdot-}$, and then generates H_2O_2 spontaneously or through superoxide dismutase (SOD) (Suzuki et al., 2011). At present, studies have reported that there are 10 RBOHs members in *Arabidopsis*, among them, RBOHD and RBOHF are the most representative members, and they play a very key role in the process of apoplast ROS synthesis, such as, caused by the interaction between plants and pathogens (Torres et al., 2002; Nühse et al., 2007; Zhang et al., 2007a). OST1 targets to bind RBOHF on the plasma membrane, phosphorylates the N-terminal Ser-13 and Ser-174 residues of RBOHF, and then generates H_2O_2 through the SOD outside the plasma membrane, whereas the regulation of ABA to NADPH through OST1 is ineffective in guard cells of the *ost1* knockout mutant, and then resulting in the hindrance of ROS production (Wang and Song, 2008; Sirichandra et al., 2009). Interestingly, H_2O_2 production through OST1 regulation can be used as a feedback signal to inactivate PP2C activity, thereby further promoting the activity of OST1, forming a positive feedback loop (Raghavendra et al., 2010).

While plants live in drought and high salt environments, osmotic stress can induce a large amount of endogenous ABA enrichment to regulate the expression of stress resistance genes and the physiological processes in cells, which plays a very important role in the response of plants to adversity stress (Shi et al., 2013; Xie et al., 2016). Research evidence shows that ROS as a second messenger is an indispensable signal molecule in the ABA signal transduction pathway (Kwak, 2003; He et al., 2012; Shi et al., 2013; Suzuki et al., 2013; Gong et al., 2020). ABA stimulates the production of ROS through the NADPH oxidase of guard cells, and then induces the closure of stomata to adapt to drought stress condition (Xie et al., 2016). However, the mechanism disappears that ABA induces H_2O_2 production and stomata closure in the *rbohD*/*rbohF* double mutant, and reduces the inhibitory effect of ABA on root growing development in *Arabidopsis* (Kwak, 2003). The downstream transcription factor ABI1 (ABA INSENSITIVE1, ABI1) of ABA signal transduction pathway forms a complex with phosphatidic acid, which interacts with RBOHD/F to stimulate the generation of ROS (Zhang et al., 2009). Nevertheless, ABA loses the ability to induce ROS generation in the guard cells of the phospholipase Da1 mutant (*PLDa1*) (Zhang et al., 2004). ABA insensitivity factor ABI2 (ABA INSENSITIVE2, ABI2) also can interact with GPX3 to regulate the redox balance in guard cells (Miao et al., 2006). Research findings have confirmed that ROS is an important second messenger in the ABA signal transduction pathway.

ABA induces the opening of Ca^{2+} channels caused by hyperpolarization in the cytoplasmic membrane of guard cells, thereby increasing the concentration of Ca^{2+} in the cytoplasm (Schroeder and Hagiwara, 1990; Pei et al., 2000). As a signal molecule, ROS, especially H_2O_2, plays a very important role in ABA-mediated activation of Ca^{2+} channels (Pei et al., 2000; Kwak, 2003). The increase of the Ca^{2+} concentration in the cytoplasm activates the anion channel which binding the plasma membrane, leading to the depolarization of the plasma membrane, resulting in inhibition of the KAT1 K^+ channel (Osakabe et al., 2014). Interestingly, OST1 can phosphorylate the C-terminal domain of KAT1, thereby inhibiting K^+ channel activity (Sato et al., 2009). Therefore, it is hypothesized that ABA and Ca^{2+} play a synergistic role in regulating K^+ channel closure. Ca^{2+} is very important for the activation of RBOHD in the cell. The combination of Ca^{2+} and EF hand is necessary for the activation of RBOHD (Ogasawara et al., 2008). In addition, the activation of CPKs promoted by Ca^{2+} is also essential for the activation of RBOHD (Boudsocq et al., 2010), such as, CPK5 phosphorylates the N-terminus of RBOHD to promote its activity (Dubiella et al., 2013). Although Ca^{2+} and CPKs are upstream of RBOHD-induced ROS generation, ROS can promote intracellular Ca^{2+} accumulation and activate CPK5 in turn (Dubiella et al., 2013). Studies have reported that ROS and Ca^{2+} may play an important role in the signal transmission between cells, and the long-distance transmission of Ca^{2+} and ROS is called Ca^{2+} wave or ROS wave, which may be effective in the system response of plants to biotic or abiotic stress as an important signaling molecule (Mittler et al., 2011; Gilroy et al., 2016).

In the ABA signal transduction pathway, OST1 is an essential signal element in the process of ABA-induced ROS

generation (Mustilli et al., 2002). Moreover, studies have found that OST1 can phosphorylate the N-terminus of RBOHF (Sirichandra et al., 2009), but it is unclear whether this phosphorylation is necessary for OST1-mediated signal transduction. The latest research shows that OST1 persulfidation can enhance ABA-induced intracytoplasmic Ca^{2+} signal transduction and stomatal closure, and this persulfidation is related to the two Cys residues of OST1, which are very close to the kinase catalytic region and phosphorylation site of OST1 (Chen et al., 2020). However, it is interesting that recent studies have found that the phosphorylation of OST1 by BAK1 (BRI1-associated receptor kinase1, BAK1) is necessary for the process of ABA-induced ROS generation in guard cells (Shang et al., 2016). Considering that BAK1 is a co-receptor for LRR-RLKs (leucine-rich repeats receptor-like kinases, LRR-RLKs), it is speculated that OST1 may also be regulated by a certain LRR-RLKs. Excitingly, the latest research has discovered for the first time that the plant H_2O_2 receptor HPCA1 is an LRR receptor kinase located on the plasma membrane of the cell with two special Cys residues in its extracellular domain. Because the sulfhydryl group on the Cys residue is the target of H_2O_2 oxidation, H_2O_2 will cause the oxidation of the extracellular Cys residues of HPCA1 in guard cells, thereby activating the intracellular kinase activity of HPCA1, and further triggering the activation of Ca^{2+} channels and causing Ca^{2+} internal flow, which subsequently causes the stomatal closure. HPCA1-mediated H_2O_2-induced activation of Ca^{2+} channels is necessary for stomatal closure in guard cells (Wu et al., 2020). This receptor kinase-mediated H_2O_2 sensing mechanism is not similar to any known H_2O_2 receptor or sensor protein reported in other organisms. However, whether the endogenous H_2O_2 regulates the Cys residue on OST1, and then regulates ABA signal pathway, or exists a feedback signal synergy pathway between ABA and H_2O_2, maybe that is a new research entry point. Meanwhile, studies have found that H_2O_2 promoted by ABA inhibits the interaction of PP45 and DMI3 in rice, thereby activating DMI3, which can combine with calmodulin induced by the ABA signal pathway to regulate the ABA signal transduction process (Ni et al., 2019). It can be seen that H_2O_2 regulates the ABA signaling pathway in different ways.

The crosstalk between ABA and ROS in plant hormones network

Plant hormones can bind and induce downstream regulatory factors to affect plant growth and development in biotic and abiotic stresses, such as ABA, ethylene and brassinolide. Among them, ABA and ethylene, as two vital plant hormones, play important roles in many aspects of plant growth and development, including the response to adversity stresses (Bleecker and Kende, 2000; Finkelstein et al., 2002; Vishwakarma et al., 2017; Yin et al., 2017). Reports have shown that the accumulation of ROS in plants can not only regulate ABA-induced stomata closure and the activity of root meristems mediated by salicylic acid, but also play an important role in the Na balance mechanism in buds regulated by ethylene (Pei et al., 2000; Jiang et al., 2012; Jiang and Bel, 2013; Xu et al., 2017a). Therefore, it is speculated that there may be an important regulatory relationship between ROS and the plant hormone network, which plays an important role in the growing development of plants (Xia et al., 2015). For example, ABA induces the accumulation of ROS, which leads to the increase of Ca^{2+} content in guard cells, regulates stomatal closure in turn (Pei et al., 2000; Wang and Song, 2008). On the contrary, exogenous ABA treatment will decrease the level of ROS in rice seed germination embryos (Ye et al., 2012) (Figure 2). Ethylene can trigger the accumulation of ROS in the ozone-induced PCD and response to pathogenic bacteria to achieve the primary response of plant immunity (Overmyer et al., 2000; Overmyer et al., 2003; Mersmann et al., 2010; Tintor et al., 2013). Recent studies have shown that ethylene can regulate the MPK pathway by promoting RBOHF-mediated accumulation of ROS, which in turn leads to the accumulation of NO in plants and promotes the closure of stomata, in addition, as an important factor in the MPK pathway, MPK3/6 plays a role in the upstream of EIN2, indicating that ROS directly participates in the signal transduction regulation of ethylene (Zhang et al., 2021). Conversely, ethylene replies to the seedlings' response to salt stress and photooxidation damage by reducing the level of ROS (Zhong et al., 2009; Peng et al., 2014). The results of these studies indicate that there may be a mechanism by which ABA and ethylene co-regulate the accumulation of ROS, which is a key element in elucidating the regulatory network between plant hormones and ROS. Interestingly, many reports show that ABA and ethylene exhibit two relationships in different biological processes, synergy and antagonism (Figure 2). For example, ethylene inhibits the growth of rice roots by promoting the biosynthesis of ABA (Yin et al., 2015). In addition, when plants respond to nutrient stress, the endoderm of roots will form plugging, while the effects of ABA and ethylene on root plugging are completely opposite (Barberon et al., 2016). ABA and ethylene jointly regulate the histone acetylation of SWI-INDEPENDENT 3 LIKE1 and SWI-INDEPENDENT 3 LIKE2 to achieve an antagonistic effect on seed dormancy (Wang et al., 2013).

Ascorbate acid (AsA), as an important and highly effective non-enzymatic antioxidant, not only removes ROS generated during photosynthesis and growing development, but also plays an important role in the response of plants to biotic and abiotic stresses (Davey et al., 2000; Smirnoff and Wheeler, 2000; Gallie, 2013; Akram et al., 2017). With the discovery of multiple roles of AsA in the growing development of various plants, AsA and its

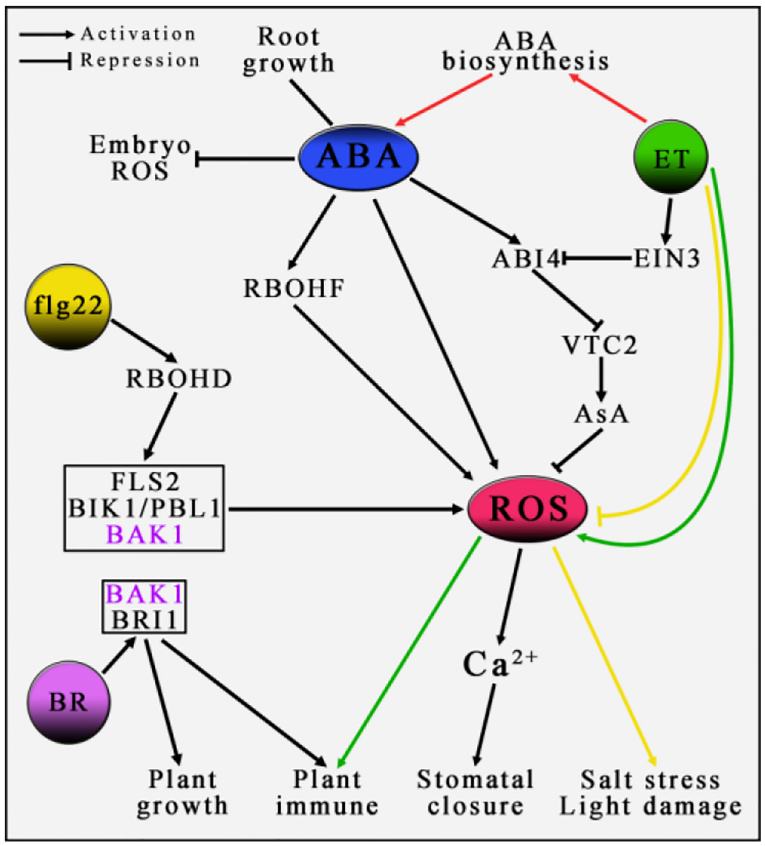

FIGURE 2
The role of ROS in the regulation of plant by hormones. In the process of plant growth and development, plant hormones play an important regulatory role, such as ABA, ET, BR, etc. In the entire regulatory network, ROS may play a central pivotal role. It remains to be elucidated whether BAK1, as an important element in the pathway of ROS generation induced by flg22, is the key site associated with the BR signaling pathway. Lines of the same color indicate the same regulatory pathway. For detailed explanation, please see text.

regulatory mechanisms have received more and more attention (De Tullio and Arrigoni, 2004; Gallie, 2013; Bulley and Laing, 2016). Studies have reported that AsA plays an important role in the antagonistic relationship between ABA and ethylene. The AsA synthesis gene *VTC2* is antagonistically regulated by the downstream factors ABI4 and EIN3 in the ABA and ethylene signal pathway respectively. EIN3 directly binds to the promoter of *ABI4* to inhibit its expression, and ABI4 directly binds to the promoter of *VTC2* to inhibit its expression, revealing a new mechanism of ABA and ethylene antagonistic regulation, that is to say, regulating the biosynthesis of AsA through the transcriptional cascade antagonism of EIN3-ABI4-VTC2, thereby affecting the accumulation of ROS in *Arabidopsis thaliana* seedlings (Yu et al., 2019) (Figure 2). With continuous literature reports, the regulatory relationship between ABA and ethylene on plant growing development has shown a complex regulatory network. However, the detailed mechanisms and key connections are still poorly understood.

Similar to the pathway that ABA induces RBOHF to trigger apoplast ROS generation, triggering apoplast ROS generation by the activation of RBOHD induced by bacterial flagellin epitope flg22 (Chinchilla et al., 2006), and the combination of receptors on the plasma membrane is composed of FLS2 (FLAGELLIN-SENSING-2), BIK1 (BOTRYTIS-INDUCED KINASE1) and PBL1 (PBS1-Like 1) (Lu et al., 2010; Zhang et al., 2010). Among them, BIK1 and PBL1 are germane cytoplasmic protein kinases, and FLS2 is a leucine-rich repeat receptor kinases (LRR-RLKs). When flg22 induces apoplast ROS generation, another LRR-RLK, BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1) acts as a co-receptor to sense flg22 signal and jointly promote the transphosphorylation of FLS2, BAK1 and BIK1/PBL1 (Chinchilla et al., 2007; Heese et al., 2007; Lu et al., 2010; Zhang et al., 2010), thereby triggering a transient burst of Ca^{2+} in the cytoplasm (Li et al., 2014; Ranf et al., 2014; Monaghan et al., 2015), apoplast ROS generation (Lu et al., 2010; Zhang et al., 2010), and resistance to bacteria and pathogenic

fungi (Veronese et al., 2006; Zhang et al., 2010). Interestingly, BAK1 acts as a brassinolide (BR) signaling regulatory element interacts with BR receptor BRI1 (Li et al., 2002; Nam and Li, 2002; Russinova et al., 2004), and co-phosphorylated to perceive and transmit BR signals (Wang et al., 2005; Wang et al., 2008), thereby mediate BR-regulated plant growth and development. Studies have found that BAK1 is not necessary in the FLS2-mediated plant immune signal pathway and BR-induced plant PAMP signal to pathogenic fungi, indicating that BAK1 may not be an intermediate element in the ABA, ROS and BR signaling networks (Figure 2). However, BAK1 can phosphorylate SnRK2.6 (Shang et al., 2016), and BIN2 kinase interacts with SnRK2.2 (Belin et al., 2006). It remains to be proved that SnRK2s and BIN2 may link ABA and BR signaling pathways, and mediate ROS generation as the key element.

Future perspective

Under normal physiological conditions, the production and consumption of ROS in cells is in a dynamic equilibrium state. An appropriate amount of ROS is of great significance to the signal transduction in plant cells and the immunity of pathogenic microorganisms. However, excessive ROS will lead to oxidative stresses, and the generation of stresses will be accompanied by a series of negative effects, such as irreversible damage to proteins, biofilms, DNA and RNA. Recent study has found that increased ROS can cause DNA breakage in maize sperm cells, resulting in haploid maize plants (Jiang et al., 2022).

There are many factors that cause plant cells to produce excessive ROS, such as ABA, high light, adversity stress, and pathogen infiltration. Among them, apoplast ROS is mainly induced by the plasma membrane binding protein NADPH oxidase (RBOH). In addition to the NADPH oxidase of apoplasts, there are also some oxidases located on the plasma membrane of chloroplasts, peroxisomes, and mitochondria, which are also related to the production of ROS, especially during the interaction between plants and pathogens (Bolwell and Wojtaszek, 1997; Apel and Hirt, 2004). The main elements involved in the generation of apoplast ROS are rich in leucine and belong to the LRR-RLK class of protein molecules. The newly discovered plant H_2O_2 receptor HPCA1 is also an LRR-RLK protein molecule located on the cytomembrane. Cys residues exposed outside the cytomembrane are covalent modification by H_2O_2, leading to HPCA1 autophosphorylation of the part inside cell, and then gets activated (Wu et al., 2020). H_2O_2 is an important molecule for cells to respond to external stresses and internal signals. H_2O_2 enters into the cell through the water channel membrane proteins and covalently modified cytoplasmic proteins to regulate signal transduction and cellular processes (Figure 3).

It has been reported in the literature that H_2S is involved in ABA-regulated ROS production and stomatal closure, conversely, excessive accumulation of ROS inhibits H_2S production, thus forming a mini-regulatory circuit (Jie et al., 2020) (Figure 3). In addition, hydrogen sulfide (H_2S) modifies SnRK2.6/OST1 through *S*-nitrosylation to participate in the molecular mechanism of regulating ABA signaling to induce

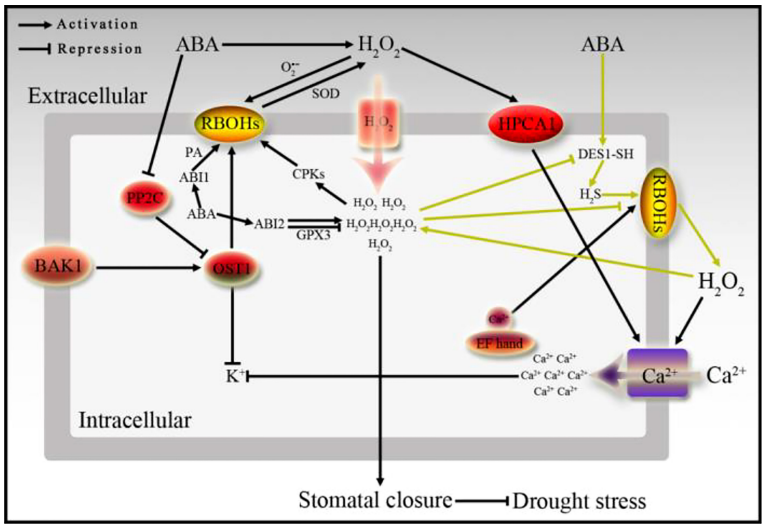

FIGURE 3
The relationship between ABA signaling and H_2O_2 in stomatal movement. ABA signal is transmitted downward through PP2C and OST1, or ABI1 and ABI2, meanwhile, ABA can promote the generation of H_2O_2 induced by RBOHs on the plasma membrane, and forms a microcirculation by H_2S. H_2O_2 is sensed by the H_2O_2 receptor HPCA1, and the H_2O_2 signal is transmitted into the cell to promote Ca^{2+} influx, inhibit K^+ accumulation, and then causes stomata to close.

plant stomatal closure (Chen et al., 2020). Interestingly, two S-sulfhydryl modification sites-Cys residues are found in SnRK2.6, when these two Cys residues are modified by S-sulfhydryl, the activity of SnRK2.6 and its interaction with transcription factors downstream of ABA signaling all will be promoted (Chen et al., 2020). Meanwhile, ABA and H_2S-induced stomatal closure and Ca^{2+} influx will be less sensitive, and leading to water loss and decreased drought resistance in the *ost1-3* mutant (Chen et al., 2020). In addition, ABA induces the DES1 enzyme in the guard cells to catalyze the production of H_2S, in turn, the H_2S positively regulates ABA signal transduction through the persulfidation of SnRK2.6 to affect calcium influx, and finally regulates stomatal movement, therefore, a new ABA signal transduction mechanism is proposed that relies on the regulation of sulfhydryl modification (Chen et al., 2020) (Figure 3). As mentioned above, the N-terminus of RBOHs contain multiple regulatory sites or motifs, including phosphorylation sites, EF hand, and phosphatidic acid binding elements, etc, which make RBOHs with binding and regulating effects (Ogasawara et al., 2008) (Figure 3). In addition to phosphorylation and sulfhydrylation, ubiquitination modification which is generally modified by E3 ubiquitin ligases is also an important regulation of ABA perception, signal transduction and response. PYR/PRLs, PP2Cs, PP2As, transcription factors and proteins encoded by ABA response genes are all targets of E3 ubiquitin ligase-mediated ubiquitination modification, and will be further degraded by the 26S proteasome. At present, it has been confirmed that a variety of E3 ubiquitin ligases are related to ABA signaling, including CRLs, RING and U-box types of ubiquitin ligases (Yu et al., 2015), and another type of E6-AP ubiquitin ligases have not been reported to be related to ABA signaling. Many ABA-related factors affect ABA signal transduction and stress resistance through the regulation of E3 ubiquitin ligases in plant growing, for example, ABA synthesis elements ABA1 and ABA2 (Lee et al., 2010), ABA receptor molecules PYL1 and PYL4 (Bueso et al., 2014), PYL8 (Irigoyen et al., 2014), ABA signaling factor ABI1 (Park et al., 2009; Kong et al., 2015), ABI2 and HAB2 (Wu et al., 2016), ABA signaling downstream transcription factor ABI3 (Kurup et al., 2000), ABI5 (Smalle et al., 2003; Lee et al., 2010; Seo et al., 2014), ABF1 (Chen et al., 2013), ABF3 (Chen et al., 2013), ATH86 (Lechner et al., 2011), SDIRIP1 (Zhang et al., 2007b; Zhang et al., 2015), and ABA downstream response gene *RD21* (Kim and Kim, 2013), etc. In addition, recent studies have reported that the new E2 ubiquitin coupling enzyme UBC27 and the RING type E3 ubiquitin ligase AIRP3 form a specific E2-E3 complex and activate the ubiquitin ligase activity of AIRP3. Meanwhile, both UBC27 and AIRP3 can directly interact with the ABA co-receptor ABI1 to regulate ABA signaling and plant drought stress response (Pan et al., 2020). Ubiquitination modification basically regulates all the related fields of ABA in plants, and further affects plant growth and development, stress resistance, and interaction with pathogens. Although the modification and regulation mechanisms of many ABA-related elements have been studied and reported, the detailed ABA signaling network still needs to be verified and supplemented.

The photosynthesis of plants will be stimulated by high light to generate ROS in the cells, thereby causing light damage to plant cells. The latest research has found that the phytochrome-related transcription factor PHYTOCHROME-INTERACTING FACTORS (PIFs) directly binds to the promoter of the *ABI5* and activating the expression of *ABI5* specifically to regulate the ABA signaling pathway in the dark, meanwhile, ABA receptors PYL8/PYL9 can directly interact with PIFs and mediate this transcriptional regulation of *ABI5* (Qi et al., 2020). PIFs are the key factors that integrate multiple signal pathways to regulate plant growth and development, and widely involved in a variety of plant hormones, such as gibberellin, ethylene, auxin, brassinolide, etc. meanwhile, mediated by external environmental factors, such as high temperature, high light, etc. in the signal regulation network (Paik et al., 2017). Whether the ROS generated by high light is involved in the signal transduction network that regulated by these plant hormones, we believe that the discovery of more ROS functions will play a very important role in the improvement of the signal regulation network. In addition, the interaction between ABA and auxin has been proven, ABA can promote auxin-mediated plant growth inhibition (Tiryaki and Staswick, 2002; Monroe-Augustus et al., 2003; Belin et al., 2009). In turn, auxin can promote ABA-mediated inhibition of seed germination and leaf senescence under different oxidative stress conditions (Liu et al., 2007; Mahmood et al., 2016). It shows that ABA and auxin co-promote the process of seed germination and seedling growth (Belin et al., 2009; Tsukagoshi et al., 2010). It is interesting to report that ABA can make use of ROS from mitochondria to antagonize the regulation of auxin in *Arabidopsis*. In turn, auxin can also attenuate the inhibitory effect of ABA on seed germination (He et al., 2012), indicating that there is both synergy and antagonism between ABA and auxin, which may be related to the concentration of plant hormones, and ROS plays an important role therein. In terms of plant defenses against pathogens, ABA may coordinate or antagonize the SA, JA, ET and other signal pathways through direct or indirect mechanisms, thereby regulating the antibacterial properties of plants (Mohr and Cahill, 2007; Robert-Seilaniantz et al., 2007; Derksen et al., 2013). This seems to be regarded as the main mechanism by which ABA induces the sensitivity of plants to a variety of pathogens. However, the role of ROS in these mechanisms has not been fully elucidated, this is also future research hotspots in plant disease resistance.

Studies have found that just restoring the expression of *RBOHD* in the xylem parenchyma or phloem cells in the *rbohD* mutant can restore the systemic ROS signal in plant. The transcriptional expression and the adaptive mechanism cause by systemic stress response to deal with the high light stimulation applied to a single leaf, indicating that RBOHD plays

a key role in regulating the generation of the vascular bundles ROS, inducing systemic signal transduction and adaptation in *Arabidopsis*. Meanwhile, the signal integration of ROS and calcium, which are important signaling molecules for long-distance transport between cells or in plants, and electricity and hydraulic pressure occurs in the vascular bundle along with the process of systemic signal transduction (Zandalinas et al., 2020) (Figure 3). A recent review article also reported that ROS is produced, enriched, converted and transported across the membrane in several organelles of plant cells, including chloroplasts, mitochondria, endoplasmic reticulum, peroxisomes and vacuoles, etc. Meanwhile, ROS is transported across the membrane between plant cells to achieve ROS signal transmission throughout the plant (Mittler et al., 2022). This study suggests that we can express RBOHD/F specifically in different tissues, cells and organelles to perfect the understanding of RBOHD/F in mediating ROS fluctuations, and understanding of plant systemic responses induced by ROS. Improving plant ABA and ROS signal regulation components and pathways will help to further understand how plants adjust their endogenous signals and regulation strategies according to environmental changes, so as to obtain better survival ability in the natural. Additionally, that will provide new insights for the elucidation of plant hormone signal network and regulatory mechanism.

Author contributions

SHL, SL and LW designed this research and wrote the manuscript. LW, QZ, MC and MZ contributed to the provided guidance of the whole manuscript. LW, SL, QZ and RW reviewed and revised the manuscript. SHL, and LW prepared the figures. NL, SW, YC and LZ checked and revised the language and format of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

AbuQamar, S., Moustafa, K., and Tran, L. S. (2017). Mechanisms and strategies of plant defense agains botrytis cinerea. *Crit. Rev. Biotechnol.* 37, 262–274. doi: 10.1080/07388551.2016.1271767

Achard, P., Renou, J. P., Berthomé, R., Harberd, N. P., and Genschik, P. (2008). Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. *Curr. Biol.* 18, 656–660. doi: 10.1016/j.cub.2008.04.034

Agurla, S., Gahir, S., Munemasa, S., Murata, Y., and Raghavendra, A. S. (2018). Mechanism of stomatal closure in plants exposed to drought and cold stress. *Adv. Exp. Med. Biol.* 1081, 215–232. doi: 10.1007/978-981-13-1244-1_12

Akram, N. A., Shafiq, F., and Ashraf, M. (2017). Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.00613

Apel, K., and Hirt, H. (2004). REACTIVE OXYGEN SPECIES: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55, 373–399. doi: 10.1146/annurev.arplant.55.031903.141701

Arif, M. A., Alseekh, S., Harb, J., Fernie, A., and Frank, W. (2018). Abscisic acid, cold and salt stimulate conserved metabolic regulation in the moss physcomitrella patens. *Plant Biol. J.* 20, 1014–1022. doi: 10.1111/plb.12871

Asad, M., Zakari, S., Zhao, Q., Zhou, L., Ye, Y., and Cheng, F. (2019). Abiotic stresses intervene with ABA signaling to induce destructive metabolic pathways leading to death: premature leaf senescence in plants. *IJMS* 20, 256. doi: 10.3390/ijms20020256

Audenaert, K., Meyer, G. B. D., and Höfte, M. M. (2002). Abscisic acid determines basal susceptibility of tomato to botrytis cinerea and suppresses salicylic acid-dependent signaling mechanisms. *Plant Physiol.* 128, 491–501. doi: 10.1104/pp.010605

Barberon, M., Vermeer, J. E. M., De Bellis, D., Wang, P., Naseer, S., Andersen, T. G., et al. (2016). Adaptation of root function by nutrient-induced plasticity of endodermal differentiation. *Cell* 164, 447–459. doi: 10.1016/j.cell.2015.12.021

Belin, C., de Franco, P. O., Bourbousse, C., Chaignepain, S., Schmitter, J. M., Vavasseur, A., et al. (2006). Identification of features regulating OST1 kinase activity and OST1 function in guard cells. *Plant Physiol.* 141, 1316–1327. doi: 10.1104/pp.106.079327

Belin, C., Megies, C., Hauserová, E., and Lopez-Molina, L. (2009). Abscisic acid represses growth of the arabidopsis embryonic axis after germination by enhancing auxin signaling. *Plant Cell* 21, 2253–2268. doi: 10.1105/tpc.109.067702

Bleecker, A. B., and Kende, H. (2000). Ethylene: A gaseous signal molecule in plants. *Annu. Rev. Cell Dev. Biol.* 16, 1–18. doi: 10.1146/annurev.cellbio.16.1.1

Bolwell, G. P., and Wojtaszek, P. (1997). Mechanisms for the generation of reactive oxygen species in plant defence–a broad perspective. *Physiol. Mol. Plant Pathol.* 51, 347–366. doi: 10.1006/pmpp.1997.0129

Boudsocq, M., Willmann, M. R., McCormack, M., Lee, H., Shan, L., He, P., et al. (2010). Differential innate immune signalling *via* Ca2+ sensor protein kinases. *Nature* 464, 418–422. doi: 10.1038/nature08794

Brandt, B., Brodsky, D. E., Xue, S., Negi, J., Iba, K., Kangasjarvi, J., et al. (2012). Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and OST1 kinases and branched ABI1 PP2C phosphatase action. *Proc. Natl. Acad. Sci. U.S.A.* 109, 10593–10598. doi: 10.1073/pnas.1116590109

Bueso, E., Rodriguez, L., Lorenzo-Orts, L., Gonzalez-Guzman, M., Sayas, E., Muñoz-Bertomeu, J., et al. (2014). The single-subunit RING-type E3 ubiquitin ligase RSL1 targets PYL4 and PYR1 ABA receptors in plasma membrane to modulate abscisic acid signaling. *Plant J.* 80, 1057–1071. doi: 10.1111/tpj.12708

Bulley, S., and Laing, W. (2016). The regulation of ascorbate biosynthesis. *Curr. Opin. Plant Biol.* 33, 15–22. doi: 10.1016/j.pbi.2016.04.010

Cao, F. Y., Yoshioka, K., and Desveaux, D. (2011). The roles of ABA in plant–pathogen interactions. *J. Plant Res.* 124, 489–499. doi: 10.1007/s10265-011-0409-y

Chen, S., Jia, H., Wang, X., Shi, C., Wang, X., Ma, P., et al. (2020). Hydrogen sulfide positively regulates abscisic acid signaling through persulfidation of SnRK2.6 in guard cells. *Mol. Plant* 13, 732–744. doi: 10.1016/j.molp.2020.01.004

Chen, Y., Liu, H., Stone, S., and Callis, J. (2013). ABA and the ubiquitin E3 ligase KEEP ON GOING affect proteolysis of the arabidopsis thaliana transcription factors ABF1 and ABF3. *Plant J.* 75, 965–976. doi: 10.1111/tpj.12259

Chinchilla, D., Bauer, Z., Regenass, M., Boller, T., and Felix, G. (2006). The arabidopsis receptor kinase FLS2 binds fig22 and determines the specificity of flagellin perception. *Plant Cell* 18, 465–476. doi: 10.2307/20076607

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nürnberger, T., Jones, J. D. G., et al. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* 448, 497–500. doi: 10.1038/nature05999

Chi, M. H., Park, S. Y., Kim, S., and Lee, Y. H. (2009). A novel pathogenicity gene is required in the rice blast fungus to suppress the basal defenses of the host. *PloS Pathog.* 5, e1000401. doi: 10.1371/journal.ppat.1000401

Choudhury, F. K., Rivero, R. M., Blumwald, E., and Mittler, R. (2017). Reactive oxygen species, abiotic stress and stress combination. *Plant J.* 90, 856–867. doi: 10.1111/tpj.13299

Darma, R., Lutz, A., Elliott, C. E., and Idnurm, A. (2019). Identification of a gene cluster for the synthesis of the plant hormone abscisic acid in the plant pathogen leptosphaeria maculans. *Fungal Genet. Biol.* 130, 62–71. doi: 10.1016/j.fgb.2019.04.015

Davey, M. W., Montagu, M. V., Inz, D., Sanmartin, M., Kanellis, A., Smirnoff, N., et al. (2000). PlantL-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agric.* 80, 825–860. doi: 10.1002/(SICI)1097-0010(20000515)80:7<825::AID-JSFA598>3.0.CO;2-6

del Pozo, J. C. (2016). Reactive oxygen species: From harmful molecules to fine-tuning regulators of stem cell niche maintenance. *PloS Genet.* 12, e1006251. doi: 10.1371/journal.pgen.1006251

Derksen, H., Rampitsch, C., and Daayf, F. (2013). Signaling cross-talk in plant disease resistance. *Plant Sci.* 207, 79–87. doi: 10.1016/j.plantsci.2013.03.004

de Torres Zabala, M., Bennett, M. H., Truman, W. H., and Grant, M. R. (2009). Antagonism between salicylic and abscisic acid reflects early host-pathogen conflict and moulds plant defence responses. *Plant J.* 59, 375–386. doi: 10.1111/j.1365-313X.2009.03875.x

de Torres-Zabala, M., Truman, W., Bennett, M. H., Lafforgue, G., Mansfield, J. W., Rodriguez Egea, P., et al. (2007). Pseudomonas syringae pv. tomato hijacks the arabidopsis abscisic acid signalling pathway to cause disease. *EMBO J.* 26, 1434–1443. doi: 10.1038/sj.emboj.7601575

De Tullio, M. C., and Arrigoni, O. (2004). Hopes, disillusions and more hopes from vitamin c. *Cell Mol. Life Sci.* 61, 209–219. doi: 10.1007/s00018-003-3203-8

Dietz, K. J., Turkan, I., and Krieger-Liszkay, A. (2016). Redox and reactive oxygen species-dependent signaling into and out of the photosynthesizing chloroplast. *Plant Physiol.* 171, 1541–1550. doi: 10.1104/pp.16.00375

Dong, W., Wang, M., Xu, F., Quan, T., Peng, K., Xiao, L., et al. (2013). Wheat oxophytodienoate reductase gene TaOPR1 confers salinity tolerance *via* enhancement of abscisic acid signaling and reactive oxygen species scavenging. *Plant Physiol.* 161, 1217–1228. doi: 10.1104/pp.112.211854

Dubiella, U., Seybold, H., Durian, G., Komander, E., Lassig, R., Witte, C. P., et al. (2013). Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. *Proc. Natl. Acad. Sci. U.S.A.* 110, 8744–8749. doi: 10.1073/pnas.1221294110

Dunand, C., Crèvecoeur, M., and Penel, C. (2007). Distribution of superoxide and hydrogen peroxide in arabidopsis root and their influence on root development: possible interaction with peroxidases. *New Phytol.* 174, 332–341. doi: 10.1111/j.1469-8137.2007.01995.x

Ensminger, I., Busch, F., and Huner, N. P. A. (2006). Photostasis and cold acclimation: sensing low temperature through photosynthesis. *Physiol. Plant* 126, 28–44. doi: 10.1111/j.1399-3054.2006.00627.x

Finkelstein, R. R., Gampala, S. S. L., and Rock, C. D. (2002). Abscisic acid signaling in seeds and seedlings. *Plant Cell* 14, S15–S45. doi: 10.1105/tpc.010441

Foreman, J., Demidchik, V., Bothwell, J. H. F., Mylona, P., Miedema, H., Torres, M. A., et al. (2003). Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 422, 442–446. doi: 10.1038/nature01485

Foyer, C. H., and Noctor, G. (2005). Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. *Plant Cell* 17, 1866–1875. doi: 10.1105/tpc.105.033589

Frank, V. B., and James, F. D. (2006). Reactive oxygen species in plant cell death. *Plant Physiol.* 141, 384–390. doi: 10.1104/pp.106.078295

Fujii, H., Verslues, P. E., and Zhu, J. K. (2007). Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in arabidopsis. *Plant Cell* 19, 485–494. doi: 10.1105/tpc.106.048538

Gallie, D. R. (2013). L-ascorbic acid: A multifunctional molecule supporting plant growth and development. *Scientifica* 2013, 1–24. doi: 10.1155/2013/795964

Gan, S., and Amasino, R. M. (1997). Making sense of senescence (Molecular genetic regulation and manipulation of leaf senescence). *Plant Physiol.* 113, 313–319. doi: 10.1104/pp.113.2.313

Gechev, T., Willekens, H., Van Montagu, M., Inzé, D., Van Camp, W., Toneva, V., et al. (2003). Different responses of tobacco antioxidant enzymes to light and chilling stress. *J. Plant Physiol.* 160, 509–515. doi: 10.1078/0176-1617-00753

Geiger, D., Scherzer, S., Mumm, P., Marten, I., Ache, P., Matschi, S., et al. (2010). Guard cell anion channel SLAC1 is regulated by CDPK protein kinases with distinct Ca2+ affinities. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8023–8028. doi: 10.1073/pnas.0912030107

Geiger, D., Scherzer, S., Mumm, P., Stange, A., Marten, I., Bauer, H., et al. (2009). Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21425–21430. doi: 10.1073/pnas.0912021106

Ghassemian, M., Lutes, J., Chang, H. S., Lange, I., Chen, W., Zhu, T., et al. (2008). Abscisic acid-induced modulation of metabolic and redox control pathways in arabidopsis thaliana. *Phytochemistry* 69, 2899–2911. doi: 10.1016/j.phytochem.2008.09.020

Gilroy, S., Białasek, M., Suzuki, N., Górecka, M., Devireddy, A. R., Karpiński, S., et al. (2016). ROS, calcium, and electric signals: Key mediators of rapid systemic signaling in plants. *Plant Physiol.* 171, 1606–1615. doi: 10.1104/pp.16.00434

Gong, Z., Xiong, L., Shi, H., Yang, S., Herrera-Estrella, L. R., Xu, G., et al. (2020). Plant abiotic stress response and nutrient use efficiency. *Sci. China Life Sci.* 63, 635–674. doi: 10.1007/s11427-020-1683-x

Gosti, F., Beaudoin, N., Serizet, C., Webb, A. A., Vartanian, N., and Giraudat, J. (1999). ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. *Plant Cell* 11, 1897–1910. doi: 10.1105/tpc.11.10.1897

Grant, M. R., and Jones, J. D. G. (2009). Hormone (Dis) harmony moulds plant health and disease. *Science* 324, 750–752. doi: 10.1126/science.1173771

Hartung, W. (2010). The evolution of abscisic acid (ABA) and ABA function in lower plants, fungi and lichen. *Funct. Plant Biol.* 37, 806. doi: 10.1071/FP10058

Haudecoeur, E., Planamente, S., Cirou, A., Tannieres, M., Shelp, B. J., Morera, S., et al. (2009). Proline antagonizes GABA-induced quenching of quorum-sensing in agrobacterium tumefaciens. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14587–14592. doi: 10.1073/pnas.0808005106

Hauser, F., Waadt, R., and Schroeder, J. I. (2011). Evolution of abscisic acid synthesis and signaling mechanisms. *Curr. Biol.* 21, R346–R355. doi: 10.1016/j.cub.2011.03.015

He, J., Duan, Y., Hua, D., Fan, G., Wang, L., Liu, Y., et al. (2012). DEXH box RNA helicase–mediated mitochondrial reactive oxygen species production in arabidopsis mediates crosstalk between abscisic acid and auxin signaling. *Plant Cell* 24, 1815–1833. doi: 10.1105/tpc.112.098707

Heese, A., Hann, D. R., Gimenez-Ibanez, S., Jones, A. M. E., He, K., Li, J., et al. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proc. Natl. Acad. Sci. U.S.A.* 104, 12217–12222. doi: 10.1073/pnas.0705306104

Hong, Z., Lakkineni, K., Zhang, Z., and Verma, D. P. S. (2000). Removal of feedback inhibition of delta(1)-pyrroline-5- carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiol.* 122, 1129–1136. doi: 10.1104/pp.122.4.1129

Hong, Y., Wang, Z., Liu, X., Yao, J., Kong, X., Shi, H., et al. (2020). Two chloroplast proteins negatively regulate plant drought resistance through separate pathways. *Plant Physiol.* 182, 1007–1021. doi: 10.1104/pp.19.01106

Hoque, M., Banu, M. N.A., Nakamura, Y., Shimoishi, Y., and Murata, Y. (2008). Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *J. Plant Physiol.* 165, 813–824. doi: 10.1016/j.jplph.2007.07.013

Huang, X., Hou, L., Meng, J., You, H., Li, Z., Gong, Z., et al. (2018). The antagonistic action of abscisic acid and cytokinin signaling mediates drought stress response in arabidopsis. *Mol. Plant* 11, 970–982. doi: 10.1016/j.molp.2018.05.001

Huang, X., Shi, H., Hu, Z., Liu, A., Amombo, E., Chen, L., et al. (2017). ABA is involved in regulation of cold stress response in bermudagrass. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.01613

Huda, K. M. K., Banu, M. S. A., Garg, B., Tula, S., Tuteja, R., and Tuteja, N. (2013). OsACA6 , a p-type IIB Ca2+ ATPase promotes salinity and drought stress tolerance in tobacco by ROS scavenging and enhancing the expression of stress-responsive genes. *Plant J.* 76, 997–1015. doi: 10.1111/tpj.12352

Hu, C. A., Delauney, A. J., and Verma, D. P. (1992). A bifunctional enzyme (delta 1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants. *Proc. Natl. Acad. Sci. U.S.A.* 89, 9354–9358. doi: 10.1073/pnas.89.19.9354

Irigoyen, M. L., Iniesto, E., Rodriguez, L., Puga, M. I., Yanagawa, Y., Pick, E., et al. (2014). Targeted degradation of abscisic acid receptors is mediated by the ubiquitin ligase substrate adaptor DDA1 in arabidopsis. *Plant Cell* 26, 712–728. doi: 10.1105/tpc.113.122234

Jiang, C., and Bel, E. J. (2013). An arabidopsis Soil-Salinity–tolerance mutation confers ethylene-mediated enhancement of Sodium/Potassium HomeostasisW. *Plant Cell* 25, 3535–3552. doi: 10.2307/23598366

Jiang, C., Belfield, E. J., Mithani, A., Visscher, A., Ragoussis, J., Mott, R., et al. (2012). ROS-mediated vascular homeostatic control of root-to-shoot soil Na delivery in arabidopsis: ROS-mediated control of Na homeostasis. *EMBO J.* 31, 4359–4370. doi: 10.1038/emboj.2012.273

Jiang, C., Sun, J., Li, R., Yan, S., Chen, W., Guo, L., et al. (2022). A reactive oxygen species burst causes haploid induction in maize. *Mol. Plant* 15, 943–955. doi: 10.1016/j.molp.2022.04.001

Jiang, M., and Zhang, J. (2002). Involvement of plasma-membrane NADPH oxidase in abscisic acid- and water stress-induced antioxidant defense in leaves of maize seedlings. *Planta* 215, 1022–1030. doi: 10.1007/s00425-002-0829-y

Jie, S., Jing, Z., Mingjian, Z., Heng, Z., Beimi, C., Cecilia, G., et al. (2020). Persulfidation-based modification of cysteine desulfhydrase and the NADPH oxidase RBOHD controls guard cell abscisic acid signaling. *Plant Cell* 32, 1000–1017. doi: 10.1105/tpc.19.00826

Kavi Kishor, P. B., and Sreenivasulu, N. (2014). Is proline accumulation perse correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant Cell Environ.* 37, 300–311. doi: 10.1111/pce.12157

Kim, J. H., and Kim, W. T. (2013). The arabidopsis RING E3 ubiquitin ligase AtAIRP3/LOG2 participates in positive regulation of high-salt and drought stress responses. *Plant Physiol.* 162, 1733–1749. doi: 10.1104/pp.113.220103

Kong, L., Cheng, J., Zhu, Y., Ding, Y., Meng, J., Chen, Z., et al. (2015). Degradation of the ABA co-receptor ABI1 by PUB12/13 U-box E3 ligases. *Nat. Commun.* 6, 1–13. doi: 10.1038/ncomms9630

Kurup, S., Jones, H. D., and Holdsworth, M. J. (2000). Interactions of the developmental regulator ABI3 with proteins identied from developing arabidopsis seeds. *Plant J.* 21, 143–155. doi: 10.1046/j.1365-313x.2000.00663.x

Kwak, J. M. (2003). NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in arabidopsis. *EMBO J.* 22, 2623–2633. doi: 10.1093/emboj/cdg277

Lechner, E., Leonhardt, N., Eisler, H., Parmentier, Y., Alioua, M., Jacquet, H., et al. (2011). MATH/BTB CRL3 receptors target the homeodomain-leucine zipper ATHB6 to modulate abscisic acid signaling. *Dev. Cell* 21, 1116–1128. doi: 10.1016/j.devcel.2011.10.018

Lee, S. C., Lan, W., Buchanan, B. B., and Luan, S. (2009). A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21419–21424. doi: 10.1073/pnas.0910601106

Lee, S. C., and Luan, S. (2012). ABA signal transduction at the crossroad of biotic and abiotic stress responses: ABA in drought and pathogen responses. *Plant Cell Environ.* 35, 53–60. doi: 10.1111/j.1365-3040.2011.02426.x

Lee, J. H., Yoon, H. J., Terzaghi, W., Martinez, C., Dai, M., Li, J., et al. (2010). DWA1 and DWA2, two arabidopsis DWD protein components of CUL4-based E3 ligases, act together as negative regulators in ABA signal transduction. *Plant Cell* 22, 1716–1732. doi: 10.1105/tpc.109.073783

Leng, Y., Yang, Y., Ren, D., Huang, L., Dai, L., Wang, Y., et al. (2017). A rice PECTATE LYASE-LIKE gene is required for plant growth and leaf senescence. *Plant Physiol.* 174, 1151–1166. doi: 10.1104/pp.16.01625

Leung, J., and Giraudat, J. (1998). Abscisic acid signal transducyion. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 199–222. doi: 10.1146/annurev.arplant.49.1.199

Li, L., Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z., et al. (2014). The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. *Cell Host Microbe* 15, 329–338. doi: 10.1016/j.chom.2014.02.009

Lim, C., Baek, W., Jung, J., Kim, J. H., and Lee, S. (2015). Function of ABA in stomatal defense against biotic and drought stresses. *Int. J. Mol. Sci.* 16, 15251–15270. doi: 10.3390/ijms160715251

Liszkay, A., van der Zalm, E., and Schopfer, P. (2004). Production of reactive oxygen intermediates (O2–, H2O2, and OH-) by maize roots and their role in wall loosening and elongation growth. *Plant Physiol.* 136, 3114–3123. doi: 10.1104/pp.104.044784

Liu, P. P., Montgomery, T. A., Fahlgren, N., Kasschau, K. D., Nonogaki, H., and Carrington, J. C. (2007). Repression of AUXIN RESPONSE FACTOR10 by microRNA160 is critical for seed germination and post-germination stages: microRNA in arabidopsis seed germination. *Plant J.* 52, 133–146. doi: 10.1111/j.1365-313X.2007.03218.x

Li, Z., Wang, F., Zhao, Q., Liu, J., and Cheng, F. (2018). Involvement of NADPH oxidase isoforms in the production of O2– manipulated by ABA in the senescing leaves of early-senescence-leaf (esl) mutant rice (Oryza sativa). *PloS One* 13, e0190161. doi: 10.1371/journal.pone.0190161

Li, J., Wen, J., Lease, K. A., Doke, J. T., Tax, F. E., and Walker, J. C. (2002). BAK1, an arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell* 110, 213–222. doi: 10.1016/S0092-8674(02)00812-7

Lu, Z., Liu, D., and Liu, S. (2007). Two rice cytosolic ascorbate peroxidases differentially improve salt tolerance in transgenic arabidopsis. *Plant Cell Rep.* 26, 1909–1917. doi: 10.1007/s00299-007-0395-7

Lu, D., Wu, S., Gao, X., Zhang, Y., Shan, L., and He, P. (2010). A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 496–501. doi: 10.1073/pnas.0909705107

Lv, X., Li, H., Chen, X., Xiang, X., Guo, Z., Yu, J., et al. (2018). The role of calcium-dependent protein kinase in hydrogen peroxide, nitric oxide and ABA-dependent cold acclimation. *J. Exp. Bot.* 69, 4127–4139. doi: 10.1093/jxb/ery212

Mahmood, K., El-Kereamy, A., Kim, S. H., Nambara, E., and Rothstein, S. J. (2016). ANAC032 positively regulates age-dependent and stress-induced senescence in arabidopsis thaliana. *Plant Cell Physiol.* 57, 2029–2046. doi: 10.1093/pcp/pcw120

Ma, Y., Szostkiewicz, I., Korte, A., Moes, D., Yang, Y., Christmann, A., et al. (2009). Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* 324, 1064–1068. doi: 10.1126/science.1172408

Matysik, J., Alia, Bhalu, B., and Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.* 82, 525–532.

Ma, L., Zhang, H., Sun, L., Jiao, Y., Zhang, G., Miao, C., et al. (2012). NADPH oxidase AtrbohD and AtrbohF function in ROS-dependent regulation of Na+/K+ homeostasis in arabidopsis under salt stress. *J. Exp. Bot.* 63, 305–317. doi: 10.1093/jxb/err280

Melotto, M., Underwood, W., Koczan, J., Nomura, K., and He, S. Y. (2006). Plant stomata function in innate immunity against bacterial invasion. *Cell* 126, 969–980. doi: 10.1016/j.cell.2006.06.054

Mersmann, S., Bourdais, G., Rietz, S., and Robatzek, S. (2010). Ethylene signaling regulates accumulation of the FLS2 receptor and is required for the oxidative burst contributing to plant immunity. *Plant Physiol.* 154, 391–400. doi: 10.1104/pp.110.154567

Miao, Y., Lv, D., Wang, P., Wang, X. C., Chen, J., Miao, C., et al. (2006). An arabidopsis glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. *Plant Cell* 18, 2749–2766. doi: 10.1105/tpc.106.044230

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7, 405–410. doi: 10.1016/S1360-1385(02)02312-9

Mittler, R. (2017). ROS are good. *Trends Plant Sci.* 22, 11–19. doi: 10.1016/j.tplants.2016.08.002

Mittler, R., and Blumwald, E. (2015). The roles of ROS and ABA in systemic acquired acclimation. *Plant Cell* 27, 64–70. doi: 10.1105/tpc.114.133090

Mittler, R., Vanderauwera, S., Suzuki, N., Miller, G., Tognetti, V. B., Vandepoele, K., et al. (2011). ROS signaling: the new wave? *Trends Plant Sci.* 16, 300–309. doi: 10.1016/j.tplants.2011.03.007

Mittler, R., Zandalinas, S. I., Fichman, Y., and Van Breusegem, F. (2022). Reactive oxygen species signalling in plant stress responses. *Nat. Rev. Mol. Cell Biol.* 23, 1–17. doi: 10.1038/s41580-022-00499-2

Mohr, P. G., and Cahill, D. M. (2007). Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in arabidopsis infected with pseudomonas syringae pv. tomato. *Funct. Integr. Genomics* 7, 181–191. doi: 10.1007/s10142-006-0041-4

Monaghan, J., Matschi, S., Romeis, T., and Zipfel, C. (2015). The calcium-dependent protein kinase CPK28 negatively regulates the BIK1-mediated PAMP-induced calcium burst. *Plant Signal Behav.* 10, e1018497. doi: 10.1080/15592324.2015.1018497

Monroe-Augustus, M., Zolman, B. K., and Bartel, B. (2003). IBR5, a dual-specificity phosphatase-like protein modulating auxin and abscisic acid responsiveness in arabidopsis. *Plant Cell* 15, 2979–2991. doi: 10.1105/tpc.017046

Muhlemann, J. K., Younts, T. L. B., and Muday, G. K. (2018). Flavonols control pollen tube growth and integrity by regulating ROS homeostasis during high-temperature stress. *Proc. Natl. Acad. Sci. U.S.A.* 115, E11188–E11197. doi: 10.1073/pnas.1811492115

Mustilli, A. C., Merlot, S., Vavasseur, A., Fenzi, F., and Giraudat, J. (2002). Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *Plant Cell* 14, 3089–3099. doi: 10.1105/tpc.007906

Nambara, E., and Marion-Poll, A. (2005). Abscisic acid biosynthesis and catabolism. *Annu. Rev. Plant Biol.* 56, 165–185. doi: 10.1146/annurev.arplant.56.032604.144046

Nam, K. H., and Li, J. (2002). BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell* 110, 203–212. doi: 10.1016/S0092-8674(02)00814-0

Neill, S. (2002). Hydrogen peroxide signalling. *Curr. Opin. Plant Biol.* 5, 388–395. doi: 10.1016/S1369-5266(02)00282-0

Ni, L., Fu, X., Zhang, H., Li, X., Cai, X., Zhang, P., et al. (2019). Abscisic acid inhibits rice protein phosphatase PP45 *via* H2O2 and relieves repression of the Ca2+/CaM-dependent protein kinase DMI3. *Plant Cell* 31, 128–152. doi: 10.1105/tpc.18.00506

Nühse, T. S., Bottrill, A. R., Jones, A. M. E., and Peck, S. C. (2007). Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses: Quantitative phosphoproteomics for signalling pathways. *Plant J.* 51, 931–940. doi: 10.1111/j.1365-313X.2007.03192.x

Ogasawara, Y., Kaya, H., Hiraoka, G., Yumoto, F., Kimura, S., Kadota, Y., et al. (2008). Synergistic activation of the arabidopsis NADPH oxidase AtrbohD by Ca2+ and phosphorylation. *J. Biol. Chem.* 283, 8885–8892. doi: 10.1074/jbc.M708106200

Oide, S., Bejai, S., Staal, J., Guan, N., Kaliff, M., and Dixelius, C. (2013). Novel role of PR2 in abscisic acid (ABA) mediated, pathogen-induced callose deposition in arabidopsis thaliana. *New Phytol.* 200, 1187–1199. doi: 10.1111/nph.12436

Osakabe, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., and Tran, L. P. (2014). ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. *New Phytol.* 202, 35–49. doi: 10.1111/nph.12613

Overmyer, K., Brosché, M., and Kangasjärvi, J. (2003). Reactive oxygen species and hormonal control of cell death. *Trends Plant Sci.* 8, 335–342. doi: 10.1016/S1360-1385(03)00135-3

Overmyer, K., Tuominen, H., Kettunen, R., Betz, C., Langebartels, C., Sandermann, H., et al. (2000). Ozone-sensitive arabidopsis rcd1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. *Plant Cell* 12, 1849–1862. doi: 10.2307/3871197

Paik, I., Kathare, P. K., Kim, J. I., and Huq, E. (2017). Expanding roles of PIFs in signal integration from multiple processes. *Mol. Plant* 10, 1035–1046. doi: 10.1016/j.molp.2017.07.002

Pan, W., Lin, B., Yang, X., Liu, L., Xia, R., Li, J., et al. (2020). The UBC27–AIRP3 ubiquitination complex modulates ABA signaling by promoting the degradation of ABI1 in arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* 117, 27694–27702. doi: 10.1073/pnas.2007366117

Park, S. Y., Fung, P., Nishimura, N., Jensen, D. R., Fujii, H., Zhao, Y., et al. (2009). Abscisic acid inhibits type 2C protein phosphatases *via* the PYR/PYL family of START proteins. *Science* 324, 1068–1071. doi: 10.1126/science.1173041

Pastori, G. M., and Foyer, C. H. (2002). Common components, networks, and pathways of cross-tolerance to stress. the central role of "redox" and abscisic acid-mediated controls. *Plant Physiol.* 129, 460–468. doi: 10.1104/pp.011021

Pei, Z. M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G. J., et al. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acidsignalling in guard cells. *Nature* 406, 731–734. doi: 10.1038/35021067

Peng, J., Li, Z., Wen, X., Li, W., Shi, H., Yang, L., et al. (2014). Salt-induced stabilization of EIN3/EIL1 confers salinity tolerance by deterring ROS accumulation in arabidopsis. *PloS Genet.* 10, e1004664. doi: 10.1371/journal.pgen.1004664

Qi, L., Liu, S., Li, C., Fu, J., Jing, Y., Cheng, J., et al. (2020). PHYTOCHROME-INTERACTING FACTORS interact with the ABA receptors PYL8 and PYL9 to orchestrate ABA signaling in darkness. *Mol. Plant* 13, 414–430. doi: 10.1016/j.molp.2020.02.001

Qi, J., Wang, J., Gong, Z., and Zhou, J. M. (2017). Apoplastic ROS signaling in plant immunity. *Curr. Opin. Plant Biol.* 38, 92–100. doi: 10.1016/j.pbi.2017.04.022

Raghavendra, A. S., Gonugunta, V. K., Christmann, A., and Grill, E. (2010). ABA perception and signalling. *Trends Plant Sci.* 15, 395–401. doi: 10.1016/j.tplants.2010.04.006

Ramirez-Parra, E., Perianez-Rodriguez, J., Navarro-Neila, S., Gude, I., Moreno-Risueno, M. A., and del Pozo, J. C. (2017). The transcription factor OBP4 controls root growth and promotes callus formation. *New Phytol.* 213, 1787–1801. doi: 10.1111/nph.14315

Ranf, S., Eschen-Lippold, L., Fröhlich, K., Westphal, L., Scheel, D., and Lee, J. (2014). Microbe-associated molecular pattern-induced calcium signaling requires the receptor-like cytoplasmic kinases, PBL1 and BIK1. *BMC Plant Biol.* 14, 1–15. doi: 10.1186/s12870-014-0374-4

Robert-Seilaniantz, A., Navarro, L., Bari, R., and Jones, J. D. (2007). Pathological hormone imbalances. *Curr. Opin. Plant Biol.* 10, 372–379. doi: 10.1016/j.pbi.2007.06.003

Rossel, J. B., Walter, P. B., Hendrickson, L., Chow, W. S., Poole, A., Mullineaux, P. M., et al. (2006). A mutation affecting ASCORBATE PEROXIDASE 2 gene expression reveals a link between responses to high light and drought tolerance. *Plant Cell Environ.* 29, 269–281. doi: 10.1111/j.1365-3040.2005.01419.x

Rubio, S., Noriega, X., and Pérez, F. J. (2019). Abscisic acid (ABA) and low temperatures synergistically increase the expression of CBF/DREB1 transcription factors and cold-hardiness in grapevine dormant buds. *Ann. Bot.* 123, 681–689. doi: 10.1093/aob/mcy201

Russinova, E., Borst, J. W., Kwaaitaal, M., Caño-Delgado, A., Yin, Y., Chory, J., et al. (2004). Heterodimerization and endocytosis of arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). *Plant Cell* 16, 3216–3229. doi: 10.1105/tpc.104.025387

Sahu, M., and Kar, R. K. (2018). Possible interaction of ROS, antioxidants and ABA to survive osmotic stress upon acclimation in vigna radiata l. wilczek seedlings. *Plant Physiol. Biochem.* 132, 415–423. doi: 10.1016/j.plaphy.2018.09.034

Sanchez-Fernandez, R., Fricker, M., Corben, L. B., White, N. S., Sheard, N., Leaver, C. J., et al. (1997). Cell proliferation and hair tip growth in the arabidopsis root are under mechanistically different forms of redox control. *Proc. Natl. Acad. Sci. U.S.A.* 94, 2745–2750. doi: 10.1073/pnas.94.6.2745

Santiago, J., Dupeux, F., Round, A., Antoni, R., Park, S. Y., Jamin, M., et al. (2009). The abscisic acid receptor PYR1 in complex with abscisic acid. *Nature* 462, 665–668. doi: 10.1038/nature08591

Sato, A., Sato, Y., Fukao, Y., Fujiwara, M., Umezawa, T., Shinozaki, K., et al. (2009). Threonine at position 306 of the KAT1 potassium channel is essential for channel activity and is a target site for ABA-activated SnRK2/OST1/SnRK2.6 protein kinase. *Biochem. J.* 424, 439–448. doi: 10.1042/BJ20091221

Schroeder, J. I., and Hagiwara, S. (1990). Repetitive increases in cytosolic Ca2+ of guard cells by abscisic acid activation of nonselective Ca2+ permeable channels. *Proc. Natl. Acad. Sci. U.S.A.* 87, 9305–9309. doi: 10.1073/pnas.87.23.9305

Seo, K. I., Lee, J. H., Nezames, C. D., Zhong, S., Song, E., Byun, M. O., et al. (2014). ABD1 is an arabidopsis DCAF substrate receptor for CUL4-DDB1–based E3 ligases that acts as a negative regulator of abscisic acid signaling. *Plant Cell* 26, 695–711. doi: 10.1105/tpc.113.119974

Shang, Y., Dai, C., Lee, M. M., Kwak, J. M., and Nam, K. H. (2016). BRI1-associated receptor kinase 1 regulates guard cell ABA signaling mediated by open stomata 1 in arabidopsis. *Mol. Plant* 9, 447–460. doi: 10.1016/j.molp.2015.12.014

Sharma, R., Sahoo, A., Devendran, R., and Jain, M. (2014). Over-expression of a rice tau class glutathione s-transferase gene improves tolerance to salinity and oxidative stresses in arabidopsis. *PloS One* 9, e92900. doi: 10.1371/journal.pone.0092900

Shi, Y., Wang, Z., Meng, P., Tian, S., Zhang, X., and Yang, S. (2013). The glutamate carboxypeptidase AMP1 mediates abscisic acid and abiotic stress responses in arabidopsis. *New Phytol.* 199, 135–150. doi: 10.1111/nph.12275

Shu, K., Zhou, W., Chen, F., Luo, X., and Yang, W. (2018). Abscisic acid and gibberellins antagonistically mediate plant development and abiotic stress responses. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00416

Silva, N. C. Q., de Souza, G. A., Pimenta, T. M., Brito, F. A. L., Picoli, E. A. T., Zsögön, A., et al. (2018). Salt stress inhibits germination of stylosanthes humilis seeds through abscisic acid accumulation and associated changes in ethylene production. *Plant Physiol. Biochem.* 130, 399–407. doi: 10.1016/j.plaphy.2018.07.025

Simmons, A. R., and Bergmann, D. C. (2016). Transcriptional control of cell fate in the stomatal lineage. *Curr. Opin. Plant Biol.* 29, 1–8. doi: 10.1016/j.pbi.2015.09.008

Sirichandra, C., Gu, D., Hu, H. C., Davanture, M., Lee, S., Djaoui, M., et al. (2009). Phosphorylation of the arabidopsis AtrbohF NADPH oxidase by OST1 protein kinase. *FEBS Lett.* 583, 2982–2986. doi: 10.1016/j.febslet.2009.08.033

Siripornadulsil, S., Traina, S., Verma, D. P. S., and Sayre, R. T. (2002). Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. *Plant Cell* 14, 2837–2847. doi: 10.1105/tpc.004853

Skubacz, A., Daszkowska-Golec, A., and Szarejko, I. (2016). The role and regulation of ABI5 (ABA-insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.01884

Smalle, J., Kurepa, J., Yang, P., Emborg, T. J., Babiychuk, E., Kushnir, S., et al. (2003). The pleiotropic role of the 26S proteasome subunit RPN10 in arabidopsis growth and development supports a substrate-specific function in abscisic acid signaling. *Plant Cell* 15, 965–980. doi: 10.1105/tpc.009217

Smirnoff, N., and Wheeler, G. L. (2000). Ascorbic acid in plants: biosynthesis and function. *Crit. Rev. Biochem. Mol. Biol.* 35, 291–314. doi: 10.1080/10409230008984166

Suzuki, N., and Katano, K. (2018). Coordination between ROS regulatory systems and other pathways under heat stress and pathogen attack. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00490

Suzuki, N., Miller, G., Morales, J., Shulaev, V., Torres, M. A., and Mittler, R. (2011). Respiratory burst oxidases: the engines of ROS signaling. *Curr. Opin. Plant Biol.* 14, 691–699. doi: 10.1016/j.pbi.2011.07.014

Suzuki, N., Miller, G., Salazar, C., Mondal, H. A., Shulaev, E., Cortes, D. F., et al. (2013). Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants. *Plant Cell* 25, 3553–3569. doi: 10.1105/tpc.113.114595

Szabados, L., and Savouré, A. (2010). Proline: a multifunctional amino acid. *Trends Plant Sci.* 15, 89–97. doi: 10.1016/j.tplants.2009.11.009

Székely, G., Abrahám, E., Cséplo, A., Rigó, G., Zsigmond, L., Csiszár, J., et al. (2008). Duplicated P5CS genes of arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. *Plant J.* 53, 11–28. doi: 10.1111/j.1365-313X.2007.03318.x

Tintor, N., Ross, A., Kanehara, K., Yamada, K., Fan, L., Kemmerling, B., et al. (2013). Layered pattern receptor signaling *via* ethylene and endogenous elicitor peptides during arabidopsis immunity to bacterial infection. *Proc. Natl. Acad. Sci. U.S.A.* 110, 6211–6216. doi: 10.1073/pnas.1216780110

Tiryaki, I., and Staswick, P. E. (2002). An arabidopsis mutant defective in jasmonate response is allelic to the auxin-signaling mutant axr1. *Plant Physiol.* 130, 887–894. doi: 10.1104/pp.005272

Tognetti, V. B., Bielach, A., and Hrtyan, M. (2017). Redox regulation at the site of primary growth: auxin, cytokinin and ROS crosstalk: Apical meristems plasticity in response to stress. *Plant Cell Environ.* 40, 2586–2605. doi: 10.1111/pce.13021

Ton, J., Flors, V., and Mauch-Mani, B. (2009). The multifaceted role of ABA in disease resistance. *Trends Plant Sci.* 14, 310–317. doi: 10.1016/j.tplants.2009.03.006

Torres, M. A., Dangl, J. L., and Jones, J. D. G. (2002). Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *PNAS* 99, 517–522. doi: 10.1073/pnas.012452499

Tsukagoshi, H., Busch, W., and Benfey, P. N. (2010). Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell* 143, 606–616. doi: 10.1016/j.cell.2010.10.020

Umezawa, T., Nakashima, K., Miyakawa, T., Kuromori, T., Tanokura, M., Shinozaki, K., et al. (2010). Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. *Plant Cell Physiol.* 51, 1821–1839. doi: 10.1093/pcp/pcq156

Umezawa, T., Sugiyama, N., Mizoguchi, M., Hayashi, S., Myouga, F., Yamaguchi-Shinozaki, K., et al. (2009). Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 17588–17593. doi: 10.1073/pnas.0907095106

Vernoux, T., Wilson, R. C., Seeley, K. A., Reichheld, J. P., Muroy, S., Brown, S., et al. (2000). The root MERISTEMLESS1/CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell* 12, 97–109. doi: 10.1105/tpc.12.1.97

Veronese, P., Nakagami, H., Bluhm, B., AbuQamar, S., Chen, X., Salmeron, J., et al. (2006). The membrane-anchored BOTRYTIS-INDUCED KINASE1 plays distinct roles in arabidopsis resistance to necrotrophic and biotrophic pathogens. *Plant Cell* 18, 257–273. doi: 10.1105/tpc.105.035576

Verslues, P. E., Kim, Y. S., and Zhu, J. K. (2007). Altered ABA, proline and hydrogen peroxide in an arabidopsis glutamate:glyoxylate aminotransferase mutant. *Plant Mol. Biol.* 64, 205–217. doi: 10.1007/s11103-007-9145-z

Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R. K., et al. (2017). Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.00161

Wang, Z., Cao, H., Sun, Y., Li, X., Chen, F., Carles, A., et al. (2013). Arabidopsis paired amphipathic helix proteins SNL1 and SNL2 redundantly regulate primary seed dormancy *via* abscisic acid–ethylene antagonism mediated by histone deacetylation. *Plant Cell* 25, 149–166. doi: 10.1105/tpc.112.108191

Wang, X., Goshe, M. B., Soderblom, E. J., Phinney, B. S., Kuchar, J. A., Li, J., et al. (2005). Identification and functional analysis of *in vivo* phosphorylation sites of the arabidopsis BRASSINOSTEROID-INSENSITIVE1 receptor kinase. *Plant Cell* 17, 1685–1703. doi: 10.1105/tpc.105.031393

Wang, X., Kota, U., He, K., Blackburn, K., Li, J., Goshe, M. B., et al. (2008). Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. *Dev. Cell* 15, 220–235. doi: 10.1016/j.devcel.2008.06.011

Wang, F., Liu, J., Chen, M., Zhou, L., Li, Z., Zhao, Q., et al. (2016). Involvement of abscisic acid in PSII photodamage and D1 protein turnover for light-induced premature senescence of rice flag leaves. *PloS One* 11, e0161203. doi: 10.1371/journal.pone.0161203

Wang, P., and Song, C. (2008). Guard-cell signalling for hydrogen peroxide and abscisic acid. *New Phytol.* 178, 703–718. doi: 10.1111/j.1469-8137.2008.02431.x

White, C. N., Proebsting, W. M., Hedden, P., and Rivin, C. J. (2000). Gibberellins and seed development in maize. i. evidence that gibberellin/abscisic acid balance governs germination versus maturation pathways. *Plant Physiol.* 122, 1081–1088. doi: 10.1104/pp.122.4.1081

Wong, H. L., Pinontoan, R., Hayashi, K., Tabata, R., Yaeno, T., Hasegawa, K., et al. (2008). Regulation of rice NADPH oxidase by binding of rac GTPase to its n-terminal extension. *Plant Cell* 19, 4022–4034. doi: 10.1105/tpc.107.055624

Wu, F., Chi, Y., Jiang, Z., Xu, Y., Xie, L., Huang, F., et al. (2020). Hydrogen peroxide sensor HPCA1 is an LRR receptor kinase in arabidopsis. *Nature* 578, 577–581. doi: 10.1038/s41586-020-2032-3

Wu, B., Li, L., Qiu, T., Zhang, X., and Cui, S. (2018). Cytosolic APX2 is a pleiotropic protein involved in H2O2 homeostasis, chloroplast protection, plant architecture and fertility maintenance. *Plant Cell Rep.* 37, 833–848. doi: 10.1007/s00299-018-2272-y

Wu, Q., Zhang, X., Peirats-Llobet, M., Belda-Palazon, B., Wang, X., Cui, S., et al. (2016). Ubiquitin ligases RGLG1 and RGLG5 regulate abscisic acid signaling by controlling the turnover of phosphatase PP2CA. *Plant Cell* 28, 2178–2196. doi: 10.1105/tpc.16.00364

Xia, X. J., Zhou, Y. H., Shi, K., Zhou, J., Foyer, C. H., and Yu, J. Q. (2015). Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. *J. Exp. Bot.* 66, 2839–2856. doi: 10.1093/jxb/erv089

Xie, Y., Mao, Y., Duan, X., Zhou, H., Lai, D., Zhang, Y., et al. (2016). Arabidopsis HY1-modulated stomatal movement: an integrative hub is functionally associated with ABI4 in dehydration-induced ABA responsiveness. *Plant Physiol.* 170, 1699–1713. doi: 10.1104/pp.15.01550

Xie, H. T., Wan, Z. Y., Li, S., and Zhang, Y. (2014). Spatiotemporal production of reactive oxygen species by NADPH oxidase is critical for tapetal programmed cell death and pollen development in arabidopsis. *Plant Cell* 26, 2007–2023. doi: 10.1105/tpc.114.125427

Xu, N., Chu, Y., Chen, H., Li, X., Wu, Q., Jin, L., et al. (2018). Rice transcription factor OsMADS25 modulates root growth and confers salinity tolerance *via* the ABA–mediated regulatory pathway and ROS scavenging. *PloS Genet.* 14, e1007662. doi: 10.1371/journal.pgen.1007662

Xue, L., Sun, M., Wu, Z., Yu, L., Yu, Q., Tang, Y., et al. (2020). LncRNA regulates tomato fruit cracking by coordinating gene expression *via* a hormone-redox-cell wall network. *BMC Plant Biol.* 20, 162. doi: 10.1186/s12870-020-02373-9

Xu, Z., Mahmood, K., and Rothstein, S. J. (2017b). ROS induces anthocyanin production via late biosynthetic genes and anthocyanin deficiency confers the hypersensitivity to ROS-generating stresses in arabidopsis. *Plant Cell Physiol.* 58, 1364–1377. doi: 10.1093/pcp/pcx073

Xu, L., Zhao, H., Ruan, W., Deng, M., Wang, F., Peng, J., et al. (2017a). ABNORMAL INFLORESCENCE MERISTEM1 functions in salicylic acid biosynthesis to maintain proper reactive oxygen species levels for root meristem activity in rice. *Plant Cell* 29, 560–574. doi: 10.1105/tpc.16.00665

Yang, D. L., Yao, J., Mei, C. S., Tong, X. H., Zeng, L. J., Li, Q., et al. (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proc. Natl. Acad. Sci. U.S.A.* 109, E1192–E1200. doi: 10.1073/pnas.1201616109

Yang, L., Zhang, J., He, J., Qin, Y., Hua, D., Duan, Y., et al. (2014). ABA-mediated ROS in mitochondria regulate root meristem activity by controlling PLETHORA expression in arabidopsis. *PloS Genet.* 10, e1004791. doi: 10.1371/journal.pgen.1004791

Yan, H., Li, Q., Park, S. C., Wang, X., Liu, Y., Zhang, Y., et al. (2016). Overexpression of CuZnSOD and APX enhance salt stress tolerance in sweet potato. *Plant Physiol. Biochem.* 109, 20–27. doi: 10.1016/j.plaphy.2016.09.003

Ye, N., Zhu, G., Liu, Y., Zhang, A., Li, Y., Liu, R., et al. (2012). Ascorbic acid and reactive oxygen species are involved in the inhibition of seed germination by abscisic acid in rice seeds. *J. Exp. Bot.* 63, 1809–1822. doi: 10.1093/jxb/err336

Yin, C. C., Ma, B., Collinge, D. P., Pogson, B. J., He, S. J., Xiong, Q., et al. (2015). Ethylene responses in rice roots and coleoptiles are differentially regulated by a carotenoid isomerase-mediated abscisic acid pathway. *Plant Cell* 27, 1061–1081. doi: 10.1105/tpc.15.00080

Yin, C. C., Zhao, H., Ma, B., Chen, S. Y., and Zhang, J. S. (2017). Diverse roles of ethylene in regulating agronomic traits in rice. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.01676

Yoshida, T., Mogami, J., and Yamaguchi-Shinozaki, K. (2014). ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. *Curr. Opin. Plant Biol.* 21, 133–139. doi: 10.1016/j.pbi.2014.07.009

Yoshida, T., Nishimura, N., Kitahata, N., Kuromori, T., Ito, T., Asami, T., et al. (2006). ABA-hypersensitive germination3 encodes a protein phosphatase 2C (AtPP2CA) that strongly regulates abscisic acid signaling during germination among arabidopsis protein phosphatase 2Cs. *Plant Physiol.* 140, 115–126. doi: 10.1104/pp.105.070128

You, J., Zong, W., Hu, H., Li, X., Xiao, J., and Xiong, L. (2014). A STRESS-RESPONSIVE NAC1-regulated protein phosphatase gene rice protein phosphatase18 modulates drought and oxidative stress tolerance through abscisic acid-independent reactive oxygen species scavenging in rice. *Plant Physiol.* 166, 2100–2114. doi: 10.1104/pp.114.251116

Yu, Q., Tian, H., Yue, K., Liu, J., Zhang, B., Li, X., et al. (2016). A p-loop NTPase regulates quiescent center cell division and distal stem cell identity through the regulation of ROS homeostasis in arabidopsis root. *PloS Genet.* 12, e1006175. doi: 10.1371/journal.pgen.1006175

Yu, Y., Wang, J., Li, S., Kakan, X., Zhou, Y., Miao, Y., et al. (2019). Ascorbic acid integrates the antagonistic modulation of ethylene and abscisic acid in the accumulation of reactive oxygen species. *Plant Physiol.* 179, 1861–1875. doi: 10.1104/pp.18.01250

Yu, F., Wu, Y., and Xie, Q. (2015). Precise protein post-translational modifications modulate ABI5 activity. *Trends Plant Sci.* 20, 569–575. doi: 10.1016/j.tplants.2015.05.004

Zandalinas, S. I., Fichman, Y., and Mittler, R. (2020). Vascular bundles mediate systemic reactive oxygen signaling during light stress. *Plant Cell* 32, 3425–3435. doi: 10.1105/tpc.20.00453

Zeng, J., Dong, Z., Wu, H., Tian, Z., and Zhao, Z. (2017). Redox regulation of plant stem cell fate. *EMBO J.* 36, 2844–2855. doi: 10.15252/embj.201695955

Zhang, C., Bousquet, A., and Harris, J. M. (2014). Abscisic acid and lateral root organ defective/numerous infections and polyphenolics modulate root elongation *via* reactive oxygen species in medicago truncatula. *Plant Physiol.* 166, 644–658. doi: 10.1104/pp.114.248542

Zhang, H., Cui, F., Wu, Y., Lou, L., Liu, L., Tian, M., et al. (2015). The RING finger ubiquitin E3 ligase SDIR1 targets SDIR1-INTERACTING PROTEIN1 for degradation to modulate the salt stress response and ABA signaling in arabidopsis. *Plant Cell* 27, 214–227. doi: 10.1105/tpc.114.134163

Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., et al. (2010). Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a pseudomonas syringae effector. *Cell Host Microbe* 7, 290–301. doi: 10.1016/j.chom.2010.03.007

Zhang, T., Li, Z., Zhao, Y., Shen, W., Chen, M., Gao, H., et al. (2021). Ethylene-induced stomatal closure is mediated *via* MKK1/3–MPK3/6 cascade to EIN2 and EIN3. *J. Integr. Plant Biol.* 63, 1324–1340. doi: 10.1111/jipb.13083

Zhang, W., Qin, C., Zhao, J., and Wang, X. (2004). Phospholipase d alpha 1-derived phosphatidic acid interacts with ABI1 phosphatase 2C and regulates abscisic acid signaling. *Proc. Natl. Acad. Sci. U.S.A.* 101, 9508–9513. doi: 10.1073/pnas.0402112101

Zhang, J., Shao, F., Li, Y., Cui, H., Chen, L., Li, H., et al. (2007a). A pseudomonas syringae effector inactivates MAPKs to suppress PAMP-induced immunity in plants. *Cell Host Microbe* 1, 175–185. doi: 10.1016/j.chom.2007.03.006

Zhang, Y., Yang, C., Li, Y., Zheng, N., Chen, H., Zhao, Q., et al. (2007b). SDIR1 is a RING finger E3 ligase that positively regulates stress-responsive abscisic acid signaling in arabidopsis. *Plant Cell* 19, 1912–1929. doi: 10.1105/tpc.106.048488

Zhang, Y., Zhu, H., Zhang, Q., Li, M., Yan, M., Wang, R., et al. (2009). Phospholipase Dα1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in arabidopsis. *Plant Cell* 21, 2357–2377. doi: 10.1105/tpc.108.062992

Zhong, S., Zhao, M., Shi, T., Shi, H., An, F., Zhao, Q., et al. (2009). EIN3/EIL1 cooperate with PIF1 to prevent photo-oxidation and to promote greening of arabidopsis seedlings. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21431–21436. doi: 10.1073/pnas.0907670106

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Polystyrene microplastics protect lettuce (*Lactuca sativa*) from the hazardous effects of $Cu(OH)_2$ nanopesticides

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Copper-based nanopesticides are released into the environment during foliar spray application, and they could, on their own or in combination with microplastics (MPs), pose threats to environmental safety and human health. In this study, $Cu(OH)_2$ nanowires greatly decreased the vigor of lettuce seeds ($p< 0.01$) and the root length of lettuce seedlings ($p< 0.01$) and significantly altered the lettuce antioxidant defence system and MDA content ($p< 0.05$). Released Cu^{2+} played a critical role in the toxicity mechanism of $Cu(OH)_2$ nanowires in lettuce seedlings, as evidenced by the substantial accumulation of Cu in the seedling roots ($p< 0.01$) rather than in the leaves. Polystyrene (PS) MPs (1 mg/L) stimulated lettuce seedling growth, as shown by the (highly) significant increase in root and leaf length and in the seed vigor index ($p< 0.01$ or 0.05). Notably, PS MPs (1 mg/L) neutralized the hazardous effects of 1 mg/L $Cu(OH)_2$ nanowire treatment on lettuce growth, as reflected by the vitality and root length of the seedlings returning to normal levels. The PS MPs (1 mg/L) absorbed on middle root surfaces and strongly hindered Cu accumulation in lettuce roots, which was the predominant mechanism by which PS MPs suppressed the hazardous effects of the $Cu(OH)_2$ nanowires. This study strengthens the understanding of the toxicity and toxicity mechanisms of Cu $(OH)_2$ nanowires with or without PS MPs in the environment.

KEYWORDS

$Cu(OH)_2$ nanopesticides, polystyrene microplastics, toxicity, lettuce, antioxidant enzymes

1 Introduction

The nanopesticide copper(II) hydroxide ($Cu(OH)_2$), as a typical engineered nanomaterial, is widely applied in agriculture for the purpose of plant growth due to its excellent antimicrobial and antifungal properties (Li et al., 2019; Bindra and Singh, 2021; Konappa et al., 2021). Inevitably, a large amount of $Cu(OH)_2$ nanopesticides will be released into the environment when they are sprayed on plants (Conway, 2015), and thus, $Cu(OH)_2$ nanopesticides could pose serious threats to environmental safety and human health (Nair and Chung, 2015). Correspondingly, the environmental fate, transport, bioavailability and toxicity of $Cu(OH)_2$ nanopesticides are now being extensively investigated to elucidate their potentially hazardous effects on different plant species (Zuverza-Mena et al., 2015; Du et al., 2018). For example, studies have shown that $Cu(OH)_2$ nanopesticides can induce oxidative stress, defence responses and enhanced Cu intake in lettuce (*Lactuca sativa*) and basil (*Ocimum basilicum*); can cause a reduction in antioxidant and defence-associated metabolites in spinach (*Spinacia oleracea*); and can bring about metabolic reprogramming in both cucumber (*Cucumis sativus*) and corn (*Zea mays*) (Zhao et al., 2016a; Zhao et al., 2016b; Zhao et al., 2017c; Tan et al., 2018). In addition to exhibiting effects on plants, $Cu(OH)_2$ nanopesticides have been found to have toxic effects on other organisms, such as *Leptocheirus plumulosus*, zebrafish, and microbes, as well as on human hepatocellular carcinoma cells (Aksakal and Sisman, 2020; Vignardi et al., 2020; Zhang et al., 2020). Notably, the environmental and human health risks due to contamination of $Cu(OH)_2$ nanopesticides in water and residues on food products are still poorly understood. Moreover, MPs have been detected extensively in soil environments, organisms, and even humans (de Souza Machado et al., 2018; Hermsen et al., 2018; Ragusa et al., 2021; Song et al., 2021; Leslie et al., 2022). However, the adverse effects of coexisting $Cu(OH)_2$ nanopesticides and microplastics (MPs) on plants have not been formally investigated, although the adverse effects of Cu^{2+} in concert with MPs on plant seedling growth have attracted attention recently (Zong et al., 2021; Zhou et al., 2022).

Interestingly, MPs have shown two different effects on plant growth: inhibition and stimulation. From the inhibition perspective, MPs showed an adverse effect on seedling growth and the antioxidant system of wheat (*Triticum aestivum*) (Liu et al., 2021), on growth, photosynthesis and essential elements in *Cucurbita pepo* (Colzi et al., 2022), and on rice seedlings (Dong et al., 2020). From the stimulation perspective, MPs can stimulate plant growth, as expressed by root length increases (Lian et al., 2020; Lozano and Rillig, 2020; Liu et al., 2022; Zeb et al., 2022). This plant growth stimulation could be explained by the MPs increasing carbon and nitrogen levels in the plants (Lian et al., 2020; Liu et al., 2022) and improving the aeration and penetration of roots (Lozano and Rillig, 2020). Due to concerted efforts by scientists, the potential effects of MPs on the environment have been determined, although high concentrations of MPs were applied in only some of these studies. Nevertheless, the effects of MPs on plant growth at environmental concentrations (e.g., 1 mg/L) have not been investigated. Given that both MPs and other contaminants can coexist in the environment for a long time, MPs can serve as vectors for other contaminants, such as Cu (Zong et al., 2021; Zhou et al., 2022), Cd (Zong et al., 2021; Zeb et al., 2022), Ag^+ (Sun et al., 2020), As(III) (Dong et al., 2020), phenanthrene (Liu et al., 2021), oxytetracycline (Bao et al., 2021), and nanomaterials (Li et al., 2020; Yang et al., 2021; Tong et al., 2022a; Tong et al., 2022b), and can modify the toxicity of these environmental contaminants to biota (Fries et al., 2013; Pacheco et al., 2018; Li et al., 2020; Yang et al., 2021; Zhang et al., 2021; Tong et al., 2022a; Tong et al., 2022b). In principle, MPs likely coexist with $Cu(OH)_2$ nanopesticides in the environment and induce coupled effects on plant growth, due to they are share the entry path into environment and share the major sink a long time in environment (Rajput et al., 2020; Wang et al., 2021). Accordingly, there are synergistic or antagonistic effects of Cu(OH)2 nanopesticides and MPs on plant growth, which are consistent with the inhibitory or stimulatory effects of MPs on plant growth. Nevertheless, it is still unclear whether environmental concentrations of polystyrene (PS) MPs drive the bioavailability and toxicity of $Cu(OH)_2$ nanopesticides in the context of plant seed germination or seedling growth.

To bridge these gaps, Lettuce (*Lactuca sativa*), which is a model plant and is widely applied in toxicity assays (Zhao et al., 2016a; Zhao et al., 2016b; Gao et al., 2021; Zeb et al., 2022), was applied in this study to explore the physiological and biological effects of $Cu(OH)_2$ nanopesticides and/or MPs on plants. Therefore, we hypothesized that $Cu(OH)_2$ nanowires could induce hazardous effects on *L. sativa* seed germination and seedling growth after $Cu(OH)_2$ nanopesticides enter the environment. Furthermore, we proposed that PS MPs that have been widely detected in realistic environments (Zhang et al., 2018; Ding et al., 2021) possibly stimulate lettuce growth at an environmental concentration (1 mg/L) and thus protect lettuce from the toxicity of $Cu(OH)_2$ nanopesticides. Accordingly, this experiment aimed to (i) determine the hazardous effects of $Cu(OH)_2$ nanowires on seed germination and seedling growth of lettuce and carry out a biological analysis, and (ii) explore the effects of PS MPs (1 mg/L) on stimulating lettuce seedling growth and suppressing $Cu(OH)_2$ nanowire toxicity to lettuce.

2 Materials and methods

2.1 Materials

PS MPs (0.1 μm) and fluorescent PS MPs were purchased from Tianjin Baseline Chromtech Research Centre, China. $Cu(OH)_2$

nanowires (Product No. CuOH-NW-40) were obtained from Beijing Dk Nano Technology Co., Ltd. Scanning electron microscopy (SEM, GeminiSEM 500, Zeiss) was employed to determine the sizes and morphologies of the PS MPs and Cu $(OH)_2$ nanowires. The hydrodynamic diameters and zeta potentials of the PS MPs and/or $Cu(OH)_2$ nanowires were determined with a Zetasizer Nano instrument (1000S, Malvern Instruments, Ltd., UK). Lettuce (*Lactuca sativa*) seeds were purchased from Nanyang Seed Inc. (Henan, China). Peroxidase (POD), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT) activity assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

2.2 Lettuce culture and Cu and/or PS MPs exposure

The lettuce seeds were surface disinfected in hydrogen peroxide (3%, v/v) for 30 min with continuous stirring, rinsed 3 times with deionized water (DI water), and immersed in DI water for 24 h. The seeds were randomly divided into eight different groups (100 seeds), including a control group (CK), a 1 mg/L $Cu(OH)_2$ nanowire group, a 10 mg/L $Cu(OH)_2$ nanowire group, a 10 mg/L Cu^{2+} group, a PS (1 mg/L) group, a PS (1 mg/L) + 1 mg/L $Cu(OH)_2$ nanowire group, a PS (1 mg/L) + 10 mg/L $Cu(OH)_2$ nanowire group, and a PS (1 mg/L) + 10 mg/L Cu^{2+} group. The 10 mg of $Cu(OH)_2$ nanowire were added into a 100 glass measuring flask and sonicated on an ultrasonic water bath (0.5 min) (ready-to-use). Certain of $Cu(OH)_2$ nanowire/Cu^{2+}($CuSO_4$) were added to certain of 100 mg/L of PS MPs solution or DI water to obtain the above different exposure groups. Four replicates for these groups were performed, and 100 seeds were placed in each glass Petri dish (⌀ 90 mm). Depending on the treatment group to which the seeds were randomly assigned, the seeds were incubated with 7 mL of the assigned $Cu(OH)_2$ nanowires or Cu^{2+} with or without PS solution and were kept in the dark at 25°C for seed germination.

2.3 Germination, lettuce seedling, biomass, oxidative stress and chlorophyll measurements

The germination rate was observed and recorded for three days. Accordingly, the germination rate, germination index, vigor index and germination energy of the seeds were calculated by equations (1) to (4). The biomass of the lettuce seedlings was measured, as well as the root length and shoot height, and the fresh weights of shoots and roots were recorded.

$$\text{Germination rate (GR,\%)} = \frac{\text{Seed germination number}}{\text{Total number of tested seeds}} \text{ x } 100 \quad (1)$$

$$\text{Germination index (GI)} = \sum \left(\frac{G_t}{D_t}\right) \quad (2)$$

$$\text{Vigor index (VI)} = \text{GI x fresh weight of seedlin} \quad 3)$$

$$\text{Germination energy (GE,\%)} = \frac{\text{Seed germination number in 3 days}}{\text{Total number of tested seeds}} \text{ x; } 100 \quad (4)$$

Where GR and GI represent the germination rate and germination index, respectively. Gt represents the germination number at t days, and Dt represents the corresponding germination time (days). VI and GE represent the vigor index and germination energy, respectively.

