REVIEW PAPER

Essential trace metals in plant responses to heat stress

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Abstract

Essential trace metals function as structural components or cofactors in many proteins involved in a wide range of physiological processes in plants. Hence, trace metal deficiency can significantly hamper plant growth and development. On the other hand, excess concentrations of trace metals can also induce phytotoxicity, for example via an enhanced production of reactive oxygen species. Besides their roles in plant growth under favourable environmental conditions, trace metals also contribute to plant responses to biotic and abiotic stresses. Heat is a stress factor that will become more prevalent due to increasing climate change and is known to negatively affect crop yield and quality, posing a severe threat to food security for future generations. Gaining insight into heat stress responses is essential to develop strategies to optimize plant growth and quality under unfavourable temperatures. In this context, trace metals deserve particular attention as they contribute to defence responses and are important determinants of plant nutritional value. Here, we provide an overview of heat-induced effects on plant trace metal homeostasis and the involvement of trace metals and trace metal-dependent enzymes in plant responses to heat stress. Furthermore, avenues for future research on the interactions between heat stress and trace metals are discussed.

Keywords: Copper, ferroptosis, glutaredoxin, heat stress, iron, micronutrient, miRNA, thermotolerance, trace metal, zinc

Introduction

Plant growth and development strongly depend on optimal environmental conditions and can be significantly hampered by abiotic stress factors. The aim of this review is to provide an overview on the impact of increased temperatures on essential trace metal homeostasis and to evaluate the involvement of those trace metals in plant responses to heat stress. We first introduce essential trace metals and heat stress before discussing their interactions in plants.

Essential trace metals

Plants require relatively large quantities of nitrogen (N), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), and magnesium (Mg), which are hence classified as macronutrients (Kumar *et al.*, 2021). In comparison, they only need trace amounts of micronutrients, which apart from chloride (Cl) and boron (B) are all transition or post-transition metals: iron (Fe),

zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), and nickel (Ni) (Clemens, 2019). These essential trace metals form the focus of this review.

Both micronutrient deficiency and toxicity can severely hamper plant growth. The negative consequences of a micronutrient shortage on plant performance are related to the role of these trace elements as structural components or cofactors in a wide variety of proteins. Zn, for example, is essential for the activity of >300 enzymes belonging to all six major Enzyme Commission (EC) classes (Clemens, 2019, 2022). Zn is a structural component of proteins interacting with nucleic acids, such as RNA polymerase and many transcription factors. Furthermore, several enzymes involved in protein, carbohydrate, and lipid metabolism also rely on Zn for their structure or function (Sharma *et al.*, 2013). The importance of Fe and Cu as enzyme cofactors largely depends on their redox properties. They are essential for cellular energy provision through their role in photosynthesis and respiration, and are crucial for the functioning of the respective electron transport chains (Clemens, 2019). In biological systems, Fe can be found in multiple configurations in the form of mono- and di-iron centres, haem, and iron–sulfur (Fe–S) clusters. Besides their role in electron transport reactions, Fe metalloproteins are also involved in other processes including nucleotide biosynthesis and repair, and the biosynthesis of amino acids, proteins, cofactors, and vitamins (Talib and Outten, 2021). Mn is essential for photosynthesis, as it is a core component of the metalloenzyme cluster of the oxygen-evolving complex in PSII (Schmidt *et al.*, 2020). Furthermore, many enzymes depend on Mn as a cofactor. It should be noted, however, that in a large proportion of these enzymes, Mn is interchangeable with other divalent cations including Ca, Mg, cobalt (Co), Cu, and Zn. Besides the oxygen-evolving complex in PSII, oxalate oxidase and Mn superoxide dismutase (Mn-SOD) are the only plant enzymes that exclusively require Mn (Alejandro *et al.*, 2020). In contrast to Zn, Cu, Fe, and Mn, which are required for the structure and/or function of many different proteins, the number of proteins relying on Mo and Ni is much smaller. Currently, urease is the only plant enzyme known to depend on Ni, whereas Mo is found in five types of molybdoenzymes, requiring a pterin-based Mo cofactor: nitrate reductase, sulfite oxidase, aldehyde oxidase, xanthine dehydrogenase, and amidoxime reducing component (Hänsch and Mendel, 2005; Huang *et al.*, 2022). In addition, it should not be neglected that micronutrients are required for the growth and function of plant-associated microorganisms, which in turn influence plant growth (Compant *et al.*, 2019), but this is outside the scope of the current review.