Antioxidant enzyme levels, including SOD, CAT and POD activity levels and MDA levels, were measured with commercial assay kits obtained from Nanjing Jiancheng Bioengineering Research Institute during the seed germination (3 d) and seedling growth (7 d) stages. The measurement processes for the antioxidant enzyme activities were in accordance with the manufacturer's instructions. Chlorophyll a (Chl a) and chlorophyll b (Chl b) were measured at the seedling growth stage (7 d). A specific weight (m) of freeze-dried cotyledons of lettuce samples was ground in a mortar with quartz sand and then extracted with 80% acetone to a specific volume (V). The obtained supernatant was centrifuged (5000 r/min for 10 min at 4 °C), and the absorbance was measured at 663 and 645 nm with UV–Vis spectrometry. Finally, Chl a and Chl b were calculated according to equations (5) and (6), respectively.

$$\text{Chl a (mg/g)} = (12.7 * \text{OD}_{663} - 2.6 * \text{OD}_{645}) * V/m \quad (5)$$

$$\text{Chl b (mg/g)} = (22.9 * \text{OD}_{645} - 4.7 * \text{OD}_{663}) * V/m \quad (6)$$

Where m is the specific weight of the lettuce cotyledon sample and V is the specific volume created by adding 80% acetone to the ground cotyledons.

2.4 Uptake of PS MPs by lettuce seeds and seedlings

Surface-disinfected lettuce seeds were immersed in DI water for 24 h, after which 100 of these seeds were selected and exposed to 1 mg/L fluorescent PS MPs. The seeds were cultured with fluorescent PS MPs (total volume of 7 mL) for seed germination. After 3 d of exposure in the dark at 25°C, the lettuce seed and seedling samples were washed three times with DI water to exclude fluorescent PS MPs adsorbed on the root surface. Then, the uptake of fluorescent PS MPs by the lettuce seeds and seedlings was examined using fluorescence microscopy (Nikon SMZ1500, Japan) with a photomicrography system (Nikon DS-Fi1c, Japan). The excitation

wavelength of the fluorescence microscope was 488 nm, and the emission wavelength was 518 nm.

2.5 Cu accumulation in lettuce seeds and seedlings

Ten of lettuce seeds (at 3 d) and seedlings (at 7 d) samples were taken and washed with EDTA-2Na (0.1 mM; pH 6.0) to exclude extracellular Cu^{2+} or $Cu(OH)_2$ on the seed and seedling surfaces. Then, the sample tissues were washed three times with DI water and oven-dried to constant weight for digestion (by adding 4 mL of a mixture (1:3) of H_2O_2 and HNO_3 and heating at 80 °C for 2 h and then at 160°C for 4 h) and Cu accumulation quantification (by inductively coupled plasma–mass spectrometry).

2.6 Statistical analysis

The experimental data were for four replicates were analyzed with SigmaPlot 12.5. The data are presented as the means ± standard deviations. These data were normally distributed and were evaluated with a *t test* and/or one-way ANOVA to explore any significant differences between treatment groups. Significant differences are marked with asterisks (* ($p< 0.05$) and ** ($p< 0.01$), denoting statistically significant and highly statistically significant differences, respectively).

3 Results and discussion

3.1 Characterization of $Cu(OH)_2$ nanowires and PS MPs

The PS MPs exhibited a spherical shape with quite a smooth surface and had an average size of ~ 100 nm (Figure 1A). They were stable in ultrapure water (1 mg/L) according to their average hydrodynamic diameter of 182.46 (± 74.34) and zeta potential of −13.33 (± 0.61) mV. The $Cu(OH)_2$ nanowires exhibited a typical wire shape with a nanosized diameter (40-80 nm) and a microsized length (1-2 μm) (Figure 1B). According to the dynamic light scattering (DLS) results, the $Cu(OH)_2$ nanowires tended to aggregate slightly in ultrapure water (1 mg/L), based on their average hydrodynamic diameter of 1584.97 (± 674.29) nm and on their average zeta potential of −21.58 (± 1.81) mV. Interestingly, the $Cu(OH)_2$ nanowires (1 mg/L) with PS MPs (1 mg/L) presented a large hydrodynamic diameter of 2365.25 (± 856.37) nm and a low zeta potential of −35.12 (± 5.47) mV and tended to aggregate in ultrapure water.

3.2 Effects of $Cu(OH)_2$ nanowires and Cu^{2+} on lettuce seed germination and seedling growth

Based on the seed germination period (3 d) data, the $Cu(OH)_2$ nanowires and Cu^{2+} stress had no significant effect on the germination energy or the germination rate of the lettuce seeds ($p > 0.05$; Figures 2A, B). It is possible that the seed husks protected the lettuce seeds from the $Cu(OH)_2$ nanowires and Cu^{2+} stress during seed germination. Indeed, Cu ions hardly accumulated during the 3 d seed germination period ($p > 0.05$; Figure 2C). Nevertheless, compared with the radicle length of germinants in the water treatment groups, the radicle length was visibly shorter in the germinants of the $Cu(OH)_2$ nanowire and Cu^{2+} treatment groups (Figures 2G(I)). Moreover, the antioxidant defence system and the MDA content in seedlings were significantly modified under Cu stress, as reflected by the significant increase in SOD activity and the (highly) significant decrease in CAT and POD activities in the 10 mg/L $Cu(OH)_2$ nanowire and Cu^{2+} treatments ($p< 0.01$ or 0.05; Figures 3A–C), respectively. It has been proposed that the release of Cu^{2+} may be

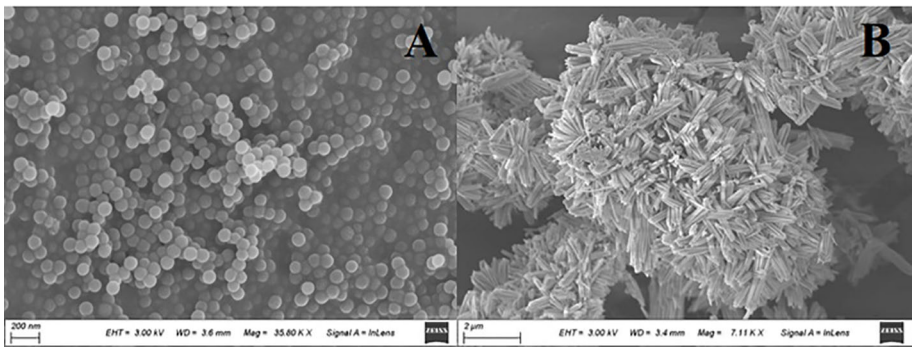

FIGURE 1
SEM images of the PS MPs **(A)** and $Cu(OH)_2$ nanowires **(B)**.

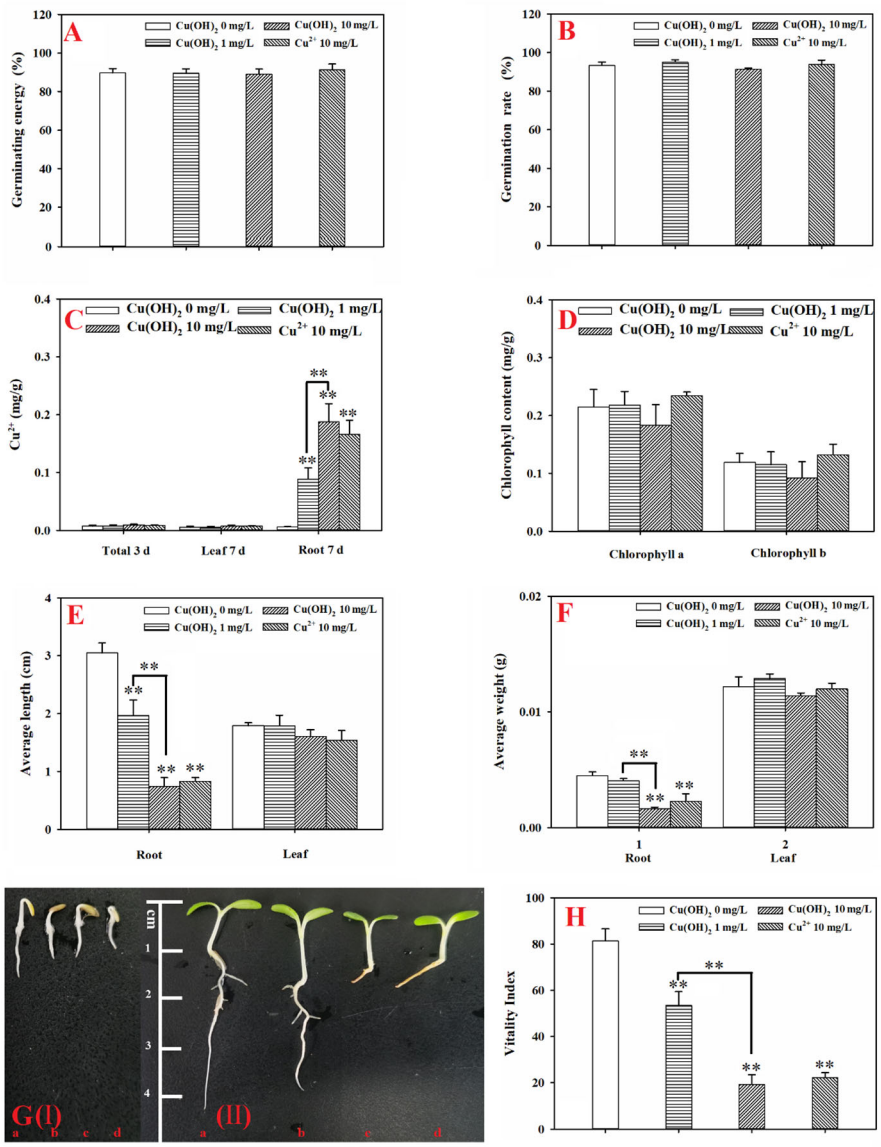

FIGURE 2
(A): Germination energy of lettuce seeds under different treatments; **(B)**: germination rate of lettuce seeds under different treatments; **(C)**: accumulation of Cu in lettuce seeds (3 d) and seedling leaves and roots (7 d) under different treatments; **(D)**: chlorophyll content in seedling leaves (7 d) under different treatments; **(E)**: average length of roots and leaves (7 d) under different treatments; **(F)**: average weight of roots and leaves (7 d) under different treatments; **(G)**: lettuce seed germination at 3 days (I) and lettuce seedling growth at 7 days (II). The letters a, b, c, and d denote the control, 1 mg/L Cu $(OH)_2$, 10 mg/L $Cu(OH)_2$, and 10 mg/L Cu^{2+} treatment groups, respectively; and **(H)** vigor index of lettuce seeds (7 d) under different treatments. The data are presented as the mean ± SD from at least four replicates. ** means extremely significant difference.

the underlying mechanism by which $Cu(OH)_2$ nanowires induce toxic effects in plants (Zhao et al., 2017c). In this study, the 10 mg/L Cu^{2+} stress treatment induced adverse effects comparable to those induced by the 10 mg/L $Cu(OH)_2$ nanowire stress treatment, suggesting that released Cu ions played a critical role in the toxicity effects induced by $Cu(OH)_2$ nanowires.

Based on the seedling growth period (7 d) data, the $Cu(OH)_2$ nanowire and Cu^{2+} treatments (highly) significantly inhibited the average root length ($p< 0.01$; Figure 2E) and weight ($p< 0.05$ or $p< 0.01$; Figure 2F). Correspondingly, the $Cu(OH)_2$ nanowire and Cu^{2+} treatments greatly decreased the vitality of the lettuce seedlings ($p< 0.01$; Figure 2H). This inhibition by the $Cu(OH)_2$ nanowires showed a dose-dependent effect (Figures 2E, F, H), which has implications for the health risk posed by $Cu(OH)_2$ nanowires in the environment. Notably, the 10 mg/L Cu^{2+} and $Cu(OH)_2$ nanowire treatments displayed similar effects, (highly)

significantly inhibiting the average root length and weight, as well as adversely affecting the vitality of the lettuce seedlings, which again emphasizes the important role of Cu^{2+} release in the hazardous effects induced by $Cu(OH)_2$ nanowires. Additionally, the antioxidant defence system and MDA content were significantly modified under Cu stress at 7 d of lettuce seedling growth. For example, compared with the SOD activity and MDA levels in seedling roots or leaves from the water treatment groups, those in the 10 mg/L $Cu(OH)_2$ nanowire or Cu^{2+} treatments were (highly) significantly increased ($p< 0.01$ or 0.05; Figures 3A, C). Furthermore, compared to the CAT and POD activities in seedling roots or leaves in the water treatment groups, those in the 10 mg/L $Cu(OH)_2$ nanowire or Cu^{2+} treatment groups were (highly) significantly decreased ($p< 0.01$ or 0.05; Figures 3B, C). Compared with the effect of Cu stress on seedling roots, Cu stress did not induce serious adverse effects on seedling leaves, as reflected by the chlorophyll a and b levels ($p > 0.05$; Figure 2D), leaf length ($p > 0.05$; Figure 2E) and leaf weight ($p > 0.05$; Figure 2F), which were not significantly modified under Cu stress. In contrast, other studies have detected biomass and chlorophyll content decrease, antioxidant-related enzyme modifications, metabolite profile alterations and antioxidant or defence-associated metabolite reductions in different plant leaves after $Cu(OH)_2$ nanopesticide or Cu^{2+} exposure through foliar spraying (Zhao et al., 2017a; Zhao et al., 2017b; Zhao et al., 2017c). It seems that the hazardous effects Cu stress induces in plants are strongly dependent on the exposure pathways. Indeed, in this study, the seedling roots were directly exposed to $Cu(OH)_2$ nanowires or Cu^{2+} solution, and thus, Cu significantly accumulated in seedling roots ($p< 0.05$; Figure 2C) rather than in seedling leaves. Notably, it has been proposed that the free Cu^{2+} released from $Cu(OH)_2$ nanopesticides may be a nonnegligible toxicity mechanism of the nanopesticides. Comparably, Cu was shown to predominantly accumulate in plant leaves but not in roots when a $Cu(OH)_2$ nanopesticide was applied through foliar spraying (Zhao et al., 2016b; Tan et al., 2018).

3.3 PS MPs (1 mg/L) stimulate lettuce seedling growth

PS MPs at an environmental concentration of 1 mg/L stimulated the seed germination and seedling growth of lettuce. To elaborate, lettuce seed germination was noticeably increased by the 1 mg/L PS MPs treatment [Figure 4G(I)],

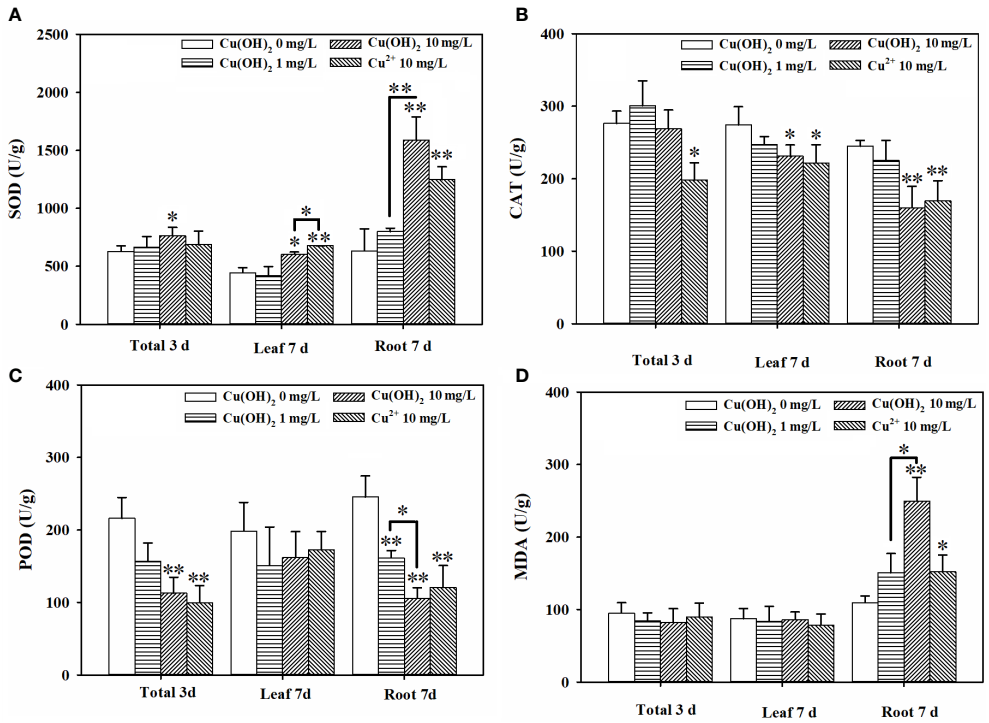

FIGURE 3
Effect of $Cu(OH)_2$ nanowire or Cu^{2+} stress on the activities of SOD **(A)**, CAT **(B)** and POD **(C)** and level of MDA **(D)** in lettuce seed (3d) and in lettuce seedling leaves and roots (7d). The data are shown as the mean ± SD from the four replicates and were analyzed with a *t test* to explore any significant differences between treatment groups; asterisks * ($p< 0.05$) and ** ($p< 0.01$) denote statistical significance and high statistical significance, respectively.

although the germination energy and rate were hardly affected ($p > 0.05$; Figures 4A, B). Moreover, the PS MPs treatment (highly) significantly increased the root and leaf length of the lettuce seedlings (Figure 4E) and significantly increased the seed vigor index ($p< 0.05$; Figure 4H). Similarly, a significant increase in root length in response to PS MPs (0.01 to 1 mg/L) and polyester microfiber treatments has also been observed in other plants (Lian et al., 2020; Lozano and Rillig, 2020; Liu et al., 2022; Zeb et al., 2022). The root length enhancements in response to MPs treatment could be interpreted as both PS MPs and cellulose cell walls are highly hydrophobic which makes PS MPs adsorb on the surface of the roots (Nel et al., 2009; Dovidat et al., 2019), increasing carbon and nitrogen levels in the plant (Lian et al., 2020; Liu et al., 2022) and improving the aeration and

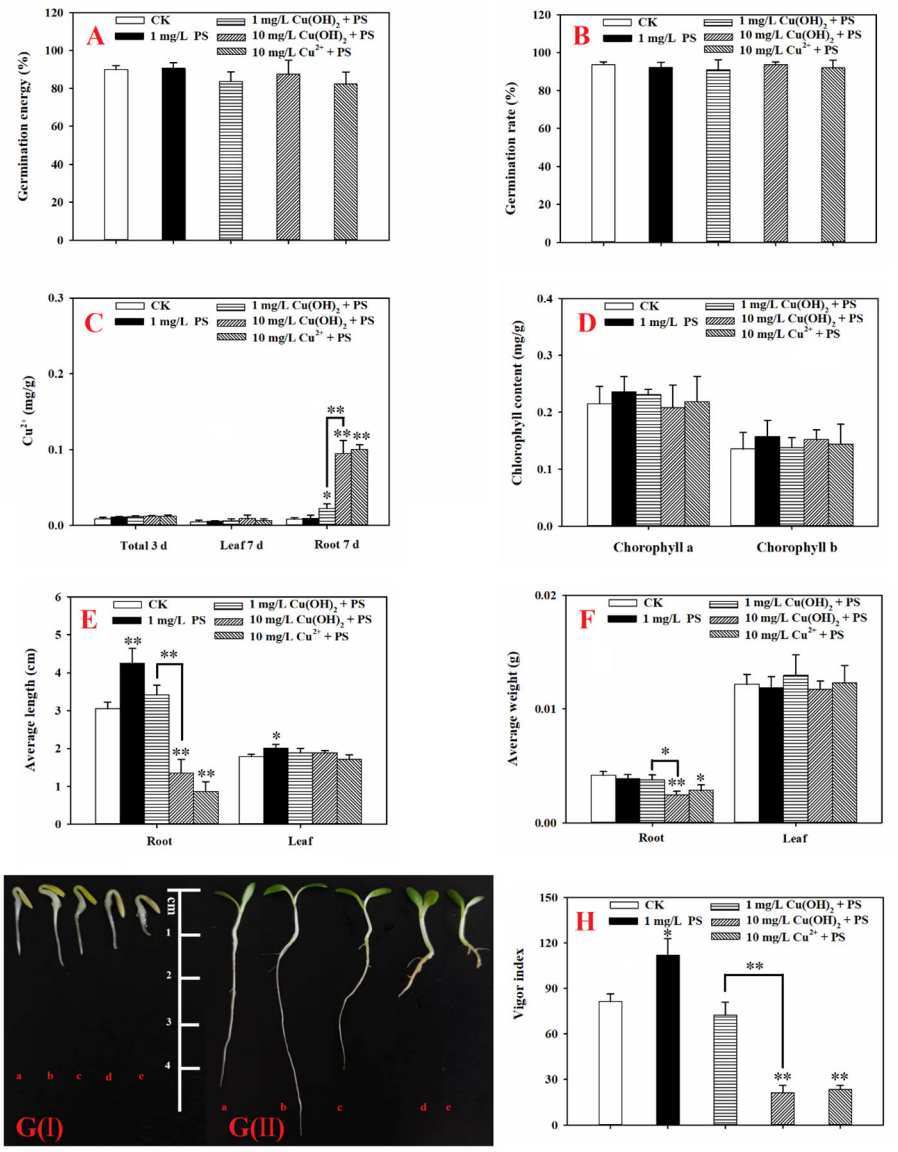

FIGURE 4
(A): Germination energy of lettuce seeds under different treatments; **(B)**: germination rates of lettuce seeds under different treatments; **(C)**: accumulation of Cu in lettuce seeds (3 d) and in seedling leaves and roots (7 d) under different treatments; **(D)**: chlorophyll content in seedling leaves (7 d) under different treatments; **(E)**: average length of roots and leaves (7 d) under different treatments; **(F)**: average weights of roots and leaves (7 d) under different treatments; **(G)**: lettuce seed germination at 3 d (I) and lettuce seedling growth at 7 d (II). The letters a, b, c, and d denote the control, 1 mg/L $Cu(OH)_2$, 10 mg/L $Cu(OH)_2$, and 10 mg/L Cu^{2+} treatment groups, respectively; and **(H)**: vigor index of lettuce seedlings (7 d) under different treatments. The data are shown as the mean ± SD from the four replicates and were analyzed with a *t test* to explore any significant differences between treatment groups; the asterisks * ($p< 0.05$) and ** ($p< 0.01$) denote statistical significance and high statistical significance, respectively.

penetration of roots (Lozano and Rillig, 2020). In this study, the PS MPs had no significant effect on the levels of chlorophyll a or b in lettuce leaves ($p > 0.05$; Figure 4D). In contrast, PE MPs and polyester microfibers have been found to increase the amount of chlorophyll in other plants (Wang et al., 2020; Liu et al., 2021; Zeb et al., 2022). Similarly, 1% PE MPs promoted carotenoids to reach the highest value in wheat leaves (Liu et al., 2021) and increased the total chlorophyll concentration in maize leaves (Wang et al., 2020). Accordingly, photosynthesis system stimulation by PE MPs could partially explain the plant growth increase (e.g., root length). In addition, the activities of SOD, CAT, and POD and the level of MDA in the lettuce leaves was hardly affected by the PS MPs (Figure 5), implying that the root, rather than the leaf, was the target organ of the PS MPs in this research. Furthermore, the adsorption of PS MPs on the middle root surface, but not on the root tips, was confirmed, as shown in Figure 6.This could partially explain why the PS MPs tended to affect the lettuce during the seedling growth stage rather than during the seed germination period (Figures 4A, B). However, significant PE MPs stimulation of SOD, CAT and POD activities ($P< 0.05$) and bottom-to-top transportation of PS MPs have been confirmed in wheat roots (Lian et al., 2020; Liu et al., 2021). It is possible that the different MPs types (PS vs. PE), sizes (nm vs. μm) and concentrations (low vs. high) are the causes of these inconsistent results.

3.4 Combined effects of PS MPs (1 mg/L) and $Cu(OH)_2$ nanowires or Cu^{2+} on lettuce seed germination and seedling growth

In this study, the 1 mg/L PS MPs treatment significantly suppressed the hazardous effects induced by $Cu(OH)_2$ nanowires or Cu^{2+} stress. Based on the seed germination period data (3 d), the PS MPs treatment helped SOD and CAT activities return to the control level ($p > 0.05$), and these activities were the dominant mechanisms for protection of lettuce seed germination against $Cu(OH)_2$ nanowire or Cu^{2+} stress (as mentioned in Section 3.2). Furthermore, when PS MPs were present, the highly decreased vigor index that was induced by the 1 mg/L $Cu(OH)_2$ nanowire treatment was reversed, and the vigor index returned to the control level ($p > 0.05$; Figure 4H). However, PS MPs treatment hardly affected the germination energy, the germination rate (Figures 4A, B), and Cu accumulation of the lettuce seeds (Figure 4C) in the $Cu(OH)_2$ nanowire or Cu^{2+} treatments.

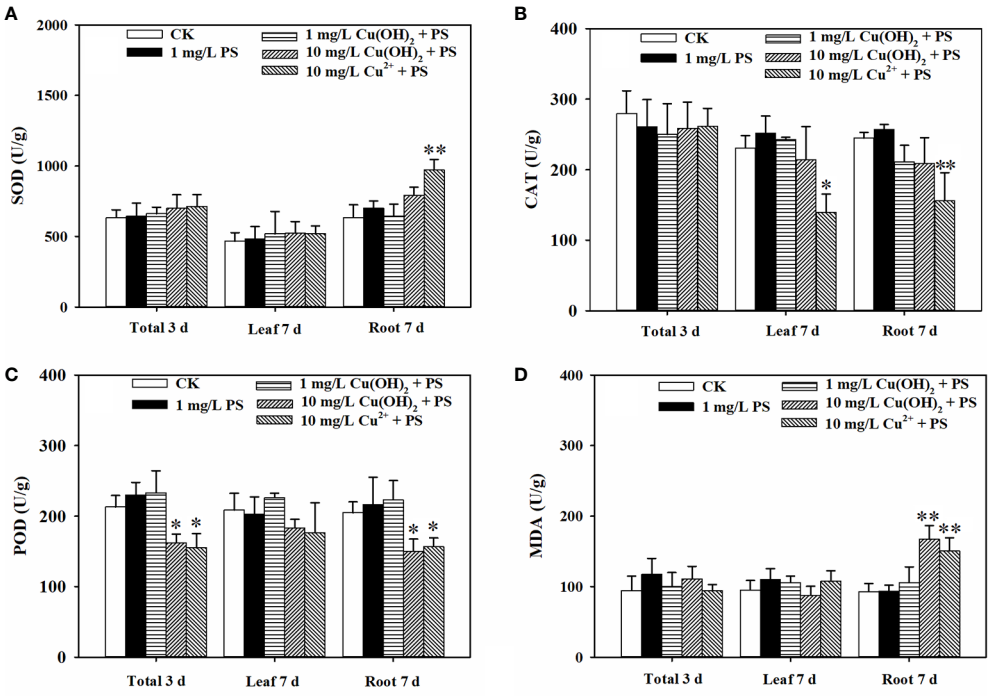

FIGURE 5
Effect of 1 mg/L PS MPs with or without $Cu(OH)_2$ nanowire or Cu^{2+} stress on the activities of SOD **(A)**, CAT **(B)** and POD **(C)** and the MDA levels **(D)** in lettuce seeds (3d) and in lettuce seedling leaves and roots (7d). The data were from four replicates and were analyzed with a *t test* to explore any significant differences between treatment groups; the asterisks * ($p < 0.05$) and ** ($p < 0.01$) denote statistical significance and high statistical significance, respectively.

Based on the seedling growth data (7 d), the decrease in lettuce seedling root length caused by the 1 mg/L $Cu(OH)_2$ nanowire treatment was significantly restrained by PS MPs treatment, as shown in Figures 4E, G(II). Moreover, the PS MP treatment partially suppressed the hazardous effects of the 10 mg/L $Cu(OH)_2$ nanowire or Cu^{2+} treatments on seedling root growth, although the average seedling root length in these treatment groups was still much shorter than that in the water treatment groups ($p< 0.01$; Figure 4E). As mentioned in Section 3.2, the dominant mechanisms of the decrease in seedling root length caused by Cu stresses were alterations of the antioxidant defence system, changes in MDA content and the accumulation of Cu. Compared with the SOD, CAT and POD activities in seedling roots or leaves in the single 10 mg/L $Cu(OH)_2$ nanowire or Cu^{2+} treatment groups, respectively, the activities in the treatment groups with 10 mg/L $Cu(OH)_2$ nanowires and Cu^{2+} with PS MPs were (highly) significantly decreased. Furthermore, the MDA level in the 10 mg/L $Cu(OH)_2$ nanowire treatment group was significantly decreased by the presence of PS MPs, although it was still much higher than that in the control (Figure 5D). Compared to the overall Cu levels in the single $Cu(OH)_2$ nanowire or Cu^{2+} treatment groups, the Cu level in seedling roots was also highly significantly decreased ($P< 0.01$) by the 1 mg/L PS MPs treatment. For example, the Cu concentration from the $Cu(OH)_2$ nanowire (1 mg/L) treatment decreased from 0.093 (± 0.017) to 0.023 (± 0.008) mg/g with PS MPs + $Cu(OH)_2$ nanowire (1 mg/L) treatment, and thus, Cu^{2+} bioavailability was decreased by PS MPs treatment in this study. Furthermore, the PS MPs were likely adsorbed on the surface of the middle root section (Figures 6C, D) rather than on the root tips (Figures 6A, B) and in turn decreased Cu^{2+} bioavailability. Notably, the uptake of nanosized MPs into plant roots has been determined previously (Jiang et al., 2019; Lian et al., 2020; Liu et al., 2021), and it has been shown that PS MPs vehicle effects on the biotic uptake of heavy metals (such as Zn^{2+} and Ag^{+}) are possible (Tong et al., 2022a; Tong et al., 2022b). However, in this study, the PS MPs bound to the lettuce root surface (Figure 6) rather than becoming embedded in the root. Thus, PS MPs treatment did not promote Cu accumulation in lettuce roots or leaves during the seedling growth period (Figure 4C). In contrast, the PS MPs might have prevented the $Cu(OH)_2$ nanowires or Cu^{2+} from entering the lettuce by binding to the lettuce root surface.

The protective effects of the MPs treatment on other plants have also been previously confirmed. For example, the presence of PE MPs reduced the accumulation of phenanthrene in wheat roots and leaves (Liu et al., 2021), decreased the dibutyl phthalate content in lettuce roots and leaves (Gao et al., 2021) and reduced Cu^{2+} toxicity on macrophyte growth (Zhou et al., 2022). PS MPs have been found to reduce the negative effects of As(III) on rice

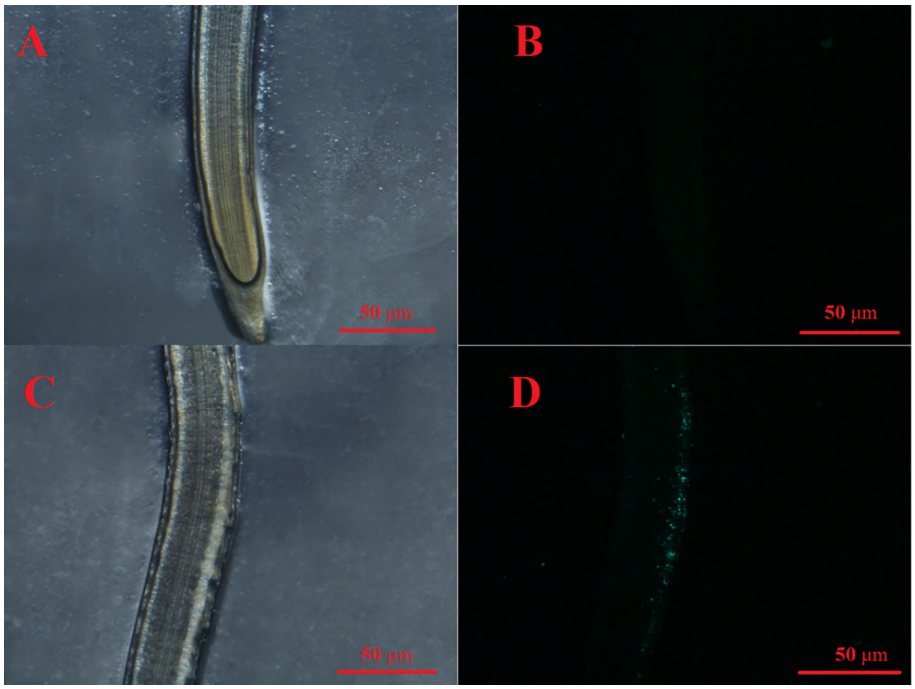

FIGURE 6
Seedling roots with 1 mg/L fluorescent PS MPs (0.1 μm) after 3 d of exposure. **(A)**: Root tip under normal light. **(B)**: Fluorescent signal in the same root tip shown in **(A, C)** Middle segment of a root under normal light. **(D)**: Fluorescent signal in the middle segment of the same root shown in **(C)**.

(Dong et al., 2020) and to mitigate the toxic effects of Cu on wheat seedlings (Zong et al., 2021). The underlying mechanisms that enable MPs to mitigate the hazardous effects of these environmental pollutants on plants are I) the reduction in environmental pollutant concentrations in water or soil through the adsorption of environmental pollutants on the MPs surface and II) the inhibition of environmental pollutant uptake into plants by MPs binding on the root surface (Dong et al., 2020; Zong et al., 2021; Zhou et al., 2022). It is possible that the different MPs types (PS vs. PE), MPs sizes (nm vs. μm), MPs exposure concentrations (low vs. high) and treatment durations (hour vs. day), as well as the plant species involved (wheat vs. lettuce), could explain why our study was inconsistent with results in previous research. In summary, $Cu(OH)_2$ nanowires greatly affected the vigor of lettuce seeds, the root length of lettuce seedlings and the lettuce antioxidant defence system and MDA content. Expectedly, released Cu^{2+} played a critical role in the toxicity mechanism of $Cu(OH)_2$ nanowires in lettuce seedlings. Interestingly, PS MPs (1 mg/L) stimulated lettuce seedling growth. Notably, treatment with PS MPs (1 mg/L) strongly hindered Cu accumulation in lettuce roots and thus neutralized the hazardous effects of the 1 mg/L $Cu(OH)_2$ nanowire treatment on lettuce growth. Therefore, PS MPs in the environment could protect lettuce from the hazardous effects of $Cu(OH)_2$ nanopesticides and decrease Cu risk in the food chain.

4 Conclusions

In this study, the hazardous effects of $Cu(OH)_2$ nanowires on lettuce seed germination and seedling growth were confirmed. These effects were highly related to the lettuce antioxidant defence system and MDA content. Released Cu^{2+} was a nonnegligible toxicity mechanism for the hazardous effects of $Cu(OH)_2$ nanowires on lettuce. PS MPs (1 mg/L) increased the lettuce seed vigor index and stimulated lettuce seedling growth. Furthermore, the PS MPs treatment (1 mg/L) partially suppressed the hazardous effects of $Cu(OH)_2$ nanowires on lettuce growth. This study provides new insights into the toxicity and toxicity mechanisms of $Cu(OH)_2$ nanowires and/or PS MPs to plants.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

GY, data curation, investigation, and writing of the original draft. YS and LY, data curation and investigation. YZ, resources and visualization. WZ, funding acquisition, formal analysis, writing-review, and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Aksakal, F. I., and Sisman, T. (2020). Developmental toxicity induced by Cu (OH)2 nanopesticide in zebrafish embryos. *Environ. Toxicol.* 35 (12), 1289–1298. doi: 10.1002/tox.22993

Bao, Y., Pan, C., Li, D., Guo, A., and Dai, F. (2021). Stress response to oxytetracycline and microplastic-polyethylene in wheat (Triticum aestivum l.) during seed germination and seedling growth stages. *Sci. Total Environ.* 806 (Pt 2), 150553. doi: 10.1016/j.scitotenv.2021.150553

Bindra, H. S., and Singh, B. (2021). *Advances in nano-fertilizers and nano-pesticides in agriculture*. Eds. S. Jogaiah, H. B. Singh, L. F. Fraceto and R.d. Lima (Woodhead Publishing) 57–84. doi: 10.1016/B978-0-12-820092-6.00003-3

Colzi, I., Renna, L., Bianchi, E., Castellani, M. B., Coppi, A., Pignattelli, S., et al. (2022). Impact of microplastics on growth, photosynthesis and essential elements in cucurbita pepo. *L. J. Hazard. Mater.* 423, 127238. doi: 10.1016/j.jhazmat.2021.127238

Conway, J. R. (2015). *Fate, transport & implications of engineered nanomaterials in the terrestrial environment* (Santa Barbara: University of California).

de Souza Machado, A. A., Lau, C. W., Till, J., Kloas, W., Lehmann, A., Becker, R., et al. (2018). Impacts of microplastics on the soil biophysical environment. *Environ. Sci. Technol.* 52 (17), 9656–9665. doi: 10.1021/acs.est.8b02212

Ding, R., Tong, L., and Zhang, W. (2021). "Microplastics in freshwater environments: Sources, fates and toxicity," in *Water, air, soil pollut*. 232. doi: 10.1007/s11270-021-05081-8

Dong, Y., Gao, M., Song, Z., and Qiu, W. (2020). Microplastic particles increase arsenic toxicity to rice seedlings. *Environ. pollut.* 259, 113892. doi: 10.1016/j.envpol.2019.113892

Dovidat, L. C., Brinkmann, B. W., Vijver, M. G., and Bosker, T. (2019). Plastic particles adsorb to the roots of freshwater vascular plant spirodela polyrhiza but do not impair growth. *Limnology Oceanography Lett.* 5 (1), 37–45. doi: 10.1002/lol2.10118

Du, W., Tan, W., Yin, Y., Ji, R., Peralta-Videa, J. R., Guo, H., et al. (2018). Differential effects of copper nanoparticles/microparticles in agronomic and physiological parameters of oregano (Origanum vulgare). *Sci. Total Environ.* 618, 306–312. doi: 10.1016/j.scitotenv.2017.11.042

Fries, E., Dekiff, J. H., Willmeyer, J., Nuelle, M. T., Ebert, M., and Remy, D. (2013). Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. *Environ. Sci. Process Impacts* 15 (10), 1949–1956. doi: 10.1039/c3em00214d

Gao, M., Liu, Y., Dong, Y., and Song, Z. (2021). Effect of polyethylene particles on dibutyl phthalate toxicity in lettuce (Lactuca sativa l.). *J. Hazard. Mater.* 401, 123422. doi: 10.1016/j.jhazmat.2020.123422

Hermsen, E., Mintenig, S. M., Besseling, E., and Koelmans, A. A. (2018). Quality criteria for the analysis of microplastic in biota samples: A critical review. *Environ. Sci. Technol.* 52 (18), 10230–10240. doi: 10.1021/acs.est.8b01611

Jiang, X., Chen, H., Liao, Y., Ye, Z., Li, M., and Klobucar, G. (2019). Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant vicia faba. *Environ. pollut.* 250, 831–838. doi: 10.1016/j.envpol.2019.04.055

Konappa, N., Krishnamurthy, S., Arakere, U. C., Chowdappa, S., Akbarbasha, R., and Ramachandrappa, N. S. (2021). "Advances in nano-fertilizers and nano-pesticides in agriculture,". Eds. S. Jogaiah, H. B. Singh, L. F. Fraceto and R.d. Lima (Woodhead Publishing) 281–330. doi: 10.1016/B978-0-12-820092-6.00012-4

Leslie, H. A., van Velzen, M. J. M., Brandsma, S. H., Vethaak, A. D., Garcia-Vallejo, J. J., and Lamoree, M. H. (2022). Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* 163, 107199. doi: 10.1016/j.envint.2022.107199

Lian, J., Wu, J., Xiong, H., Zeb, A., Yang, T., Su, X., et al. (2020). Impact of polystyrene nanoplastics (PS NPs) on seed germination and seedling growth of wheat (Triticum aestivum l.). *J. Hazard. Mater.* 385, 121620. doi: 10.1016/j.jhazmat.2019.121620

Liu, S., Wang, J., Zhu, J., Wang, J., Wang, H., and Zhan, X. (2021). The joint toxicity of polyethylene microplastic and phenanthrene to wheat seedlings. *Chemosphere* 282, 130967. doi: 10.1016/j.chemosphere.2021.130967

Liu, Y., Xiao, M., Shahbaz, M., Hu, Z., Zhu, Z., Lu, S., et al. (2022). Microplastics in soil can increase nutrient uptake by wheat. *J. Hazard. Mater.* 438, 129547. doi: 10.1016/j.jhazmat.2022.129547

Li, L., Xu, Z., Kah, M., Lin, D., and Filser, J. (2019). Nanopesticides: A comprehensive assessment of environmental risk is needed before widespread agricultural application. *Environ. Sci. Technol.* 53, 7923-7924. doi: 10.1021/acs.est.9b03146

Li, P., Zou, X., Wang, X., Su, M., Chen, C., Sun, X., et al. (2020). A preliminary study of the interactions between microplastics and citrate-coated silver nanoparticles in aquatic environments. *J. Hazard. Mater.* 385, 121601. doi: 10.1016/j.jhazmat.2019.121601

Lozano, Y. M., and Rillig, M. C. (2020). Effects of microplastic fibers and drought on plant communities. *Environ. Sci. Technol.* 54 (10), 6166–6173. doi: 10.1021/acs.est.0c01051

Nair, P. M. G., and Chung, I. M. (2015). Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (Brassica juncea l.). *Ecotoxicol. Environ. Saf.* 113, 302–313. doi: 10.1016/j.ecoenv.2014.12.013

Nel, A. E., Mädler, L., Velegol, D., Xia, T., Hoek, E. M., Somasundaran, P., et al. (2009). Understanding biophysicochemical interactions at the nano-bio interface. *Nat. Mater* 8 (7), 543–557. doi: 10.1038/nmat2442

Pacheco, A., Martins, A., and Guilhermino, L. (2018). Toxicological interactions induced by chronic exposure to gold nanoparticles and microplastics mixtures in daphnia magna. *Sci. Total Environ.* 628-629, 474–483. doi: 10.1016/j.scitotenv.2018.02.081

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., et al. (2021). Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* 146, 106274. doi: 10.1016/j.envint.2020.106274

Rajput, V., Minkina, T., Ahmed, B., Sushkova, S., Singh, R., Soldatov, M., et al. (2020). Interaction of copper-based nanoparticles to soil, terrestrial, and aquatic systems: Critical review of the state of the science and future perspectives. *Rev. Environ. Contam. Toxicol.* 252, 51–96. doi: 10.1007/398_2019_34

Song, K., Ding, R., Sun, C., Yao, L., and Zhang, W. (2021). Microparticles and microplastics released from daily use of plastic feeding and water bottles and plastic injectors: potential risks to infants and children in China. *Environ. Sci. pollut. Res. Int.* 28 (42), 59813–59820. doi: 10.1007/s11356-021-14939-7

Sun, C., Zhang, W., Ding, R., Wang, J., and Yao, L. (2020). Mechanism of low concentrations of polystyrene microplastics influence the cytotoxicity of Ag ions to escherichia coli. *Chemosphere* 253, 126705. doi: 10.1016/j.chemosphere.2020.126705

Tan, W., Gao, Q., Deng, C., Wang, Y., Lee, W.-Y., Hernandez-Viezcas, J. A., et al. (2018). Foliar exposure of Cu(OH)2 nanopesticide to basil (Ocimum basilicum): Variety-dependent copper translocation and biochemical responses. *J. Agric. Food Chem.* 66 (13), 3358–3366. doi: 10.1021/acs.jafc.8b00339

Tong, L., Duan, P., Tian, X., Huang, J., Ji, J., Chen, Z., et al. (2022a). Polystyrene microplastics sunlight-induce oxidative dissolution, chemical transformation and toxicity enhancement of silver nanoparticles. *Sci. Total Environ.* 827, 154180. doi: 10.1016/j.scitotenv.2022.154180

Tong, L., Song, K., Wang, Y., Yang, J., Jun, J., Lu, J., et al. (2022b). Zinc oxide nanoparticles dissolution and toxicity enhancement by polystyrene microplastics under sunlight irradiation. *Chemosphere* 299, 134421. doi: 10.1016/j.chemosphere.2022.134421

Vignardi, C. P., Muller, E. B., Tran, K., Couture, J. L., Means, J. C., Murray, J. L. S., et al. (2020). Conventional and nano-copper pesticides are equally toxic to the estuarine amphipod leptocheirus plumulosus. *Aquat. Toxicol.* 224, 105481. doi: 10.1016/j.aquatox.2020.105481

Wang, L., Wu, W. M., Bolan, N. S., Tsang, D. C. W., Li, Y., Qin, M., et al. (2021). Environmental fate, toxicity and risk management strategies of nanoplastics in the environment: Current status and future perspectives. *J. Hazard. Mater.* 401, 123415. doi: 10.1016/j.jhazmat.2020.123415

Wang, F., Zhang, X., Zhang, S., Zhang, S., and Sun, Y. (2020). Interactions of microplastics and cadmium on plant growth and arbuscular mycorrhizal fungal communities in an agricultural soil. *Chemosphere* 254, 126791. doi: 10.1016/j.chemosphere.2020.126791

Yang, W., Cheng, P., Adams, C. A., Zhang, S., Sun, Y., Yu, H., et al. (2021). Effects of microplastics on plant growth and arbuscular mycorrhizal fungal communities in a soil spiked with ZnO nanoparticles. *Soil Biol. Biochem.* 155, 108179. doi: 10.1016/j.soilbio.2021.108179

Zeb, A., Liu, W., Meng, L., Lian, J., Wang, Q., Lian, Y., et al. (2022). Effects of polyester microfibers (PMFs) and cadmium on lettuce (Lactuca sativa) and the rhizospheric microbial communities: A study involving physio-biochemical properties and metabolomic profiles. *J. Hazard. Mater.* 424 (Pt C), 127405. doi: 10.1016/j.jhazmat.2021.127405

Zhang, K., Shi, H., Peng, J., Wang, Y., Xiong, X., Wu, C., et al. (2018). Microplastic pollution in china's inland water systems: A review of findings, methods, characteristics, effects, and management. *Sci. Total Environ.* 630, 1641–1653. doi: 10.1016/j.scitotenv.2018.02.300

Zhang, W., Song, K., Ding, R., Han, H., Yao, L., Ji, M., et al. (2021). Role of polystyrene microplastics in sunlight-mediated transformation of silver in aquatic environments: Mechanisms, kinetics and toxicity. *J. Hazard. Mater.* 419, 126429. doi: 10.1016/j.jhazmat.2021.126429

Zhang, X., Xu, Z., Qian, X., Lin, D., Zeng, T., Filser, J., et al. (2020). Assessing the impacts of Cu(OH)2 nanopesticide and ionic copper on the soil enzyme activity and bacterial community. *J. Agric. Food Chem.* 68 (11), 3372–3381. doi: 10.1021/acs.jafc.9b06325

Zhao, L., Huang, Y., Adeleye, A. S., and Keller, A. A. (2017c). Metabolomics reveals Cu(OH)2 nanopesticide-activated anti-oxidative pathways and decreased beneficial antioxidants in spinach leaves. *Environ. Sci. Technol.* 51 (17), 10184–10194. doi: 10.1021/acs.est.7b02163

Zhao, L., Huang, Y., Hannah-Bick, C., Fulton, A. N., and Keller, A. A. (2016a). Application of metabolomics to assess the impact of Cu(OH)2 nanopesticide on the nutritional value of lettuce (Lactuca sativa): Enhanced Cu intake and reduced antioxidants. *NanoImpact* 3-4, 58–66. doi: 10.1016/j.impact.2016.08.005

Zhao, L., Hu, Q., Huang, Y., Fulton, A. N., Hannah-Bick, C., Adeleye, A. S., et al. (2017a). Activation of antioxidant and detoxification gene expression in cucumber plants exposed to a Cu(OH)2 nanopesticide. *Environ. Science: Nano* 4 (8), 1750–1760. doi: 10.1039/C7EN00358G

Zhao, L., Hu, Q., Huang, Y., and Keller, A. A. (2017b). Response at genetic, metabolic, and physiological levels of maize (Zea mays) exposed to a Cu(OH)2

nanopesticide. *ACS Sustain. Chem. Eng.* 5 (9), 8294–8301. doi: 10.1021/acssuschemeng.7b01968

Zhao, L., Ortiz, C., Adeleye, A. S., Hu, Q., Zhou, H., Huang, Y., et al. (2016b). Metabolomics to detect response of lettuce (Lactuca sativa) to Cu(OH)2 nanopesticides: Oxidative stress response and detoxification mechanisms. *Environ. Sci. Technol.* 50 (17), 9697–9707. doi: 10.1021/acs.est.6b02763

Zhou, J., Liu, X., Jiang, H., Li, X., Li, W., and Cao, Y. (2022). Antidote or Trojan horse for submerged macrophytes: Role of microplastics in copper toxicity in aquatic environments. *Water Res.* 216, 118354. doi: 10.1016/j.watres.2022.118354

Zong, X., Zhang, J., Zhu, J., Zhang, L., Jiang, L., Yin, Y., et al. (2021). Effects of polystyrene microplastic on uptake and toxicity of copper and cadmium in hydroponic wheat seedlings (Triticum aestivum l.). *Ecotoxicol Environ. Saf.* 217, 112217. doi: 10.1016/j.ecoenv.2021.112217

Zuverza-Mena, N., Medina-Velo, I. A., Barrios, A. C., Tan, W., Peralta-Videa, J. R., and Gardea-Torresdey, J. L. (2015). Copper nanoparticles/compounds impact agronomic and physiological parameters in cilantro (Coriandrum sativum). *Environ. Science: Processes Impacts* 17 (10), 1783–1793. doi: 10.1039/C5EM00329F

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Genome-wide analysis and characterization of *Dendrocalamus farinosus SUT* gene family reveal *DfSUT4* involvement in sucrose transportation in plants

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Sucrose is the main transported form of photosynthetic products. Sucrose transporter (SUT) participates in the translocation of sucrose from source to sink, which is important for the growth and development of plants. *Dendrocalamus farinosus* is an important economic crop in southwestern China because of its high growth rate, high fiber content, and dual usage for food and timber, but the mechanism of sucrose transportation in *D. farinosus* is unclear. In this study, a total of 12 *SUT* transporter genes were determined in *D. farinosus* by whole-genome identification. *DfSUT2*, *DfSUT7*, and *DfSUT11* were homologs of rice *OsSUT2*, while *DfSUT4* was a homolog of *OsSUT4*, and these four *DfSUT* genes were expressed in the leaf, internode, node, and bamboo shoots of *D. farinosus*. In addition, *DfSUT* family genes were involved in photosynthetic product distribution, ABA/MeJA responses, and drought resistance, especially *DfSUT4*. The function of *DfSUT4* was then verified in *Nicotiana tabacum*. DfSUT4 was localized mainly in the leaf mesophyll and stem phloem of *pDfSUT4::GUS* transgenic plant. The overexpression of *DfSUT4* gene in transgenic plant showed increases of photosynthetic rate, above-ground biomass, thousand grain weight, and cellulose content. Our findings altogether indicate that *DfSUT4* can be a candidate gene that can be involved in phloem sucrose transportation from the source leaves to the sink organs, phytohormone responses, abiotic stress, and fiber formation in plants, which is very important in the genetic improvement of *D. farinosus* and other crops.

KEYWORDS

***Dendrocalamus farinosus*, *SUT* gene, phytohormone responses, abiotic stress, genome-wide analysis, sucrose transportation, fiber formation**

Introduction

Sucrose is the major long-distance-transported form of carbohydrate metabolites in plants (Deol et al., 2013; Wang et al., 2021). After having been synthesized by photosynthesis in mature leaves (source), sucrose is further transported to a variety of heterotrophic organs (sink) such as roots, developing leaves, flowers, and seeds (Barker et al., 2000; Sun et al., 2010). Sucrose transporters (SUTs; also called SUCs) are sucrose–H^+ symporters that are localized in the vacuole membrane or plasma membrane (Zhu et al., 2015), and they are members of the major facilitator superfamily that contains 12 transmembrane-spanning helices (Aoki et al., 2012). In addition, the *SUT* family genes play key roles in sucrose loading and unloading from t source tissue to sink cells through the phloem to effect multiple processes of plant growth and development, such as phytohormone biosynthesis, abiotic stresses, photosynthesis, flower development, and fiber synthesis in many species (Srivastava et al., 2009; Slewinski and Braun, 2010; Payyavula et al., 2011; Wang et al., 2021). According to the direction and type of transmembrane transport of sucrose, SUT transporters in plants can be divided into three types: plasma membrane efflux carriers are active in the efflux of sucrose from the mesophyll cells to the apoplast, plasma membrane influx carriers function in the entry of sucrose from the apoplast into phloem cells, and tonoplast carriers are involved in the transport of sucrose between the vacuole and the cytoplasm (Ward et al., 1998; Lemoine, 2000).

To date, there are numerous studies that have shown SUTs as crucial not only for carbohydrate partitioning but also for responses to various phytohormones, abiotic stresses, and environmental factors in plants—for example, *Arabidopsis suc2* seedlings were smaller than the wild type in the absence of sucrose, and ABA treatment on *Arabidopsis suc2* mutant could increase the sucrose content in shoots but decrease the sucrose content in roots (Gottwald et al., 2000), suggesting that AtSUC2 is crucial for plant development and sucrose loading in *Arabidopsis* root (Gong et al., 2015). In contrast to other AtSUC transporters, AtSUC4 is localized in the tonoplast and participate in the translocation of sucrose from the vacuole into the cytoplasm (Weise et al., 2000). Consistently with *SUC4* from *Arabidopsis*, *PtaSUT4* from poplar and *OsSUT2* from rice are also localized to the tonoplast and mediate sucrose efflux from the source leaves (Eom et al., 2011). Moreover, PtaSUT4-repressed poplar showed increased leaf-to-stem biomass ratio and sucrose content in the source leaves because of the translocation of sucrose to the sink organs which was inhibited in transgenic plants (Payyavula et al., 2011). The plasma membrane-localized *OsSUT4* functions in the apoplastic phloem loading of sucrose. The rice *sut4* mutant showed a dwarf phenotype, and the yield of rice was decreased (Chung et al., 2014).

In addition, *AtSUC2*, *AtSUC4*, and *AtSUC9* are all associated with ABA treatment and drought/cold stress (Gong et al., 2015; Jia et al., 2015). *OsSUT2* promotes photosynthesis and sucrose distribution in plants under drought and salt stress (Ibraheem et al., 2011). *PtaSUT4* transporters also function in photosynthesis (Frost et al., 2012) and drought response (Harding et al., 2020). Furthermore, *PaSUT* and *OsSUT1* transporters are associated with phytohormone (auxin and cytokinin) signaling pathways in the translocation of sucrose in *Petunia axillaris* flower (Rolland et al., 2002; Iftikhar et al., 2020) and rice seeds (Zhao et al., 2022), respectively. More importantly, *PttSUT3* transporters are responsible for carbon delivery and formation of wood fibers in a hybrid aspen (Mahboubi et al., 2013).

Bamboo is widely scattered in China, and products made of bamboo have been utilized by humans since the ancient times (Qi et al., 2015). *D. farinosus* is one of the essential economic bamboo species in southwestern China, which has many advantages including high growth rate and disease resistance, cold and drought tolerance, and dual usage for food and timber (Zakikhani et al., 2014; Chen et al., 2021; Pei et al., 2022). In particular, *D. farinosus* is also an excellent paper pulp bamboo species because of its high fiber content, stiffness, and low density (Xiong et al., 2012; Imran et al., 2022). Sucrose is the predominant form of transported carbon, which is necessary to synthesize fiber (Rennie and Turgeon, 2009), and *SUT* transporter genes are crucial to carbon delivery and fiber formation in some plants (Zhang et al., 2017; Yadav et al., 2022). However, the mechanisms of sucrose translocation between the source leaves and the sink organs of *D. farinosus* are unclear. In this study, we reported the phylogenetic analysis and the whole-genome identification of *SUT* genes as well as their chromosomal localization and expression pattern in *D. farinosus*. Most *DfSUT* genes were involved in ABA/MeJA signaling pathways and drought resistance. We then verified the transport function of *D. farinosus DfSUT4* as a rice *OsSUT4* homolog in transgenic *Nicotiana tabacum*. Our results indicated that *DfSUT4* is a candidate gene in carbohydrate transport and improving the photosynthetic rate as well as involved in the phloem transportation of sucrose from the source leaves to the sink organs, phytohormone responses, abiotic stress, and fiber formation in *D. farinosus* and other crops.

Results

Identification of *SUT* genes in *D. farinosus*

The identified *SUT* sequences in rice were searched for the corresponding *SUT* homologous gene in *D. farinosus*. According to the chromosomal location, we identified 12 genes of the

DfSUT transporter family (Table 1), namely *DfSUT1*–*DfSUT12*. Detailed information about the 12 *DfSUT* genes, including the predicted length of CDSs, encoded proteins, and physicochemical parameters, are presented in Table 1. The length of *DfSUT* CDSs ranged from 291 to 1,803 bp, with an average length of 1,481 bp. The protein size of *DfSUT* was an average of 493 aa, which ranged from 96 to 600 aa. The molecular weight ranged from 10.54 kDa (*DfSUT12*) to 64.06 kDa (*DfSUT9*). The pI for all *DfSUT* genes was below 10.0, with an average value of 8.1; only one gene product (*DfSUT1*) was below 5.0. *DfSUT* genes were predicted to localize to the plasma membrane or tonoplast. Among them, *DfSUT1* was not assembled on the chromosome of Liang Shan Cichlid, so it was not discussed in the following analysis.

Analysis of the phylogenetic relationships, chromosomal localization, and gene structure of *SUT* families in *D. farinosus*

To comprehensively analyze the evolutionary relationships between SUT families in *D. farinosus*, *Phyllostachys edulis*, *D. latiflorus munro*, *Arabidopsis thaliana*, rice, and *Populus* L., we constructed a neighbor-joining (NJ) phylogenetic tree of *SUTs* based on 55 full-length SUT protein sequences, including 12 sequences from *D. farinosus*, 10 sequences from *Phyllostachys edulis*, 13 sequences from *D. latiflorus munro*, nine sequences from *Arabidopsis thaliana*, five sequences from rice, and six sequences from *Populus* L. (Figure 1A). All three woody bamboos contain more *SUT* genes than *Arabidopsis thaliana*, rice, and *Populus* L. According to the phylogenetic analysis, plant SUTs are divided into three types. Type I and type II SUTs are localized to the plasma membrane, and type III SUTs are localized to the vacuolar membrane (Reinders et al., 2012). The phylogenetic tree showed that the 55 SUT proteins could be divided into three distinct groups (clade I–clade III; Figure 1A). All 12 *SUT* genes in *D. farinosus* could be divided into two clades (clade II and clade III), such as three *DfSUTs* (*DfSUT2*, *DfSUT7*, and *DfSUT11*) that belonged to clade II. The other nine *DfSUTs* belonged to clade III. However, none of the *SUTs* in all three woody bamboos and rice belonged to clade I. *DfSUT2*, *DfSUT7*, and *DfSUT11* were homologs of rice *OsSUT2*, *Arabidopsis AtSUC4*, and *Populus* L. *PtaSUT4*. While *DfSUT4*, *DfSUT9*, and *DfSUT12* were homologs of *AtSUC3*, *OsSUT4*, *PtaSUT5*, and *PtaSUT6*, suggesting that they might have a similar function.

TABLE 1 Detailed information about the 12 predicted *DfSUT* genes in *D. farinosus*.

Locus name	Gene name	Clade	Os ortho-logs	pI	Molecular weight (Da)	Open reading frame length (bp)	Protein length (aa)	Location	Subcellular localization[a]
Dfa0G064610.1	*DfSUT1*	III	OsSUT3	4.77	59,552.72	1,683	560	Contig01867	Plasma membrane
DfaA10G000160.1	*DfSUT2*	II	OsSUT2	8.96	53,294.26	1,500	499	DfA01	Tonoplast
DfaB03G013150.1	*DfSUT3*	III	OsSUT5	8.66	53,560.78	1,500	499	DfB03	Plasma membrane
DfaB03G024800.1	*DfSUT4*	III	OsSUT4	6.18	63,938.51	1,803	600	DfB03	Plasma membrane
DfaB08G004380.1	*DfSUT5*	III	OsSUT1	8.84	55,356.67	1,575	524	DfB08	Plasma membrane
DfaB09G006030.1	*DfSUT6*	III	OsSUT3	8.32	51,342.23	1,464	487	DfB09	Plasma membrane
DfaB10G000010.1	*DfSUT7*	II	OsSUT2	9.28	53,431.54	1,503	500	DfB10	Tonoplast
DfaC03G011900.1	*DfSUT8*	III	OsSUT5	8.99	55,931.41	1,578	525	DfC03	Plasma membrane
DfaC03G022010.1	*DfSUT9*	III	OsSUT4	6.63	64,062.84	1,800	599	DfC03	Plasma membrane
DfaC08G007580.1	*DfSUT10*	III	OsSUT1	8.95	54,922.15	1,566	521	DfC08	Plasma membrane
DfaC08G021670.1	*DfSUT11*	II	OsSUT2	9.19	53,556.58	1,512	503	DfC08	Tonoplast
DfaC11G004310.1	*DfSUT12*	III	OsSUT4	8.77	10,535.14	291	96	DfC11	Plasma membrane

[a]Subcellular localization of *D. farinosus* sucrose transporters (SUTs) based on the subcellular localization of rice homologous SUTs (Hu et al., 2021).

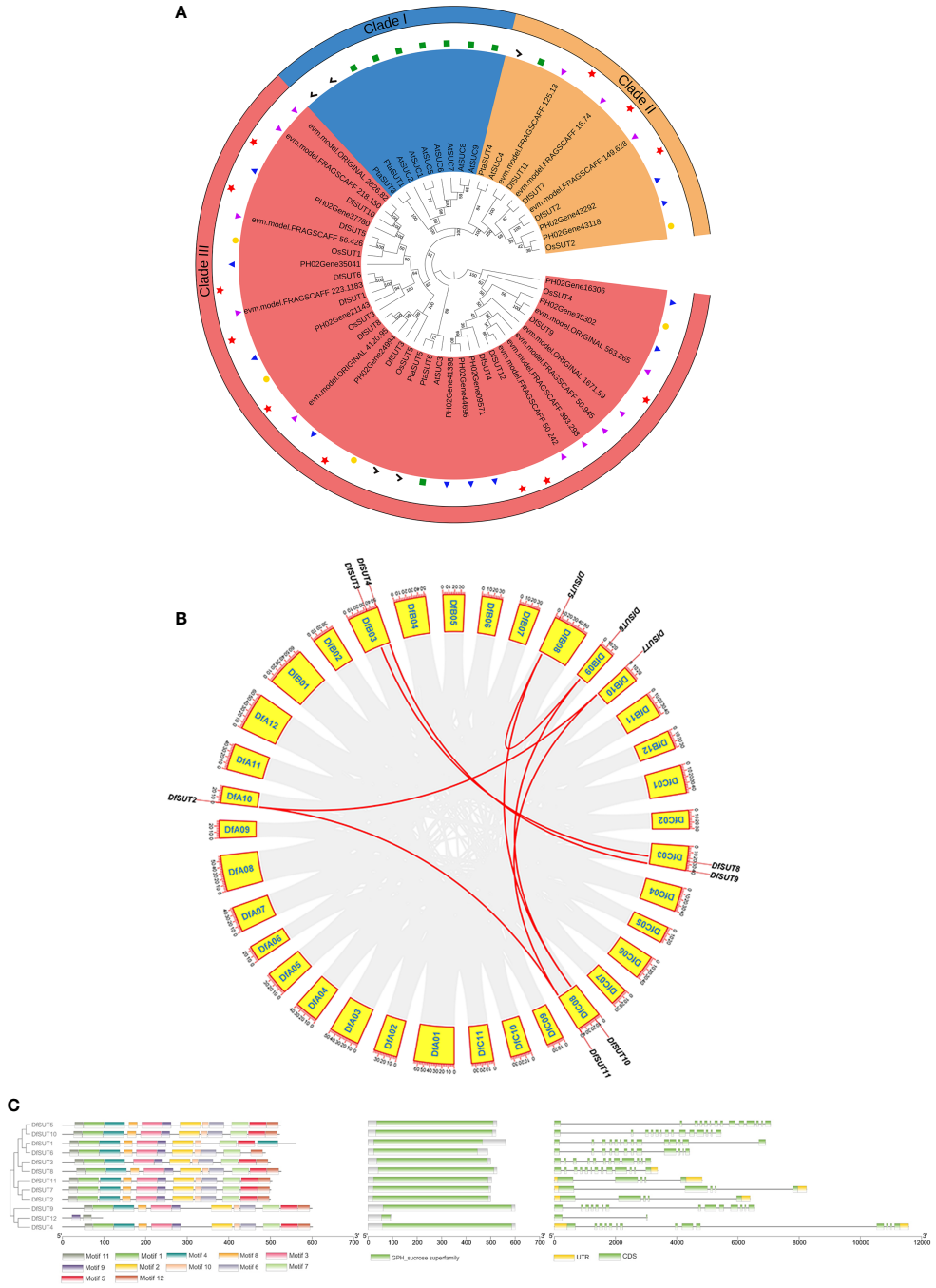

FIGURE 1
Analysis of the phylogenetic evolutionary, chromosomal localization and gene structure of the *SUT* family in *D. farinosus*. **(A)** Phylogenetic tree of the *SUT* family in *D. farinosus*. Based on the amino acid sequences of *D. farinosus*, *Phyllostachys edulis*, *D. latiflorus munro*, *Arabidopsis*, rice, and *Populus* L., phylogenetic trees were generated by the neighbor joining method using MEGA 7.0.21. The *SUT* members are divided into three branches: clades I, II, and III. The species are labeled with different colors: Df, *D. farinosus* (red); *Phyllostachys edulis* (blue); *D. latiflorus munro* (purple); At, *Arabidopsis* (green); Os, rice (yellow); Pta, *Populus* L. (black). **(B)** Chromosomal localization and gene duplication analysis of *DfSUT* genes in the genome of *D. farinosus*. The *DfSUT* genes are localized on different chromosomes. Chromosome numbers are indicated in the yellow box. The numbers on the chromosome boxes represent the sequence length in megabases. Gene pairs with sibling relationships are connected by a red line. **(C)** Conserved motif and gene structure analysis of the *SUT* family in *D. farinosus*. The three maps of conserved motif analysis, conserved structural domains, and exon/intron structure of *SUT* genes were merged in the order of phylogenetic tree by the Gene Structure View online software analysis of TBtools software.

Interestingly, we found that all three woody bamboos contained almost an equal number of *SUT* genes, and they all had more *SUT* genes than the other species.

The chromosomal distribution showed that 10 of the 12 *DfSUT* genes were mapped on seven chromosomes in *D. farinosus* (Figure 1B) (Supplementary Table S4). The result showed that four chromosomes (DfA10, DfB08, DfB09, and DfB10) harbored only one gene, and they were *DfSUT2*, *DfSUT5*, *DfSUT6*, and *DfSUT7*, respectively. Three chromosomes (DfB03, DfC03, and DfC08) contained two genes, such that chromosome DfB03 contained *DfSUT3* and *DfSUT4*, chromosome DfC03 contained *DfSUT8* and *DfSUT9*, and chromosome DfC08 contained *DfSUT10* and *DfSUT11*. In addition, among the 12 *DfSUT* family genes, there were 10 genes with existing gene duplication. Seven gene pairs connected with each other, representing fragment duplication, such as *DfSUT2* and *DfSUT7*, *DfSUT2* and *DfSUT11*, *DfSUT7* and *DfSUT11*, *DfSUT3* and *DfSUT8*, *DfSUT4* and *DfSUT9*, *DfSUT5* and *DfSUT6*, *DfSUT5* and *DfSUT10* (Figure 1B). In addition, we estimated the approximate dates of *DfSUT* gene duplication events and the gene evolutionary selection pressure by measuring the Ka and Ks values and the Ka/Ks ratios (Supplementary Table S5). The Ka/Ks ratios of the *DfSUT* genes were all less than 1, indicating that they were all in a purifying selection condition.

We then analyzed the conserved motif and gene structure of the *SUT* family in *D. farinosus* (Figure 1C). The amino acid sequences of DfSUT in Table 1 were used to construct the phylogenetic tree separately. The conserved motifs and structural domains of the family were identified using MEME and GSDS online tools, respectively. The structural analysis maps of the above-mentioned three sequences were combined and plotted using TBtools (Figure 1C). The result showed that all DfSUT proteins contain 11 or 12 motifs and have similar conserved structural domains, except for DfSUT12 which has only two motifs. In the *SUT* family, eight *SUT* genes contain 14 exons and 13 introns, while three *SUT* genes contain five exons and four introns. Interestingly, we found that *DfSUT12* only has two exons and one intron, and seven *SUT* genes including *DfSUT12* have no upstream and downstream untranslated regions (Figure 1C).

Analysis of the expression patterns of *SUT* genes and C content in different developmental phases/tissues in *D. farinosus*

To determine the dynamic gene expression of *SUT* genes in *D. farinosus*, we performed RNA-Seq analysis of 12 *SUT* protein-coding genes in 14 organs and developmental stages of *D. farinosus*, including branch, culm sheath (CSh), internode (Inod), lateral bud (Labud), mature leaf (MLeaf), node, root, sheath of bamboo shoot (ShB), young leaf (Yleaf), 10-cm shoot (C10), 50-cm shoot (C50), 200-cm shoot (C200), 400-cm shoot (C400), and 800-cm shoot (C800). According to the transcriptome heat map, *DfSUT2*, *DfSUT4*, *DfSUT7*, and *DfSUT11* showed a wide range of expression levels and distinct regulation during *D. farinosus* development (Figure 2A). These four *SUT*-encoding genes (*DfSUT2*, *DfSUT4*, *DfSUT7*, and *DfSUT11*) were abundantly expressed in the branch, leaf, and root (Figure 2A), suggesting that they were associated with the transportation of sucrose from the source leaf to the sink organs. They were all highly expressed in bamboo shoots (Figure 2A), such as *DfSUT2* and *DfSUT11* in 400-cm-tall shoot, *DfSUT4* in 200-cm- and 800-cm-tall shoots, and *DfSUT7* in 200-cm-tall shoot, suggesting that they were all associated with the rapid growth of shoots. Interestingly, *DfSUT4*, *DfSUT7*, and *DfSUT11* were abundantly expressed in internode and node, suggesting that they were involved in sucrose accumulation in the node and internode of *D. farinosus*. *DfSUT8* was only highly expressed in the lateral bud and 10-cm shoot, suggesting that it is important for the development of buds and shoots.

We then measured the C content in different developmental phases and tissues in *D. farinosus* (Figure 2B). Consistent with the expression patterns of *SUT* genes, the high C content in the mature internode suggested that carbohydrate transportation by DfSUT proteins was necessary to synthesize cellulose in the internode of *D. farinosus*. More importantly, the new leaf (base), new node (base), and mature root of *D. farinosus* contained the same high C content (Figure 2B). These results showed that, except the leaf source and the root sink, the bamboo node that consists of complex vascular bundles in *D. farinosus* might be a temporary sink for the accumulation of sucrose for some specific functions.

Prediction of cis-regulatory elements in the *SUT* families of *D. farinosus*

To investigate the responses to various factors by *DfSUT* members, the promoter (2 kb upstream) of these genes was submitted to the PlantCARE server to predict its promoter cis-regulatory elements. We identified 20 cis-regulatory elements in *DfSUT* gene promoter, such as phytohormone response elements (abscisic acid, auxin, methyl jasmonate, gibberellin, and salicylic acid), abiotic stress response elements (anaerobic, low-temperature, drought, defense, wound, and light), and growth and development response elements (meristem expression, circadian control, mesophyll cells differentiation, cell cycle regulation, and seed-specific regulation) (Figure 3). The result showed that most *DfSUT* genes were associated with plant hormones, stress, growth, and development, suggesting that *DfSUT* genes are involved in multiple physiological processes through a variety of environmental adaptations. In addition, ABRE-motif, CGTCA-motif, TGACG-motif, MBS-motif, and G-Box-motif were most enriched in the *DfSUT* promoter regions (Figure 3), which indicated that most *DfSUT* genes might be involved in the responses of ABA, MeJA, drought, and light—for example, six *DfSUT* genes (*DfSUT2*, *DfSUT4*, *DfSUT5*, *DfSUT6*, *DfSUT8*, and *DfSUT10*) were related to the responses of

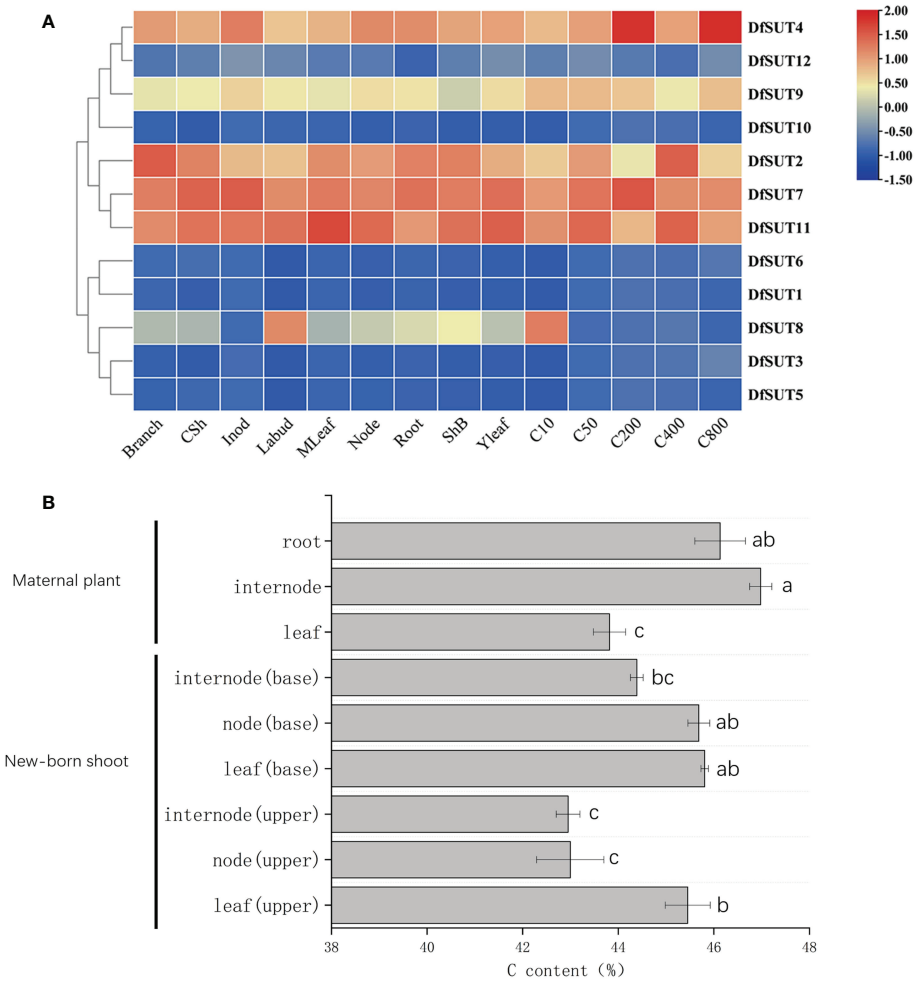

FIGURE 2
Analysis of transcriptome expression profiles of *SUT* and C content in different developmental phases/tissues of *D. farinosus*. **(A)** Transcriptome expression profiles of *SUT* at different developmental stages and various parts in *D. farinosus*. The red and blue colors correspond to the strong and weak expression of the genes, respectively. **(B)** C content in different developmental phases and tissues in *D. farinosus*. Data are detected as the mean ± SD from three independent experiments. The *P*-values of Student's test for different developmental phases and tissues in *D. farinosus* are denoted with letters ($P < 0.05$).

ABA, and five *DfSUT* genes (*DfSUT2*, *DfSUT4*, *DfSUT5*, *DfSUT7*, and *DfSUT11*) were related to the responses of MeJA. Moreover, *DfSUT2* and *DfSUT5* included more than 10 motifs in the promoter that were associated with ABA and light, respectively. *DfSUT4* contained about five motifs in the promoter that were associated with ABA, MeJA, and light, respectively.

Analysis of the photosynthetic rate and response to phytohormone treatment and drought stress of the *SUT* family in *D. farinosus*

To determine the transport of photosynthetic products by SUT proteins, we measured the transcript levels and net photosynthetic reaction rate in *D. farinosus* leaves every 3 h (from 6 a.m. to 9 p.m.). The results showed that the expression of *DfSUT* remained basically consistent with the change in the net photosynthetic reaction rate, except *DfSUT7* (Figure 4A). The transcript levels of five *DfSUT* genes (*DfSUT2*, *DfSUT4*, *DfSUT8*, *DfSUT9*, and *DfSUT11*) increased with the increase of the net photosynthetic reaction rate in *D. farinosus*, and both of them reached the highest levels at 9 a.m. These results indicated that the expression of these *DfSUT* genes was involved in the transport of photosynthetic products.

More importantly, we determined the response of *DfSUT* genes to phytohormone treatment (ABA and MeJA) and drought stress. Consistent with the analysis of the cis-regulatory elements of the *DfSUT* gene promoter, the results showed that the expression of nine *DfSUT* genes decreased

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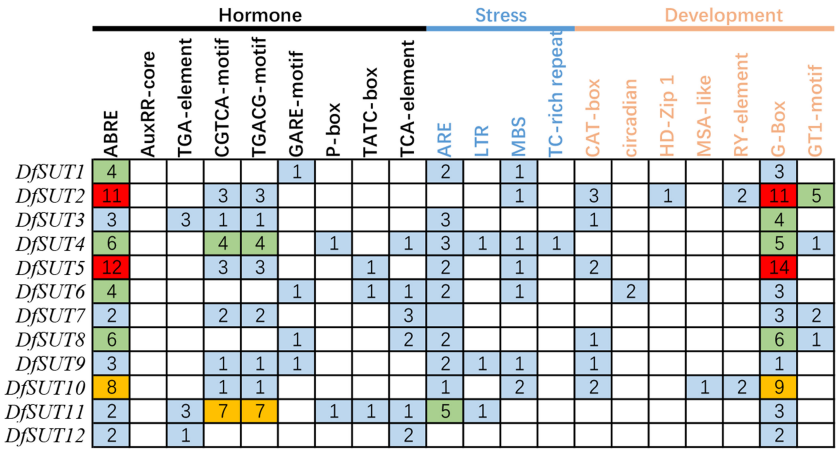

FIGURE 3
The *cis*-regulatory elements involved in phytohormone, development, and stress responses in the upstream regions of *DfSUT* gene promoters. ABRE, abscisic acid-responsive element; AuxRR core and TGA element, auxin-responsive element; CGTCA-motif and TGACG-motif, MeJA-responsive elements; GARE-motif, P-box and TATC-box, gibberellin-responsive elements; TCA element, salicylic acid-responsive elements; ARE, involved in anaerobic induction; LTR, low temperature-responsive element; MBS, TC-rich repeats, involved in defense and stress response; CAT box, circadian, HD-Zip I, MSA-like, and RY-element involved in meristem expression, circadian control differentiation of the palisade mesophyll, cell cycle regulation, and seed-specific regulation, respectively; G-box, GT1-motif, light-responsive elements.

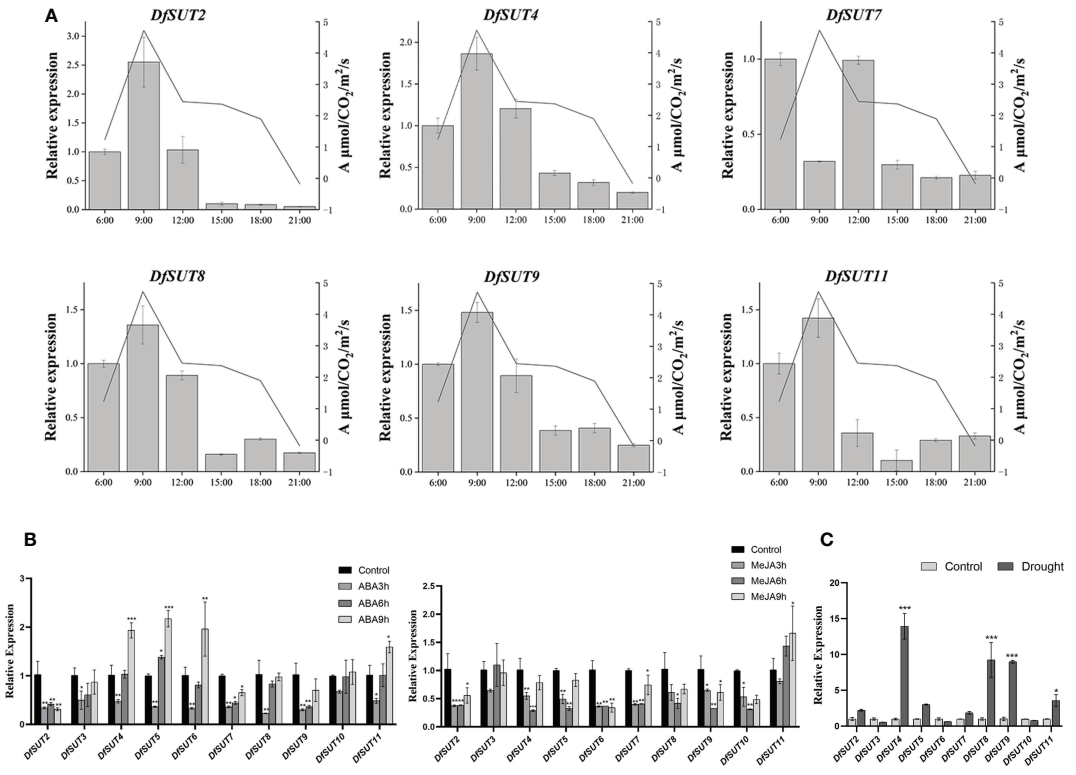

FIGURE 4
Analysis of photosynthetic rate and response to different treatments of the *SUT* family in *D. farinosus*. **(A)** Relative expression of *DfSUT* at different times of the day and the net photosynthetic reaction rate at the corresponding time periods. Total RNA was extracted from *D. farinosus* leaves every 3 h (from 6 a.m. to 9 p.m.) during the day. **(B)** Relative expression of *DfSUT* in response to 100 mM ABA treatment and 100 mM MeJA treatment for 3, 6, and 9 h, respectively. **(C)** Relative expression of *DfSUT* under drought treatment. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, Student's t-test.

within 3 h and then increased within 9 h under the treatment of exogenous ABA, except *DfSUT2*. Compared with the control that was untreated, the expression of *DfSUT4*, *DfSUT5*, and *DfSUT6* was much higher in response to ABA within 9 h, but the transcript levels of *DfSUT2* and *DfSUT7* showed significant declines within 9 h in response to ABA (Figure 4B). However, the transcript levels of six *DfSUT* genes (*DfSUT2*, *DfSUT3*, *DfSUT4*, *DfSUT5*, *DfSUT7*, and *DfSUT11*) decreased within 3 h and then showed a rebound within 9 h in response to MeJA. Compared with the control that was untreated, the transcript levels of *DfSUT2*, *DfSUT6*, and *DfSUT10* showed significant declines, but the expression of *DfSUT11* showed an increase within 9 h in response to MeJA (Figure 4B). Moreover, the transcript levels of *DfSUT4*, *DfSUT8*, *DfSUT9*, and *DfSUT11* showed significant increases in response to the drought treatment (Figure 4C).

Overexpression of *DfSUT4* gene in *N. tabacum* showed increases of photosynthesis, biomass, and cellulose content

Since we have found that the *DfSUT4* gene was involved in the photosynthetic product transport and the responses to various phytohormone treatment (ABA and MeJA) and drought stress, we then verified the transport function of *DfSUT4* in transgenic *N. tabacum* lines. The result showed that GUS activity was mainly detected in the mesophyll of leaves, primary roots, lateral roots, and root hair in *pDfSUT4::GUS* transgenic plant, but not in flower organ (Figures 5A–C). The *DfSUT4* gene was also found to be expressed in the phloem of the stem section in *N. tabacum* (Figure 5D).

Moreover, the result showed that, compared with CK, *DfSUT4* transgenic *N. tabacum* showed phenotypes of taller stem and noticeably larger leaves, flowers, and fruits (Figures 6A–D, F–I). The net photosynthetic reaction rate increased nearly twofold in transgenic *N. tabacum* (Figure 6E), suggesting that *DfSUT4* genes might be involved in the distribution of carbohydrate from the source leaves. Carbohydrates can be synthesized by photosynthesis in mature leaves and then transported to other sink tissues (Barker et al., 2000; Sun et al., 2010). To explore the distribution mechanism of the increased photosynthetic products, we measured the above-ground biomass and cellulose content of stem in three transgenic lines. The results showed that, compared with the control line, the above-ground biomass in transgenic *N. tabacum* line 3 has increased nearly twofold compared with the control lines (Figure 6J), and the cellulose content of the stem in line 3 was increased by more than threefold (Figure 6K). Therefore, these results suggested that *DfSUT4* is important for the translocation of sucrose from the leaf to the sink organs through the phloem and promotes cellulose synthesis in plants.

Discussion

In higher plants, sucrose is produced by leaf photosynthesis and then metabolized into hexoses (glucose and fructose), which are necessary to synthesize cellulose, proteins, and starch and generate energy in the sink tissues (Ruan, 2014). The SUT proteins play key roles in the translocation of sucrose from the

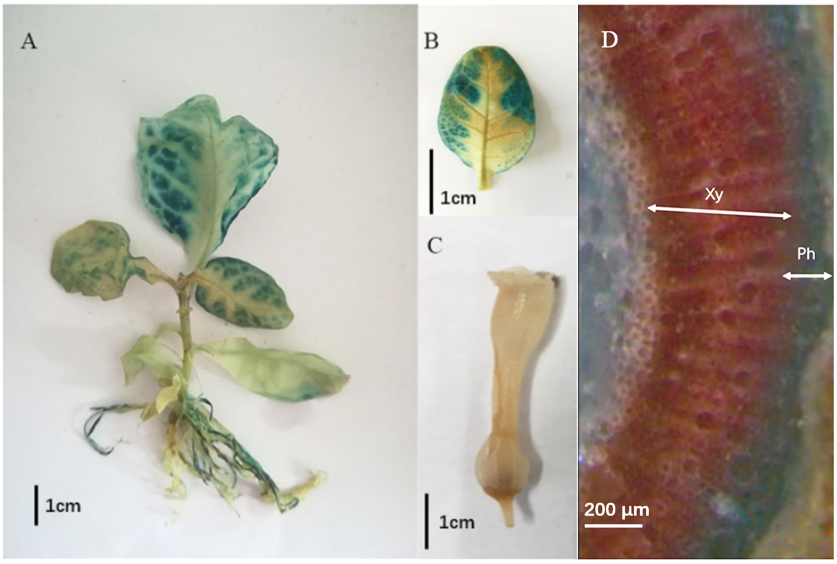

FIGURE 5
Expression of *pDfSUT4::GUS* in *N. tabacum*. **(A)** Intact plant. **(B)** Leaf. **(C)** Flower. **(D)** Stem section. Xy, xylem, Ph, phloem.

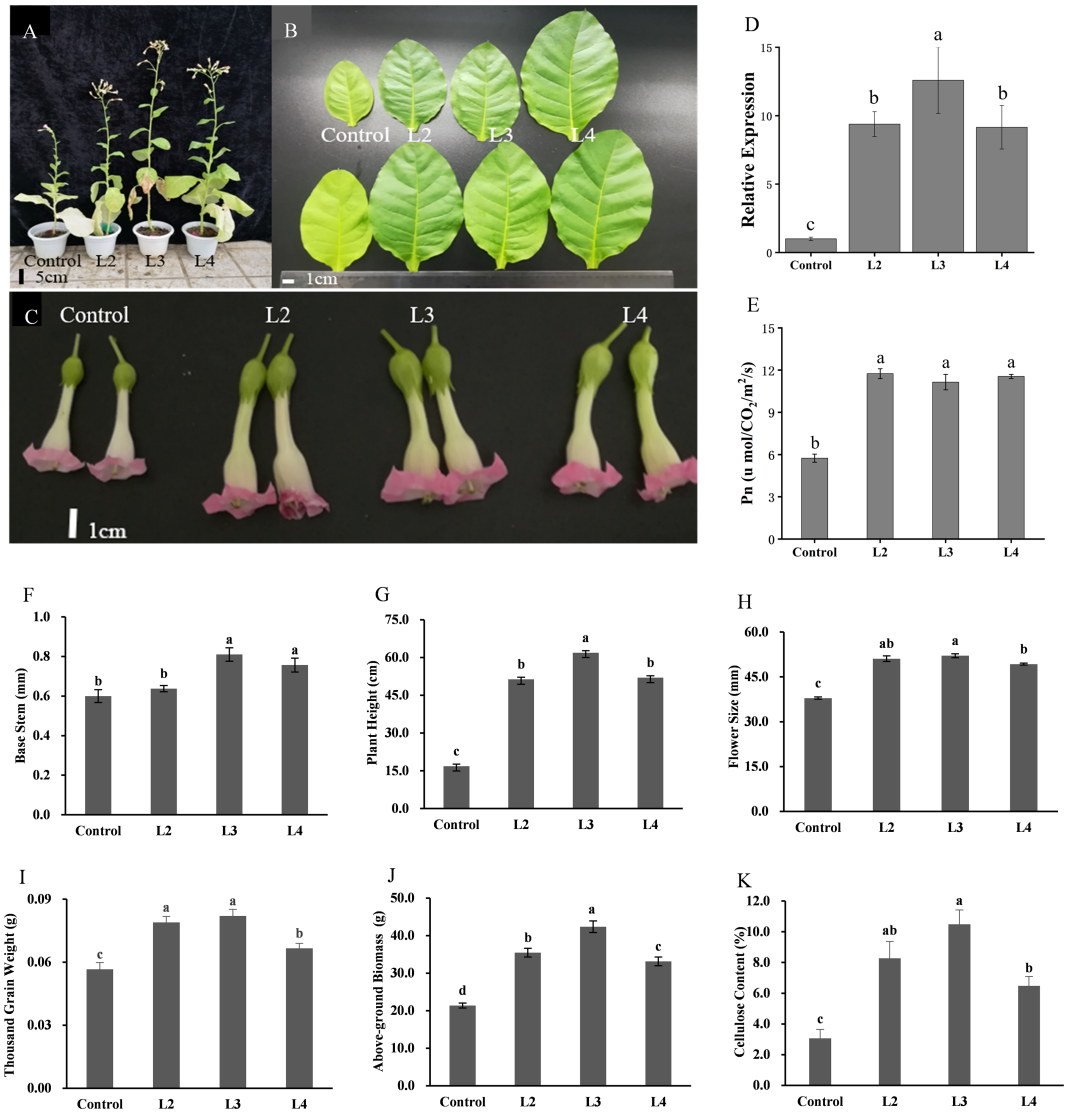

FIGURE 6
Morphological phenotypes, statistics, and above-ground biomass/cellulose content of control and three transgenic *N. tabacum* lines. **(A)** 3-month-old plants. **(B)** Leaf blades of 2-month-old plants. **(C)** Phenotype of the flower. **(D)** Relative expression of *DfSUT4* in transgenic *N. tabacum*. **(E)** Net photosynthetic reaction rate of control and transgenic lines. **(F–I)** Morphologic index of control and transgenic lines. **(J, K)** Above-ground biomass and cellulose content of control and transgenic lines. Data are expressed as the mean ± SD from six independent experiments. The *P*-values of Student's test for control and transgenic lines are denoted with letters ($P < 0.05$).

source to the sink through the phloem to support plant growth and development (Slewinski and Braun, 2010; Deol et al., 2013). *D. farinosus* is an ideal economic crop in southwestern China because of its high fiber content and dual usage for food and paper pulp (Chen et al., 2021; Imran et al., 2022; Pei et al., 2022). Sucrose is the main substrate of fiber synthesis, and studying the function of *SUT* genes in regulating sucrose transportation helps to illustrate the synthesis mechanism of fiber in plants (Yadav et al., 2022)—for example, *GhSUT1* and *GhSUT3* are crucial to regulate sucrose accumulation in cotton fibers (Zhang et al., 2017; Yadav et al., 2022). *PttSUT3* transporters are responsible for the synthesis of wood fibers in a hybrid aspen (Mahboubi et al., 2013).

Herein we identified 12 main *SUT* transporter genes in *D. farinosus*, which were distributed in eight chromosomes in groups A, B, and C of *D. farinosus*, respectively (Table 1; Figure 1B). We analyzed the phylogenetic relationships of *SUT* genes from three woody bamboos including *D. farinosus*, *Phyllostachys edulis*, and *D. latiflorus munro* and found that some *SUT* genes from *Phyllostachys edulis* and *D. latiflorus munro* clustered together, respectively. However, the *SUT* genes from *D. farinosus* were relatively scattered (Figure 1A).

All three woody bamboos contained more *SUT* genes than *Arabidopsis* and rice, suggesting that *SUT* genes were vital to synthesize fiber with high quality and content in woody bamboos. The phylogenetic analysis also showed that *DfSUT2*, *DfSUT7*, and *DfSUT11* were homologs of *OsSUT2* and *PtaSUT4*, and *DfSUT4* is a homolog of *OsSUT4* (Figure 1A); all of these four *DfSUT* genes were mainly expressed in the leaves of *D. farinosus* (Figure 2A). These results suggested that *DfSUT2*, *DfSUT7*, and *DfSUT11* might be important for sucrose efflux from the vacuole to the apoplast in the source leaves of *D. farinosus* (Eom et al., 2011), and *DfSUT4* might function in the apoplastic loading of sucrose from the leaf mesophyll to the phloem (Chung et al., 2014). Chen et al. (2022) revealed that the bamboo node consists of sophisticated porous vascular bundles, microfibers, and twist-aligned nanofibers, and they are essential to the structural stability maintenance and body growth of bamboo. We found that *DfSUT4*, *DfSUT7*, and *DfSUT11* were highly expressed in both the internode and node of *D. farinosus* (Figure 2A). The same high C content was detected in the new leaf (base), new node (base), and mature root in *D. farinosus* (Figure 2B), suggesting that the directional channels, including multiscale sieve tubes and vessels in the node, might serve as a temporary sink for the accumulation of sucrose for some specific functions, such as supply of nutrients for the rapid growth of bamboo at night, and these *DfSUT* genes were also involved in fiber formation in the internode of *D. farinosus*.

The involvement of sucrose transporters in phytohormone signaling and abiotic responses was already described in other species—for example, SlSUT2 and StSUT4 proteins were found to interact with brassinosteroid signaling, ethylene sensing, gibberellic acid responses, and auxin transport in tomato and potato (Bitterlich et al., 2014; Garg et al., 2022a). PaSUT and OsSUT1 were found to interact with hormone (auxin and cytokinin) signaling pathways to regulate the carbohydrate partitioning in *Petunia axillaris* flower (Rolland et al., 2002; Iftikhar et al., 2020) and the reproductive organ of rice (Zhao et al., 2022), respectively. Consistent with these conclusions, we found that *DfSUT4*, *DfSUT5*, and *DfSUT6* showed significant increases in expression in response to ABA treatment within 9 h (Figure 4B). *DfSUT2*, *DfSUT6*, and *DfSUT10* showed significant decreases in response to MeJA treatment within 9 h (Figure 4B). The drought treatment also increased the transcript levels of *DfSUT4*, *DfSUT8*, *DfSUT9*, and *DfSUT11* (Figure 4C), so these results showed that the *DfSUT* family genes interacted with ABA and MeJA signaling pathways and drought resistance, which helped the plants to be more adaptable to environmental changes, especially the *DfSUT4* gene (Figure 4). We also found that the *DfSUT* genes were resistant to *Puccinia striiformis* (not shown).

Then, we verified the transport function of *DfSUT4* in *N. tabacum*. The OsSUT4 transporters were localized in the plasma membrane (PM), functioned in apoplastic phloem loading of sucrose, and increased the rice yield (Chung et al., 2014). Consistently, we found that *DfSUT4* was localized in the plasma membrane (not shown), and the GUS activity was expressed in the mesophyll, roots, and stem phloem of *pDfSUT4::GUS* transgenic plant (Figure 5). The overexpression of the *DfSUT4* gene in transgenic plant showed increases of photosynthesis capacity, above-ground biomass, thousand grain weight, and cellulose content and promoted the development of flower and seed in transgenic plant (Figure 6). These results suggested that *DfSUT4* can be a candidate gene to regulate the phloem transportation of sucrose from the source leaves to the sink organs, phytohormone responses, abiotic stress, and fiber formation in the internode of *D. farinosus*.

Partially functional redundancy existed in nine different *Arabidopsis AtSUC* genes (Garg et al., 2022b), such as the PM-localized AtSUC1 protein which interacted with the vacuolar AtSUC4 protein to mediate sucrose allocation in plants (Schulze et al., 2003; Kruegel et al., 2012). Recent studies revealed that SUT proteins are dynamic within cells, and SNARE protein is important to the subcellular movement of SUT transporters (Garg et al., 2020). At present, the interacting proteins of DfSUT, the functional redundancy between different DfSUT proteins, and the mechanisms of the *DfSUT4* genes involved in phytohormone responses, abiotic stress, and fiber formation in *D. farinosus* need to be analyzed further.

Materials and methods

Identification of the *SUT* genes of *D. farinosus*

The *SUT* family genes of rice were queried using the Uniprot database (https://www.uniprot.org/), and the candidate *SUT* sequences were obtained from the whole genomic data of *D. farinosus* (https://www.ncbi.nlm.nih.gov/, PRJNA923443) using HMMER 3.0 ($E \leq 10–20$). The Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) online software in NCBl was used to remove the sequences that did not include the GPH sucrose superfamily domain. There were 12 *SUT* family members annotated in *D. farinosus* for further study (Table 1).

Phylogenetic analysis of the *SUT* family of *D. farinosus*

The SUT family protein sequences of *Arabidopsis thaliana*, rice, *Populus* L., *Phyllostachys edulis*, and *Dendrocalamus latiflorus munro* were downloaded from UniProt (http://www.uniprot.org) (Supplementary Table S1). By using the Clustal W program in MEGA 6.06 software, we performed a multiple sequence alignment analysis of the SUT protein between *D. farinosus* and other species and constructed a phylogenetic tree

by using the NJ method using MEGA 7.0.21 with bootstrap option n = 1,000 (Chen et al., 2020).

Chromosomal localization and syntenic analysis of *DfSUT* genes

The Multiple Collinearity Scan toolkit (MCScanX) was used to analyze the internal synteny relationship and obtain syntenic gene pairs in the *SUT* family genes of *D. farinosus* (Wang et al., 2012). The syntenic analysis maps were then constructed using the Dual Systeny Plotter software (https://github.com/CJ-Chen/TBtools) (Liu et al., 2017).

Gene structure and conserved motif analysis of the *SUT* family of *D. farinosus*

The conserved motifs of *DfSUTs* were analyzed using the MEME online tool (version 5.3.0, http://meme-suite.org/tools/meme) (Bailey et al., 2015), and the maximum motif search value was set at 10. Motif analysis and gene structure visualization were performed *via* TBtools. The Gene Structure Display Server 2.0 online tool (http://gsds.gao-lab.org/index.php) (Hu et al., 2015) was used to display the structure of *DfSUT* genes and the genomic length and organization of introns/exons. The 2,000-bp upstream promoter sequences of *DfSUT* genes were uploaded to the PlantCARE database (Lescot et al., 2002) (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/), and the cis-elements were subsequently screened manually.

Carbon content measurement

One-year-old *D. farinosus* materials were potted in a growing chamber wherein the condition was kept at 16-h light/8-h dark, and the temperature was 23–25°C. The samples from maternal plants and newborn shoots which included root, internode, leaf, and node were collected for the analysis of C content. All samples were de-enzymed at 100°C, dried at 85°C to a constant weight, and then ground. The total C content was measured using the potassium dichromate–sulfuric acid oxidation method (Cai et al., 2011). The experiment was repeated three times.

Plant materials and hormone treatment

All materials used in this study were grown in a greenhouse at the Institute of Bamboo Research, Southwest University of Science and Technology. The leaves of 1-year-old *D. farinosus* were collected every 3 h (from 6:00 a.m. to 9:00 p.m.) during the day for the measurement of net photosynthetic reaction rate by using LCpro-SD photosynthesis equipment, and six independent biological replicates were performed. In addition, the young leaves of 1-year-old *D. farinosus* were sprayed with 100 mM ABA and MeJA, respectively, and the leaf samples were taken before treatment and after 3, 6, and 9 h of treatment.

RNA extraction and qRT-PCR

Total RNA was extracted from *D. farinosus* leaves by using TRIzol reagent (Tiangen, Beijing, China), and DNase I was used to purify potentially genomic DNA. The quality of total RNA was checked by 1% denaturing agarose gel and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Beijing, China). First-strand cDNA synthesis was performed by using the FastKing cDNA First Strand Synthesis Kit (Tiangen, Beijing, China). Specific primers were designed by using Primer Premier 5.0 (Supplementary Table S2). The internal reference gene was *TUBLIN*. The transcript levels of *DfSUT* were analyzed by real-time quantitative PCR assay using SYBR qPCR Master MIX kit (Vazyme, Nanjing, China) and CFX96TM Real-Time System thermal cycler (Bio-Rad, CA, USA). The relative expression levels of the *SUT* gene were calculated by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The analysis included three biological replicates, and each had three technical replicates. The expression levels under different treatments were shown by histograms using the mean values.

RNA sequencing

One-year-old *D. farinosus* materials potted in the growing chamber were used for RNA sequencing. The samples included branch, culm sheath, internode, lateral bud, young and mature leaves, node, root, sheath of bamboo shoot, and shoots of different height, which were collected for RNA sequencing to be performed. Each sample had three biological replicates. Total RNA was extracted by using TRIzol reagent (Tiangen, Beijing, China), and DNase I was used to purify potentially genomic DNA. The quantity and the quality of total RNA were checked by using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Beijing, China). All 14 samples were subjected to the HiSeq 2500 platform (Illumina, Beijing, China), and the data of RNA sequencing (Supplementary Table S3) was used to construct the heat map of the *DfSUT* genes.

Overexpression of *DfSUT4* in *N. tabacum*

The recombinant plasmid of pCAMBIA1303-N-DfSUT4 with CaMV 35S promoter was transferred into *Agrobacterium tumefaciens* EHA105. Transgenic tobacco overexpression lines were obtained by the leaf disc method (Zhang et al., 2019). Infected tobacco leaf discs were cultivated in the dark on MS

medium containing 9 mg/L Hyg, 400 mg/L cephalexin, 0.5 mg/L 6-BA, and 0.1 mg/L NAA at 27 ± 1°C for 2 days. The regenerated shoots were transferred to 1/2 MS medium with 9 mg/L Hyg, 400 mg/L cephalexin, and 0.1 mg/L NAA for the formation of whole plants. Genomic DNA was extracted from the leaves of transgenic plants and wild-type and amplified under the following conditions: preheating at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 2 min, and finally extension at 72°C for 10 min. The PCR products were detected by 1% agarose gel electrophoresis to confirm the insertion of *DfSUT4* into the transgenic plants. Then, qRT-PCR was used to detect the expression of *DfSUT4* in transgenic plants. The internal reference gene was *N. tabacum NtActin* (Wang et al., 2022).

GUS staining

Fresh plant organs of transgenic tobacco were fixed by 90% acetone on ice for 20 min and then placed in GUS staining solution containing 2 mM X-Gluc in GUS assay buffer (200 mM phosphate buffer, pH 7.0, 0.1% Triton X-100, 20 mM EDTA, and 0.5 mM potassium ferricyanide and potassium ferrocyanide), vacuum infiltrated, and incubated at 37°C overnight. The stained samples were then dehydrated in an ethanol series. Photographs were taken with a stereomicroscope.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA923443.

Author contributions

SHu and YC planned and designed the research. BD, MZ, SC, SHa, and LW performed all experiments and analyzed the data. XG and BD wrote the original manuscript. SHu and YC proofread the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1118398/full#supplementary-material

References

Aoki, N., Hirose, T., and Furbank, R. T. (2012). Sucrose transport in higher plants: From source to sink. *In Photosynthesis Springer* 34, 703–729. doi: 10.1007/978-94-007-1579-0_28

Bailey, T. L., Johnson, J., Grant, C. E., and Noble, W. S. (2015). The MEME suite. *Nucleic Acids Res.* 43 (W1), W39–W49. doi: 10.1093/nar/gkv416

Barker, L., Kuhn, C., Weise, A., Schulz, A., Gebhardt, C., Hirner, B., et al. (2000). SUT2, a putative sucrose sensor in sieve elements. *Plant Cell* 12 (7), 1153–1164. doi: 10.1105/tpc.12.7.1153

Bitterlich, M., Kruegel, U., Boldt-Burisch, K., Franken, P., and Kuehn, C. (2014). The sucrose transporter SlSUT2 from tomato interacts with brassinosteroid functioning and affects arbuscular mycorrhiza formation. *Plant J.* 78 (5), 877–889. doi: 10.1111/tpj.12515

Cai, Y., Peng, C., Qiu, S., Li, Y., and Gao, Y. (2011). Dichromate digestion-spectrophotometric procedure for determination of soil microbial biomass carbon in association with fumigation-extraction. *Commun. Soil Sci. Plant Anal.* 42 (22), 2824–2834. doi: 10.1080/00103624.2011.623027

Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., et al. (2020). TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* 13 (8), 1194–1202. doi: 10.1016/j.molp.2020.06.009

Chen, S. M., Zhang, S. C., Gao, H. L., Wang, Q., Zhou, L., Zhao, H. Y., et al. (2022). Mechanically robust bamboo node and its hierarchically fibrous structural design. *Natl. Sci. Rev.* nwac195. doi: 10.1093/nsr/nwac195

Chen, P., Zheng, R. H., Wang, Y., and Hu, Y. B. (2021). Study on clonal selection of dendrocalamus farinosus in south sichuan. *J. Sichuan Forestry Sci. Technol.* 42 (1), 109–113. doi: 10.12172/202007270002

Chung, P., Hsiao, H. H., Chen, H. J., Chang, C. W., and Wang, S. J. (2014). Influence of temperature on the expression of the rice sucrose transporter 4 gene,

OsSUT4, in germinating embryos and maturing pollen. *Acta Physiologiae Plantarum* 36 (1), 217–229. doi: 10.1007/s11738-013-1403-x

Deol, K. K., Mukherjee, S., Gao, F., Brule-Babel, A., Stasolla, C., and Ayele, B. T. (2013). Identification and characterization of the three homeologues of a new sucrose transporter in hexaploid wheat (Triticum aestivum l.). *BMC Plant Biol.* 13, 181. doi: 10.1186/1471-2229-13-181

Eom, J. S., Cho, J. I., Reinders, A., Lee, S. W., Yoo, Y., Pham Quoc, T., et al. (2011). Impaired function of the tonoplast-localized sucrose transporter in rice, OsSUT2, limits the transport of vacuolar reserve sucrose and affects plant growth. *Plant Physiol.* 157 (1), 109–119. doi: 10.1104/pp.111.176982

Frost, C. J., Nyamdari, B., Tsai, C. J., and Harding, S. A. (2012). The tonoplast-localized sucrose transporter in populus (PtaSUT4) regulates whole-plant water relations, responses to water stress, and photosynthesis. *PloS One* 7 (8), e44467. doi: 10.1371/journal.pone.0044467

Garg, V., Hackel, A., and Kuehn, C. (2020). Subcellular targeting of plant sucrose transporters is affected by their oligomeric state. *Plants* 9 (2), 158. doi: 10.3390/plants9020158

Garg, V., and Kuhn, C. (2022b). Subcellular dynamics and protein-protein interactions of plant sucrose transporters. *J. Plant Physiol.* 273, 1–8. doi: 10.1016/j.jplph.2022.153696

Garg, V., Reins, J., Hackel, A., and Kuhn, C. (2022a). Elucidation of the interactome of the sucrose transporter StSUT4: sucrose transport is connected to ethylene and calcium signaling. *J. Exp. Bot.* 73 (22), 7401–7416. doi: 10.1093/jxb/erac378

Gong, X., Liu, M., Zhang, L., Ruan, Y., Ding, R., Ji, Y., et al. (2015). Arabidopsis AtSUC2 and AtSUC4, encoding sucrose transporters, are required for abiotic stress tolerance in an ABA-dependent pathway. *Physiologia Plantarum* 153 (1), 119–136. doi: 10.1111/ppl.12225

Gottwald, J. R., Krysan, P. J., Young, J. C., Evert, R. F., and Sussman, M. R. (2000). Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. *Proc. Natl. Acad. Sci. U. S. A.* 97 (25), 13979–13984. doi: 10.1073/pnas.250473797

Harding, S. A., Frost, C. J., and Tsai, C. J. (2020). Defoliation-induced compensatory transpiration is compromised in SUT4-RNAi populus. *Plant Direct* 4 (9), e00268. doi: 10.1002/pld3.268

Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., and Gao, G. (2015). GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics* 31 (8), 1296–1297. doi: 10.1093/bioinformatics/btu817

Hu, Z., Tang, Z., Zhang, Y., Niu, L., Yang, F., Zhang, D., et al. (2021). Rice SUT and SWEET transporters. *Int. J. Mol. Sci.* 22 (20), 11198. doi: 10.3390/ijms222011198

Ibraheem, O., Dealtry, G., Roux, S., and Bradley, G. (2011). The effect of drought and salinity on the expressional levels of sucrose transporters in rice (Oryza sativa nipponbare) cultivar plants. *Plant Omics* 4 (2), 68–74. doi: 10.1105/tpc.110.081794

Iftikhar, J., Lyu, M., Liu, Z., Mehmood, N., Munir, N., Ahmed, M. A. A., et al. (2020). Sugar and hormone dynamics and the expression profiles of SUT/SUC and SWEET sugar transporters during flower development in petunia axillaris. *Plants-Basel* 9 (12) 1770. doi: 10.3390/plants9121770

Imran, M., Luo, X., Hu, S., Cao, Y., and Long, Z. (2022). Epigenetic and somaclonal divergence in dendrocalamus farinosus for physiological augmentation and lignin degradation. *Biotechnol. Appl. Biochem.* 69 (4), 1545–1556. doi: 10.1002/bab.2226

Jia, W., Zhang, L., Wu, D., Liu, S., Gong, X., Cui, Z., et al. (2015). Sucrose transporter AtSUC9 mediated by a low sucrose level is involved in arabidopsis abiotic stress resistance by regulating sucrose distribution and ABA accumulation. *Plant Cell Physiol.* 56 (8), 1574–1587. doi: 10.1093/pcp/pcv082

Kruegel, U., He, H.-X., Gier, K., Reins, J., Chincinska, I., Grimm, B., et al. (2012). The potato sucrose transporter StSUT1 interacts with a DRM-associated protein disulfide isomerase. *Mol. Plant* 5 (1), 43–62. doi: 10.1093/mp/ssr048

Lemoine, R. (2000). Sucrose transporters in plants: update on function and structure. *Biochim. Et Biophys. Acta-Biomembranes* 1465 (1-2), 246–262. doi: 10.1016/s0005-2736(00)00142-5

Lescot, M., Dehais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., et al. (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* 30 (1), 325–327. doi: 10.1093/nar/30.1.325

Liu, C., Xie, T., Chen, C., Luan, A., Long, J., Li, C., et al. (2017). Genome-wide organization and expression profiling of the R2R3-MYB transcription factor family in pineapple (Ananas comosus). *BMC Genomics* 18 (1), 503. doi: 10.1186/s12864-017-3896-y

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. *Methods* 25 (4), 402–408. doi: 10.1006/meth.2001.1262

Mahboubi, A., Ratke, C., Gorzsas, A., Kumar, M., Mellerowicz, E. J., and Niittyla, T. (2013). Aspen SUCROSE TRANSPORTER3 allocates carbon into wood fibers. *Plant Physiol.* 163 (4), 1729–1740. doi: 10.1104/pp.113.227603

Payyavula, R. S., Tay, K. H. C., Tsai, C. J., and Harding, S. A. (2011). The sucrose transporter family in populus: the importance of a tonoplast PtaSUT4 to biomass and carbon partitioning. *Plant J.* 65 (5), 757–770. doi: 10.1111/j.1365-313X.2010.04463.x

Pei, J., Wang, Y., Zhuo, J., Gao, H., Vasupalli, N., Hou, D., et al. (2022). Complete chloroplast genome features of dendrocalamus farinosus and its comparison and evolutionary analysis with other bambusoideae species. *Genes* 13 (9) 1519. doi: 10.3390/genes13091519

Qi, J., Xie, J., Yu, W., and Chen, S. (2015). Effects of characteristic inhomogeneity of bamboo culm nodes on mechanical properties of bamboo fiber reinforced composite. *J. Forestry Res.* 26 (4), 1057–1060. doi: 10.1007/s11676-015-0106-0

Reinders, A., Sivitz, A. B., and Ward, J. M. (2012). Evolution of plant sucrose uptake transporters. *Front. Plant Sci.* 3 (22). doi: 10.3389/fpls.2012.00022

Rennie, E. A., and Turgeon, R. (2009). A comprehensive picture of phloem loading strategies. *Proc. Natl. Acad. Sci. U. S. A.* 106 (33), 14162–14167. doi: 10.1073/pnas.0902279106

Rolland, F., Moore, B., and Sheen, J. (2002). Sugar sensing and signaling in plants. *Plant Cell* 14, 185–205. doi: 10.1105/tpc.010455

Ruan, Y. L. (2014). Sucrose metabolism: Gateway to diverse carbon use and sugar signaling. *Annu. Rev. Plant Biol.* 65, 33–67. doi: 10.1146/annurev-arplant-050213-040251

Schulze, W. X., Reinders, A., Ward, J., Lalonde, S., and Frommer, W. B. (2003). Interactions between co-expressed arabidopsis sucrose transporters in the split-ubiquitin system. *BMC Biochem.* 4, 3. doi: 10.1186/1471-2091-4-3

Slewinski, T. L., and Braun, D. M. (2010). Current perspectives on the regulation of whole-plant carbohydrate partitioning. *Plant Sci.* 178 (4), 341–349. doi: 10.1016/j.plantsci.2010.01.010

Srivastava, A. C., Ganesan, S., Ismail, I. O., and Ayre, B. G. (2009). Effective carbon partitioning driven by exotic phloem-specific regulatory elements fused to the arabidopsis thaliana AtSUC2 sucrose-proton symporter gene. *BMC Plant Biol.* 9, 7. doi: 10.1186/1471-2229-9-7

Sun, Y., Reinders, A., LaFleur, K. R., Mori, T., and Ward, J. M. (2010). Transport activity of rice sucrose transporters OsSUT1 and OsSUT5. *Plant Cell Physiol.* 51 (1), 114–122. doi: 10.1093/pcp/pcp172

Wang, Y., Chen, Y., Wei, Q., Wan, H., and Sun, C. (2021). Phylogenetic relationships of sucrose transporters (SUTs) in plants and genome-wide characterization of SUT genes in orchidaceae reveal roles in floral organ development. *Peer J.* 9, e11961. doi: 10.7717/peerj.11961

Wang, Y., Tang, H., DeBarry, J. D., Tan, X., Li, J., Wang, X., et al. (2012). MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.* 40 (7), e49. doi: 10.1093/nar/gkr1293

Wang, C., Wang, L., Lei, J., Chai, S., Jin, X., Zou, Y., et al. (2022). IbMYB308, a sweet potato R2R3-MYB gene, improves salt stress tolerance in transgenic tobacco. *Genes* 13 (8), 1476. doi: 10.3390/genes13081476

Ward, J. M., Kuhn, C., Tegeder, M., and Frommer, W. B. (1998). Sucrose transport in higher plants. *Int. Rev. Cytology* 178, 41–71. doi: 10.1016/s0074-7696(08)62135-x

Weise, A., Barker, L., Kuhn, C., Lalonde, S., Buschmann, H., Frommer, W. B., et al. (2000). A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. *Plant Cell* 12 (8), 1345–1355. doi: 10.1105/tpc.12.8.1345

Xiong, Z., Fu., Z. H., Yan, W. X., Gan, S. X., Cao, X. J., and Feng, S. J. (2012). The preliminary studies on production status and cultivation techniques of dendrocalamus farinosus. *World Bamboo Rattan* 10 (5), 7–11. doi: 10.13640/j.cnki.wbr.2012.05.008

Yadav, U. P., Evers, J. F., Shaikh, M. A., Ayre, B. G., and Hancock, R. (2022). Cotton phloem loads from the apoplast using a single member of its nine-member sucrose transporter gene family. *J. Exp. Bot.* 73 (3), 848–859. doi: 10.1093/jxb/erab461

Zakikhani, P., Zahari, R., Sultan, M. T. H., and Majid, D. L. (2014). Extraction and preparation of bamboo fibre-reinforced composites. *Materials Design* 63, 820–828. doi: 10.1016/j.matdes.2014.06.058

Zhang, H., Gao, X., Zhi, Y., Li, X., Zhang, Q., Niu, J., et al. (2019). A non-tandem CCCH-type zinc-finger protein, IbC3H18, functions as a nuclear transcriptional activator and enhances abiotic stress tolerance in sweet potato. *New Phytol.* 223 (4), 1918–1936. doi: 10.1111/nph.15925

Zhang, Z., Ruan, Y. L., Zhou, N., Wang, F., Guan, X., Fang, L., et al. (2017). Suppressing a putative sterol carrier gene reduces plasmodesmal permeability and activates sucrose transporter genes during cotton fiber elongation. *Plant Cell* 29 (8), 2027–2046. doi: 10.1105/tpc.17.00358

Zhao, Z., Wang, C., Yu, X., Tian, Y., Wang, W., Zhang, Y., et al. (2022). Auxin regulates source-sink carbohydrate partitioning and reproductive organ development in rice. *Proc. Natl. Acad. Sci. U. S. A.* 119 (36), 1–11. doi: 10.1073/pnas.2121671119

Zhu, W., Ma, S., Zhang, G., Liu, H., Ba, Q., Li, Z., et al. (2015). Carbohydrate metabolism and gene regulation during anther development disturbed by chemical hybridizing agent in wheat. *Crop Sci.* 55 (2), 868–876. doi: 10.2135/cropsci2014.04.0333

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The role of nitric oxide and hydrogen sulfide in regulation of redox homeostasis at extreme temperatures in plants

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Nitric oxide and hydrogen sulfide, as important signaling molecules (gasotransmitters), are involved in many functions of plant organism, including adaptation to stress factors of various natures. As redox-active molecules, NO and H_2S are involved in redox regulation of functional activity of many proteins. They are also involved in maintaining cell redox homeostasis due to their ability to interact directly and indirectly (functionally) with ROS, thiols, and other molecules. The review considers the involvement of nitric oxide and hydrogen sulfide in plant responses to low and high temperatures. Particular attention is paid to the role of gasotransmitters interaction with other signaling mediators (in particular, with Ca^{2+} ions and ROS) in the formation of adaptive responses to extreme temperatures. Pathways of stress-induced enhancement of NO and H_2S synthesis in plants are considered. Mechanisms of the NO and H_2S effect on the activity of some proteins of the signaling system, as well as on the state of antioxidant and osmoprotective systems during adaptation to stress temperatures, were analyzed. Possibilities of practical use of nitric oxide and hydrogen sulfide donors as inductors of plant adaptive responses are discussed.

KEYWORDS

nitric oxide, hydrogen sulfide, reactive oxygen species, redox regulation, calcium, hypothermia, hyperthermia, stress-protective systems

Temperature is a factor that plants cannot effectively control. In this regard, it is the ambient temperature that limits growth, productivity and distribution of plants in climatic zones. For example, high temperatures are now believed to be a major determinant of crop growth and yields at the global level (Ali et al., 2020). According to predictive models, the average temperature on the planet may increase by 3-6°C by the middle of this century, significantly increasing the likelihood of thermal damage to plants (Mohapatra, 2019). This challenge is especially relevant for Europe, where temperature increase trends come out three to four times faster than in the other northern latitudes (Rousi et al., 2022). However, despite the increase in the mean annual temperature in recent decades, the relevance of plant resistance to cold and frost for many countries of the world not only lessens, but becomes

greater (Chervenkov and Slavov, 2022). Winter thaws alternating with sudden frosts damage plants and decrease their productivity. Winter cereals are most affected by the cold, causing the loss of their productivity.

The effects of low and high temperatures on plants at the cellular and molecular levels have both fundamental differences and significant similarities (Zhen and Ungerer, 2008). For instance, a decrease in temperature causes a very rapid increase in the rigidity of cell membranes due to phase transitions of the lipid bilayer (Kazemi-Shahandashti and Maali-Amiri, 2018). On the other hand, exposure to high temperatures leads to fluidization of the lipid part of the membranes (Li et al., 2018). At the same time, however, both types of exposure disrupt the native state of cell membranes and enhance the stochastic formation of reactive oxygen species (ROS), primarily in the inner membranes of chloroplasts and mitochondria (Asada, 1999; Han et al., 2013; Bernfur et al., 2017; Choudhury et al., 2017). Along with this, both low- and high-temperature exposures cause an increase in the ROS generation on the cell surface by NADPH oxidase (Piotrovskii et al., 2011; Kolupaev et al., 2013; Yao et al., 2017). ROS generated under stressful temperatures are considered to be components of a signaling network that ensures the formation of the adaptive plant responses to these stress factors (Zhang et al., 2016).

The action of low and high temperatures on cell membranes also leads to changes in calcium homeostasis. Opening of calcium channels is one of the earliest responses of plant cells to cooling (Jian et al., 1999). The obtained experimental data indicate the possibility of cold-induced calcium release into cytosol by several pathways: involving COLD1-RGA1 complex (Ma et al., 2015b), *via* cyclic nucleotide-gated channels (CNGCs) (Chen et al., 2021), as well as Ca^{2+}-permeable transporter Annexin1 (AtANN1) (Liu et al., 2021). Changes in the cytosolic calcium concentration due to the effect of high temperatures on membranes are primarily associated with the opening of specific thermosensitive CNGCs (Saidi et al., 2009; Gao et al., 2012; Finka and Goloubinoff, 2014). Thus, changes in calcium homeostasis are considered to be a key element in the transduction of cold and heat stress signals.

Along with such universal signaling mediators as ROS and calcium ions, the main gasotransmitters, nitric oxide (NO) (Fancy et al., 2017; Yemets et al., 2019) and hydrogen sulfide (H_2S) (Fu et al., 2013; Kolupaev et al., 2022; Raza et al., 2022), are involved in signal transduction caused by extreme temperatures. To date, experiments with plants of different taxonomic affiliations have revealed increases in the content of NO and H_2S in response to both low (Liu et al., 2013b; Du et al., 2017; Fancy et al., 2017; Liu et al., 2019) and high (Christou et al., 2014; Karpets et al., 2015a; Chen et al., 2016) temperatures. However, the causal relationships of such effects with changes in cellular content of other signaling mediators, including such important ones as ROS, remain largely understudied. Moreover, information on the role of gasotransmitters in cold adaptation of plants is quite contradictory. Even for the best studied signaling molecule NO, both positive (Zhao et al., 2009; Puyaubert and Baudouin, 2014) and negative (Costa-Broseta et al., 2018) regulation of gene expression critical for adaptation to extreme temperatures has been reported on the same objects.

Proteomics and bioinformatics methods have identified a large number of proteins that can undergo post-translational modification by NO, H_2S, and ROS (Valderrama et al., 2019; Corpas et al., 2022). However, this vast body of information is still poorly interpreted in the context of specific physiological processes and their role in plant adaptation to extreme temperatures. In many cases, such interpretation becomes more complicated due to parallel processes of direct interaction between redox active molecules and proteins and their mediated effect on the expression of genes important for adaptation. Thus, understanding of the mechanisms of functional interaction of NO, H_2S, and ROS as signal mediators involved in the induction of adaptive responses to extreme temperatures is far from being fully elaborated. The analysis of data on the role of functional relationships between two key gasotransmitters (nitrogen oxide and hydrogen sulfide) and ROS in plant adaptation to low and high temperatures has been the main objective of this review. Since the effects of extreme high and low temperatures on plants are accompanied by a disruption of the water regime, we also discuss in some cases the processes of functional interaction of signaling molecules in the formation of protective responses to dehydration.

Nitric oxide synthesis in plants at stress temperatures

Main pathways of NO synthesis in plants

Despite intensive research that has revealed the diversity of nitric oxide functions in plants, its synthesis still remains one of the most difficult challenges in this field (Astier et al., 2018). There are two main ways of NO synthesis in plants: reductive, based on the reduction of nitrites to NO, and oxidative, related to the oxidation of molecules containing amino groups (Kumar and Ohri, 2023).

The reductive pathway is a well-described and proven pathway for nitric oxide synthesis in plants. Nitrate reductase (NR), a multifunctional cytoplasmic enzyme involved in nitrogen assimilation and metabolism, is considered one of the key enzymes for NO synthesis. It is responsible for the first limiting step in nitrate assimilation, catalyzing reduction of nitrate to nitrite using NADH or NADPH as an electron donor. Active enzymatic homodimeric complex requires the presence of molybdopterin, heme, and FAD as cofactors (Astier et al., 2018). In addition to its primary activity, NR also possessed nitrite-NO reductase activity (Ni-NR activity) (Rockel et al., 2002). Under normal conditions, this activity is only 1% of nitrate-reducing capacity of NR (Astier et al., 2018). However, factors such as an anoxic or acidic environment contribute to NO formation by NR. Despite such specific conditions, the significant contribution of NR-mediated NO production in plant physiology has been convincingly demonstrated using both pharmacological and genetic approaches (Mur et al., 2013).

A specific mechanism for NR-mediated NO formation was discovered in the case of *Chlamydomonas reinhardtii*. In this unicellular alga, NR can interact with its partner protein, the amidoxime reducing component (ARC), forming a catalytic complex with it (Chamizo-Ampudia et al., 2017). The ARC protein within such a complex was named nitric oxide-forming nitrite reductase (NOFNiR). It is the one that catalyzes NO formation from nitrite. It was shown that gene expression patterns and enzymatic activity of NR and NOFNiR correlate with each other

(Chamizo-Ampudia et al., 2017). We assume that detection of such enzymatic system in higher plants will help to better explain the crucial role of NR in NO synthesis by plants (Astier et al., 2018).

Thus, the functions of NR and its partner proteins can be complex and diverse. It is notable that many plants, in particular *Arabidopsis thaliana*, *Nicotiana tabacum*, *Hordeum vulgare*, *Zea mays*, *Brassica napus*, *Glycine max*, *Oryza sativa*, have two or more NR isoforms that can perform different functions (Mohn et al., 2019). When comparing the functional properties of *A. thaliana* NR isoforms, NIA2 has been found to function in nitrate reduction process, whereas NIA1 is mainly involved in NO synthesis (Mohn et al., 2019).

Another possible reductive pathway for NO synthesis is the one associated with xanthine oxidoreductase localized in peroxisomes (Farnese et al., 2016).

In addition to the reductive pathways for NO synthesis from nitrite/nitrate listed above, several lines of evidence demonstrate the existence of an oxidative pathway for NO generation in plants similar to the one described in animals. However, to date, animal NO synthase homologues have been identified only in green algae (*Ostreococcus tauri* and *Ostreococcus lucimarinus*), but not in higher plants (Roszer, 2014; Hancock and Neill, 2019). It is assumed that the NO synthase gene (NOS) was lost during the evolution of plants (Jeandroz et al., 2016). NOS has not been detected in any of the more than 1000 screened transcriptomes of terrestrial plants (Jeandroz et al., 2016).

Nevertheless, the presence of NOS-like activity in plants has been confirmed by other methods, including those that allow direct measurement of NO generation (Chaki et al., 2009). This enzymatic activity has been termed NOS-like since it has been reported to be strictly dependent on the presence of arginine and NADPH, as well as several NOS cofactors, as in the case of the animal enzyme. This NOS-like activity has been found in chloroplasts, mitochondria, and peroxisomes (Corpas and Barroso, 2014; Farnese et al., 2016). However, clear evidence of the existence of the corresponding protein in higher plants is still lacking (Astier et al., 2018).

Recently, not only L-arginine, but also polyamines and hydroxylamine have been considered as substrates for NO formation in oxidative pathway (Wimalasekera et al., 2001; Hancock and Whiteman, 2014). It has been suggested that these transformations can be catalyzed by di- and polyamine oxidases localized mainly in cell walls (Saha et al., 2015).

Nitric oxide can also be obtained non-enzymatically from nitrite in the presence of a reducing agent such as ascorbate. Since this reaction requires an undissociated acid, it can occur in certain microenvironments under acidic conditions, in particular in apoplast, vacuole, and cellular compartments under unbalanced redox conditions (Grossi and Casadei, 2021). Another possible pathway for non-enzymatic nitric oxide production is NO released from S-nitrosoglutathione (GSNO) (Kumar and Ohri, 2023).

It should be noted that GSNO is the most significant reservoir of NO (Gupta et al., 2011). As is known, nitric oxide can react with glutathione (GSH) to form GSNO, which in turn can again serve as a NO donor in the cell. GSNO content is regulated by S-nitrosoglutathione reductase (GSNOR), which reduces GSNO to glutathione sulfinamide ($GS(O)NH_2$) using NADH (Gupta et al., 2011).

Nitric oxide synthesis in plants during adaptation to cold

An increase in NO content in response to cold exposure was found in organs of plants of different taxonomic affiliation (*Arabidopsis thaliana*, *Pisum sativum*, *Triticum aestivum*, *Citrus aurantium*) (Yemets et al., 2019). It is noteworthy that such an effect was caused by both relatively short-term (1-4 hours) and long-term (7-14 days) exposures of plants to low temperatures (Ziogas et al., 2013; Puyaubert and Baudouin, 2014; Baudouin and Jeandroz, 2015; Fancy et al., 2017). At the same time, the increase in the content of NO in the tissues was stable. For example, in Arabidopsis, a gradual increase in NO content in leaves was recorded, which reached a maximum on the 14th day of exposure at 4°C (Zhao et al., 2009).

The main enzyme of NO synthesis induced by hypothermia in plants is probably nitrate reductase. Thus, in Arabidopsis *nia1nia2* double mutants, at low positive temperatures the NO content almost did not change. At the same time, their resistance to negative temperatures did not develop after cold exposure to 4°C, which indicates the role of endogenous nitric oxide in plant adaptation to cold (Zhao et al., 2009).

A significant increase in nitrate reductase activity and transcripts of its genes was shown in Arabidopsis and citruses exposed to low positive temperatures (Puyaubert and Baudouin, 2014). The effect of increasing the content of nitric oxide in Arabidopsis was leveled by the action of the nitrate reductase inhibitor tungstate, but not by treatment with an inhibitor of enzymes of the oxidative pathway of NO synthesis L-NAME (N^G-nitro-L-arginine methyl ester) (Cantrel et al., 2011). On the other hand, in pea (*Pisum sativum*), cold-induced NO formation was suppressed by L-NAME (Puyaubert and Baudouin, 2014). Therefore, in general, cold stress-induced nitric oxide generation in plants, apparently, can be carried out by different enzyme systems and have specific features (Yemets et al., 2019).

Nitric oxide synthesis in plants exposed to high temperatures

In response to high temperatures, an increase in nitric oxide content in plants of different species has also been recorded. For example, even at moderately raised temperatures (+30°C), pea plants showed an increase in nitric oxide in tissues (Corpas et al., 2008). In rice (*Oryza sativa*) leaves, increased nitric oxide content was recorded after two hours of exposure to +38°C (Song et al., 2013). When exposed to +42°C, an increase in NO amount was found in *Citrus aurantium* (Ziogas et al., 2013) and strawberry (*Fragaria* × *ananassa*) (Christou et al., 2014). After a 1-minute hardening heating of wheat (*Triticum aestivum*) seedlings at +42°C, a rise in nitric oxide content was noted in the roots with a maximum after 30-60 min (Karpets et al., 2015a). This effect was almost completely eliminated by animal NOS inhibitor L-NAME and partially by nitrate reductase inhibitor sodium tungstate, indicating the likely involvement of both the oxidative and reductive NO synthesis pathways under the action of high temperature.

Heat-induced NO fluorescence has been observed in cell of *Nicotiana tabacum*. The earliest increase in NO content was noted

in plastids with a subsequent effect in the nucleus and cytosol (Gould et al., 2003). At the same time, some studies report a decrease in the amount of nitric oxide in plant cells some time after the action of stress factors (Parankusam et al., 2017). This may be due to the transient effect of increasing the NO content, which is characteristic of signaling processes.

The role of nitric oxide in the development of heat resistance in plants is indicated by the elimination of the effects of thermal hardening of wheat seedlings by the action of the NO scavenger PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide), as well as inhibitors of NO synthesis enzymes (Karpets et al., 2015b).

Hydrogen sulfide synthesis in plants at extreme temperatures

Enzymatic systems for synthesis of hydrogen sulfide

One of the main pathways for hydrogen sulfide synthesis in plants is the conversion of L-cysteine into pyruvate with the release of hydrogen sulfide and ammonium (Romero et al., 2013). This reaction is catalyzed by L-cysteine desulfhydrase (L-CD). This enzyme has been detected in plants of various taxonomic groups (Zhang et al., 2021). An enzyme with this activity is localised in the cytoplasm, plastids, and mitochondria (Li, 2013). Proteins with chloroplast and mitochondrial localisation are regarded as a special kind of L-CDes – NifS-like proteins (Zhang et al., 2021). Hydrogen sulfide can also be formed from D-cysteine by D-cysteine desulfhydrase in the cytoplasm (Guo et al., 2016).

It is known that H_2S can be a by-product of cysteine biosynthesis and an intermediate product in sulfate assimilation (Birke et al., 2015). The process of cysteine biosynthesis involves two steps. First, serine acetyltransferase (SAT) catalyzes the biosynthesis of the intermediate O-acetylserine (OAS) from acetyl-CoA and serine (Birke et al., 2012). Then, O-acetylserine (thiol) lyase (OASTL) enables sulfide incorporation into OAS to form cysteine (Álvarez et al., 2010). However, OASTL, which is a cysteine synthase (CS)-like protein, is involved predominantly in L-cysteine degradation rather than in its biosynthesis, because the K_m value associated with L-cysteine degradation is 13 times higher than that for L-cysteine biosynthesis (Álvarez et al., 2010). This protein is also found in the cytoplasm and is considered as another desulfhydrase, AtDES1 (Álvarez et al., 2010; Zhang et al., 2021).

It is also assumed that CBL protein is involved in hydrogen sulfide synthesis in plants (Wang et al., 2022b). The endogenous H_2S content was shown to be lower in *Arabidopsis thaliana cbl* mutants than in the wild type.

Cyanoalanine synthase (CAS) is also considered to be one of the enzymes for H_2S synthesis in plants. This mitochondria-localized enzyme generates cyanoalanine, produced alongside hydrogen sulfide, with cyanide and cysteine as substrates (Zhang et al., 2021). It is assumed that the main function of cyanoalanine synthase is associated with the control of toxic cyanide (Li, 2015).

In addition, H_2S can be synthesized by sulfite reduction involving sulfite reductase (Li, 2013). This process requires reduced ferredoxin as a sulfur reducing agent. Finally, hydrogen sulfide can also be released during the decomposition of carbonyl sulfide by carbonic anhydrase contained in chloroplasts (Zhang, 2016).

In general, it is assumed that the relevance of different enzymatic properties depends on the specific subcellular localisation of hydrogen sulfide-producing enzymes (Zhang et al., 2021). The existence of complex and diverse mechanisms regulating H_2S production and cysteine biosynthesis among plant species may in itself indicate the involvement of hydrogen sulfide in complex regulatory processes in plant cells (Li, 2020).

Hydrogen sulfide synthesis in response to low and high temperatures

Several reports have shown that low temperatures increased expression of L/D-cysteine disulfhydrase genes and increased H_2S content in leaves in Arabidopsis plants (Shi et al., 2015), increased synthesis of hydrogen sulfide in grapes (Fu et al., 2013) and raised H_2S levels in Bermuda grass seedlings (Shi et al., 2013).

It was also reported that in *Lamiophlomis rotate*, a plant growing in cold mountain conditions, the expression of genes for enzymes involved in H_2S biosynthesis (*OASTL*, *CAS*, *L/DCD*) increased with increasing altitude (4350, 4800, and 5200 m) (Ma et al., 2015a). The authors believe that this phenomenon indicates a role for H_2S in cold tolerance of plants at high altitudes. The effect of increased hydrogen sulfide content has also been found in some warm weather species. Thus, H_2S synthesis was increased in cucumber leaves in response to 4°C (Liu et al., 2019). Also, on example of cucumber plants, it was shown that endogenous and exogenous hydrogen sulfide induced a signal chain involving plant hormone indole-3-acetic acid (IAA) and hydrogen peroxide (Zhang et al., 2021). One function of this signal chain during cold stress may be to induce expression of transcription factor CBF1 gene and, consequently, cold-sensitive genes *COR47* (Zhang et al., 2021).

There is still little data on the involvement of endogenous hydrogen sulfide in plant adaptation to high temperatures. Prolonged exposure of plants to moderately high temperatures resulted in an increase in hydrogen sulfide content in the cells of strawberry (Christou et al., 2014), tobacco (*Nicotiana tabacum*) (Chen et al., 2016), and rice (Gautam et al., 2022). In roots of wheat seedlings, a transient increase in hydrogen sulfide content was noted after one-minute heating at a hardening temperature of 42°C (Havva et al., 2022). Such an effect was not observed when seedlings were treated with hydrogen sulfide scavenger hypotaurine or sodium pyruvate, a L-cysteine desulfhydrase inhibitor. At the same time, these H_2S antagonists also eliminate the effect of increasing heat resistance of wheat seedlings.

Post-translational modifications of proteins as the primary mechanism for the biological effects of NO, H_2S and ROS

Following the biosynthesis in ribosomes, proteins can undergo numerous post-translational modifications (PTMs), which occur

through the interaction of specific amino acid residues with certain compounds contained in the cellular environment (Corpas et al., 2022). The spectrum of PTMs is very diverse and includes phosphorylation, ubiquitination, methylation, glycosylation, acylation, alkylation, hydroxylation as well as specific reactions with protein thiol groups: S-sulfenylation, S-glutathionylation, S-nitrosation, persulfidation, S-cyanylation, and S-acylation. According to the UniProtKB/Swiss-Prot database, about 450 different PTMs have been identified.

PTMs can be either reversible or irreversible, in some cases they are carried out by specific modifying enzymes. Also, PTMs may occur spontaneously (non-enzymatic) depending on the physico-chemical properties of the reactive amino acids and characteristics of the cellular environment (e.g. pH, metabolites, etc.) (Valderrama et al., 2019). Notably, Cys, Lys, and N-terminal residues are targets for several PTMs (Friso and van Wijk, 2015).

Among the compounds that interact with proteins, gasotransmitters and ROS play a special role. PTMs serve as a mechanism for regulating functional activity of proteins due to the direct influence of signaling molecules on them, as well as the main mechanism for realizing signaling potential of gasotransmitters and ROS.

ROS-induced protein PTMs

ROS means a set of mutually convertible reactive oxygen species, most of which exist for a short time. These include free radical particles – superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($ON^{\bullet}$), peroxide radicals ($RO_2^{\cdot-}$, etc), and neutral molecules such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), etc (Mittler et al., 2022). Among ROS, hydrogen peroxide has the highest signaling potential. It is believed to play a crucial role in oxidative signaling (Mittler et al., 2022). The main participants in $O_2^{\cdot-}/H_2O_2$ generation in plants are photosynthetic electron transport chain (chloroplasts), photorespiration process (peroxisomes), respiratory electron transport chain in mitochondria, and plasmalemma-localized NADPH oxidase (Gautam et al., 2017).

ROS-induced PTMs are usually more common in those compartments where ROS are produced. Cys-oxidation processes play a vital role in redox homeostasis of plant cells. The initial oxidation of Cys with hydrogen peroxide leads to the formation of sulfenic acid (R-SOH), which can later be oxidized to sulfinic (R-SO_2H) and sulfonic (R-SO_3H) acids (Valderrama et al., 2019). The oxidation of thiols to sulfinic acid is believed to be largely irreversible; however, these groups can be reduced by the ATP-dependent enzyme sulfiredoxin (Rey et al., 2007). Oxidation to sulfonic acid is definitely irreversible.

S-sulfenylation of Cys by hydrogen peroxide is primarily regarded as a mechanism for regulating functional activity of proteins. Under *in vitro* conditions, S-sulfenylation of about 800 polypeptides, including such important regulatory proteins as protein kinases, phosphatases, acetyltransferases, deacetylases, and deubiquitinases, has been demonstrated using human colon carcinoma cell line (Yang et al., 2014). In plants, 1394 potentially S-sulfenylation-sensitive targets were detected using a special highly reactive BTD probe (1-(pent-4-yn-1-yl)-1 Hbenzo[c][1,2]thiazin-4(3H)-one 2,2-dioxide) (Huang et al., 2019).

Despite the considerable progress in understanding the mechanism of post-translational modification of proteins under the ROS action, the contribution of this mechanism to the regulation of the state of specific proteins important for the adaptation of plants to stressful temperatures has not yet been sufficiently studied. It is a fact that in prokaryotes, H_2O_2 can oxidize thiol groups directly in transcription factor proteins (for example, Oxy R) (Vranova et al., 2002). In eukaryotes, the regulation mechanism of transcriptional activity involving ROS is more complex and includes a complex of proteins and peptides (Ndamukong et al., 2007; Lushchak, 2011).

However, experimental data accumulated over the last decade indicate the possibility of direct ROS-mediated activation of HSF (heat shock factors) under heat stress not only in prokaryotes but also in higher plants (Haider et al., 2021). For example, the transcription factor HSFA2 has been shown to be strongly induced at the transcriptional level in response to heat stress and H_2O_2 treatment (Nishizawa et al., 2006). It was also found that HSFA1a was activated by trimerization not only in response to heat stress, but also to the H_2O_2 action both *in vivo* and *in vitro* (Liu et al., 2013a). Heat stress-induced expression of HSP17.7 and HSP21 was found to be impaired in RBOH (respiratory burst oxidase homolog –catalytic subunit of NADPH oxidase) mutants (*rbohB*, *rbohD*, and *rbohB/D*), indicating a heat stress-induced HSF-ROS interaction (Wang et al., 2014). However, more research is still required to directly identify which amino acid residues of HSF interact with ROS, leading to their activation.

A specific example of the regulatory role of hydrogen peroxide-induced PTM is the oxidation of Cys in transcription factor protein (ethylene-sensitive group VII factor, ERFVII), which plays a key role in altering gene expression under hypoxia and probably heat stress. In the presence of oxygen, cysteine residues of ERFVII are oxidized to sulfenic acid, conjugated with arginine, and sent to proteasomes for degradation. However, under conditions of low oxygen content, ERFVII is released from the plasma membrane and moves to the nucleus, where it activates the expression of hypoxia response genes (Dietz, 2014). On the other hand, such a PTM may be a positive regulator of gene transcription, promoting the translocation of heat shock protein transcription factors (HSF) from the cytosol to the nucleus following the oxidation of Cys by hydrogen peroxide (Habibi, 2014).

A review by Sun and Guo (2016) provides many examples of the induction of various HSPs in chloroplasts and mitochondria under the influence of H_2O_2. Using genome-wide analysis of the Arabidopsis catalase-deficient mutant, a number of genes encoding transcription factors regulating synthesis of specific small HSP were found to be activated by hydrogen peroxide (Vandenabeele et al., 2004).

The influence of ROS on the state of transcription factors can also be realized indirectly, primarily through the processes of phosphorylation/dephosphorylation. The effect of ROS oxidation of cysteine residues in protein kinases and protein phosphatases is well known (Gupta and Luan, 2003). The participation of H_2O_2 in the control of tyrosine phosphorylation of plant proteins has also been shown (Karimova and Petrova, 2007). At the same time, according to

the authors, endogenous hydrogen peroxide affects both the activity of tyrosine protein phosphatases (inhibits them by oxidising the SH-groups of the catalytic centre) and the activity of tyrosine protein kinases (oxidation of sulfhydryl groups activates these enzymes).

NO-induced protein PTMs

Nitric oxide can act directly or *via* derivative molecules (reactive nitrogen species, RNS) to induce various PTMs, including tyrosine (Tyr) nitration, metal nitrosylation, and S-nitrosation of Cys (Mishra et al., 2021) (Figure 1).

Protein tyrosine nitration is a selective process consisting of the accession of a nitro group (-NO_2) to one of the two equivalent *ortho*-carbons in the aromatic ring of tyrosine residues to form 3-nitrotyrosine (Valderrama et al., 2019). Tyrosine nitration has traditionally been considered an irreversible mechanism and a marker of nitrosative stress (Corpas and Barroso, 2013). However, the existence of tyrosine denitrase, which reduces 3-nitrotyrosine in mammalian cells, points to a role for tyrosine nitration in NO-mediated signaling in these cells. Yet, the specific denitrase protein has not been identified in plants and there is currently no information on these issues (Valderrama et al., 2019).

Nevertheless, in recent years, it has been possible to show that protein nitration can be involved in regulatory processes. Analysis of the nitration of different organs of pea (*Pisum sativum* L.) plants during development and senescence indicates a specific protein nitration pattern for each organ (Begara-Morales et al., 2013). In addition, site-directed mutagenesis confirmed that Tyr-198 of NPR1 from Arabidopsis is the major nitration site responsible for the inhibition of its enzymatic activity by peroxynitrite ($ONOO^-$). Based on these results, the authors suggested that peroxisomal NO metabolism may contribute to the regulation of physiological processes in the absence of stress (Mata-Pérez et al., 2016a; Mata-Pérez et al., 2016b). The effect of tyrosine nitration in molecules of α-tubulin, the main protein of cytoskeleton components in plant cells, was also found, which may be associated with the regulation of dynamic properties of microtubules, directly involved in growth and division of plant cells (Blume et al., 2013).

S-nitrosation is the covalent attachment of the NO fragment to the reactive cysteine (Cys) of a protein to form S-nitrosothiol (SNO) (Figure 1). S-nitrosation is a reversible process considered to be a cellular switch that regulates functions of target proteins (Mishra et al., 2021). Since the SNO bond is redox sensitive, it can be cleaved by intracellular reducing agents such as glutathione and ascorbate, as well as reduced metal ions (Zhang and Liao, 2019). Moreover, S-nitrosation of target cysteine residues promotes the formation and breaking of intermolecular disulfide bridges, causing conformational changes in target proteins, and also affects cofactor binding (Astier et al., 2012). This type of PTM can also affect protein activity, stability, subcellular localization, and protein-protein interactions necessary for the regulation of physiological functions under certain conditions (Zhang and Liao, 2019).

In Arabidopsis plants, using a site-specific nitrosoproteomic approach, 926 proteins were found that can be targets for S-nitrosation (Hu et al., 2015). The effect of S-nitrosation of 44 proteins was detected in Arabidopsis under cold stress (Puyaubert et al., 2014). However, in general, data on S-nitrosation of specific proteins involved in adaptation to stress temperatures are still insufficient.

Another NO-induced PTM is metal nitrosylation. This effect consists of an interaction of NO with transition metals such as iron or copper present in metalloproteins, including phytohemoglobin, catalase, or cytochrome oxidase (Mishra et al., 2021). Formation of metal nitrosyl complexes causes reversible conformational changes in proteins and changes their structure and/or functional activity (Arora et al., 2016). However, little is known about the significance of this PTM in higher plants.

One of the best studied enzymatic proteins whose activity is regulated by nitric oxide post-translational modifications is cytosolic ascorbate peroxidase (cAPX) (Correa-Aragunde et al., 2015). Thus, S-nitrosylation of Cys-32 was found to stimulate cAPX activity. Cys-32 is present in 100% of cAPX described so far and is part of the pocket that binds ascorbate (Correa-Aragunde et al., 2013). At the same time, the reversible binding of NO to the heme prosthetic group is known to inhibit cAPX (Clark et al., 2000). Another potential redox modification of cAPX is the nitration of tyrosine residues (Lozano-Juste et al., 2011). This modification occurs in Tyr-5 and Tyr-235 of cAPX, causing irreversible inhibition of enzyme activity (Begara-Morales et al., 2013). In addition to the action of these modifications separately, simultaneous S-nitrosylation, tyrosine nitration, and carbonylation of cAPX are also possible. This phenomenon can occur under the influence of severe oxidative stress, and it leads to the degradation of the enzyme molecules by ubiquitin

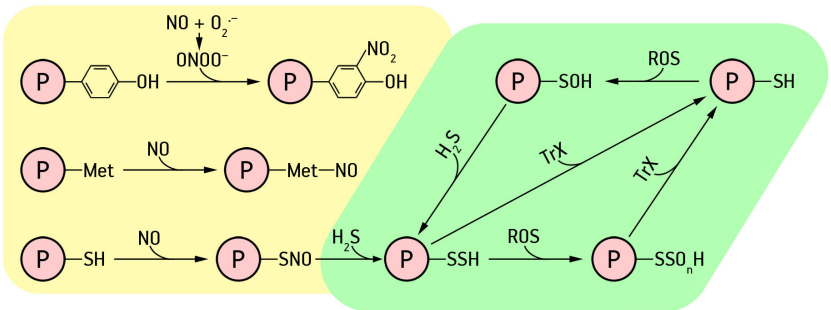

FIGURE 1
Post-translational modifications of proteins by the action of hydrogen sulfide and nitric oxide. Explanations in the text.

(Correa-Aragunde et al., 2015). This is precisely the phenomenon recorded in the heat shock effect on Bright Yellow-2 tobacco cells (de Pinto et al., 2013).

Catalase has been studied in considerable detail as a possible target of modifications by NO. It was found that NO can bind to iron in the heme, which prevents the binding of hydrogen peroxide from to the metal ion, thereby inhibiting catalase activity (Arora et al., 2016). On the other hand, the activation of catalase by nitric oxide through the S-nitrosylation process has also been reported (Bai et al., 2011).

There is also evidence that nitric oxide can inhibit NADPH-oxidase through S-nitrosylation of cysteine (Cys-890) (Yun et al., 2011). This may be another mechanism of NO involvement in regulating cell redox homeostasis.

Nitric oxide PTMs of NADPH-generating enzyme molecules have been reported in recent years (Corpas et al., 2021), in particular, of ferredoxin-NADP reductase (FNR). This enzyme is considered one of the main sources of NADPH in chloroplasts. Various proteomic analyses identified FNR as a target for Tyr nitration and S-nitrosation.

Increased FNR protein synthesis in sunflower seedlings under high-temperature stress was shown. However, FNR activity was wherein reduced, and *in vitro* analysis of FNR activity in the presence of ONOO-donor SIN-1 showed inhibition of enzyme activity (Chaki et al., 2011). In this work it was found that sunflowers were subjected to nitro-oxidative stress at high temperatures, resulting in impaired NADPH generation. Thus, tyrosine nitration inhibits FNR (Chaki et al., 2011). At the same time, it remains unclear how FNR activity will change due to the S-nitrosation of cysteine residues (Niu et al., 2019).

NADP-glyceraldehyde-3-phosphate dehydrogenase (NADP-GAPDH) is another enzyme involved in the formation of NADPH, various forms of which are localized in the cytoplasm and chloroplasts (Corpas et al., 2021). Both tyrosine nitration and S-nitrosation of the enzyme lead to the inhibition of its activity. The S-nitrosation at Cys-149 was found to be reversible; the reverse reaction is mediated by GSH (Zaffagnini et al., 2013).

Another mechanism of NADPH synthesis is related to the activity of enzymes in the oxidative phase of the pentose phosphate pathway, primarily glucose-6-phosphate dehydrogenase (G6PDH). Using nitric oxide donors and proteomics techniques, G6PDH inhibition of pea (*Pisum sativum*) leaves was shown to be possible both through nitration of tyrosine residues and through S-nitrosation of cysteine (Corpas et al., 2021). However, *in vivo* experiments have shown increased activity and expression of G6PDH genes in soybean (*Glycine max*) roots under drought conditions when treated with NO donor (Wang et al., 2020).

There is also evidence of nitric oxide donors reducing the activity of several other enzymes involved in NADPH synthesis. In particular, a decrease in the activity of the NADP-malic enzyme during Tyr-73 nitration has been shown (Begara-Morales et al., 2019). Moreover, this effect is considered a component of the development of nitrosative damage in *A. thaliana* under cold stress. A decrease in NADP-isocitrate dehydrogenase activity has also been shown in the presence of NO donors causing the effects of tyrosine nitration and S-nitrosation of cysteine residues (Corpas et al., 2021). Thus, *in vitro* NO modifications of the enzyme molecules involved in NADPH generation mainly cause their inhibition. However, *in vivo* it is possible for nitric oxide to activate the gene expression of individual enzymes involved in maintaining the NADPH pool.

H_2S-induced protein PTMs

It is currently believed that the signaling effects of hydrogen sulfide, which constitute many physiological and pathological processes in mammals and plants, are related to protein persulfidation – conversion of cysteine thiol group (-SH) into corresponding persulfide (-SSH) (Yuan et al., 2017; Filipovic et al., 2018; Paul and Snyder, 2018). However, the mechanism for this process is still debated. It is assumed that H_2S or its ionic forms HS^- and S^{2-} cannot react directly with protein thiols. This interaction requires the presence of oxidizing agents (Aroca et al., 2021). It is more likely that H_2S can react directly with oxidised cysteine residues (R-SOH). Protein nitrosothiols (RSNO) SNO groups can also react with H_2S to form protein persulfides (Paul and Snyder, 2012; Xuan et al., 2020). However, there is evidence that this process is thermodynamically unfavourable (Filipovic et al., 2012). Therefore, reaction of H_2S with sulfenic acid residues to form protein persulfides is considered the most likely effect of H_2S.

Oxidation of cysteine residues is a method of redox control of proteins functional activity (Cuevasanta et al., 2015). Thus, it is assumed that the process of protein modification is triggered by ROS signal, i.e., oxidation of thiol group of cysteine to sulfenic acid with H_2O_2 (Aroca et al., 2021). Sulfenic residues are easily persulfided with hydrogen sulfide to form persulfide groups (R-SSH). Protein sulfenic residues have been shown to react two orders of magnitude faster with H_2S than with glutathione (Cuevasanta et al., 2015). Persulfidation is also a mechanism to protect proteins from oxidative damage. Persulfided residues involving the thioredoxin system (Trx/TrxR) can be converted into conventional sulfhydryl groups (Filipovic et al., 2018). Schematically, the processes of formation of sulfene and persulfide groups, as well as reduction of thiol groups, can be depicted as follows:

$$\text{protein} - \text{SH} + \text{ROS} \rightarrow \text{protein} - \text{SOH} \quad (1)$$

$$\text{protein} - \text{SOH} + \text{H}_2\text{S} \rightarrow \text{protein} - \text{SSH} \quad (2)$$

$$\text{protein} - \text{SSH} + \text{Trx/TrxR} \rightarrow \text{protein} - \text{SH} \quad (3)$$

In the proteome of *A. thaliana* treated with NaHS, 106 persulfided proteins have been identified that are mainly involved in photosynthesis, protein synthesis, cellular organization, and a primary metabolism (Aroca et al., 2018). Using another technique of proteomic analysis of endogenous persulfated proteins in *A. thaliana* wild-type and *des1*-mutant leaves, 2015 persulfated proteins were identified, which were mainly involved in the regulation of the primary metabolism, abiotic and biotic stress responses, plant growth, and development processes (Aroca et al., 2017).

The possibility of regulating through the persulfidation process the activity of several enzymes involved in maintaining the pool of a key cellular reducing agent, NADPH, is being considered (Corpas

et al., 2021). In particular, NADP-GAPDH activity was found to be increased by H_2S (Aroca et al., 2015). On the other hand, inhibition of NADP-isocitrate dehydrogenase and NADP-malic enzyme due to persulfidation has been shown (Muñoz-Vargas et al., 2018; Muñoz-Vargas et al., 2020).

Persulfidation is probably a part of the toolkit for regulation of gene expression. A transcriptomic study carried out on Arabidopsis plants showed that treatment with exogenous H_2S resulted in significant changes in expression of numerous genes. When plants were treated with hydrogen sulfide, in particular, the expression of genes encoding various regulatory transcription factors was enhanced (Sen et al., 2012; Aroca et al., 2017). A study of tomato gene expression during NaHS root treatment showed that 5349 genes were activated and 5536 ones inhibited (Guo et al., 2018). A number of studies have also shown a role for sulfide in modifying histones and altering chromatin structure that constitutes epigenetic regulation (Shivaraj et al., 2020).

Influence of gasotransmitters on functioning of plant protective systems at extreme temperatures

Modification of stress-protective systems with nitric oxide

As already mentioned, cold stress induces increased nitric oxide synthesis in plants. This effect appears in most cases to be one of the signals needed to trigger adaptive responses. Thus, the expression of specific cold-sensitive genes such as *CBF1*, *CBF2*, *CBF3*, *LTI30*, *LTI78*, *COR15a* has been shown to be NO-status dependent (Puyaubert et al., 2014; Baudouin and Jeandroz, 2015). The cold-induced enhancement of expression of these genes was suppressed by NO scavenger PTIO and was weakly manifested in mutants defective in nitrate reductase genes. During cold acclimation of tomato, expression of the protein kinase CPK27 gene turned out to be dependent on NO (Lv et al., 2018).

Zhang et al. (2019) investigated the role of NO signals in cold acclimatization of two alfalfa species, *Medicago falcata* (resistant) and less resistant *Medicago truncatula*. The cold acclimatization effect of both species was suppressed when the plants were treated with nitrate reductase inhibitor tungstate and the NO scavenger PTIO. Cold exposure increased the number of NIA1 NR isoform transcripts, but not NIA2. However, in the more resistant species *M. falcata*, this effect was more pronounced. Treatment with NO donors, as well as cold acclimatisation, led to an increase in cold resistance of both species. Wherein the expression of key antioxidant enzyme genes, *Cu/Zn-SOD2*, *Cu/Zn-SOD3*, catalase, and chloroplast ascorbate peroxidase (*APX1*), was induced under the influence of cold and NO donor. These effects were more pronounced in *M. falcata* than in *M. truncatula*. Thus, the authors have shown an association between NO generation, antioxidant system performance, and cold tolerance of alfalfa species (Zhang et al., 2019).

The positive effect of NO on plant cold resistance may be due to S-nitrosation of target proteins, including antioxidant enzymes. Thus, in *Brassica juncea*, Fe-SOD was identified as a target for S-nitrosation under cold stress conditions (Sehrawat et al., 2013). Overall, *B. juncea* showed the effect of differential S-nitrosation of 10 proteins, among which, apart from SOD, also dehydroascorbate reductase and glutathione-S-transferase (Sehrawat and Deswal, 2014). The possibility of increasing the activity of catalase and non-specific peroxidases due to their post-translational modifications by NO under cold stress and the association of these effects with resistance of plants to hypothermia has also been reported (Sougrakpam et al., 2018).

Processes associated with S-nitrosation of antioxidant enzymes also occur in the apoplast. It is assumed that the PTM of apoplastic ascorbate peroxidase, which leads to an increase in its activity, may be associated with the further development of cold resistance in plants (Sehrawat and Deswal, 2014). Importantly, NO not only interacts directly with protein molecules of antioxidant enzymes, but is also involved in enhancing gene expression of these proteins during cold adaptation (Puyaubert et al., 2014). This allows us to consider NO as one of the key signaling mediators involved in the maintenance of redox homeostasis under cold stress.

Treatment of Bermuda grass (*Cynodon dactylon*) plants with the NO donor sodium nitroprusside (SNP) reduced the electrolyte release from tissues caused by cold and prevented an increase in the lipid peroxidation product malondialdehyde content in cells. At the same time, the NO-donor-treated plants showed higher values of SOD, peroxidase, and catalase activity at low temperatures (Fan et al., 2015). Priming of seeds of winter wheat and rye with SNP contributed to an increase in activity of SOD and guaiacol peroxidase (Kolupaev et al., 2020). Protective effects of the nitric oxide donor were clearly evident in seedlings subjected to cold hardening. After cold hardening and especially after freezing, the content of malondialdehyde (lipid peroxidation product) in seedlings grown from seeds primed with SNP was lower compared to the corresponding controls. Consequently, there is reason to believe that nitric oxide enhances cold-induced activation of cereal antioxidant system (Kolupaev et al., 2020).

It is noteworthy that NO can affect many components of antioxidant defense. Thus, exposure to NO mitigated the negative effects of cold stress on peach fruit by stimulating alternative oxidase and reducing oxidative damage (Song et al., 2021).

Cold-induced Δ^1-pyrroline-5-carboxylate synthase gene expression and proline accumulation in *A. thaliana* were also found to be mediated by NO (Zhao et al., 2009). These effects were weakly expressed in the *nia1nia2* mutants and were suppressed by the NO scavenger PTIO.

As mentioned above, the adaptation of plants to the stress effect of high temperatures, similarly to adaptation to low temperatures, includes the activation of gene expression and increased activity of antioxidant enzymes as well as accumulation of low-molecular-weight antioxidants and multifunctional compounds that also exhibit antioxidant properties. Nitric oxide can enhance such effects. The example of wheat plants shows an increase in the activity of SOD, catalase, guaiacol peroxidase, ascorbate peroxidase, glutathione reductase under heat stress as a result of pretreatment of plant objects with NO donor SNP (Karpets et al., 2011; El-Beltagi et al., 2016). Under prolonged heat stress, SOD, catalase, ascorbate peroxidase, and glutathione reductase activities increased in wheat treated with SNP (Iqbal et al., 2022). The activities of SOD, ascorbate

peroxidase, and glutathione reductase increased in callus tissues of wheat under the influence of SNP in addition to the increase in these indicators caused by heat stress (El-Beltagi et al., 2016). The increase in heat resistance of *Phaseolus radiatus* and *Phragmites communis* under the action of NO donors was also accompanied by an increase in activity of antioxidant enzymes (Yang et al., 2006; Song et al., 2008). Treatment of strawberry plants with a nitric oxide donor increased their heat resistance, which was manifested in a decrease in H_2O_2 accumulation, a decrease in lipid peroxidation under stress conditions, and an increase in activity of enzymatic antioxidants (Manafi et al., 2021).

In *Zea mays* plants, an increase in ascorbate peroxidase activity under normal conditions and under heat stress has been shown by treatment with the NO donor SNP (Sun et al., 2022). However, the nitric oxide donor did not affect *APX1* gene expression. At the same time, both an increase in glutathione reductase activity and an increase in *GR1* gene expression under the influence of exogenous NO were shown.

An increase in the content of multifunctional protective compounds that also have antioxidant activity – proline and trehalose – was found under the influence of SNP treatment in wheat plants exposed to long-term heat stress (Ju and Ma, 2011; Han et al., 2013; Iqbal et al., 2022). Spraying rice plants with SNP mitigated the manifestation of oxidative stress under high temperature conditions by increasing the content of ascorbate, reduced glutathione, and increasing the accumulation of proline (Gautam et al., 2021). Alamri et al. (2019) revealed an endogenous NO-dependent increase in proline content in horse beans (*Vicia faba*) in response to high temperature. This effect was inhibited when plants were treated with NO scavenger cPTIO (Alamri et al., 2019).

In general, a fairly large pool of results has been accumulated in the literature, indicating an increase in the enzymatic antioxidant system of plants and the accumulation of low-molecular antioxidants under the action of NO. Such effects may be due to both post-translational modifications and the effect of nitric oxide on the expression of genes for antioxidant enzymes and enzymes involved in synthesis of low-molecular-weight protective compounds.

However, there are also a large number of contrary phenomena, without understanding the reasons for which it is impossible to build a complete picture of NO participation in the regulation of the antioxidant system. For example, the NO donor SNP has been shown to reduce ascorbate peroxidase and glutathione reductase activity in cotton callus (Vital et al., 2008). The possible mechanisms of this phenomenon were not explained in the above work, but it is possible that that effect took place due to the predominance of those post-translational modifications by NO that inhibit these enzymes. On the other hand, along with numerous reports on a decrease in the activity of antioxidant enzymes when plants were treated with NO antagonists, some studies have revealed an increase in the activity of antioxidant enzymes under the influence of nitric oxide scavengers. Using roots of intact wheat seedlings, treatment with NO donor, as well as a one-minute hardening at 42°C, was found to cause an increase in SOD, catalase, and guaiacol peroxidase activities (Karpets et al., 2015b). At the same time, treatment of such seedlings with NO scavenger PTIO and L-NAME, an inhibitor of nitric oxide synthesis along the oxidative pathway, also increased the activity of these enzymes, although it did not affect the increasing activity of these enzymes under the influence of hardening temperature (Karpets et al., 2015b). Thus, the opposite effects – a decrease in nitric oxide content when plants are treated with PTIO or L-NAME and an increase in nitric oxide (when plants are exposed to hardening heat or NO donor treatments) – can cause a phenomenologically similar effect: an increase in activity of antioxidant enzymes. Since different post-translational modifications of antioxidant enzyme molecules by nitric oxide can lead to different results (increased or decreased activity, see above), it is likely that, depending on the experimental conditions, both increased and decreased antioxidant enzyme activities are possible under the influence of NO donors.

Unusual aspects of nitric oxide regulation of protective systems were found in studies using NO-deficient *nia1,2noa1-2* mutants of Arabidopsis (Costa-Broseta et al., 2018). Unlike wild-type plants, these mutants exhibited constitutive frost resistance and were characterised by increased levels of low-molecular-weight antioxidants – ascorbate, reduced glutathione, flavonoids, and sugars. The authors concluded that NO action can attenuate synthesis and accumulation of antioxidants, osmoprotective and regulatory metabolites. It is noteworthy that rice mutants with a reduced activity of one of nitrate reductase forms (*nia2*) turned out to be more drought-resistant, with higher expression of the *APX2* and *CATA* genes of antioxidant enzymes (Zhou et al., 2021). Based on these data, the authors concluded that the *NIA2* gene negatively regulates drought tolerance in rice. However, it should be noted, that the *NIA2* form of nitrate reductase is not considered the main producer of NO and its content was not monitored in this work, making it impossible to unambiguously associate the observed phenomena with NO deficiency.

On the other hand, *hot5* mutants of Arabidopsis, defective in GSNOR activity, are known to be extremely sensitive to high temperatures (Lee et al., 2008). This effect has been attributed to an excess of nitric oxide in their cells when exposed to high temperatures. Notably, the sensitivity of *hot5* mutants to high temperatures was partially mitigated by the NO scavenger cPTIO treatment.

Despite the existence of such examples of negative regulation of plant adaptive responses by nitric oxide, in general, the literature accumulates significantly more examples of positive regulation of plant stress-protective systems by the action of NO. One mechanism of their activation may be related to the effect of nitric oxide on the expression of genes from the families of transcription factors controlling the complex defense response. Thus, when rice was treated with the nitric oxide donor SNP, an increase in the expression of genes of several transcription factors of the MYB and WRKY families was detected, which provide resistance to various stresses, including dehydration and extreme temperatures (Singh et al., 2017).

In general, it is likely that modifications in the NO status of plants, or even more broadly in nitrogen metabolism, can lead to significant changes in functioning of protective systems, including antioxidant ones (Hancock and Veal, 2021). Apparently, its activation can occur either through the direct involvement of NO or through the action of other signaling mediators, including calcium and hydrogen sulfide. H_2S can function both synergistically and antagonistically with NO (see below). It is possible that, under certain conditions, a deficiency of NO or other nitrogen compounds may be a signal to activate other

mechanisms that effectively regulate the functioning of the protective systems.

Modifications of stress-protective systems with hydrogen sulfide

As noted earlier, the plant response to low temperatures is usually accompanied by an increase in gene expression and an increase in L/D-cysteine disulfhydrase activity and, consequently, endogenous hydrogen sulfide content. Using Arabidopsis mutants defective in L/D-cysteine desulfhydrase genes, the role of hydrogen sulfide in the activation of cold-sensitive *COR15* and *CBF3* genes has been shown (Du et al., 2017). One of the mechanisms of hydrogen sulfide involvement in the Arabidopsis cold stress response involves persulfidation of at least one component of the MAP-kinase signaling cascade (MAP4), resulting in the increased activity of this enzyme and activation of related signaling processes (Du et al., 2017).

Evidence for the stimulation by hydrogen sulfide of protective systems important for providing plant cold resistance is obtained mainly by the application of exogenous hydrogen sulfide donors (mainly NaHS). For example, NaHS treatment of *C. dactylon* increased plant survival after exposure to sub-zero temperatures, which was accompanied by the activation of antioxidant enzymes, such as SOD, catalase, guaiacol peroxidase, and glutathione reductase, and the accumulation of low-molecular-weight antioxidants of ascorbate-glutathione cycle, and osmolytes (Shi et al., 2013).

An increase in survival after freezing of winter wheat seedlings was observed when they were pretreated with 0.1 or 0.5 mM NaHS. One of the components of stress-protective effect of H_2S donor was the phenylalanine ammonia lyase-dependent accumulation of flavonoid compounds with high antioxidant activity and reduction of the effects of oxidative stress (Kolupaev et al., 2018; Kolupaev et al., 2019a). Grapes (*Vitis vinifera*) under the influence of exogenous hydrogen sulfide at low temperatures showed an increase in SOD activity (Fu et al., 2013). In Arabidopsis plants, treatment with H_2S donor under stressful conditions reduced not only ROS but also the content of active forms of nitrogen by increasing the activity of S-nitrosoglutathione reductase (GSNOR) (Shi et al., 2015).

A number of studies have shown an increase in resistance of fruits with hydrogen sulfide during low-temperature storage. Thus, cucumber fruits treated with hydrogen sulfide exhibited lower ROS levels and higher antioxidant enzyme activity during low-temperature storage (Wang et al., 2022a). Also, hydrogen sulfide treatment increased activity of main enzymes involved in energy metabolism, including cytochrome *c* oxidase, succinate dehydrogenase, H^+-ATPase, and Ca^{2+}-ATPase. In addition, H_2S was found to induce Δ^1-pyrroline-5-carboxylate synthase activity and cause a decrease in proline dehydrogenase activity, which promotes proline accumulation. An increase in total content of phenolic compounds and the activity of phenylalanine ammonium lyase (was recorded in banana (*Musa*) fruits during low-temperature storage under the influence of the hydrogen sulfide donor NaHS (Luo et al., 2015). A total content of flavonoids and anthocyanins under the NaHS action was also increased during low-temperature storage of hawthorn (*Crataegus*) fruits (Aghdam et al., 2018).

As already mentioned, under the conditions of high as well as low temperatures, expression of genes and activity of enzymes involved in hydrogen sulfide synthesis was shown to increase, which, in turn, caused an increase in endogenous H_2S content in plants of different species. Also, high temperatures cause an increase in gene expression and activity of GSNOR, which contributes to the removal of excess reactive nitrogen species and ROS, ultimately increasing the tolerance to a high temperature stress. Using poplar plants (*Populus trichocarpa*) as an example, it has been shown that inhibition of H_2S biosynthesis reduces GSNOR activity with a consequent increase in oxidative damage in leaves induced by RNS and ROS (Cheng et al., 2018). These results suggest that one of the mechanisms of H_2S effects on plant heat resistance may be related to the activation of GSNOR, which contributes to the reduction of oxidative stress induced by RNS and ROS.

There is also evidence that hydrogen sulfide enhances the complex of major antioxidant enzymes in plants under high-temperature stress. Thus, treatment of maize seedlings under normal cultivation conditions with hydrogen sulfide donor NaHS stimulated the activity of antioxidant enzymes (catalase, guaiacol peroxidase, SOD, and glutathione reductase) and increased antioxidants content (GSH and ascorbate) compared to control (Li et al., 2014). Under heat stress, all of the above physiological parameters decreased, but the treatment of corn seedlings with NaHS helped to maintain the activity of antioxidant enzymes and the content of low-molecular-weight antioxidants at a higher level. An increase in activity of Δ^1-pyrroline-5-carboxylate synthetase and a decrease in activity of proline dehydrogenase with subsequent accumulation of proline were also shown on corn plants (Li et al., 2013a).

In heat-stressed strawberry plants, pretreatment with NaHS enhanced the expression of genes encoding enzymes for the synthesis of antioxidants (ascorbic acid and glutathione) and maintained a higher redox potential of ascorbate AsA and glutathione GSH (AsA/GSH). NaHS treatment of plants also increased gene transcription of antioxidant enzymes (ascorbate peroxidase, catalase, SOD, and glutathione reductase), as well as chaperones (HSP 70, HSP 80, and HSP 90) and aquaporins (Christou et al., 2014). It is noteworthy that treatment with exogenous hydrogen sulfide eliminated the heat stress-induced increase in NO content. Perhaps, this effect was aimed at preventing the development of nitrosative stress.

Functional interaction of NO, H_2S, ROS, and Ca^{2+} in the formation of plant adaptive responses to stress temperatures and other adverse factors

Signaling and regulatory effects of gasotransmitters and ROS are largely determined by their functional interaction with each other. These interactions can be divided into several levels: (a) ordinary chemical interaction of molecules with each other; (b) competition for common binding targets with biomacromolecules; (c) influencing on each other's synthesis, which may involve many other signaling mediators (Kolupaev et al., 2019b).

Direct reactions between H_2S and NO include formation of nitroxyl (HNO) and nitrosothiols (RSNO), which in turn can interact with biomacromolecules (Aroca et al., 2020). It has also recently been discovered that persulfides are able to produce NO using nitrite through intermediates such as polysulfide, SNO^- (thionitrite) and S_2NO^- (perthionitrite, nitrosodisulfide) (Bailey et al., 2016; Marcolongo et al., 2016; Marcolongo et al., 2019). Consequently, the interaction of H_2S and NO produces intermediates that may also be involved in cellular signaling (Aroca et al., 2020). However, the mechanisms of formation and, moreover, the biological activity of these compounds in plant cells is not yet fully understood.

Hydrogen sulfide also directly interacts with some ROS. For example, the removal of hydroxyl radicals in the reaction with hydrogen sulfide ($HO^\bullet + H_2S/HS^- \rightarrow S^{\bullet-} + H_2O$), and the interaction of hydrogen peroxide with the hydrosulfide anion ($H_2O_2 + HS^- \rightarrow HSOH + OH^-$) are considered possible (Carballal et al., 2011). Such interactions can reduce the signaling potential of relevant molecules and thereby modulate cellular signals (Hancock, 2019). However, the real contribution of these processes to signal damping remains largely unexplored.

The second level of interaction between gasotransmitters and ROS is determined by the presence of common interaction sites with target proteins s. Most often, thiol groups play this role. Sun et al. (2020) proposed a model according to which an excess of nitric oxide in the cellular system creates conditions for the conversion of individual thiol groups of proteins into nitrosothiol ones. At high concentrations of hydrogen sulfide in cells, persulfidation of proteins is activated, and finally, with a certain balance of H_2S and NO, the persulfidated groups can be converted into the original thiols using glutathione and ascorbate as reducing agents. As mentioned above, ROS are also involved in the interaction of hydrogen sulfide with thiol groups in proteins: oxidation of thiols by hydrogen peroxide to sulfenes promotes the interaction of molecules with H_2S to form –SSH. It is assumed that the type of thiol group conversion and eventually protein activity will be determined by the probability of the above interactions and will depend on the local concentrations of H_2S, NO, and ROS (Hancock, 2019). Not only enzymatic and signaling proteins, but also some transcription factors can undergo such 'probabilistic' modifications (Zhou et al., 2020). Specific examples of proteins modified by ROS, NO, and H_2S, in particular antioxidant enzymes, are given above in the review.

Probably, the regulation of the activity of enzymes that are involved in the maintenance of the NADPH pool is also mediated *via* the crosstalk between NO and H_2S. Thus, glyceraldehyde-3-phosphate dehydrogenase (NADP-GAPDH) is negatively regulated by tyrosine nitration and cysteine S-nitrosation, whereas cysteine persulfidation causes enzyme activation (Aroca et al., 2015; Corpas et al., 2021). Therefore, it is likely that local concentrations of NO and H_2S may influence NADP-GAPDH activity and NADPH formation in specific cellular compartments.

The most difficult to study and interpret the results are the mechanisms of functional interaction of NO, H_2S, and ROS related to their mutual influence on each other's synthesis. Such effects involve both direct modification by active molecules of functional groups of enzymes that synthesize signal mediators, and the indirect influence of these molecules on the expression of genes encoding the corresponding enzymes. There seems to be a constant direct and reverse regulation mechanism at work in these processes. Thus, several channels of interaction between ROS and hydrogen sulfide as mediators are known. Hydrogen sulfide directly activates the key enzyme that generates the ROS signaling pool, NADPH oxidase, by persulfidation of two cysteine residues (Cys-825 and Cys-890) in the catalytic subunit (RBOH*D*) (Shen et al., 2020). On the other hand, Arabidopsis plants showed increased expression of L/D-cysteine desulfhydrase in response to H_2O_2 treatment (Shi et al., 2015). The *atrbohD*, *atrbohF*, *atrbohD/F* mutants, unlike wild-type plants, did not show increased formation of hydrogen sulfide in response to drought stress, indicating the role of ROS generated by NADPH oxidase in the activation of stress-induced H_2S synthesis (Shi et al., 2015).

Very ambiguous functional relationships were also found between H_2S and NO. There was evidence of reduced stress-induced NO accumulation in strawberry leaves under the influence of hydrogen sulfide (Christou et al., 2014). It is believed that this effect is due to the stimulating effect of H_2S on the expression of the S-nitrosoglutathione reductase gene (Li, 2020). On the other hand, hydrogen sulfide has been shown to cause persulfidation of cysteine residues of nitrate reductase and reduce its activity (Zhou et al., 2021). Thus, on the one hand, the effects of antagonism between nitric oxide and hydrogen sulfide are possible, and on the other hand, NO may mediate the signal transduction induced by H_2S. The involvement of ROS and NO as mediators in stress-protective action of the hydrogen sulfide donor is also indicated by the data obtained on wheat seedlings subjected to heating. Induction of wheat seedlings heat tolerance by the hydrogen sulfide donor NaHS was accompanied by a transient increase in hydrogen peroxide and nitric oxide content in the roots as well as by an increase in nitrate reductase activity (Karpets et al., 2020). The H_2S donor-induced increase in NO content was not evident in the presence of the antioxidant dimethylthiourea and the NADPH oxidase inhibitor imidazole. At the same time, the increase in H_2O_2 in roots occurring during treatment with exogenous hydrogen sulfide was not eliminated by the NO scavenger PTIO and the nitrate reductase inhibitor tungstate (Karpets et al., 2020). It can therefore be assumed that H_2O_2 is above NO in the hydrogen sulfide signaling chain inducing the development of heat resistance.

However, there is also evidence that hydrogen sulfide mediates the effects of nitric oxide. For example, the treatment of maize seedlings with a nitric oxide donor, which induced the development of heat tolerance, was accompanied by increased expression of the gene encoding L-cysteine desulfhydrase (*LCD1*) and increased enzyme activity (Sun et al., 2022). Wherein, the hydrogen sulfide scavenger hypotaurine abolished the effect of the NO donor on L-cysteine desulfhydrase and the development of heat resistance in maize seedlings.

An ambiguous functional interaction between H_2S and NO is also evident in their regulation of plant stomatal responses, important for adaptation to drought and stress temperatures. Thus, the stomata-closing effect caused by hydrogen sulfide can be mediated by nitric oxide. NO scavenger PTIO has been shown to eliminate stomatal closure induced by NaHS treatment in *Ipomoea batatas* plants (Hu et al., 2014). In Arabidopsis, the slow-acting hydrogen sulfide donor CYY4137 induced a stomatal aperture-reducing effect that was also reversed by NO antagonists (Honda et al., 2015). The assumption of a

mediating role of nitric oxide in the stomatal effects of hydrogen sulfide is also consistent with the absence of sodium hydrosulfide effects on stomata of Arabidopsis mutants defective in activity of two forms of nitrate reductase (*nia1/nia2*), the main enzyme generating NO in plant cells (Scuffi et al., 2014). On the other hand, there is also evidence of reduced H_2S accumulation in Arabidopsis epidermal cells after treatment with NO donors (Lisjak et al., 2010).