Whereas essential trace metals are indispensable for a plethora of physiological processes, they become detrimental to plant growth and function when present in concentrations that are too high. Excessive Mn concentrations, for example, can interfere with the uptake and translocation of other essential elements and disturb chlorophyll production and photosynthesis (Alejandro *et al.*, 2020). Phytotoxic Zn concentrations influence plant growth via interference with auxin biosynthesis and redistribution, water status, mineral nutrition, photosynthesis, and respiration. In addition, surplus Zn is able to bind unspecifically to thiol groups in proteins, which alters their structure and enhances their degradation, ultimately triggering senescence. Furthermore, Zn has also been reported to activate lipoxygenase enzymes involved in lipid peroxidation reactions that negatively affect membrane stability (Kaur and Garg, 2021). The phytotoxicity of Cu and Fe is largely related to their redox-active properties. Whereas the ability of these metals to donate and accept electrons is crucial for their function in many cellular processes, it is also the reason for their participation in Fenton and Haber–Weiss reactions which enhance the production of highly reactive hydroxyl radicals from hydrogen peroxide (H_2O_2). Although it is well known that reactive oxygen species (ROS) play important roles in signalling processes, they can damage nucleic acids, lipids, and proteins when present in excess (Waszczak *et al.*, 2018; Kollist *et al.*, 2019; Smirnoff and Arnaud, 2019). To prevent toxicity, plants have developed strategies to fine-tune the delivery of redox-active metals to specific metalloproteins while preventing uncontrolled reactivity by keeping cellular free metal ion concentrations low. This is achieved by the chelation of metals to ligands, chaperones, and storage proteins, as well as sequestration to vacuoles for storage (Ravet and Pilon, 2013). Furthermore, plants rely on an extensive antioxidative defence system consisting of enzymatic and non-enzymatic components to tightly control ROS levels. Interestingly, many enzymatic antioxidants depend on trace metals for their function (Ravet and Pilon, 2013). For example, SODs catalyse the dismutation of superoxide (O_2^-) into O_2 and H_2O_2, and are subdivided into Cu/Zn-SODs, Mn-SODs, and Fe-SODs based on their metal cofactor. These different SOD categories are also characterized by different subcellular localizations (Dumanović *et al.*, 2021). Furthermore, catalase and certain peroxidases such as ascorbate peroxidase and guaiacol peroxidase, which mediate the reduction of H_2O_2 to H_2O, typically depend on an Fe-containing haem cofactor (Mhamdi *et al.*, 2010).

Plant responses to heat stress

Due to their sessile nature, the ability of plants to escape from unfavourable environmental conditions is strongly limited in comparison with that of many other organisms. As such, they frequently fall prey to different biotic stresses such as pathogen attack and abiotic stresses such as drought, salinity, soil contamination with organic and inorganic compounds, and unfavourable temperatures (Choudhury *et al.*, 2017; Huang *et al.*, 2019). Since climate change is predicted to entail the occurrence of climatological extremes including high temperatures during summer, heat is a stress factor that will become increasingly prevalent in the future (Bita and Gerats, 2013; Zandalinas *et al.*,