Overall, the available evidence suggests that the location of NO and H_2S as mediators in signaling circuits may be different (Li et al., 2013b). Thus, the hydrogen sulfide scavenger hypotaurine abrogates the stress-protective effect of the NO donor SNP on rice plants under prolonged heat stress, indicating the H_2S role as a mediator of exogenous NO physiological effects (Gautam et al., 2022). The possibility of a positive (synergistic) effect of NO and H_2S on the resistance of wheat plants to long-term heat stress is evidenced by data on the strengthening of the positive effect of the donors of these gasotransmitters on the antioxidant system (activity of SOD, catalase, and ascorbate-glutathione cycle enzymes) when used together (Iqbal et al., 2021).

Overall, ROS, nitric oxide, and hydrogen sulfide form a complex signaling network that ensures that appropriate adaptive responses are triggered. Another integral component of such a network is calcium as a universal intracellular messenger (Neill et al., 2008). As already noted, fluidization of membranes under heat stress and the opposite process of increasing their rigidity under cold stress led to a rapid entry of calcium into the cytosol. These changes in calcium homeostasis may be primary to changes in cellular ROS, NO, and H_2S (Kolupaev et al., 2022) (Figure 2).

For instance, calcium ions are able to induce NO accumulation. The effects of plant nitrate reductase activation by calcium ions and inhibition in the presence of a calcium chelator *in vitro* have long been demonstrated (Sane et al., 1987). An increase in activity of this NO-synthesizing enzyme in intact plants under the influence of exogenous calcium has been also observed (Gao et al., 2011). Calcium entry into the cytosol can also be a stimulus for hydrogen sulfide synthesis. Thus, under the influence of exogenous calcium and calmodulin, activity of L-cysteine desulfhydrase was increased in cultured tobacco (*Nicotiana tabacum*) cells, which led to an increase in endogenous H_2S formation (Li et al., 2015a). In addition, it is well known that calcium directly and indirectly activates NADPH oxidase, one of the main enzymes of ROS signaling (Baxter et al., 2014). Thus, temperature stress effects are likely to induce various components of the signaling network, including gasotransmitters and ROS (Figure 2).

These signaling mediators can promote the opening of calcium channels of different types. For example, potential-dependent calcium

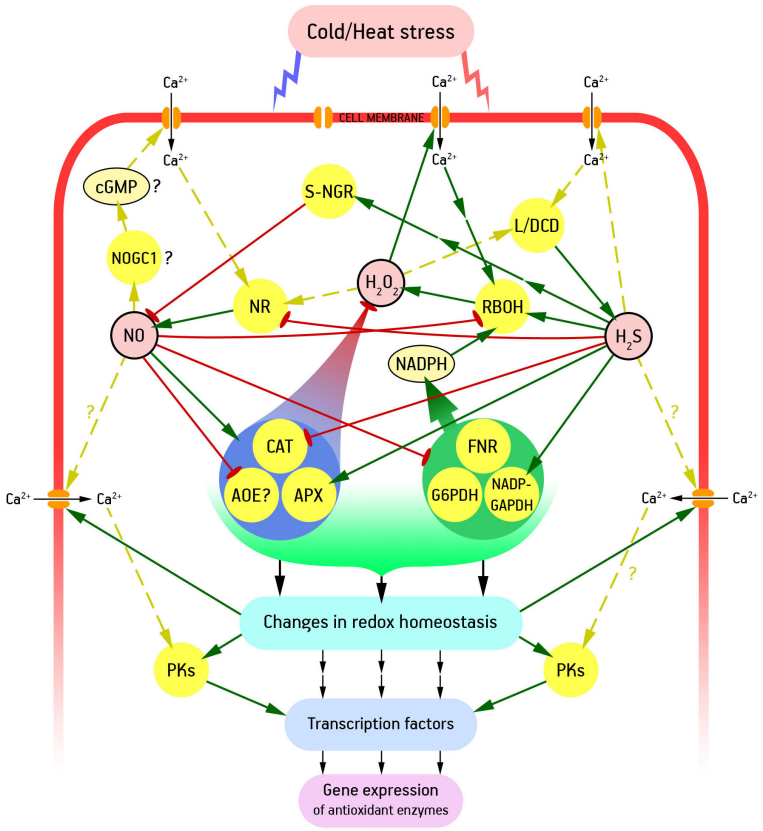

FIGURE 2
Pathways of functional interaction between H_2S, NO, ROS and Ca^{2+} in formation of plant adaptive responses to low and high temperatures. AOE, antioxidant enzymes; APX, ascorbate peroxidase; CAT, catalase; cGMP, cyclic guanosine 3′-5′-monophosphate; FNR, ferredoxin-NADP reductase; G6PDH, glucose-6-phosphate dehydrogenase; L/DCD, L/D-cysteine desulfhydrase; NADP-GAPDH, NADP-dependent glyceraldehyde-3-phosphate dehydrogenase; NOGC1, NO-dependent guanilate cyclase 1; NR, nitrate reductase; PKs, protein kinases; RBOH, respiratory burst oxidase homolog (catalytic subunit of NADPH oxidase); S-NGR, S-nitrosoglutathione reductase. Interrupted arrows show indirect interactions between signalling participants, and dashed arrows show interactions whose mechanisms are poorly understood. Other explanations in the text.

channels are known to be activated by ROS (Mori and Schroeder, 2004). Under the influence of NO, calcium channel proteins can undergo S-nitrosation, which also leads to increased calcium entry into the cytosol (Laxalt et al., 2007).

In addition to its possible direct effect on calcium channel status, nitric oxide may be involved in signaling chains as a secondary messenger functionally linked to calcium *via* cyclic guanosine 3′-5′-monophosphate (cGMP), cyclic adenosine diphosphate ribose (cADPR), and probably several other mediators (Singhal et al., 2021) (Figure 2). Despite the lack of clear molecular genetic evidence for the functioning of a cGMP-dependent signaling pathway in higher plants (Hancock and Veal, 2021), which connects NO and Ca^{2+} signaling in green algae (Singh et al., 2022), bioinformatics methods have provided evidence for the functioning of NO-dependent guanylate cyclase 1 (NOGC1) in *A. thaliana*. The recombinant NOGC1 protein has subsequently been shown to be able to synthesize cGMP in a NO-dependent manner, albeit in very small amounts (Gross and Durner, 2016). Overall, there is considerable experimental evidence indirectly suggesting the existence of NO-mediated cGMP formation in plant cells and the regulation of calcium homeostasis with its participation. A number of relevant examples are provided in recent reviews (Kolupaev et al., 2022; Praveen, 2022). It is *via* cGMP and calcium that many of the stress-protective effects of nitric oxide can be realized, in particular an increase in the heat resistance of plants (Karpets et al., 2016). A component of the latter is, in particular, a calcium-dependent increase in the activity of antioxidant enzymes (Karpets, 2017).

Calcium also appears to be involved in the transduction of H_2S signals that induce the development of plant heat tolerance. The increase in heat resistance of tobacco suspension cell culture by the action of the H_2S donor NaHS was eliminated by various calcium and calmodulin antagonists (Li et al., 2012). These results are consistent with the inhibition by calcium antagonists of the effect of hydrogen sulfide on the heat resistance of wheat coleoptiles and the activity of antioxidant enzymes (Kolupaev et al., 2017).

ROS and gasotransmitters are capable of both stimulating and inhibiting each other's effects. A direct post-translational modification of key enzymes involved in regulation of redox homeostasis – NADPH oxidase, ascorbate peroxidase, catalase, and probably several others – which, as already noted, can be positive and negative, may be a component of hydrogen sulfide and nitric oxide competitive action (Aroca et al., 2015; Correa-Aragunde et al., 2015; Corpas et al., 2019a). In turn, ROS can be inducers of NO formation with the participation of nitrate reductase (Dubovskaya et al., 2011) and H_2S synthesis under the influence of L/D-cysteine desulfhydrase (Shi et al., 2015). These gasotransmitters carry out both antagonism and synergy in a complex regulation of redox homeostasis by direct effect on the corresponding proteins as well as by forming signals that alter the state of transcription factors and expression of corresponding genes (Figure 2).

Conclusion and prospects

Two key gasotransmitters, nitric oxide and hydrogen sulfide, are involved in the regulation of adaptive responses to low and high temperatures and are involved in a complex overall cellular signaling-regulatory network. Many of their effects are related to functional interactions with ROS, which are also key signaling mediators in formation of plant responses to extreme impacts. The close interaction between NO, H_2S, and ROS is largely due to the presence of common targets for chemical modification. The main such target is the thiol groups of proteins. The probability of the type of protein modifications depends largely on the local concentrations of nitric oxide, hydrogen sulfide, hydrogen peroxide and other active molecules, as well as other conditions affecting these interactions (e.g. pH, redox potential, etc.). In other words, there may be competition between ROS, NO, and H_2S for binding targets in proteins. At the same time, active concentrations of these molecules may change due to their direct chemical interaction with each other.

However, in addition to direct modifications of protein molecules, ROS, NO, and H_2S are involved in complex processes of signal formation and transmission to the genetic apparatus. Modifications to individual protein molecules can have both immediate and long-term effects, which can be directed in different ways. For example, inhibition of individual enzymes by the direct NO or H_2S action can result in the formation of a signal that induces gene expression of these enzymes. The most obvious example would be the induction of antioxidant enzymes gene expression involving ROS accumulated when these enzymes are inhibited by nitric oxide or hydrogen sulfide. The possibility of post-translational modifications by nitric oxide and hydrogen sulfide of key antioxidant enzymes – ascorbate peroxidase, catalase, SOD, and some others – has now been studied in detail (Begara-Morales et al., 2014; Arora and Bhatla, 2015; Correa-Aragunde et al., 2015; Corpas et al., 2019b). These modifications can either increase or decrease activity of these enzymes, which in turn leads to changes in cellular redox homeostasis and formation of signals involving ROS. NO and H_2S are also known to modulate key ROS-generating enzymes, NADPH oxidase and certain forms of peroxidases (Clark et al., 2000; Arora et al., 2016; Shen et al., 2020). Thus, the above examples in themselves demonstrate the close and complex interaction of gasotransmitters with ROS. In turn, ROS and gasotransmitters realize a significant part of their signaling potential with calcium as a universal secondary messenger. As noted, direct and indirect effects of ROS, NO, and H_2S on the calcium channel status and cytosolic calcium content are possible.

Signals involving ROS, NO, H_2S, and Ca^{2+} are critical for plant adaptation to extreme temperatures. As already noted, they affect the state and expression of genes of transcription factors important for adaptation to high and low temperatures. NO, H_2S, and ROS are involved in the activation of gene expression of antioxidant enzymes, enzymes of synthesis of proline, soluble carbohydrates, phenolic compounds, polyamines, and many other low-molecular-weight compounds that exhibit membrane-protective, chaperone, and antioxidant effects in plant cells. Although these effects have been studied for quite a long time, and are sometimes perceived as obvious, we are still far from a full understanding of the mechanisms of NO and H_2S involvement in the regulation of adaptive responses. Suffice it to mention some of the paradoxical effects discovered in recent years and discussed in this review. In particular, the effect of negative nitric oxide regulation of plant adaptation to low temperatures and drought manifested in increased resistance in mutants of nitrate reductase

genes (Costa-Broseta et al., 2018; Zhou et al., 2021). Apparently, the effects of increased antioxidant enzyme activity under the influence of scavengers and inhibitors of nitric oxide synthesis seem to be of the same series (Talukdar, 2013; Karpets et al., 2015b). A possible cause of these phenomena could be the negative effect of excess NO on plant cells (nitrosative stress). On the other hand, increased resistance in mutants defective in NO synthesis or increased plant resistance to stressors in the presence of NO antagonists may be due to the triggering of alternative signaling mechanisms which ensure successful activation of defense responses but are not seen in the presence of 'basic' NO signaling.

While the NO and ROS involvement in the formation of plant stress-protective reactions is complicated by the possibility of nitrosative and/or oxidative stress in excess of these compounds, the activation of plant defense reactions by the hydrogen sulfide action seems more unambiguous. However, this compound is also highly toxic. Suffice it to mention the well-known severe inhibition of heme-containing enzymes by hydrogen sulfide. In general, the effects of hydrogen sulfide in plant cells are much less studied compared to NO. It is worth mentioning the very contradictory information on the H_2S effect on state of stomata. It seems that the influence of hydrogen sulfide on physiological processes depends very much on the different types of its interaction with reactive nitrogen species (direct interaction, competition for protein targets, influence on each other's synthesis, and involvement in hormonal signaling). Many aspects of this interaction remain unexplored. Meanwhile, works showing a synergistic stress-protective effect on plants when they are treated simultaneously with exogenous NO and H_2S indicate additional possibilities for the practical use of gasotransmitter donors in crop production. Another understudied application may be the use of endogenous gasotransmitter assays to test the resistance of breeding material. So far, such studies have been sporadic, focusing only on nitric oxide (Zhang et al., 2019). All in all, there is no doubt that the gasotransmitters NO and H_2S have great potential for developing new techniques to practically increase the resistance of cultivated plants to temperature and other environmental stresses.

Author contributions

YK: Writing original draft. AY: review and editing. TY: review and editing, YB: Review, editing and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Aghdam, M. S., Mahmoudi, R., Razavi, F., Rabiei, V., and Soleimani, A. (2018). Hydrogen sulfide treatment confers chilling tolerance in hawthorn fruit during cold storage by triggering endogenous H_2S accumulation, enhancing antioxidant enzymes activity and promoting phenols accumulation. *Sci. Horticult.* 238, 264–271. doi: 10.1016/j.scienta.2018.04.063

Alamri, S. A., Siddiqui, M. H., Al-Khaishany, M. Y., Khan, M. N., Ali, H. M., and Alakeel, K. A. (2019). Nitric oxide-mediated cross-talk of proline and heat shock proteins induce thermotolerance in *Vicia faba* l. *Environ. Exp. Bot.* 161, 290–302. doi: 10.1016/j.envexpbot.2018.06.012

Ali, S., Rizwan, M., Arif, M. S., Rehan, A., Hasanuzzaman, M., Ali, B., et al. (2020). Approaches in enhancing thermotolerance in plants: An updated review. *J. Plant Growth Regul.* 39, 456–480. doi: 10.1007/s00344-019-09994-x

Álvarez, C., Calo, L., Romero, L. C., García, I., and Gotor, C. (2010). An O-acetylserine (thiol)lyase homolog with l-cysteine desulfhydrase activity regulates cysteine homeostasis in arabidopsis. *Plant Physiol.* 152, 656–669. doi: 10.1104/pp.109.147975

Aroca, A., Benito, J. M., Gotor, C., and Romero, L. C. (2017). Persulfidation proteome reveals the regulation of protein function by hydrogen sulfide in diverse biological processes in arabidopsis. *J. Exp. Bot.* 68 (17), 4915–4927. doi: 10.1093/jxb/erx294

Aroca, A., Gotor, C., Bassham, D. C., and Romero, L. C. (2020). Hydrogen sulfide: From a toxic molecule to a key molecule of cell life. *Antioxid. (Basel)* 9 (7), 621. doi: 10.3390/antiox907062

Aroca, A., Gotor, C., and Romero, L. C. (2018). Hydrogen sulfide signaling in plants: Emerging roles of protein persulfidation. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.01369

Aroca, A., Serna, A., Gotor, C., and Romero, L. C. (2015). S-sulfhydration: A cysteine posttranslational modification in plant systems. *Plant Physiol.* 168, 334–342. doi: 10.1104/pp.15.00009

Aroca, A., Zhang, J., Xie, Y., Romero, L. C., and Gotor, C. (2021). Hydrogen sulfide signaling in plant adaptations to adverse conditions: Molecular mechanisms. *J. Exp. Bot.* 72 (16), 5893–5904. doi: 10.1093/jxb/erab239

Arora, D., and Bhatla, S. C. (2015). Nitric oxide triggers a concentration-dependent differential modulation of superoxide dismutase (FeSOD and Cu/ZnSOD) activity in sunflower seedling roots and cotyledons as an early and long. *Plant Signal. Behav.* 10 (10), e1071753. doi: 10.1080/15592324.2015.1071753

Arora, D., Jain, P., Singh, N., Kaur, H., and Bhatla, S. C. (2016). Mechanisms of nitric oxide crosstalk with reactive oxygen species scavenging enzymes during abiotic stress tolerance in plants. *Free Radical Res.* 50 (3), 291–303. doi: 10.3109/10715762.2015.1118473

Asada, K. (1999). The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 601–639. doi: 10.1146/annurev.arplant.50.1.601

Astier, J., Gross, I., and Durner, J. (2018). Nitric oxide production in plants: an update. journal of experimental botany. *J. Exp. Bot.* 69 (14), 3401–3411. doi: 10.1093/jxb/erx420

Astier, J., Kulik, A., Koen, E., Besson-Bard, A., Bourque, S., Jeandroz, S., et al. (2012). Protein s-nitrosylation: what's going on in plants? *Free Radic. Biol. Med.* 53 (5), 1101–1110. doi: 10.1016/j.freeradbiomed.2012.06.032

Bailey, T. S., Henthorn, H. A., and Pluth, M. D. (2016). The intersection of NO and H_2S: Persulfides generate NO from nitrite through polysulfide formation. *Inorg. Chem.* 55 (24), 12618–12625. doi: 10.1021/acs.inorgchem.6b01660

Bai, X., Yang, L., Tian, M., Chen, J., Shi, J., Yang, Y., et al. (2011). Nitric oxide enhances desiccation tolerance of recalcitrant *Antiaris toxicaria* seeds *via* protein s-nitrosylation and carbonylation. *PloS One* 6 (6), e20714. doi: 10.1371/journal.pone.0020714

Baudouin, E., and Jeandroz, S. (2015). "Nitric oxide as a mediator of cold stress response: a transcriptional point of view," in *Nitric oxide action in abiotic stress responses in plants* (Switzerland: Springer), 129–139. doi: 10.1007/978-3-319-17804-2_8

Baxter, A., Mittler, R., and Suzuki, N. (2014). ROS as key players in plant stress signalling. *J. Exp. Bot.* 65 (5), 1229–1240. doi: 10.1093/jxb/ert375

Begara-Morales, J. C., Chaki, M., Sánchez-Calvo, B., Mata-Pérez, C., Leterrier, M., Palma, J. M., et al. (2013). Protein tyrosine nitration in pea roots during development and senescence. J. *Exp. Bot.* 64 (4), 1121–1134. doi: 10.1093/jxb/ert006

Begara-Morales, J. C., Sánchez-Calvo, B., Chaki, M., Valderrama, R., Mata-Pérez, C., López-Jaramillo, J., et al. (2014). Dual regulation of cytosolic ascorbate peroxidase (APX) by tyrosine nitration and s-nitrosylation. *J. Exp. Bot.* 65 (2), 527–538. doi: 10.1093/jxb/ert396

Begara-Morales, J. C., Sánchez-Calvo, B., Gómez-Rodríguez, M. V., Chaki, M., Valderrama, R., Mata-Pérez, C., et al. (2019). Short-term low temperature induces nitro-oxidative stress that deregulates NADP-malic enzyme function by tyrosine nitration in *Arabidopsis thaliana*. *Antioxid. (Basel)* 8 (10), 448. doi: 10.3390/antiox8100448

Bernfur, K., Rutsdottir, G., and Emanuelsson, C. (2017). The chloroplast-localized small heat shock protein Hsp21 associates with the thylakoid membranes in heat-stressed plants. *Protein Sci.* 26, 1773–1784. doi: 10.1002/pro.3213

Birke, H., Haas, F. H., Kok, L. J., Balk, J., Wirtz, M., and Hell, R. (2012). Cysteine biosynthesis, in concert with a novel mechanism, contributes to sulfide detoxification in mitochondria of *Arabidopsis thaliana*. *Biochem. J.* 445 (2), 275–283. doi: 10.1042/BJ20120038

Birke, H., Kok, L. J., Wirtz, M., and Hell, R. (2015). The role of compartment-specific cysteine synthesis for sulfur homeostasis during H_2S exposure in arabidopsis. *Plant Cell Physiol.* 56 (2), 358–367. doi: 10.1093/pcp/pcu166

Blume, Y. B., Krasylenko, Y. A., Demchuk, O. M., and Yemets, A. I. (2013). Tubulin tyrosine nitration regulates microtubule organization in plant cells. *Front. Plant Sci.* 4. doi: 10.3389/fpls.2013.00530

Cantrel, C., Vazquez, T., Puyaubert, J., Rezé, N., Lesch, M., Kaiser, W. M., et al. (2011). Nitric oxide participates in cold-responsive phosphosphingolipid formation and gene expression in *Arabidopsis thaliana*. *New Phytol.* 189, 415–427. doi: 10.1111/j.1469-8137.2010.03500.x

Carballal, S., Trujillo, M., Cuevasanta, E., Bartesaghi, S., Möller, M. N., Folkes, L. K., et al. (2011). Reactivity of hydrogen sulfide with peroxynitrite and other oxidants of biological interest. *Free Radic. Biol. Med.* 50 (1), 196–205. doi: 10.1016/j.freeradbiomed.2010.10.705

Chaki, M., Fernández-Ocaña, A. M., Valderrama, R., Carreras, A., Esteban, F. J., Luque, F., et al. (2009). Involvement of reactive nitrogen and oxygen species (RNS and ROS) in sunflower-mildew interaction. *Plant Cell Physiol.* 50 (2), 265–279. doi: 10.1093/pcp/pcn196

Chaki, M., Valderrama, R., Fernández-Ocaña, A. M., Carreras, A., Gómez-Rodríguez, M. V., Pedrajas, J. R., et al. (2011). Mechanical wounding induces a nitrosative stress by down-regulation of GSNO reductase and an increase in s-nitrosothiols in sunflower (*Helianthus annuus*) seedlings. *J. Exp. Bot.* 62 (6), 1803–1813. doi: 10.1093/jxb/erq358

Chamizo-Ampudia, A., Sanz-Luque, E., Llamas, A., Galvan, A., and Fernandez, E. (2017). Nitrate reductase regulates plant nitric oxide homeostasis. *Trends Plant Sci.* 22 (2), 163–174. doi: 10.1016/j.tplants.2016.12.001

Chen, X., Chen, Q., Zhang, X., Li, R., Jia, Y., Ef, A. A., et al. (2016). Hydrogen sulfide mediates nicotine biosynthesis in tobacco (*Nicotiana tabacum*) under high temperature conditions. *Plant Physiol. Biochem.* 104, 174–179. doi: 10.1016/j.plaphy.2016.02.033

Chen, X., Ding, Y., Yang, Y., Song, C., Wang, B., Yang, S., et al. (2021). Protein kinases in plant responses to drought, salt, and cold stress. *J. Integr. Plant Biol.* 63 (1), 53–78. doi: 10.1111/jipb.13061

Cheng, T., Shi, J., Dong, Y., Ma, Y., Peng, Y., Hu, X., et al. (2018). Hydrogen sulfide enhances poplar tolerance to high-temperature stress by increasing s-nitrosoglutathione reductase (GSNOR) activity and reducing reactive oxygen/nitrogen damage. *Plant Growth Regul.* 84, 11–23. doi: 10.1007/s10725-017-0316-x

Chervenkov, H., and Slavov, K. (2022). Inter-annual variability and trends of the frost-free season characteristics over central and southeast Europe in 1950-2019. *J. Cent. Eur. Agricult.* 23 (1), 154–164. doi: 10.5513/JCEA01/23.1.3394

Choudhury, F. K., Rivero, R. M., Blumwald, E., and Mittler, R. (2017). Reactive oxygen species, abiotic stress and stress combination. *Plant J.* 90, 856–867. doi: 10.1111/tpj.13299

Christou, A., Filippou, P., Manganaris, G., and Fotopoulos, V. (2014). Sodium hydrosulfide induces systemic thermotolerance to strawberry plants through transcriptional regulation of heat shock proteins and aquaporin. *BMC Plant Biol.* 14, 42. doi: 10.1186/1471-2229-14-42

Clark, D., Durner, J., Navarre, D. A., and Klessig, D. F. (2000). Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. *Mol. Plant Microbe Interact.* 13 (12), 1380–1384. doi: 10.1094/MPMI.2000.13.12.1380

Corpas, F. J., and Barroso, J. B. (2013). Nitro-oxidative stress vs oxidative or nitrosative stress in higher plants. *New Phytol.* 199, 633–635. doi: 10.1111/nph.12380

Corpas, F. J., and Barroso, J. B. (2014). Peroxisomal plant nitric oxide synthase (NOS) protein is imported by peroxisomal targeting signal type 2 (PTS2) in a process that depends on the cytosolic receptor PEX7 and calmodulin. *FEBS Lett.* 588 (12), 2049–2054. doi: 10.1016/j.febslet.2014.04.034

Corpas, F. J., Barroso, J. B., González-Gordo, S., Muñoz-Vargas, M. A., and Palma, J. M. (2019a). Hydrogen sulfide: A novel component in arabidopsis peroxisomes which triggers catalase inhibition. *J. Integr. Plant Biol.* 61 (7), 871–883. doi: 10.1111/jipb.12779

Corpas, F. J., González-Gordo, S., Canas, A., and Palma, J. M. (2019b). Nitric oxide (NO) and hydrogen sulfide (H_2S) in plants: Which is first? *J. Exp. Bot.* 70 (17), 4391–4404. doi: 10.1093/jxb/erz031

Corpas, F. J., González-Gordo, S., and Palma, J. M. (2021). Nitric oxide and hydrogen sulfide modulate the NADPH-generating enzymatic system in higher plants. *J. Exp. Bot.* 72 (3), 830–847. doi: 10.1093/jxb/eraa440

Corpas, F. J., González-Gordo, S., Rodríguez-Ruiz, M., Muñoz-Vargas, M. A., and Palma, J. M. (2022). Thiol-based oxidative posttranslational modifications (OxiPTMs) of plant proteins. *Plant Cell Physiol.* 63 (7), 889–900. doi: 10.1093/pcp/pcac036

Corpas, F. J., Palma, J. M., Sandalio, L. M., Valderrama, R., Barroso, J. B., and del Rio, L. A. (2008). Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisum sativum* l.) leaves. *J. Plant Physiol.* 165 (13), 1319–1330. doi: 10.1016/j.jplph.2008.04.004

Correa-Aragunde, N., Foresi, N., Delledonne, M., and Lamattina, L. (2013). Auxin induces redox regulation of ascorbate peroxidase 1 activity by s-nitrosylation/denitrosylation balance resulting in changes of root growth pattern in arabidopsis. *J. Exp. Bot.* 64 (11), 3339–3349. doi: 10.1093/jxb/ert172

Correa-Aragunde, N., Foresi, N., and Lamattina, L. (2015). Nitric oxide is a ubiquitous signal for maintaining redox balance in plant cells: Regulation of ascorbate peroxidase as a case study. *J. Exp. Bot.* 66 (10), 2913–2921. doi: 10.1093/jxb/erv073

Costa-Broseta, Á., Perea-Resa, C., Castillo, M. C., Ruíz, M. F., Salinas, J., and León, J. (2018). Nitric oxide controls constitutive freezing tolerance in arabidopsis by attenuating the levels of osmoprotectants, stress-related hormones and anthocyanins. *Sci. Rep.* 8 (1), 9268. doi: 10.1038/s41598-018-27668-8

Cuevasanta, E., Lange, M., Bonanata, J., Coitiño, E. L., Ferrer-Sueta, G., Filipovic, M. R., et al. (2015). Reaction of hydrogen sulfide with disulfide and sulfenic acid to form the strongly nucleophilic persulfide. *J. Biol. Chem.* 290, 26866–26880. doi: 10.1074/jbc.M115.672816

de Pinto, M. C., Locato, V., Sgobba, A., Romero-Puertas, M. C., Gadaleta, C., Delledonne, M., et al. (2013). S-nitrosylation of ascorbate peroxidase is part of programmed cell death signaling in tobacco bright yellow-2 cells. *Plant Physiol.* 163 (4), 1766–1775. doi: 10.1104/pp.113.222703

Dietz, K. J. (2014). Redox regulation of transcription factors in plant stress acclimation and development. *Antioxid. Redox Signal.* 21 (9), 1356–1372. doi: 10.1089/ars.2013.5672

Dubovskaya, L. V., Bakakina, Y. S., Kolesneva, E. V., Sodel, D. L., McAinsh, M. R., Hetherington, A. M., et al. (2011). cGMP-dependent ABA-induced stomatal closure in the ABA-insensitive arabidopsis mutant *abi1-1*. *New Phytol.* 191 (1), 57–69. doi: 10.1111/j.1469-8137.2011.03661.x

Du, X., Jin, Z., Liu, D., Yang, G., and Pei, Y. (2017). Hydrogen sulfide alleviates the cold stress through MPK4 in *Arabidopsis thaliana*. *Plant Physiol. Biochem.* 120, 112–119. doi: 10.1016/j.plaphy.2017.09.028

El-Beltagi, H. S., Ahmed, O. K., and Hegazy, A. E. (2016). Protective effect of nitric oxide on high temperature induced oxidative stress in wheat (*Triticum aestivum*) callus culture. *Not. Sci. Biol.* 8 (2), 192–198. doi: 10.15835/nsb.8.2.9807

Fan, J., Chen, K., Amombo, E., Hu, Z., Chen, L., and Fu, J. (2015). Physiological and molecular mechanism of nitric oxide (NO) involved in bermudagrass response to cold stress. *PloS One* 10 (7), e0132991. doi: 10.1371/journal.pone.0132991

Fancy, N. N., Bahlmann, A. K., and Loake, G. J. (2017). Nitric oxide function in plant abiotic stress. *Plant Cell Environ.* 40 (4), 462–472. doi: 10.1111/pce.12707

Farnese, F. S., Menezes-Silva, P. E., Gusman, G. S., and Oliveira, J. A. (2016). When bad guys become good ones: The key role of reactive oxygen species and nitric oxide in the plant responses to abiotic stress. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.00471

Filipovic, M. R., Miljkovic, J. L., Nauser, T., Royzen, M., Klos, K., Shubina, T., et al. (2012). Chemical characterization of the smallest s-nitrosothiol, HSNO; cellular cross-talk of H_2S and s-nitrosothiols. *J. Amer. Chem. Soc* 134, 12016–12027. doi: 10.1021/ja3009693

Filipovic, M. R., Zivanovic, J., Alvarez, B., and Banerjee, R. (2018). Chemical biology of H_2S signaling through persulfidation. *Chem. Rev.* 118 (3), 1253–1337. doi: 10.1021/acs.chemrev.7b00205

Finka, A., and Goloubinoff, P. (2014). The CNGCb and CNGCd genes from *Physcomitrella patens* moss encode for thermosensory calcium channels responding to fluidity changes in the plasma membrane. *Cell Stress Chaperones.* 19 (1), 83–90. doi: 10.1007/s12192-013-0436-9

Friso, G., and van Wijk, K. J. (2015). Update: Post-translational protein modifications in plant metabolism. *Plant Physiol.* 169 (3), 1469–1487. doi: 10.1104/pp.15.01378

Fu, P. N., Wang, W. J., Hou, L. X., and Liu, X. (2013). Hydrogen sulfide is involved in the chilling stress response in *Vitis vinifera* l. *Acta Societatis Botanicorum Poloniae.* 82 (4), 295–302. doi: 10.5586/asbp.2013.031

Gao, F., Han, X., Wu, J., Zheng, S., Shang, Z., Sun, D., et al. (2012). A heat activated calcium permeable channel – arabidopsis cyclic nucleotide gated ion channel6 – is

involved in heat shock responses. *Plant J.* 70 (6), 1056–1069. doi: 10.1111/j.1365-313X.2012.04969.x

Gao, H., Jia, Y., Guo, S., Lv, G., Wang, T., and Juan, L. (2011). Exogenous calcium affects nitrogen metabolism in root-zone hypoxia-stressed muskmelon roots and enhances short-term hypoxia tolerance. *J. Plant Physiol.* 168 (11), 1217–1225. doi: 10.1016/j.jplph.2011.01.022

Gautam, H., Fatma, M., Sehar, Z., Mir, I. R., and Khan, N. A. (2022). Hydrogen sulfide, ethylene, and nitric oxide regulate redox homeostasis and protect photosynthetic metabolism under high temperature stress in rice plants. *Antioxidants* 11, 1478. doi: 10.3390/antiox11081478

Gautam, V., Kaur, R., Kohli, S. K., Verma, V., Kaur, P., Singh, R., et al. (2017). "ROS compartmentalization in plant cells under abiotic stress condition," in *Reactive oxygen species and antioxidant systems in plants: Role and regulation under abiotic stress* (Singapore: Springer), 89–114. doi: 10.1007/978-981-10-5254-5_4

Gautam, H., Sehar, Z., Rehman, M. T., Hussain, A., AlAjmi, M. F., and Khan, N. A. (2021). Nitric oxide enhances photosynthetic nitrogen and sulfur-use efficiency and activity of ascorbate-glutathione cycle to reduce high temperature stress-induced oxidative stress in rice (*Oryza sativa* l.) plants. *Biomolecules* 11, 305. doi: 10.3390/biom11020305

Gould, K. S., Lamotte, O., Klinguer, A., Pugin, A., and Wendehenne, D. (2003). Nitric oxide production in tobacco leaf cells: A generalized stress response? *Plant Cell Environ.* 26, 1851–1862. doi: 10.1046/j.1365-3040.2003.01101.x

Gross, I., and Durner, J. (2016). In search of enzymes with a role in 3', 5'-cyclic guanosine monophosphate metabolism in plants. *Front. Plant Sci.* 7, 576. doi: 10.3389/fpls.2016.00576

Grossi, L., and Casadei, R. (2021). The crucial role of non-enzymatic NO-production in plants. an EPR study. *Phytochem.* 188, 112794. doi: 10.1016/j.phytochem.2021.112794

Guo, Z., Liang, Y., Yan, J., Yang, E., Li, K., and Xu, H. (2018). Physiological response and transcription profiling analysis reveals the role of H_2S in alleviating excess nitrate stress tolerance in tomato roots. *Plant Physiol. Biochem.* 124, 59–69. doi: 10.1016/j.plaphy.2018.01.006

Guo, H., Xiao, T., Zhou, H., Xie, Y., and Shen, W. (2016). Hydrogen sulfide: a versatile regulator of environmental stress in plants. *Acta Physiol. Plant* 38, 16. doi: 10.1007/s11738-015-2038-x

Gupta, K. J., Fernie, A. R., Kaiser, W. M., and van Dongen, J. T. (2011). On the origins of nitric oxide. *Trends Plant Sci.* 16 (3), 160–168. doi: 10.1016/j.tplants.2010.11.007

Gupta, R., and Luan, S. (2003). Redox control of protein tyrosine phosphatases and mitogen-activated protein kinases in plants. *Plant Physiol.* 132 (3), 1149–1152. doi: 10.1104/pp.103.020792

Habibi, G. (2014). "Hydrogen peroxide (H2O2) generation, scavenging and signaling in plants," in *Oxidative damage to plants: Antioxidant networks and signaling* (Amsterdam: Academic Press), 557–584. doi: 10.1016/B978-0-12-799963-0.00019-8

Haider, S., Iqbal, J., Naseer, S., Yaseen, T., Shaukat, M., Bibi, H., et al. (2021). Molecular mechanisms of plant tolerance to heat stress: Current landscape and future perspectives. *Plant Cell Rep.* 40 (12), 2247–2271. doi: 10.1007/s00299-021-02696-3

Hancock, J. T. (2019). Hydrogen sulfide and environmental stresses. *Environ. Exp. Bot.* 161, 50–56. doi: 10.1016/j.envexpbot.2018.08.034

Hancock, J. T., and Neill, S. J. (2019). Nitric oxide: Its generation and interactions with other reactive signaling compounds. *Plants (Basel)* 8 (2), 41. doi: 10.3390/plants8020041

Hancock, J. T., and Veal, D. (2021). Nitric oxide, other reactive signalling compounds, redox, and reductive stress. *J. Exp. Bot.* 72 (3), 819–829. doi: 10.1093/jxb/eraa331

Hancock, J. T., and Whiteman, M. (2014). Hydrogen sulfide and cell signaling: Team player or referee? *Plant Physiol. Biochem.* 78, 37–42. doi: 10.1016/j.plaphy.2014.02.012

Han, Y., Fan, S., Zhang, Q., and Wang, Y. (2013). Effect of heat stress on the MDA, proline and soluble sugar content in leaf lettuce seedlings. *Agricult. Sci.* 4 (5B), 112–115. doi: 10.4236/as.2013.45B021

Havva, E. N., Yu., E., Shkliarevskyi, M., Kokorev, A. I., and Dmitriev, A. P. (2022). Hydrogen sulfide participation in the formation of wheat seedlings' heat resistance under the action of hardening temperature. *Cytol. Genet.* 56 (3), 218–225. doi: 10.3103/S0095452722030045

Honda, K., Yamada, N., Yoshida, R., Ihara, H., Sawa, T., Akaike, T., et al. (2015). 8-Mercapto-Cyclic GMP mediates hydrogen sulfide-induced stomatal closure in arabidopsis. *Plant Cell Physiol.* 56 (8), 1481–1489. doi: 10.1093/pcp/pcv069

Huang, J., Willems, P., Wei, B., Tian, C., Ferreira, R. B., Bodra, N., et al. (2019). Mining for protein s-sulfenylation in arabidopsis uncovers redox-sensitive sites. *Proc. Natl. Acad. Sci. U.S.A.* 116 (42), 21256–21261. doi: 10.1073/pnas.1906768116

Hu, J., Huang, X., Chen, L., Sun, X., Lu, C., Zhang, L., et al. (2015). Site-specific nitrosoproteomic identification of endogenously s-nitrosylated proteins in arabidopsis. *Plant Physiol.* 167 (4), 1731–1746. doi: 10.1104/pp.15.00026

Hu, K. D., Tang, J., Zhao, D. L., Hu, L. Y., Li, Y. H., Liu, Y. S., et al. (2014). Stomatal closure in sweet potato leaves induced by sulfur dioxide involves H_2S and NO signaling pathways. *Biol. Plant* 58 (4), 676–680. doi: 10.1007/s10535-014-0440-7

Iqbal, N., Sehar, Z., Fatma, M., Umar, S., Sofo, A., and Khan, N. A. (2022). Nitric oxide and abscisic acid mediate heat stress tolerance through regulation of osmolytes and antioxidants to protect photosynthesis and growth in wheat plants. *Antioxid.* 11, 372. doi: 10.3390/antiox11020372

Iqbal, N., Umar, S., Khan, N. A., and Corpas, F. J. (2021). Nitric oxide and hydrogen sulfide coordinately reduce glucose sensitivity and decrease oxidative stress *via* ascorbate-glutathione cycle in heat-stressed wheat (*Triticum aestivum* l.) plants. *Antioxidants* 10, 108. doi: 10.3390/antiox10010108

Jeandroz, S., Wipf, D., Stuehr, D. J., Lamattina, L., Melkonian, M., Tian, Z., et al. (2016). Occurrence, structure, and evolution of nitric oxide synthase-like proteins in the plant kingdom. *Sci. Signal.* 9, re2. doi: 10.1126/scisignal.aad4403

Jian, L. C., Li, J. H., Chen, W. P., Li, P. H., and Ahlstrand, G. G. (1999). Cytochemical localization of calcium and Ca^{2+}-ATPase activity in plant cells under chilling stress: a comparative study between the chillingsensitive maize and the chilling-insensitive winter wheat. *Plant Cell Physiol.* 40 (10), 1061–1071. doi: 10.1093/oxfordjournals.pcp.a029488

Ju, A., and Ma, H. (2011). Evaluation of seedling proline content of wheat genotypes in relation to heat tolerance. *Bangladesh J. Bot.* 40 (1), 17–22. doi: 10.3329/bjb.v40i1.7991

Karimova, F. G., and Petrova, N. V. (2007). Effect of H_2O_2 on tyrosine phosphorylation of pea proteins. *Russ. J. Plant Physiol.* 54 (3), 322–328. doi: 10.1134/S1021443707030053

Karpets, Y. V. (2017). Role of calcium ions and reactive oxygen species in induction of antioxidant enzymes and heat resistance of plant cells by nitric oxide donor. *Visn. Hark. nac. agrar. univ. Ser. Biol.* 3 (42), 52–61. doi: 10.35550/vbio2017.03.052

Karpets, Y. V., Kolupaev, Y. E., Lugovaya, A. A., Shvidenko, N. V., Shkliarevskyi, M. A., and Yastreb, T. O. (2020). Functional interaction of ROS and nitric oxide during induction of heat resistance of wheat seedlings by hydrogen sulfide donor. *Russ. J. Plant Physiol.* 67 (4), 653–660. doi: 10.1134/S1021443720030140

Karpets, Y. V., Kolupaev, Y. E., and Vayner, A. A. (2015a). Functional interaction between nitric oxide and hydrogen peroxide during formation of wheat seedling induced heat resistance. *Russ. J. Plant Physiol.* 62 (1), 65–70. doi: 10.1134/S1021443714060090

Karpets, Y. V., Kolupaev, Y. E., and Yastreb, T. O. (2011). Effect of sodium nitroprusside on heat resistance of wheat coleoptiles: dependence on the formation and scavenging of reactive oxygen species. *Russ. J. Plant Physiol.* 58 (6), 1027–1034. doi: 10.1134/S1021443711060094

Karpets, Y. V., Kolupaev, Y. E., Yastreb, T. O., and Oboznyi, A. I. (2015b). Effects of NO-status modification, heat hardening, and hydrogen peroxide on the activity of antioxidant enzymes in wheat seedlings. *Russ. J. Plant Physiol.* 62 (3), 292–298. doi: 10.1134/S1021443715030097

Karpets, Y. V., Kolupaev, Y. E., Yastreb, T. O., and Oboznyi, A. I. (2016). Induction of heat resistance in wheat seedlings by exogenous calcium, hydrogen peroxide, and nitric oxide donor: functional interaction of signal mediators. *Russ J. Plant Physiol.* 63 (4), 490–498. doi: 10.1134/S1021443716040075

Kazemi-Shahandashti, S.-S., and Maali-Amiri, R. (2018). Global insights of protein responses to cold stress in plants: Signaling, defence, and degradation. *J. Plant Physiol.* 226, 123–135. doi: 10.1016/j.jplph.2018.03.022

Kolupaev, Y. E., Firsova, E. N., Yastreb, T. O., and Lugovaya, A. A. (2017). The participation of calcium ions and reactive oxygen species in the induction of antioxidant enzymes and heat resistance in plant cells by hydrogen sulfide donor. *Appl. Biochem. Microbiol.* 53 (5), 573–579. doi: 10.1134/S0003683817050088

Kolupaev, Y. E., Horielova, E. I., Yastreb, T. O., Popov, Y., and Ryabchun, N. I. (2018). Phenylalanine ammonia-lyase activity and content of flavonoid compounds in wheat seedlings at the action of hypothermia and hydrogen sulfide donor. *Ukr. Biochem. J.* 90 (6), 12–20. doi: 10.15407/ubj90.06.012

Kolupaev, Y. E., Horielova, E. I., Yastreb, T. O., Ryabchun, N. I., and Kirichenko, V. V. (2019a). Stress-protective responses of wheat and rye seedlings whose chilling resistance was induced with a donor of hydrogen sulfide. *Russ. J. Plant Physiol.* 66 (4), 540–547. doi: 10.1134/S1021443719040058

Kolupaev, Y. E., Horielova, E. I., Yastreb, T. O., Ryabchun, N. I., and Reznik, A. M. (2020). Nitrogen oxide donor enhances cold-induced changes in antioxidant and osmoprotective systems of cereals. *Appl. Biochem. Microbiol.* 56 (2), 219–225. doi: 10.1134/S000368382002009X

Kolupaev, Y. E., Karpets, Y. V., Beschasniy, S. P., and Dmitriev, A. P. (2019b). Gasotransmitters and their role in adaptive reactions of plant cells. *Cytol. Genet.* 53 (5), 392–406. doi: 10.3103/S0095452719050098

Kolupaev, Y. E., Oboznyi, A. I., and Shvidenko, N. V. (2013). Role of hydrogen peroxide in generation of a signal inducing heat tolerance of wheat seedlings. *Russ. J. Plant Physiol.* 60 (2), 227–234. doi: 10.1134/S102144371302012X

Kolupaev, Y. E., Karpets, Y. V., Shkliarevskyi, M. A., Yastreb, T. O., Plohovska, S. H., Yemets, A. I., et al. (2022). Gasotransmitters in plants: Mechanisms of participation in adaptive responses. *Open Agricult. J.* 16 (Suppl-1, M5), e187433152207050. doi: 10.2174/18743315-v16-e2207050

Kumar, D., and Ohri, P. (2023). Say "NO" to plant stresses: Unravelling the role of nitric oxide under abiotic and biotic stress. *Nitric. Oxide* 130, 36–57. doi: 10.1016/j.niox.2022.11.004

Laxalt, A. M., Raho, N., Ten Have, A., and Lamattina, L. (2007). Nitric oxide is critical for inducing phosphatidic acid accumulation in xylanase-elicited tomato cells. *J. Biol. Chem.* 282 (29), 21160–21168. doi: 10.1074/jbc.M701212200

Lee, U., Wie, C., Fernandez, B. O., Feelisch, M., and Vierling, E. (2008). Modulation of nitrosative stress by s-nitrosoglutathione reductase is critical for thermotolerance and plant growth in arabidopsis. *Plant Cell.* 20 (3), 786–802. doi: 10.1105/tpc.107.052647

Li, Z. G. (2013). Hydrogen sulfide: A multifunctional gaseous molecule in plants. *Russ. J. Plant Physiol.* 60 (6), 733–740. doi: 10.1134/S1021443713060058

Li, Z. G. (2015). Analysis of some enzymes activities of hydrogen sulfide metabolism in plants. *Methods Enzymol.* 555, 253–269. doi: 10.1016/bs.mie.2014.11.035

Li, Z.-G. (2020). "Hydrogen sulfide: A novel signaling molecule in plant cross-stress tolerance," in *Priming-mediated stress and cross-stress tolerance in crop plants* (Elsevier Inc), 215–229. doi: 10.1016/B978-0-12-817892-8.00014-3

Li, Z. G., Ding, X. J., and Du, P. F. (2013a). Hydrogen sulfide donor sodium hydrosulfide-improved heat tolerance in maize and involvement of proline. *J. Plant Physiol.* 170 (8), 741–747. doi: 10.1016/j.jplph.2012.12.018

Li, B., Gao, K., Ren, H., and Tang, W. (2018). Molecular mechanisms governing plant responses to high temperatures. *J. Integr. Plant Biol.* 60 (9), 757–779. doi: 10.1111/jipb.12701

Li, Z. G., Gong, M., Xie, H., Yang, L., and Li, J. (2012). Hydrogen sulfide donor sodium hydrosulfideinduced heat tolerance in tobacco (*Nicotiana tabacum* l) suspension cultured cells and involvement of Ca^{2+} and calmodulin. *Plant Sci.* 185/186, 185–189. doi: 10.1016/j.plantsci.2011.10.006

Li, Z. G., Long, W. B., Yang, S. Z., Wang, Y. C., Tang, J. H., Wen, L., et al. (2015). Endogenous hydrogen sulfide regulated by calcium is involved in thermotolerance in tobacco *Nicotiana tabacum* l. suspension cell cultures. *Acta Physiol. Plant* 37, 219. doi: 10.1007/s11738-015-1971-z

Lisjak, M., Srivastava, N., Teklic, T., Civale, L., Lewandowski, K., Wilson, I., et al. (2010). A novel hydrogen sulfide donor causes stomatal opening and reduces nitric oxide accumulation. *Plant Physiol. Biochem.* 48 (12), 931–935. doi: 10.1016/j.plaphy.2010.09.016

Liu, Q., Ding, Y., Shi, Y., Ma, L., Wang, Y., Song, C., et al. (2021). The calcium transporter ANNEXIN1 mediates cold-induced calcium signaling and freezing tolerance in plants. *EMBO J.* 40 (2), e104559. doi: 10.15252/embj.2020104559

Liu, Z., Li, Y., Cao, C., Liang, S., Ma, Y., Liu, X., et al. (2019). The role of H_2S in low temperature-induced cucurbitacin c increases in cucumber. *Plant Mol. Biol.* 99 (6), 535–544. doi: 10.1007/s11103-019-00834-w

Liu, W., Yu, K., He, T., Li, F., Zhang, D., and Liu, J. (2013b). The low temperature induced physiological responses of *Avena nuda* l., a cold-tolerant plant species. *Sci. World J.* 2013, 658793. doi: 10.1155/2013/658793

Liu, Y., Zhang, C., Chen, J., Guo, L., Li, X., Li, W., et al. (2013a). Arabidopsis heat shock factor HsfA1a directly senses heat stress, pH changes, and hydrogen peroxide *via* the engagement of redox state. *Plant Physiol. Biochem.* 64, 92–98. doi: 10.1016/j.plaphy.2012.12.013

Li, Z. G., Yang, S. Z., Long, W. B., Yang, G. X., and Shen, Z. Z. (2013b). Hydrogen sulfide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* l.) seedlings. *Plant Cell Environ.* 36 (8), 1564–1572. doi: 10.1111/pce.12092

Li, Z. G., Yi, X. Y., and Li, Y. T. (2014). Effect of pretreatment with hydrogen sulfide donor sodium hydrosulfide on heat tolerance in relation to antioxidant system in maize (*Zea mays*) seedlings. *Biologia.* 69, 1001–1009. doi: 10.2478/s11756-014-0396-2

Lozano-Juste, J., Colom-Moreno, R., and Leon, J. (2011). *In vivo* protein tyrosine nitration in *Arabidopsis thaliana*. *J. Exp. Bot.* 62, 3501–3517. doi: 10.1093/jxb/err042

Luo, Z., Li, D., Du, R., and Mou, W. (2015). Hydrogen sulfide alleviates chilling injury of banana fruit by enhanced antioxidant system and proline content. *Sci. Horticult.* 183, 144–151. doi: 10.1016/j.scienta.2014.12.021

Lushchak, V. I. (2011). Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 153 (2), 175–190. doi: 10.1016/j.cbpc.2010.10.004

Lv, X., Li, H., Chen, X., Xiang, X., Guo, Z., Yu, J., et al. (2018). The role of calcium-dependent protein kinase in hydrogen peroxide, nitric oxide and ABA-dependent cold acclimation. *J. Exp. Bot.* 69 (16), 4127–4139. doi: 10.1093/jxb/ery212

Ma, Y., Dai, X., Xu, Y., Luo, W., Zheng, X., Zeng, D., et al. (2015b). COLD1 confers chilling tolerance in rice. *Cell.* 160 (6), 1209–1221. doi: 10.1016/j.cell.2015.01.046

Manafi, H., Baninasab, B., Gholami, M., and Talebi, M. (2021). Nitric oxide induced thermotolerance in strawberry plants by activation of antioxidant systems and transcriptional regulation of heat shock proteins. *J. Horticult. Sci. Biotechnol.* 96 (6), 1–14. doi: 10.1080/14620316.2021.1927206

Marcolongo, J. P., Morzan, U. N., Zeida, A., Scherlis, D. A., and Olabe, J. A. (2016). Nitrosodisulfide $[S_2NO]^-$ (perthionitrite) is a true intermediate during the "cross-talk" of nitrosyl and sulfide. *Phys. Chem. Chem. Phys.* 18, 30047–30052. doi: 10.1039/C6CP06314D

Marcolongo, J. P., Venâncio, M. F., Rocha, W. R., Doctorovich, F., and Olabe, J. A. (2019). NO/H_2S "crosstalk" reactions. the role of thionitrites (SNO^-) and perthionitrites ($SSNO^-$). *Inorg. Chem.* 58 (22), 14981–14997. doi: 10.1021/acs.inorgchem.9b01978

Mata-Pérez, C., Sánchez-Calvo, B., Begara-Morales, J. C., Carreras, A., Padilla, M. N., Melguizo, M., et al. (2016a). Nitro-linolenic acid is a nitric oxide donor. *Nitric. Oxide* 57, 57–63. doi: 10.1016/j.niox.2016.05.003

Mata-Pérez, C., Sánchez-Calvo, B., Begara-Morales, J. C., Padilla, M. N., Valderrama, R., and Corpas, F. J. (2016b). Nitric oxide release from nitro-fatty acids in arabidopsis roots. *Plant Signal. Behav.* 11 (3), e1154255. doi: 10.1080/15592324.2016.1154255

Ma, L., Yang, L., Zhao, J., Wei, J., Kong, X., Wang, C., et al. (2015a). Comparative proteomic analysis reveals the role of hydrogen sulfide in the adaptation of the alpine plant *Lamiophlomis rotata* to altitude gradient in the northern Tibetan plateau. *Planta.* 241, 887–906. doi: 10.1007/s00425-014-2209-9

Mishra, V., Singh, P., Tripathi, D. K., Corpas, F. J., and Singh, V. P. (2021). Nitric oxide and hydrogen sulfide: An indispensable combination for plant functioning. *Trends Plant Sci.* 26 (12), 1270–1285. doi: 10.1016/j.tplants.2021.07.016

Mittler, R., Zandalinas, S. I., Fichman, Y., and Van Breusegem, F. (2022). Reactive oxygen species signalling in plant stress responses. *Nat. Rev. Mol. Cell Biol.* 23 (10), 663–679. doi: 10.1038/s41580-022-00499-2

Mohapatra, T. (2019). "Foreword," in *Heat stress tolerance in plants physiological, molecular and genetic perspectives*, vol. xix. (Wiley).

Mohn, M. A., Thaqi, B., and Fischer-Schrader, K. (2019). Isoform-specific NO synthesis by *Arabidopsis thaliana* nitrate reductase. *Plants.* 8, 67. doi: 10.3390/plants8030067

Mori, I. C., and Schroeder, J. S. (2004). Reactive oxygen species activation of plant Ca^{2+} channels. A signaling mechanism in polar growth, hormone transduction, stress signaling, and hypothetically mechanotransduction. *Plant Physiol.* 135 (2), 702–708. doi: 10.1104/pp.104.042069

Muñoz-Vargas, M. A., González-Gordo, S., Cañas, A., López-Jaramillo, J., Palma, J. M., and Corpas, F. J. (2018). Endogenous hydrogen sulfide (H_2S) is up-regulated during sweet pepper (*Capsicum annuum* l.) fruit ripening. *In vitro* analysis shows that NADP-dependent isocitrate dehydrogenase (ICDH) activity is inhibited by H_2S and NO. *Nitric. Oxide* 81, 36–45. doi: 10.1016/j.niox.2018.10.002

Muñoz-Vargas, M. A., González-Gordo, S., Palma, J. M., and Corpas, F. J. (2020). Inhibition of NADP-malic enzyme activity by H_2S and NO in sweet pepper (*Capsicum annuum* l.) fruits. *Physiol. Plant* 168 (92), 278–288. doi: 10.1111/ppl.13000

Mur, L. A., Mandon, J., Persijn, S., Cristescu, S. M., Moshkov, I. E., Novikova, G. V., et al. (2013). Nitric oxide in plants: An assessment of the current state of knowledge. *AoB Plants.* 5, pls052. doi: 10.1093/aobpla/pls052

Ndamukong, I., Al Abdallat, A., Thurow, C., Fode, B., Zander, M., Weigel, R., et al. (2007). SA-Inducible arabidopsis glutaredoxin interacts with TGA factors and suppresses JA responsive PDF1.2 transcription. *Plant J.* 50 (1), 128–139. doi: 10.1111/j.1365-313X.2007.03039.x

Neill, S., Barros, R., Bright, J., Desikan, R., Hancock, J., Harrison, J., et al. (2008). Nitric oxide, stomatal closure and abiotic stress. *J. Exp. Bot.* 59 (2), 165–176. doi: 10.1093/jxb/erm293

Nishizawa, A., Yabuta, Y., Yoshida, E., Maruta, T., Yoshimura, K., and Shigeoka, S. (2006). Arabidopsis heat shock transcription factor A2 as a key regulator in response to several type so environ mental stress. *Plant J.* 48 (4), 535–547. doi: 10.1111/j.1365-313X.2006.02889.x

Niu, L., Yu, J., Liao, W., Xie, J., Yu, J., Lv, J., et al. (2019). Proteomic investigation of s-nitrosylated proteins during NO-induced adventitious rooting of cucumber. *Int. J. Mol. Sci.* 20 (21), 5363. doi: 10.3390/ijms20215363

Parankusam, S., Adimulam, S. S., Bhatnagar-Mathur, P., and Sharma, K. K. (2017). Nitric oxide (NO) in plant heat stress tolerance: Current knowledge and perspectives. *Front. Plant Sci.* 13 (8), 1582. doi: 10.3389/fpls.2017.01582

Paul, B. D., and Snyder, S. H. (2012). H_2S signalling through protein sulfhydration and beyond. *Nat. Rev. Mol. Cell Biol.* 13 (8), 499–507. doi: 10.1038/nrm3391

Paul, B. D., and Snyder, S. H. (2018). Gasotransmitter hydrogen sulfide signaling in neuronal health and disease. *Biochem. Pharmacol.* 149, 101–109. doi: 10.1016/j.bcp.2017.11.019

Piotrovskii, M. S., Shevyreva, T. A., Zhestkova, I. M., and Trofimova, M. S. (2011). Activation of plasmalemmal NADPH oxidase in etiolated maize seedlings exposed to chilling temperatures. *Russ. J. Plant Physiol.* 58 (2), 290–298. doi: 10.1134/S1021443711020154

Praveen, A. (2022). Nitric oxide mediated alleviation of abiotic challenges in plants. *Nitric. Oxide* 128, 37–49. doi: 10.1016/j.niox.2022.08.005

Puyaubert, J., and Baudouin, E. (2014). New clues for a cold case: nitric oxide response to low temperature. *Plant Cell Environ.* 37, 2623–2630. doi: 10.1111/PCE.12329

Puyaubert, J., Fares, A., Reze, N., Peltier, J. B., and Baudouin, E. (2014). Identification of endogenously s-nitrosylated proteins in arabidopsis plantlets: Effect of cold stress on cysteine nitrosylation level. *Plant Sci.* 215–216, 150–156. doi: 10.1016/j.plantsci.2013.10.014

Raza, A., Tabassum, J., Mubarik, M. S., Anwar, S., Zahra, N., Sharif, Y., et al. (2022). Hydrogen sulfide: an emerging component against abiotic stress in plants. *Plant Biol. J.* 24, 540–558. doi: 10.1111/plb.13368

Rey, P., Bécuwe, N., Barrault, M. B., Rumeau, D., Havaux, M., Biteau, B., et al. (2007). The *Arabidopsis thaliana* sulfiredoxin is a plastidic cysteine-sulfinic acid reductase involved in the photooxidative stress response. *Plant J.* 49, 505–514. doi: 10.1111/j.1365-313X.2006.02969.x

Rockel, P., Strube, F., Rockel, A., Wildt, J., and Kaiser, W. M. (2002). Regulation of nitric oxide (NO) production by plant nitrate reductase *in vivo* and *in vitro*. *J. Exp. Bot.* 53 (366), 103–110. doi: 10.1093/jexbot/53.366.103

Romero, L. C., García, I., and Gotor, C. (2013). L-cysteine desulfhydrase 1 modulates the generation of the signaling molecule sulfide in plant cytosol. *Plant Signal. Behav.* 8 (5), 4621–4634. doi: 10.4161/psb.24007

Roszer, T. (2014). "Biosynthesis of nitric oxide in plants," in *Nitric oxide in plants: Metabolism and role in stress physiology* (Switzerland: Springer), 17–32. doi: 10.1007/978-3-319-06710-0_2

Rousi, E., Kornhuber, K., Beobide-Arsuaga, G., Luo, F., and Coumou, D. (2022). Accelerated western European heatwave trends linked to more-persistent double jets over Eurasia. *Nat. Commun.* 13, 3851. doi: 10.1038/s41467-022-31432-y

Saha, J., Brauer, E. K., Sengupta, A., Popescu, S. C., Gupta, K., and Gupta, B. (2015). Polyamines as redox homeostasis regulators during salt stress in plants. *Front. Environ. Sci.* 3. doi: 10.3389/fenvs.2015.00021

Saidi, Y., Finka, A., Muriset, M., Bromberg, Z., Weiss, Y. G., Maathuis, F. J. M., et al. (2009). The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma membrane. *Plant Cell.* 21 (9), 2829–2843. doi: 10.1105/tpc.108.065318

Sane, P. V., Kumar, N., Baijal, M., Singh, K. K., and Kochhar, V. K. (1987). Activation of nitrate reductase by calcium and calmodulin. *Phytochem.* 26 (5), 1289–1291. doi: 10.1016/S0031-9422(00)81796-3

Scuffi, D., Alvarez, C., Laspina, N., Gotor, C., Lamattina, L., and García-Mata, C. (2014). Hydrogen sulfide generated by l-cysteine desulfhydrase acts upstream of nitric oxide to modulate abscisic acid-dependent stomatal closure. *Plant Physiol.* 166 (4), 2065–2076. doi: 10.1104/pp.114.245373

Sehrawat, A., Abat, J. K., and Deswal, R. (2013). RuBisCO depletion improved proteome coverage of cold responsive s-nitrosylated targets in *Brassica juncea*. *Front. Plant Sci.* 4. doi: 10.3389/fpls.2013.00342

Sehrawat, A., and Deswal, R. (2014). S-nitrosylation analysis in *Brassica juncea* apoplast highlights the importance of nitric oxide in cold-stress signaling. *J. Proteome Res.* 13 (5), 2599–2619. doi: 10.1021/pr500082u

Sen, N., Paul, B. D., Gadalla, M. M., Mustafa, A. K., Sen, T., Xu, R., et al. (2012). Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. *Mol. Cell.* 45 (1), 13–24. doi: 10.1016/j.molcel.2011.10.021

Shen, J., Zhang, J., Zhou, M., Zhou, H., Cui, B., Gotor, C., et al. (2020). Persulfidation-based modification of cysteine desulfhydrase and the NADPH oxidase RBOHD controls guard cell abscisic acid signaling. *Plant Cell.* 32 (4), 1000–1017. doi: 10.1105/tpc.19.00826

Shivaraj, S. M., Vats, S., Bhat, J. A., Dhakte, P., Goyal, V., Khatri, P., et al. (2020). Nitric oxide and hydrogen sulfide crosstalk during heavy metal stress in plants. *Physiol. Plant* 168 (2), 437–455. doi: 10.1111/ppl.13028

Shi, H., Ye, T., and Chan, Z. (2013). Exogenous application of hydrogen sulfide donor sodium hydrosulfide enhanced multiple abiotic stress tolerance in bermudagrass (*Cynodon dactylon* (L.). pers.). *Plant Physiol. Biochem.* 71, 226–234. doi: 10.1016/j.plaphy.2013.07.021

Shi, H., Ye, T., Han, N., Bian, H., Liu, X., and Chan, Z. (2015). Hydrogen sulfide regulates abiotic stress tolerance and biotic stress resistance in arabidopsis. *J. integr. Plant Biol.* 57 (7), 628–640. doi: 10.1111/jipb.12302

Singhal, R. K., Jatav, H. S., Aftab, T., Pandey, S., Mishra, U. N., Chauhan, J., et al. (2021). Roles of nitric oxide in conferring multiple abiotic stress tolerance in plants and crosstalk with other plant growth regulators. *J. Plant Growth Regul.* 40, 2303–2328. doi: 10.1007/s00344-021-10446-8

Singh, P. K., Indoliya, Y., Chauhan, A. S., Singh, S. P., Singh, A. P., Dwivedi, S., et al. (2017). Nitric oxide mediated transcriptional modulation enhances plant adaptive responses to arsenic stress. *Sci. Rep.* 7 (1), 3592. doi: 10.1038/s41598-017-03923-2

Singh, G., Patel, A., Tiwari, S., Gupta, D., and Prasad, S. M. (2022). Signaling molecules hydrogen sulfide (H_2S) and nitric oxide (NO): Role in microalgae under adverse environmental conditions. *Acta Physiol. Plant* 44, 68. doi: 10.1007/s11738-022-03404-8

Song, L., Ding, W., Shen, J., Zhang, Z., Bi, Y., and Zhang, L. (2008). Nitric oxide mediates abscisic acid induced thermotolerance in the calluses from two ecotypes of reed under heat stress. *Plant Sci.* 175, 826–832. doi: 10.1016/j.plantsci.2008.08.005

Song, L., Zhao, H., and Hou, M. (2013). Involvement of nitric oxide in acquired thermotolerance of rice seedlings. *Russ. J. Plant Physiol.* 60 (6), 785–790. doi: 10.1134/S1021443713060149

Song, C., Zhao, Y., Li, A., Qi, S., Lin, Q., and Duan, Y. (2021). Postharvest nitric oxide treatment induced the alternative oxidase pathway to enhance antioxidant capacity and chilling tolerance in peach fruit. *Plant Physiol. Biochem.* 167, 113–122. doi: 10.13560/j.cnki.biotech.bull.1985.2020-0084

Sougrakpam, Y., Babuta, P., and Deswal, R. (2018). "Current scenario of NO (S-nitrosylation) signaling in cold stress," in *Metabolic adaptations in plants during abiotic stress* (Boca Raton: CRC Press), 351–360.

Sun, A. Z., and Guo, F. Q. (2016). Chloroplast retrograde regulation of heat stress responses in plants. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.00398

Sun, Y., Qiu, X., Ye, X., and Li, Z. (2020). Crosstalk between hydrogen sulfide and nitric oxide signaling in plants. *Biotechnol. Bull.* 36 (8), 153–161. doi: 10.13560/j.cnki.biotech.bull.1985.2020-0084

Sun, Y.-Y., Wang, J.-Q., Xiang, R.-H., and Li, Z.-G. (2022). Key role of reactive oxygen species-scavenging system in nitric oxide and hydrogen sulfide crosstalk-evoked thermotolerance in maize seedlings. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.967968

Talukdar, D. (2013). Arsenic-induced oxidative stress in the common bean legume, *Phaseolus vulgaris* l. seedlings and its amelioration by exogenous nitric oxide. *Physiol. Mol. Biol. Plants.* 19 (1), 69–79. doi: 10.1007/s12298-012-0140-8

Valderrama, R., Begara-Morales, J. C., Chaki, M., Mata-Pérez, C., Padilla, M. N., and Barroso, J. B. (2019). "Hydrogen peroxide (H2O2)- and nitric oxide (NO)-derived posttranslational modifications," in *Nitric oxide and hydrogen peroxide signaling in higher plants* (Switzerland: Springer), 37–67. doi: 10.1007/978-3-030-11129-8_3

Vandenabeele, S., Vanderauwera, S., Vuylsteke, M., Rombauts, S., Langebartels, C., Seidlitz, H. K., et al. (2004). Catalase deficiency drastically affects gene expression induced by high light in *Arabidopsis thaliana*. *Plant J.* 39, 45–58. doi: 10.1111/j.1365-313X.2004.02105.x

Vital, S. A., Fowler, R. W., Virgen, A., Gossett, D. R., Banks, S. W., and Rodriguez, J. (2008). Opposing roles for superoxide and nitric oxide in the NaCl stress-induced upregulation of antioxidant enzyme activity in cotton callus tissue. *Environ. Exp. Bot.* 62 (1), 60–68. doi: 10.1016/j.envexpbot.2007.07.006

Vranova, E., Inze, D., and Breusegem, F. (2002). Signal transduction during oxidative stress. *J. Exp. Bot.* 53 (372), 1227–1236. doi: 10.1093/jexbot/53.372.1227

Wang, L., Guo, Y., Jia, L., Chu, H., Zhou, S., Chen, K., et al. (2014). Hydrogen peroxide acts upstream of nitric oxide in the heat shock pathway in arabidopsis seedlings. *Plant Physiol.* 164 (4), 2184–2196. doi: 10.1104/pp.113.229369

Wang, X., Ruan, M., Wan, Q., He, W., Yang, L., Liu, X., et al. (2020). Nitric oxide and hydrogen peroxide increase glucose-6-phosphate dehydrogenase activities and expression upon drought stress in soybean roots. *Plant Cell Rep.* 39 (1), 63–73. doi: 10.1007/s00299-019-02473-3

Wang, J., Zhao, Y., Ma, Z., Zheng, Y., and Jin, P. (2022a). Hydrogen sulfide treatment alleviates chilling injury in cucumber fruit by regulating antioxidant capacity, energy metabolism and proline metabolism. *Foods.* 11, 2749. doi: 10.3390/foods11182749

Wang, Z., He, FY., Mu, Y., Zhang, L., Liu, Z., and Liu, D. (2022b). Identification and functional characterization of a cystathionine β-lyase (CBL) enzyme for H2S production in Arabidopsis thaliana. *Plant Physiol. Biochem.* 182, 76–89. doi: 10.1016/j.plaphy.2022.04.008

Wimalasekera, R., Villar, C., Begum, T., and Scherer, G. F. (2001). Coper amine oxidase1 (CuAO) of *Arabidopsis thaliana* contributes to abscisic acid- and polyamine-induced nitric oxide biosynthesis and abscisic acid signal transduction. *Mol. Plant* 4, 663–678. doi: 10.1093/mp/ssr023

Xuan, L., Li, J., Wang, X., and Wang, C. (2020). Crosstalk between hydrogen sulfide and other signal molecules regulates plant growth and development. *Int. J. Mol. Sci.* 21 (13), 4593. doi: 10.3390/ijms21134593

Yang, J., Gupta, V., Carroll, K. S., and Liebler, D. C. (2014). Site-specific mapping and quantification of protein s-sulphenylation in cells. *Nat. Commun.* 5, 4776. doi: 10.1038/ncomms5776

Yang, J. D., Yun, J. Y., Zhang, T. H., and Zhao, H. L. (2006). Presoaking with nitric oxide donor SNP alleviates heat shock damages in mung bean leaf discs. *Bot. Stud.* 47 (2), 129–136.

Yao, Y., He, R. J., Xie, Q. L., Zhao, X. H., Deng, X. M., He, J. B., et al. (2017). ETHYLENE RESPONSE FACTOR 74 (ERF74) plays an essential role in controlling a respiratory burst oxidase homolog d (RbohD) dependent mechanism in response to different stresses in arabidopsis. *New Phytol.* 213 (4), 1667–1681. doi: 10.1111/nph.14278

Yemets, A. I., Karpets, Y. V., Kolupaev, Y. E., and Blume, Y. B. (2019). "Emerging technologies for enhancing ROS/RNS homeostasis," in *Reactive oxygen, nitrogen and sulfur species in plants: Production, metabolism, signaling and defense mechanisms*, vol. 2. (John Wiley & Sons Ltd), 873–922. doi: 10.1002/9781119468677.ch39

Yuan, S., Shen, X., and Kevil, C. G. (2017). Beyond a gasotransmitter: Hydrogen sulfide and polysulfide in cardiovascular health and immune response. *Antioxid. Redox Signal.* 27, 634–653. doi: 10.1089/ars.2017.7096

Yun, B. W., Feechan, A., Yin, M., Saidi, N. B. B., Le Bihan, T., Yu, M., et al. (2011). S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. *Nature* 478, 264–268. doi: 10.1038/nature10427

Zaffagnini, M., Morisse, S., Bedhomme, M., Marchand, C. H., Festa, M., Rouhier, N., et al. (2013). Mechanisms of nitrosylation and denitrosylation of cytoplasmic glyceraldehyde-3-phosphate dehydrogenase from *Arabidopsis thaliana*. *J. Biol. Chem.* 288 (31), 22777–22789. doi: 10.1074/jbc.M113.475467

Zhang, H. (2016). "Hydrogen sulfide in plant biology," in *Signaling and communication in plants. vol. gasotransmitters in plants. the rise of a new paradigm in cell signaling* (Switzerland: Springer), 23–51. doi: 10.1007/978-3-319-40713-5

Zhang, J., and Liao, W. (2019). Protein s-nitrosylation in plant abiotic stresses. *Funct. Plant Biol.* 47 (1), 1–10. doi: 10.1071/FP19071

Zhang, P., Li, S., Zhao, P., Guo, Z., and Lu, S. (2019). Comparative physiological analysis reveals the role of NR-derived nitric oxide in the cold tolerance of forage legumes. *Int. J. Mol. Sci.* 20 (6), 1368. doi: 10.3390/ijms20061368

Zhang, J. Y., Luo, W., Zhao, Y., Xu, Y. Y., Song, S. H., and Chong, K. (2016). Comparative metabolomic analysis reveals a reactive oxygen species-dominated dynamic model underlying chilling environment adaptation and tolerance in rice. *New Phytol.* 211 (4), 1295–1310. doi: 10.1111/nph.14011

Zhang, J., Zhou, M., Zhou, H., Zhao, D., Gotor, C., Romero, L. C., et al. (2021). Hydrogen sulfide, a signaling molecule in plant stress responses. *J. Integr. Plant Biol.* 63 (1), 146–160. doi: 10.1111/jipb.13022

Zhao, M. G., Chen, L., Zhang, L. L., and Zhang, W. H. (2009). Nitric reductase-dependent nitric oxide production is involved in cold acclimation and freezing tolerance in arabidopsis. *Plant Physiol.* 151, 755–767. doi: 10.1104/pp.109.140996

Zhen, Y., and Ungerer, M. C. (2008). Relaxed selection on the CBF/DREB1 regulatory genes and reduced freezing tolerance in the southern range of *Arabidopsis thaliana*. *Mol. Biol. Evol.* 25 (12), 2547–2555. doi: 10.1093/molbev/msn196

Zhou, H., Zhang, J., Shen, J., Zhou, M., Yuan, X., and Xie, Y. (2020). Redox-based protein persulfidation in guard cell ABA signaling. *Plant Signal. Behav.* 15 (5), 1741987. doi: 10.1080/15592324.2020.1741987

Zhou, H., Zhou, Y., Zhang, F., Guan, W., Su, Y., Yuan, X., et al. (2021). Persulfidation of nitrate reductase 2 is involved in l-cysteine desulfhydrase-regulated rice drought tolerance. *Int. J. Mol. Sci.* 22, 12119. doi: 10.3390/ijms222212119

Ziogas, V., Tanou, G., Filippou, P., Diamantidis, G., Miltiadis, V., Fotopoulos, V., et al. (2013). Nitrosative responses in citrus plants exposed to six abiotic stress conditions. *Plant Physiol. Biochem.* 68, 118–126. doi: 10.1016/j.plaphy.2013.04.004

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Subsurface aeration mitigates organic material mulching-induced anaerobic stress *via* regulating hormone signaling in *Phyllostachys praecox* roots

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Organic material mulching has been used extensively to allow *Phyllostachys praecox* to promote growth and development of shoots. However, the bamboo forest always showed a significant degradation, probably due to anaerobic damage caused by the mulching after several years. Therefore, we have innovatively proposed an improvement measure to aerate the underground pipes for the first time. We investigated the role of subsurface pipe aeration in regulating root hypoxia to reduce the stress and to identify the degradation mechanism. Results showed that aeration increased oxygen concentration, shoot yield and root growth compared with mulching, and the aeration enhanced the concentration of indole-3-acetic acid (IAA) and the expression of *Aux/IAAs* (*Aux1*, *Aux2*, *Aux3*, and *Aux4*). Aeration reduced gibberellin (GA), ethylene (ETH), and abscisic acid (ABA) contents as well as anaerobic enzyme activities (alanine transaminase, AlaAT; alcohol dehydrogenase, ADH; pyruvate decarboxylase, PDC; and lactate dehydrogenase, LDH), which alleviated root damage in anoxic conditions. Furthermore, correlation showed that the activities of ADH, LDH, PDC, and AlaAT showed significant linear correlations with soil oxygen levels. RDA analyses showed that ABA, IAA, and ETH were found as the key driving hormones of *Aux/IAAs* in the root of the forest mulched with organic material. Here we show that subsurface aeration increases soil oxygen concentration, shoot yield, root growth and regulates phytohormone concentrations and *Aux/IAAs* expression, which reduces anaerobic enzyme activities. Consequently, subsurface pipe aeration is an effective measure to mitigate the degradation of bamboo forests caused by soil hypoxia that results from organic material mulching.

KEYWORDS

mulching, Aux/IAAs, hormones, anaerobic enzyme, soil aeration

1 Introduction

Phyllostachys praecox f. *preveynalis* is a bamboo species in the family Gramineae that is widespread in southern of China (Gui et al., 2013). The shoots of *P. praecox* are known as "the best bamboo shoots in China" because of their delicious taste, early season harvesting, high yield, and economic impact (Huang et al., 2007; Guo et al., 2015). The most effective way to obtain greater benefits is to mulch the bamboo forest with organic material in winter, which allows the farmer to harvest the shoots in the March earlier than expected (Mulumba and Lal, 2008). However, after 3-4 years of continuous mulch management, the *P. praecox* forest will inevitably experience an overall decline, resulting from degradation of the underground rhizome system, reductions in bamboo shoot production and quality, and flowering of the bamboo (Guo et al., 2011; Chen et al., 2014). According to prior studies, organic material mulching is the critical factor in the forest degradation since the fermentation of organic matter increased the temperature, blocked air exchange, and depleted the oxygen in the rhizosphere soil, resulting a low oxygen environment (Gui et al., 2013; Qian et al., 2020). Therefore, figuring out how to improve soil oxygen is the key to alleviating the bamboo degradation during the mulching period. Most of the methods currently used to alleviate root hypoxia are chemical methods that involve adding calcium peroxide (CaO_2) or magnesium peroxide (MgO_2), or quick release formulations like hydrogen peroxide (H_2O_2) or carbamide peroxide ($CH_4N_2O{\cdot}H_2O_2$) (Liu et al., 2012; Liu and Porterfield, 2015), which largely changes the soil structure and the original living environment of the plants. Other methods require manually inserting tubes into the soil to increase soil oxygen levels, which is time-consuming and labor-intensive (Qian et al., 2022). Therefore, we devised a method using subsurface pipe aeration to investigate whether it could effectively increase oxygen levels in the root zone, improve plant physiological and biochemical performance, and thus mitigate degradation of the bamboo forest.

Hypoxic stress occurs frequently in nature (Drew, 1997; Bailey-Serres and Voesenek, 2008; Gibbs et al., 2011b). In order to survive, cells switch from aerobic respiration to anaerobic fermentation, generating harmful metabolites including lactic acid, acetaldehyde, and ethanol (Bailey-Serres and Voesenek, 2008c; Visser and Voesenek, 2006). Ethanol and lactic acid fermentation are the two primary metabolic routes for energy production under hypoxic conditions (Fukao and Bailey-Serres, 2004). The pyruvate content then increases, and the anaerobic enzymes alanine aminotransferase (AlaAT), pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), and lactate dehydrogenase (LDH), are produced in these "hypoxic" cells (Armstrong et al., 2019). PDC and ADH catalyse the conversion of pyruvate PDC and ADH to ethanol, followed by the conversion of pyruvate to lactate by LDH and the simultaneous oxidation of NADH to NAD+ (Robertson et al., 1994; Kathleen et al., 2003). The reversible interconversion of alanine and 2-ketoglutarate to pyruvate and glutamate is catalyzed by AlaAT in the presence of higher pyruvate concentrations (Ricoult et al., 2006). Hu et al. (2005) found that ADH and PDC activities increased in roots of cucumber under anaerobic condition. In *Arabidopsis* and *Medicago truncatula*, LDH and AlaAT fermentation are enhanced in anoxic and hypoxic cells (Bray et al., 2002; Dolferus et al., 2008). Plant tolerance to hypoxia stress can be improved by increasing anaerobic enzyme activity (Gibbs et al., 2000; Morimoto and Yamasue, 2007). Therefore, anaerobic enzyme activity is also an important indicator.

Plant hormones including gibberellin (GA), abscisic acid (ABA), ethylene (ETH) and the growth hormone, indoleacetic acid (IAA), interact with each other to regulate biochemical and physiological processes (Davies, 2005; Achard et al., 2008; Bari and Jones, 2009; Santner et al., 2009; Peleg and Blumwald, 2011). Ethylene is the most sensitive hormone under hypoxic stress. Under anoxic conditions such as flooding, ethylene production is induced in the plant, which to some extent stimulates the formation of adventitious roots and air chambers, creating favorable conditions for nutrient and water uptake as well as oxygen input. Moreover, ethylene alleviates the toxic effects of secondary metabolites (Fukao et al., 2006; Hartman et al., 2021). The precursor of ETH, 1-aminocyclopropane-1-carboxylate (ACC), is formed by the action of ACC synthase (ACS) using methionine as the substrate, and ETH is then formed by the reactions catalyzed of ACC oxidase (ACO) with molecular oxygen as the coenzyme (Chae and Kieber, 2005; Rieu et al., 2005; Xu and Zhang, 2015). ACO and ACS are important rate-limiting steps in ETH synthesis (Barry et al., 1996; Vriezen et al., 1999). In addition, GA has been shown to stimulate seed germination and root elongation, while ABA is a potent inhibitor of GA activity (Holdsworth et al., 2008). ETH promotes elongation plant development and adventitious root production by managing the dynamic stability of ABA and GA concentration to expand the contact area of plants with air for more oxygen (Steffens and Sauter, 2006; Rzewuski and Sauter, 2008). Plant growth hormones known as auxins, such as IAA, increase root initiation and postpone plant senescence (Nguyen et al., 2018). Tognetti et al. (2012) found interaction between IAA and other hormonal signals during stress adaptation, which might be mediated by changes in plant growth and development. In addition, it was also found that the high IAA/ABA ratio is associated to the activity of rhizome buds in *P. praecox* (Hu et al., 1996). Jasim and Merhij (2013) suggest that there are improvements in IAA, GA3, and Zeatine while a reduction in ABA under mulch + fertilizers. These findings suggest that endogenous hormones are vital in the formation of bamboo roots. The key hormonal changes that lead to bamboo forest degradation after long-term mulching with organic materials and the relationship between them are poorly studied and deserve further investigation.

Auxin/indole-3-acetic acid (Aux/IAA) proteins are transcription factors (TFs) that control auxin-responsive gene expression during plant development (Remington et al., 2004; Szemenyei et al., 2008; Chandler, 2016). *Aux/IAA* family member genes are often homologous in the same plant; for example, *SlIAA2*, *SlIAA13*, *SlIAA15*, *SlIAA16*, and *SlIAA20* genes in tomato are functionally similar and are significantly expressed during root development (Wu et al., 2012). Other studies have also found specificity in *Aux/IAA* gene functions, such as the expression of *AetIAA3*, *AetIAA11*, and *AetIAA26* in *Aegilops tauschii*, which are tissue-specific genes that are expressed specifically in pistils, seeds, and roots, respectively (Qiao et al., 2014). The majority members of

the Aux/IAA TF family have been associated with lateral root growth. For example, the *AtIAA14* gene controls lateral root development in Arabidopsis (Fukaki et al., 2010). In wheat, *TaIAA1* regulates the development of important organs such as roots and tillers, and also flowering and leaf patterns (Singla et al., 2006). In addition, there is also crosstalk between the *Aux/IAA* family and ethylene, and it thus regulates plant growth. According to Li et al. (2015), ETH modifies alkaline stress-mediated root development inhibition by increasing the expression of *Aux1* and auxin-related genes, which enhances auxin accumulation. So yet, only four members of the *Auxin* family, *Aux1*, *Aux2*, *Aux3*, and *Aux4*, have been discovered in *P. praecox*. Whether the *Aux/IAA* gene family has crosstalk with GA and/or ABA to regulate root growth is still unknown for the organic material mulching system used in *P. praecox*.

In this study, the effects of subsurface pipe aeration on soil oxygen and root physiology and biochemistry of a *P. praecox* forest under organic material mulching were investigated. The aims of the study were to examine the hypotheses: aeration improves the soil condition and associated physiological and biochemical properties of bamboo forests. The obtained evidences are expected to provide a new direction for the sustainable cultivation of bamboo forests.

2 Materials and methods

2.1 Site location

The research was performed at the Panmugang Modern Forestry Demonstration Base of Zhejiang Agriculture and Forestry University, Zhejiang Province, China (119°58′ E, 30°29′ N). This area has a subtropical monsoon climate with an average annual temperature of 17.8°C, an average relative humidity of 70.3%, an annual precipitation of 1,454 mm, a frost-free period of 234 d, and 1,765 hours of sunshine per year. The relevant weather data is in Table S1. The agricultural area has a hilly environment with hills that are typically less than 150 meters high. The soil is classified as a Ferralsol since it is largely originated from quaternary sandstone parent material. Natural precipitation and soil water storage are the primary sources of agricultural productivity (Xu et al., 2017).

2.2 Experimental design

The experimental *P. praecox* plot had a stand density of 15,000 plants per hectare, the average diameter at breast height of the bamboo culm was 3.89 cm, and the ratio of the number of bamboo culms in each year was year 1: year 2: year 3 = 1:1.89:0.58. The experimental area of the forest was split into twelve 50 m × 50 m plots, with the treatment arrangement being a full block with three replicates for each treatment. The treatments were (1) control; (2) mulching; (3) control + aeration (aeration) and (4) mulching + aeration (M+A). On December 17, 2020, the surface of the bamboo forest was mulched with organic material to increase the temperature and moisture content, and the hulled bran that had not decayed was removed on March 24, 2021. Once mulch has been removed, the shoot yield was recorded. The following was the mulching procedure: Initially, 4,500 kg·ha^{-1} of chicken manure was spread to the soil surface. The chicken dung was subsequently covered with rice straw (3,750 kg·ha^{-1}). Finally, rice bran (412.5 t·ha^{-1}) was sprinkled on top to provide 15 cm of thickness. We randomly selected three plots that had been mulched for many years as aerated plots. The aeration measures were as follows: the holes were drilled in a straight line parallel below the ground at a depth of 50 cm (the bamboo rhizomes are mainly present in the 20-30 cm layer) in each plot at a spacing of 60 cm, and plastic ventilation pipes with an external diameter of 21 mm and a wall thickness of 1 mm were then inserted and connected in sequence, with small holes of 0.2 mm diameter every 30 cm in the wall for ventilation. An air pump was connected to the main pipe in each plot and the air was delivered by a compressor (AS7.5Hi, Quanzhou Jinba, China). The plots with aeration were aerated for the whole day. Samples were collected in March, June, September, and December 2021. In each sample plot, the bamboo root was sampled from a depth of 20-30 cm and taken to the laboratory for analysis. At the end of the experiment, the root system was scanned with the Winrhizo root analysis system and calculated for root length density, surface area density and volume density and record the diameter at breast height of the bamboo. Root samples were cleaned with distilled water, instantly dried, frozen in liquid nitrogen, and kept at -80°C until tested.

2.3 Sample analysis

2.3.1 Determination of soil oxygen content and temperature

Soil oxygen concentration and temperature were measured using a fiber-optic oxygen meter and a soil temperature probe (Firesting O_2, Pyro Science, Germany), calibrated at two points using saturated air (21% oxygen) and saturated Na_2SO_3 solution (0% oxygen) before use. For the test, the measuring probe and the soil temperature measuring probe were mounted on the oxygen meter at the same time. After selecting the measuring point at the soil profile (25 cm), the two probes were slowly and accurately inserted into the soil, covered with soil, and the soil was then allowed to return to its original state after one week before the oxygen content and temperature measurements were taken. Three replicate measurements were taken for each sample plot. The oxygen meter recorded both soil oxygen concentration and soil temperature, with the probes buried in the same way.

2.3.2 Root activity assay

The 2, 3, 5-triphenyltetrazolium chloride (TTC) redox technique was applied to assess root activity (Li et al., 2004). In the dark at 37°C, 0.5 g root pieces were pulverized with 5 mL PBS (pH 7.0) and 5 mL 0.4% TTC. To finish the incubation, 2 mL 1 M H_2SO_4 was supplied after 2 h. After wiping the roots with filter paper, they were homogenized in a mortar with 5 ml ethylacetate and fixed to 10 ml with ethylacetate. After that, a spectrophotometer

was used to measure absorbance at 485 nm (UVmini-1280, Shimadzu, Japan), the root activity was expressed by TTC reduction ($mg{\cdot}g^{-1}h^{-1}$).

2.3.3 Enzyme activity assays

Fresh root samples were extracted with LDH, AlaAT, ADH and PDC using 9 ml of 0.1 M phosphate buffer (pH 7.0). After centrifuging the mixtures at 14,000 g for 15 minutes at 4°C, the supernatant was collected and the anaerobic enzyme activity was determined using the appropriate assay [LDH (A020-2); AlaAT (C009-2-1); ADH (A083-2-1); PDC (A141-1-1), Nanjing Jiancheng Bioengineering Institute, China] (Qian et al., 2020; Gao et al., 2022). The formula is as follows:

$$\text{ADH activity (U/g FW)} = \frac{\Delta A_m - \Delta A_b}{6.22 \times 0.5} \times \frac{V_t}{V_s} \div T \times 1000 \div FW$$

ΔAm: A_2-A_1 (OD value of sample)
ΔAb: A_2-A_1 (OD value of blank)
V_t: Total volume of reaction solution (1.5mL);
V_S: Sample size (0.05mL);
T: Reaction time (10 minutes);
FW: sample fresh weight.

$$\text{PDC activity (U/g FW)} = \frac{\Delta A_m - \Delta A_b}{\epsilon \times d} \times \frac{V_t \times 10^6}{W \times V_s \div V_{ts}} \div T$$
$$= 1.61 \times (\Delta A_m - \Delta A_b) \div w$$

ΔAm: A_2-A_1 (OD value of sample)
ΔAb: A_2-A_1 (OD value of blank)
V_t: Total volume of reaction system, 1 mL=0.001 L;

$$\text{LDH activity(U/g FW)} = \frac{A_m - A_c}{A_s - A_b} \times C_s \div FW$$

A_m: Measured vials OD value;
A_c: Control vials OD value;
A_s: Standard vials OD value;
A_b: Blank vials OD value.
C_s: Standard solution concentration, 0.2 μmol/mL

$$\textit{ALT} \text{ activity of sample (U/g FW)} = U_h \div FW$$

U_h: The ALT activity of the protein homogenate to be tested is obtained through the standard curve;
FW: sample fresh weight.

2.3.4 Hormone analysis

ELISA plant hormones assay kit were used to determine the concentrations of GA, IAA, ABA, ACO and ACS (Shanghai Enzyme-linked Biotechnology Co., Ltd., China). Horseradish peroxidase enzyme-catalyzed label-antibody complexes were formed by combining antibodies directed against GA, IAA, ABA, ACO and ACS with enzyme-catalyzed label and hormones, which generates a blue material when combined with TMB substrate solution. Spectrophotometric measurements were then performed at 450 nm (Infinite M200 pro, Tecan, Switzerland) (Gao et al., 2022; Li et al., 2022). In the Excel worksheet, the linear regression curve was plotted using the standard concentration as the horizontal coordinate and the corresponding OD value as the vertical coordinate, and the concentration value of each sample was calculated according to the curve equation.

Based on Gao et al. (2022), root samples of *P. praecox* were put in 15-mL glass vials with 1mL 0.6% water agar and closed instantly. Following a 4-hour dark incubation period at 30°C, 1 mL of gas was attracted from the air space of each vial with an air-tight syringe (Focus GC, Thermo, Massachusetts, USA) and infused into a gas chromatograph (Focus GC, Thermo) fitted with a capillary column (CP-CarboPLOT P7, California, USA) and flameion. The ETH production was then determined using the fresh weight (f.wt) of bamboo roots (Wu et al., 2011; Zhu et al., 2016).

2.3.5 Quantitative real-time PCR (qRT–PCR) analysis

The OminiPlant RNA Kit was used to extract total RNA (CWBIO, CW2598, China). A spectrophotometer was applied to determine the concentration and purity of RNA (Nano Drop 2000c, Thermo Scientific, USA). To generate cDNA, the Prime ScriptTM RT reagent Kit with gDNA Eraser was utilized (Takara Bio, RR047A, Japan). Primers of *Actin*, *Aux1*, *Aux2*, *Aux3*, and *Aux4* came from Gao et al. (2022); primer of *PeNTB* was cited from Fan et al. (2013). In qRT-PCR assays, gene-specific primers of *Actin*, *Aux1*, *Aux2*, *Aux3*, and *Aux4* were utilized (Table 1). Ct values of *Actin* were used as internal controls. Values reported represent the averages of three biological replicates with two independent trials. Sangon Biotech produced the primers (Shanghai, China). The Ultra SYBR Mixture (Takara, RR820A) fluorescent dye was utilized for qRT-PCR (Applied Biosystems QuantStudio 6, USA). The $2^{-\Delta\Delta CT}$ approach was then used to determine the relative gene expression levels (Livak and Schmittgen, 2001; Gao et al., 2022).

2.4 Statistical analysis

Using SPSS 20.0, all data were statistically assessed utilizing ANOVA and Duncan's Multiple Range test (IBM Corp., Armonk, NY, USA). Correlation and redundancy analyses were carried out using R program v3.6.3. Origin v8.0 was used to create the figures (Origin Lab Corporation, Northampton, USA).

2.4.1 Redundancy analysis (RDA)

RDA is a method that combination of correspondence analysis and multiple regression analysis, each step of the calculation is regression with environmental factors, also known as multiple direct gradient analysis (Borcard et al., 1992; Larkin et al., 2007). This analysis is used to reflect the relationship between genetic (*Auxs/IAA*) and enzyme and hormones factors in this study. Results were visualized by RDA biplot using CANOCO (version 4.5), where the position, angle, and length of arrows indicated the direction, degree, and scope of response of the genetic (enzyme and hormones) to enzyme and hormones (genetic) variables. The

TABLE 1 Specific primers used for qRT-PCR.

Gene name	Primer sequence (5′-3′)	Amplicon size (bp)
PeNTB	F: TCTTGTTTGACACCGAAGAGGAG R: AATAGCTGTCCCTGGAGGAGTTT	133
Actin	F: CGTCAAAGCCCCAAGAACAC R: GCTAGGAAAGACAGCCCTGG	129
Aux1	F: GTTCGTGAAGGTGAGCATGG R: CGTTCATGCCGTTCATCCCT	155
Aux2	F: TCTGAGGATGTACGGAGGGT R: GCATCAGATCGCCGTCCTTG	125
Aux3	F: AAGGGCATGAACGAGAGCAA R: CGACTCGACGAACATCTCCC	126
Aux4	F: TGACCAGCCGATGACGAAG R: GCTGCTTGGAAGGTGTTCCT	186

main function of the Monte Carlo test (Julian and Peter, 1989) is to test the significance of the constrained ranking method.

2.4.1 Correlation analyses

Pearson correlation analyses between enzyme and hormones and gene characteristics were performed using SPSS Statistics v20.0 (IBM Corp., USA) and illustrated using the " pheatmap" package in R v 4.0.2. Before variance analysis, we used Shapiro-Wilk and Levene tests to assay the data normality and the equality of variances, respectively. We conducted a one-way Analysis of Variance to explore the effects of aeration on plant enzyme and hormones and gene characteristics using SPSS Statistics v20.0. F values were derived from ANOVA at $p< 0.05$, $p< 0.01$, and $p< 0.001$ using SPSS v20.0.

3 Results

3.1 Effect of aeration on bamboo growth under coditions

Compared to the control, shoot yield was significantly increased by 60.5%, 20.23% and 115.1% for mulching, aeration and M+A respectively, and by 34.0% for M+A compared to mulched (Figure 1A). As for diameter at breast height, the diameter at breast height in the aeration group was significantly increased compared to the mulched group (Figure 1B). Mulching significantly reduced root length density (67.9%), root surface area density (39.4%), and root volume density (73.0%), respectively, compared to the control (Figures 1C–E). But M+A significantly increased root length density (39.3%), root surface area density (22.7%), and root volume density (50.6%), respectively, compared to mulching. It showed that mulching combined with aeration techniques has a positive effect on the growth of bamboo.

3.2 Effect of aeration on soil oxygen concentration, soil temperature, and root activity under mulching conditions

When the bamboo mulched, soil oxygen concentration decreased rapidly, reaching its lowest level after two months (Figure 2A). Three months after mulching, soil oxygen concentration began to recover when the mulch was removed, but it was still lower than that of the control plots. There were no significant differences in oxygen concentration between the treatments of M+A and control in June, September and December. During the mulching period, soil temperature in the mulched treatments was significantly higher than the control, while aeration significantly reduced the soil temperature compared to the mulched (Figure 2B). When the mulch was removed in March, soil temperature increased and then decreased with time that was consistent with the air temperature. From June, there were no significant differences among all treatments. As indicated by Figure 2C, the mulching resulted in a lower root activity compared to the control, while aeration improved the activity significantly during the mulching peroid.

3.3 Effect of aeration on anaerobic enzyme activity in roots under mulching conditions

LDH, AlaAT, PDC, and ADH activities of mulching treatment were significantly elevated and the M+A treatment significantly lowered the activities of these anaerobic enzymes compared with mulching throughout the year (Figures 3A–D). There was no significant difference between the M+A and the control regarding anaerobic enzyme activity. Anaerobic enzyme activity of aeration group was significantly decreased compared to the control.

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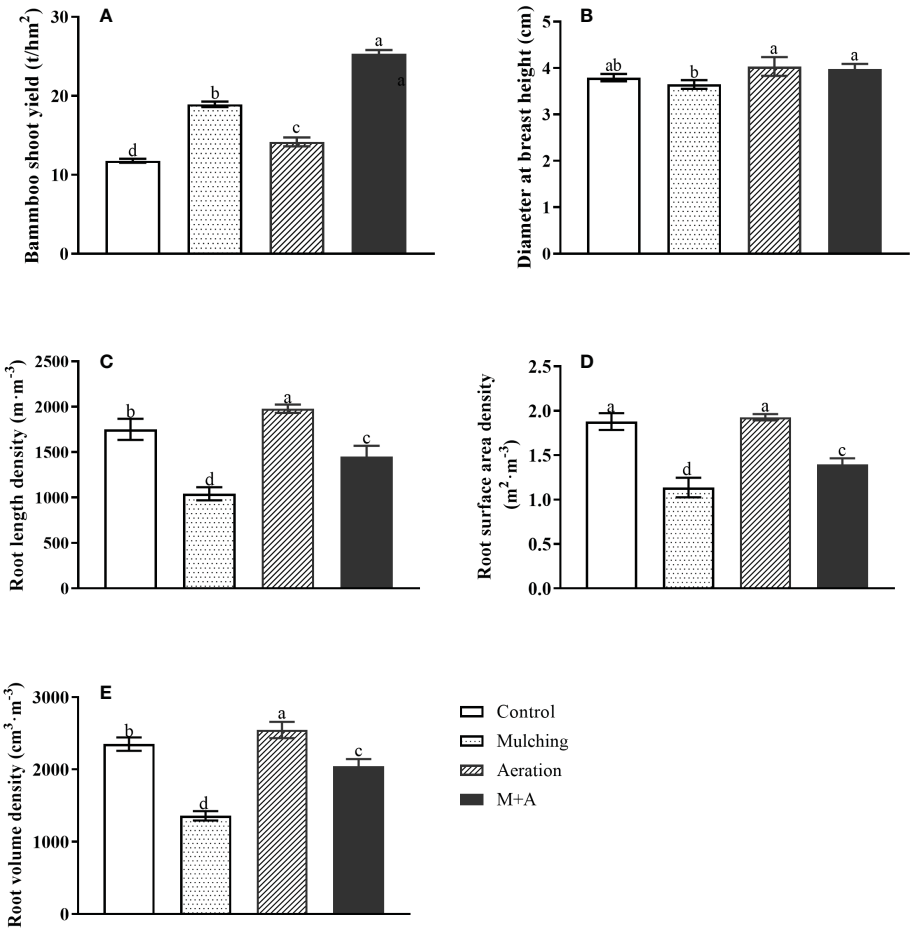

FIGURE 1
Effects of aeration on shoot production **(A)**, diameter at breast height **(B)**, root length density **(C)**, surface area density **(D)** and root volume density **(E)** in the bamboo forest under mulching conditions (Control, Mulching, Aeration, M + A). The vertical bars ± reflect the standard deviation of the mean. Different letters in the same period represent different significance ($p< 0.05$).

3.4 Effect of aeration on the activities of ACO and ACS in roots under mulching conditions

Mulching significantly improved the activities of ACO and ACS, aeration significantly reduced both activities (Figures 3E, F). For all four seasons, there were no statistically significant changes between the aeration treatment and the control. The activities of ACS and ACO were lower overall in June when compared to the other months of the year.

3.5 Effects of aeration on the hormone content in roots under mulching conditions

In March and September, mulching significantly increased ABA, GA, and ETH contents in the roots and decreased the IAA content compared to the control, while aeration significantly decreased the contents of ABA, GA, and ETH and increased the IAA content in the M+A group (Figures 4A–D). ABA, GA, and ETH contents were significantly reduced and IAA was enhanced in aeration treatment compared to the control.

3.6 Effect of aeration on *Aux/IAAs* gene expression in roots under mulching conditions

Mulching significantly reduced the expression of *Aux1*, *2*, *3*, and *4* compared to the control throughout the year, while soil aeration significantly enhanced *Aux* gene expression in the M+A group (Figures 5A–D). *Aux/IAAs* gene expression of aeration was significantly increased compared to the control.

3.7 Analyses of correlations between enzyme and hormones and gene expression traits

As shown in Figure 6, the activities of ADH, PDC, LDH, and AlaAT all showed a linear correlation with soil oxygen concentration, and the activities of anaerobic enzyme increased as the oxygen concentration decreased.

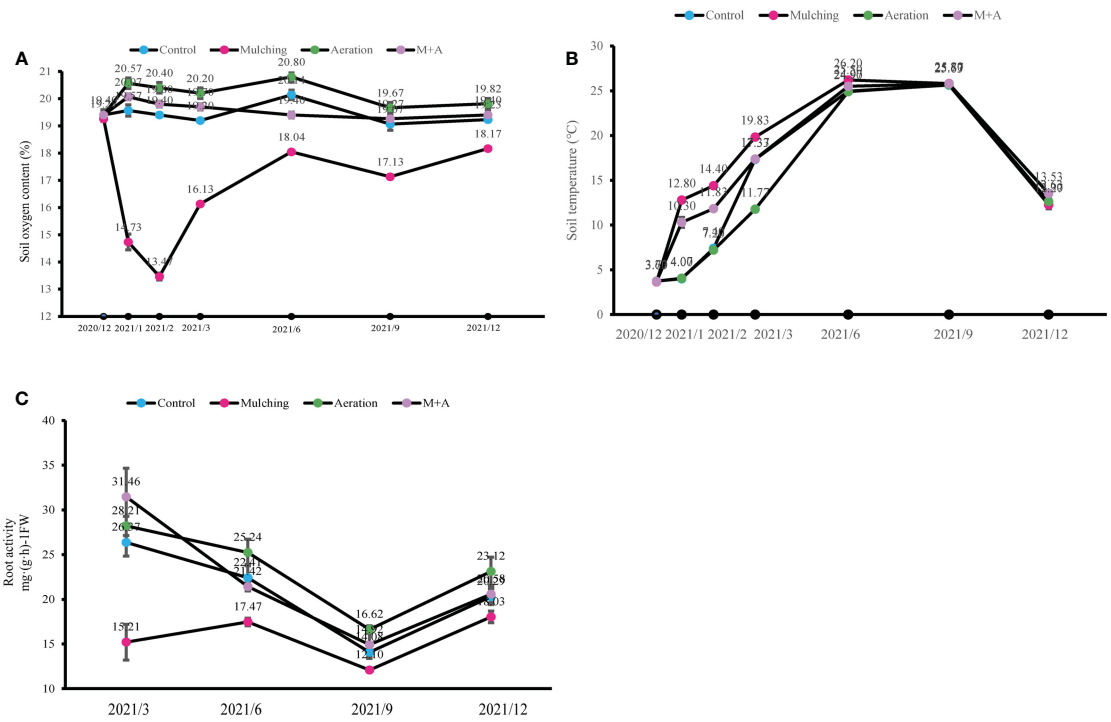

FIGURE 2
Effects of aeration on soil oxygen content **(A)**, temperature **(B)** and root respiratory activity **(C)** in the bamboo forest under mulching conditions (Control, Mulching, Aeration, M + A). The vertical bars ± reflect the standard deviation of the mean. Different letters in the same period represent different significance ($p< 0.05$).

Pearson's correlation tests were also carried out to assess the correlations between each enzyme and hormones and genetic attribute under mulching and aeration (Figure 7). The expression of *Aux1*, *2*, *3*, and *4* was substantially positively associated with IAA levels, and *Aux/IAAs* expression was significantly negatively associated with ETH, ABA and GA concentration ($p< 0.05$). Moreover, ETH was significantly positively related with the activities of PDC, ADH, LDH, AlaAT, and also with ABA and GA concentration. ABA concentration was significantly positively correlated with anaerobic enzyme activities and ETH and GA concentration, and significantly negatively correlated with *Aux/IAAs* expression and IAA concentration. As for anaerobic enzyme activity, LDH activity was significantly positively associated with the activities of PDC, AlaAT, and ADH, and the concentrations of ABA, ETH, and GA and significantly negatively correlated with IAA concentration and *Aux/IAAs* gene expression ($p< 0.05$). ADH, LDH and AlaAT activity was significantly positively associated with anaerobic respiration enzymes activities and ETH, GA, and ABA concentrations, and significantly negatively associated with IAA and *Aux/IAAs* gene expression ($p< 0.05$). The activity of PDC was significantly positively correlated with ETH, ABA and GA concentrations and the activities of anaerobic respiration enzymes, and it was significantly and negatively associated with *Aux/IAAs* gene expression ($p< 0.05$). The analysis showed that plant hormones have an important role in mulch-induced root hypoxia in *P. praecox*, influencing changes in *Aux/IAAs* expression and anaerobic enzymes activities.

3.8 Redundancy analysis of enzyme and hormones and gene parameters

We did redundancy analysis (RDA) to see whether there were any commonalities among the treatments in terms of enzyme and hormones (ADH, AlaAT, LDH, and PDC activities, hormone concentrations of ABA, IAA, GA, and ETH) and genetic characteristics (*Aux* gene expression) (Figure 8). As a result, we found that enzyme and hormones and genetic characteristics interact with one another. The activities of ABA, PDC, and LDH, as well as the concentrations of ETH and IAA, had a significant influence on plant genetic composition ($p< 0.05$), with RDA1 and RDA2 exhibiting variances across all treatments, accounting for 56.15 and 35.88% of the variation, respectively (Figure 8A). Furthermore, *Aux1*, *Aux2*, *Aux3*, and *Aux4* expression was strongly associated to plant enzyme and hormones parameters, with the first and second major axis accounting for 53.01 and 20.82% of the variance, respectively (Figure 8B).

4 Discussion

4.1 Aeration improved soil condition and bamboo growth under mulching

In natural environments, soil hypoxia is caused by factors such as heavy rainfall, poor soil structure, and little drainage, which

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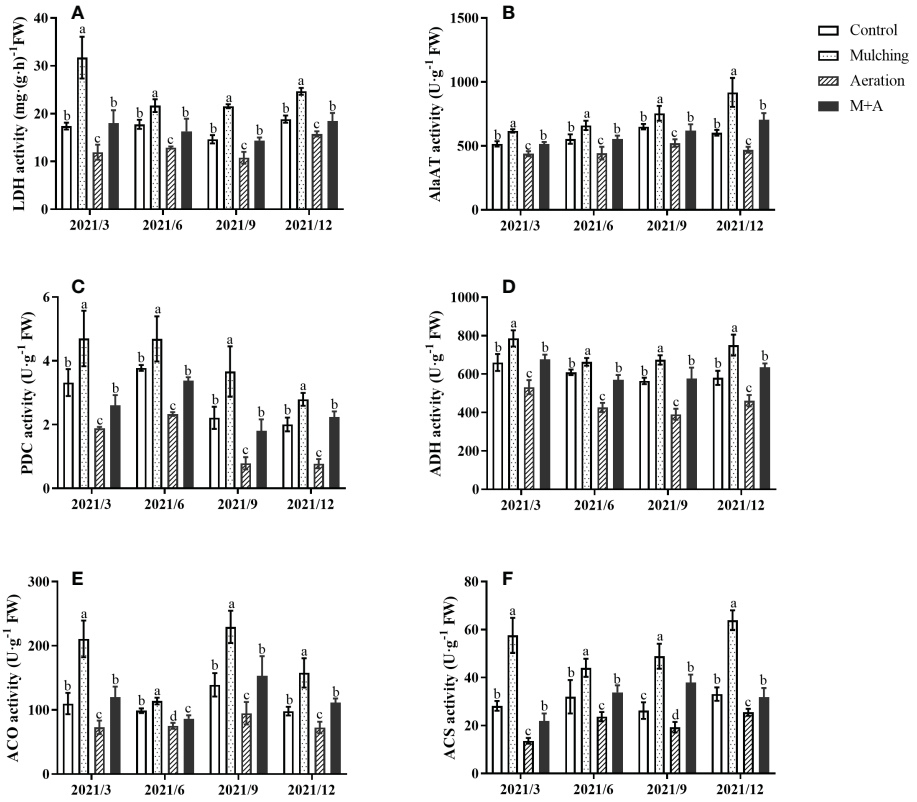

FIGURE 3
Effects of aeration on the activities of LDH **(A)**, AlaAT **(B)**, PDC **(C)**, ADH **(D)** ACO **(E)** and ACS **(F)** in the bamboo forest under mulching conditions (Control, Mulching, Aeration, M + A). The vertical bars ± reflect the standard deviation of the mean. Different letters in the same period represent different significance (p< 0.05). LDH, lactate dehydrogenase; AlaAT, alanine transaminase; PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase; ACO (ACC oxidase) and ACS (ACC synthase).

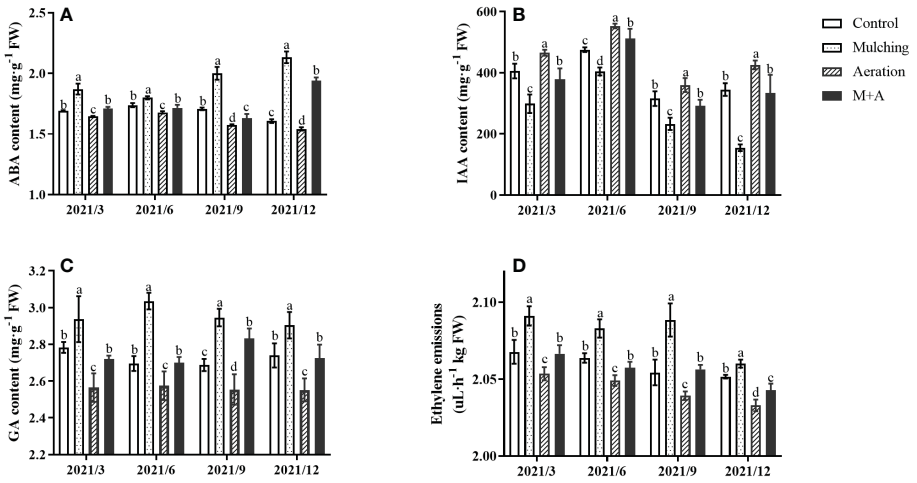

FIGURE 4
Effects of aeration on the contents of the phytohormones ABA **(A)**, IAA **(B)**, GA **(C)**, and ETH **(D)** in the bamboo forest under mulching conditions (Control, Mulching, Aeration, M + A). The vertical bars ± reflect the standard deviation of the mean. Different letters in the same period represent different significance (p< 0.05). ETH, ethylene; ABA, abscisic acid; GA, gibberellic acid; IAA, indole-3-acetic acid.

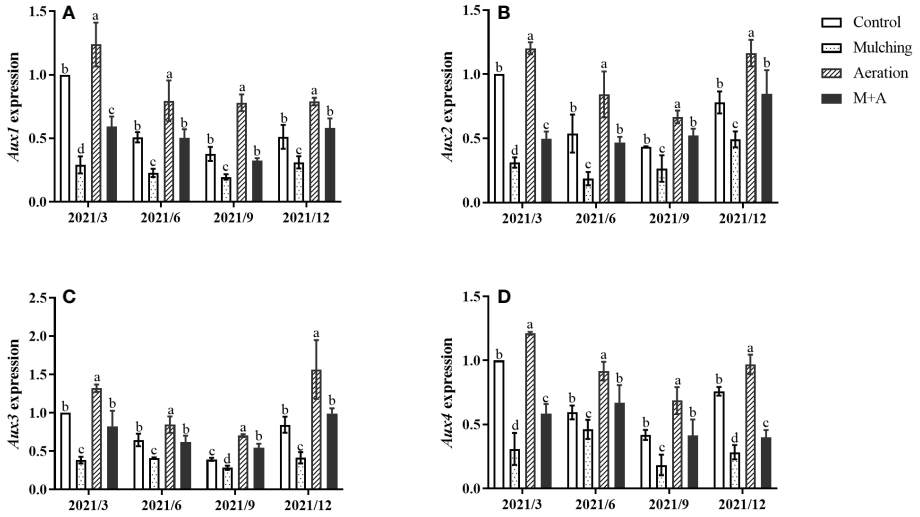

FIGURE 5
Effects of aeration on the expression of the *Aux1* **(A)**, *Aux2* **(B)**, *Aux3* **(C)** and *Aux4* **(D)** genes in the bamboo forest under mulching conditions (Control, Mulching, Aeration, M + A). The expression levels of *PeNTB* and *Actin* were used to normalize the expression for each sample. The vertical bars ± reflect the standard deviation of the mean. Different letters in the same period represent different significance ($p < 0.05$).

generate unfavorable porosity and ventilation interactions in the soil, restricting root growth and crop output considerably (Brookes et al., 1982; Weits et al., 2019; Strudley et al., 2008). Nevertheless, when bamboo forest grove is mulched, the organic material heats and ferments. The aerobic microorganisms consume oxygen directly from the soil, and the thick mulching material stops ambient oxygen from entering the soil, which causes a lack of oxygen at the root level, unlike flooding and soil slumping (Jiang et al., 2009; Qian et al., 2020). We discovered in the investigation that soil temperatures increased and soil oxygen concerntration decreased during the mulching period (Figure 2A, B; Table 2), which is in line with previous research findings (Qian et al., 2020).

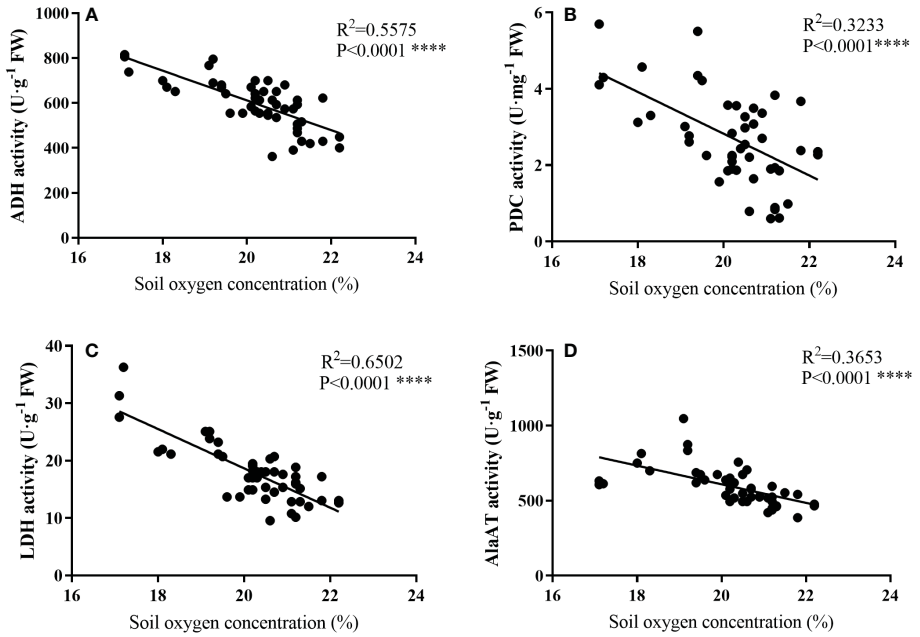

FIGURE 6
Relationships between soil oxygen concerntration (%) and the activities of ADH **(A)**, PDC **(D)**, LDH **(C)**, and AlaAT **(D)** of bamboo roots under mulching conditions (Control, Mulching, Aeration, M + A). Data are from Figures 1 and 3 and represent mean values. Regression equations are $Y = -66.22X_1 + 1936$, $Y = -0.5478X_2 + 13.78$, $Y = -3.426X_3 + 87.18$ and $Y = -62.03X_4 + 1850$, respectively, for **(A–D)** (Y is the soil oxygen concerntration, and X_1, X_2, X_3, and X_4 represent ADH, PDC, LDH, and AlaAT, respectively). R^2 is determined as the coefficient of determination. ($p < 0.01$, n=48).

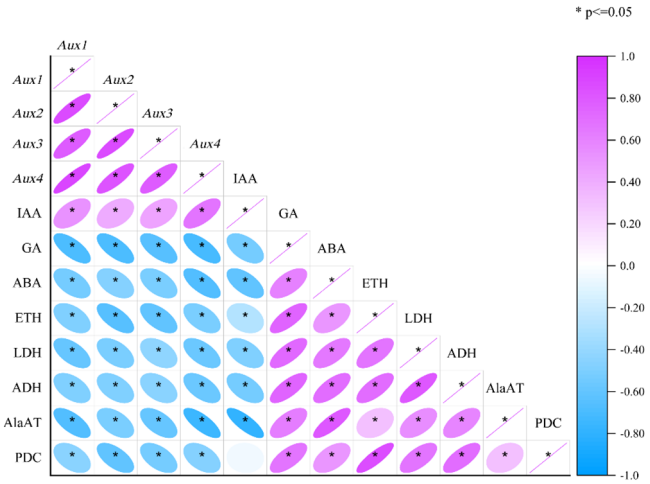

FIGURE 7
Correlation matrix of physiological and gene factors of the roots cultivated with and without mulching and aeration. PDC: pyruvate decarboxylase; AlaAT: alanine aminotransferase; LDH: lactate dehydrogenase; ADH: alcohol dehydrogenase; IAA: indole-3-acetic acid; ETH: ethylene; ABA: abscisic acid; GA: gibberellic acid and *Aux1, 2, 3, 4*: auxin-related genes 1, 2, 3 and 4, respectively.

However, aeration significantly reduced soil temperature, increased oxygen content, diameter at breast height, shoot production and root growth, suggesting that soil aeration using buried pipes is effective in improving soil condition and bamboo growth. After removal of the mulch, soil oxygen concentrations recovered substantially but remained slightly lower than in the control. Also, soil temperatures did not differ significantly between the three treatments from June onwards. It is possible that mulching changed the soil microbial populations and soil structure, but after the organic material was removed, the soil repaired itself and gradually returned to the control level. Root growth is known to be limited by low soil oxygen availability (Christianson et al., 2010; Cruz et al., 2019). Hence, we found that mulching reduced root activity while aeration increased it. Also, it has stronger root activity in March than the other months (Figure 2C; Table 2). It is similar to previous research findings, Holthausen and Caldwell (1980) suggested that root system's breathing capacity varies seasonally, meaning that respiratory capacity peaks in spring and a respiratory minimum occurs in late summer. This tendency might be attributed to environmental pretreatment together with an overall genetic-based program to extend the length of root activity and reduce the root system's carbon requirement (Holthausen and Caldwell, 1980).

4.2 Aeration changed anaerobic enzyme activity under mulching

Pyruvate produced by glycolysis will undergo anaerobic respiration (also called fermentation) in plant cells once the oxygen content is low (Yazdani and Gonzalez, 2007). The reversible conversion of alanine and 2-ketoglutarate to pyruvate and glutamate is catalyzed by AlaAT (Ricoult et al., 2005). Fermentation may be classified into two types: lactic acid fermentation, in which LDH is responsible for catalyzing the transformation of lactate to pyruvate then back, with the end product being lactate; and alcoholic fermentation, in which ADH catalyzes the acetaldehyde-to-ethanol conversion. PDC catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA and NADH, with carbon dioxide and ethanol as byproducts (Bailey-Serres and Chang, 2005; Arbona et al., 2010; Armstrong et al.,

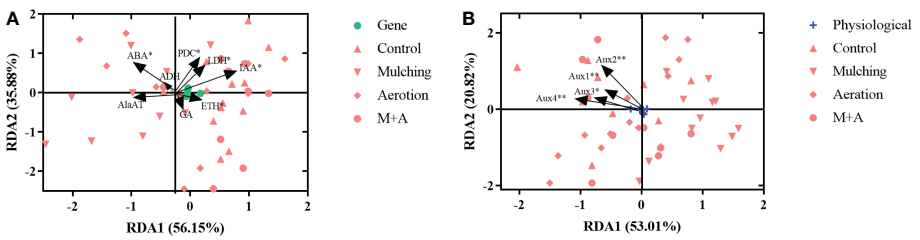

FIGURE 8
Redundancy analysis results reveal the correlations between physiological **(A)** and gene **(B)** factors in roots under mulching and aeration conditions. PDC: pyruvate decarboxylase; AlaAT: alanine aminotransferase; LDH: lactate dehydrogenase; ADH: alcohol dehydrogenase; IAA: indole-3-acetic acid; ETH: ethylene; ABA: abscisic acid; GA: gibberellic acid and *Aux1, 2, 3, 4*: auxin-related genes 1, 2, 3 and 4, respectively.

TABLE 2 The F value is obtained from the analysis of variance (ANOVA) on the data of the factors in the roots when different aeration measures were applied under mulching. *, **, *** significant at 0.05, 0.01 and 0.001 probability, respectively.

Source of variation	df	Soil O_2 content	Soil temperature	Root activity	PDC	AlaAT	ADH	LDH	ETH	ABA	GA	IAA	*Aux1*	*Aux2*	*Aux3*	*Aux4*
Time-M	3	22.87***	7.05***	11.82**	0.81	4.86*	3.07	4.25*	7.31*	20.21***	0.26	7.31**	6.50**	5.09*	9.04***	7.32**
Mulching	1	428.03***	30.20***	86.20***	30.2***	59.03***	61.21***	136.33***	63.61***	125.17***	49.66***	134.01***	105.60***	60.97***	109.34***	184.88***
Time-A	3	0.95*	0.37	0.14	3.03*	0.73	1.10*	3.95*	0.23	9.17**	0.12	1.22*	1.42*	4.12*	5.81**	6.67**
Aeration	1	69.55***	0.01	27.92***	226.09***	82.17***	119.04***	122.14***	60.57***	180.26***	35.73***	64.62***	51.69***	71.41***	66.79***	117.67***
Time-M+A	3	25.81***	10.35***	6.38**	2.35*	0.73	0.14	4.77**	1.96*	9.62**	0.80	6.98**	1.36*	1.93*	3.09**	1.84*
M+A	1	423.01***	2.05*	70.77***	36.36***	55.56***	51.30***	89.80***	114.40***	126.28***	39.12***	54.90***	97.97***	68.27***	93.18***	31.53***
Mulching × time	3	17.58***	1.05*	11.86***	1.05*	5.131*	2.78	11.09***	4.75*	18.74***	0.37	5.89**	9.40***	3.07	10.93***	8.55***
Aeration × time	3	0.81	0.46	0.49	0.47	0.97*	0.44	1.63*	0.22	9.26**	0.11	0.14	0.21	3.14	6.78**	1.33*
M+A × time	3	26.84***	9.83***	11.74***	1.97*	3.09**	0.33	4.68**	1.99*	9.77**	0.70	2.40**	24.60***	1.92***	4.46**	0.822*

2019). Neither type of fermentation produces ATP molecules and both are detrimental to cell survival (Bailey-Serres and Chang, 2005). Our results showed that the activities of PDC, LDH, AlaAT and ADH were significantly increased of bamboo root during the mulching period (Figure 3A-D; Table 2). This discovery is in line with the findings of Qian et al. (2020). After the mulch was removed, anaerobic enzyme activity of mulching remained greater than in the control, but aeration drastically decreased anaerobic enzyme activity. It occurred because the mulching technique lowered soil oxygen concentration and enhanced anaerobic enzyme activity. There was a linear relationship between the activities of the anaerobic enzymes ADH, PDC, LDG, and AlaAT and soil oxygen concerntration (Figure 6; Table 2). Extensive molecular and biochemical analyses revealed the mechanism behind these relationships. In hypoxic tissues in barley (*Hordeum vulgare*) and *M. truncatula*, AlaAT activity and gene expression are stimulated (Muench and Good, 1994; Bray et al., 2002; Ricoult et al., 2005). Previous studies have shown that flood-tolerant plants accumulate alanine by activating AlaAT, and that the alanine is carried *via* the xylem and becomes a transportable energy source (De Sousa and Sodek, 2003). AlaAT is essential for plant life not only in hypoxia, but also throughout the reoxygenation period following hypoxia (Nakamura and Noguchi, 2020). Additionally, when the oxygen content was inadequate in the root zone, the activities of LDH, PDC, and ADH, as well as the expression of the genes that encode these enzymes, were elevated in cucumber (Xu et al., 2014). Increased anaerobic enzyme activity may enhance plant tolerance to hypoxia (Kato-Noguchi, 2001). For example, plants of white clover with strong ADH activity, demonstrate better flood tolerance under flood stress than plants with weak ADH activity (Chan and Burton, 1992). Generally, subsurface buried pipe aeration reduced the anaerobic enzyme activity of bamboo roots caused by mulching with organic materials.

4.3 Aeration regulated hormone variation under mulching

The levels of some phytohormones in *P. praecox* are highly susceptible to external environmental conditions, and the insulating effect of mulching disrupts the balance of endogenous hormones. Endogenous plant hormones, including IAA, GA, ABA, and ETH, are the "switches" that modulate and control plant growth (Davies, 2004). A previous study showed that lateral shoots at the base of bamboo plants had significantly higher IAA/ABA and ZT/ABA levels one year after mulching than did plants grown without mulching, thus promoting early differentiation of lateral shoots (Huang et al., 2002). However, this does not correspond with our experimental results, where we found significant increases in GA and ABA contents and a reduction in growth hormone content of roots treated with organic material mulch for consecutive years, and this was also found in degraded *P. praecox* stands (Figure 4; Table 2). The ABA, GA, and cytokinin (CTK) contents of flowering bamboo in the degraded *P. praecox* forest were all higher than in unflowered bamboo, with the most significant increase being in ABA content (He et al., 2005). It

might be because long-term mulching inhibited plant root growth, but the anoxic environment caused by mulching allowed the roots of bamboo to stretch towards soil surface to find more oxygen, thus increasing the contents of ABA, which promotes the formation of plant organ separation, and GA, which promotes cell elongation and division, ultimately leading to degradation of the bamboo forest (Davies, 2004). Previously, Xu et al. (2017) also found that long-term mulching caused roots to grow toward the ground in search of oxygen, which promoted root elongation. This phenomenon is also observed in rice. Deep-water rice leaves and internodes may stretch and grow above the water surface under flood circumstances to gather oxygen and prevent drowning (Ayano et al., 2015). We also found that the higher ABA contents in September and December and the higher IAA contents in March and June may be related to the growth habit of the plant (Figure 4A, B; Table 2), where the plant grows vigorously in spring and summer, while abscisic acid inhibits germination and promotes dormancy and plant organ separation in autumn and winter (Baktir et al., 2004; Davies, 2004). In addition, the actions of ABA and IAA are antagonistic, and one study showed that ABA may function as an inhibitor of GA and restrict root development, allowing the plant to survive during flooding (Wu and Hong, 2021). Our results suggest that there may be some antagonistic effects between ABA and IAA and GA in the hypoxic environment caused by organic material mulching (Figure 7; Table 2). In addition to this, it has been shown that the fast buildup of ethylene in submerged tissues (through physical trapping and active synthesis) under anoxic circumstances, causes alterations in branch lengthening, glucose metabolism, and adventitious root development (Steffens and Sauter, 2006; Xu et al., 2006; Hattori et al., 2009). At the same time, the equilibrium of GA and ABA contents is likewise coordinated by ETH under anoxic conditions caused by submergence (Xu et al., 2006). In this investigation, we discovered significant increases in ETH content and the activities of enzymes involved in ethylene synthesis (ACO, ACS), and also a significant positive correlation between ETH and GA contents under organic material cover (Figures 3E, F; 4D; 7; Table 2). It is due to the synergy established by the combination of ETH and GA. From this we can infer that ETH perception is essential for adventitious root development, and GA substantially promotes the ensuing ETH-induced adventitious root growth (Steffens and Sauter, 2006). Aeration from the buried pipes provided oxygen to alleviate soil hypoxia caused by mulching, thus changing the hormone contents in the bamboo roots by reducing the ABA, GA, and ETH contents, decreasing the activities of ACS and ACO, increasing the IAA content, and finally improving metabolic and physiological alterations in roots.

4.4 Aeration regulated *Aux/IAAs* gene expression under mulching

It is well known that auxin is the key regulator during plant growth (Wu et al., 2012; Eysholdt-Derzso and Sauter, 2017). IAA, a most abundant hormone in higher plants, is a weak acid, and growth hormone influx and efflux carriers promote its intercellular movement (Wu et al., 2012; Van and Licausi, 2015) Auxin transporters are necessary for the transfer of auxin into various cells. AUXIN1 (encoded by *Aux1*) is an auxin influx carrier. AUXIN1 is the major transporter for auxin uptake in root hairs and it controls root gravitropism, root hair formation, and leaf phyllotaxy (Ori, 2019). In this study, we found that mulching reduced the expression of *Aux1* (Figure 5A), and that *Aux1* expression was highly associated with growth hormone and unfavorably related to ETH concentrations (Li et al., 2015). *Aux2* and *Aux3* have been implicated in processes like as hypocotyl elongation and foliar growth in Arabidopsis and rice. *Aux3* regulates lateral root growth and root hair production, whereas *Aux4* regulates plant tiller height (Liscum and Reed, 2002; Overvoorde, 2005; Song and Xu, 2013). In the present study, mulching reduced gene expression of *Aux* genes, while aeration increased *Aux* gene expression (Figure 5). *Aux/IAAs* expression was favorably linked with IAA and negatively associated with ethylene (Figures 7, 8). It is because ETH can control IAA synthesis by regulating *Aux1* expression and growth hormone synthesis-related genes, which in turn regulate root development under adverse situations (Li et al., 2015).

Following aeration, the correlations between each enzyme and hormones indicator and gene expression were also investigated. *Aux/IAAs* gene expression was highly related to many enzyme and hormones factors, and the expression of *Aux1*, *2*, *3*, and *4* was closely associated with root development factors (Figure 8). The results imply that *Aux/IAAs* genes are engaged in the management of hormone levels as well as the regulation of anaerobic enzymes and root respiration activity to keep proper root development, while *Aux/IAAs* contributing in this mechanism (Gao et al., 2022). Abiko et al. (2012) showed the expression of *Aux/IAA* in *Zea nicaraguensis* of hypoxic circumstances altered dramatically, and it may also govern the development of adventitious roots and the production of vented tissue. Mulching caused fast alterations in a number of critical enzyme and hormones markers in *P. praecox*. Here, LDH, PDC, ABA, IAA, and ETH all had significant impacts on the expression of *Aux/IAAs* (Figure 8). It demonstrates that the overlay affects *Aux/IAAs* expression in plants, which in turn regulates changes in endogenous hormone levels that are involved in regulating anaerobic respiratory enzymes and ultimately improving ability of plant roots to cope with hypoxia caused by organic materials.

Overall, the findings of our investigation demonstrate that mulching with organic material degrades *P. praecox* forests (Figure 9), which consistent with the phenomenon observed informally by local farmers (Xu et al., 2017). We are here for the first time to demonstrate the mechanism of underground pipeline aeration to mitigate the degradation of bamboo forest. We also explain for the first time that hormones crosstalk with *Aux/IAAs* and thus regulate changes in enzyme and hormones indicators under bamboo forest mulching. We therefore are of the opinion that our subsurface aeration strategy will help to mitigate soil

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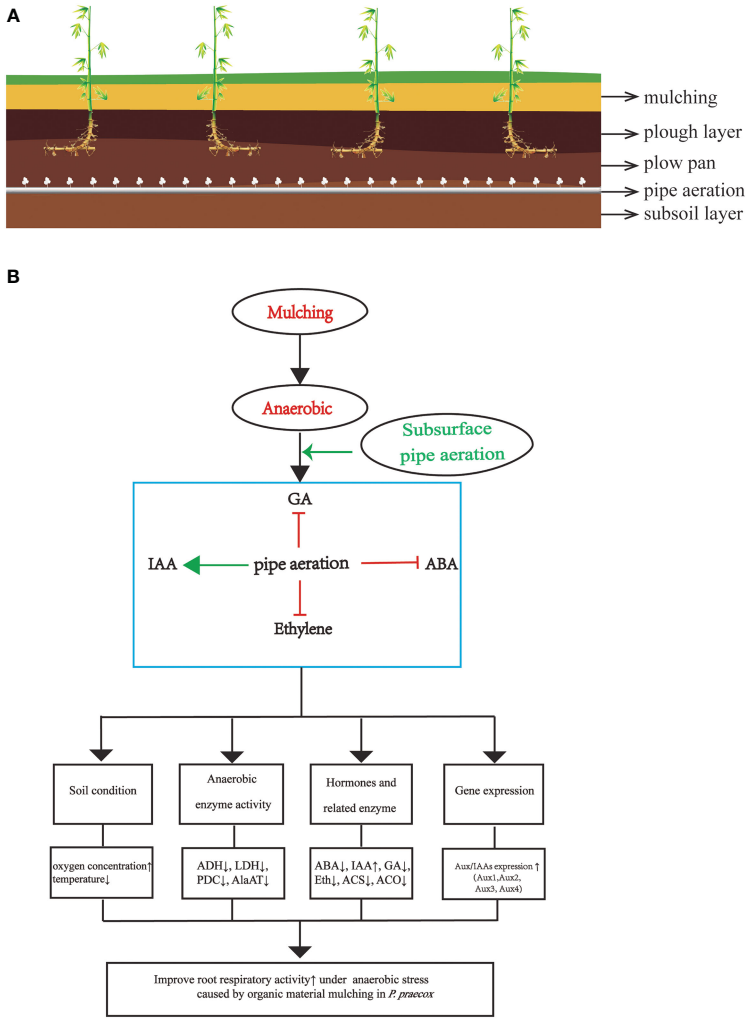

FIGURE 9
(A) the scheme of soil horizons including plants and air-pipe system; **(B)** a model for root aeration through hormone in *P. praecox*. Aeration promotes downstream protective systems in mulching *via* interacting with hormones (decreasing ABA, GA and ethylene content as well as increaseing IAA content), for instance, lowering soil temperature, anaerobic enzyme activity, increasing soil oxygen concentration, changing hormone synthase related activity, as well as increasing the expression of *Aux/IAAs* to stimulate root growth. Green arrow represents promotion, whereas red t-bars denote inhibition.

hypoxia and, in turn, improve the growth of bamboo. However, the intensity and time of the aeration needs to be studied in detail in the future.

There are certain limitations to this study, namely that we only studied the effects of short-term aeration on plant biochemistry. Different aeration times may also have inconsistent effects on plant growth. Moreover, our experiment lasted for one year, which is a short period of time compared to a long-term bamboo mulch, and long-term monitoring of soil changes to determine physiological and biochemical changes in bamboo roots could be conducted in future studies. Due to the lack of research on the application of aeration systems to alleviate soil hypoxia caused by organic mulching, many scientific and technical problems remain. Our study could serve as a representative example of this research area and generate interest in further research on the role of post-mulching aeration in various cropping systems.

5 Conclusions

Mulching with organic matter resulted in a decline in soil oxygen content and a reduction in shoot yield and root growth accompanied with increasing activities of anaerobic enzymes (ADH, LDH, PDC, and AlaAT). Here we innovatively propose a mechanism for improving the degradation of bamboo forest by underground pipeline aeration and find the crosstalk between hormones and *Aux/IAAs* under mulching and thus regulate the changes of enzyme and hormones indicators. Moreover, subsurface pipe aeration increased the expression of *Aux/IAAs* genes (*Aux1*, *Aux2*, *Aux3*, and *Aux4*) and IAA concentration, and reduced ABA, GA, and ETH concentrations and limited ETH synthesis enzyme activity (ACS and ACO) in the roots. The increased soil oxygen content improved root growth and shoot yield and reduced anaerobic enzyme activity, thus enhancing root resistance to

organic material mulching-induced hypoxia. These findings suggest that subsurface pipe aeration helps to mitigate mulch-induced root hypoxia in bamboo and support sustainable bamboo production.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding author.

Author contributions

JG and SZ conceived and designed the experiments. JG performed the experiments. JG and RG analyzed the data. JG drafted the manuscript. SZ and RG modified the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1121604/full#supplementary-material

References

Abiko, T., Kotula, L., Shiono, K., Malik, A. I., Colmer, T. D., and Nakazono, M. (2012). Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of zea nicaraguensis contribute to its waterlogging tolerance as compared with maize (*Zea mays ssp. mays*). *Plant Cell Environ.* 35, 1618–1630. doi: 10.1111/j.1365-3040.2012.02513.x

Achard, P., Gong, F., Cheminant, S., Alioua, M., Hedden, P., and Genschik, P. (2008). The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins *via* its effect on gibberellin metabolism. *Plant Cell* 20, 2117–2129. doi: 10.1105/tpc.108.058941

Arbona, V., Hossain, Z., López-Climent, M. F., Pérez-Clemente, R. M., and Gómez-Cadenas, A. (2010). Antioxidant enzymatic activity is linked to waterlogging stress tolerance in citrus. *Physiol. Planta.* 132, 452–466. doi: 10.1111/j.1399-3054.2007.01029.x

Armstrong, W., Beckett, P. M., Colmer, T. D., Setter, T. L., and Greenway, H. (2019). Tolerance of roots to low oxygen: 'Anoxic' cores, the phytoglobin-nitric oxide cycle, and energy or oxygen sensing. *J. Plant Physiol.* 239, 92–108. doi: 10.1016/j.jplph.2019.04.010

Ayano, M., Kani, T., Kojima, M., Sakakibara, H., and Ashikari, M. (2015). Gibberellin biosynthesis and signal transduction is essential for internode elongation in deepwater rice. *Plant Cell Environ.* 37, 2313–2324. doi: 10.1111/pce.12377

Bailey-Serres, J., and Chang, R. (2005). Sensing and signalling in response to oxygen deprivation in plants and other organisms. *Ann. BOT-London* 96 (4), 507–518. doi: 10.1093/aob/mci206

Bailey-Serres, J., and Voesenek, L. A. C. J. (2008). Flooding stress: acclimations and genetic diversity. *Annu. Rev. Plant Biol.* 59, 313–339. doi: 10.1146/annurev.arplant.59.032607.092752

Baktir, I., Ulger, S., and Kaynak, L. (2004). Relationship of seasonal changes in endogenous plant hormones and alternate bearing of olive trees. *HortScience* 39, 987–990. doi: 10.21273/HORTSCI.39.5.987

Bari, R., and Jones, J. D. G. (2009). Role of plant hormones in plant defence responses. *Plant Mol. Biol.* 69, 473–488. doi: 10.1007/s11103-008-9435-0

Barry, C. S., Blume, B., Bouzayen, M., Cooper, W., Hamilton, A. J., and Grierson, D. (1996). Differential expression of the 1-aminocyclo-propane-1-carboxylate oxidase gene family of tomato. *Plant J.* 9, 525–535. doi: 10.1046/j.1365-313x.1996.09040525.x

Bray, E. A., Bailey-Serres, J. W., and Weretilnyk, E. E. (2002). "Responses to abiotic stresses," in *Biochemistry and molecular biology of plants*. Ed. B. Buchanan, W. Gruissem and R. Jones (USA: Courier Companies Inc), 1158–1203.

Borcard, D., Legendre, P., and Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology* 73, 1045–1055. doi: 10.2307/1940179

Brookes, P. C., Powlson, D. S., and Jenkinson, D. S. (1982). Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14, 319–329. doi: 10.1016/0038-0717(82)90001-3

Chae, H. S., and Kieber, J. J. (2005). Eto brute? role of ACS turnover in regulating ethylene biosynthesis. *Trends Plant Sci.* 10, 291–296. doi: 10.1016/j.tplants.2005.04.006

Chan, J. W. Y., and Burton, R. S. (1992). Variation in alcohol dehydrogenase activity and flood tolerance in white clover, *Trifolium repens*. *Evolution* 46, 721–734. doi: 10.1111/j.1558-5646.1992.tb02078.x

Chandler, W. J. (2016). Auxin response factors. *Plant Cell Environ.* 39, 1014–1028. doi: 10.1111/pce.12662

Chen, S., Chen, S. L., and Fan, Y. R. (2014). Correlations between soil nutrient contents and nutrient characteristics of *Phyllostachys violascens* leaves under mulching management. *Guihaia* 34 (6), 793–798. doi: 10.3969/j.issn.1000-3142.2014.06.011

Christianson, J. A., Wilson, I. W., Llewellyn, D. J., and Dennis, E. S. (2010). The low-oxygen-induced NAC domain transcription factor ANAC102 affects viability of arabidopsis seeds following low-oxygen treatment. *Plant Physiol.* 149, 1724–1738. doi: 10.1104/pp.108.131912

Cruz, J., Lukasz, K., Veneklaas, E. J., and Colmer, T. D. (2019). Root-zone hypoxia reduces growth of the tropical forage grass *Urochloa humidicola* in high-nutrient but not low-nutrient conditions. *Ann. Bot-London* 6, 1019–1032. doi: 10.1093/aob/mcz071

Davies, P. J. (2004). "Introduction," in *Plant hormones, biosynthesis, signal transduction, action*. Ed. P. J. Davies (Dordrecht, The Netherlands: Springer), 1–35.

Davies, P. J. (2005). Plant hormones. *Br. Med. J.* 2, 1-15. doi: 10.1007/978-1-4020-2686-7

De Sousa, C., and Sodek, L. (2003). Alanine metabolism and alanine aminotransferase activity in soybean (*Glycine max*) during hypoxia of the root system and subsequent return to normoxia. *Environ. Exp. Bot.* 50, 1–8. doi: 10.1016/S0098-8472(02)00108-9

Dolferus, R., Wolansky, M., Carroll, R., and Miyashita, Y. (2008). Functional analysis of lactate dehydrogenase during hypoxic stress in *Arabidopsis*. *Funct. Plant Biol.* 35, 131–140. doi: 10.1071/FP07228

Drew, M. C. (1997). Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Biol.* 48, 223–250. doi: 10.1146/annurev.arplant.48.1.223

Eysholdt-Derzso, E., and Sauter, M. (2017). Root bending is antagonistically affected by hypoxia and ERF-mediated transcription *via* auxin signaling. *Plant Physiol.* 175 (1), 412–423. doi: 10.1104/pp.17.00555

Fan, C., Ma, J., Guo, Q., Li, X., Wang, H., and Lu, M. (2013). Selection of reference genes for quantitative real-time PCR in bamboo (*Phyllostachys edulis*). *PloS One* 8 (2), 1–8. doi: 10.1371/journal.pone.0056573

Fukaki, H., Tameda, S., Masuda, H., and Tasaka, M. (2010). Lateral root formation is blocked by a gain-of-function mutation in the *solitary-root/IAA14* gene of arabidopsis. *Plant J.* 29, 153–168. doi: 10.1046/j.0960-7412.2001.01201.x

Fukao, T., and Bailey-Serres, J. (2004). Plant responses to hypoxia is survival a balancing act? *Trends Plant Sci.* 9, 449–456. doi: 10.1016/j.tplants.2004.07.005

Fukao, T., Xu, K., and Bailey-Serres, R. J. (2006). A variable cluster of ethylene response factor–like genes regulates metabolic and developmental acclimation responses to submergence in rice. *Plant Cell* 18, 2021–2034. doi: 10.1105/tpc.106.043000

Gao, J. S., Qian, Z. Z., Zhang, Y. H., and Zhuang, S. Y. (2022). Exogenous spermidine regulates the anaerobic enzyme system through hormone concentrations and related-gene expression in *Phyllostachys praecox* roots under flooding stress. *Plant Physiol. Bioch.* 186, 182–196. doi: 10.1016/j.plaphy.2022.07.002

Gibbs, D. J., Lee, S. C., Isa, N. M., Corbineau, F., and Bailey-Serres, J. (2011). Homeostatic response to hypoxia is regulated by the n-end rule pathway in plants. *Nature* 479, 415–418. doi: 10.1038/nature10534

Gibbs, J., Morrell, S., Valdez, A., Setter, T. L., and Greenway, H. (2000). Regulation of alcoholic fermentation in coleoptiles of two rice cultivars differing in tolerance to anoxia. *J. Exp. Bot.* 51, 785–796. doi: 10.1093/jexbot/51.345.785

Gui, R. Y., Sun, X., and Zhuang, S. Y. (2013). Soil acidification in *Phyllostachys praecox* f. preveynalis cultivation with intensive management. *Commun. Soil Sci. Plant* 44 (22), 3235–3245. doi: 10.1080/00103624.2013.841915

Guo, Z. W., Hu, J. J., and Yang, Q. (2015). Influence of mulching management on the relationships between foliar non-structural carbo-hydrates and n, p concentrations in *Phyllostachys violascens* stand. *J. Appl. Ecol.* 26 (4), 1064–1070. doi: CNKI:SUN:YYSB.0.2015-04-014

Guo, Z. W., Yu, M. Z., and Zheng, L. X. (2011). Stoichiometry of c, n and p in *Phyllostachys iridescens* leaves under long-term application of different fertilizers. *J. Ecol.* 30 (12), 2667–2671. doi: CNKI:SUN:STXZ.0.2011-12-002

Hartman, S., Sasidharan, R., and Voesenek, L. A. C. J. (2021). The role of ethylene in metabolic acclimations to low oxygen. *New Phytol.* 229 (1), 64–70. doi: 10.1111/nph.16378

Hattori, Y., Nagai, K., and Furukawa, S. (2009). The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deep water. *Nature* 460, 1026–1030. doi: 10.1038/nature08258

He, Q., Wang, K. H., Hua, X. Q., and Tong, X. Q. (2005). Change of endogenous hormones, amino-acid and nutrition in flowering stage of *Phyllostachys praecox* f. prevernalis. *Sci. Silvae Sinicae.* 41, 169–173. doi: 10.3321/j.issn:1001-7488.2005.02.029

Holdsworth, M. J., Bentsink, L., and Soppe, W. J. J. (2008). Molecular networks regulating arabidopsis seed maturation, after-ripening, dormancy and germination. *New Phytol.* 179, 33–54. doi: 10.1111/j.1469-8137.2008.02437.x

Holthausen, R. S., and Caldwell, M. M. (1980). Seasonal dynamics of root system respiration in *Atriplex confertifolia*. *Plant Soil* 55, 307–317. doi: 10.1007/bf02181810

Hu, X. H., Guo, S. R., and Jing, L. I. (2005). Effects of hypoxia stress on anaerobic respiratory enzyme and antioxidant enzyme activities in roots of cucumber seedlings. *J. Wuhan Botanical Res.* 23 (4), 337–341. doi: 10.1017/S0308210500024860

Hu, C. Z., Jin, A. W., and Zhang, Z. W. (1996). Change of endohormone in mixed bud on lei bamboo rhizome during differentiation. *J. Zhejiang For Coll.* 13, 1–4. doi: 10.1007/BF02951625

Huang, M. Z., Chen, J. H., and Wang, L. Z. (2007). Reconstruction technology of degraded forest stand. *Cultivation Technique* 11, 12–13. doi: 10.3969/j.issn.1671-4938.2007.11.005

Huang, J. Q., Liu, L., Zhang, B. S., and Qiu, L. Z. (2002). Dynamic changes of endophytohormones in rhizomal buds of *Phyllostachys Praecox*. *Scientia Silvae Sinicae* 38 (3), 38–41. doi: 10.3321/j.issn:1001-7488.2002.03.007

Jasim, A. H., and Merhij, E. I. (2013). Effect of soil mulch and fertilizers on alleviating of salt stress of chlorophyll, leaf area and hormones content of broccoli plants (*Brassica oleracea* var. italica). *Euphrates J. Agric. Sci.* 5, 48–58.

Jiang, P. K., Wang, H. L., and Zhou, G. M. (2009). Winter mulch increases soil CO_2 efflux under *Phyllostachys praecox* stands. *J. Soil Sediment* 9 (6), 511–514. doi: 10.1007/s11368-009-0134-5

Julian, B., and Peter, C. (1989). Generalized Monte Carlo significance tests. *Biometrika* 76 (4), 633–643. doi: 10.1093/biomet/76.4.633

Kathleen, P. I., Rudy, D., Mary, D. P., Elizabeth, S. D., and Allen, G. G. (2003). Enhanced low oxygen survival in arabidopsis through increased metabolic flux in the fermentative pathway. *Plant Physiology* 132, 3, 1292–1302. doi: 10.1104/pp.103.022244

Kato-Noguchi, H. (2001). The importance of ethanolic fermentation for primary root growth of germinating rice under anoxia. *Plant Growth Regul.* 35, 181–185. doi: 10.1023/A:1014439432584

Larkin, M. A., Blackshields, G., and Brown, N. P. (2007). Clustal W and clustal X version 2.0. *Bioinformatics* 23, 2947–2948. doi: 10.1093/bioinformatics/btm404

Li, J., Xu, H., Liu, W., Zhang, X., and Lu, Y. T. (2015). Ethylene inhibits root elongation during alkaline stress through *AUXIN1* and associated changes in auxin accumulation. *Plant Physiol.* 168 (4), 1777–1791. doi: 10.1104/pp.15.00523

Li, C., Yin, C., and Liu, S. (2004). Different responses of two contrasting *Populus* davidiana populations to exogenous abscisic acid application. *Environ. Exp. Bot.* 51, 237–246. doi: 10.1016/j.envexpbot.2003.11.001

Li, L. H., Xia, T., Lin, B., and Yang, H. Q. (2022). Hormone and carbohydrate metabolism associated genes play important roles in rhizome bud full-year germination of *Cephalostachyum pingbianense*. *Physiol Plantarum* 174 (2), 1–19. doi: 10.1111/ppl.13674

Liscum, E., and Reed, J. W. (2002). Genetics of *Aux/IAA* and *ARF* action in plant growth and development. *Plant Mol. Biol.* 49, 387–400. doi: 10.1007/978-94-010-0377-3_10

Liu, G., and Porterfield, D. M. (2015). Oxygen enrichment with magnesium peroxide for minimizing hypoxic stress of flooded corn. *J. Plant Nutr. Soil Sci.* 177, 733–740. doi: 10.1002/jpln.201300424

Liu, G., Porterfield, D. M., and Klassen, K. (2012). Increased oxygen bioavailability improved vigor and germination of aged vegetable seeds. *Hort Sci.* 47, 1714–1721. doi: 10.21273/HORTSCI.47.12.1714

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Morimoto, K., and Yamasue, Y. (2007). Differential ability of alcohol fermentation between the seeds of flooding-tolerant and flooding-susceptible varieties of *Echinochloa crus-galli (L.) beauv*. *Weed Biol. Manage.* 7, 62–69. doi: 10.1111/j.1445-6664.2006.00229.x

Muench, D. G., and Good, A. G. (1994). Hypoxically inducible barley alanine aminotransferase: cDNA cloning and expression analysis. *Plant Mol. Biol.* 24, 417–427. doi: 10.1007/BF00024110

Mulumba, L. N., and Lal, R. (2008). Mulching effects on selected soil physical properties. *Soil Till. Res.* 98, 106–111. doi: 10.1016/j.still.2007.10.011

Nakamura, M., and Noguchi, K. (2020). Tolerant mechanisms to O_2 deficiency under submergence conditions in plants. *J. Plant Res.* 133, 343–371. doi: 10.1007/s10265-020-01176-1

Nguyen, T. N., Tuan, P. A., Mukherjee, S., Son, S., and Ayele, B. T. (2018). Hormonal regulation in adventitious roots and during their emergence under waterlogged conditions in wheat. *J. Exp. Bot.* 69 (16), 4065–4082. doi: 10.1093/jxb/ery190

Ori, N. (2019). Dissecting the biological functions of *ARF* and *Aux/IAA* genes. *Plant Cell* 31 (6), 1210–1211. doi: 10.1105/tpc.19.00330

Overvoorde, P. J. (2005). Functional genomic analysis of the *AUXIN/INDOLE-3-ACETIC ACID* gene family members in *Arabidopsis thaliana*. *Plant Cell* 17, 3282–3300. doi: 10.1105/tpc.19.00330

Peleg, Z., and Blumwald, E. (2011). Hormone balance and abiotic stress tolerance in crop plants. *Curr. Opin. Plant Biol.* 14 (3), 290–295. doi: 10.1016/j.pbi.2011.02.001

Qian, Z. Z., Zhuang, S. Y., Gui, R. Y., and Tang, L. Z. (2020). Effect of soil aeration treatment on the physiological and biochemical characteristics of *Phyllostachys praecox* under the organic material mulching. *Plant Soil* 459, 357–369. doi: 10.1007/s11104-020-04770-3

Qian, Z. Z., Zhuang, S. Y., Gao, J. S., and Tang, L. Z. (2022). Can aeration improve bamboo soil fertility of soil below bamboo and fungal diversity under mulching conditions? *Land Degrad. Dev.* 33 (13), 2353–2365. doi: 10.1002/ldr.4311

Qiao, L. Y., Li, X., and Chang, Z. J. (2014). Whole-genome sequence isolation, chromosome location, and characterization of primary auxin-responsive *Aux/IAA* gene family in *Aegilops tauschii*. *Acta Agronomica Sin.* 40, 2059–2069. doi: 10.3724/SP.J.1006.2014.02059

Remington, D. L., Vision, T. J., Guilfoyle, T. J., and Reed, J. W. (2004). Contrasting modes of diversification in the *Aux/IAA* and *ARF* gene families. *Plant Physiol.* 135 (3), 1738–2004. doi: 10.1104/pp.104.039669

Ricoult, C., Cliquet, J. B., and Limami, A. M. (2005). Stimulation of *alanine amino transferase* (*AlaAT*) gene expression and alanine accumulation in embryo axis of the model legume *Medicago truncatula* contribute to anoxia stress tolerance. *Physiol. Planta.* 123, 30–39. doi: 10.1111/j.1399-3054.2005.00449.x

Ricoult, C., Echeverria, L. O., Cliquet, J. B., and Limami, A. M. (2006). Characterization of *alanine amino transferase* (*AlaAT*) multigene family and hypoxic response in young seedlings of the model legume *Medicago truncatula*. *J. Exp. Bot.* 57, 3079–3089. doi: 10.1093/jxb/erl069

Rieu, I., Cristescu, S. M., and Harren, F. J. (2005). *RP-ACS1*, a flooding-induced 1-aminocyclopropane-1-carboxylate synthase gene of rumex palustris, is involved in rhythmic ethylene production. *J. Exp. Bot.* 56, 841–849. doi: 10.1093/jxb/eri078

Robertson, A. J., Ishikawa, M., Gusta, L. V., and MacKenzie, S. L. (1994). Abscisic acid-induced heat tolerance in bromus inermis leyss cell-suspension cultures: Heat-stable, abscisic acid-responsive polypeptides in combination with sucrose confer enhanced thermostability. *Plant Physiol.* 105, 181–190. doi: 10.1104/pp.105.1.181

Rzewuski, G., and Sauter, M. (2008). Ethylene biosynthesis and signaling in rice. *Plant Sci.* 175, 32–42. doi: 10.1016/j.plantsci.2008.01.012

Santner, A., Calderon-Villalobos, L. I. A., and Estelle, M. (2009). Plant hormones are versatile chemical regulators of plant growth. *Nat. Chem. Biol.* 5 (5), 301–307. doi: 10.1038/nchembio.165

Singla, B., Chugh, A., Jitendra, P., and Khurana, P. K. (2006). An early auxin-responsive *Aux/IAA* gene from wheat (*Triticum aestivum*) is induced by epibrassinolide and differentially regulated by light and calcium. *J. Exp. Bot.* 57 (15), 4059–4070. doi: 10.1093/jxb/erl182

Song, Y. L., and Xu, Z. F. (2013). Ectopic overexpression of an AUXIN/INDOLE-3-ACETIC ACID (*Aux/IAA*) gene *OsIAA4* in rice induces morphological changes and reduces responsiveness to auxin. *Int. J. Mol. Sci.* 14, 13646–13656. doi: 10.3390/ijms140713645

Steffens, B., and Sauter, W. M. (2006). Interactions between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious roots in deepwater rice. *Planta* 223, 604–612. doi: 10.1007/s00425-005-0111-1

Strudley, M. W., Green, T. R., and James, I. I. (2008). Tillage effects on soil hydraulic properties in space and time: State of the science. *Soil Till. Res.* 99, 4–48. doi: 10.1016/j.still.2008.01.007

Szemenyei, H., Hannon, M., and Long, J. A. (2008). Topless mediates auxin-dependent transcriptional repression during arabidopsis embryogenesis. *Science* 319, 1384–1386. doi: 10.1126/science.1151461

Tognetti, V. B., Muhlenbock, P., and Van, B. F. (2012). Stress homeostasis the redox and auxin perspective. *Plant Cell Environ.* 35, 321–333. doi: 10.1111/j.1365-3040.2011.02324.x

Van, D. J. T., and Licausi, F. (2015). Oxygen sensing and signaling. *Annu. Rev. Plant Biol.* 66, 345–367. doi: 10.1146/annurev-arplant-043014-114813

Visser, E. J. W., and Voesenek, L. A. C. J. (2006). Acclimation to soil flooding-sensing and signal-transduction. *Plant Soil* 274, 197–214. doi: 10.1007/s11104-004-1650-0

Vriezen, W. H., Hulzink, R., Mariani, C., and Voesenek, L. A. C. J. (1999). 1-Aminocyclopropane-1-carboxylate oxidase activity limits ethylene biosynthesis in *Rumex palustris* during submergence. *Plant Physiol.* 121, 189–195. doi: 10.1104/pp.121.1.189

Weits, D. A., Kunkowska, A. B., and Kamps, N. C. W. (2019). An apical hypoxic niche sets the pace of shoot meristem activity. *Nature* 569, 714–717. doi: 10.1038/s41586-019-1203-6

Wu, C. Y., and Hong, C. Y. (2021). An *in vivo* GA- and ABA-responsive dual-luciferase reporter system for simultaneous detection of GA and ABA responses, hormone crosstalk and heat stress response in rice. *Plant Biotechnol. J.* 19, 1486–1488. doi: 10.1111/pbi.13630

Wu, J., Peng, Z., Liu, S., He, Y., Cheng, L., and Kong, F. (2012). Genome-wide analysis of *Aux/IAA* gene family in *Solanaceae* species using tomato as a model. *Mol. Genet. Genomics* 287, 295–311. doi: 10.1007/s00438-012-0675-y

Wu, J. J., Wang, C. A., Zheng, L. Q., Wang, L., and Chen, Y. L. (2011). Ethylene is involved in the regulation of iron homeostasis by regulating the expression of iron-acquisition-related genes in *Oryza sativa*. J. *Exp. Bot.* 62, 667–674. doi: 10.1093/jxb/erq301

Xu, X. W., Wang, H. H., and Qi, X. H. (2014). Waterlogging-induced increase in fermentation and related gene expression in the root of cucumber (*Cucumis sativus* l.). *Sci. Hortic-Amsterdam* 179, 388–395. doi: 10.1016/j.scienta.2014.10.001

Xu, K., Xu, X., Fukao, T., Canlas, P., and Maghirang-Rodriguez, R. (2006). *Sub1A* is an ethylene-response-factor-like gene that confers submergence tolerance to rice. *Nature* 442, 705–708. doi: 10.1038/nature04920

Xu, J., and Zhang, S. (2015). Ethylene biosynthesis and regulation in plants. *Ethylene Plants*, 1–25. doi: 10.1007/978-94-017-9484-8_1

Xu, M. J., Zhuang, S. Y., and Gui, R. Y. (2017). Soil hypoxia induced by an organic-material mulching technique stimulates the bamboo rhizome up-floating of *Phyllostachys praecox*. *Sci. Rep-UK* 7, 1–6. doi: 10.1038/s41598-017-14798-8

Yazdani, S. S., and Gonzalez, R. (2007). Anaerobic fermentation of glycerol: a path to economic viability for the biofuels industry. *Curr. Opin. Biotechnol.* 18, 213–219. doi: 10.1016/j.copbio.2007.05.002

Zhu, X. F., Zhu, C. Q., Zhao, X. S., Zheng, S. J., and Shen, R. F. (2016). Ethylene is involved in root phosphorus remobilization in rice (*Oryza sativa*) by regulating cell-wall pectin and enhancing phosphate translocation to shoots. *Ann. Bot.* 118, 645–653. doi: 10.1093/aob/mcw044

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Individual and combined effects of arbuscular mycorrhizal fungi and phytohormones on the growth and physiobiochemical characteristics of tea cutting seedlings

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Both arbuscular mycorrhizal fungi (AMF) and phytohormones collectively regulate plant growth and root development, but their individual and combined effects on tea [*Camellia sinensis* (L.) O. Kuntze] cutting seedings remain unclear. This study examined the individual and combined effects of two species of AMF (*Rhizophagus intraradices*, RI and *Funneliformis mosseae*, FM) and two types of palnt hormones (strigolactones, SLs; polyamines, PAs) on tea cutting seedings, by evaluating the growth and physiobiochemical characteristics of plants treated with the AMFs and/or hormones. The results showed that inoculation with either AMF individually or hormones treatment alone could significantly enhanced mycorrhizal colonization, growth target and physiobiochemical characteristics of tea cutting seedlings. Interestingly, the addition of a combination of AMFs and hormones showed superior effects, while SL and RI exhibited the most improvements to the colonization rate, plant growth, root-morphological traits, root DHA activity, photosynthesis, chlorophyll content, soluble sugar content in leaves, and the activities of antioxidant enzymes (SOD, POD, and CAT), compared to other treatment combinations (SL + FM, PA + RI, and PA + FM). Correlation analyses revealed a significantly ($p < 0.05$) positive correlation of root AMF colonization with root-related traits (e.g., DHA, root total length, surface area, and volume) and leaf-related traits (e.g., leaf area, shoot biomass, total chlorophyll, and antioxidant enzyme activities). This study demonstrated that while the apllication of individual AMF or plant hormones had a certain good effects on most growth and physiobiochemical characteristics parameters of tea cutting seedings, the additive effect was from specific combined of AMF and plant hormones. These results highlight the possibility for combined of AMF and plant hormones to improve the asexual reproduction of tea plants *via* cuttings.

KEYWORDS

***Camellia sinensis*, mycorrhiza, antioxidase, polyamines, strigolactone**

1 Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze] is a widely consumed aromatic beverage throughout the world and an important cash crop in China (Liu et al., 2021). As a perennial evergreen woody plant that generally grows in acidic soil (Singh et al., 2010), tea leaves are rich in polyphenols, amino acids, polysaccharides, flavonoids, and other natural active substances, which have multiple health benefits, including promoting immunity and the mental health of humans (Senanayake, 2013).

Though tea plant propagation can occur *via* sexual or asexual reproduction, asexual reproduction is mainly preferred as it ensures the inheritance of the mother plant's excellent characteristics and facilitates rapid propagation (Koyuncu and Balta, 2004; Romero, 2004). Tea plants are mainly propagated using the tea-cuttings method, which has been used for over 200 years in China (Wang et al., 2022). This asexual propagation method allows for a long breeding season and maintains the high purity of the tea variety (Wang et al., 2011). However, tea cutting seedlings with limited rooting have low survival rates. Therefore, it is important to improve the survival of tea cutting seedlings and promote their growth.

Arbuscular mycorrhizal fungus (AMFs) is a type of beneficial soil microorganism that benefits the host plant by improving root growth, nutrient absorption, soil properties, and stress resistance (Huang et al., 2011; Wipf et al., 2019). Tea plants live in symbiosis with AMFs and strongly depend on the capacity of AMFs for P uptake Shao et al, 2021. The AMF resources in the rhizosphere of tea are abundant, while *Acaulospora* and *Glomus* are the dominant AMF genera, and form good symbiotic relationships with tea plants (Liu et al., 2021). A previous study revealed that inoculation with four AMFs (*Claroideoglomus etunicatum*, *Diversispora spurca*, *D. versiformis*, and mixed-AMF) could promote the growth and biomass of tea seedlings (Shao et al., 2018). Meanwhile, inoculation with *C. etunicatum* significantly improved the leaf water content and antioxidant enzyme activity of tea plants under drought stress (Liu et al., 2020). In addition, AMF inoculation promoted the flavor and quality (e.g., catechins, amino acids, and tea polyphenols) of tea under phosphorus stress conditions (Shao et al., 2018; Cao et al., 2021a).

Increasing evidence has shown that AMF spore germination, hyphal growth, and root colonization were initiated by plant hormones (Requena et al., 2007; Sun et al., 2012; Liao et al., 2018; Pei et al., 2020). Plant hormones are known to be signaling molecules that act as important regulators of plant growth and root development, and have also been shown to play crucial roles in modulating the interactions between plants and AMFs.

Strigolactones (SLs), a new class of plant hormone, are synthesized in the plant roots and play an important role in in the regulation of plant growth, root development, and the overall morphological structure of plants (Akiyama et al., 2005). SLs are also involved in enhancing plant resistance to biotic and abiotic stresses (Sedaghat et al., 2017; Min et al., 2019). Recently, it has become clear that SLs not only stimulate seed germination, but also promote hyphae branching, activate mitochondrial function, release small molecular functional proteins of AMFs (Akiyama et al., 2005; Besserer et al., 2008; Waters et al., 2017), and improve the symbiotic associations between plant roots and soil microorganisms. SLs also enhance plant resistance to abiotic stresses (Xu et al., 2018; Yao and Waters, 2020).

Similarly, polyamines (PAs) [e.g., diamine putrescine (Put), triamine spermidine (Spd), and tetraamine spermine (Spm)]) are another class of exogenous hormones that can regulate plant growth and development, and enhance stress tolerance (Wang et al., 2015). Exogenic PAs are considered to be associated with mycorrhizal development (Jiménez Bremont et al., 2014) and were shown to significantly increase mycorrhizal colonization (Wu et al., 2012), improve antioxidant enzyme activity (SOD, POD and APX), and reduce malondialdehyde (MDA) levels in *Elymus nutans* and *Elymus sibiricus* under drought stress (Liang et al., 2020). In addition, phytohormones are important regulatory signaling factors involved in the symbiosis between AMFs and plants (Hull et al., 2021). Meanwhile, the concentration and chemical structure of SLs can control many aspects of shoot and root growth due to varied recognition by the root affecting the branching of arbuscular mycorrhizal hyphae (Waters et al., 2017).

Both AMF and plant hormones play important roles in plant growth and development, and also in enhanceing plants resistance, which showed a probably to compensate for the disadvantage of current tea cutting technology, but few studies have examined the individual and combined effects of AMF and plant hormones on the growth and propagation of tea cutting seedlings. In this study, we studied two species of AMF (*R. intraradices* and *F. mosseae*) with two types of exogenous plant hormone (GR24 and Spm) to examine their effects on the propagation, root development, photosynthesis, and antioxidant pathways in tea cutting seedings (*C. sinensis* cv. Fuding-Dabaicha).

2 Materials and methods

2.1 Experimental site and conditions

The experiment was carried out from October 2017 to November 2018, at the Guiyang Tea Garden Base (106° 39'24.09"E, 26°30'52.07"N, 1127.10 m) of the Guizhou Tea Research Institute, Guizhou Province Academy of Agricultural Science, Guizhou, China. Tea cuttings were grown in a greenhouse at 30/22°C (day/night) with a relative humidity of 60% and a 14/10 h (light/dark) cycle. A seedbed (8–10 m length × 1.2–1.3 m width × 20–25 cm height) was prepared in a deep furrow field, in which a width of 10–15 cm was reserved for each region separated by furrows.

2.2 Test materials

Fuding white tea (*C.sinensis* (L.) O. Kuntze cv. Fuding-Dabaicha), from the Guiyang Tea Germplasm Garden (106° 39'15.86"E, 26°30'10.53"N, 1116.4m) at the Guizhou Tea Research Institute, Guizhou Province Academy of Agricultural Science, China, and were most widely cultivated variety of tea tree

of Guizhou provience (planting rate is more than 65%) and planted ~20 years ago, were used as the experimental materials. From October to November of 2017, when the lateral buds of the mother tea tree grew to 10–15 cm, spikes were cut. The spikes were half-lignified, annual, and strong, with long internodes, and were full axillary buds. The spikes were also free of disease and insect pests. Each cutting was 3–4 cm long with a portion of the mature leaf and plump axillary bud.

Based on the morphological identification of AMF spores, the dominant strains, *Rhizophagus intraradices* and *Funneliformis mosseae*, were used as the fungal materials. Fungi were isolated from the soil of the Guiyang Germplasm Tea Garden using the wet sieving and decanting method described by Gerdemann and Nicolson (1963). The isolated AMF spores were propagated in a greenhouse with white clover (*Trifolium repens*) hosts, and grown in a sterilized sand-soil substrate (mass ratio = 1: 1) at 30/22°C (day/night) with a relative humidity of 60% and a 14/10 h (light/dark) cycle in a greenhouse. After three months of cultivation, root segments, spores, hyphae, and the substrate containing white clover colonization were collected as the inocula (the spores density of RI and FM were 19.7 ± 1.0 and 19.4 ± 1.4 spores per 1 g of soil, respectively, using the wet sieving and decanting method).

2.3 Experimental design

The study was a two-factor experiment. The first factor was the fungal inoculation with *R. intraradices* (+RI), *F. mosseae* (+FM), or non-AMF inoculations (-AMF). The second factor was the application of the SL analog, GR24 (+SL), spermidine (+PA), or no hormone (-HOR). In total, there were nine treatments: -AMF – HOR, -AMF + SL, -AMF + PA, +RI – HOR, +RI + SL, +RI + PA, +FM – HOR, +FM + SL and +FM + PA. Each treatment contained three replicate plots (1.2 m in width and 5 m in length).

2.4 Preparation and experimental execution

Tea cuttings were grown in a seedling bed of acidic soil by laying them flat on a black plastic film and treating them with 5% formaldehyde for disinfection for 24 h before the test. The physicochemical properties of the soil were: pH, 4.51; Olsen-P, 4.52 mg/kg; available K, 275.67 mg/kg; and alkali-hydrolyzed N, 31.09 mg/kg. A layer of matrix containing AMF inocula of about 2.0 kg was spread on the furrowed field and covered with a 3–5 cm of subsoil. The subsoil was collected from a barren mountain, was mildly acidic, and was also treated with 5% formaldehyde.

The tea cuttings were disinfected by immersion in 1% carbendazim for 5 min, followed by three washes in distilled water. The planting density included a 0.5–1 cm plant spacing and 3–5 cm row spacing. After planting ~45 days, the base of tea cuttings were sprayed with 5 L of SL (1 μmol/L GR24) or PA (1 mmol/L triamine spermidine) in the hormone-treatment groups. GR24 (1 μmol/L; Umehara et al., 2008) and triamine spermidine (1 mmol/L; Wu and Zou, 2009) were dissolved in about 3-5 mL of acetone and absolute ethanol, respectively, and then prepared at 2x the desired final concentrations. The control treatments (-AMF + -HOR, -AMF + SL, -AMF + PA) were sprayed with the same weight sterilization inocula, which contained the same concentration of acetone or absolute ethanol as the solvent, respectively.

2.5 Measurements

After ~90 days of AMF inoculation, the survival rate, germination rate, and callus formation rate of 1,000 randomly selected tea seedlings were estimated. After ~160 days of AMF inoculation, the tea roots were stained for microscopic observation, according to the method of Phillips and Hayman (1970). The mycorrhizal colonization rate was estimated in terms of the number of roots planted with fungi and the percentage of the total number of roots observed.

After ~240 days of AMF inoculation, the tea cuttings were harvest. Then the height and leaf surface area of the plants were determined, and the above-ground and below-ground biomasses were determined after 48 h of drying at 80°C. The roots were immediately scanned with an Epson Scanner (J221A, Seiko Epson Cop., Tokyo, Japan), and root pictures were analyzed using WinRHIZO Software (2007b) (Regent Instruments, Montreal, QC Canada) to identify morphological traits.

During the sampling time between 9:00–11:00 am on sunny days, three fully functional and expanded leaves were selected for each treatment (the third leaf from the top). The gas exchange parameters of the tea seedling leaves were determined using the Li-6400 portable photosynthetic instrument (LI-COR, United States) to determine the net photosynthetic rate (Pn), stomatal conductivity (Cond), intercellular CO_2 concentration (Ci), and transpiration rate (Tr). Each measurement was repeated three times. The chlorophyll index of the second leaf from the top of tea cuttings were estimated with the Dualex Portable Plant Polyphenol Chlorophyll Meter (Dualex Scientific+)(Zhang et al., 2022).

Root dehydrogenase activity was measured using the triphenyltetrazazole chloride (TTC) colorimetry method (Chu et al., 2007). The soluble sugar content in leaves was determined using anthrone colorimetry (Li, 2006). The leaf MDA content was measured at 532 nm and 600 nm following the thiobarbituric acid method described by Sudhakar et al. (2001). Peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) activities in leaves were determined colorimetrically, as described by He et al. (2020).

2.6 Statistical analysis

The data (means ± SD) obtained in this study were analyzed by two-way analysis of variance (ANOVA) in SAS software (v9.1.3) (SAS Institute Inc., Cary, NC, USA). Significant differences between treatments were compared using the Duncan's Multiple Range

Tests at the $P < 0.05$ level. The Pearson's correlation coefficients between selective variables were analyzed using SAS software. Data were graphed using SigmaPlot software (v10.0) (Systat Software, Inc., Chicago, IL, USA).

3 Results

3.1 AMF-hormone combinations improved the mycorrhizal colonization of tea cutting seedings

AMF colonization was observed in the RI-inoculated, FM-inoculated, and non-inoculated treatment groups (Figure 1A). Compared to -AMF – HOR and +FM + PA treatments, different treatments of RI and FM significantly improved the mycorrhizal colonization rate of tea cutting seedlings (Figure 1B). In addition, SL treatment increased the mycorrhizal colonization rates of non-inoculated and RI-inoculated tea cutting seedlings by 22.2% and 35.8%, respectively, but did not affect the AMF colonization rate in the FM-alone group. Irrespective of AMF inoculation, the application of PA had no significant effect on the mycorrhizal colonization rate. Two-way ANOVA was used to determine the effect of inoculated AMFs and plant hormones on the differences between variables after treatment, and a significant interaction between AMF and plant hormones was found in the mycorrhizal colonization of tea cutting seedlings (Table 1).

3.2 AMF-hormone combinations improved the quality and growth indices of tea cutting seedings

Indices such as the survival rate, callus formation rate, height, leaf area, shoot biomass, and root biomass are closely associated with the quality and growth of tea cutting seedings. In this study, the survival rate of tea cutting seedings in all treatments was more than 95%, and the callus formation rate was more than 94% (Table 2). Compared to the -AMF - HOR treatment, the +RI + SL treatment group had the highest survival rate (98.6%) and callus formation rate (97.9%), and increased plant height of tea cutting seedings by 38.9%. None of the other treatments exhibited significant differences in plant height ($P < 0.05$). In the absense of the hormone application, RI and FM inoculations significantly increased the tea leaf surface area by 12.0% and 6.7%, respectively. Such inoculations also increased the shoot biomass and root biomass by 12.5% and 1.8%, 22.6% and 6.5% in the RI alone group and SL alone group, respectively.Without AMF inoculation, SL alone significantly increased the tea leaf surface area and shoot biomass of tea cutting seedings by 6.8% and 12.5%, respectively. PA alone significantly increased the survival rate ($P < 0.05$). Regardless of AMF inoculation, the application of PA had no significant effect on the leaf surface area. Interestingly, compared to the -AMF - HOR treatment, AMF or hormone treatments alone did not significantly affect the plant height or root biomass, while the combination of AMF and hormone treatment significantly

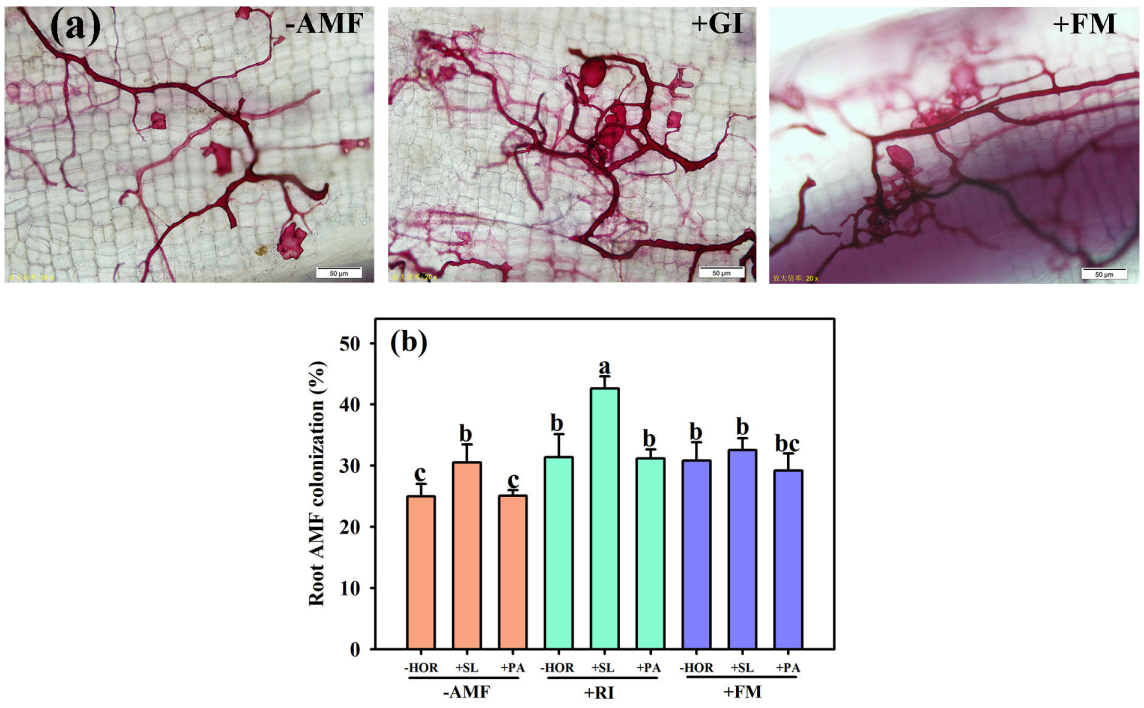

FIGURE 1
AMFs and plant hormones interaction improved the mycorrhizal colonization of tea cutting seedings. **(A)** The root system colonization structures of AMF. **(B)** Interaction effect of AMF inoculation and plant hormones on AMF colonization rates. Data bars (means ± SD, $n = 3$) indicated by different letters suggest significant differences ($P < 0.05$). AMF, arbuscular mycorrhizal fungi; RI, *Rhizophagus intraradices*; FM, *Funneliformis mosseae*; HOR, hormone; SL, strigolactones; PA, spermidine. The same treatments apply to other Figures/Tables. Different lowercase letters in a figure indicate that the mean values are significantly different (P < 0.05) from each other according to LSD test.

TABLE 1 Analysis of variance (ANOVA) of different variables after treatment with AMFs and hormones.

	Root colonization	Root DHA	Total chlorophyll	Leaf soluble sugar	MDA	SOD	POD	CAT
AMFs	**<0.0001**	**<0.0001**	0.1486	**<0.0001**	**0.0001**	**0.0392**	**<0.0001**	**<0.0001**
Hormones	**<0.0001**	**0.0008**	**0.0018**	0.0614	**0.0055**	**0.0003**	**0.0471**	**<0.0001**
AMFs×Hormones	**0.0363**	**0.0156**	**0.0178**	**0.0008**	0.9947	0.2869	**0.0031**	**<0.0001**

Bold values denote statistical significance at the p < 0.05 level. The "×" symbolizes the interaction of the two factors (AMFs and Hormones) within ANOVA. The bold values means there are significant or very significant differences. The same below.

increased the survival rate, callus formation rate, height, shoot biomass, and root biomass. In all AMF-hormone treatment groups, the best quality and growth indices of tea cutting seedings were observed in the +RI + SL treatment group. Two-way ANOVAs revealed that AMF-hormone interactions had significant effects on the leaf area and the shoot biomass of the tea cutting seedings root systems ($P < 0.05$).