2021). This constitutes a severe threat to food security as the global population continues to grow, while heat stress significantly reduces the yield of major crops such as wheat, maize, rice, and soybean (Wang *et al.*, 2020). It has been estimated, for example, that wheat and rice yield will decrease by 6% and 10%, respectively, for each additional °C of temperature increase during the grain–filling stage (Asseng *et al.*, 2015; Khan *et al.*, 2020; Haider *et al.*, 2021). This poses a major problem, as grains constitute an important source of micronutrients in the human diet, and malnutrition due to micronutrient deficiency (mainly Fe and Zn) affects a substantial part of the global population (Gupta *et al.*, 2021). Hence, increasing our knowledge on plant responses to heat stress is of crucial importance for the development of strategies to enhance plant growth and crop yield under unfavourable temperature conditions. The detrimental impact of heat stress on plant growth and crop productivity is a consequence of several effects at the cellular level including disturbances of membrane fluidity, cytoskeleton organization, protein folding, transport processes, and enzymatic reactions, and the induction of oxidative stress (Hayes *et al.*, 2021; Ashraf, 2021; Haider *et al.*, 2021). High temperatures also damage thylakoid membranes, which subsequently disturbs photosynthetic reactions and hence cellular energy provision, essential for optimal plant growth and function (Hu *et al.*, 2020). Pollen development in particular is highly sensitive to heat, rendering plants particularly vulnerable to high temperatures during the reproductive stage (Chaturvedi *et al.*, 2021). Furthermore, seed germination is also negatively impacted by heat stress (Haider *et al.*, 2021). In order to increase their tolerance to high temperatures—also termed 'thermotolerance'—plants activate different molecular pathways ultimately aimed at preventing and restoring heat-induced damage.

Currently, our knowledge of how plants 'feel the heat' is far from complete (Vu *et al.*, 2019). Nevertheless, several players involved in the perception of high temperature have been identified. For example, certain light sensors such as phytochromes are known to play a role in thermosensing (Jung *et al.*, 2016; Lamers *et al.*, 2020; Murcia *et al.*, 2021). Phytochromes undergo conformational changes in response to light, with red light causing a shift to the active form (Pfr) and far red light promoting reversion to the inactive form (Pr). The role of these light sensors in temperature sensing is explained by the fact that the rate of spontaneous reversal from Pfr to Pr is higher at warmer temperatures (Hayes *et al.*, 2021). A recent study identified early flowering 3 (ELF3) as another temperature sensor in plants. This protein is a component of the circadian clock evening complex that functions as a transcriptional repressor. The circadian clock is believed to control about one-third of the transcriptome in plants (Covington *et al.*, 2008). In response to warm temperatures, ELF3 forms 'speckles' (i.e. liquid droplets) in the nucleus through its prion-like domain and is thereby inactivated (Jung *et al.*, 2020). In addition, RNA switches could also serve a role in plant thermosensing, as Chung *et al.* (2020) demonstrated that increased temperatures

alter the mRNA hairpin structure of phytochrome-interacting factor 7 [PIF7; a basic helix–loop–helix (bHLH) transcription factor interacting with phytochromes and regulating plant growth], enhancing its translation. These thermosensing mechanisms are mainly involved in thermomorphogenesis, a process during which plants alter their morphology to avoid exposure to potentially harmful temperatures. This includes changes in leaf shape, root growth, and induction of flowering (Casal and Balasubramanian, 2019; Hayes *et al.*, 2021).