3.3 AMF-hormone combinations improved the root system architecture of tea cutting seedings

AMF and hormone interactions had different effects on the root architecture of tea cutting seedings (Figure 2, 3). Compared to the -AMF - HOR treatment, RI or FM alone significantly increased the projected root area (RI, 17.2%; FM, 20.2%), average root diameter (RI, 27.3%; FM, 29.5%), and root volume (RI, 53.2%; FM, 47.7%) (Table 3), but had no significant impact on the total length and root surface area. Without the AMF inoculation, SL only significantly increased the average root diameter by 27.2%, while PA significantly increased the projected area and average root diameter by 17.2% and 18.2%, respectively. In combination with RI inoculation, SL significantly increased the total length, average root diameter, and root volume of tea cuttings by 61.9%, 39.3%, and 42.5%, respectively. However, the application of PA in combination with RI inoculation had no significant effect on root-system configuration. Following FM inoculation, SL significantly improved the average root diameter and root volume by 19.3% and 28.0%, respectively, while the application of PA only increased the average root diameter by 15.8%. Overall, the effect of the combination of AMF and hormones on the root system architecture was not always better than AMF inoculation or hormone treatment alone; only the specified combination of +RI + SL significantly improved root system architecture in this study ($P < 0.05$). Two-way ANOVA showed that the AMF-hormone interaction had significant effects on the total length, average diameter, and volume of the root systems of tea cutting seedings ($P < 0.05$).

TABLE 2 Effects of AMFs and hormones on the quality and growth of tea cutting seedings.

AMF treatments	Hormone treatment	Surviving rate (%)	Callus formation rate (%)	Height (cm)	Leaf area (cm^2)	Shoot biomass (g/plant)	Root biomass (g/plant)
-AMF	-HOR	95.3 ± 0.4d	94.7 ± 1.1c	10.8 ± 2.0b	13.3 ± 0.3d	5.6 ± 0.5c	3.1 ± 0.3b
	+SL	96.1 ± 0.3cd	95.4 ± 0.5bc	10.9 ± 0.8b	14.2 ± 0.1bc	6.3 ± 0.1ab	3.2 ± 0.1ab
	+PA	96.8 ± 0.2bc	95.3 ± 0.2bc	11.0 ± 0.7b	13.6 ± 0.4cd	6.0 ± 0.2abc	3.2 ± 0.5ab
+RI	-HOR	97.6 ± 0.6ab	95.9 ± 1.0bc	12.8 ± 2.0ab	14.9 ± 0.4b	6.3 ± 0.5ab	3.4 ± 0.2ab
	+SL	98.6 ± 0.4a	97.9 ± 0.5a	15.0 ± 0.8a	16.1 ± 0.7a	6.5 ± 0.6a	3.8 ± 0.4a
	+PA	97.8 ± 1.1ab	96.6 ± 0.3abc	13.0 ± 1.5ab	14.3 ± 0.2bc	5.7 ± 0.2bc	3.0 ± 0.5b
+FM	-HOR	96.4 ± 1.1bcd	96.4 ± 1.7abc	11.3 ± 1.7b	14.2 ± 0.7bc	5.7 ± 0.2bc	3.3 ± 0.4ab
	+SL	95.2 ± 1.3d	96.7 ± 1.3ab	12.8 ± 2.7ab	14.7 ± 0.5b	5.8 ± 0.6bc	3.5 ± 0.3ab
	+PA	96.6 ± 0.8bcd	96.4 ± 1.1abc	12.6 ± 1.1ab	14.5 ± 0.3b	6.1 ± 0.5abc	3.0 ± 0.2b
Significance							
AMF		**<0.0001**	**<0.0001**	0.0058	**<0.0001**	0.2313	0.4027
Hormones		0.2318	**<0.0001**	0.2042	**0.0005**	0.1744	0.0654
AMF×Hormones		0.1784	0.1802	0.5719	**0.0470**	**0.0400**	0.5006

Data are presented as mean ± SE (n = 3). Different letters within each parameters indicate that the mean values are significantly different (P < 0.05) from each other according to LSD test. The same below.
The bold values mean that there are significant or very significant differences.

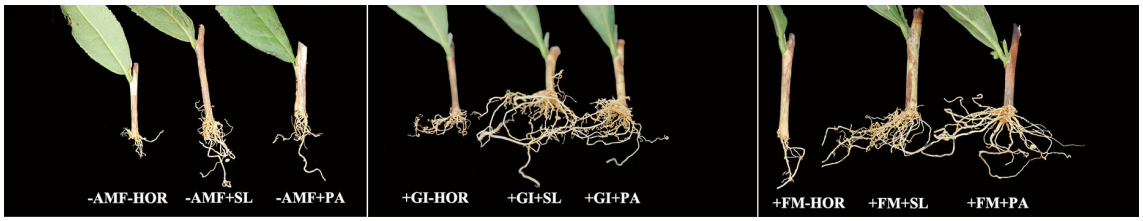

FIGURE 2
Root morphological responses of tea cutting seedings to different treatments. Compared with other treatments, +RI+SL treatment significantly improved the root-system configuration.

3.4 AMF-hormone combinations increased root DHA activity

In the absence of plant hormone treatment, inoculation with RI or FM had no significant effect on root DHA activity (Figure 4). However, following inoculation with AMF, PA significantly increased root DHA activity by 15.1% ($P < 0.05$), while SL had no significant effect compared to the corresponding control (-HOR + -AMF). Compared to the other combined treatments, the +RI + SL group exhibited the highest increase in root DHA activity at 24.3% compared to the corresponding control (-HOR + RI). The +RI + PA and +FM + PA treatment groups also had significantly higher root DHA activities than the controls ($P < 0.05$). This turned out to be the case as the specified combination of AMFs and hormones had a significant effect on the root DHA activity.

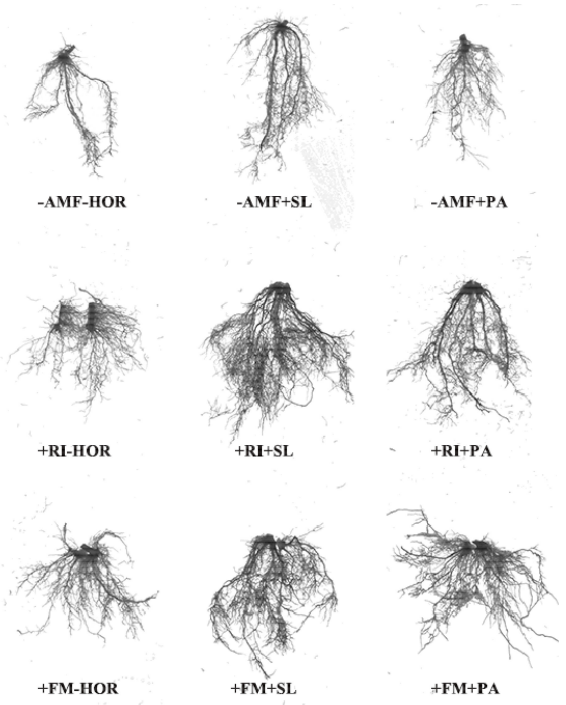

FIGURE 3
Root system architecture responses of tea cutting seedings to different treatments.

3.5 AMF-hormone combinations affected the photosynthesis of tea cutting seedings

As shown in Table 4, in the absence of hormone treamtents, RI inoculation significantly improved the net photosynthetic rate and stomatal conductance of tea cutting seedlings by 36.2% and 47.3%, respectively. Additionally, FM inoculation significantly improved the Pn, Cond, and Tr by 32.6%, 39.6%, and 41.3%, respectively. In the absence of AMF inoculation, SL had no significant effect on photosynthetic parameters, while PA only significantly increased Cond (30.8%). Treatement with +RI + SL significantly improved the Pn, Cond, and Tr by 43.8%, 58.2%, and 89.9%, rewpectively, while +RI + PA significantly improved Pn, Cond, and Tr by 37.5%, 29.9%, and 51.9%, respectively. Treatment with +FM + SL increased Pn by 21.2%, while treatment with +FM + PA significantly improved the Cond and the transpiration rate by 27.6% and 26.5%, respectively. No significant difference in Ci was observed in any treatment. Two-way ANOVA revealed that AMF-hormone interactions had significant effects on stomatal conductance and the transpiration rate of tea cutting seedings ($P < 0.05$).

3.6 AMF-hormone combinations affected chlorophyll and soluble sugar contents in tea cutting seedings leaves

As shown in Figure 5A, compared to the -AMF - HOR treatment group, RI inoculation alone had no significant effect on the chlorophyll content, while FM inoculation alone significantly increased the chlorophyll content by 28.2%. Treatment with -AMF + SL and -AMF + PA significantly increased the chlorophyll content by 31.9% and 23.4%, respectively. Treatment with +RI + SL significantly increased the chlorophyll content by 34.6%, while +RI + PA had no significant effect. Treatment with +FM + SL and +FM + PA did not affect the chlorophyll content.

Concerning the soluble sugar content in the leaves of tea cutting seedings, the application of SL and PA without AMF inoculation significantly reduced the soluble sugar contents of leaves (Figure 5B) by 32.8% and 43.2%, respectively. Treatment with +RI + SL significantly increased the soluble sugar content by 16.0%, while +RI+PA had no significant effect. Treatment with +FM + SL and +FM + PA significantly increased the soluble sugar content of leaves by 28.0% and 23.2%, respectively. In the absence of hormone treatment, RI

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TABLE 3 Interaction effect of AMFs and hormones on the root system architecture of tea cutting seedings.

AMF treatments	Hormone treatment	Total length (cm)	Projected area (cm^2)	Surface area (cm^2)	Average diameter (mm)	Volume (cm^3)
-AMF	-HOR	135.8 ± 10.9c	9.9 ± 0.9c	12.3 ± 1.0b	0.44 ± 0.04d	1.09 ± 0.09d
	+SL	152.4 ± 15.0c	11.1 ± 1.2bc	13.1 ± 0.1ab	0.54 ± 0.01c	1.21 ± 0.03d
	+PA	158.0 ± 22.5bc	11.6 ± 0.7ab	13.4 ± 1.3ab	0.52 ± 0.04c	1.23 ± 0.15d
+RI	-HOR	149.7 ± 8.1c	11.6 ± 0.4ab	13.0 ± 0.6ab	0.56 ± 0.04c	1.67 ± 0.05c
	+SL	242.3 ± 31.7a	13.0 ± 0.9a	14.9 ± 1.4a	0.78 ± 0.05a	2.38 ± 0.16a
	+PA	154.0 ± 16.8bc	11.7 ± 1.1ab	13.1 ± 1.8ab	0.55 ± 0.03c	1.77 ± 0.12c
+FM	-HOR	167.9 ± 16.1bc	11.9 ± 0.7ab	12.5 ± 0.7b	0.57 ± 0.05c	1.61 ± 0.15c
	+SL	189.3 ± 22.0b	12.0 ± 0.4ab	13.4 ± 1.0ab	0.68 ± 0.06b	2.06 ± 0.20b
	+PA	145.1 ± 18.3c	11.1 ± 1.0bc	12.7 ± 0.4b	0.66 ± 0.02b	1.73 ± 0.16c
Significance						
AMF		**0.0061**	**0.0293**	0.2537	**<0.0001**	**<0.0001**
Hormones		**0.0001**	0.0827	0.0752	**<0.0001**	**<0.0001**
AMF×Hormones		**0.0025**	0.1306	0.5409	**0.0013**	**0.0062**

Data are presented as the mean ± SE (n = 3).
The bold values mean that there are significant or very significant differences.

inoculation significantly increased the leaf content of soluble proteins by 35.9%, while FM inoculation had no effect. In addition, AMF and hormone interactions had significant effects on the total chlorophyll and soluble sugar contents of tea cutting seedings (Table 1).

3.7 AMF-hormone combinations increased the POD, SOD, and CAT activities of tea cutting seedings

Compared to the -AMF - HOR treatment, +RI + SL or +RI + PA treatments did not change the MDA content, while FM inoculation significantly reduced MDA concentrations by 19.7% (Figure 6A). When inoculated with RI and FM, spraying SL or PA had no significant effect on MDA concentration.

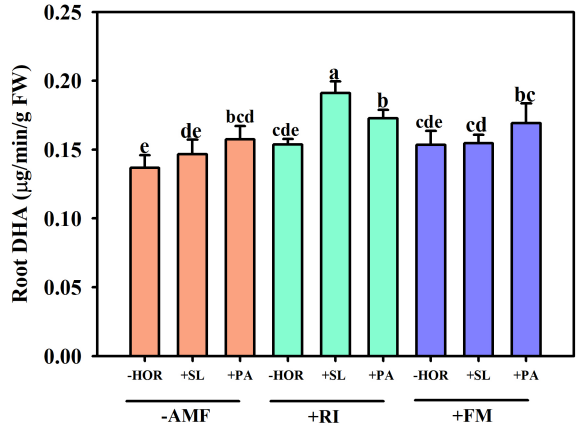

FIGURE 4
AMFs and plant hormones interaction significantly increased the root DHA activity. Different lowercase letters in a figure indicate that the mean values are significantly different (P < 0.05) from each other according to LSD test. The same below.

Similar to MDA concentrations, RI inoculation and FM inoculation alone did not affect leaf SOD activities compared to the -AMF - HOR treatment group (Figure 6B). However, the application of SL significantly increased SOD activities by 32.8%, while PA treatment caused no change. Treatment with +RI + SL significantly increased SOD activity by 26.7%, while treatment with +RI + PA caused no change. Neither +FM + SL nor +FM + PA treatments affected SOD activities.

In the absence of hormone treatments, RI inoculation alone significantly increased POD activity by 45.2%, while FM inoculation caused no effect (Figure 6C). Without AMF or with FM inoculation, the application of SL or PA had no significant effect on POD activities, while treatment with +RI + SL significantly improved POD activity by 40.1%.

In the absence of hormone treatment, RI inoculation or FM inoculations significantly improved the leaf CAT activity by 62.2% and 71.1%, respectively (Figure 6D). Treatment with -AMF + SL significantly improved leaf CAT activity by 38.2%, while -AMF + PA had no effect. When combined with RI inoculation, both SL and PA treatment significantly improved the leaf CAT activity by 87.1% and 20.3%, respectively. When combined with FM inoculation, SL or PA treatment exhibited no significant changes in leaf CAT activity.

Compared to the other treatments, +RI + SL treatment caused the highest increase in POD, SOD, and CAT activities. Thus, AMF-hormone interactions significantly ($P < 0.01$) affected the leaf POD and CAT activities of tea cutting seedings (Table 1).

3.8 Correlation analysis

Analyses of Pearson's correlation coefficients by correlation heat map showed that the AMF colonization rate had a

TABLE 4 Effects of AMFs and hormones on photosynthesis in tea cutting seedings.

AMF treatments	Hormone treatments	Pn(μmol/m^2/s)	Cond (mmol/m^2/s)	Ci (μmol/mol)	Tr (mmol/m^2/s)
-AMF	-HOR	4.70 ± 1.40e	0.091 ± 0.035f	317.3 ± 21.5a	1.04 ± 0.33f
	+SL	5.59 ± 0.74de	0.101 ± 0.026ef	309.4 ± 19.3a	1.13 ± 0.37f
	+PA	5.64 ± 0.87de	0.119 ± 0.015de	322.7 ± 9.0a	1.20 ± 0.18ef
+RI	-HOR	6.40 ± 0.74cd	0.134 ± 0.016d	313.3 ± 26.0a	1.29 ± 0.25ef
	+SL	9.20 ± 0.81a	0.212 ± 0.051a	316.3 ± 17.1a	2.45 ± 0.43a
	+PA	8.80 ± 1.22a	0.174 ± 0.031b	311.1 ± 16.2a	1.96 ± 0.45b
+FM	-HOR	6.23 ± 1.57cd	0.127 ± 0.019d	314.2 ± 14.0a	1.47 ± 0.46de
	+SL	7.55 ± 1.49b	0.140 ± 0.017cd	311.4 ± 8.1a	1.61 ± 0.33cd
	+PA	7.08 ± 2.20bc	0.162 ± 0.030bc	318.1 ± 14.3a	1.86 ± 0.40bc
Significance					
AMF		**<0.0001**	**<0.0001**	0.7625	**<0.0001**
Hormone		**<0.0001**	**<0.0001**	0.4883	**<0.0001**
AMF×Hormone		0.0676	**0.0001**	0.4635	**<0.0001**

Data are presented as the mean ± SE (n = 10). Pn, net photosynthetic rate; Cond, stomatal conductivity; Ci, intercellular CO_2 concentration; Tr, transpiration rate.
The bold values mean that there are significant or very significant differences.

significant positive correlation with the root-related indices, such as the root DHA activity, total root length, average root diameter, root volume, and root surface area (Figure 7). Root biomass was also significantly positively correlated with the total root length and root volume. Root DHA activity was significantly positively correlated with the total length, projected area, average diameter, and volume of roots.

The AMF colonization rate was also positively correlated with leaf-related indices, such as leaf surface area, total chlorophyll, leaf SOD, POD, and CAT activity, shoot biomass, and leaf soluble sugar content (Figure 8). Similarly, the leaf area exhibited significant positive correlations with the soluble leaf sugar content, leaf SOD, POD and CAT activity, shoot biomass, and total chlorophyll, while a significant negative correlation was observed between leaf area and MDA content. The total chlorophyll content was significantly positively correlated with leaf SOD and CAT activities. The soluble leaf sugar content was significantly positively correlated with leaf POD and CAT activity, while it was negatively correlated with leaf MDA content.

Collectively, the AMF colonization rate was significantly positively correlated with most of plant growth and physiobiochemical characteristics and could be one of the key factors affecting the growth status of tea cutting seedings.

4 Discussion

Both PAs and SLs can regulate the symbiosis between AMF and plants in various ways (Wu et al., 2012; Mitra et al., 2021a). This point of view was indirectly confirmed in our study. This study showed that regardless of AMF inoculation, the AMF structure was observed in the rhizosphere of Fuding-Dabaicha in all treatment groups, while the RI or FM inoculations promoted AMF colonization compared to the corresponding control treatments (Cao et al., 2021b). This was because in the field, although the seedling bed was treated with 5% formaldehyde for disinfection over 24 h before the test, a small quantity of AMF still survived. However, the effect of the surviving AMF was weaker than that of the additional AMF inocula, since the spore density of the inocula was quite higher than that in the treated seedling soil bed. The soil survived AMF could not meet the crop demands, and therefore, additional AMF inoculations are required to improve the mycorrhizal benefits to plants.

AMF-hormone interactions had a significant effect on the mycorrhizal colonization of the tea rhizosphere, which was consistent with the results of Kountche et al. (2018). SLs can induce AMF spore germination, mycelium elongation, and branching, and play a key role in the communication between plants and fungi (Mostofa et al., 2018). However, in this study, SL spraying significantly promoted the AMF colonization of rhizospheres in both the non-inoculated and RI-inoculated treatment groups, but did not promote the root colonization of FM, thus, indicating that the effect of hormones could be unique to specific AMF strains. In addition, exogenous PA treatment had no significant effect on the AMFs colonizing Fuding-Dabaicha roots, which was inconsistent with the results of Wu and Zou (2010) in citrus. Those findings could be attributed to differences in test materials. Mycorrhizal regulation by PAs has also been shown to depend on the types of polyamines and roots (Wu and Zou, 2010; Wu et al., 2012).

In this study, RI or FM inoculation, and the application of SL and PA, improved the quality and growth status of tea cutting seedlings to differing degrees. Once AMF achieve a symbiotic relationship with host plants, they expand the roots of hosts through mycelia, promoting nutrient and water absorption, stress resistance, and growth of the host plant (Wang et al., 2016;

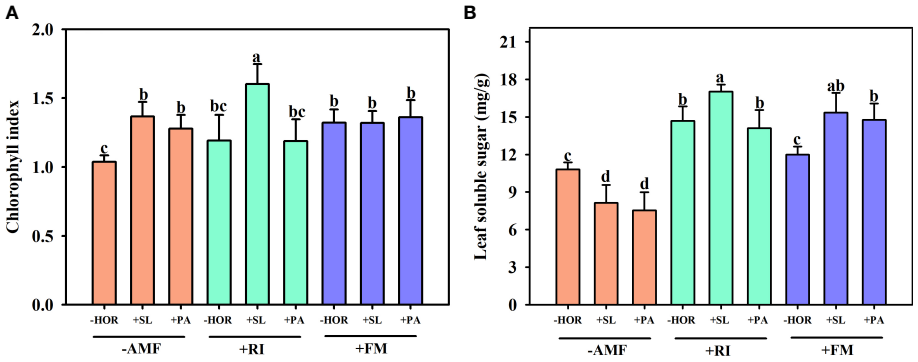

FIGURE 5
AMFs and plant hormones interaction had significant effects on chlorophyll indexes and soluble sugar contents in tea cutting seedings leaves. Effects of AMFs and hormones on **(A)** total chlorophyll index and **(B)** leaf soluble sugar content. Different lowercase letters in a figure indicate that the mean values are significantly different ($P < 0.05$) from each other according to LSD test.

Chandrasekaran et al., 2021). SLs can promote seed germination, in shaping root architecture (Ruyter-Spira et al., 2011), regulate plant branching, and improve plant biomass (Kapulnik et al., 2011; Sharifi and Bidabadi, 2020). Similarly, PAs, as common low-molecular-weight biostimulation agents, promote plant growth, development, and defense under stress conditions (Wu et al., 2012; Sharma et al., 2021). In this study, PA spraying with RI or FM inocula had no significant impacts on the growth of tea cutting seedlings, while the interaction between RI and SL improved tea cutting seedling growth. Notably, SL induces spore germination and mycelium branching in AMFs, facilitating its symbiosis with the host plant (Mitra et al., 2021a). Hormone treatments can promote the benefits of AMF to tea plants by improving plant growth to different degrees. However, the degree of the effect varies based on the AMF species and hormones. Our results suggest that +RI + SL treatment may have a strong potential for the development of tea cuttings seedings and improving asexual propagation.

Adventitious root formation is a prerequisite for successful cutting propagation (Kharal and Shrestha, 2020), and a good root system architecture could provides sufficient nutrients to tea cutting seedings (Campos et al., 2021). In this study, the single inoculation of RI or FM significantly increased the projected root area, and the average diameter and volume of tea roots. In addition, spraying SL with the RI inocula further promoted the positive effect, because SL promoted the symbiotic relationship with the host plant (Mitra et al., 2021a). The SL synthesized in roots can then be transported to the soil over a short distance, so it could further promote mycorrhizal colonization (Shen et al., 2022). The root architecture was consistent with the growth quality of tea cuttings among all treatments. Correlation analyses also showed significantly positive correlations between AMF root colonization and root morphological traits and biomass($P < 0.05$), thus, indicating that the SL-RI interaction promoted the growth of tea cutting seedlings related to the AMF colonization.

The root activity reflects the metabolic capacity of plant roots (Liu et al., 2009; Huang et al., 2015). AMF or SL treatments alone did not show a significant effect on root DHA activities (an indicator of root activity), while PA treatments alone elevated DHA activity. Notably, PAs are an important regulator of root growth and function (Sharma et al., 2021). The combination of SL or PA with RI inoculation significantly improved root DHA activity, along with providing better effects under the +RI + SL combined treatment. The DHA activity was significantly positively correlated with the total root length, projected area, and average diameter and volume, suggesting that SL conferred good root activity in mycorrhizal plants.

Plant growth is driven by photosynthesis (Moustakas et al., 2020). In this study, AMF inoculation increased the Pn and Cond of tea cutting seedlings, which was consistent with the results of Wu and Zou (2010) in citrus seedlings. Moreover, we found that the individual SL or PA treatments alone failed to change the photosynthetic activity of tea cutting seedlings, as the photosynthetic activity in combination with AMF treatments promoted almost the same change to the varying degrees. Notably, the +RI + SL treatment group showed the highest increase in the photosynthetic capacity of tea cutting seedlings. The increased photosynthetic capacity accelerates the accumulation of plant carbohydrates, promotes the growth of tea cutting seedlings, and increases the supply of organic carbon to AMFs, promoting mycorrhizal symbiosis with tea plants (Kountche et al., 2018). In addition, mycorrhiza-induction enhances root structure and plant hormones promote the absorption of soil nutrients, which increases photosynthesis in tea seedlings (Mitra et al., 2021b).

Root activity showed a significant positive correlation with the photosynthetic rate (Wei et al., 2004). Strong root activity also delays the senescence of above-ground plant parts and promotes the synthesis and partitioning of photosynthates (Wang et al., 2013). Therefore, the +RI + SL treatment group had higher chlorophyll and soluble sugar contents in leaves. Additionally, SL or PA alone significantly increased the total chlorophyll content, but reduced the soluble sugar content in tea leaves. In general, leaf-produced carbohydrates are first used for the leaf and then transferred to other parts of the plant (Zhang et al., 2020b). Thus, spraying SL or PA may have promoted the leaf carbohydrate distribution to the roots, increasing root development and thereby reducing leaf soluble sugar content.

The morphological and physiological adaptation of the root system are important factors for plants to access soil resources.

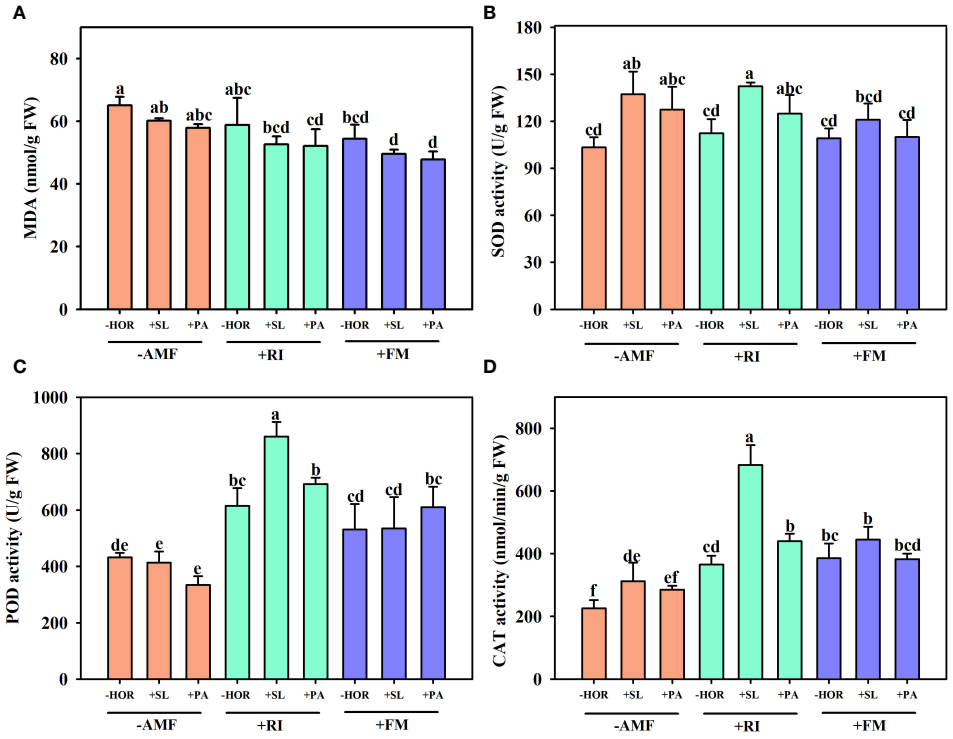

FIGURE 6
AMFs and plant hormones interaction showed the highest increase in POD, SOD, and CAT activities. Effects of AMFs and hormones on **(A)** MDA content and the leaf activities of **(B)** SOD, **(C)** POD, and **(D)** CAT. Different lowercase letters in a figure indicate that the mean values are significantly different ($P < 0.05$) from each other according to LSD test.

Cutting seedings may experience poor nutrient absorption and poor stress tolerance, which will lead to the substantial production of ROS and affect photosynthesis because of the shallow root system and weak nutrient absorption (Mutui et al., 2015; Yang et al., 2016). AMF could improve the stress resistance of host plants by promoting antioxidase activities; reducing the MDA content is also well known (Yan et al., 2020). In this study, single RI inoculation noticeably promoted POD and CAT activities, while single FM inoculation promoted CAT activities and reduced MDA content. SL treatment alone promoted SOD and CAT activities, which was consistent with the findings of Sharifi and Bidabadi (2020). The synthetic exogenous SLs are mainly SL analogs (e.g., germination releaser, GR). GR24 is the most active and commonly used synthetic SL (Jiang et al., 2013) and its exogenous application has been shown to improve cell viability, photosynthesis, and antioxidant enzyme activities in tomato and rape seedlings (Lu et al., 2019; Zhang et al., 2020a). In this study, the foliar spray containing GR24 significantly improved the antioxidant enzyme activity and effectively alleviated oxidative damage in salvia. PA alone showed no significant effects on MDA content or antioxidase activity. However, PA treatment could maintain the cell pH and ionic homeostasis to ensure the maintenance of cell health (Zhang et al., 2020c). In addition, SL in combination with AMF further promoted the SOD, POD, and CAT activities of tea, thereby improving their survival rate and growth performance.

In addition, Pearson's correlation analyses showed that the degree of root mycorrhizal colonization was significantly positively correlated with most plant growth and physiobiochemical characteristics parameters of tea cutting seedings, such as significantly positively correlated with root-related indicators (eg. root DHA activity, total root length, average root diameter, root volume) and leaf-related indicators(eg. leaf surface area, total

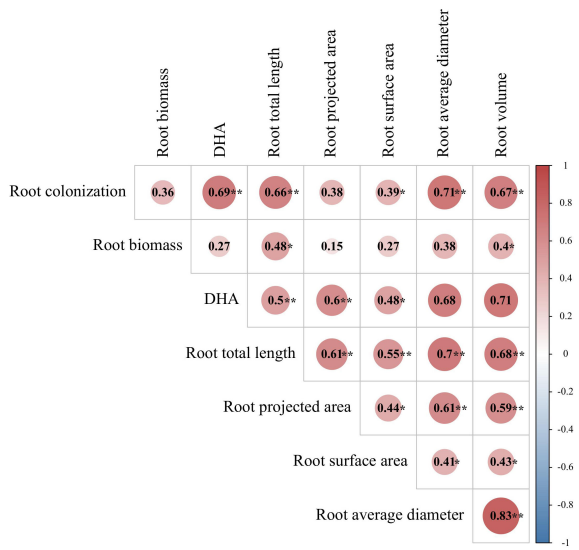

FIGURE 7
Correlation heat map of root-related indicators. **Significant at $p < 0.01$; *significant at $p < 0.05$.The same below.

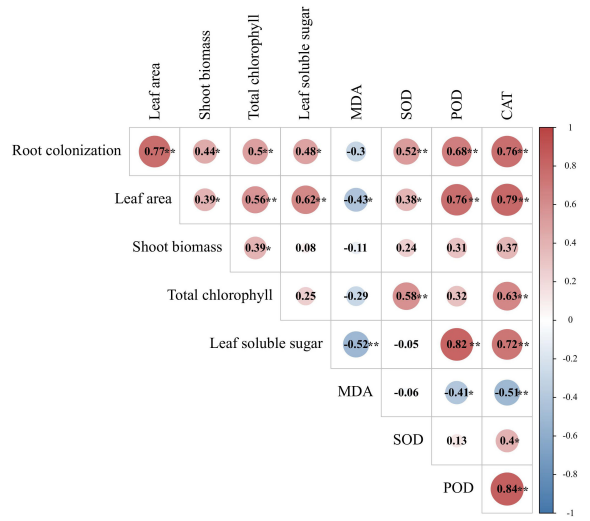

FIGURE 8
Correlation heat map between AMFs colonization and leaf-related indicators. **Significant at $p < 0.01$; *significant at $p < 0.05$.

chlorophyll index, and leaf SOD, POD, and CAT activity), so mycorrhizal colonization could be considered as one of the key factors reflecting the growth status of tea cutting seedings.

SLs, as host-derived precolonization signals (Akiyama et al., 2005), can stimulate the hyphal branching of AMF, and consequently promote symbiotic interactions between AMF and plants (Banasiak et al., 2020). Furthermore, during the symbiosis phase, SLs in root secretions enhance AMF spore germination, metabolic activity, and mycelium branching, thus, improving the interactions between AMFs and host plants (López-Ráez et al., 2017). The improved growth performance observed in +RI + SL treated tea cutting seedings can be attributed to the good symbiotic relationship between AMF and tea cutting seedings as the mycorrhizal colonization observed following this treatment was the highest compared to the corresponding AMF treatment alone. This good performance required to select a combination of specific AMF and specific plant hormone, for the mycorrhizal colonization in other AMF and hormone treatment(eg. +RI + PA, FM + SL) were not always higher or significantly increasing compared to corresponding AMF alone treatment. This could potentially be specific combination being case specific. For AMF-hormone interactions, especially between specific AMFs and the specific plant hormones, in our study were RI and SL, their effects on improving the growth performance many well by SL promoting the RI colonization to from good symbiosis with tea cutting seedings, then take this to improve their root architecture and photosynthetic characteristics, coupled with great antioxidant defense systems. The mechanisms needed to further study.

In conclusion, although the apllication of individual AMF or plant hormones had a certain good effects on most growth and physiobiochemical characteristics parameters of tea cutting seedings, the additive effect was from specific combined of AMF and plant hormone, in our study was the combined of SL and RI. These results highlight the possibility for combined of AMF and plant hormone to improve the asexual reproduction of tea plants *via* cuttings, and our findings provide a provided a practical and feasible strategy for further research to improve the asexual reproduction of tea plants *via* cuttings.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

XG designed the experiment. YL and CL wrote the manuscript and revised the manuscript. CG and YZ prepared the materials for the experiment. CM and XD analyzed the data. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Akiyama, K., Matsuzaki, K. I., and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular arbuscular mycorrhizal fungi. *Nature* 435 (7043), 824–827. doi: 10.1038/nature03608

Banasiak, J., Borghi, L., Stec, N., Martinoia, E., and JasiÅski, M. (2020). The full-size ABCG transporter of medicago truncatula is involved in strigolactone secretion, affecting arbuscular mycorrhiza. *Front. Plant Sci.* 11 (18). doi: 10.3389/fpls.2020.00018

Besserer, A., Bécard, G., Jauneau, A., Roux, C., and Séjalon-Delmas, N. (2008). GR24, a synthetic analog of strigolactones, stimulates the mitosis and growth of the arbuscular mycorrhizal fungus Gigaspora rosea by boosting its energy metabolism. *Plant Physiology*. 148 (1), 402–413. doi: 10.1104/pp.108.121400

Campos, P. M. B. S., Borie, F., Cornejo, P., Meier, S., López-Ráez, J. A., López-Garcia, A., et al. (2021). Wheat root trait plasticity, nutrient acquisition and growth responses are dependent on specific arbuscular arbuscular mycorrhizal fungus and plant genotype interactions. *J. Plant Physiol.* 256, 153297–153314. doi: 10.1016/j.jplph.2020.153297

Cao, J. L., Shao, Y. D., Zou, Y. N., Wu, Q. S., Yang, T. Y., and Kuˇca, K. (2021a). Inoculation with clariodeoglomus etunicatum improves leaf food quality of tea exposed to p stress. *Not. Bot. Horti Agrobo* 49, 12166. doi: 10.15835/nbha49112166

Cao, M. A., Wang, P., Hashem, A., Wirth, S., Abd-Allah, E. F. A., and Wu, Q. S. (2021b). Field inoculation of arbuscular arbuscular mycorrhizal fungi improves fruit quality and root physiological activity of citrus. *Agriculture* 11, 1297. doi: 10.3390/agriculture11121297

Chandrasekaran, M., Boopathi, T., and Manivannan, P. (2021). Comprehensive assessment of ameliorative effects of AMF in alleviating abiotic stress in tomato plants. *J. Fungi.* 7, 303. doi: 10.3390/jof7040303

Chu, H. Y., Lin, X. G., Fujii, T. S., Morimoto, S., Yagi, K., Hu, J. L., et al. (2007). Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. *Soil Biol. Biochem.* 39, 11: 2971–2976. doi: 10.1016/j.soilbio.2007.05.031

Gerdemann, J. W., and Nicolson, T. H. (1963). Spores of mycorrhizal endogone species extracted from soil by wet-sieving and decanting. *Trans. Br. Mycological Soc.* 46 (2), 235–244. doi: 10.1016/S0007-1536(63)80079-0

He, J. D., Zou, Y. N., Wu, Q. S., and Kua, K. (2020). Mycorrhizas enhance drought tolerance of trifoliate orange by enhancing activities and gene expression of antioxidant enzymes. *Scientia Horticulturae* 262, 108745. doi: 10.1016/j.scienta.2019.108745

Huang, J., Huang, Z., Jia, X., Hu, R., and Xiang, C. (2015). Long-term reduction of nitrogen fertilizer use through knowledge training in rice production in China. *Agric. Systems.* 135, 105–111. doi: 10.1016/j.agsy.2015.01.004

Huang, Z., Zou, Z., He, C., He, Z. Q., Zhang, Z. B., and Li, J. M. (2011). Physiological and photosynthetic responses of melon (*Cucumis melo* l.) seedlings to three glomus species under water deficit. *Plant Soil* 339 (s1-2), 391–399. doi: 10.1007/s11104-010-0591-z

Hull, R., Choi, J., and Paszkowski, U. (2021). Conditioning plants for arbuscular arbuscular mycorrhizal symbiosis through DWARF14-LIKE signalling. *Curr. Opin. Plant Biol.* 62, 102071. doi: 10.1016/j.pbi.2021.102071

Jiang, L., Liu, X., Xiong, G. S., Liu, H. H., Chen, F. L., Wang, L., et al. (2013). DWARF 53 acts as a repressor of strigolactone signalling in rice. *Nature* 504, 401–405. doi: 10.1038/nature12870

Jiménez Bremont, J. F., Marina, M., de la Luz Guerrero-González, M., Rossi, F. R., Sánchez-Rangel, D., Rodríguez-Kessler, M., et al. (2014). Physiological and molecular implications of plant polyamine metabolism during biotic interactions. *Front. Plant Sci.* 5. doi: 10.3389/fpls.2014.00095

Kapulnik, Y., Delaux, P. M., Resnick, N., Mayzlish-Gati, E., Wininger, S., and Bhattacharya, C. (2011). Strigolactones affect lateral root formation and root-hair elongation in arabidopsis. *Planta* 233 (1), 209–216. doi: 10.1007/s00425-010-1310-y

Kharal, S., and Shrestha, B. (2020). Effect of indole-butyric acid (IBA) and wounding on rooting ability and vegetative characteristics of apple rootstock cuttings under Nepal conditions. *J. Agric. Sci. Practice.* 5, 184–192. doi: 10.31248/JASP2020.220

Kountche, B. A., Novero, M., Jamil, M., Asami, T., Bonfante, P., and Al-Babili, S. (2018). Effect of the strigolactone analogs methyl phenlactonoates on spore germination and root colonization of arbuscular arbuscular mycorrhizal fungi. *Heliyon* 4 (11), e00936. doi: 10.1016/j.heliyon.2018.e00936

Koyuncu, F., and Balta, F. (2004). Adventitious root formation in leaf-bud cuttings of tea (*Camellia sinensis* l.). *Pakistan J. Bot.* 36 (4), 763–768. doi: 10.1111/j.1469-8137.2004.01195.x

Li, H. S. (2006). *Principles and techniques of plant physiological and biochemical experiments* (Beijing, China: Higher Education Press), 195–197.

Liang, K. L., Wang, M. Y., Sun, J. H., and Li, X. J. (2020). Effect of exogenous polyamines on drought tolerance physiological indicators of 2 kinds of forage. *Mol. Plant Breed.* 18 (11), 3751–3758. doi: 10.13271/j.mpb.018.003751

Liao, D. H., Wang, S. S., Cui, M. M., Liu, J. H., Chen, A. Q., and Xu, G. H. (2018). Phytohormones regulate the development of arbuscular mycorrhizal symbiosis. *Int. J. Mol. Sci.* 19 (10), 3146. doi: 10.3390/ijms19103146

Liu, C. Y., Wang, Y. J., Wu, Q. S., Yang, T. Y., and Kuˇca, K. (2020). Arbuscular arbuscular mycorrhizal fungi improve the antioxidant capacity of tea (*Camellia sinensis*) seedlings under drought stress. *Not. Bot. Horti Agrobot.* 48, 1993–2005. doi: 10.15835/nbha48412066

Liu, R. C., Xiao, Z. Y., Hashem, A., Abd-Allah, E. F., Xu, Y. J., and Wu, Q. S. (2021). Unraveling the interaction between arbuscular arbuscular mycorrhizal fungi and camellia plants. *Horticulturae* 7, 322. doi: 10.3390/horticulturae7090322

Liu, X. G., Zhang, F. C., Yang, Q. L., and Li, Z. J. (2009). Response of chlorophyll, proline and root activity of maize to regulated deficit irrigation and n fertilization treatment. *Acta Agriculturae Boreali-Sinica* 24 (4), 106–111. doi: 10.7668/hbnxb.2009.04.022

López-Ráez, J. A., Shirasu, K., and Foo, E. (2017). Strigolactones in plant interactions with beneficial and detrimental organisms: The yin and yang. *Trends Plant Sci.* 22, 527–537. doi: 10.1016/j.tplants.2017.03.011

Lu, T., Yu, H. J., Li, Q., Chai, L., and Jiang, W. J. (2019). Improving plant growth and allevia- ting photosynthetic inhibition and oxidative stress from low-light stress with exogenous GR24 in tomato (*Solanum lycopersicum* l.) seedlings. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00490

Min, Z., Li, R. Y., Chen, L., Zhang, Y., Li, Z. Y., Lu, Y. L., et al. (2019). Alleviation of drought stress ingrapevine by foliar-applied strigolactones. *Plant Physiol. Biochem.* 135, 99–110. doi: 10.1016/j.plaphy.2018.11.037

Mitra, D., Djebaili, R., Pellegrini, M., Mahakur, B., Sarker, A., Chaudhary, P., et al. (2021b). Arbuscular arbuscular mycorrhizal symbiosis: plant growth improvement and induction of resistance under stressful conditions. *J. Plant Nutr.* 44 (13), 1993–2028. doi: 10.1080/01904167.2021.1881552

Mitra, D., Rad, K. V., Chaudhary, P., Ruparelia, J., Sagarika, M. S., Boutaj, H., et al. (2021a). Involvement of strigolactone hormone in root development, influence and interaction with arbuscular mycorrhizal fungi in plant: mini-review. *Curr. Res. Microbial Sci.* 2, 100026–100035. doi: 10.1016/j.crmicr.2021.100026

Mostofa, M. G., Li, W., Nguyen, K. H., Fujita, M., and Lam-Son, P. T. (2018). Strigolactones in plant adaptation to abiotic stresses:an emerging avenue of plant research. *Plant Cell Environ.* 41 (10), 2227–2243. doi: 10.1111/pce.13364

Moustakas, M., Bayçu, G., Sperdouli, I., Eroglu, H., and Eleftheriou, E. P. (2020). Arbuscular arbuscular mycorrhizal symbiosis enhances photosynthesis in the medicinal herb salvia fruticosa by improving photosystem II photochemistry. *Plants* 9, 962. doi: 10.3390/plants9080962

Mutui, T. M., Mibus, H., and Serek, M. (2015). Cytokinins inhibit leaf senescence in pelargonium cuttings. *Acta Hortic.* 1079, 325–330. doi: 10.17660/ActaHortic.2015.1079.40

Pei, Y., Siemann, E., Tian, B., and Ding, J. (2020). Root flavonoids are related to enhanced AMF colonization of an invasive tree. *AoB Plants* 12 (1), plaa002. doi: 10.1093/aobpla/plaa002

Phillips, J. M., and Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular arbuscular mycorrhizal fungi for rapid assessment of colonization. *Trans. Br. Mycol Soc* 55, 158–161. doi: 10.1016/S0007-1536(70)80110-3

Requena, N., Serrano, E., Ocón, A., and Breuniger, M. (2007). Plant signals and fungal perception during arbuscular mycorrhiza establishment. *Phytochem* 68, 33–40. doi: 10.1016/j.phytochem.2006.09.036

Romero, J. L. (2004). A review of propagation programs for *Gmelina arborea*. *New Forest* 28 (2–3), 245–254. doi: 10.1023/B:NEFO.0000040951.93838.6d

Ruyter-Spira, C., Kohlen, W., Charnikhova, T., Zeijl, A., Bezouwen, L., Ruijter, N. C. A. D., et al. (2011). Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis:another belowground role for strigolactones? *Plant Physiol.* 155 (2), 721–734. doi: 10.1104/PP.110.166645

Sedaghat, M., Tahmasebi-Sarvestani, Z., Emam, Y., and Mokhtassi-Bidgoli, A. (2017). Physiological and antioxidant responses of winter wheat cultivars to strigolactone and salicylic acid in drought. *Plant Physiol. Biochem.* 119, 59–69. doi: 10.1016/j.plaphy.2017.08.015

Senanayake, N. P. J. S. (2013). Green tea extract: Chemistry, antioxidant properties and food applications-a review. *J. Funct. Foods.* 5 (4), 1529–1541. doi: 10.1016/j.jff.2013.08.011

Shao, Y. D., Hu, X. C., Wu, Q. S., Yang, T. Y., Srivastava, A. K., Zhang, D. J., et al. (2021). Mycorrhizas promote p acquisition of tea plants through changes in root morphology and p transporter gene expression. *South Afr. J. Botany.* 137, 455–462. doi: 10.1016/j.sajb.2020.11.028

Shao, Y. D., Zhang, D. J., Hu, X. C., Wu, Q. S., Jiang, C. J., Xia, T. J., et al. (2018). Mycorrhiza-induced changes in root growth and nutrient absorption of tea plants. *Plant Soil Environ.* 64, 283–289. doi: 10.17221/126/2018-PSE

Sharifi, P., and Bidabadi, S. S. (2020). Strigolactone could enhances gas-ex- change through augmented antioxidant defense system in *Salvia nemorosa* l. plants subjected to saline conditions stress. *Ind. Crops Products* 151, 112460. doi: 10.1016/j.indcrop.2020.112460

Sharma, K., Gupta, S., Thokchom, S. D., Jangir, P., and Kapoor, R. (2021). Arbuscular mycorrhiza-mediated regulation of polyamines and aquaporins during abiotic stress: deep insights on the recondite players. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.642101

Shen, Y., Tao, B. J., Hua, X., Lv, B., Liu, L. J., and Chen, Y. (2022). Research progress of strigolactone and hormones to regulate root growth. *Biotechnol. Bull.* 38 (8), 24–31. doi: 10.13560/j.cnki.biotech.bull.1985.2022-0102

Singh, S., Pandey, A., Kumar, B., and Palni, L. M. S. (2010). Enhancement in growth and quality parameters of tea [*Camellia sinensis* (L.) o. kuntze] through inoculation

with arbuscular arbuscular mycorrhizal fungi in an acid soil. *Biol. Fertil Soils* 46 (5), 427–433. doi: 10.1007/s00374-010-0448-x

Sudhakar, C., Lakshmi, A., and Giridarakumar, S. (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* l.) under NaCl salinity. *Plant Sci.* 161, 613–619. doi: 10.1016/S0168-9452(01)00450-2

Sun, S., Wang, J., Zhu, L., Liao, D., Gu, M., Ren, L., et al. (2012). An active factor from tomato root exudates plays an important role in efficient establishment of mycorrhizal symbiosis. *PloS One* 7 (8), e43385. doi: 10.1371/journal.pone.0043385

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., et al. (2008). Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 455 (7210), 195–200. doi: 10.1038/nature07272

Wang, A. J., Huang, C. M., Liu, H. Y., and Zou, G. (2011). Discussion on the reproduction of tea tree seeds and the optimal cultivation of seedlings. *Seed* 30 (08), 105–107. doi: 10.16590/j.cnki.1001-4705.2011.08.057

Wang, X. R., Liu, L. L., Chai, X. J., Li, S. T., and Zhang, G. W. (2015). Effects of exogenous spermide on photosynthesis and carbohydrate accumulation of millet seedlings under drought stress. *Crop Magazine* 05, 100–106. doi: 10.16035/j.issn.1001-7283.2015.05.019

Wang, Y. X., Pang, D. D., Ruan, L., Liang, J. B., Zhang, Q., Qian, Y. H., et al. (2022). Integrated transcriptome and hormonal analysis of naphthalene acetic acid-induced adventitious root formation of tea cuttings(*Camellia sinensis*). *BMC Plant Biol.* 22, 319. doi: 10.1186/s12870-022-03701-x

Wang, P., Wu, S. H., Wen, M. X., Wang, Y., and Wu, Q. S. (2016). Effects of combined inoculation with *Rhizophagus intraradices* and *Paenibacillus mucilaginosus* on plant growth, root morphology, and physiological status of trifoliate orange (*Poncirus trifoliata* l. raf.) seedlings under different levels of phosphorus. *Sci. Hortic.* 205, 97–105. doi: 10.1016/j.scienta.2016.04.023

Wang, F. F., Zhang, S. P., Shao, L. J., Li, G., Chen, X. L., Liu, P., et al. (2013). Effect of root in different soil layers on plant growth and yield formation after anthesis in summer maize. *Scientia Agricultura Sin.* 46 (19), 4007–4017. doi: 10.3864/j.issn.0578-1752.2013.19.006

Waters, M. T., Gutjahr, C., Bennett, T., and Nelson, D. C. (2017). Strigolactone signaling and evolution. *Annu. Rev. Plant Biol.* 68, 291–322. doi: 10.1146/annurev-arplant-042916-040925

Wei, D. Z., Ning, S. J., and Lin, W. X. (2004). Relationship between wheat root activity and left senescence. *Chin. J. Appl. Ecol.* 15 (9), 1565–1569. doi: 10.1088/1009-0630/6/5/011

Wipf, D., Krajinski, F., Tuinen, D., Recorbet, G., and Courty, P. E. (2019). Trading on the arbuscular mycorrhiza market: from arbuscules to common arbuscular mycorrhizal networks. *New Phytol.* doi: 10.1111/nph.15775

Wu, Q. S., He, X. H., Zou, Y. N., Liu, C. Y., Xiao, J., and Li, Y. (2012). Arbuscular mycorrhizas alter root system architecture of *Citrus tangerine* through regulating metabolism of endogenous polyamines. *Plant Growth Regul.* 68 (1), 27–35. doi: 10.1007/s10725-012-9690-6

Wu, Q. S., and Zou, Y. N. (2009). The effect of dual application of arbuscular mycorrhizal fungi and polyamines upon growth and nutrient uptake on trifoliate orange (*Poncirus trifoliata*) seedlings. *Not Bot. Horti Agrobo.* 37, 95–98. doi: 10.15835/nbha3723237

Wu, Q. S., and Zou, Y. N. (2010). Beneficial roles of arbuscular mycorrhizas in citrus seedlings at temperature stress. *Sci. Hortic.* 125, 289–293. doi: 10.1016/j.scienta.2010.04.001

Xu, Y., Miyakawa, T., Nosaki, S., Nakamura, A., Lyu, Y., Nakamura, H., et al. (2018). Structural analysis of HTL and D14 proteins reveals the basis for ligand selectivity in striga. *Nat. Communation.* 9 (1), 3947. doi: 10.1038/s41467-018-06452-2

Yan, P., Geng, L., Sun, H. Q., Zhang, Z. H., Yang, R. Y., and Sun, J. N. (2020). Can arbuscular arbuscular mycorrhizal fungi and biochar enhance plant resistance to low-temperature stress? *Agron. J.* 113 (2), 1457–1466. doi: 10.1002/agj2.20520

Yang, H., Hu, J., Long, X., Liu, Z., and Rengel, Z. (2016). Salinity altered root distribution and increased diversity of bacterial communities in the rhizosphere soil of Jerusalem artichoke. *Sci. Rep.* 6, 20687. doi: 10.1038/srep20687

Yao, J., and Waters, M. T. (2020). Perception of karrikins by plants: A continuing enigma. *J. Exp. Bot.* 71 (6), 1774–1781. doi: 10.1093/jxb/erz548

Zhang, C. F., Pan, C. D., Chen, H., and Wang, S. W. (2020b). The relationship between carbohydrate translocation and the leaf-to-fruit ratio in walnut trees. *Hortic. Sci. Technol.* 38, 308–322. doi: 10.7235/HORT.20200030

Zhang, X., Sun, H., Qiao, X., Yan, X. B., Feng, M. C., Xiao, L. J., et al. (2022). Hyperspectral estimation of canopy chlorophyll of winter wheat by using the optimized vegetation indices. *Comput. Electron. Agriculture.* 193, 106654–106654. doi: 10.1016/j.compag.2021.106654

Zhang, X., Zhang, L., Sun, Y., Zheng, S., and Zhang, T. (2020a). Hydrogen peroxide is involved in strigolactone induced low temperature stress tolerance in rape seedlings (*Brassica rapa* l.). *Plant Physiol. Biochem.* 157, 402–415. doi: 10.1016/j.plaphy.2020.11.006

Zhang, F., Zou, Y. N., Wu, Q. S., and Kuca, K. (2020c). Arbuscular mycorrhizas modulate root polyamine metabolism to enhance drought tolerance of trifoliate orange. *Environ. Exp. Bot.* 171, 103926. doi: 10.1016/j.envexpbot.2019.103926

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Morphology, photosynthetic physiology and biochemistry of nine herbaceous plants under water stress

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Global climate warming and shifts in rainfall patterns are expected to trigger increases in the frequency and magnitude of drought and/or waterlogging stress in plants. To cope with water stress, plants develop diverse tactics. However, the adoption capability and mechanism vary depending upon the plant species identity as well as stress duration and intensity. The objectives of this study were to evaluate the species-dependent responses of alpine herbaceous species to water stress. Nine herbaceous species were subjected to different water stresses (including moderate drought and moderate waterlogging) in pot culture using a randomized complete block design with three replications for each treatment. We hypothesized that water stress would negatively impact plant growth and metabolism. We found considerable interspecies differences in morphological, physiological, and biochemical responses when plants were exposed to the same water regime. In addition, we observed pronounced interactive effects of water regime and plant species identity on plant height, root length, root/shoot ratio, biomass, and contents of chlorophyll a, chlorophyll b, chlorophyll (a+b), carotenoids, malondialdehyde, soluble sugar, betaine, soluble protein and proline, implying that plants respond to water regime differently. Our findings may cast new light on the ecological restoration of grasslands and wetlands in the Qinghai-Tibetan Plateau by helping to select stress-tolerant plant species.

KEYWORDS

plant physiology, drought, waterlogging, morphology, chlorophyll content, lipid oxidation, malodehyde, osmoprotectant

1 Introduction

Grasslands and wetlands play a critical role in water and soil conservation, flood storage, maintaining productivity, cycling and storing carbon and sustaining biodiversity (Wang et al., 2021; Wang Y. et al., 2022). The Qinghai–Tibetan Plateau is dominated by alpine grassland, which accounts for more than 60% of the total area of the Qinghai–Tibetan Plateau (Li et al., 2023) and is important for its contributions to the aforementioned processes (Wang Y. et al., 2022). However, approximately 70% of alpine grassland has been degraded in recent decades (Peng et al., 2019). The degradation of grasslands has resulted in soil erosion (Liu et al., 2018; Li et al., 2019; Dai et al., 2021; Li et al., 2023) and biodiversity loss (Wang et al., 2009; Yu et al., 2022).

Ongoing climate change is expected to induce extremes in drought and flooding (Bailey-Serres et al., 2012), particularly in the Qinghai-Tibetan Plateau region, and thus, the plants here are likely to be subjected to frequent drought and waterlogging stress concurrently under global warming (Luo et al., 2022). To reduce and eliminate the deleterious effects of degrading grasslands on ecosystem functioning, it is of utmost importance to develop and adopt germplasms that have a better capacity to endure abiotic stress. A comprehensive understanding of the developmental, morphological, and physiological responses of plants to extremes in water availability is a prerequisite to identify high-quality germplasm resources. Waterlogging stress or drought stress, the major environmental stresses that plants encounter during their growth and development stages (Bello et al., 2022), limits plant survival, reproduction and yield (Kar, 2011). Previous studies have demonstrated that water stress induces changes in morphological, physiological, and biochemical plant characteristics to counteract potential harm (Hsiao, 1973; Nautiyal et al., 1994; Fernández et al., 2002). However, the degree of adaptation of plants to water stress may vary considerably among environmental conditions (including soil fertility and climate) and within species. Moreover, there are complex interactions across plant species, water stress intensity and duration and environmental factors. Therefore, the response and adaptation mechanisms of plants to water stress remain unclear. However, the response and adaptation mechanisms of plants are very important for breeding water-tolerant varieties (Wu et al., 2022).

Plant height is an important trait used to indicate competition capability, plant growth and production (Jiang et al., 2020). Plant roots are also an important component of the drought stress response (Guo et al., 2020). The root/shoot ratio is an alternative measurement method and is frequently employed to capture the biomass allocation of plants (Poorter et al., 2012) or reflect the differential investment of photosynthesis between the aboveground and belowground organs (Titlyanova et al., 1999). An increased root/shoot ratio suggests more investment of photosynthesis into belowground parts. According to the "optimal partitioning theory" (Gedroc et al., 1996), plants preferentially allocate biomass and nonstructural carbohydrates to acquire the resource that most limits their growth (Kobe et al., 2010). For example, drought increased the root/shoot ratio in rice (Xu et al., 2015) and Sage (Caser et al., 2017), and waterlogging reduced the root/shoot ratio in winter wheat (Shao et al., 2013) and maize (Herzog et al., 2016). Photosynthetic pigments play a role in the absorption, transmission and transformation of light energy during photosynthesis (Arjenaki et al., 2012; Reinbothe et al., 2010). Chlorophyll a mainly converts the collected light energy into chemical energy for photochemistry, while chlorophyll b mainly collects light energy. Although both chlorophyll a and chlorophyll b can receive and transmit light energy, only part of chlorophyll a can act as the central pigment of photosynthetic reactions (Reddy et al., 2004). Chlorophyll concentration is known as an indicator for the evaluation of photosynthesis (Zobayed et al., 2005), and its decline has been considered a nonstomatal limiting factor and a kind of protection mechanism for photosynthetic structures under abiotic stress (Du et al., 2012; Jumrani and Bhatia, 2019; Bhusal et al., 2020a). The contents of chlorophyll a and chlorophyll b directly affect the photosynthesis and growth status of plants to a certain extent (Paknejad et al., 2006). Carotenoids overcome the effects of stress on plant growth by helping maintain photosynthesis and reducing the degree of membrane oxidative damage (Paknejad et al., 2006). Photosynthetic activity is inhibited in plant tissues due to an imbalance between light capture and its utilization under drought stress (Foyer and Noctor, 2000). Malondialdehyde (MDA) can well indicate the degree of membrane lipid peroxidation (Rui et al., 2013; Zhou et al., 2022), and its content has been used to reflect the response of plants to stress (Gill and Tuteja, 2010; Wang X. et al., 2022; Zhou et al., 2022). Osmotic adjustment substances such as proline (Pro), soluble sugars (SS), and soluble protein (SP) can effectively reduce the water potential of plant cells under drought conditions and prevent cell dehydration to ensure normal plant growth (Ozturk et al., 2021; Zhou et al., 2022). For instance, Pro accumulates as an adaptive response under stress conditions (Maggio et al., 2002; Zulfiqar and Ashraf, 2022) to protect plants from the deleterious impact of water deficiency-mediated oxidative stress by increasing ROS quenching efficiency *via* different mechanisms, including maintaining GSH/GSSG balance. In addition, its accumulation aids in retaining membrane integrity by decreasing lipid oxidation by guarding the cellular redox potential and scavenging free radicals (Shinde et al., 2016; Nadeem et al., 2019). Finally, inhibited activities of Pro dehydrogenase and Pro oxidase by water stress slow the incorporation of Pro into protein (Kumar et al., 2020).

In the present study, we compared the performance of nine herbaceous species in different soil water conditions stimulating global climate change. As environmental stress induces multiple responses in plants, from subcellular to structural levels, major morphological, physiological, and biochemical parameters were assessed. We aimed to answer the following questions: (1) Is plant species identity an important factor determining the morphological, physiological and biochemical responses to environmental change? (2) Does environmental stress change the inherent differences in growth and metabolism across plant species? We hypothesized that water stress would impact plant growth and metabolism. We also expected that the effect of moderate water stress would be less pronounced than that of plant species identity.

2 Materials and methods

2.1 Substrate

To exclude the potential effects of soil texture, fertility and soil biota on plant growth and metabolism, all soil used in the present study was of the same source. The soil was collected from an alpine meadow in Dawu Town, Maqing County, Golog Tibetan Ethnic Minority Autonomous Prefecture, Qinghai Province, while the sands were purchased from a building materials market nearby. As previously described (Luo et al., 2022), the soil of the alpine meadow was classified as Mat Cry-gelic Cambisol, and its chemical properties are as follows: soil organic matter 14.53 mg/g, total nitrogen 3.12 mg/g, total phosphorus 0.26 mg/g, total potassium 19.58 mg/g, pH 7.63 (water/soil at 1:1 weight/volume) and CEC 225.52 μS/cm (water/soil at 5:1 weight/volume).

2.2 Plant material

In the present study, nine herbaceous species including *Deschampsia caespitosa*, *Poa crymophila* Keng, *Poa pratensis* L. cv. Qinghai, *Festuca sinensis* Keng ex S. L. Lu, *Puccinellia tenuiflora* (Griseb.) Scribn. et Merr. cv. Tongde, *Elymus nutans* Griseb., *Kobresia tibetica*, *Blysmus sinocompressus* Tang et Wang, and *Carex moorcroftii* Falc. Ex Boott were tested. All selected plants were supplied with root nutrients. *D. caespitosa*, *P. crymophila*, *P. pratensis*, *F. sinensis*, *P. tenuiflora*, and *E. nutans* were obtained from the seed breeding fields of the Grassland Research Institute, Academy of Animal Husbandry and Veterinary Sciences, Qinghai University, which is located in Dawu Town, Maqin County, Golog Tibetan Autonomous Prefecture, Qinghai Province, whereas *K. tibetica*, *B. sinocompressus* and *C. moorcroftii* were collected from the nearby alpine meadow of the seed breeding field. In early May 2018, the plants with rootstalk were dug up, and then litter were removed. The plants were carefully divided into small clusters with approximately the same amount of root and aboveground biomass and kept at moderate moisture for later use.

2.3 Experimental setup

The study was conducted at the Chengbei Campus of Qinghai Normal University (36°44′N, 101°44′E), Xining city, Qinghai Province, China. In May 2018, each cluster of the nine plant species was transplanted into a pot (20 cm in diameter, 25 cm in height) containing 3.0 kg of a mixture of alpine meadow soil and sand (sand/soil at 1:1 weight/volume). Seedlings were kept at 10 individual plants per pot after seedlings survived. During this period, the plants were kept in the greenhouse and watered when needed. To ensure that all replicates of each treatment had similarly healthy and representative individuals, only those growing well were kept for later use. In July 2018, water stress treatment was carried out using a completely randomized design.

During water treatment, the canopy was erected in situ. Both sides of the canopy were ventilated, which did not affect the temperature and humidity. Thereafter, the daytime and nighttime temperatures of the greenhouse were continuously managed to mimic environmental field conditions. During the experiment, the daytime temperature was (20 ± 2)°C, and the nighttime temperature was (5 ± 2)°C. The day length of the interior climate was that of the outside environmental conditions since the transparent, clear glass chamber structure was completely exposed to the outside environment. The structure was also equipped with automatic vents and fans. Air temperature and relative humidity inside the canopy were monitored with a portable meteorological meter (Holder HED-SQ, China).

Three water treatments were set up as follows: moderate waterlogging (only the root and neck of the plant was flooded, that is, the depth of the water was approximately 3 cm, MW), normal plant water requirement (70%-80% of field water capacity, control (CK), and moderate drought (30%-40% of field water capacity, MD). There were 10 replicates for each treatment. Soil moisture was monitored by the combined weighting method and soil moisture sensor (ProCheck, USA), and the lost water was replenished every two days to ensure that the plants were living under the given soil moisture. Watering was performed between 18:00~19:00, and a plant-free pot was set as a control to estimate water loss due to evaporation. The water stress treatments lasted for 35 days.

2.4 Sampling and assaying

2.4.1 Determination of biomass

Sampling was conducted on the 36th day after treatment. Five individual plants were randomly selected from each pot to measure height and root length using a measuring tape. Then, three pots of each treatment were randomly selected and harvested manually, and the biomass of aboveground parts, including stems and leaves, and underground root biomass were collected separately. The plants were cut with scissors at 5 cm above the soil. Stems and leaves were collected and put into a kraft bag, and roots were removed carefully and washed. All the collected plant materials were desiccated at 105°C and oven-dried at 80°C to determine the dry weight. The plants of the remaining seven pots were collected, snap-frozen and stored at –80°C for biochemical assays (contents of photosynthetic pigment, MDA and osmoprotectant). The root/shoot ratio was estimated by dividing the total dry root biomass by the total dry shoot biomass of each pot.

2.4.2 Photosynthetic pigment determination

Chlorophyll was extracted using acetone and anhydrous ethanol. Briefly, approximately 0.1 g of fresh leaves was weighed, cut and put into a calibration test tube. Then, 10 mL of a mixture of 95% ethanol and 80% acetone at a volume ratio of 1:1 was added and incubated in the dark for 48 hours until the green leaves became colorless. A mixture of 95% ethanol and 80% acetone was used as a blank control. The absorbance values were measured at 470 nm, 645 nm and 663 nm by an enzyme-labeled instrument (Bole xMark). Absorbance values were calculated using the following equations:

$$Chlorophyll\ a\ =\ [(12.72\ A_{663}-2.59\ A_{645})\times V\times N/W$$

$$Chlorophyll\ b\ =\ [(22.88\ A_{645}-4.67\ A_{663})\times V\times N/W$$

$$Chlorophyll\ =\ chlorophyll\ a\ +\ chlorophyll\ b$$

$$Carotenoid\ =\ (1000\ A_{470}-2.05\ Chlorophyll\ a-114.8$$
$$Chlorophyll\ b)/245\times V\times N/W$$

where V represents the volume of the extract, N represents the dilution, and W represents the fresh weight of the sample (g).

2.4.3 MDA assaying

Lipid peroxidation, an indicator of oxidative damage to the cell membranes (Girotti, 1990), was estimated by measuring MDA production (Dhindsa and Matowe, 1981). Briefly, frozen leaf samples (0.5 g) were ground to a powder in a mortar with liquid nitrogen and homogenized with 2 mL phosphate buffer (PBS, pH 7.8). The resulting residue was washed three times with 1 mL PBS each time and pooled into a centrifuge tube. The homogenate was centrifuged at 6000 rpm for 20 min. The supernatant was used to measure MDA. The supernatant (1 mL) was added to 5 mL of 0.5% TCA containing 0.6% thiobarbituric acid (TBA). The solution was boiled for 10 min and then centrifuged at 12000 rpm for 10 min after cooling. The absorbance of the mixture was measured at 450 nm, 532 nm, and 600 nm. The MDA content was estimated according to the following equation:

$$C(MDA)/\mu mol\cdot L^{-1}$$
$$=\ [6.452\times(A_{532}-A_{600})\ -0.559\times A_{450}]\ \times V1/(W\times V2)$$

where V1 represents the total volume of the extract; V2 represents the volume of sample solution during measurement; and W is the fresh weight of the sample (g).

2.4.4 Determination of osmotic adjustment substances

The SS content was determined using the anthrone method (John et al., 1950). The content of SP was determined using the G-250 Coomassie brilliant blue method (Bradford, 1976). A 1 g frozen sample was ground with 1.5 mL 80% ethanol (adding a little quartz sand) in the precooling bowl, and the volume was fixed to 5 mL with 80% ethanol solution. The extract was transferred into the test tube at 80°C for 20 min. Then, the extract was filtered twice through filter paper with activated carbon. The filtrate was placed in the test tube with 0.2 × the weight of zeolite and oscillated for 5 min. The supernatant was centrifuged at 4°C for 10 min at 5000 × g, and Procontent was determined by acid ninhydrin colorimetry (Bates et al., 1973).

2.5 Statistical analysis

Before analysis, the normality and variance homogeneity of variables were examined by using the Shapiro–Wilk normality test and Levene's test, respectively. When the assumption was met, means were compared with two-way ANOVAs, and multiple comparisons were carried out using Tukey's HSD test. Otherwise, means were compared with the Kruskal–Wallis test, and multiple comparisons were performed using the Wilcox test with the Benjamini method to adjust the *P* values. All statistics were performed using Statistical Product and Service Solutions (SPSS v22.0, IBM Corporation, United States). The figures were produced using OriginPro 2017 (OriginLab Corp, Northampton, United States).

3 Results

3.1 Morphology and biomass

We found a significant effect of water stress on plant height, root length, total biomass, and root/shoot ratio and distinct interspecies differences in plant height, total biomass, and root/shoot ratio (Tables 1, A1). Additionally, we also observed significant interactive effects of water stress and species identity on plant height, biomass and root/shoot ratio (Tables 1, A1).

In comparison with CK, moderate waterlogging significantly increased the plant height of *C. moorcroftii*, whereas moderate drought significantly decreased the plant heights of *C. moorcroftii*, *E. nutans* and *F. sinensis*. Additionally, the heights of *C. moorcroftii*, *D. caespitosa*, and *F. sinensis* under moderate waterlogging were significantly higher than those under moderate drought. The opposite was the case for *E. nutans* and *P. crymophila*.

In comparison with CK, the root length of nine selected plant species under moderate drought did not significantly change. In contrast, the response of root length to moderate waterlogging varied greatly depending on plant species identity. In comparison with CK, moderate waterlogging significantly decreased the root length of *E. nutans*, whereas moderate drought exerted no significant effect on the root length of *E. nutans*. However, the root length of *E. nutans* under moderate waterlogging was significantly shorter than that under moderate drought.

In comparison with CK, both moderate waterlogging and moderate drought significantly decreased the biomasses of *D. caespitosa*, *E. nutans*, *F. sinensis* and *P. crymophila*. In contrast, moderate waterlogging significantly increased the biomasses of *B. sinocompressus* and *C. moorcroftii* in comparison with CK. In addition, the biomasses of *D. caespitosa*, *E. nutans* and *P. crymophila* under moderate waterlogging were significantly less than those under moderate drought. The opposite pattern was observed for *B. sinocompressus*, *C. moorcroftii*, *K. tibetica and P. pratensis*. However, only the biomasses of *D. caespitosa*, *E. nutans* and *P. crymophila* showed significant differences across water regimes.

In comparison with CK, moderate waterlogging significantly decreased the root/shoot ratios of *E. nutans* and *K. tibetica*, and moderate drought significantly increased the root/shoot ratios of *B. sinocompressus*, *D. caespitosa*, *E. nutans*, *F. sinensis and P. tenuiflora*, while significantly decreasing that of *P. crymophila*. However, only the root/shoot ratio of *E. nutans* showed significant differences across water regimes.

TABLE 1 Plant height, root length, plant biomass and root/shoot ratio of nine selected herbaceous plant species under different water regimes, including control (CK), moderate drought (MD), and moderate waterlogging (MW).

Index	Water regime	*Deschampsia caespitosa*	*Poa crymophila*	*Poa pratensis*	*Festuca sinensis*	*Puccinellia tenuiflora*	*Elymus nutans*	*Kobresia tibetica*	*Blysmus sinocompressus*	*Carex moorcroftii*
Plant height (cm)	MW	43.09 ± 2.29 ABa	37.67 ± 4.33 ABCb	34.33 ± 2.91 BCa	46.55 ± 1.13 Aa	42.89 ± 3.40 ABa	42.00 ± 4.19 ABa	24.11 ± 0.73 Da	32.22 ± 0.78 Cab	43.89 ± 1.16 Aa
	CK	38.40 ± 0.86 ABab	38.78 ± 0.73 ABab	33.66 ± 3.38 ABCa	41.89 ± 1.42 Aa	33.89 ± 7.53 ABCa	37.22 ± 1.06 ABa	25.89 ± 3.31 Ca	36.89 ± 2.63 ABa	29.33 ± 0.38 BCc
	MD	32.12 ± 4.64 ABCb	41.33 ± 5.77 Aa	36.33 ± 1.00 ABa	33.45 ± 3.01 ABCb	41.33 ± 1.00 Aa	26.89 ± 1.22 Cb	30.50 ± 0.87 BCa	30.50 ± 1.25 BCb	38.66 ± 1.54 ABb
Root length (cm)	MW	16.77 ± 0.41 ABCa	15.78 ± 2.44 ABCa	10.78 ± 1.46 Ca	18.72 ± 1.16 ABa	12.44 ± 1.49 BCa	13.52 ± 0.75 ABCb	18.56 ± 3.51 ABa	12.72 ± 1.45 BCa	19.83 ± 2.77 Aa
	CK	18.29 ± 1.25 ABa	16.55 ± 1.18 ABCa	10.89 ± 2.02 Da	12.73 ± 1.83 CDa	10.28 ± 0.94 Da	17.22 ± 0.87ABCa	14.00 ± 1.76 BCDa	12.56 ± 0.11 CDa	19.50 ± 1.83 Aa
	MD	16.73 ± 1.61 ABa	18.00 ± 0.51 ABa	16.00 ± 1.07ABCa	18.13 ± 3.09 ABa	12.11 ± 0.73 Ca	16.78 ± 0.59 ABa	17.73 ± 1.19 ABa	14.17 ± 1.44 BCa	19.50 ± 0.48 Aa
Total biomass (g/pot)	MW	5.74 ± 0.32 Cc	4.44 ± 0.25 Cc	8.17 ± 0.68 Ba	4.86 ± 0.38 Cb	8.80 ± 0.96 ABa	4.26 ± 0.32 Cc	8.07 ± 0.21 Ba	9.18 ± 0.44 Ba	12.00 ± 0.63 Aa
	CK	8.47 ± 0.23 ABa	6.43 ± 1.66 BCa	5.41 ± 0.57 Cb	6.72 ± 0.21BCa	7.71 ± 1.08 ABCa	9.78 ± 0.36 Aa	7.42 ± 0.25 ABCa	6.41 ± 0.72 BCb	8.81 ± 0.87 ABb
	MD	6.87 ± 0.18 ABb	8.18 ± 0.52 Ab	5.75 ± 0.47 BCb	4.13 ± 0.20 Db	6.10 ± 0.38 Ba	7.71 ± 0.39 Ab	6.09 ± 0.21Bb	4.70 ± 0.67 CDb	7.08 ± 0.57 ABb
Root/shoot ratio	MW	0.60 ± 0.06 Bb	1.45 ± 0.19 Aa	0.75 ± 0.05 Ba	0.54 ± 0.12 Bb	0.53 ± 0.06 Bab	0.56 ± 0.10 Bc	0.63 ± 0.05 Bb	1.41 ± 0.05 Ab	0.58 ± 0.05 Ba
	CK	0.60 ± 0.03 Db	1.06 ± 0.07 Aa	0.65 ± 0.05 CDa	0.56 ± 0.09 Db	0.35 ± 0.07 Eb	0.98 ± 0.06 ABb	0.99 ± 0.09 ABa	1.12 ± 0.06 Ab	0.82 ± 0.06 BCa
	MD	1.60 ± 0.13 Ba	0.87 ± 0.18 Db	0.72 ± 0.08 Da	1.31 ± 0.07 BCa	0.73 ± 0.05 Da	1.34 ± 0.09 BCa	1.08 ± 0.10 CDa	2.94 ± 0.18 Aa	0.81 ± 0.09 Da

Different lowercase letters within the same row indicate that there are significant differences between water regimes for the same plant species at α=0.05; Different capital letters within the same column indicate that there are significant differences between plant species under the same water condition at α=0.05. Data are expressed as means ± SEM (n=3).

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3.2 Photosynthetic pigments

Water regime, species identity and their interactive effect significantly affected the contents of chlorophyll a, chlorophyll b, chlorophyll (a+b), and carotenoids (Tables 2, 3), suggesting that photosynthetic pigment contents in plants respond differently to water stress.

In comparison with CK, the content of chlorophyll a in leaves of *B. sinocompressus*, *C. moorcroftii* and *K. tibetica* significantly increased under moderate waterlogging but significantly decreased under moderate drought. In contrast, the content of chlorophyll a in leaves of *P. crymophila* significantly increased under water stress, whereas those in leaves of *F. sinensis* significantly decreased. Finally, the chlorophyll a content in the leaves of *D. caespitosa* significantly decreased under moderate drought compared to that of CK. Overall, we observed significant differences in chlorophyll a content in leaves of *B. sinocompressus*, *C. moorcroftii*, *K. tibetica* and *P. crymophila* across water regimes.

In comparison with CK, the content of chlorophyll b in leaves of *B. sinocompressus*, *C. moorcroftii* and *K. tibetica* significantly increased under moderate waterlogging, whereas the opposite held true for those in leaves of *B. sinocompressus*, *C. moorcroftii* and *K. tibetica* under moderate drought. Additionally, the chlorophyll b content in the leaves of *D. caespitosa* significantly decreased under both moderate waterlogging and moderate drought. Finally, the content of chlorophyll b in leaves of *P. crymophila* significantly decreased under moderate waterlogging in comparison with those of CK. Overall, we observed significant differences in the content of chlorophyll b in leaves of *B. sinocompressus*, *C. moorcroftii*, *K. tibetica* and *D. caespitosa* across water regimes.

Moderate waterlogging significantly increased the chlorophyll content in the leaves of *B. sinocompressus*, *C. moorcroftii* and *K. tibetica* in comparison with CK. In contrast,moderate drought significantly decreased the chlorophyll content in the leaves of *B. sinocompressus*, *C. moorcroftii* and *K. tibetica*. However, both moderate waterlogging and moderate drought significantly increased the chlorophyll content in leaves of *P. crymophila*. Finally, chlorophyll content in leaves of *P. crymophila* significantly decreased under moderate drought compared to that of CK. Overall, we observed significant differences in chlorophyll content in leaves of *B. sinocompressus*, *C. moorcroftii*, *K. tibetica* and *P. crymophila* across water regimes. The carotenoid content in leaves of *B. sinocompressus* and *D. caespitosa* significantly increased under moderate waterlogging in comparison with CK. In contrast, the carotenoid contents in leaves of *B. sinocompressus* and *D. caespitosa* significantly decreased under moderate drought in comparison with CK. Additionally, the carotenoid content in leaves of *P. crymophila* significantly increased under moderate drought compared to that of CK. Overall, we only observed significant differences in carotenoid content in leaves of *B. sinocompressus* across water regimes.

3.3 Lipid peroxidation

MDA content in both the shoots and roots of the nine selected plants was changed by the water regime. In addition, MDA content showed a remarkable interspecies difference. Furthermore, we observed significant interactive effects of plant species identity and water regime on MDA contents in shoots of the nine selected plants (Table A2; Figure 1).

3.4 Osmoprotective compounds

The water regime exerted significant effects on the contents of soluble sugar, betaine, soluble protein and proline in both the shoots and roots of the nine selected plants. Plant species identity exerted significant effects on the contents of soluble sugar and proline in the shoots of selected plants as well as on the contents of soluble sugar, betaine, soluble protein and proline in the roots of selected plants (Table A3; Figure 2). Additionally, the interactive effects of water regime and plant species identity significantly affected the contents of soluble sugar, betaine, soluble protein and proline in plant shoots and roots, suggesting that osmoprotective compound contents in plants respond differently to water stress (Table A3; Figure 2).

4 Discussion

4.1 Morphology and biomass

In the present study, water stress including drought and waterlogging, evidently decreased the biomasses of *D. caespitosa*, *E. nutans* and *P. crymophila*, but did not significantly change the biomass of *P. tenuiflora*. However, moderate waterlogging significantly increased the biomasses of *B. sinocompressus* and *C. moorcroftii* (Table 2). Our results are in agreement with previous studies that demonstrated that drought stress inhibits plant growth and biomass accumulation (*e.g.*, Loggini et al., 1999; Lenhart et al., 2015; Caser et al., 2019; El-Beltagi et al., 2020; Moreno-Galván et al., 2020; Wang et al., 2021), whereas waterlogging decreases (de San Celedonio et al., 2014; Imaz et al., 2015; Doupis et al., 2017) or increases plant biomass (Rubio et al., 1995). The likely reason why does plant increase biomass under waterlogging is that this plant has a tight regulation of water and carbon relations under severe soil-oxygen deficiency (Insausti et al., 2001). In light of the stability of biomass, we argue that *P. tenuiflora* rather than *D. caespitosa* is a promising species in grassland restoration in the Qinghai-Tibetan Plateau.

In our study, the responses of plants to drought and waterlogging were dependent on plant species identity. Similar results have been reported elsewhere (Reents et al., 2021). In addition, *E. nutans* showed a marked increase in root/shoot ratio when exposed to drought and pronounced lower in root/shoot ratio when exposed to waterlogging (Table 1). As proposed, an increased root/shoot ratio would increase absorbent root surface and further improve water and nutrient use to enhance tolerance of plants under stress conditions (Hebeisen et al., 1997; Lazzarotto et al., 2009). Besides, previous studies have reported resource allocation was a response of plant to water stress (Blanch et al., 1999; Reents et al., 2021). However, the allocation of photosynthetic carbon in the underground part is likely to change with soil physicochemical properties (Wang et al., 2019). For instance, soil nutrient shortages

TABLE 2 Chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenoid contents of nine herbaceous plant species under different water regimes, including control (CK), moderate drought (MD), and moderate waterlogging (MW).

Index	Water regime	*Deschampsia caespitosa*	*Poa crymophila*	*Poa pratensis*	*Festuca sinensis*	*Puccinellia tenuiflora*	*Elymus nutans*	*Kobresia tibetica*	*Blysmus sinocompressus*	*Carex moorcroftii*
chl.a (mg/g FW)	MW	1.28 ± 0.11Ca	1.41 ± 0.01Ca	0.63 ± 0.07Da	0.74 ± 0.20Db	0.47 ± 0.05Da	0.57 ± 0.08Da	2.32 ± 0.09ABa	2.15 ± 0.23Ba	2.57 ± 0.08Aa
	CK	1.42 ± 0.08BCa	1.07 ± 0.02Dc	0.73 ± 0.10Ea	1.34 ± 0.09BCa	0.50 ± 0.07Ea	0.73 ± 0.06Ea	1.57 ± 0.08ABb	1.32 ± 0.09Cb	1.73 ± 0.06Ab
	MD	0.90 ± 0.04Bb	1.25 ± 0.07Ab	0.84 ± 0.02Ba	0.74 ± 0.10Bb	0.32 ± 0.09Ca	0.71 ± 0.07Ba	0.73 ± 0.06Bc	0.41 ± 0.05Cc	0.81 ± 0.12Bc
chl.b (mg/g FW)	MW	0.55 ± 0.04BCb	0.50 ± 0.01Ca	0.22 ± 0.02DEa	0.27 ± 0.06Da	0.16 ± 0.02Ea	0.21 ± 0.03DEa	0.85 ± 0.03Aa	0.62 ± 0.01Ba	0.49 ± 0.04Ca
	CK	0.63 ± 0.07Aa	0.42 ± 0.02Bb	0.25 ± 0.03Ca	0.24 ± 0.08Ca	0.24 ± 0.08Ca	0.25 ± 0.02Ca	0.59 ± 0.04Ab	0.27 ± 0.03Cb	0.29 ± 0.02BCb
	MD	0.33 ± 0.01Bc	0.44 ± 0.02Aab	0.30 ± 0.01BCa	0.26 ± 0.04BCDa	0.13 ± 0.02Ea	0.20 ± 0.05DEa	0.25 ± 0.02BCDc	0.13 ± 0.02Ec	0.23 ± 0.03CDc
Chl (mg/g FW)	MW	1.83 ± 0.12Ba	1.91 ± 0.23Ba	0.85 ± 0.09Ca	1.00 ± 0.27Ca	0.63 ± 0.07Ca	0.79 ± 0.10Ca	3.17 ± 0.10Aa	2.76 ± 0.23Aa	3.06 ± 0.08Aa
	CK	2.05 ± 0.09Aa	1.49 ± 0.03Bc	0.98 ± 0.13Ca	1.57 ± 0.01Ba	0.74 ± 0.16Ca	0.98 ± 0.08Ca	2.16 ± 0.08Ab	1.59 ± 0.07Bb	2.01 ± 0.04Ab
	WD	1.23 ± 0.06Bb	1.69 ± 0.08Ab	1.14 ± 0.03BCa	1.00 ± 0.14BCa	0.45 ± 0.11Da	0.91 ± 0.11Ca	0.98 ± 0.08BCc	0.53 ± 0.06Dc	1.04 ± 0.13BCc
Cx.c (mg/g FW)	MW	0.39 ± 0.04Ba	0.43 ± 0.02Ba	0.18 ± 0.01CDb	0.23 ± 0.07Ca	0.16 ± 0.01CDa	0.17 ± 0.02CDa	0.12 ± 0.02Db	0.58 ± 0.03Aa	0.38 ± 0.03Ba
	CK	0.39 ± 0.03ABa	0.32 ± 0.04ABCb	0.23 ± 0.02Cab	0.21 ± 0.08Ca	0.21 ± 0.08Ca	0.21 ± 0.01Ca	0.17 ± 0.02Cab	0.45 ± 0.03Ab	0.29 ± 0.03BCab
	MD	0.26 ± 0.01Bb	0.42 ± 0.05Aa	0.26 ± 0.01Ba	0.23 ± 0.03BCa	0.12 ± 0.02Da	0.21 ± 0.02BCa	0.21 ± 0.01BCa	0.12 ± 0.02Dc	0.18 ± 0.04CDb

Different lowercase letters within the same row indicate that there are significant differences between water regimes for the same plant species at α=0.05; Different capital letters within the same column indicate that there are significant differences between plant species under the same water condition at α=0.05.

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TABLE 3 Results of two-way ANOVAs examining the major and interactive effects of water regime and plant species identity on the contents of photosynthetic pigments.

Source of variation	*df*	Chlorophyll a		Chlorophyll b		Chlorophyll (a+b)		Carotenoid	
		F	*P*	F	*P*	F	*P*	F	*P*
Water regime (W)	2	60.821	<0.001	42.405	<0.001	68.274	<0.001	21.619	<0.001
Species identity (S)	8	97.901	<0.001	55.472	<0.001	115.663	<0.001	10.630	<0.001
Interaction (W×S)	16	22.358	<0.001	12.941	<0.001	24.868	<0.001	8.000	<0.001

will increase the proportion of plant photosynthates to roots in wetlands (Cronin and Lodge, 2003). Further studies are warranted to explore the potential effects of soil physicochemical properties in shaping how plants respond to water stress.

4.2 Photosynthetic pigments

Our study agrees with previous studies suggesting that the contents of chlorophyll a and b showed a species-dependent response to water stress (Zaefyzadeh et al., 2009; Bhusal et al., 2020b). We observed that photosynthetic pigments in the *P. crymophila* significantly increased under water stress (Table 2). Previous studies demonstrated that drought stress and water deficit decreased (Din et al., 2011; Sperdouli and Moustakas, 2012; Chen et al., 2016; Meher et al., 2018; El-Beltagi et al., 2020) or increased (Balbaa et al., 2022) the chlorophyll content of leaves. In addition, waterlogging also exerts a negative effect on chlorophyll content and photosynthesis (Du et al., 2012; Bai et al., 2013; Barickman et al., 2019; Bhusal et al., 2020a). The decrease in chlorophyll content may be because drought or waterlogging induced the production of reactive oxygen species (ROS), such as O_2 and H_2O_2, which led to lipid peroxidation and consequently chlorophyll destruction. We observed that moderate drought decreased chlorophyll a and b contents as well as carotenoid content. In previous studies, drought decreased chlorophyll a and b contents as well as carotenoid contents in green grams (Anosheh et al., 2012), and moderate drought also decreased chlorophyll a and b in *Salvia officinalis* (Caser et al., 2019).

4.3 MDA content

Earlier studies suggested that drought did not change (Loggini et al., 1999) or increased lipid peroxidation (Moreno-Galván et al.,

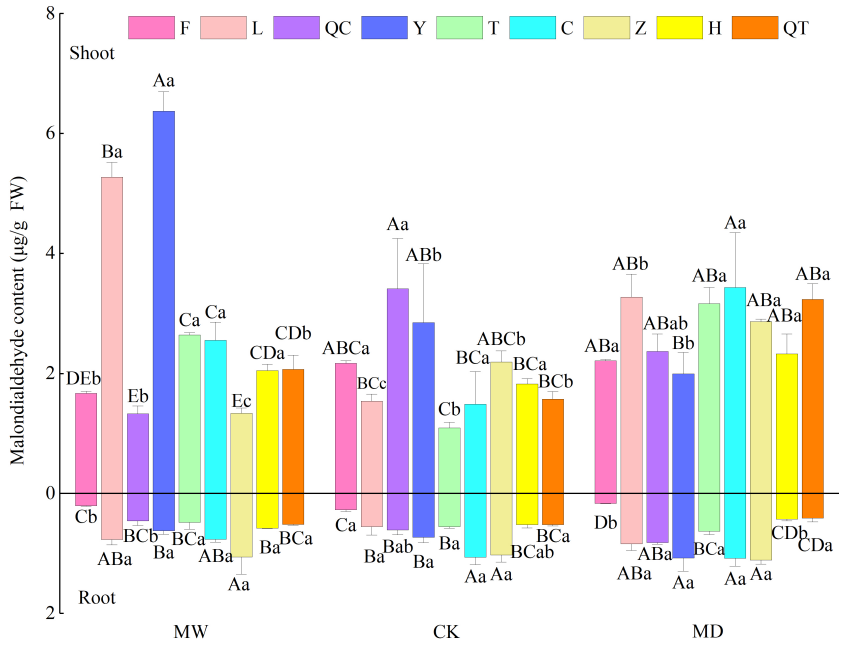

FIGURE 1
Malonaldehyde contents in leaves of nine selected plant species under different water regimes, including control (CK), moderate drought (MD) and moderate waterlogging (MW). Plant species include *Deschampsia caespitosa* (F), *Poa crymophila* Keng (L), *Poa pratensis* L. cv. Qinghai (QC), *Festuca sinensis* Keng ex S. L. Lu (Y), *Puccinellia tenuiflora* (Griseb.) Scribn.et Merr.cv. Tongde (T), *Elymus nutans* Griseb. (C), *Kobresia tibetica* (Z), *Blysmus sinocompressus* Tang et Wang (H), and *Carex moorcroftii* Falc. Ex Boott (QT). Different lowercase letters indicate that there are significant differences between water regimes for the same plant species at $P<0.05$, different capital letters indicate that there are significant differences between plant species under the same water condition at $P<0.05$. Bars represent the standard error (n = 3).