Thermomorphogenesis takes place at temperatures that exceed the optimum for growth, but are still within the physiological range. However, plants also need mechanisms to sense and respond to more severe temperature increases (i.e. heat stress). Although it is still largely unclear how plants sense such extreme temperatures, accumulation of unfolded proteins and alterations in membrane fluidity have been proposed to play key roles (Hayes *et al.*, 2021). Signalling pathways operating in heat-stressed plants can be triggered by activation of Ca^{2+} channels at the plasma membrane. In *Arabidopsis thaliana*, heat induces an increase in cAMP levels, which triggers Ca^{2+} influx into the cytosol via cyclic nucleotide-gated channel 6 (CNGC6) (Gao *et al.*, 2012). Although the exact underlying molecular mechanism for heat-induced cAMP accumulation is still unclear, activation of a membrane-associated adenylyl cyclase by increased membrane fluidity has been proposed to play a role (Hayes *et al.*, 2021). Other Ca^{2+} channels besides CNGC6 are probably involved in Ca^{2+} influx as well, but have not been identified so far (Hayes *et al.*, 2021). The elevated cytosolic Ca^{2+} concentrations can subsequently recruit annexin 1 (ANN1) to membranes where it could either form a Ca^{2+} channel itself or activate other Ca^{2+} channels to further enhance Ca^{2+} influx, required to trigger multiple downstream signalling pathways (Wang *et al.*, 2015). As well as Ca^{2+}, ROS also play key roles as signalling molecules in heat stress responses. Heat induces the activation of respiratory burst oxidase homologue D (RBOHD), a plasma membrane-localized NADPH oxidase that catalyses the formation of O_2^- in the apoplast. The O_2^- produced is subsequently converted to H_2O_2, which can enter cells and activate multiple signalling pathways such as those mediated by mitogen-activated protein kinases (MAPKs) (Miller *et al.*, 2009; Haider *et al.*, 2021). Interestingly, ROS and Ca^{2+} signals are closely intertwined, as RBOHD activation depends on Ca^{2+} binding to its EF-hand motifs as well as phosphorylation by calcium-dependent protein kinases (CDPKs) (Ogasawara *et al.*, 2008; Steinhorst and Kudla, 2013). Lipid signals, which also play crucial roles in plant responses to heat stress, depend on ROS and Ca^{2+} as well. In heat-stressed guard cells, H_2O_2 oxidizes cysteine residues in the C2 domain of phospholipase Dδ (PLDδ), which subsequently promotes Ca^{2+} binding to this enzyme, resulting in microtubule depolymerization, which in turn influences stomatal movement (Zhang *et al.*, 2017; Song *et al.*, 2020). Furthermore, PLDδ catalyses the production of phosphatidic acid (PA), which functions as a signalling molecule through its

interaction with different cytosolic target proteins. Other lipid signals such as phosphatidylinositol-4,5-bisphosphate (PIP$_2$) and D-myoinositol-1,4,5-trisphosphate (IP$_3$) are also involved in heat stress responses (Hayes *et al.*, 2021). Downstream pathways triggered by heat-induced Ca^{2+}, ROS, and lipid signals ultimately induce transcriptional responses, many of which are the result of activation of heat shock factors (HSFs) (Hayes *et al.*, 2021). These form a class of conserved transcription factors regulating the expression of a broad array of stress-inducible genes including those encoding heat shock proteins (HSPs). The latter function as molecular chaperones that promote correct protein folding and prevent aggregation of misfolded proteins. Among plant HSFs, those of the A1 type are considered master regulators of the heat stress response, as they control the expression of many other HSFs as well as DREB2A, another key transcription factor regulating plant responses to heat stress (Andrási *et al.*, 2020).

Heat-induced accumulation of unfolded and misfolded proteins in the endoplasmic reticulum (ER) lumen due to overloading of the protein quality control system (i.e. ER stress) triggers the activation of the so-called 'unfolded protein response' (UPR) (Deng *et al.*, 2016). The plant UPR consists of two arms that depend on the basic leucine zipper 28 (bZIP28) transcription factor and inositol-requiring enzyme 1 (IRE1), respectively. Upon activation, IRE1 mediates alternative splicing of the mRNA encoding the bZIP60 transcription factor, allowing translocation of bZIP60 from the ER membrane to the nucleus. Both bZIP28 and bZIP60 induce the transcription of a plethora of genes to enhance protein folding capacity and suppress translation with the ultimate aim of restoring protein homeostasis (Liu and Howell, 2016; Depaepe *et al.*, 2021).

Besides transcriptional responses, heat stress is also known to induce epigenetic alterations. It has been shown, for example, that histone H3 lysine 4 trimethylation (H3K4me3) of specific *HSP* genes in Arabidopsis induces thermomemory and enables a strong induction of these genes in response to repeated heat stress (Lämke *et al.*, 2016). In addition, nucleosome remodelling and miRNAs are also involved in heat stress memory (Haider *et al.*, 2021). The ability of plants to memorize previous heat stress episodes contributes to the maintenance of 'acquired thermotolerance' over time. This allows plants to survive otherwise lethal temperatures after a period of acclimation to a sublethal temperature (Sharma *et al.*, 2019). Although most of this memory disappears several days after the heat stress ends, some aspects of epigenetic heat stress memory can be passed on to the next generation (Haider *et al.*, 2021). An overview of the most important signalling mechanisms in plants subjected to increased temperatures is provided in Fig. 1. For a detailed overview of recent insights into plant heat stress responses, readers are referred to Haider *et al.* (2021) and Hayes *et al.* (2021).

Although many of the molecular mechanisms underlying plant heat stress responses have been characterized, knowledge of the involvement of essential trace metals is currently scarce.

To gain more insight into this topic, the next sections of this review summarize how heat stress influences plant uptake and translocation of trace metals and how trace metals take part in plant responses to heat stress.

Heat stress influences plant metal micronutrient levels

Research has demonstrated that increased temperatures affect Cu, Fe, Mn, and Zn concentrations in a wide variety of plant species (Table 1). The studies performed in this context have used a broad range of experimental approaches, ranging from tightly controlled set-ups with plant exposure to specific, predetermined temperature conditions (Giri *et al.*, 2017), to observational studies comparing nutrient levels between plants grown in a greenhouse during different seasons (Darawsheh *et al.*, 2006). Whereas in some studies, analyses were performed on plants grown in climate chambers (Giri *et al.*, 2017) or greenhouses (Darawsheh *et al.*, 2006; Dias and Lidon, 2009), others employed field-grown plants (Impa *et al.*, 2019). It should be noted that although studies in greenhouses and field conditions can yield interesting insights into plant nutrient homeostasis, they do not allow completely separating temperature-induced effects from those of other variables such as light and humidity.

Whether plant levels of trace metals increase or decrease in response to heat stress depends on many factors including the plant species, genotype, organ, and developmental stage, as well as the heat stress severity and duration. However, knowledge of the mechanisms underlying heat-induced alterations of plant trace metal concentrations is currently scarce and deserves more attention in the framework of future climate change scenarios. Heat-induced effects on trace metal uptake can either rely on intrinsic plant characteristics or can be indirectly caused by changes in external conditions.