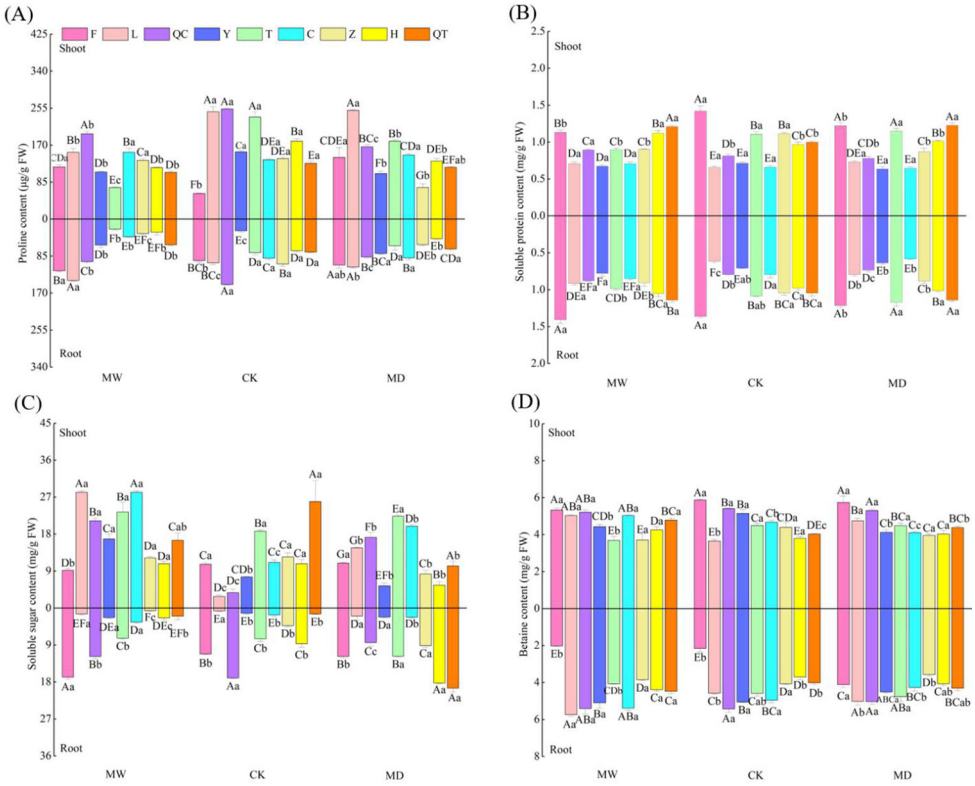

FIGURE 2
Proline **(A)**, soluble protein **(B)**, soluble sugar **(C)** and betaine **(D)** contents in shoots of nine selected plant species under different water regimes, including control (CK), moderate drought (MD) and moderate waterlogging (MW). Plant species include *Deschampsia caespitosa*(F), *Poa crymophila* Keng (L), *Poa pratensis* L. cv. Qinghai (QC), *Festuca sinensis* Keng ex S. L. Lu (Y), *Puccinellia tenuiflora* (Griseb.) Scribn.et Merr.cv. Tongde (T)>, *Elymus nutans* Griseb. (C), *Kobresia tibetica* (Z), *Blysmus sinocompressus* Tang et Wang (H), and *Carex moorcroftii* Falc. Ex Boott (QT). Different lowercase letters within the same row indicate that there are significant differences between water regimes for the same plant species at $P<0.05$ and different capital letters within the same column indicate that there are significant differences between plant species under the same water condition at $P<0.05$. Bars represent the standard error (n = 3).

2020), whereas waterlogging increased lipid peroxidation (Tan et al., 2008; Jumrani and Bhatia, 2019). In our study, we found that there were significant plant species effect and interactive effects of water regime and plant species on lipid peroxidation (Figure 1; Table A2), implying that plant tolerance to abiotic stress can be context dependent; the interspecies inherent difference in adoption tactics would change with living conditions. From the perspective of lipid peroxidation, *D. caespitosa* seems to be suitable for both waterlogging and water deficit conditions. The possible reason why the content of MDA remained fairly stable under water stresses may be related to either effective scavenging of free radicals by the antioxidant system or the prevention of free radical production (Tokarz et al., 2020).

4.4 Osmoprotective compounds

Our findings imply that the interspecies differences in the contents of soluble sugar, betaine, soluble protein and proline in plant shoots and roots changed greatly with their habitats (Table A; Figure 2). Specifically, we observed divergent effects of water stress on soluble sugar in plants (Figure 2C). Previous studies found that drought stress significantly increased the levels of sugars, betaines and proline (Chaves and Oliveira, 2004; Anosheh et al., 2012). Additionally, plants can also cope with water or osmotic stress by increasing the synthesis of osmoprotectants, such as proline (Sperdouli and Moustakas, 2012), an amino acid, exhibiting a dual function as an osmolyte compound and as an antioxidant when plants are exposed to various stresses (Hayat et al., 2012; Sperdouli and Moustakas, 2012; El-Beltagi et al., 2020). Studies have proposed that drought triggers modifications in proline metabolism that impair plant stress tolerance. Our findings are in agreement with studies suggesting that the proline content of plants under drought stress significantly increased compared with that of untreated plants (Hare et al., 1998; Anosheh et al., 2012; Sperdouli and Moustakas, 2012; Jayant and Sarangi, 2014; Jumrani and Bhatia, 2019; El-Beltagi et al., 2020).

5 Conclusions

In summary, we found significant effects of water stress on plant height, root length, total biomass, root/shoot ratio, and contents of chlorophyll a, chlorophyll b, chlorophyll (a+b), carotenoids, malondialdehyde and soluble sugar. We also observed apparent interspecies differences in plant height, root length, total biomass, root/shoot ratio, and contents of chlorophyll a, chlorophyll b,

chlorophyll (a+b), carotenoids, malondialdehyde, soluble sugar, betaine, soluble protein and proline. Finally, we observed significant interactive effects of water stress and species identity on plant height, root length, total biomass, root/shoot ratio, and contents of chlorophyll a, chlorophyll b, chlorophyll (a+b), carotenoids, malondialdehyde, soluble sugar, betaine, soluble protein and proline. However, the interactive effects of water stress and plant species identity on some examined parameters changed with plant tissue. Our results yield important implications for our understanding of ecosystem resilience to water stresses as well as plant species distribution in the Qinghai-Tibetan Plateau. We argue that these findings could provide a fundamental basis for the identification of tolerant germplasm resources to restore the degraded grassland and wetlands under future intensive global climate change.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

YSM designed the experiments. QL, HX and YGM collected the samples. QL and HY performed the laboratory work. QL, ZC and BY analyzed the data. BY contributed to manuscript revision. QL wrote the first version of the manuscript, which was then edited by all co-authors. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1147208/full#supplementary-material

References

Anosheh, H. P., Emam, Y., Ashraf, M., and Foolad, M. R. (2012). Exogenous application of salicylic acid and chlormequat chloride alleviates negative effects of drought stress in wheat. *Adv. Stud. Biol.* 4, 501–520.

Arjenaki, F. G., Jabbari, R., and Morshedi, A. (2012). Evaluation of drought stress on relative water content, chlorophyll content and mineral elements of wheat (Triticum aestivum L.) varieties. *Int. J. Agric. Crop. Sci.* 4, 726–729.

Bai, T., Li, C., Li, C., Liang, D., and Ma, F. (2013). Contrasting hypoxia tolerance and adaptation in *Malus* species is linked to differences in stomatal behavior and photosynthesis. *Physiol. Plant* 147, 514–523. doi: 10.1111/j.1399-3054.2012.01683.x

Bailey-Serres, J., Lee, S. C., and Brinton, E. (2012). Waterproofing crops: effective flooding survival strategies. *Plant Physiol.* 160, 1698–1709. doi: 10.1104/pp.112.208173

Balbaa, M. G., Osman, H. T., Kandil, E. E., Javed, T., Lamlom, S. F., Ali, H. M., et al. (2022). Determination of morpho-physiological and yield traits of maize inbred lines (*Zea mays* l.) under optimal and drought stress conditions. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.959203

Barickman, T. C., Simpson, C. R., and Sams, C. E. (2019). Waterlogging causes early modification in the physio1ogica1 perfonnance carotenoids ch1orophylls proline and soluble sugars of cucumber plants. *Plants (Basel)* 8, 160. doi: 10.3390/plants8060160

Bates, L. S., Waldren, R. A., and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant Soil* 39, 205–207.

Bello, Z. A., van Rensburg, L. D., Dlamini, P., Tfwala, C. M., and Tesfuhuney, W. (2022). Characterisation and effects of different levels of water stress at different growth stages in malt barley under water-limited conditions. *Plants (Basel)* 11, 578. doi: 10.3390/plants11050578

Bhusal, N., Kim, H. S., Han, S. G., and Yoon, T. M. (2020a). Photosynthetic traits and plant–water relations of two apple cultivars grown as bi-leader trees under long-term waterlogging conditions. *Environ. Exp. Bot.* 176, 104111. doi: 10.1016/j.envexpbot.2020.104111

Bhusal, N., Lee, M., Reum Han, A., Han, A., and Kim, H. S. (2020b). Responses to drought stress in *Prunus sargentii* and *Larix kaempferi* seedlings using morphological and physiological parameters. *For. Ecol. Manag* 465, 118099. doi: 10.1016/j.foreco.2020.118099

Blanch, S. J., Ganf, G. G., and Walker, K. F. (1999). Growth and resource allocation in response to flooding in the emergent sedge *Bolboschoenus medianus*. *Aquat. Bot.* 63, 145–160. doi: 10.1016/S0304-3770(98)00109-0

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72 (1-2), 248–254. doi: 10.1016/0003-2697(76)90527-3

Caser, M., Angiolillo, F., Chitarra, W., Lovisolo, C., Ruffoni, B., Pistelli, L., et al. (2017). Ecophysiological and phytochemical responses of *Salvia sinaloensis* fern. to drought stress. *Plant Growth Regul.* 84, 383–394. doi: 10.1007/s10725-017-0349-1

Caser, M., Chitarra, W., D'Angiolillo, F., Perrone, I., Demasi, S., Lovisolo, C., et al. (2019). Drought stress adaptation modulates plant secondary metabolite production in *Salvia dolomitica* codd. *Ind. Crop Prod* 129, 85–96. doi: 10.1016/j.indcrop.2018.11.068

Chaves, M. M., and Oliveira, M. M. (2004). Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J. Exp. Bot.* 55, 2365–2384. doi: 10.1093/jxb/erh269

Chen, Y. E., Liu, W. J., Su, Y. Q., Cui, J. M., Zhang, Z. W., Yuan, M., et al. (2016). Different response of photosystem II to short and long-term drought stress in *Arabidopsis thaliana*. *Physiol. Plant* 158, 225–235. doi: 10.1111/ppl.12438

Cronin, G., and Lodge, D. M. (2003). Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia* 137, 32–41. doi: 10.1007/s00442-003-1315-3

Dai, L., Guo, X., Ke, X., Du, Y., Zhang, F., and Cao, G. (2021). The variation in soil water retention of alpine shrub meadow under different degrees of degradation on

northeastern qinghai-Tibetan plateau. *Plant Soil* 458, 231–244. doi: 10.1007/s11104-020-04522-3

de San Celedonio, R. P., Abeledo, L. G., and Miralles, D. J. (2014). Identifying the critical period for waterlogging on yield and its components in wheat and barley. *Plant Soil* 378, 265–277. doi: 10.1007/s11104-014-2028-6

Dhindsa, R. S., and Matowe, W. (1981). Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. *J. Exp. Bot.* 32 (1), 79–91. doi: 10.1093/jxb/32.1.79

Din, J., Khan, S. U., Ali, I., and Gurmani, A. R. (2011). Physiological and agronomic response of canola varieties to drought stress. *J. Anim. Plant Sci.* 21, 78–82. doi: 10.4081/ijas.2011.e11

Doupis, G., Kavroulakis, N., Psarras, G., and Papadakis, I. E. (2017). Growth, photosynthetic performance and antioxidative response of 'Hass' and 'Fuerte' avocado (*Persea americana* mill.) plants grown under high soil moisture. *Photosynthetica* 55, 655–663. doi: 10.1007/s11099-016-0679-7

Du, K., Xu, L., Wu, H., Tu, B., and Zheng, B. (2012). Ecophysiological and morphological adaption to soil flooding of two poplar clones differing in flood-tolerance. *Flora* 207 (2), 96–106. doi: 10.1016/j.flora.2011.11.002

El-Beltagi, H. S., Mohamed, H. I., and Sofy, M. R. (2020). Role of ascorbic acid, glutathione and proline applied as singly or in sequence combination in improving chickpea plant through physiological change and antioxidant defense under different levels of irrigation intervals. *Molecules* 25 (7), 1702, 2-17. doi: 10.3390/molecules25071702

Fernández, R. J., Wang, M. B., and Reynolds, J. F. (2002). Do morphological changes mediate plant responses to water stress? a steady-state experiment with two C4 grasses. *New Phytol.* 155, 79–88. doi: 10.1046/j.1469-8137.2002.00438.x

Foyer, C. H., and Noctor, G. (2000). Oxygen processing in photosynthesis: regulation and signalling. *New Phytol.* 146, 359–388. doi: 10.1046/j.1469-8137.2000.00667.x

Gedroc, J. J., McConnaughay, K. D. M., and Coleman, J. S. (1996). Plasticity in root/shoot partitioning: optimal, ontogenetic, or both? *Funct. Ecol.* 10, 44–50. doi: 10.2307/2390260

Gill, S. S., and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 48, 909–930. doi: 10.1016/j.plaphy.2010.08.01

Girotti, A. W. (1990). Photodynamic lipid peroxidation in biological systems. *Photochem. Photobiol.* 51, 497–509. doi: 10.1111/j.1751-1097.1990.tb01744.x

Guo, J., Li, C., Zhang, X., Li, Y., Zhang, D., Shi, Y., et al. (2020). Transcriptome and GWAS analyses reveal candidate gene for seminal root length of maize seedlings under drought stress. *Plant Sci.* 292, 110380. doi: 10.1016/j.plantsci.2019.110380

Hare, P. D., Cress, W. A., and Van Staden, J. (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ.* 21 (6), 535–553. doi: 10.1046/j.1365-3040.1998.00309.x

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., and Ahmad, A. (2012). Role of proline under changing environments: A review. *Plant Signal Behav.* 7, 1456–1466. doi: 10.4161/psb.21949

Hebeisen, T., Lüscher, A., Zanetti, S., Fischer, B., Hartwig, U., Frehner, M., et al. (1997). Growth response of trifolium repens l. and *Lolium perenne* l. as monocultures and bi-species mixture to free air CO_2 enrichment and management. *Global Change Biol.* 3 (2), 149–160. doi: 10.1046/j.1365-2486.1997.00073.x

Herzog, M., Striker, G. G., Colmer, T. D., and Pedersen, O. (2016). Mechanisms of waterlogging tolerance in wheat – a review of root and shoot physiology. *Plant Cell Environ.* 39, 1068–1086. doi: 10.1111/pce.12676

Hsiao, T. C. (1973). Plant responses to water stress. *Ann. Rev. Plant Physio.* 24, 519–570. doi: 10.1146/annurev.pp.24.060173.002511

Imaz, J. A., Giménez, D. O., Grimoldi, A. A., and Striker, G. G. (2015). Ability to recover overrides the negative effects of flooding on growth of tropical grasses *Chloris gayana* and *Panicum coloratum*. *Crop Pasture Sci.* 66 (1), 100–106. doi: 10.1071/CP14172

Insausti, P., Grimoldi, A. A., Chaneton, E. J., and Vasellati, V. (2001). Flooding induces a suite of adaptive plastic responses in the grass *Paspalum dilatatum*. *New Phytol.* 152 (2), 291–299. doi: 10.1111/j.0028-646X.2001.257_1.x

Jayant, K. S., and Sarangi, S. K. (2014). Effect of drought stress on proline accumulation in peanut genotypes. *Int. J. Adv. Res.* 2 (10), 301–309.

Jiang, T., Liu, J., Gao, Y., Sun, Z., Chen, S., Yao, N., et al. (2020). Simulation of plant height of winter wheat under soil water stress using modified growth functions. *Agr Water Manage* 232, 106066. doi: 10.1016/j.agwat.2020.106066

John, A., Barnett, G., and Miller, T. B. (1950). The determination of soluble carbohydrate in dried samples of grass silage by the anthrone method. *J. Sci. Food Agr* 1 (11), 336–339.

Jumrani, K., and Bhatia, V. S. (2019). Interactive effect of temperature and water stress on physiological and biochemical processes in soybean. *Physiol. Mol. Biol. Pla* 25, 667–681. doi: 10.1007/s12298-019-00657-5

Kar, R. K. (2011). Plant responses to water stress: role of reactive oxygen species. *Plant Signal Behav.* 6 (11), 1741–1745. doi: 10.4161/psb.6.11.17729

Kobe, R. K., Iyer, M., and Walters, M. B. (2010). Optimal partitioning theory revisited: Nonstructural carbohydrates dominate root mass responses to nitrogen. *Ecology* 91, 166–179. doi: 10.1890/09-0027.1

Kumar, S., Kumar, D., Kumar, P., Malik, P. S., and Kumar, M. (2020). Proline accumulation in the leaves of four potato cultivars in response to water stress. *Plant Arch.* 20 (2), 3510–3514.

Lazzarotto, P., Calanca, P., and Fuhrer, J. (2009). Dynamics of grass–clover mixtures–an analysis of the response to management with the PROductive GRASsland simulator (PROGRASS). *Ecol. Model.* 220 (5), 703–724. doi: 10.1016/j.ecolmodel.2008.11.023

Lenhart, P. A., Eubanks, M. D., and Behmer, S. T. (2015). Water stress in grasslands: dynamic responses of plants and insect herbivores. *Oikos* 124, 381–390. doi: 10.1111/oik.01370

Li, W., Shang, X., Yan, H., Xu, J., Liang, T., and Zhou, H. (2023). Impact of restoration measures on plant and soil characteristics in the degraded alpine grasslands of the qinghai Tibetan plateau: A meta-analysis. *Agr Ecosyst. Environ.* 347, 108394. doi: 10.1016/j.agee.2023.108394

Li, Y., Li, J., Are, K. S., Huang, Z., Yu, H., and Zhang, Q. (2019). Livestock grazing significantly accelerates soil erosion more than climate change in Qinghai-Tibet Plateau: Evidenced from 137Cs and 210Pbex measurements. *Agr. Ecosyst. Environ.* 285, 106643. doi: 10.1016/j.agee.2019.106643

Liu, S., Zamanian, K., Schleuss, P.-M., Zarebanadkouki, M., and Kuzyakov, Y. (2018). Degradation of Tibetan grasslands: consequences for carbon and nutrient cycles. *Agr Ecosyst. Environ.* 252, 93–104. doi: 10.1016/j.agee.2017.10.011

Loggini, B., Scartazza, A., Brugnoli, E., and Navari-Izzo, F. (1999). Antioxidative defence system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol.* 119, 1091–1099. doi: 10.1104/pp.119.3.1091

Luo, Q., Ma, Y., Chen, Z., Xie, H., Wang, Y., Zhou, L., et al. (2022). Biochemical responses of hairgrass (*Deschampsia caespitosa*) to hydrological change. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.987845

Maggio, A., Miyazaki, S., Veronese, P., Fujita, T., Ibeas, J. I., Damsz, B., et al. (2002). Does proline accumulation play an active role in stress-induced growth reduction? *Plant J.* 31, 699–712. doi: 10.1046/j.1365-313X.2002.01389.x

Meher,, Shivakrishna, P., Ashok Reddy, K., and Manohar Rao, D. (2018). Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. *Saudi J. Biol. Sci.* 25, 285–289. doi: 10.1016/j.sjbs.2017.04.008

Moreno-Galván, A. E., Cortés-Patiño, S., Romero-Perdomo, F., Uribe-Vélez, D., Bashan, Y., and Bonilla, R. R. (2020). Proline accumulation and glutathione reductase activity induced by drought-tolerant rhizobacteria as potential mechanisms to alleviate drought stress in Guinea grass. *Appl. Soil Ecol.* 147, 103367. doi: 10.1016/j.apsoil.2019.103367

Nadeem, M., Li, J., Yahya, M., Sher, A., Ma, C., Wang, X., et al. (2019). Research progress and perspective on drought stress in legumes: A review. *Int. J. Mol. Sci.* 20 (10), 2541, 1–32. doi: 10.3390/ijms20102541

Nautiyal, S., Badola, H. K., Pal, M., and Negi, D. S. (1994). Plant responses to water stress: changes in growth, dry matter production, stomatal frequency and leaf anatomy. *Biol. Plantarum* 36 (1), 91–97.

Ozturk, M., Turkyilmaz Unal, B., García-Caparrós, P., Khursheed, A., Gul, A., and Hasanuzzaman, M. (2021). Osmoregulation and its actions during the drought stress in plants. *Physiol. plantarum* 172 (2), 1321–1335. doi: 10.1111/ppl.13297

Paknejad, F., Majidi, H. E., Nourmohammadi, G., Siadat, A., and Vazan, S. (2006). Effects of drought stress on chlorophyll fluorescence parameters, chlorophyll content and grain yield of wheat cultivars. *J. Biol. Sci.* 7, 841–847. doi: 10.3923/jbs.2007.841.847

Peng, F., Xue, X., You, Q., Sun, J., Zhou, J., Wang, T., et al (2020). Change in the trade-off between aboveground and belowground biomass of alpine grassland: Implications for the land degradation process. *Land Degrad. Dev.* 31 (1), 105–117. doi: 10.1002/ldr.3432

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., and Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.* 193, 30–50. doi: 10.1111/j.1469-8137.2011.03952.x

Reddy, A. R., Chaitanya, K. V., and Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* 161, 1189–1202. doi: 10.1016/j.jplph.2004.01.013

Reents, S., Mueller, P., Tang, H., Jensen, K., and Nolte, S. (2021). Plant genotype determines biomass response to flooding frequency in tidal wetlands. *Biogeosciences* 18 (2), 403–411. doi: 10.5194/bg-18-403-2021

Reinbothe, C., El Bakkouri, M., Buhr, F., Muraki, N., Nomata, J., Kurisu, G., et al. (2010). Chlorophyll biosynthesis: spotlight on protochlorophyllide reduction. *Trends Plant Sci.* 15 (11), 614–624. doi: 10.1016/j.tplants.2010.07.002

Rubio, G., Casasola, G., and Lavado, R. S. (1995). Adaptations and biomass production of two grasses in response to waterlogging and soil nutrient enrichment. *Oecologia* 102, 102–105.

Rui, H. Y., Wang, L. N., Jin, L., Li, L., Pan, H. L., Li, J. Y., et al. (2013). Effect of drought stress at seedling on protective enzyme activity and MDA content of different soybeans. *Soybean Sci.* 32, 647–649.

Shao, G. C., Lan, J. J., Yu, S. E., Liu, N., Guo, R. Q., and She, D. L. (2013). Photosynthesis and growth of winter wheat in response to waterlogging at different growth stages. *Photosynthetica* 51, 429–437. doi: 10.1007/s11099-013-0039-9

Shinde, S., Villamor, J. G., Lin, W. D., Sharma, S., and Verslues, P. E. (2016). Proline coordination with fatty acid synthesis and redox metabolism of chloroplast and mitochondria. *Plant Physiol.* 172 (2), 1074–1088. doi: 10.1104/pp.16.01097

Sperdouli, I., and Moustakas, M. (2012). Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of *Arabidopsis thaliana* to drought stress. *J. Plant Physiol.* 169, 577–585. doi: 10.1016/j.jplph.2011.12.015

Tan, W., Liu, J., Dai, T., Jing, Q., Cao, W., and Jiang, D. (2008). Alterations in photosynthesis and antioxidant enzyme activity in winter wheat subjected to post-anthesis water-logging. *Photosynthetica* 46, 21–27. doi: 10.1007/s11099-008-0005-0

Titlyanova, A. A., Romanova, I. P., Kosykh, N. P., and Mironycheva-Tokareva, N. P. (1999). Pattern and process in above-ground and below-ground of grassland ecosystems. *J. Veg Sci.* 10, 307–320.

Tokarz, B., Wójtowicz, T., Makowski, W., Jędrzejczyk, R. J., and Tokarz, K. M. (2020). What is the difference between the response of grass pea (*Lathyrus sativus* l.) to salinity and drought stress? —a physiological study. *Agronomy* 10 (6), 833. doi: 10.3390/agronomy10060833

Wang, X., He, Y., Zhang, C., Tian, Y. A., Lei, X., Li, D., et al. (2021). Physiological and transcriptional responses of phalaris arundinacea under waterlogging conditions. *J. Plant Physiol.* 261, 153428. doi: 10.1016/j.jplph.2021.153428

Wang, C. T., Long, R. J., Wang, Q. L., Jing, Z. C., and Shi, J. J. (2009). Changes in plant diversity, biomass and soil c, in alpine meadows at different degradation stages in the headwater region of three rivers, China. *Land Degrad Dev.* 20 (2), 187–198. doi: 10.1002/ldr.879

Wang, Y., Lv, W., Xue, K., Wang, S., Zhang, L., Hu, R., et al. (2022). Grassland changes and adaptive management on the qinghai–Tibetan plateau. *Nat. Rev. Earth Env.* 3, 668–683. doi: 10.1038/s43017-022-00330-8

Wang, X., Wu, Z., Zhou, Q., Wang, X., Song, S., and Dong, S. (2022). Physiological response of soybean plants to water deficit. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.809692

Wang, C., Xiao, R., Cui, Y., Ma, Z., Guo, Y., Wang, Q., et al. (2019). Photosynthate-^{13}C allocation in the plant-soil system after ^{13}C–pulse labeling of *Phragmites australis* in different salt marshes. *Geoderma* 347, 252–261. doi: 10.1016/j.geoderma.2019.03.045

Wu, J., Wang, J., Hui, W., Zhao, F., Wang, P., Su, C., et al. (2022). Physiology of plant responses to water stress and related genes: A review. *Forests* 13, 324. doi: 10.3390/f13020324

Xu, W., Cui, K., Xu, A., Nie, L., Huang, J., and Peng, S. (2015). Drought stress condition increases root to shoot ratio *via* alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. *Acta Physiol. Plant* 37 (2), 1–11. doi: 10.1007/s11738-014-1760-0

Yu, J., Wan, L., Liu, G., Ma, K., Cheng, H., Shen, Y., et al. (2022). A meta-analysis on degraded alpine grassland mediated by climate factors: Enlightenment for ecological restoration. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.821954

Zaefyzadeh, M., Quliyev, R. A., Babayeva, S., and Abbasov, M. A. (2009). The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces. *Turk J. Biol.* 33 (1), 1–7. doi: 10.3906/biy-0801-12

Zhou, Q., Li, Y., Wang, X., Yan, C., Ma, C., Liu, J., et al. (2022). Effects of different drought degrees on physiological characteristics and endogenous hormones of soybean. *Plants (Basel)* 11 (17), 2282. doi: 10.3390/plants11172282

Zobayed, S. M., Afreen, F., and Kozai, T. (2005). Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in st. john's wort. *Plant Physiol. Biochem.* 43, 977–984. doi: 10.1016/j.plaphy.2005.07.013

Zulfiqar, F., and Ashraf, M. (2022). Proline alleviates abiotic stress induced oxidative stress in plants. *J. Plant Growth Regul.* 1 (1) 1–23. doi: 10.1007/s00344-022-10839-3

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Regulation of reactive oxygen species and phytohormones in osmotic stress tolerance during seed germination in *indica* rice

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Climate change due to global warming is now affecting agricultural production worldwide. In rice, one of the most important crops, water limitation due to irregular rainfall in rainfed lowlands during crop growth limits yield. Dry direct-sowing has been proposed as a water-efficient approach to cope with water stress during rice growth, but poor seedling establishment due to drought during germination and emergence is a problem. Here, we germinated *indica* rice cultivars Rc348 (drought tolerant) and Rc10 (drought sensitive) under osmotic stress induced by PEG to elucidate mechanisms of germination under drought. Rc348 had higher germination rate and germination index under severe osmotic stress of −1.5 MPa, above those of Rc10. Rc348 showed up-regulated GA biosynthesis, down-regulated ABA catabolism, and up-regulated α-amylase gene expression in imbibed seeds under PEG treatment compared to that of Rc10. During germination, reactive oxygen species (ROS) play important roles in antagonism between gibberellic acid (GA) and abscisic acid (ABA). Embryo of Rc348 treated with PEG had significantly greater expression of NADPH oxidase genes and higher endogenous ROS levels, together with significantly increased endogenous GA_1, GA_4 and ABA contents compared to that of Rc10. In aleurone layers treated with exogenous GA, expression of α-amylase genes was higher in Rc348 than in Rc10, and expression of NADPH oxidase genes was enhanced with significantly higher ROS content in Rc348, suggesting higher sensitivity of GA to ROS production and starch degradation in aleurone cells of Rc348. These results suggest that the osmotic stress tolerance of Rc348 is due to enhancement of ROS production, GA biosynthesis, and GA sensitivity, resulting in a higher germination rate under osmotic stress.

KEYWORDS

germination, reactive oxygen species, gibberellic acid, abscisic acid, osmotic stress, rice

1 Introduction

Rice (*Oryza sativa* L.) is one of the most important staple crops, feeding a third of the world's population. It is produced mainly in Asia, largely in flooded conditions. Increasing demand for rice production and diminishing rainfall due to climate change profoundly affect rice production, for which reliable irrigation is crucial (Vries et al., 2010). Rainfed lowland covers more than 30% of rice cultivation areas globally and in major rice producing countries (Matloob et al., 2015; Gadal et al., 2019). However, weather fluctuations due to climate change in recent years and the irregular rainfall in rainfed lowlands, often delay transplantation (Ohno et al., 2018). Prolonged water stress during the transition from vegetative stage to reproductive stage delays heading and significantly reduces yield (Pantuwan et al., 2002). To cope with water stress in rice, dry direct-sowing (DDS) has been proposed as a water-efficient approach, since it uses much less water than transplantation into puddled fields (Haefele et al., 2016). DSS method has been adopted in many countries (Shekhawat et al., 2020), which more than 25% of total rice production in tropical regions in Asia, and more than 90% of rice cultivated areas in the United States and Sri Lanka depend on DDS (Kumar and Ladha, 2011; Subedi et al., 2019). DDS is a promising approach for rainfed rice cropping, using less labor and having no need for irrigation or seedling preparation (Hayashi et al., 2007; Kato and Katsura, 2014). However, it faces problems of weed infestation and poor seedling establishment if drought occurs during germination and emergence (Yamane et al., 2017; Ohno et al., 2018).

Germination is a crucial developmental stage and is regulated by many factors, including the phytohormones gibberellic acid (GA), which induces germination, and abscisic acid (ABA), which suppresses germination (Liu et al., 2010; Ishibashi et al., 2012; Jacobsen et al., 2020). Biosynthesis of GA involves many catalytic enzymes, including ent-kaurene acid oxidase (KAO), GA 20-oxidase (GA20ox), and GA 3-oxidase (GA3ox) (Hedden and Phillips, 2000). ABA is synthesized by the enzyme 9-cis epoxycarotenoid dioxygenase (NCED) and is biodegraded by a cytochrome P450 monooxygenase or ABA 8'-hydroxylase *(ABA8' OH)* (Millar et al., 2006). Reactive oxygen species (ROS) as developmental and stress-signaling molecules are also involved *via* an 'oxidative window', wherein ROS homeostasis regulates germination (Bailly et al., 2008). ROS produced by NADPH oxidases during seed imbibition induce the production of GA and inhibit ABA to promote germination, which ROS homeostasis is important for abiotic stress responses *via* phytohormone signaling in many species (Oracz et al., 2007; Bailly et al., 2008; Ishibashi et al., 2010; Ishibashi et al., 2012; Ye et al., 2012; El-Maarouf-Bouteau et al., 2015; Shi et al., 2020; Wu et al., 2020). To degrade stored starch, the production of α-amylases, starch-hydrolyzing enzymes, are regulated by GA and ABA signaling factors such as GAMYB (GA-induced MYB-like transcription factor) and PKABA (ABA-induced protein kinase ABA-responsive protein kinase) which induces and inhibits expression of α-amylases, respectively, in cereal aleurone layers (Gubler et al., 1999; Gomez-Cadenas et al., 2001; Kaneko et al., 2002; Woodger et al., 2003; Ishibashi et al., 2012). Under osmotic stress caused by polyethylene glycol (PEG), α-amylase activity is inhibited, and germination is impaired (Bialecka and Kepczynski, 2010; Muscolo et al., 2013).

Indica rice Rc348 is a newly released drought-tolerant DDS cultivar that has a higher seedling emergence rate than the common and widely grown drought-sensitive cultivar Rc10, resulting in higher yield under drought stress on farm experiments in the Philippines (Yamane et al., 2017; Ohno et al., 2018). Both germination ability and seedling establishment are crucial for later growth and development (Yamane et al., 2017; Ohno et al., 2018). Although many studies have suggested drought-tolerant traits and cultivars for DDS cropping, the molecular mechanisms underlying drought responses of tolerant cultivars, especially in germination, are not yet well studied.

Here, we focused on germination ability of Rc348 under osmotic stress. We aimed at elucidating how different rice cultivars respond to osmotic pressure, an important component of drought stress, at the transcriptional, hormonal, and ROS levels during seed imbibition.

2 Materials and methods

2.1 Plant materials and growth conditions

Three-week-old seedlings of *indica* rice (*Oryza sativa* L.) cvv. Rc348, Rc10, Rc420, Rc222, and Dular were transplanted into 1/2000-a Wagner pots (5 plants per pot) with 32.8 g of basal dressing compound fertilizer (N–P–K: 4%–4%–4%) and 3.2 g of sigmoid-type controlled-release coated urea. Topdressing of 1.88 g of ammonium sulfate (21% N) per pot was applied during the tiller development stage and the panicle booting stage. Plants were grown under natural conditions at Kyushu University, Fukuoka, Japan, from mid-May to late-October in 2019. Anthesis, the day when spikelets on the upper primary rachis branches flowered on >50% of the population, was set as the day of flowering (0 DAF; days after flowering). Plants were harvested at 49 DAF. Harvested seeds were dried at room temperature for 1 week and stored at −30°C to maintain dormancy. Seed morphology of all cultivars are shown in Supplemental Figure 1.

2.2 Seed germination test under osmotic stress and exogenous chemical treatments

Seeds of all cultivars underwent dormancy break treatment at 45°C in the dark for 2 weeks to ensure a uniform degree of seed dormancy. Seeds were rested at room temperature for 1 h, sterilized in 0.2% NaClO for 20 min and washed thoroughly in sterilized distilled water; 30 seeds were placed in 9-cm Petri dishes with 10 mL of sterilized distilled water (control) or −0.5, −1.0, or −1.5 MPa of PEG 4000 solution (Nacalai Tesque inc., Kyoto, Japan) to germinate at 28°C in the dark. Germination rates were recorded every 6 h until 144 h after imbibition (HAI). A seed was recorded as germinated when shoot length was ≥0.2 cm. The germination index of each sample was calculated as described by Coolbear and Grierson (1984).

In the experiment with exogenous GA and ABA, embryoless half-seeds were imbibed in 10 mL of 1 μM GA_3 in −1.5-MPa PEG solution on a filter paper in a Petri dish at 28°C in the dark, with or without 5 μM ABA, and transcript levels of *GAMYB, SAPK*, α-amylase, and NADPH oxidase genes were analyzed at 24 HAI and endogenous ROS content was measured at 36 HAI.

In the GA sensitivity experiment, embryoless half-seeds were imbibed in 10 mL of 1 μM GA_3 in −1.5-MPa PEG solution on a filter paper in a Petri dish at 28°C in the dark and sampled at 36 HAI for endogenous hydrogen peroxide content measurement.

In the experiment with exogenous sodium ascorbate, seeds were imbibed in 10 mL of -1.5 MPa PEG or 5, 15 and 25 mM of sodium ascorbate dissolved in -1.5MPa PEG solution. Germination percentage, gene expression and endogenous hormonal levels were analyzed at 84 HAI.

Seeds were imbibed with 6 mL of -1.5 MPa PEG supplied with exogenous 100 μM Diphenyleneiodonium chloride (DPI) or 10, 20, and 50 mM H_2O_2 with equal amount of DMSO to that of DPI solution. Germination rates were recorded with the same methods above.

2.3 RNA extraction and quantitative real-time PCR analysis

Total RNA from whole seeds, embryos, and embryoless half-seeds was extracted from frozen materials by the SDS/phenol/LiCl method (Chirgwin et al., 1979). cDNA was synthesized from extracted RNA with ReverTra Ace reverse transcriptase (Toyobo co., Ltd., Osaka, Japan) according to the manufacturer's instructions. Quantitative real-time PCR was performed on a CFX Connect Optics Module Real-time PCR detector system (Bio-Rad) with SYBR Green dye (Toyobo) as described in the manufacturer's instructions. PCR thermal cycling conditions were as follows: initial denaturation at 94°C for 2 min; 40 cycles of denaturation at 94°C for 20 s, annealing at a primer-specific temperature for 20 s (Table S1), and extension at 72°C for 20 s; followed by melting and plate reading. The data were normalized to the expression of *OsActin*.

2.4 Endogenous GA and ABA contents

Endogenous GA_1, GA_4, and ABA contents in embryos imbibed in −1.5-MPa PEG at 72 HAI were analyzed by LC-MS/MS (Exion LC and X500B, AB Sciex) as described by Xin et al. (2020). Three biological replicates were measured, each comprising embryos from 300 seeds. Isotope internal standards of GA_1, GA_4, and ABA were purchased from OlChemIm (Olomouc, Czech Republic).

2.5 NADPH oxidase enzyme activity

Embryos of 30 seeds from Rc348 and Rc10 (−1.5 MPa PEG at 48 HAI) were ground into fine power with liquid nitrogen. Ice cold 2 mL of Na-phosphate buffer (pH 8.0) was added to the sample and the contents were mixed and sonicate for 15 s prior to centrifugation at 16,000 *g* for 15 min at 4°C. Crude embryo homogenates were precipitated with acetone (9:1, acetone: homogenate) at −30°C for 15 min. Precipitated proteins were collected from centrifugation at 12,500 rpm for 10 min at 4°C. Protein pallets were resuspended in reaction buffer (50 mM Tris-HCl pH 8.0, 0.1 mM $MgCl_2$, 0.25 M sucrose and 0.1% Triton-X100) and used for enzyme activity assay. The reaction of NADPH-dependent superoxide generation was measured using NBT (nitro blue tetrazolium chloride) at 530 nm in a spectrophotometer (Gynesys 40, Thermofisher Scientific) as previously described (Van Gestelen et al., 1997; Sarath et al., 2007; Ishibashi et al., 2010). Monoformazan concentrations were calculated using an extinction coefficient of 12.8 mM^{-1} cm^{-1}.

2.6 Endogenous hydrogen peroxide content

Embryos of 20 seeds imbibed in −1.5-MPa PEG and embryoless half-seeds imbibed in 1 μM GA_1 in −1.5-MPa PEG were sampled at 24 and 36 HAI, respectively. Samples were snap-frozen in liquid nitrogen and stored at −80°C before analysis. Samples were homogenized in 2 mL of 0.2 M perchloric acid on ice and centrifuged at 13,000 rpm at 4°C for 15 min. Supernatant (0.5 mL) was mixed with 0.5 mL of 4 M KOH, and samples were centrifuged at 1000× *g* at 4°C for 5 min. The H_2O_2 content was measured by peroxidase-based assay as described by Ishibashi et al. (2015) and O'Kane et al. (1996).

2.7 Statistical analysis

Statistical analyses in this study were performed in SPSS statistical software version 28.0.0.0 (IBM). Differences among treatments were analyzed by one-tailed Student's *t*-test and Tukey's test with biological replications described in figure legends.

3 Results

3.1 Delayed germination under osmotic stress

Imbibition of seeds of all five cultivars in PEG suppressed germination in a concentration-dependent manner (Figure 1A). Under severe osmotic pressure of −1.5 MPa, Rc348 had the fastest germination and the highest final germination rate of about 50%, whereas those of the other cultivars were ≤20%. Rc348 had a significantly higher GI than the other cultivars at all PEG concentrations, and about double that of the other cultivars at −1.5 MPa (Figure 1B). Therefore, we used this PEG concentration in all other experiments.

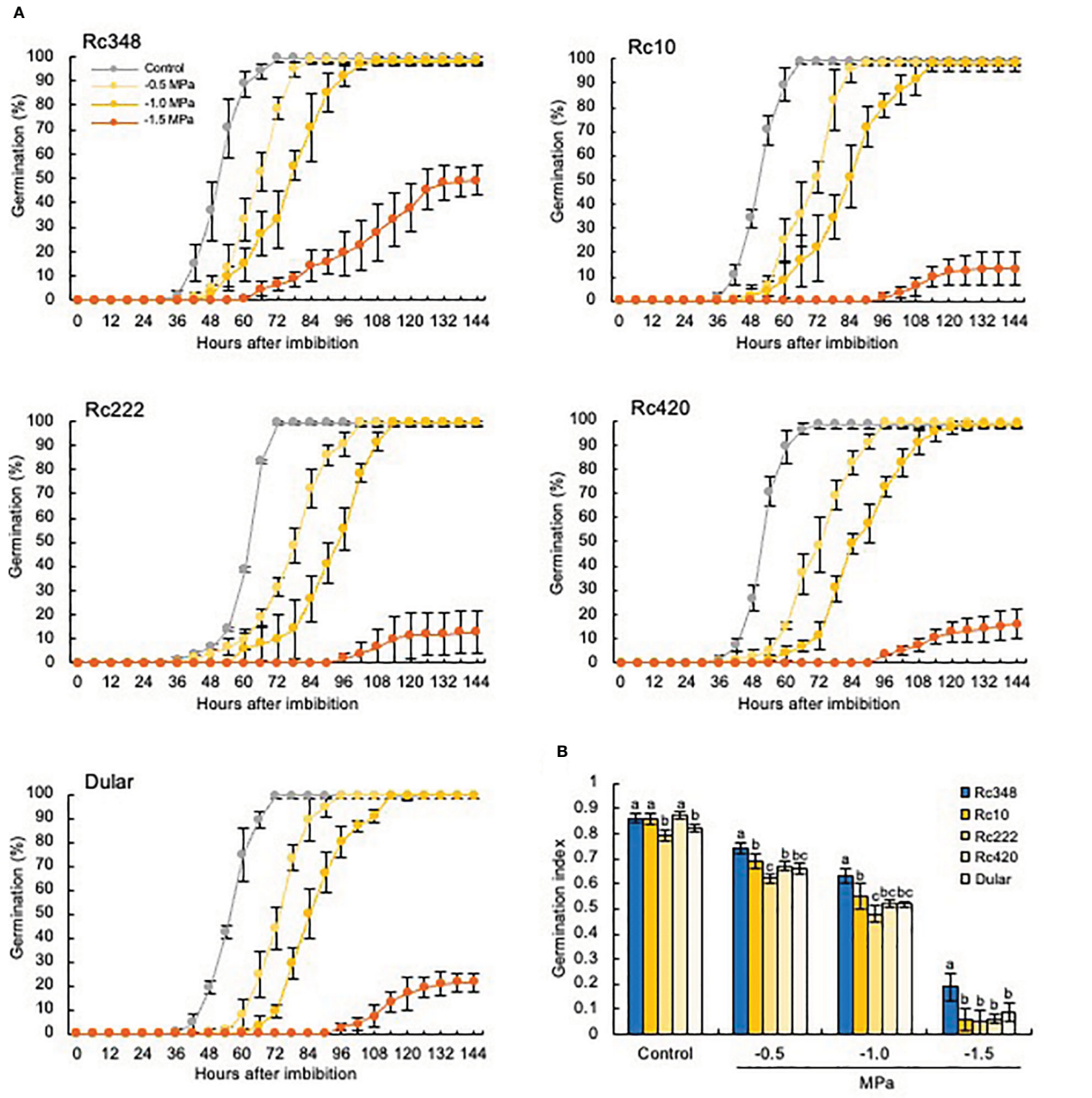

FIGURE 1
(A) Germination rates and **(B)** germination index of five *indica* rice cultivars under osmotic stress imposed by PEG. Control, germination in distilled water. Values with the same letter within a treatment are not significantly different at $P < 0.05$ by Tukey's test ($n = 5$).

3.2 Expression of GA and ABA related genes in imbibed seeds under osmotic stress

As a reference, we chose the widely grown drought-tolerant cultivar Rc348 and drought-sensitive cultivar Rc10 within all examined cultivars (Supplemental Figure 1). We analyzed transcript levels of GA- and ABA-metabolism-related genes and contents of GA and ABA in seeds during imbibition at 24, 48 and 72 HAI. Among genes for GA biosynthesis, despite the significantly lower expression of *OsKAO* in Rc348 at 48 HAI (1/1.9×), and no significant difference in *OsGA3ox2* expression, Rc348 had significantly higher *OsGA20ox1* expression at 48 HAI (3.0× that of Rc10), and marginally higher at 24 HAI. Significantly higher *OsGA3ox1* expression of Rc348 compared to that of Rc10 at 24 HAI (2.1×) and 48 HAI (2.1×) was also observed (Figure 2). Transcript levels of *OsGA3ox2* remained stable and showed no significant difference over time in both cultivars.

Among genes for ABA biosynthesis (*OsNCEDs*), *OsNCED1* and *OsNCED3* expression gradually increased from 24 to 72 HAI in both Rc10 and Rc348 under osmotic stress. On the other hands, changes of *OsNCED5* expression overtime from 24 to 72 HAI were

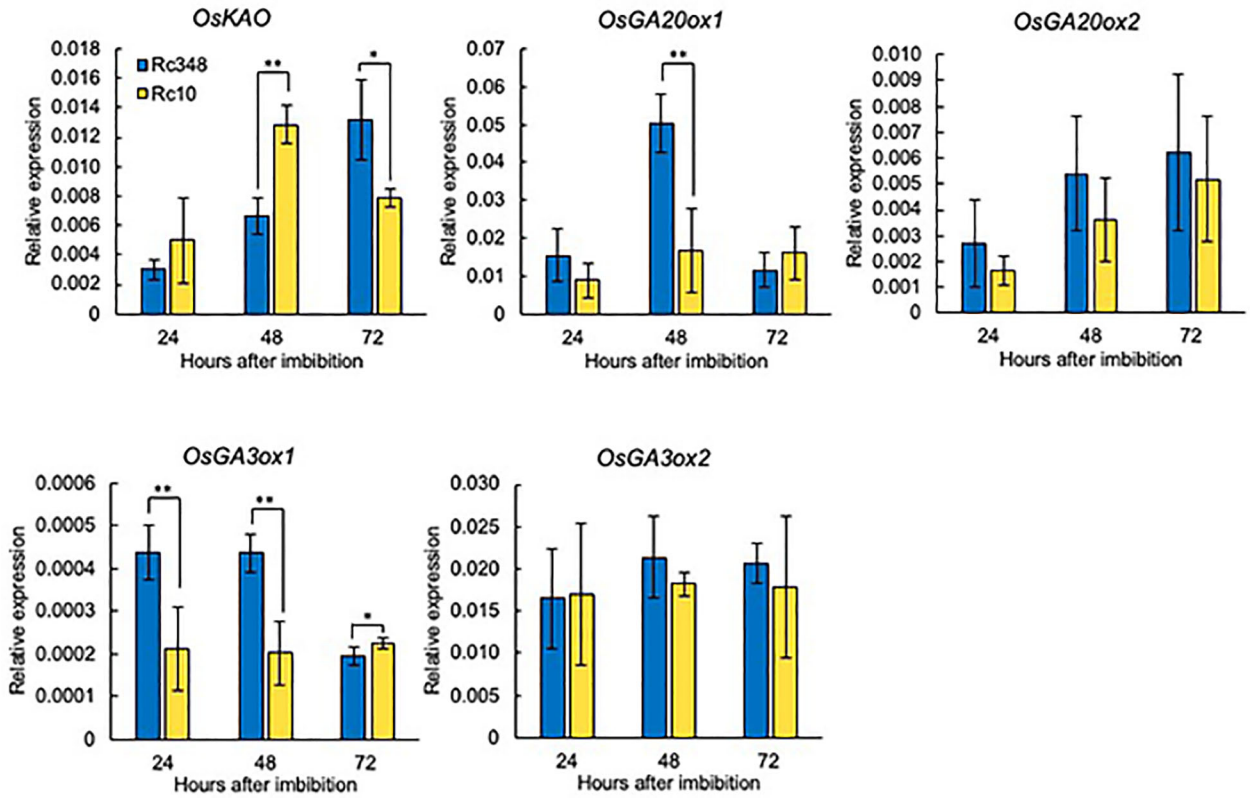

FIGURE 2
Relative expression of GA biosynthesis genes at 24, 48, and 72 HAI during seed imbibition under osmotic stress imposed by −1.5 MPa PEG. Significant differences: *$P < 0.05$, **$P < 0.01$ by Student's *t*-test ($n = 3$).

barely observed. Rc10 had significantly higher *OsNCED1* and *OsNCED5* expression at 24 HAI. Rc348 showed higher expression of *OsNCED3* and *OsNCED5* at 48 HAI, together with marginally higher expression of *OsNCED1* and *OsNCED3* at 72 HAI (Figure 3A). Despite no change in *OsABA8'OH1* expression, Rc348 had significantly lower *OsABA8'OH3* expression at 24 HAI (1/2.4×) and 72 HAI (1/2.2×). Overall, with fluctuations during germination time course of ABA biosynthesis genes, significant downregulation of *OsABA8'OH3* for ABA catabolism in Rc348 was observed (Figure 3B).

3.3 NADPH oxidase gene expression, ROS and hormone contents in embryos under osmotic stress

Since GA and ABA are known to be regulated by ROS in embryos, we then analyzed the transcript levels of nine *NADPH oxidase* genes (*Respiratory burst oxidase homologs*, *OsRboh*s) in embryos during imbibition in −1.5-MPa PEG at 48 HAI (Figure 4A). Expression of *OsRbohA*, *OsRbohC*, *OsRbohF*, *OsRbohG*, *OsRbohH*, and *OsRbohI* was significantly higher in Rc348 than in Rc10 (Figure 2A); *OsRbohH* had the highest transcript level in *OsRboh*s (2.8× that in Rc10). NADPH oxidase activity in embryos of Rc348 was also 2.0× significantly higher than that in Rc10 (Figure 4B), resulting in significantly enhanced endogenous ROS content in Rc348 embryos for 3.1× that in Rc10 (Figure 4C). These results show that osmotic stress enhanced NADPH oxidase gene expression and increased ROS content in Rc348 embryos. We also showed that inhibition of NADPH oxidase by DPI significantly reduced germination rate of Rc348, where exogenous H_2O_2 significantly improved Rc10 seed germination under −1.5-MPa PEG (Supplemental Figure 2), suggesting the role of ROS on seed germination under osmotic stress. We also analyzed endogenous GA_1, GA_4 and ABA in imbibed embryos at 72 HAI. Rc348 had significantly higher content of endogenous GA_1 (1.9×), GA_4 (1.9×), and ABA (2.0×) than Rc10, which is explained by upregulated GA biosynthesis and downregulated ABA catabolism transcript levels during imbibition in Rc348 seeds under osmotic stress (Table 1). Since enhanced endogenous ROS stimulated GA production without decreasing ABA content to promote germination in Rc348, exogenous sodium ascorbate (AsA), an antioxidant to decrease endogenous ROS, was applied to elucidate the role of ROS on GA and ABA production under osmotic stress in Rc348 (Supplemental Figure 3). As a result, exogenous AsA significantly reduced germination rate in dose dependent manner under −1.5 MPa PEG (Supplemental Figure 1A). Despite no obvious change in ABA metabolism gene expression, Rc348 seeds imbibed with 25 mM AsA showed significantly reduced expression of GA biosynthesis, *OsGA20ox1*

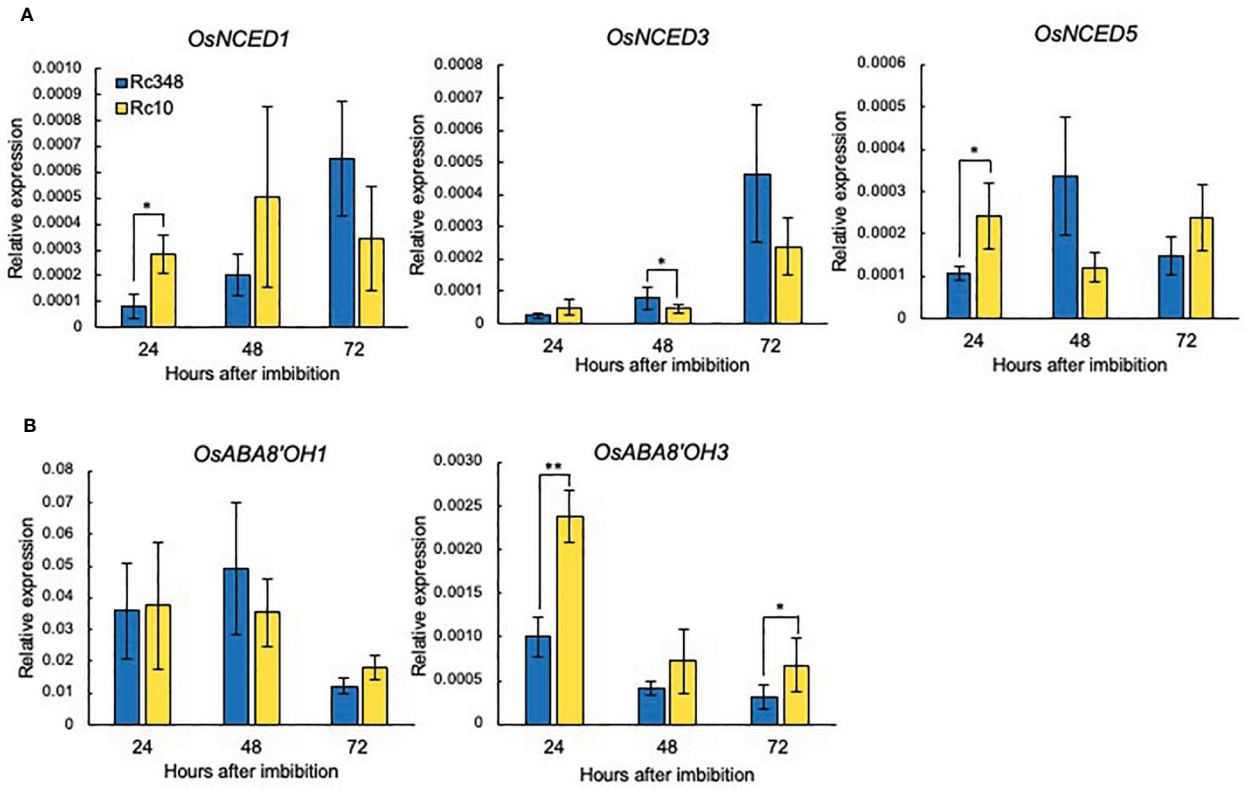

FIGURE 3
Expression of **(A)** ABA biosynthesis and **(B)** ABA catabolism genes at 24, 48, and 72 HAI during imbibition under osmotic stress imposed by −1.5 MPa PEG. Significant differences: *$P < 0.05$, **$P < 0.01$ by Student's *t*-test ($n = 3$).

(1/4.3×) and *OsGA3ox2* (1/33.8×) compared to that of −1.5 MPa PEG only during imbibition (Supplemental Figure 3B–C). Consequently, endogenous GA_1 content was significantly reduced by exogenous AsA, while ABA and GA_4 content remained unchanged (Supplemental Figure 3D–F). Thus, these results suggest that enhancement of ROS rather induce GA production than inhibiting ABA to promote seed germination in Rc348 under osmotic stress.

3.4 α-Amylase gene expression in imbibed seeds under osmotic stress

α-Amylase is induced by GA and suppressed by ABA in cereal aleurone cells (Woodger et al., 2003). During seed imbibition, the expression of α-amylase genes (*OsAmy1A*, *OsAmy1C*, *OsAmy3B*, and *OsAmy3E*) is induced by GA, and they are highly expressed in rice endosperm after imbibition (Chen, 2006). We analyzed the expression of these genes in imbibed seeds during germination under osmotic stress (Figure 5). Rc348 had significantly higher α-amylase gene expression than Rc10 at 48 HAI (*OsAmy3B*, 4.8×; *OsAmy3E*, 2.6×) and 72 HAI (*OsAmy1A*, 1.6×; *OsAmy1C*, 3.4×; *OsAmy3B*, 1.8×; *OsAmy3E*, 1.5×). These results suggest that α-amylase upregulation during imbibition of Rc348 seeds facilitates germination under osmotic stress.

3.5 Responses of starch degradation and ROS accumulation in aleurone cells to exogenous GA and ABA

Rc348 imbibed seeds had significantly higher expression of α-amylase genes (Figure 5). In aleurone cells, the expression of GAMYB and its downstream target α-amylase is induced by GA and suppressed by ABA through PKABA induction (Gomez-Cadenas et al., 2001; Woodger et al., 2003; Ishibashi et al., 2012). It has been shown that rice SAPK8 and SAPK10 of SAPK family genes in rice are orthologous to PKABA1 in barley, which expression of both is induced by ABA (Li et al., 2007). We investigated the effects of exogenous GA with/without of ABA on GAMYB, PKABA and α-amylase gene expression in aleurone cells at 24 HAI (Figure 6). Exogenous GA alone significantly increased expression of Os*GAMYB* (1.3×) relative to level in Rc10 (Figure 6A). Presence of exogenous ABA inhibited the expression of *OsGAMYB* in both Rc348 and Rc10, however, the expression was significantly increased in Rc348 (1.4×) relative to levels in Rc10. Exogenous ABA induced the expression of *OsSAPK8* and *OsSAPK10* in aleurone cells. Despite no change between Rc348 and Rc10 in OsSAPK10 expression, Rc348 showed significantly reduced expression of *OsSAPK8* (1/1.5×) compared to level in RC10. Exogenous GA alone significantly increased expression of α-amylase genes (*OsAmy1A*, 4.9×; *OsAmy1C*, 4.2×; *OsAmy3B*, 9.5×;

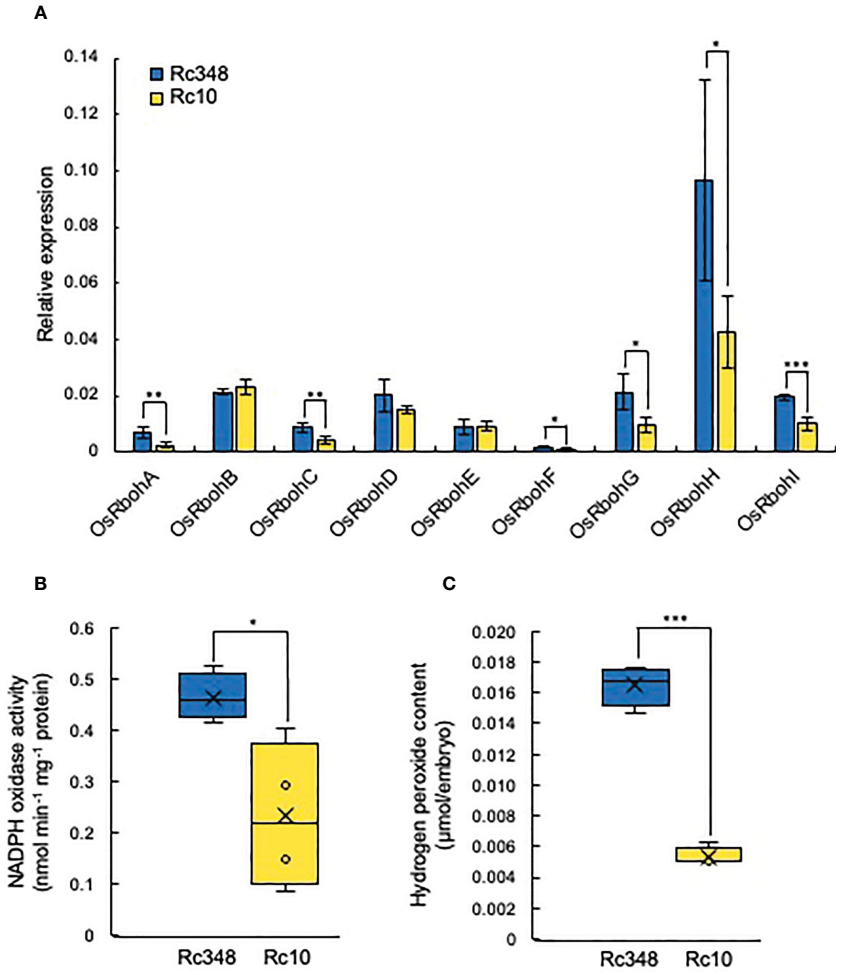

FIGURE 4
Expression of rice NADPH oxidases, NADPH oxidase enzyme activity, and endogenous hydrogen peroxide content in embryos in –1.5 MPa PEG. **(A)** Relative expression of *OsRbohA* to *OsRbohI* at 48 HAI. **(B)** Endogenous NADPH oxidase activity and **(C)** hydrogen peroxide content in embryo at 48 HAI. Significant differences: **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by Student's *t*-test [*n* = 3 for A, *n* = 4 for (**B**, **C**)].

OsAmy3E, 6.1×) relative to levels in Rc10 (Figure 6A). α-amylase gene expression was also increased by GA even in the presence of ABA in Rc348 (3.9×, 4.1×, 7.3×, and 4.6×, respectively). Therefore, the suppressive effect of ABA was countered by the inductive effect of GA on signaling and starch degradation in aleurone cells of Rc348. GA induces *RboH* gene expression to regulate α-amylase activity in barley aleurone cells (Ishibashi et al., 2015). We analyzed the transcript levels of NADPH oxidase genes in aleurone cells treated with exogenous GA and found that *OsRbohA*, *OsRbohD*, *OsRbohE*, *OsRbohG*, and *OsRbohI* expression (Figure 6B) and endogenous ROS levels in aleurone cells (Figure 6C) were significantly higher in Rc348 than in Rc10. These results suggest that Rc348 also had higher sensitivity to exogenous GA in terms of ROS induction in aleurone cells.

4 Discussion

We propose that Rc348, a newly developed drought-stress-tolerant cultivar bred for DDS (Yamane et al., 2017; Ohno et al., 2018), gains its capacity for a high germination rate under osmotic stress *via* the regulation of ROS and phytohormones. Loss of

TABLE 1 Endogenous GA_1, GA_4, and ABA contents in imbibed seeds under osmotic stress imposed by –1.5 MPa PEG.

Cultivar	GA_1 content(pg/seed)	GA_4 content (pg/seed)	ABA content (pg/seed)
Rc348	0.185 ± 0.053	0.550 ± 0.129	8.840 ± 1.824
Rc10	0.099 ± 0.037	0.293 ± 0.114	4.391 ± 2.134
Student's *t*-test (n=3)	*P*=0.046*	*P*=0.031*	*P*=0.026*

Values are means ± SD of 3 biological replicates. Significant differences by Student's t-test.

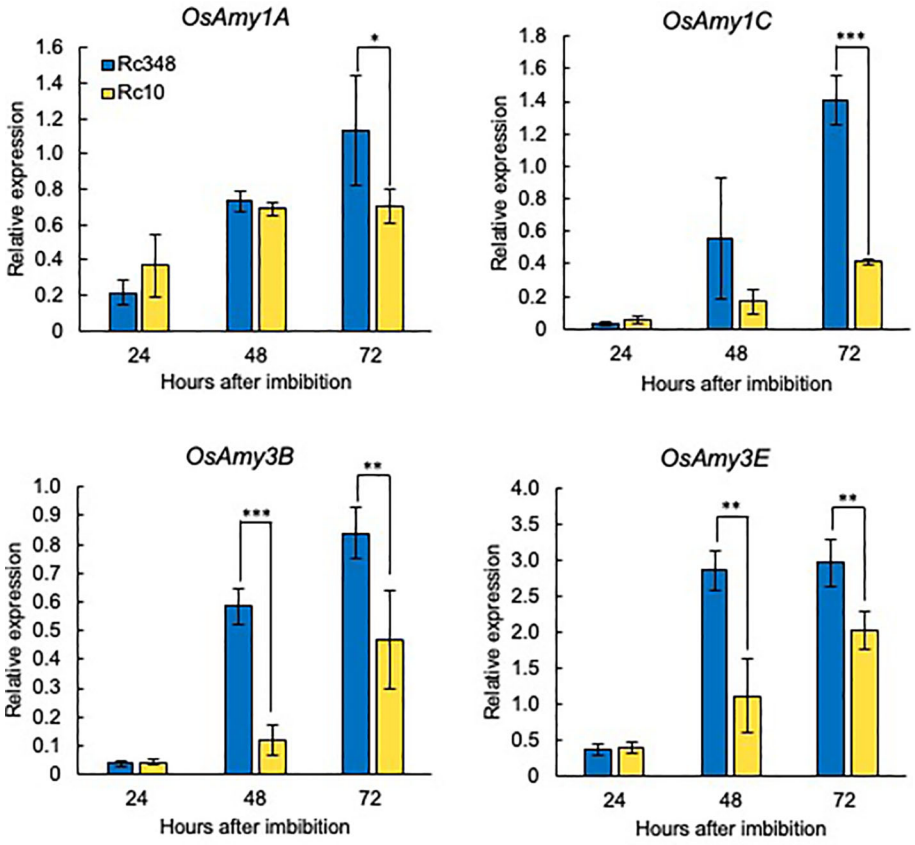

FIGURE 5
Relative expression of α-amylase genes at 24, 48, and 72 HAI during imbibition under osmotic stress imposed by –1.5 MPa PEG. Significant differences: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by Student's *t*-test ($n = 3$).

function of NADPH oxidase of *osrbohb* mutant results in reduced osmotic stress tolerance due to lower levels of ROS and ABA contents in rice seedlings and resulted in impaired seed germination (Shi et al., 2020). In plants, *Rboh* genes not only function in responses to stress signaling and development (Kaur and Pati, 2016; Suriyasak et al., 2017; Chapman et al., 2019), but also promote germination (Muller et al., 2009; Ishibashi et al., 2010; Ishibashi et al., 2015; Kai et al., 2016; Ishibashi et al., 2017). After imbibition, ROS produced in seeds induce GA and inhibit ABA production to initiate germination (Liu et al., 2010; Ishibashi et al., 2015). We showed that Rc348 had the highest ability to germinate under a severe osmotic stress of -1.5 MPa, when compared to other cultivars tested. ROS induce production of GA (which promotes germination) and inhibit production of ABA (which suppresses germination) (Oracz et al., 2007; Ishibashi et al., 2012; El-Maarouf-Bouteau et al., 2015; Ishibashi et al., 2015). In barley embryos treated with diphenylene iodonium chloride (DPI), an NADPH oxidase inhibitor, endogenous GA was significantly reduced while ABA was enhanced resulting in inhibited germination (Ishibashi et al., 2015). In our results, we observed increased ROS content together with higher endogenous GA_1, GA_4 and ABA contents in Rc348, which were due to up-regulated *OsGA20ox1*, *OsGA3ox1* and *OsNCED3*, and down-regulated *OsABA8'OH3*. For ABA biosynthesis, previous studies have reported that *OsNCED1* plays a role in salinity stress response (Zhang et al., 2022) and heat stress tolerance (Zhou et al., 2022), where *OsNCED3* expression is highly induced by PEG and other osmotic stresses, contributing to ABA accumulation for stress responses (Huang et al., 2018). Here, we observed overall increase in *OsNCED1* and *OsNCED3* expression overtime upon germination under osmotic stress in both cultivars. This suggests the possibility of ABA accumulation due to osmotic stress response in both cultivars. For *OsNCED5*, we could not observe obvious difference between cultivars from 24 to 72 HAI, which might be due to that its expression drops rapidly after imbibition and stays at the same basal level from 18 HAI onward (Suriyasak et al., 2020). Additionally, *OsABA8'OH3* expression in Rc348 was lower than that in Rc10 under osmotic stress. High endogenous ABA level in RC348 under osmotic stress might be attributed to the *OsABA8'OH3* expression. For GA biosynthesis, we did not observe change in *OsGA3ox2* expression over time, which might be due to its rapid peak at the very early stage of imbibition as reported in previous study (Kaneko et al., 2002), whereas *OsGA20ox1* expression peaks at the later phase of germination (Liu et al., 2014). A previous study has shown that expression of GA biosynthetic genes, including *OsGA20ox1* and *OsGA3ox1*, was suppressed by ABA in rice (Ye et al., 2012). In our study, enhancement of endogenous ABA did not inhibit expression of *OsGA20ox1* and *OsGA3ox1* in Rc348 under osmotic stress. Our

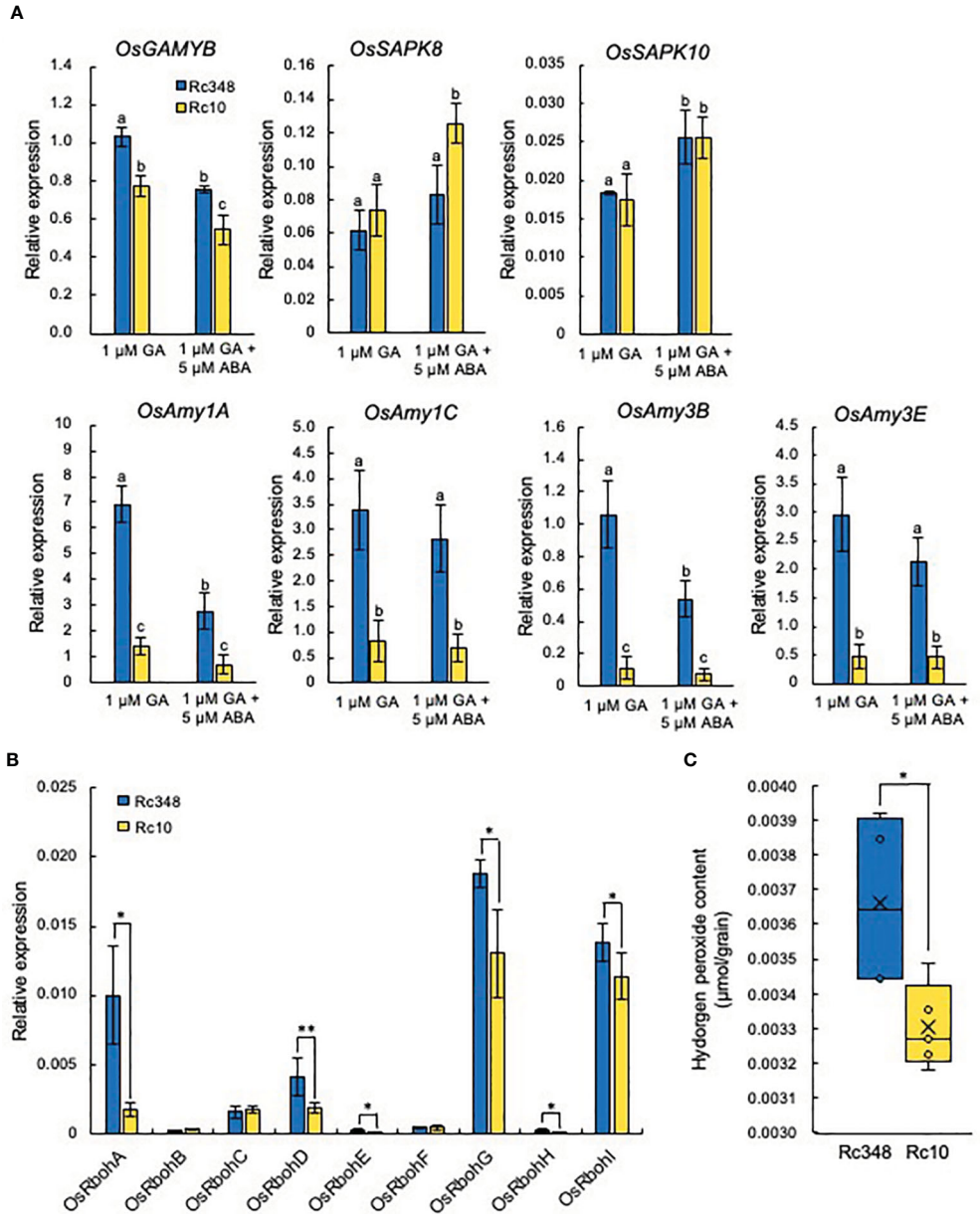

FIGURE 6
Induction of GA and ABA signaling and NADPH oxidase genes in aleurone layers under osmotic stress. **(A)** Relative expression of *OsGAMYB*, *OsSAPK8,10*, and α-amylase genes at 24 HAI in aleurone layers of embryoless seeds in −1.5 MPa PEG + 1 μM GA or −1.5 MPa PEG + 1 μM GA + 5 μM ABA. **(B)** Relative expression of NADPH oxidase genes at 24 HAI in −1.5 MPa PEG + 1 μM GA. **(C)** Endogenous hydrogen peroxide content in aleurone layers of embryoless seeds at 36 HAI in −1.5 MPa PEG + 1 μM GA. In A, values with the same letter are not significantly different at $P < 0.05$ by Tukey's test ($n = 3$). **(B, C)** Significant differences: $^{*}P < 0.05$, $^{**}P < 0.01$ by Student's *t*-test ($n = 3$ for **A**, **B**, $n = 4$ *for* **C**).

results showed that enhanced ROS rather promote GA than act to suppress ABA in Rc348. To explain this phenomenon, we observed that exogenous sodium ascorbate (AsA), an antioxidant, significantly inhibited seed germination of Rc348 *via* suppressing GA biosynthesis genes, *OsGA20ox1* and *OsGA3ox2*, rather than affecting ABA metabolism genes (Supplemental Figure 3A–C). Consequently, endogenous GA_1 level in seeds was not detected by AsA treatment, without affecting endogenous ABA and GA_4 contents (Supplemental Figure 3D–F), which confirmed our results that enhancement of ROS mainly induces GA production to promote germination under osmotic stress of Rc348. Additionally, drought-tolerant maize and *Medicago sativa* L. seedlings accumulated more endogenous ABA in leaves under osmotic stress induced by PEG than drought-intolerant seedlings (Yao et al., 2019; Liu et al., 2022). Since ABA is known to accumulate under osmotic stress and enhance stress responses (Kuromori et al., 2018), in this study also, enhancement of ABA under osmotic stress in Rc348 might be involved in osmotic stress tolerance, with better seedling establishment under drought, as described in our previous study (Yamane et al., 2017).

In cereal aleurone cells, GAMYB is a transcription factor that is upregulated by GA and downregulated by ABA (Gomez-Cadenas

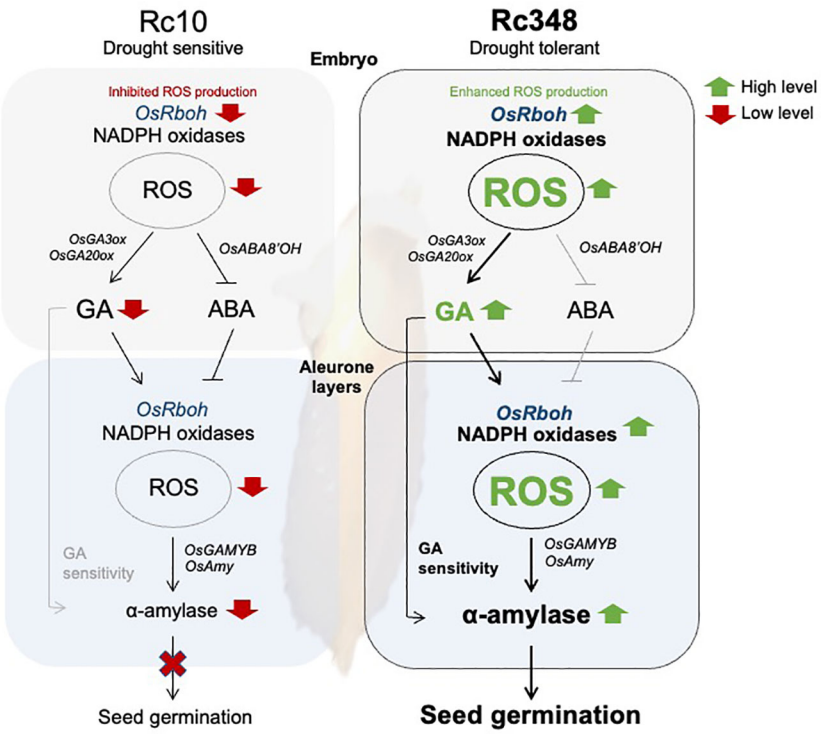

FIGURE 7
Scheme of regulations of reactive oxygen species in osmotic stress tolerance during seed germination suggested in this study. Pathways in black lines are shown to be more effective than that in grey lines in this study.

et al., 2001; Washio, 2003; Woodger et al., 2003; Ishibashi et al., 2012), and binds to GARE boxes in α-amylase promoters to induce starch degradation (Kaneko et al., 2002). Osmotic stress reduces α-amylase activity and thus impairs germination (Muscolo et al., 2013). Here, we showed that Rc348 gains its osmotic tolerance *via* upregulation of α-amylase gene expression in aleurone cells due to higher endogenous bioactive GA levels in embryos under osmotic stress. In aleurone cells of Rc348, expression of both *GAMYB* and α-amylase genes was highly induced by exogenous GA, suggesting its higher responses to GA than Rc10's. In barley aleurone cells, PKABA induced by ABA inhibits *GAMYB* and α-amylase expression (Ishibashi et al., 2012). Here we showed that ABA induction of SAPK8 was significantly lower in Rc348 aleurone cells. Consequently, GAMYB and α-amylase induction in aleurone cells was still significantly higher in Rc348 than in Rc10 even in the presence of exogenous ABA, suggesting the importance of enhanced GA signaling in Rc348 for starch degradation to fuel germination. We previously showed that GA stimulates NADPH oxidase gene expression for ROS production in aleurone layer of barley seed, which inhibits PKABA to promote α-amylase expression (Ishibashi et al., 2012; Ishibashi et al., 2015). Rc348 also had higher sensitivity than Rc10 to exogenous GA in terms of higher *Rboh* expression and endogenous ROS content in aleurone cells, which consequently led to α-amylase induction in aleurone cells *via* up-regulation of GAMYB and down-regulation of PKABA. Despite an increase in endogenous ROS contents in both embryos and aleurone cells, expression patterns of *OsRboh* genes differed between the two: *OsRbohH* was expressed mainly in embryos, while *OsRbohA*, *OsRbohG*, and *OsRbohI* were highly expressed in GA-treated aleurone. In summary, the osmotic stress tolerance in seed germination of Rc348 is caused by enhancement of ROS production, GA biosynthesis, and GA sensitivity (Figure 7).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

YK, KY, KS, CS, and YI designed the experiments; RK, CS, RM, YSaw, YSak, NH, and YI performed the experiments; RK, CS, and YI performed data analysis; CS, CB, and YI wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1186960/full#supplementary-material

References

Bailly, C., El-Maarouf-Bouteau, H., and Corbineau, F. (2008). From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. *C. R. Biol.* 331, 806–814. doi: 10.1016/j.crvi.2008.07.022

Bialecka, B., and Kepczynski, J. (2010). Germination, α-, β-amylase and total dehydrogenase activities of *Amaranthus caudatus* seeds under water stress in the presence of ethephon or gibberellin A3. *Acta Biol. Crac. Ser. Bot.* 52, 7–12. doi: 10.2478/v10182-010-0001-0

Chapman, J. M., Muhlemann, J. K., Gayomba, S. R., and Muday, G. K. (2019). RBOH-dependent ROS synthesis and ROS scavenging by plant specialized metabolites to modulate plant development and stress responses. *Chem. Res. Toxicol.* 32, 370–396. doi: 10.1021/acs.chemrestox.9b00028

Chen, P. W. (2006). Interaction between rice MYBGA and the gibberellin response element controls tissue-specific sugar sensitivity of α-amylase genes. *Plant Cell* 18, 2326–2340. doi: 10.1105/tpc.105.038844

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18, 5294–5299. doi: 10.1021/bi00591a005

Coolbear, P., and Grierson, D. (1984). The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. *J. Exp. Bot.* 35, 1609–1617. doi: 10.1093/jxb/35.11.1609

El-Maarouf-Bouteau, H., Sajjad, Y., Bazin, J., Langlade, N., Cristescu, S. M., Balzergue, S., et al. (2015). Reactive oxygen species, abscisic acid and ethylene interact to regulate sunflower seed germination. *Plant Cell Env.* 38, 364–374. doi: 10.1111/pce.12371

Gadal, N., Shrestha, J., Poudel, M. N., and Pokharel, B. (2019). A review on production status of growing environments of rice in Nepal and in the world. *AAES* 4, 83–87. doi: 10.26832/24566632.2019.0401013

Gomez-Cadenas, A., Zentella, R., Walker-Simmons, M. K., and Ho, T. H. D. (2001). Gibberellin/abscisic acid antagonism in barley aleurone cells: site of action of the protein kinase PKABA1 in relation to gibberellin signaling molecules. *Plant Cell* 13, 667–669. doi: 10.1105/tpc.13.3.667

Gubler, F., Racentos, D., Keys, M., Watts, R., Mundy, J., and Jacobsen, J. V. (1999). Target genes and regulatory domain of the GAMYB transcriptional activator in cereal aleurone. *Plant J.* 17, 1–9. doi: 10.1046/j.1365-313X.1999.00346.x

Haefele, S. M., Kato, Y., and Singh, S. (2016). Climate ready rice: augmenting drought tolerance with best management practices. *Field Crops Res.* 190, 60–69. doi: 10.1016/j.fcr.2016.02.001

Hayashi, S., Kamoshita, A., Yamagishi, J., Kotchasatit, A., and Jongdee, B. (2007). Genotypic differences in grain yield of transplanted and direct-seeded rainfed lowland rice (*Oryza sativa* l.) in northeastern Thailand. *Field Crops Res.* 102, 9–21. doi: 10.1016/j.fcr.2007.01.001

Hedden, P., and Phillips, A. L. (2000). Gibberellin metabolism: new insights revealed by the genes. *Trends Plant Sci.* 5, 523–530. doi: 10.1016/S1360-1385(00)01790-8

Hossain, M., Bhattacharjee, S., Armin, S–M., Qian, P., Xin, W., Li, H–Y., et al. (2015). Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00420

Huang, Y., Guo, Y., Liu, Y., Zhang, F., Wang, Z., Wang, H., et al. (2018). 9-*cis*-Epoxycarotenoid dioxygenase 3 regulates plant growth and enhances multi-abiotic stress tolerance in rice. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00162

Ishibashi, Y., Aoki, N., Kasa, S., Sakamoto, M., Kai, K., Tomokiyo, R., et al. (2017). The interrelationship between abscisic acid and reactive oxygen species plays a key role in barley seed dormancy and germination. *Front. Plant Sci.* 8, 275. doi: 10.3389/fpls.2017.00275

Ishibashi, Y., Kasa, S., Sakamoto, M., Aoki, N., Kai, K., Yuasa, T., et al. (2015). A role for reactive oxygen species produced by NADPH oxidases in the embryo and aleurone cells in barley seed germination. *PloS One* 10, e0143173. doi: 10.1371/journal.pone.0143173

Ishibashi, Y., Tawaratsumida, T., Kondo, K., Kasa, S., Sakamoto, M., Aoki, N., et al. (2012). Reactive oxygen species are involved in gibberellin/abscisic acid signaling in barley aleurone cells. *Plant Physiol.* 158, 1705–1714. doi: 10.1104/pp.111.192740

Ishibashi, Y., Tawaratsumida, T., Zheng, S. H., Yuasa, T., and Iwaya-Inoue, M. (2010). NADPH oxidases act as key enzyme on germination and seedling growth in barley (*Hordeum vulgare* l.). *Plant Prod. Sci.* 13, 45–52. doi: 10.1626/pps.13.45

Jacobsen, J. V., Pearce, D. W., Poole, A. T., Pharis, R. P., and Mander, L. N. (2002). Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination in barley. *Physiol. Plant* 115, 428–441. doi: 10.1034/j.1399-3054.2002.1150313.x

Jira-anunkul, W., and Pattanakul, W. (2021). Effects of hydrogen peroxide application on agronomic traits of rice (*Oryza sativa* l.) under drought stress. *Plant Soil Environ.* 67, 221–229. doi: 10.17221/628/2020-PSE

Kai, K., Kasa, S., Sakamoto, M., Aoki, N., Watabe, G., Yuasa, T., et al. (2016). Role of reactive oxygen species produced by NADPH oxidase in gibberellin biosynthesis during barley seed germination. *Plant Signal. Behav.* 11, e1180492. doi: 10.1080/15592324.2016.1180492

Kaneko, M., Itoh, H., Ueguchi-Tanaka, M., Ashikari, M., and Matsuoka, M. (2002). The alpha-amylase induction in endosperm during rice seed germination is caused by gibberellin synthesized in epithelium. *Plant Physiol.* 128, 1264–1270. doi: 10.1104/pp.010785

Kato, Y., and Katsura, K. (2014). Rice adaptation to aerobic soils: physiological considerations and implications for agronomy. *Plant Prod. Sci.* 17, 1–12. doi: 10.1626/pps.17.1

Kaur, G., and Pati, P. K. (2016). Analysis of cis-acting regulatory of respiratory burst oxidase homolog (*Rboh*) gene families in arabidopsis and rice provides clues for their diverse functions. *Comput. Biol. Chem.* 62, 104–118. doi: 10.1016/j.compbiolchem.2016.04.002

Kumar, V., and Ladha, J. K. (2011). Chapter six – direct seeding of rice: recent developments and future research needs. *Adv. Agron.* 111, 297–413. doi: 10.1016/B978-0-12-387689-8.00001-1

Kuromori, T., Seo, M., and Shinosaki, K. (2018). ABA transport and plant water stress responses. *Trends Plant Sci.* 23, 513–522. doi: 10.1016/j.tplants.2018.04.001

Li, G., Lin, F., and Xue, H. W. (2007). Genome-wide analysis of the phospholipase d family in *Oryza sativa* and functional characterization of PLDβ1 in seed germination. *Cell Res.* 17, 881–894. doi: 10.1038/cr.2007.77

Liu, Y., Fang, J., Xu, F., Chu, J., Yan, C., Schlappi, M. R., et al. (2014). Expression patterns of ABA and GA metabolism genes and hormone levels during rice seed development and imbibition: a comparison of dormant and non-dormant rice cultivars. *J. Genet. Genomics* 41, 327–338. doi: 10.1016/j.jgg.2014.04.004

Liu, Y., Jiang, D., Yan, J., Wang, K., Lin, S., and Zhang, W. (2022). ABA-insensitivity of alfalfa (*Medicago sativa* l.) during seed germination associated with plant drought tolerance. *Env. Exp. Bot.* 203, 105069. doi: 10.1016/j.envexpbot.2022.105069

Liu, Y., Ye, N., Liu, R., Chen, M., and Zhang, J. (2010). H_2O_2 mediates the regulation of ABA catabolism and GA biosynthesis in *Arabidopsis* seed dormancy and germination. *J. Exp. Bot.* 61, 2979–2990. doi: 10.1093/jxb/erq125

Matloob, A., Khaliq, A., and Chauhan, B. S. (2015). Weeds of rice in Asia: problems and opportunities. *Adv. Agron.* 130, 291–336. doi: 10.1016/bs.agron.2014.10.003

Millar, A. A., Jacobsen, J. V., Ross, J. J., Helliwell, C. A., Poole, A. T., Scofield, G., et al. (2006). Seed dormancy and ABA metabolism in Arabidopsis and barley: the role of ABA 8′-hydroxylase. *Plant J.* 45, 942–954. doi: 10.1111/j.1365-313X.2006.02659.x

Muller, K., Carstens, A. C., Linkies, A., Torres, M. A., and Leubner-Metzger, G. (2009). The NADPH-oxidase *AtrbohB* plays a role in arabidopsis seed after-ripening. *New Phytol.* 184, 885–897. doi: 10.1111/j.1469-8137.2009.03005.x

Muscolo, A., Sidari, M., Anastasi, U., Santonoceto, C., and Maggio, A. (2013). Effect of PEG-induced drought stress on seed germination of four lentil genotypes. *J. Plant Interact.* 9, 354–363. doi: 10.1080/17429145.2013.835880

Ohno, H., Banayo, N. P. M. C., Bueno, C., Kashiwagi, J., Nakashima, T., Iwama, K., et al. (2018). On-farm assessment of new early-maturing drought-tolerant rice cultivar for dry direct seeding in rainfed lowlands. *Field Crops. Res.* 219, 222–228. doi: 10.1016/j.fcr.2018.02.005

O'Kane, D., Gill, V., and Burdon, R. (1996). Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. *Planta* 198, 371–377. doi: 10.1007/BF00620053

Oracz, K., El-Maarouf-Bouteau, H., Farrant, J. M., Cooper, K., Belghazi, M., and Claudette, J. (2007). ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. *Plant J.* 50, 452–465. doi: 10.1111/j.1365-313X.2007.03063.x

Pantuwan, G., Fukai, S., Cooper, M., Rajarasereekul, S., and O'Toole, J. C. (2002). Yield response of rice (*Oryza sativa* l.) genotypes to different types of drought under rainfed lowlands part 1. grain yield and yield components. *Field Crops Res.* 73, 153–168. doi: 10.1016/S0378-4290(01)00187-3

Sarath, G., Hou, G., Baird, L. M., and Mitchell, R. B. (2007). Reactive oxygen species, ABA and nitric oxide interactions on the germination of warm-season C_4-grasses. *Planta* 226, 697–708. doi: 10.1007/s00425-007-0517-z

Shekhawat, K., Rathore, S. S., and Chauhan, B. S. (2020). Weed management in dry direct-seeded rice: a review on challenges and opportunities for sustainable rice production. *Agronomy* 10, 1264. doi: 10.3390/agronomy10091264

Shi, Y., Chang, Y.-L., Wu, H.-T., Shalmani, A., Liu, W.-T., Li, W.-Q., et al. (2020). OsRbohB-mediated ROS production plays a crucial role in drought stress tolerance of rice. *Plant Cell Rep.* 39, 1767–1784. doi: 10.1007/s00299-020-02603-2

Subedi, S. R., Sandhu, N., Singh, V. K., Sinha, P., Kumar, S., Singh, S. P., et al. (2019). Genome-wide association study reveals significant genomic regions for improving yield, adaptability of rice under dry direct seeded cultivation condition. *BMC Genomics* 20, 471. doi: 10.1186/s12864-019-5840-9

Suriyasak, C., Harano, K., Tanamachi, K., Matsuo, K., Tamada, A., Iwaya-Inoue, M., et al. (2017). Reactive oxygen species induced by heat stress during grain filling of rice (*Oryza sativa* l.) are involved in occurrence of grain chalkiness. *J. Plant Physiol.* 216, 52–57. doi: 10.1016/j.jplph.2017.05.015

Suriyasak, C., Oyama, Y., Ishida, T., Mashiguchi, K., Yamaguchi, S., Hamaoka, N., et al. (2020). Mechanism of delayed seed germination caused by high temperature stress during grain filling in rice (*Oryza sativa* l.). *Sci. Rep.* 10, 17378. doi: 10.1038/s41598-020-74281-9

Van Gestelen, P., Asard, H., and Caubergs, R. J. (1997). Solubilization and separation of a plant plasma membrane NADPH-O_2^- synthase from other NAD(P)H oxidoreductases. *Plant Physiol.* 115, 543–550. doi: 10.1104/pp.115.2.543

Vries, M. E. D., Rodenburg, J., Bado, B. V., Sow, A., Leffelaar, A., and Giller, K. E. (2010). Rice production with less irrigation water is possible in a sahelian environment. *Field Crops Res.* 116, 154–164. doi: 10.1016/j.fcr.2009.12.006

Washio, K. (2003). Functional dissections between GAMYB and dof transcription factors suggest a role for protein-protein associations in the gibberellin-mediated expression of the RAmy1A gene in the rice aleurone. *Plant Physiol.* 133, 850–863. doi: 10.1104/pp.103.027334

Woodger, F. J., Millar, A., Murray, F., Jacobsen, J. V., and Gubler, F. (2003). The role of GAMYB transcription factors in GA-regulated gene expression. J. *Plant Growth Regul.* 22, 176–184. doi: 10.1007/s00344-003-0025-8

Wu, J., Zhang, N., Liu, Z., Liu, S., Liu, C., Lin, J., et al. (2020). The *AtGSTU7* gene influences glutathione-dependent seed germination under ABA and osmotic stress in arabidopsis. *BBRC* 528, 538–544. doi: 10.1016/j.bbrc.2020.05.153

Xin, P., Guo, Q., Li, B., Cheng, S., Yan, J., and Chu, J. (2020). A tailored high-efficiency sample pretreatment method for simultaneous quantification of 10 classes of known endogenous phytohormones. *Plant Commun.* 1, 100047. doi: 10.1016/j.xplc.2020.100047

Yamane, K., Gracia, R., Imayoshi, K., Mabesa-Telosa, R. C., Banayo, N. P. M. C., Vergara, G., et al. (2017). Seed vigor contributes to yield improvement in dry direct-seeded rainfed lowland rice. *Ann. Appl. Biol.* 172, 100–110. doi: 10.1111/aab.12405

Yao, C., Zhang, F., Sun, X., Shang, D., He, F., Li, X., et al. (2019). Effects of s-abscisic acid (S-ABA) on seed germination, seedling growth, and *Asi1* gene expression under drought stress in maize. *J. Plant Growth. Reg.* 38, 1300–1313. doi: 10.1007/s00344-019-09934-9

Ye, N., Zhu, G., Liu, Y., Zhang, A., Li, Y., Liu, R., et al. (2012). Ascorbic acid and reactive oxygen species are involved in the inhibition of seed germination by abscisic acid in rice seeds. *J. Exp. Bot.* 63, 1809–1822. doi: 10.1093/jxb/err336

Zhang, Q., Liu, Y., Jiang, Y., Li, A., Cheng, B., and Wu, J. (2022). *OsASR6* enhances salt stress tolerance in rice. *Int. Mol. Sci.* 23, 9340. doi: 10.3390/ijms23169340

Zhou, H., Wang, Y., Zhang, Y., Xiao, Y., Liu, X., Deng, H., et al. (2022). Comparative analysis of heat-tolerant and heat-susceptible rice highlights the role of *OsNCED1* gene in heat stress tolerance. *Plants* 11, 1062. doi: 10.3390/plants11081062

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Spermidine enhances heat tolerance of rice seeds during mid-filling stage and promote subsequent seed germination

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Introduction: Heat stress is a vital factor which restricts rice seed quality and yield. However, the response mechanism to heat stress in the mid filling stage of rice seed is unclear.