León-Sánchez *et al.* (2020) proposed that the desiccating effect of heat could induce drying of the fertile top soil, thereby lowering nutrient availability. In addition, increased temperatures might cause roots to grow into deeper soil layers, which contain more water but are less fertile. Finally, the authors suggest that heat-induced disturbances of photosynthesis can result in carbon limitation of nutrient uptake due to reductions in fine root growth and a decreased abundance and activity of mycorrhizal fungi (León-Sánchez *et al.*, 2020). It should be taken into account, however, that decreases in plant nutrient concentrations could also be a consequence of so-called 'growth dilution' when plant growth is favoured in response to small temperature increases (Menzel *et al.*, 1987; Darawsheh *et al.*, 2006; León-Sánchez *et al.*, 2020). On the other hand, a positive impact of increased temperatures on root development could also enhance the ability of plants to take up nutrients from the soil (Viciedo *et al.*, 2021). Besides influencing the uptake of nutrients in roots, high temperatures can alter their translocation to various plant organs. Darawsheh *et al.*
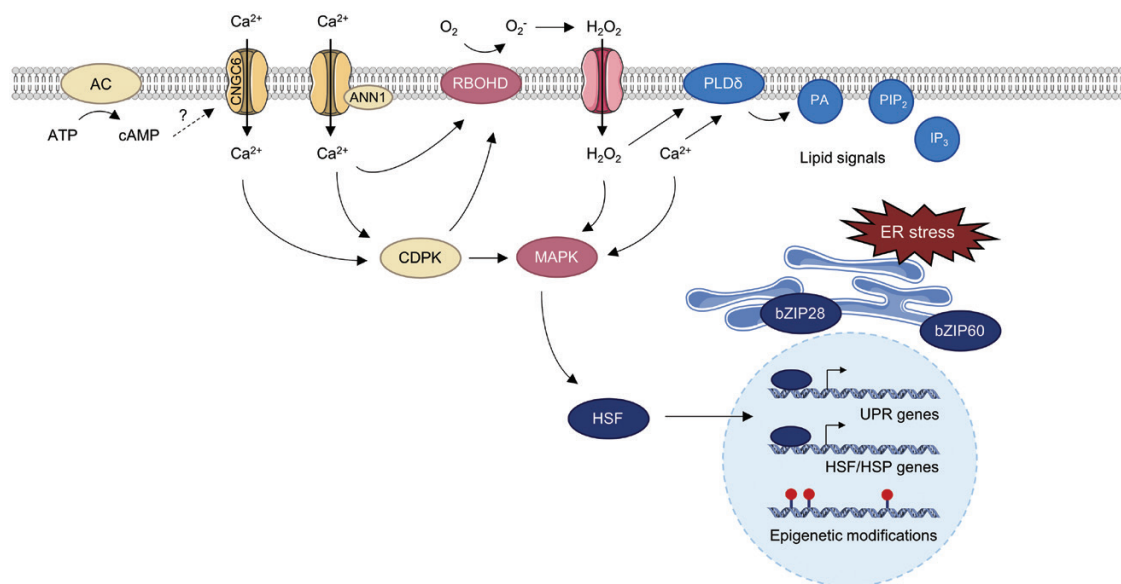
Fig. 1. Simplified overview of heat-induced signalling responses in plants. Calcium (Ca^{2+}), reactive oxygen species (ROS), and lipid signals are key players in plant responses to heat stress. Upon heat stress perception (via largely unidentified mechanisms), cytosolic Ca^{2+} influx is mediated by cyclic nucleotide-gated channel 6 (CNGC6) and other Ca^{2+} transporters. Activation of CNGC6 is suggested to be triggered by cAMP, generated by adenylyl cyclise (AC). The latter might be activated by heat-induced changes in membrane fluidity. Heat induces recruitment of annexin 1 (ANN1) to the plasma membrane, where it can either function as a Ca^{2+} channel itself or enhance the activity of other Ca^{2+} channels. Activation of respiratory burst oxidase homologue D (RBOHD) causes apoplastic generation of superoxide (O_2^-), which is converted to hydrogen peroxide (H_2O_2) that can subsequently enter the cell and trigger downstream signalling pathways. RBOHD is activated by Ca^{2+} binding to its EF-hand motifs and phosphorylation by calcium-dependent protein kinases (CDPKs). In addition, H_2O_2 oxidizes cysteines in the C2 domain of phospholipase Dδ (PLDδ), promoting Ca^{2+} binding. This enzyme generates phosphatidic acid (PA) which, together with PIP_2 and IP_3, constitutes an important lipid signal involved in plant heat stress signalling. Different signals trigger a mitogen-activated protein kinase (MAPK) cascade, which ultimately causes activation of heat shock factors (HSFs). These transcription factors regulate the expression of other HSFs as well as heat shock proteins (HSPs) that function as protein chaperones. Accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum (ER), also known as 'ER stress', triggers the unfolded protein response (UPR) mediated by bZIP28 and bZIP60 transcription factors. In addition to transcriptional changes, epigenetic modifications are also implied in plant responses to heat stress. Organelles except the nucleus and the ER are not shown. The figure was created using templates from Servier Medical Art, licensed under a Creative Commons Attribution 3.0 Unported License (http://smart.servier.com).

Table 1. Overview of research articles reporting effects of increased temperatures on metal micronutrients in plants

Plant species	Organ	Effect on metal micronutrients	Reference
Asparagus officinalis	Shoot	Fe ↓	Yeasmin *et al.* (2019)
Citrus sinensis with *Citrus volkameriana* root stock	Leaf	Cu, Fe. and Zn ↑	Abd El-Naby *et al.* (2020)
Coffea arabica and *Coffea canephora*	Leaf	Cu, Fe, Mn. and Zn ↑	Martins *et al.* (2014)
Different shrub species	Leaf	Overall Cu, Fe and Zn ↓	Léon-Sánchez *et al.* (2020)
Lens culinaris Medikus	Grains	Fe and Zn ↓	Choukri *et al.* (2020)
Megathyrsus maximus	Leaf	Cu, Fe, Mn, and Zn ↑	Viciedo *et al.* (2021)
Passiflora edulis f. *edulis*×*P. edulis* f. *flavicarpa*	Shoot	Cu, Fe, Mn, and Zn ↓	Menzel *et al.* (1987)
Solanum lycopersicum	Root and leaf	Total and free Fe ↓	Rivero *et al.* (2003)
Solanum lycopersicum	Stem, root, and leaf	Zn translocation ↓	Darawsheh *et al.* (2006)
Solanum lycopersicum	Root and leaf	Cu, Fe, and Mn ↑	Darawsheh *et al.* (2006)
Solanum lycopersicum	Fruit	Cu, Fe, Mn, and Zn ↓	Maboko *et al.* (2013)
Solanum lycopersicum	Root	Fe uptake rate ↓	Giri *et al.* (2017)
Sorghum bicolor	Grains	Cu, Fe, Mn, and Zn ↓	Impa *et al.* (2019)
Triticum aestivum	Tillers	Cu, Fe, Mn, and Zn ↓	Cabral *et al.* (2016)
Triticum aestivum and *Triticum turgidum*	Root, shoot, and spike	Cu and Zn ↑	Dias and Lidon (2009)
Triticum aestivum and *Triticum turgidum*	Root, shoot, and spike	Fe and Mn translocation ↑	Dias *et al.* (2009)

↑ and ↓ symbols indicate increases and decreases, respectively. For detailed information on the specific temperature conditions and experimental set-up, readers are referred to the respective publications.

(2006), for example, reported that during winter the extractable Zn concentration in the top part of the stem of tomato plants was significantly higher as compared with that in the bottom part of the stem. The opposite was observed during summer, indicating a strong seasonal impact on Zn translocation (Darawsheh *et al.*, 2006). However, heat might also promote mineral uptake and translocation when plants increase their transpiration rate to promote leaf cooling (Martins *et al.*, 2014).

Interestingly, several studies have addressed the impact of increased temperatures in the root zone only. Du and Tachibana (1994) investigated the effects of supraoptimal root zone temperatures on cucumber plants via the use of a heated nutrient solution. Their results showed that Fe and Mn concentrations in the leaves decreased with increasing root temperatures. Similar results were observed for most of the other micro- and macronutrients studied, suggesting a general effect of root temperature on nutrient uptake capacity (Du and Tachibana, 1994). Tan *et al.* (2002) showed that growth-related increases in root and shoot concentrations of Cu, Fe, Mn, and Zn were more pronounced in *Lactuca sativa* plants grown in a nutrient solution with a temperature of 20 °C as compared with those grown in nutrient solutions with a higher temperature. These responses are probably related to temperature-dependent alterations in root morphology, as a supraoptimal temperature of the nutrient solution inhibited root length and surface area, while increasing the root diameter. The authors proposed that this could in turn affect root nutrient uptake dynamics. Nevertheless, the involvement of root temperature-mediated alterations in enzyme activities and/or phytohormone signalling in determining mineral levels in roots and shoots cannot be excluded (Tan *et al.*, 2002).