Methods: In the present study we integrated phenotypic analysis with biochemical, hormone, and gene expression analysis in order to explore technologies for improving rice seeds heat tolerance and subsequent seed germination.

Results: Spermidine (Spd) application effectively alleviated the damage of heat stress treatment during mid-filling stage (HTM, 12-20 days after pollination) on seed development, promoted subsequent seed germination and seedlings establishment. Spd significantly increased seed dry weight, starch and amylose contents during seed development under heat stress, and improved seed germinate, seedlings establishment and seedling characteristics during germination time. Biochemical analysis indicated that, HTM significantly decreased the activities of several starch synthase enzymes and led to a decrease in starch content. While Spd treatment significantly enhanced the activities of ADP-glucose pyrophosphorylas and granule-bound starch synthase, as well as the corresponding-genes expressions in HTM rice seeds, resulting in the increases of amylose and total starch contents. In addition, Spd significantly increased the catalase and glutathione reductase activities together with corresponding-genes expressions, and lowered the overaccumulation of H2O2 and malondialdehyde in HTM seeds. In the subsequent seed germination process, HTM+Spd seeds exhibited dramatically up-regulated levels of soluble sugars, glucose, ATP and energy charges. Consistently, HTM+Spd seeds showed significantly increased of α-amylose and α-glucosidase activities as well as corresponding-genes expressions during early germination. Moreover, HTM evidently increased the abscisic acid (ABA) content, decreased the gibberellin (GA) content, and accordingly significantly declined the GA/ABA ratio during early rice seeds germination. However, Spd treatment did not significantly affect the metabolism of GA and ABA in seed germination stage.

Discussion: The present study suggested that Spd treatment could effectively alleviate the negative impact of HTM on seed development and the subsequent seed germination, which might be closely correlated with starch synthesis and antioxidant defense during seed filling period, starch decomposition and energy supply in seed germination period.

KEYWORDS

heat stress, rice, seed development, seed germination, spermidine, starch, antioxidant defense

Introduction

Rice is planted globally as a critical staple food (Valipour et al., 2014). Rice originated from tropical atmosphere and subtropics, and the extreme high temperature will influence its yield and quality. Under global warming, heat stress frequently occurred in China, which had become one of the major catastrophic climatic factors for rice production (Xiong et al., 2016; Zhao et al., 2017; Yang et al., 2020). It was estimated that, the rice yield will decrease by 41% by the end of the 21st century due to the frequent heat stress (Peng et al., 2004). Meanwhile, heat stress will also impair the rice seed quality. Heat stress during the rice seed filling increased the chalkiness of rice seeds, and lower the thousand seed weight and germination rate, thereby severely affecting the production of rice seed (Tang et al., 2017). Heat stress has become the bottleneck that restricts rice production in the southern rice region of China. However, the research on the heat stress effect on rice seed development is still lacking. It is worth noting that the reports concerning the heat stress effect on rice seed development mainly concentrates on the flowering and early seed development stage (Madan et al., 2012; Fu et al., 2019; Chen et al., 2021). Nevertheless, severe heat stress frequently occurs during rice seed mid-filing stage (august to october) in the major rice production areas in yangtze river basin of China (including Hubei, Anhui, Jiangxi, Jiangsu and Hunan provinces) (Zhang et al., 2018). Notably, the seed mid-filling stage is a key period for seed vigor formation (Iwai et al., 2012; Martínez-Eixarch and Ellis, 2015; Zhu et al., 2016). Accordingly, investigating the heat resistance mechanism during seed mid-development stage, mitigating the heat stress damage to improve the seed quality are of great practical significance for rice production in China. Starch accumulates in rice endosperm and offers energy to achieve seed germination and seedling establishment; Besides, rice provide over 22% of global energy intake to humans (Tabassum et al., 2021). Starch is comprised by two D-glucose homopolymers, the branched amylopectin and linear amylose. Starch accumulated in rice consists of about 1/4 of amylose and 3/4 of amylopectin (Zhu et al., 2021). Starch biosynthesis represents the complicated biochemical process involving the reactions of various enzymes. Generally, ADP-glucose pyrophosphorylas (ADPGase), soluble starch synthases (SSs), granule-bound starch synthase (GBSS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) are critical starch biosynthesis-related enzymes in higher plants, which are widely investigated in detail (Cai et al., 2006; Huang et al., 2021). Amylose synthesis from glucosyl monomers is catalyzed by GBSS. Starch has the highest abundance in seed endosperm during cereal seed development. Heat stress significantly suppressed starch synthesis during rice seed development. Yamakawa and Hakata (2010) reported that the activities of several AGPases isoforms were down-regulated in Nipponbare rice seeds under heat stress. Heat stress significantly lowered the *OsGBSSI* expression and amylose content of rice seeds during late filling stage (Lin et al., 2010). During rice seed early-filling process, high temperature resulted in the decreased expressions of *SBEI and SBEII*, whereas the increased expression of *SBEIV* (Su et al., 2009). However, the effects of high temperature on starch metabolism during mid-filling process remains to be further explored. Starch degradation to soluble sugars plays a crucial role in supporting seed germination and early seedlings growth. Two enzymic routes for starch degradation had been elucidated in cereals, hydrolysis with amylase and phospholysis with starch phosphorylase. Wang et al. (2022) reported that hydrolysis, rather than phosphorlysis, is the main process for cereal starch degradation during seed germination. It was found that α-amylase was crucial in the starch hydrolysis during rice and maize seeds germination (Zhao and Wang, 2001). It was well known that α-amylase expression in germinating cereal grains was regulated by phytohormone. As discovered by Kim et al. (2006), gibberellic acid (GA) promoted the *de novo* α-amylase synthesis within aleurone layer cells.

Polyamines (PAs) are small aliphatic polycationic nitrogenous compounds, mainly including putrescine (Put), spermidine (Spd) and spermine (Spm) (Liu et al., 2015). Spd is the major triamine, and proved be crucial in regulating plant growth and development, such as flowers differentiation, fruits development and senescence, and seed germination (Carolina et al., 2015; Kamrun et al., 2016; Tao et al., 2018; Alcázar et al., 2020). Spd also plays a vital role in the plant response to heat stress (Ma et al., 2020; Zhang L et al., 2017; Sagor et al., 2013). Spd application mitigated the plant heat damage by inducing the antioxidases defense, maintaining cell membrane stability and stabilizing the photosynthetic system function (Zhang L et al., 2017). Sang et al. (2017) found that exogenous Spd increased the heat tolerance of tomato seedlings through inducing defensive response, protein folding and protein degradation. The overexpression of Spm synthase gene (*AtSPMS*) induced the expression of heat shock factor (*HSF*) and heat shock protein (*HSP*) to mitigate the heat damage to *Arabidopsis thaliana*

seedlings (Sagor et al., 2013). Besides, the overexpression of Spd synthase gene (*AtSPDS*) in *Arabidopsis thaliana* up-regulated the expressions of several key stress-response factors (including *WRKY*, *bZIP* and *rd29A*), thereby enhancing the plant resistance to high temperature, drought and flood stress (Kasukabe, 2004). During seed filing process, the endogenous Spd in rice and maize seeds were extremely significantly positively correlated with the seed weight (Wang et al., 2017). Under heat stress, the endogenous Spd in heat-resistant rice varieties and superior grains were more stable than those in heat-sensitive varieties and inferior grains (Chen et al., 2010). Moreover, exogenous Spd induced the expressions of stress-associated proteins (*SAPs*) in the seed early-development stage, and alleviated the heat damage to the hybrid rice seed quality (Fu et al., 2019). The above reports suggested that Spd played an important role in the plant response to heat stress.

However, the study regarding the molecular mechanism of Spd involvement in seed development and seed vigor formation of rice with heat stress treatment during mid-filling stage (HTM) is still lacking. Therefore, the present study aims to explore the potential role of Spd in the regulation of metabolism involved in seed weight accumulation, seed vigor formation and heat stress response process, and elucidate further the mechanism of Spd promoting the seed development of rice.

Materials and methods

Materials

Rice seeds of 'Zhegeng 100' (*Oryza sativa* L. *ssp. japonica*) were used in present study, which has been widely planted in Zhejiang province due to its high yield, better flavor and wide adaptability. The rice plants were grown under normal conditions at the experimental farm of Zhejiang Academy of Agricultural Science (Hangzhou, China). At 8-12 days after pollination (DAP), 10 mL of 0.5 mM Spd rice plants on the spikelets per plant every day for 5 days continuously. The Spd concentration were determined by preliminary experiments. Plants sprayed with distilled water were used as the control. Thereafter, rice plant was placed in the high-temperature growth chambers under the 16-h/8-h light (40°C)/dark (30°C) photoperiod (60% relative humidity) at 12-20 DAP (HTM). Meanwhile, plants grown under16-h/8-h light (30°C)/dark (20°C) photoperiod (60% relative humidity) served as controls (NT). All rice plants were placed to normal environment under 16-h/8-h light (30°C)/dark (20°C) photoperiod at 20-28 DAP.

Determination of seed characteristics

Seeds dry weight was recorded after 24-h drying under 80°C at 12, 16, 20 and 28 DAP, respectively. Rice seeds were sampled at 28 DAP for determination of seed length, width and thickness using the vernier caliper.

Seed germination and seedling establishment tests

Rice seeds sampled at 28 DAP were dried to target moisture (14%) at room temperature and then used for seed germination and seedling establishment tests (n=100 under each treatment in four replicates). Seed germination was performed within the germination chambers under the 8-h/16-h light/dark cycle and 25°C conditions. Seed germination was determined in the case of radicle reaching 2/3 of seed length. The germinated seed number was measured daily. Seeds samples were collected at 1, 3, and 5 days of germination for subsequent analyses.

For seedling establishment test, 100 rice seeds were sowed in sands under 25°C and 12-h/12-h light/dark cycle conditions. After 14 days, seedling emergence rate, seedlings height, seedling dry weight, and total chlorophyll content were analyzed in line with experimental requirements.

Amylose and amylopectin content analysis by dual-wavelength spectrophotometry

Amylose and amylopectin extraction from seed was determined by dual-wavelength spectrophotometry according to the method of Zhu et al. (2021). Briefly, 0.1 g seed starch sample was dissolved in 0.1 mL absolute alcohol and 1 mL sodium hydroxide at 100°C. The mixture was diluted to 10 mL with distilled water. Afterwards, 0.5 mL mixture was sampled and mixed with 0.42 mL 0.1 M hydrochloride solution, 1.08 mL distilled water and 0.1 mL iodine solution, followed by 10 min incubation. The amylose content was determined by the sample absorbance at 607 nm and 433 nm. The amylopectin content was determined by sample absorbance at 729 nm and 543 nm.

Assay of starch, antioxidant enzymes, PAs metabolism-related enzymes activity

The enzyme activity analysis of ADP-glucose pyrophosphorylase (AGPase), soluble starch synthases (SSs), granule-bound starch synthase (GBSS), starch branching enzyme (SBE), starch debranching enzyme (DBE), α-amylase, β-amylase, α-glucosidase, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and glutathione reductase (GR) were performed using enzyme-linked immune kit (Mlbio, Shanghai, China). The color change was determined by spectrophotometry using an enzyme mark instrument at the 450 nm wavelength. The enzyme activity was determined through comparison of sample O.D. to standard curve.

Soluble sugar and glucose contents analysis

Anthrone-H_2SO_4 colorimetry was conducted to determine total soluble sugar level of rice seeds (Zhu et al., 2016). In brief, 0.5 g seed sample was dissolved in 10 mL distilled water. The mixture was transformed to 30 mL test tube with stopper, followed by 30 min

extraction with at 100°C, dilution till 25 mL with distilled water. The soluble sugar content was determined through comparison of sample absorbance at 620 nm to standard curve.

The glucose content of rice seeds was determined by high performance liquid chromatography (HPLC) with the method of Bailly et al. (2010). In brief, 10 µl glucose extraction was injected into the Spherisorb-NH_2 column (Thermo Separation Products, France), followed by elution using 75/25 (v/v) acetonitrile/H_2O_2 with the Spectra Physics 8700 pump at the 0.8 ml·min^{-1} flow rate. Glucose contents were analyzed through comparison of sample peak area to standard curve.

ATP and energe charge analysis

ATP and energe charge were determined by HPLC, as described by Liu et al. (2006) with minor modifications (Liu et al., 2006). ATP in the samples were identified by comparison with retention time of standards, while the concentrations of ATP were determined using the external standard method. Data of ATP and energy charge analysis were expressed as means of four replicate determinations.

Real-Time Quantitative PCR (RT-qPCR)

Total sample RNA was extracted with RNeasy Mini Kit (HuaYueYang, Beijing, China). Seeds RNA (500 ng) was reverse-transcribed into cDNA with PrimeScript RT reagent Kit (Takara, Dalian, China). Gene-specific primers were list in the Supplementary Table 1. Briefly, the 20-µl reaction system was used in PCR amplifications, which included 1 µl diluted cDNA, 0.8 µl primers, 10 µl AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China) and 8.2 µl ddH_2O. Three biological replicates were conducted and each biological replicate was technically repeated three times. All data were expressed as the mean SD after normalization.

Statistical analysis

Data were analyzed by Statistical Analysis System (SAS) software through analysis of variance (ANOVA). The multiple comparison for mean values were performed by Tukey'S Honestly Significant Difference (HSD) test (P<0.05). Prior to ANOVA, percentage data was converted in line with y = arcsin [sqrt (x/100)].

Results

Spermidine treatment increased seeds characteristics of HTM during rice seed development

During rice seed development, the seed fresh and dry weight rapidly increased at 12-20 DAP (Supplementary Figure 1). Accordingly, heat stress treatment was performed at 12-20 DAP to explore the heat tolerance mechanism of rice during the middle stage of seed filling.

At 28 DAP, the seed length, width and volume of HTM seeds were significantly lower than those of NT (Figure 1). While HTM+Spd seeds showed significant higher seed length, seed width and volume as compared with HTM seeds. Moreover, Spd treatment markedly increased rice seeds dry weight at 12, 16, and 28 DAP, respectively (Table 1). While Spd treatment made no significantly difference on seed characteristics in NT seeds.

Spd treatment during rice seed development under heat stress improved subsequent seed germination and seedling emergence

At 5 and 6 days of germination time, HTM-seeds showed lower germination ability with germination percentage of 31.50% and 55.25% compared with NT. While HTM+Spd seeds germinated much faster than HTM-seeds. Seed germination percentage of HTM+Spd had reached 47.50% and 67.25% at 5 and 6 days of germination, which was 1.51- and 1.23-folds of HTM-seeds, respectively. After 14 days of germination, HTM significantly decreased seedlings establishment rate compared with NT. HTM+Spd treatment showed significant higher seedlings establishment rate compared with HTM (Figures 2A–D).

On the other hand, seedling growth was inhibited in HTM seeds, of which seedlings dry weight, seedlings height and total chlorophyll content reached significant levels (Figures 2E, F, Supplementary Figure 2). On the contrary, the seedlings dry weight, seedlings height, and total chlorophyll content in HTM+Spd were obviously higher than those of HTM. In addition, no significant difference on seed germination rate, seedlings establishment and seedling characteristics were detected between NT and NT+Spd treatments during germination time. After clarifying the role of Spd treatment during seed filling under heat stress in promoting the seed development and subsequent seed germination, only NT, HTM, and HTM+Spd seeds were used in subsequent experiments.

Spermidine treatment promoted starch synthesis of HTM seeds during rice seed development

It was shown that the contents of total starch, amylase and amylopectin of HTM-seeds were significantly lower than those in NT at 16, 20 and 28 DAP (Figures 3A–C). Spd application significantly increased the total starch and amylase contents in HTM seeds at 16, 20 and 28 DAP. While there was no remarkable difference in amylopectin content between HTM and HTM+Spd seeds during seed development. In consistent with the above results, enzymes activity analysis revealed that HTM significantly decreased the activities of AGPase, SSs, and GBSS, and increased SBE activity at 16 and 20 DAP. Besides, HTM+Spd seeds showed significant higher activities of AGPase and GBSS at DAP 16 compared with

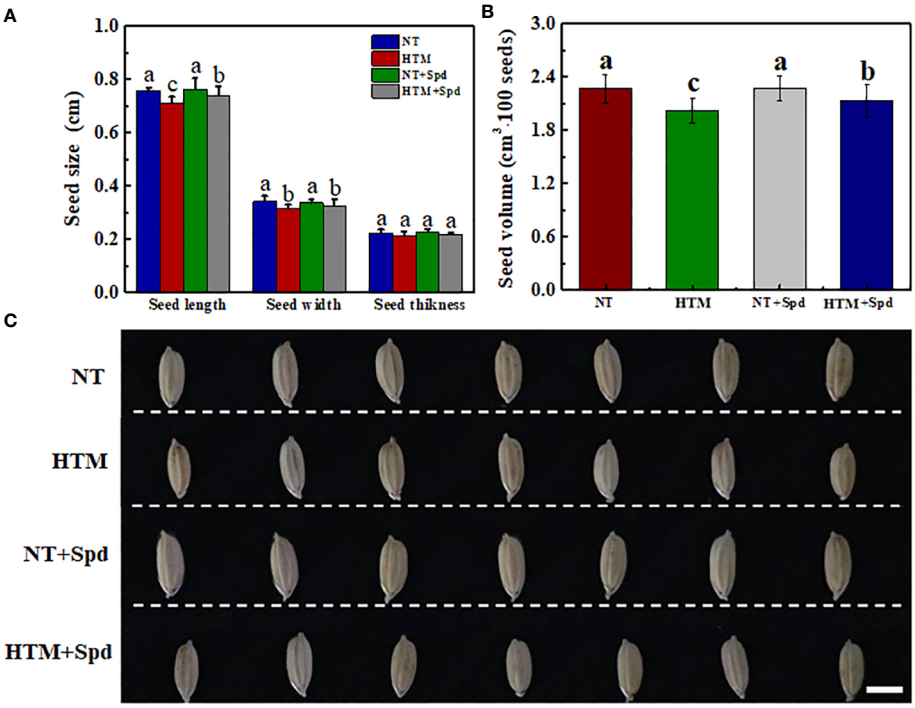

FIGURE 1
Effects of exogenous Spd on seed characteristics in rice under heat stress. **(A)** Seed size; **(B)** Seed volume; **(C)** Typical images of rice seeds. Seeds were sampled at 28 days after pollination time. NT, normal temperature + distilled water treatment; HTM, heat stress treatment + distilled water treatment; NT + Spd, normal temperature + 0.5 mM Spd treatment; HTM + Spd, heat stress +0.5 mM Spd treatment. Rice plants were treated with Spd solution during 8-12 days after pollination. Heat stress treatment was performed at 12-20 days after pollination. Scale bar, 5 mm. Different lowercase(s) above the bars indicate significant differences ($p < 0.05$, Tukey's HSD) among treatments.

HTM. The activities of SSs, SBE and DBE were not obviously affected by Spd in HTM seeds (Figures 3D–H). The RT-qPCR indicated that the transcripts of starch-synthesis related genes were significantly down-regulated by HTM at 16 and 20 DAP, including *OsAGPls2*, *OsAGPss1*, *OsGBSSI*, *OsSSI*, *OsSSIIc*, and *OsSBE3*. On the contrary, HTM+Spd significantly increased the transcript of *OsAGPls2*, *OsGBSSI*, *OsSSI*, and *OsSBE3* at 16 or 20 DAP compared with HTM (Figure 4).

Spermidine treatment induced antioxidant defense and alleviated excessive ROS during HTM rice seed development

The excessive MDA and ROS contents in rice seeds induced by heat stress were the critical causes of the decreased seed vigor. For further investigated the mechanism of Spd in improving germination and seedling establishment of HTM rice seeds, we

TABLE 1 Effects of exogenous Spd on seed dry weight in rice under heat stress.

Treatment	Seeds dry weight (g/1000 seeds)			
	DAP 12	DAP 16	DAP 20	DAP 28
NT	6.87 ± 0.482a*	18.25 ± 1.121a	20.33 ± 1.253a	24.39 ± 1.177a
HTM	6.57 ± 0.451a	15.31 ± 1.113c	17.37 ± 1.034c	21.59 ± 2.022c
NT+Spd	6.66 ± 0.358a	18.87 ± 1.343a	20.57 ± 1.387a	24.18 ± 1.023a
HTM+Spd	6.74 ± 0.471a	17.68 ± 0.929b	18.35 ± 1.993b	23.18 ± 1.542b

*Values followed by a different letter within a column are significantly different at the 0.05 probability level. NT: normal temperature + distill water treatment; HTM: heat stress treatment + distill water treatment; NT+Spd: normal temperature+0.5 mM Spd treatment; HTM+Spd: heat stress +0.5 mM Spd treatment. Rice plants were treated with Spd solution during 8-12 days after pollination. Heat stress treatment was application at 12-20 days after pollination. DAP, days after pollination.

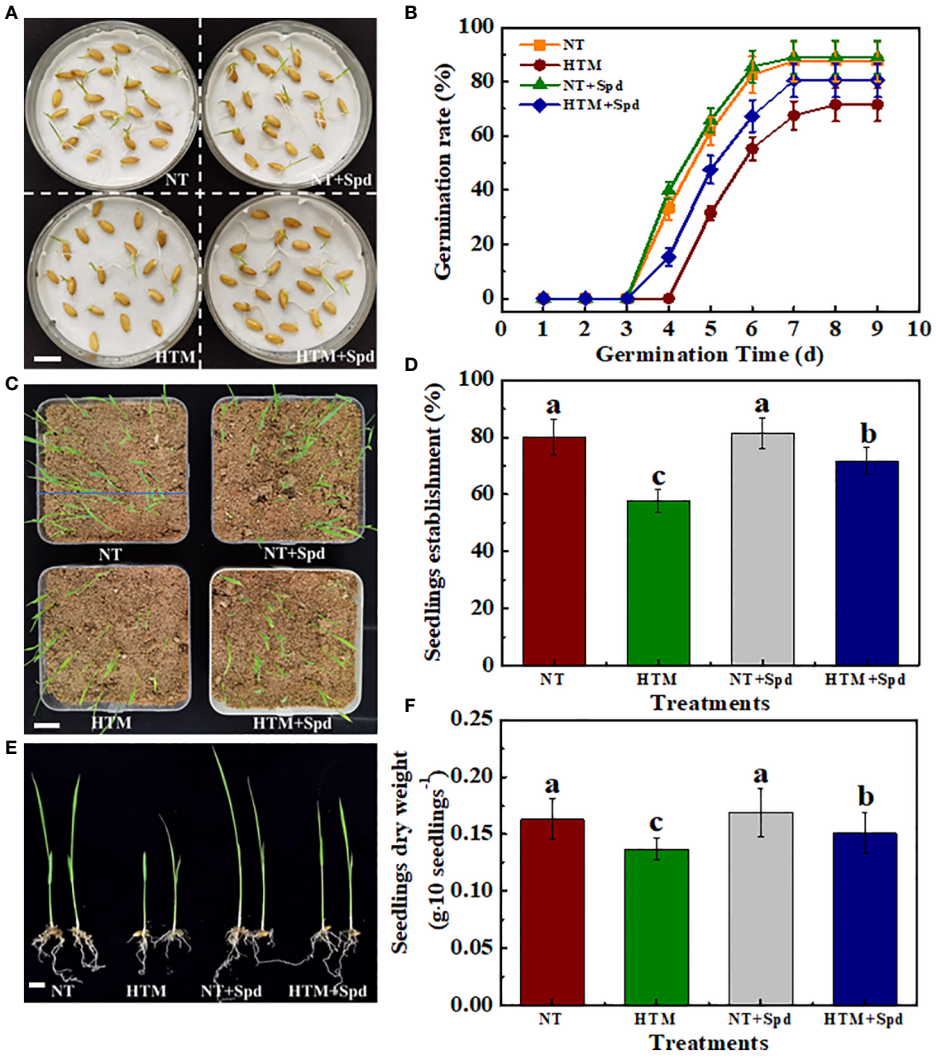

FIGURE 2
Spermidine treatment remarkably promoted seed germination and seedling emergence of HTM seeds. **(A)** Characteristic images of rice seed samples at 5 days of germination. Scale bar, 10 mm. **(B)** The time courses showing seeds sample germination rates. **(C)** Characteristic images of seedling emergence at 14 days of germination. Scale bar, 20 mm. **(D)** Seedling emergence rate in **(C)** is presented. **(E)** Characteristic images of rice seedlings. Scale bar, 10 mm. **(F)** Quantification of dry weight of rice seedlings. NT: normal temperature + distilled water treatment; HTM: heat stress + distilled water treatment; HTM + Spd: heat stress + 0.5 mM Spd treatment. Rice plants were treated with Spd solution during 8-12 days after pollination. Heat stress treatment was application at 12-20 days after pollination. Four biological replicates each with 100 seeds for each treatment were set in seed germination and seedling emergence tests. The asterisk (*) or different lowercase(s) above the bars indicate significant differences ($p < 0.05$, Tukey's HSD) among treatments.

determined the H_2O_2, O_2^-, and MDA contents in rice seeds during seed development (Figure 5). HTM significantly increased the H_2O_2, O_2^-, and MDA contents at 16 and 20 DAP. By contrast, the contents of H_2O_2 (20 and 28 DAP) and MDA (16 and 20 DAP) in HTM+Spd seeds were apparently lower than those in HTM seeds. However, exogenous Spd did not significantly affect O_2^- level in HTM seeds at 16, 20, 28 DAP.

Given that Spd treatment decreased contents of H_2O_2 and MDA in HTM seeds during seed development, the Spd role in antioxidant enzyme activities, including SOD, CAT, OPD, APX, and GR, was examined (Figure 6). As a result, HTM apparently enhanced SOD, CAT, and POD activities at 16 and 20 DAP compared with NT seeds. Besides, Spd remarkably increased the CAT (16 DAP) and GR (16 and 20 DAP) activities in HTM seeds. While no significant effect of Spd on SOD, POD activities was observed in HTM seeds during seed development. Consistent with the above results, RT-qPCR showed that the transcriptional levels of antioxidant enzyme related-genes were markedly up-regulated by HTM, such as *OsCu-ZnSOD*, *OsCAT1*, *OsCAT3*, *OsPOD3*, *OsAPX2*, and *OsGR* (Figure 7). Besides, significantly higher transcriptional levels of *OsCu-ZnSOD*, *OsCAT1*, *OsCAT3*, *OsGR* were observed in HTM+Spd seeds compared with HTM seeds. However, it was shown that Spd decreased the transcriptional levels of *OsPOD3* and *OsAPX2* at 16 and 20 DAP.

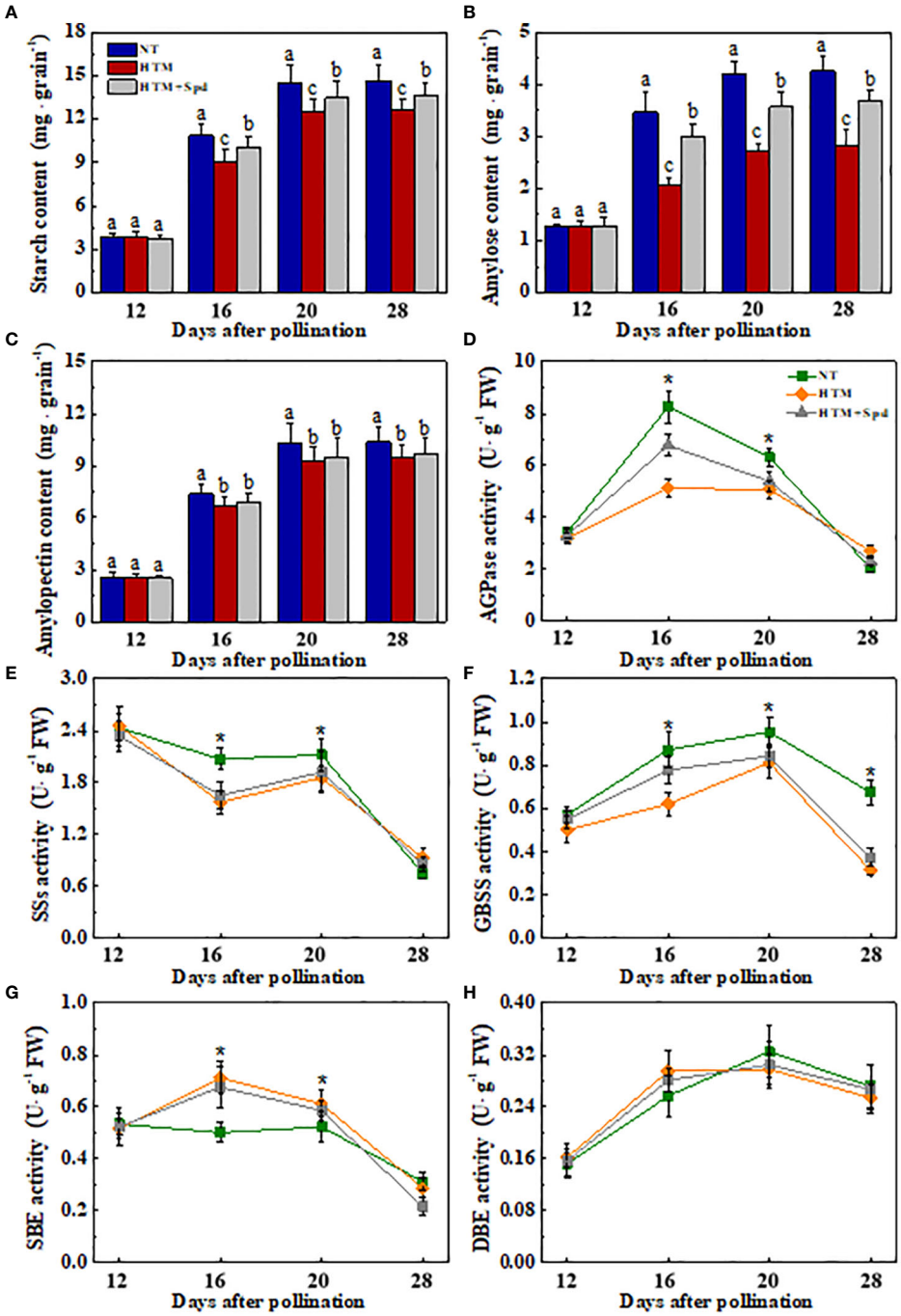

FIGURE 3
Effect of spermidine treatment on content of starch **(A)**, amylose **(B)**, amylopectin **(C)** and activities of ADPGase **(D)**, SSs **(E)**, GBSS **(F)**, SBE **(G)**, DBE **(H)** in HTM seeds during rice seed development. NT: normal temperature + distilled water treatment; HTM: heat stress + distilled water treatment; HTM + Spd: heat stress + 0.5 mM Spd treatment. AGPase: ADP-glucose pyrophosphorylase; SSs: soluble starch synthases; GBSS: granule-bound starch synthase (GBSS); SBE: starch branching enzyme; DBE: starch debranching enzyme. Rice plants were treated with Spd solution during 8-12 days after pollination. Heat stress treatment was application at 12-20 days after pollination. Results are representative of four independent experiments. The asterisk (*) or different lowercase(s) above the bars indicate significant differences ($p < 0.05$, Tukey's HSD) among treatments. .

Spd treatment improved starch hydrolysis in HTM rice seeds during early germination time

Seed storage substances are the main energy sources in early seed germination and seedling emergence, while soluble sugar is the major nutrient generated via storage substance decomposition. For better analyzing the mechanism by which Spd promoted germination and seedling emergence from HTM seeds, the levels of soluble sugar, glucose, ATP, and energy charge were determined during early germination (Figure 8). Compared with NT, HTM remarkably lowed the levels of soluble sugar, glucose, ATP and energy charge

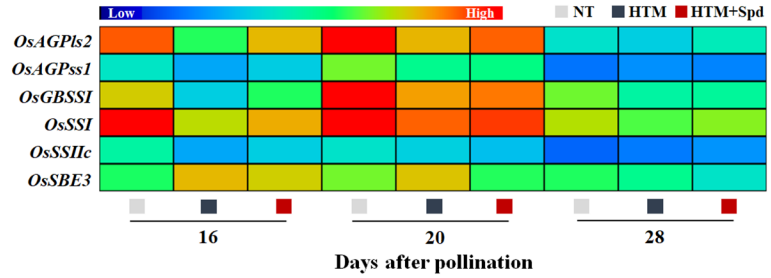

FIGURE 4
Spd treatment increased the expressions of starch synthesis-related genes in rice seeds during seed development under heat stress. NT, normal temperature + distilled water treatment; HTM, heat stress + distilled water treatment; HTM + Spd, heat stress + 0.5 mM Spd treatment. AGPls, ADP-glucose pyrophosphorylase large subunit; AGPss, ADP-glucose pyrophosphorylase small subunit; GBSS, granule-bound starch synthase; SSs, soluble starch synthases; SBE, starch branching enzyme. RT-qPCR was carried out in three biological replicates, each containing three technical replicates. Heat map was created with Illustrator software. Gene expression from lowest (L) to highest (H) stands for diverse gene levels in the entire database.

at 3 and 5 days of germination. In contrast, Spd treatment remarkably increased the contents of soluble sugar and glucose in HTM seeds at 3 and 5 days of germination. Besides, ATP and energy charge levels apparently increased in HTM+Spd seeds at 3 and 5 days of germination relative to HTM seeds. The above results suggested that Spd treatment promoted germination and seedling emergence of HTM seeds by increasing ATP and soluble sugar contents.

During seed germination and early seedling emergence, starch is hydrolyzed by α-amylose and β-amylose to produce maltose, which then converted into glucose through α-glucosidase. In present study, soluble sugar content elevated in HTM+Spd seeds, and its underlying mechanism was explored by measuring the activities of α-amylose, β-amylose, and α-glucosidase (Figures 9A–C). It was shown that HTM significantly lowered the activities of α-amylose and α-glucosidase during rice seeds germination. By contrast, remarkably up-regulated activities of α-amylose and α-glucosidase were observed in HTM+Spd seeds compared with HTM seeds. However, no significant difference of

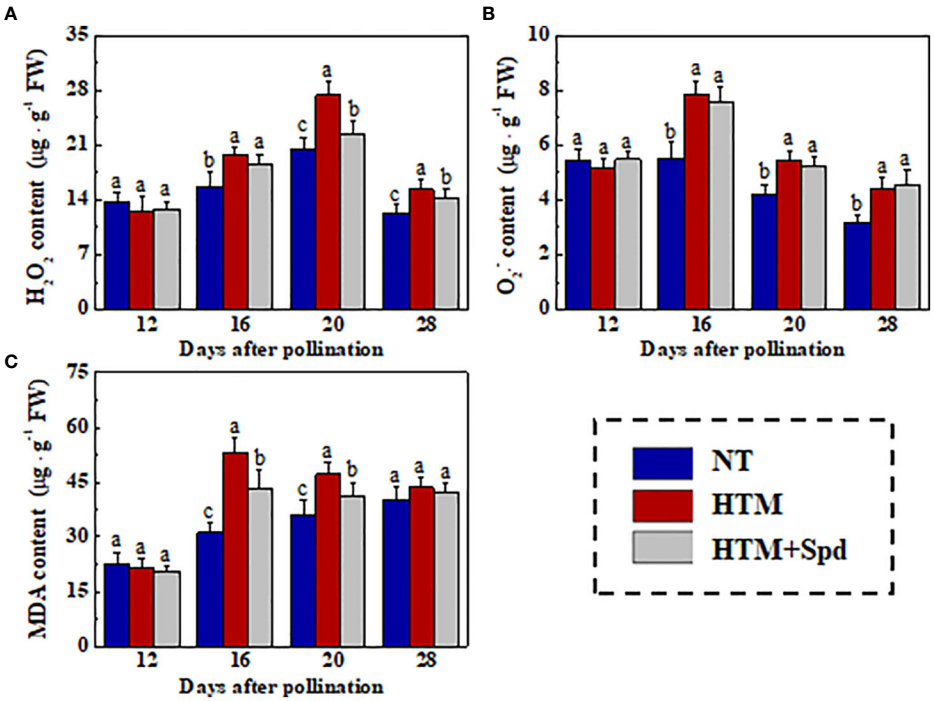

FIGURE 5
Spd treatment lowered the contents of H_2O_2 **(A)**, O_2^- **(B)**, and MDA **(C)** of HTM rice seeds during seed development. NT, normal temperature + distilled water treatment; HTM, heat stress + distilled water treatment; HTM + Spd, heat stress + 0.5 mM Spd treatment. H_2O_2, hydrogen peroxide; O_2^-, superoxide anion; MDA, malondialdehyde; The determination of H2O2, O_2^-, and MDA were performed with four biological replicates. Diverse lowercase letters stand for significant differences across treatments ($p < 0.05$, Tukey's HSD).

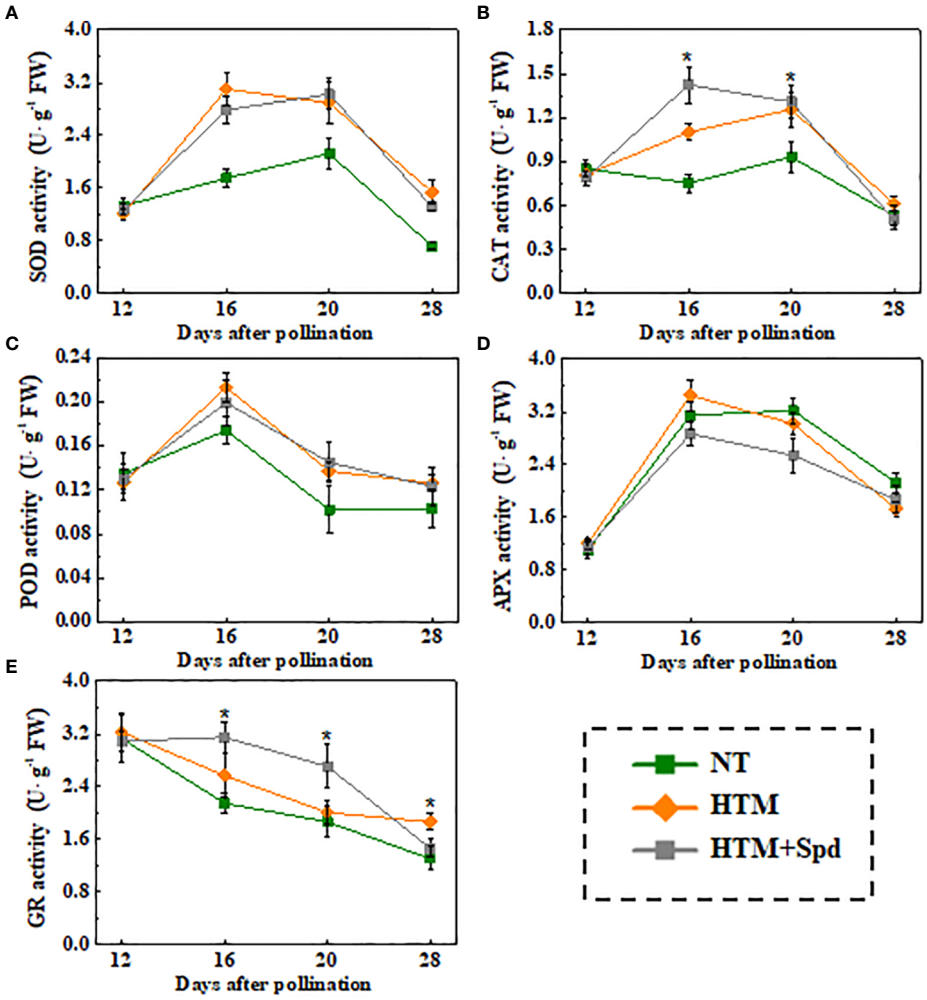

FIGURE 6
Spd treatment enhanced SOD **(A)**, CAT **(B)**, POD **(C)**, APX **(D)**, and GR **(E)** activities of HTM rice seeds during seed development. NT, normal temperature + distilled water treatment; HTM, heat stress + distilled water treatment; HTM + Spd, heat stress + 0.5 mM Spd treatment. SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase; GR, glutathione reductase. The enzymes activity analysis was performed with four biological replicates. The asterisk (*) indicates significant differences ($p < 0.05$, Tukey's HSD) across treatments.

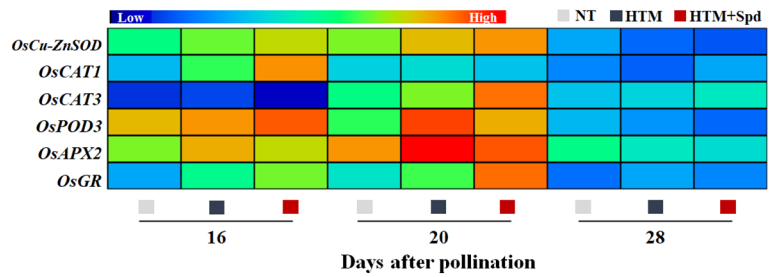

FIGURE 7
Spd treatment up-regulated the expressions of antioxidant enzyme genes in HTM seeds during seed development. NT, normal temperature + distilled water treatment; HTM, heat stress + distilled water treatment; HTM + Spd, heat stress + 0.5 mM Spd treatment. SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; APX, ascorbate peroxidase; GR, glutathione reductase. RT-qPCR was carried out in three biological replicates, each containing three technical replicates. Heat map was made with Illustrator software. Gene expression from lowest (L) to highest (H) stands for diverse gene levels in the entire database.

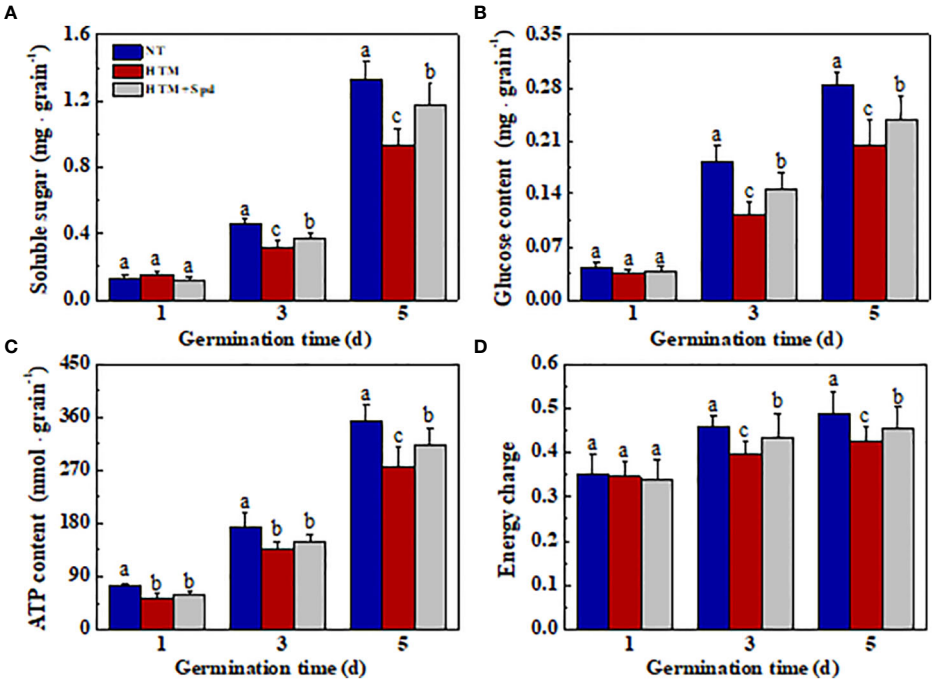

FIGURE 8
Spermidine treatment during rice seed development under heat stress increased contents of soluble sugar **(A)**, glucose **(B)**, ATP **(C)**, and energy charge **(D)** in rice seeds during germination time. NT, normal temperature + distilled water treatment; HTM, heat stress + distilled water treatment; HTM + Spd, heat stress + 0.5 mM Spd treatment. Percentages stand for averages of four tests ± SE. Different lowercase(s) above the bars stand for statistical significance ($p<0.05$, Tukey's HSD) among treatments.

β-amylose activity was detected between three treatments. These observations conformed to positive role of Spd in soluble sugar and glucose contents during early seed germination.

Additionally, significant lower transcriptional levels of *OsAmy1, OsAmy3, OsGlu2* were detected in HTM seeds at 1, 3, 5 DAP compared with NT seeds (Figure 9D). It was worth noting that Spd application up-regulated the transcriptional levels of *OsAmy1*, *OsAmy3* in HTM seeds at 3 and 5 days of germination. In addition, significant higher transcriptional level of *OsGlu2* was detected in HTM+Spd seeds at 1 and 3 days of germination. Such results consistent with the role of Spd in α-amylose and α-glucosidase activities of HTM+Spd rice seeds during germination.

Role of Spd in GA and ABA levels in HTM rice seeds in early germination

As gibberellin (GA) and abscisic acid (ABA) are important for the seed germination (Shu et al., 2016; Shu et al., 2018), we further analyzed the relation of Spd role in rice seed heat tolerance with GA/ABA pathways in rice seed germination (Figure 10). It was shown that ABA content slightly declined, whereas GA content evidently elevated in NT seeds during early germination. HTM significantly enhanced the ABA level and lowered the GA level at 3 and 5 days of germination, resulting in significant lower GA/ABA ratio at 1, 3, 5 DAP. However, the ABA, GA contents and GA/ABA ratio in HTM+Spd seeds showed no significant difference with those in HTM seeds during seed germination.

Discussion

Rice plants are vulnerable to heat stress during grain filling process, which seriously affect the seed yield and quality (Peng et al., 2004; Zhao et al., 2017; Yang et al., 2020). However, most studies on the rice heat tolerance focused on the roles of endogenous or environmental cues specifically during flowering, pollination and early grain filling stages (Madan et al., 2012; Fu et al., 2019; Chen et al., 2021). Although some reports had been published on the effects of heat stress during mid or late seed filling stages on the seed development (Morita et al., 2005; Beáta et al., 2008; Lin et al., 2010), the detailed regulatory mechanism remains largely unknown, especially the role of exogenous substances to rice heat tolerance during seed mid-filling stage on subsequent seed germination. According to our results, HTM remarkably suppressed the seed development, germination and seedling establishment. Spd treatment markedly mitigated heat injury, improved HTM rice seed germination and seedling establishment.

Spd alleviated the heat damage of HTM on rice seed development

Several reports found that heat stress during rice pollination and early grain filling stage remarkably decreased the seed maturing rate, seed dry weight, and seed size (Tang et al., 2017; Zhang, 2022). Seed size and shape are determined by endosperm cell number and size. It was reported that heat stress (34°C) decreased cell size

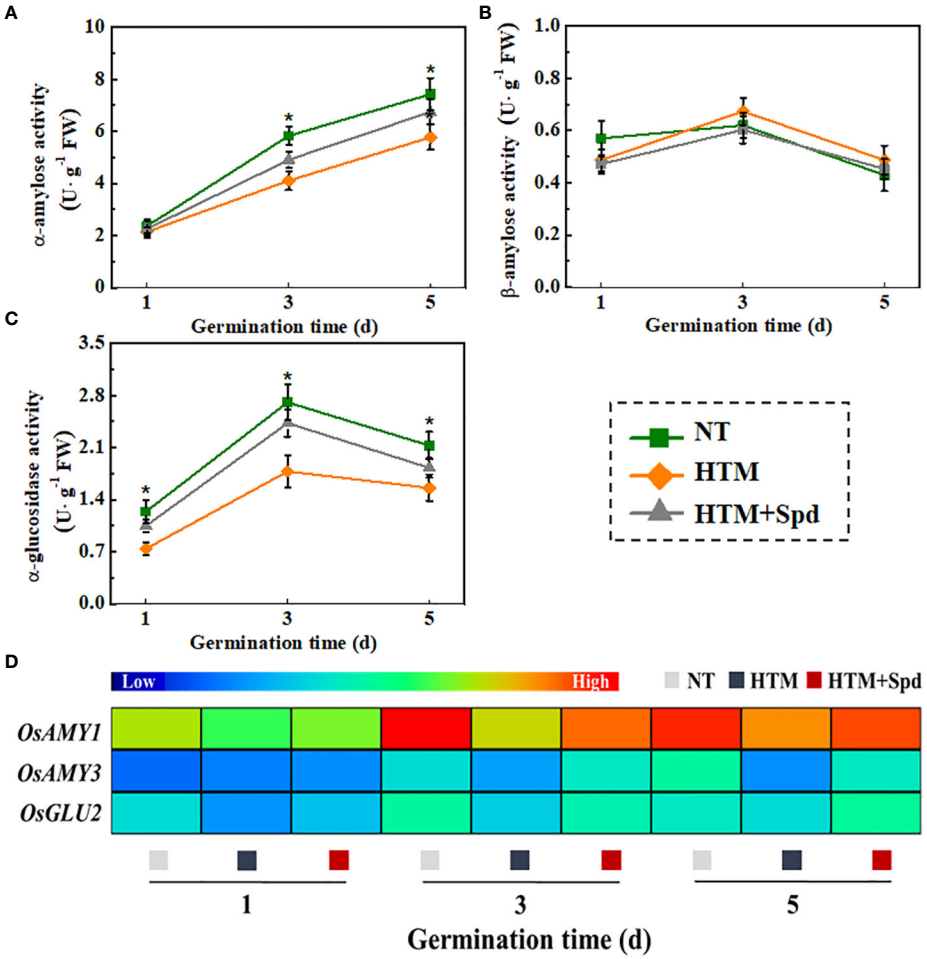

FIGURE 9
Spermidine treatment increased the activities of α-amylose **(A)**, β-amylose **(B)**, α-glucosidase **(C)** and expressions of starch metabolism-related genes **(D)**. NT, normal temperature + distilled water treatment; HTM, heat stress + distilled water treatment; HTM + Spd, heat stress + 0.5 mM Spd treatment. Four biological replicates for each treatment were set in enzymes activity assay. Realtime quantitative PCR was performed with three biological replications, and each was made in three technical replicates. The asterisk (*) was indicative of significant differences ($p<0.05$, Tukey's HSD) across treatments. The Illustrator software was used for creating the heat map. The gene levels from low (L) to high (H) indicated the lowest and highest levels in the whole database.

midway between endosperm surface and central point, resulting in the reduce of rice seed filling rate and seed thickness (Morita et al., 2005). Similarly, the present study found that HTM significantly suppressed rice seed development, which manifested as the lower seed width, seed length, seed volume and dry weight. However, HTM did not dramatically influence the seed thickness. Fu et al. (2019) found that high temperature treatment in the early grain filling stage (7-11 DAP) evidently decreased the rice seed thickness, but not significantly affected seed length or width. It was proposed that the impact of high temperature on rice seed size varied depending on the treatment period and rice variety. It was noting that Spd application notably improved the length, width and dry weight of HTM seeds. Consistently, several studies revealed a similar effect of Spd application on seed heat tolerance during blooming or early filling stage (Fu et al., 2019; Chen et al., 2021).

Spd effectively promoted the germination and seedling establishment of HTM rice seeds

Certain reports investigated the effect of parental environmental factors in subsequent seed germination (Nonogaki et al., 2014; Postma and Agren, 2015; Awan et al., 2018), while the detailed regulatory mechanism is still unknown. Brunel-Muguet et al. (2016) reported that oilseed rape seeds development under heat stress (33°C/day, 19°C/night) during seed filling are associated with high sprouting rate after harvesting, reduced ABA level and high aberrant seedling rate. In present study, HTM remarkably lowered rice seed germination speed and decreased the seedlings dry weight, seedlings height and total chlorophyll content. By contrast, Spd application effectively promoted seed germination

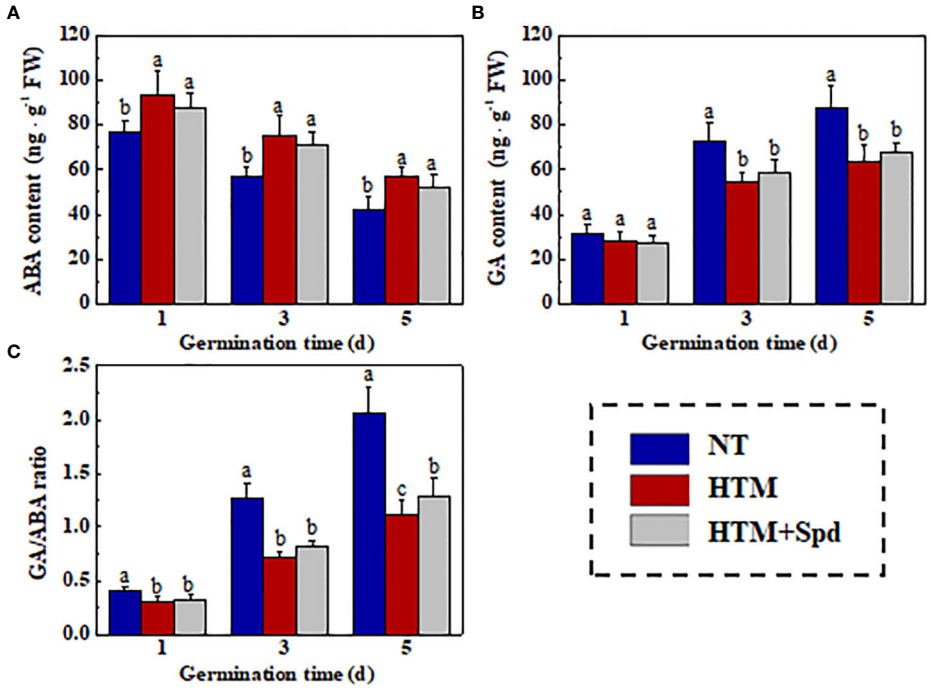

FIGURE 10
The effect of spermidine treatment on contents of ABA **(A)**, GA **(B)**, and GA/ABA ratio **(C)** in HTM seed during early germination time. NT, normal temperature + distilled water treatment; HTM, heat stress + distilled water treatment; HTM + Spd, heat stress + 0.5 mM Spd treatment. Percentages indicate the averages of four tests ± SE. Different lowercase(s) above the bars stand for statistical significance ($p<0.05$, Tukey's HSD) among treatments.

and seedling growth in HTM seeds. HTM+Spd treatment showed significant higher seed germination rate, seedlings establishment rate, and seedlings characteristics compared with HTM. Chen et al. (2021) found that 1.5 mM Spd treatment remarkably enhanced rice seed germination index in the early filling stage upon heat stress condition, while *OsSAP5* was the potentially important gene related to heart resistance of rice treated by Spd. Therefore, Spd possibly has a critical effect on the evolution of rice seed vigor upon heat stress conditions in diverse seed filling stages.

Spd promoted the starch synthesis of HTM rice seeds during seed development

Several studies explored the adverse impacts of heat stress on starch synthesis during crops seed development (Su et al., 2009; Lin et al., 2010; Yamakawa and Hakata, 2010; Sreenivasulu et al., 2015). Consistently, our results revealed that HTM significantly decreased contents of amylose, amylopectin, and total starch through down-regulating the activities of several key starch biosynthesis enzymes and corresponding-genes expressions. Spd was proved to be involved in starch metabolism. It was found that Spd treatment remarkably enhanced starch content in wheat grains during post-anthesis process under drought stress (Yang et al., 2014). Wang et al. (2012) found that starch accumulation rate was positively correlated with Spd content in superior and inferior spikelets of rice. Herein, Spd application efficiently increased AGPase and GBSS activities and transcripts of corresponding-genes expressions (OsAGPls2, OsAGPss1, and OsGBSSI), resulting in significant higher contents of starch and amylose in HTM seeds. While the amylopectin content was not significantly affected by Spd in rice seeds under heat stress. Consistent with the above results, Fu et al. (2019) found that the Spd-treated rice seeds showed improved seed development with higher amylose level under heat stress during early seed development. It was suggested that the effect of Spd on starch accumulation response to heat stress at different stages of rice seed development was consistent. Moreover, it was proved that amylose level made greater effects on seed germination compared with total starch and amylopectin, and high vigor hybrid rice seeds always showed higher amylose content and lower amylopectin content (Zhang, 2014). It was speculated that amylose plays a critical role in the role of Spd in improving heat tolerance of rice seeds during filling stage.

Spd induced the antioxidant defense and alleviated the over-accumulation of H_2O_2 and MAD in HTM rice seeds during seed development

Reactive oxygen species (ROS) mainly including hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radical

(OH)(Sachdev et al., 2021). ROS exert dual functions, to be specific, ROS at the suitable levels can positively affect seed development, germination, and environmental stress resistance, while excessive ROS contents generated toxicity to plant cells and tissues (Barba-Espin et al., 2011; Oracz and Karpinski, 2016; Sachdev et al., 2021). Consistently, we found HTM induced overaccumulation of H_2O_2, O_2^-, and MDA in rice seed during mid-filling stage (Figure 5). HTM +Spd markedly mitigated the excessive MAD and H_2O_2 contents at 16 and 20 DAP. It was suggested that maintaining ROS homeostasis in seed development process is of great significance for guaranteeing the evolution of seed vigor (Rajjou et al., 2012; Rashid et al., 2020). Our results revealed that Spd application could enable better ROS scavenging ability in rice seeds during mid-filling stage under heat stress. Such results were consistent with prior findings that maintenance of ROS homeostasis accounts for a mechanism related to Spd in improving seed vigor during seed filling or early germination upon several abiotic stresses (Pan et al., 2014; Yang et al., 2014; Sheteiwy et al., 2017; Tao et al., 2018).

Antioxidases, mainly including SOD, POD, CAT, APX, and GR, are crucial to the ROS scavenging system (Sachdev et al., 2021). POD activity showed positive relation to seed vigor index, whereas MDA level displayed negative relation (Cui et al., 2014). Exogenous Spd could eliminates ROS through enhancing CAT, SOD and POD activities in tomato cells under upon stress conditions (Diao et al., 2015). During rice flowering period, Spd-treated leaves exhibited reduced MDA level and enhanced SOD and POD activities (Tang et al., 2018). Likewise, this study suggested that activities of SOD, CAT, POD, GR and related genes expressions were enhanced by HTM. In addition, HTM+Spd seeds displayed remarkably enhanced SOD, CAT, and GR activities and corresponding-genes (including OsCu-ZnSOD1, OsCAT1, OsCAT3, and OsGR) expressions. CAT functions in the decompose of H_2O_2 to water and oxygen, while SOD could disproportion superoxide anion free radicals for producing H_2O_2 and oxygen (Sachdev et al., 2021). In addition, GR is responsible for the reduction of oxidaized glutathione disulfide to reduced glutathione, which provides reducing power for ROS scavenging (Smiri et al., 2010). Our results conformed to prior research suggesting that Spd protected macromolecules and biofilms and maintained organelle integrity upon stresses (Diao et al., 2015). The positive role of Spd on activating of antioxidant defense was also proved by several previous studies (Liu et al., 2014; Li et al., 2016; Zhang YP et al., 2017; Sang et al., 2017).

Spd promoted the starch degradation during early germination and improved the energy supply of HTM rice seeds

Starch is the main storage polysaccharide in rice seeds (Gorim and Asch, 2014; Kaneko et al., 2004). Starch level is tightly linked to rice seed vigor and germination (Kim et al., 2006). The present results showed that HTM resulted in significant lower starch content and poor seed germination; while Spd application improved the seed germination of HTM seeds. However, whether starch degradation was crucial to the improvement of Spd on seed germination was unclear. Rather than β-amylose, α-amylase and α-glucosidase were proved to be closely related with seed germination in rice and maize (Zhao and Wang, 2001). α-amylase and α-glucosidase are mostly detected in cells of aleurone layer and scutellar epithelium in germinated cereal seeds (Gorim and Asch, 2014; Kaneko et al., 2004). α-amylase is secreted via aleurone layer and released in endosperm for catalyzing stored starch hydrolysis into maltose and maltotriose, which were then converted to glucose by α-glucosidase (Catusse et al., 2011). It is well known that the ATP supplied by glycolytic process is crucial to support seed germination and seedling establishment (Rajjou et al., 2012). Our results revealed that HTM resulted in inhibitory effects on the induction of α-amylase and α-glucosidase activities, soluble sugar, glucose and ATP content, consequently, the germination of rice seeds at early imbibition time. While Spd application during seed development could alleviated the inhibitory effects of HTM on starch degradation and subsequent seed germination.

GA and ABA represent the important phytohormones related to seed germination and seedling establishment (An and Lin, 2011; Boccaccini et al., 2016; Wang et al., 2021). To be specific, GA contributes to breaking seed dormancy and inducing seed germination, while the high ABA content caused seed dormancy and suppressing seed germination (Jia et al., 2012; Arc et al., 2013). However, weather the GA and ABA pathways were related to the regulation of Spd on starch hydrolase and germination in HTM seeds remain poorly understood. In this study, HTM was found to decrease GA level and increased ABA level in rice seeds during early germination. However, it was unexpected that Spd made no significant effect on GA and ABA level in germinated HTM rice seeds. Typically, GA and ABA metabolism is possibly modulated via signals other than Spd in rice seed resistance to heat stress. GA facilitated seed dormancy breaking and seed germination induction by activating the α-amylose and α-glucosidase activities from aleurone layer, thereby promoting the decomposition of stored starch (Zhang et al., 2011; Liu et al., 2016). It was proposed that the GA metabolism might not be involved in the improvement of Spd on starch hydrolysis during HTM seed germination.

Conclusion

In summary, the present study revealed that the excess ROS levels, block of starch biosynthesis during seed filling, as well as the inhibited amylohydrolysis pathway and metabolic imbalance of GA/ABA, might be the potential causes of deterioration vigor of HTM rice seeds. Spd treatment markedly mitigated heat injury, improved HTM rice seed germination and seedling establishment, and this was possibly closely related to antioxidant defense and starch metabolism (Figure 11). This work sheds more lights on the

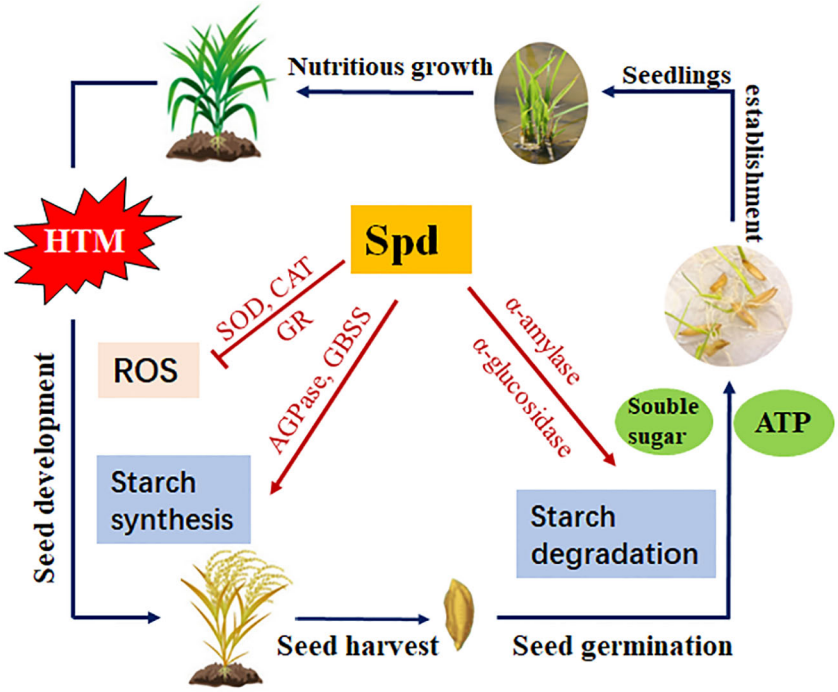

FIGURE 11
Proposed scheme for the role of Spd in heat tolerance of rice seeds during mid-filling stage and subsequent seed germination. HTM, heat stress treatment during mid-filling stage (12-20 days after pollination); Spd, spermidine; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; GR, glutathione reductase; AGPase, ADP-glucose pyrophosphorylase; GBSS, granule-bound starch synthase.

theoretical and practical foundation for the application of Spd in enhancing the production of crop seeds.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization, YH and YQ; Investigation, YH and GM; resources, YH and YQ; writing-original draft preparation, YH and LY; writing-review and editing, YQ; supervision, DC, LY, and XR; All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author LY and XR were employed by the Zhejiang Nongke Seed Co.Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1230331/full#supplementary-material

References

Alcázar, R., Fortes, A. M., and Tiburcio, A. F. (2020). Editorial: Polyamines in plant biotechnology, food nutrition, and human health. *Front. Plant Sci.* 11, 120. doi: 10.3389/fpls.2020.00120

An, Y. Q., and Lin, L. (2011). Transcriptional regulatory programs underlying barley germination and regulatory functions of gibberellin and abscisic acid. *BMC Plant Biol.* 11, 105. doi: 10.1186/1471-2229-11-105

Arc, E., Sechet, J., Corbineau, F., Rajjou, L., and Marion-Poll, A. (2013). ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. *Front. Plant Sci.* 4, 63. doi: 10.3389/fpls.2013.00063

Awan, S., Footitt, S., and Finch-Savage, W. E. (2018). Interaction of maternal environment and allelic differences in seed vigour genes determines seed performance in Brassica oleracea. *Plant J.* 94, 1098–1108. doi: 10.1111/tpj.13922

Bailly, C., Audigier, C., Ladonne, F., Wagner, M. H., Coste, F., Corbineau, F., et al. (2010). Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. *J. Exp. Bot.* 52, 701–708. doi: 10.1093/jexbot/52.357.701

Barba-Espin, G., Díaz-Vivancos, P., Job, D., Belghazi, M., Job, C., and Hernandez, J. A. (2011). Understanding the role of H_2O_2 during pea seed germination: a combined proteomic and hormone profiling approach. *Plant Cell Environ.* 34, 1907–1919. doi: 10.1111/j.1365-3040.2011.02386.x

Beáta, B., Katalin, J., and Attila, F. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ.* 31, 11–38. doi: 10.1111/j.1365-3040.2007.01727.x

Boccaccini, A., Lorrai, R., Ruta, V., Frey, A., Mercey-Boutet, S., Marion-Poll, A., et al. (2016). The DAG1 transcription factor negatively regulates the seed-to-seedling transition in Arabidopsis acting on ABA and GA levels. *BMC Plant Biol.* 16, 198. doi: 10.1186/s12870-016-0890-5

Brunel-Muguet, S., D'Hooghe, P., Bataillé, M. P., Larré, C., Kim, T., Trouverie, J., et al. (2016). Heat stress during seed filling interferes with sulfur restriction on grain composition and seed germination in oilseed rape (*Brassica napus* L.). *Front. Plant Sci.* 6, 1236. doi: 10.3389/fpls.2015.01236

Cai, Y. X., Wang, W., Zhu, Z. W., Zhang, Z. J., Yang, J. C., and Zhu, Q. S. (2006). The physiochemical characteristics of amylopectin and their relationships to pasting properties of rice flour in different varieties. *Scientia Agricult. Sin.* 39 (6), 1122–1129.

Carolina, E. P., Thomas Duge, D. B., Galle, G., Olivier, P., Nicolas, P., Vincent, C., et al. (2015). Characterization of a spermidine hydroxycinnamoyltransferase in *Malus domestica* highlights the evolutionary conservation of trihydroxycinnamoyl spermidines in pollen coat of core Eudicotyledons. *J. Exp. Bot.* 22, 7271–7285. doi: 10.1093/jxb/erv423

Catusse, J., Meinhard, J., Job, C., Strub, J. M., Fischer, U., Pestsova, E., et al. (2011). Proteomics reveals potential biomarkers of seed vigor in sugarbeet. *Proteomics* 11, 1569–1580. doi: 10.1002/pmic.201000586

Chen, M., Fu, Y. Y., Mou, Q. S., An, J. Y., Zhu, X. B., Ahmed, T., et al. (2021). Spermidine induces expression of stress associated proteins (SAPs) genes and protects rice seed from heat stress-indued damage during grain-filling. *Antioxidants* 10 (10), 1544–1553. doi: 10.3390/antiox10101544

Chen, X. C., Wang, S. M., Zhu, Q. S., Wang, W. L., Yang, Q. J., Zhang, D. W., et al. (2010). Correlation analysis between high temperature resistance and pertinent biochemical indexes among different rice varieties. *J. Agro-Environ. Sci.* 29 (9), 1633–1639.

Cui, K., Wang, H. Y., Li, K., Liao, S. X., Li, L., and Zhang, C. (2014). Physiological and biochemical effects of ultra-dry storage on Barbados nut seeds. *Crop Sci.* 54, 1748–1755.

Diao, Q., Song, Y., and Qi, H. (2015). Exogenous spermidine enhances chilling tolerance of tomato (*Solanum lycopersicum* L.) seedlings via involvement in polyamines metabolism and physiological parameter levels. *Acta Physiol. Plant* 37, 1–15. doi: 10.1007/s11738-015-1980-y

Fu, Y. Y., Gu, Q. Q., Dong, Q., Zhang, Z. H., Lin, C., Hu, W. M., et al. (2019). Spermidine enhances heat tolerance of rice seeds by modulating endogenous starch and polyamine metabolism. *Molecules* 24, 1395. doi: 10.3390/molecules24071395

Gorim, L., and Asch, F. (2014). Seed coating reduces respiration losses and affects sugar metabolism during germination and early seedling growth in cereals. *Funct Plant Biol.* 42 (2), 209–218. doi: 10.1071/FP14142

Huang, L. C., Tan, H. Y., Zhang, C. Q., Li, Q. F., and Liu, Q. Q. (2021). Starch biosynthesis in cereal endosperms: An updated review over the last decade. *Plant Commun.* 2 (5), 100237. doi: 10.1016/j.xplc.2021.100237

Iwai, T., Takahashi, M., Oda, K., Terada, Y., and Yoshida, K. T. (2012). Dynamic changes in the distribution of minerals in relation to phytic acid accumulation during rice seed development. *Plant Physiol.* 160 (4), 2007–2014. doi: 10.1104/pp.112.206573

Jia, L., Wu, Q., Ye, N., Liu, R., Shi, L., Xu, W., et al. (2012). Proanthocyanidins inhibit seed germination by maintaining a high level of abscisic acid in Arabidopsis thaliana. *J. Integr. Plant Biol.* 54, 663–673. doi: 10.1111/j.1744-7909.2012.01142.x

Kasukabe, Y. (2004). Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic Arabidopsis thaliana. *Plant Cell Physiol.* 45 (6), 712–722. doi: 10.1093/pcp/pch083

Kim, S. K., Son, T. K., Park, S. Y., Lee, I. J., Lee, B. H., Kim, H. Y., et al. (2006). Influences of gibberellin and auxin on endogenous plant hormone and starch mobilization during rice seed germination under salt stress. *J. Environ. Biol.* 27, 181–186.

Kaneko, M., Inukai, Y., Ueguchi-Tanaka, M., Itoh, H., Izawa, T., Kobayashi, Y., et al. (2004). Loss-of-Function mutations of the rice GAMYB gene impair α-Amylase expression in aleurone and flower development. *Plant Cell.* 16, 33–44. doi: 10.1105/tpc.017327

Li, Z., Zhang, Y., Zhang, X. Q., Peng, Y., Merewitz, E. B., Ma, X., et al. (2016). The alterations of endogenous polyamines and phytohormones induced by exogenous application of spermidine regulate antioxidant metabolism, metallothionein and relevant genes conferring drought tolerance in white clover. *Environ. Exp. Bot.* 124, 22–38. doi: 10.1016/j.envexpbot.2015.12.004

Lin, C. J., Li, C. Y., Lin, S. K., Yang, F. H., Huang, F. H., Liu, Y. H., et al. (2010). Influence of high temperature during grain filling on the accumulation of storage proteins and grain quality in rice (*Oryza sativa* L.). *J. Agric. Food Chem.* 58 (19), 10545–10552. doi: 10.1021/jf101575j

Liu, X., Hu, P., Huang, M., Tang, Y., Li, Y., Li, L., et al. (2016). The NF-YCRGL2 module integrates GA and ABA signaling to regulate seed germination in Arabidopsis. *Nat. Commun.* 7, 12768. doi: 10.1038/ncomms12768

Liu, H., Jiang, Y. M., Luo, Y. B., and Jiang, W. B. (2006). A simple and rapid determination of ATP, ADP and AMP concentrations in pericarp tissue of litchi fruit by high performance liquid chromatography. *Food Technol. Biotech.* 4, 531–534.

Liu, M. Y., Sun, J., Wang, K. Y., Liu, D., Li, Z. Y., and Zhang, J. (2014). Spermidine enhances waterlogging tolerance via regulation of antioxidant defence, heat shock protein expression and plasma membrane H+-ATPase activity in Zea mays. *J. Agron. Crop Sci.* 200 (3), 199–211. doi: 10.1111/jac.12058

Liu, J. H., Wang, W., Wu, H., Gong, X. Q., and Moriguchi, T. (2015). Polyamines function in stress tolerance: from synthesis to regulation. *Front. Plant Sci.* 6, 827. doi: 10.3389/fpls.2015.00827

Ma, G. J., Zhang, M. D., Xu, J. L., Zhou, W. X., and Cao, L. W. (2020). Transcriptomic analysis of short-term heat stress response in *Pinellia ternata* provided novel insights into the improved thermotolerance by spermidine and melatonin. *Ecotoxicol. Environ. Saf.* 202, 110877. doi: 10.1016/j.ecoenv.2020.110877

Madan, P., Jagadish, S., Craufurd, P. Q., Fitzgerald, M., Lafarge, T., and Wheeler, T. R. (2012). Effect of elevated CO_2 and high temperature on seed-set and grain quality of rice. *J. Exp. Bot.* 63 (10), 3843–3852. doi: 10.1093/jxb/ers077

Martínez-Eixarch, M., and Ellis, R. H. (2015). Temporal sensitivities of rice seed development from spikelet fertility to viable mature seed to extreme-temperature. *Crop Sci.* 55 (1), 354–364. doi: 10.2135/cropsci2014.01.0042

Morita, S., Yonemaru, J., and Takanashi, J. (2005). Grain growth and endosperm cell size under high night emperatures in rice (*Oryza sativa* L.). *Ann. Bot.* 95, 695–701. doi: 10.1093/aob/mci071

Nahar, K., Hasanuzzaman, M., Rahman, A., Alam, M. M., Mahmud, J., Suzuki, T., et al. (2016). Polyamines confer salt tolerance in Mung Bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems. *Front. Plant Sci.* 7, 1104. doi: 10.3389/fpls.2016.01104

Nonogaki, M., Sall, K., Nambara, E., and Nonogaki, H. (2014). Amplification of ABA biosynthesis and signaling through a positive feedback mechanism in seeds. *Plant J.* 78, 527–539. doi: 10.1111/tpj.12472

Oracz, K., and Karpinski, S. (2016). Phytohormones signaling pathways and ROS involvement in seed germination. *Front. Plant Sci.* 7, 864. doi: 10.3389/fpls.2016.00864

Pan, M. H., Li, Z., Peng, D. D., et al. (2014). Effects of seed-soaking with spermidine on seed germination and starch metabolism of white clover (*Trifolium repens* L.) under osmotic stress. *Plant Physiol. J.* 50 (4), 426–432. doi: 10.13592/j.cnki.ppj.2013.0418

Peng, S. B., Huang, J. L., Sheehy, J. E., Laza, R. C., Visperas, R. M., Zhong, X. H., et al. (2004). Rice yields decline with higher night temperature from global warming. *Proc. Natl. Acad. Sci.* 101 (27), 9971–9975. doi: 10.1073/pnas.0403720101

Postma, F. M., and Agren, J. (2015). Maternal environment affects the genetic basis of seed dormancy in Arabidopsis thaliana. *Mol. Ecol.* 24, 785–797. doi: 10.1111/mec.13061

Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C., et al. (2012). Seed germination and vigor. *Ann. Rev. Plant Biol.* 63, 507–533. doi: 10.1146/annurev-arplant-042811-105550

Rashid, M., Hampton, J. G., Shaw, M. L., Rolston, R., Khan, K. M., and Saville, D. J. (2020). Oxidative damage in forage rape (*Brassica napus* L.) seeds following heat stress during seed development. *J. Agron. Crop Sci.* 206 (1), 101–117. doi: 10.1111/jac.12372

Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., and Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. *Antioxidants* 10 (2), 277. doi: 10.3390/antiox10020277

Sagor, G. H. M., Berberich, T., Takahashi, Y., Niitsu, M., and Kusano, T. (2013). The polyamine spermine protects Arabidopsis from heat stress-induced damage by increasing expression of heat shock-related genes. *Transgenic Res.* 22 (3), 595–605. doi: 10.1007/s11248-012-9666-3

Sang, Q. Q., Shan, X., An, Y. H., Shu, S., Sun, J., and Guo, S. R. (2017). Proteomic analysis reveals the positive effect of exogenous spermidine in tomato seedlings'

response to high-temperature stress. *Front. Plant Sci.* 8, 120. doi: 10.3389/fpls.2017.00120

Sheteiwy, M., Shen, H. Q., Xu, J. G., Guan, Y. J., Song, W. J., and Hu, J. (2017). Seed polyamines metabolism induced by seed priming with spermidine and 5-aminolevulinic acid for chilling tolerance improvement in rice (*Oryza sativa* L.) seedlings. *Environ. Exp. Bot.* 137, 58–72. doi: 10.1016/j.envexpbot.2017.02.007

Shu, K., Liu, X. D., Xie, Q., and He, Z. H. (2016). Two faces of one seed: hormonal regulation of dormancy and germination. *Mol. Plant* 9, 34–45. doi: 10.1016/j.molp.2015.08.010

Shu, K., Zhou, W., and Yang, W. (2018). APETALA 2-domain-containing transcription factors: focusing on abscisic acid and gibberellins antagonism. *New Phytol.* 217, 977–983. doi: 10.1111/nph.14880

Smiri, M., Chaoui, A., Rouhier, N., Gelhaye, E., Jacquot, J. P., and Ferjani, E. E. (2010). RETRACTED: Redox regulation of the glutathione reductase/iso-glutaredoxin system in germinating pea seed exposed to cadmium. *Plant Sci.* 179 (5), 423–436. doi: 10.1016/j.plantsci.2010.06.015

Sreenivasulu, N., Butardo, V. M., Misra, J. G., Cuevas, R. P., Anacleto, R., and Kishor, P. B. K. (2015). Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. *J. Exp. Bot.* 66 (7), 1737–1748. doi: 10.1093/jxb/eru544

Su, K. W., Cheng, F. M., Zhang, Q. F., and Liu, K. G. (2009). Differential response of three isoform genes encoding starch branching enzymes (SBE) in rice endosperms to high temperature stress at the filling stage. *Chin. J. Rice Sci.* 23 (1), 19–24.

Tabassum, J., Ahmad, S., Hussain, B., Mawia, A. M., Zeb, A., and Ju, L. (2021). Applications and potential of genome-editing systems in rice improvement: Current and future perspectives. *Agronomy* 11 (7), 1359. doi: 10.3390/agronomy11071359

Tang, T., Chen, Y. Y., Yang, Y., Yang, Y. Z., Meng, G. Y., and Zhou, J. (2017). Research progress of rice resistance to high temperature. *Mol. Plant Breed.* 15 (9), 3694–3700.

Tang, S., Zhang, H., Li, L., Liu, X., Chen, L., Chen, W., et al. (2018). Exogenous spermidine enhances the photosynthetic and antioxidant capacity of rice under heat stress during early grain-filling period. *Funct. Plant Biol.* 45, 911–921. doi: 10.1071/FP17149

Tao, Y. J., Wang, J., Miao, J., Chen, J., Wu, S. J., Zhu, J. Y., et al. (2018). The spermine synthase *OsSPMS1* regulates seed germination, grain size, and yield. *Plant Physiol.* 178 (4), 1522–1536. doi: 10.1104/pp.18.00877

Valipour, M., Ahmadi, M. Z., Raeini-Sarjaz, M., Sefidkouhi, M. A. G., and Shahnazari, A. (2014). Agricultural water management in the world during past half century. *Arch. Agron. Soil Sci.* 61 (5), 1–22.

Wang, Y. L., Bai, Y. X., Ji, H. Y., Dong, J. J., Li, X. X., Liu, J. L., et al. (2022). Insights into rice starch degradation by maltogenic α-amylase: Effect of starch structure on its rheological properties. *Food hydrocolloids* 124, 107289. doi: 10.1016/j.foodhyd.2021.107289

Wang, H., Hou, Y., Wang, S., Tong, X. H., Tang, L., Abolore, A. A., et al. (2021). WRKY72 negatively regulates seed germination through interfering gibberellin pathway in rice. *Rice Sci.* 28 (1), 1–5.

Wang, Z. G., Wei, S. L., Yang, Z. L., et al. (2017). Relationship of sink activity with endogenous hormones and polyamine contents in inferior kernels of maize. *Acta Agro. Sin.* 043 (8), 1196–1204. doi: 10.3724/SP.J.1006.2017.01196

Wang, Z., Xu, Y., Wang, J., and Yang, J. (2012). Polyamine and ethylene interactions in grain filling of superior and inferior spikelets of rice. *Plant Growth Regul.* 66, 215–228. doi: 10.1007/s10725-011-9644-4

Xiong, W., Feng, L. Z., Ju, H., and Yang, D. (2016). Possible impacts of high temperatures on China's rice yield under climate change. *Adv. Earth Sci.* 5, 515–528. doi: 10.11867/j.issn.1001-8166.2016.05.0515

Yamakawa, H., and Hakata, M. (2010). Atlas of rice grain filling-related metabolism under high temperature: joint analysis of metabolome and transcriptome demonstratedn, inhibition of starch accumulation and induction of amino acid accumulation. *Plant Cell Physiol.* 51 (5), 795–809. doi: 10.1093/pcp/pcq034

Yang, W. B., Yong, L. I., Yin, Y. P., Jiang, W. W., Peng, D. L., Cui, Z. Y., et al. (2014). Ethylene and spermidine in wheat grains in relation to starch content and granule size distribution under water deifcit. *J. Agric. Sci.* 13, 2141–2153. doi: 10.1016/S2095-3119(13)60726-1

Yang, J., Zhan, Y. Z., He, H. H., Li, Y. C., He, X. R., Bian, J. M., et al. (2020). Current status and research advances of high-temperature hazards in rice. *Chin. J. Appl. Ecol.* 31 (8), 2817–2830. doi: 10.13287/j.1001-9332.202008.027

Zhang, X. (2014). *Preliminary study on the effects of starch, protein and fat content on the vigor of hybrid rice seeds* (Hangzhou, China: Zhejiang Agriculture and Forestry University).

Zhang, S. (2022). Spatiotemporal change of heat stress and its impacts on rice growth in the middle and lower reaches of the yangtze river. *Agriculture* 12 (8), 1–13. doi: 10.3390/agriculture12081097

Zhang, L., Hou, Y. Y., Yang, B. Y., and Huang, D. P. (2018). Spatial-temporal characteristics and risk analysis of heat stress hazard of single-season rice across Yangtze River reaches. *J. Natural Disasters* 27 (2), 78–85. doi: 10.13577/j.jnd.2018.0213

Zhang, L., Hu, T., Amombo, E., et al. (2017). The alleviation of heat damage to photosystem II and enzymatic antioxidants by exogenous spermidine in Tall Fescue. *Front. Plant Sci.* 8, 1747. doi: 10.3389/fpls.2017.01747

Zhang, Z. L., Ogawa, M., Fleet, C. M., Zentella, R., Hu, J. H., Heo, J., et al. (2011). SCARECROW-LIKE 3 promotes gibberellin signaling by antagonizing master growth repressor DELLA in Arabidopsis. *Proc. Natl. Acad. Sci. United States America* 108, 2160–2165. doi: 10.1073/pnas.1012232108

Zhang, Y. P., Shuang, X. U., Yang, S. J., Lu, S. J., and Chen, Y. Y. (2017). Effect of exogenous spermidine on the growth and antioxidant system of melon seedlings under low temperature stress. *Plant Physiol. J.* 53 (6), 1087–1096. doi: 10.13592/j.cnki.ppj.2017.0049

Zhao, C., Liu, B., Piao, S., et al. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *Proc. Natl. Acad. Sci. United States America* 114, 9326–9331. doi: 10.1073/pnas.1701762114

Zhao, Y. J., and Wang, T. (2001). Analysis of the relationship between α-amylase and germinating rate of rice seeds during the process of seed germination. *Chin. Bull. Bot.* 18, 226–230.

Zhu, L. W., Cao, D. D., Hu, Q. J., Guan, Y. J., Hu, W. M., Nawaz, A., et al. (2016). Physiological changes and *sHSPs* genes relative transcription in relation to the acquisition of seed germination during maturation of hybrid rice seed. *J. Sci. Food Agric.* 96 (5), 1764–1771. doi: 10.1002/jsfa.7283

Zhu, L., Zhang, H., Wu, G., et al. (2021). Effect of structure evolution of starch in rice on the textural formation of cooked rice. *Food Chem.* 342, 128205. doi: 10.1016/j.foodchem.2020.128205

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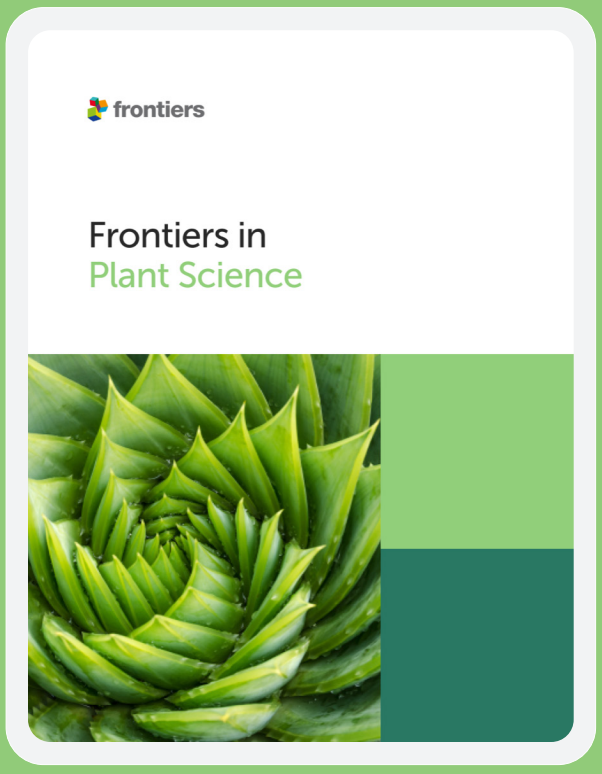

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