Changes in micronutrient levels upon exposure to increased temperatures could also be due to effects on proteins involved in their uptake, assimilation, and metabolism. For example, Giri *et al.* (2017) demonstrated that exposure of tomato plants to a temperature of 42 °C for 6 d significantly decreased the rate of Fe uptake in roots. This effect coincided with lower root levels of the iron reductase LeFRO1, which is one of the two main proteins responsible for Fe reduction (Giri *et al.*, 2017). In Strategy I (i.e. non-graminaceous) plants, reduction of Fe at the root surface is required for its uptake (Kobayashi and Nishizawa, 2012). As knowledge of heat-induced effects on specific proteins involved in trace metal homeostasis is currently very scarce, it is important to further explore this topic in future studies. In this context, it would be particularly interesting to investigate whether alternative splicing of mRNAs encoding regulators of trace metal homeostasis occurs under heat stress conditions. As recently reviewed by John *et al.* (2021), mild and severe temperature variations cause alternative splicing of primary transcripts of many genes, resulting in their degradation or translation to alternative protein products with different functions or activities. Interestingly, alternative splicing has also been reported to play a role in rice responses to Cu, Mn, and

Zn deficiency (Dong *et al.*, 2018), and Fe deficiency led to alternative splicing of mRNAs encoding proteins involved in Fe acquisition and homeostasis in *A. thaliana* roots (Li *et al.*, 2013).

Even though the underlying mechanisms have not been fully elucidated, it is clear that heat stress influences plant uptake and translocation of trace metals. The fact that plant levels of other micronutrients and macronutrients are often affected by increased temperatures in a similar manner suggests that heat causes a general impact on the plant nutrient uptake capacity (Menzel *et al.*, 1987; Maboko *et al.*, 2013; Cabral *et al.*, 2016; Giri *et al.*, 2017; León-Sánchez *et al.*, 2020; Viciedo *et al.*, 2021). Heat-induced alterations of root morphology due to an increased soil temperature probably play an important role in this process. However, more targeted heat-induced effects on specific nutrient uptake and transport systems at the transcriptional, translational, or post-translational level cannot be excluded. This topic deserves further attention in future research, as altered mineral contents can affect the development of plants as well as their nutritional quality (Martins *et al.*, 2014). As such, the more frequent occurrence of heat waves due to climate change could have a negative impact on both crop yield and quality. Therefore, increasing our knowledge on how heat stress affects plant uptake and translocation of specific nutrients can aid in the development of strategies to safeguard food security for future generations.

Metal micronutrients influence plant responses to heat stress

Metal micronutrients are highly likely to play important roles in plant responses to heat stress due to their function as cofactors for a wide variety of proteins involved in physiological processes and defence responses against stressful conditions. These might include SOD enzymes involved in antioxidative defence, as increased ROS production is a well-known consequence of plant exposure to heat stress. Shiraya *et al.* (2015) reported that MSD1, an Mn-dependent SOD of rice plants, plays a key role in their thermotolerance. Rice MSD1 has been proposed to be targeted to the plastid stroma from the Golgi apparatus via the secretory pathway, effectively leading to a dual Golgi/plastid localization. While knockdown mutants of *MSD1* were more sensitive to heat, rice plants constitutively expressing this gene had a higher grain quality than wild-type plants when grown under heat stress. The authors infer that constitutive high expression of Golgi/plastid-type *MSD1* improves the detoxification of O_2^- together with increased formation of H_2O_2, which in turn may induce changes in expression of several other antioxidant genes. In addition, MSD1 is proposed to control the redox state in the endomembrane system, leading to the normal programmed formation of protein bodies (Shiraya *et al.*, 2015). Metal micronutrients might also contribute to plant heat stress responses via their involvement in phytohormone signalling. For example, Cu serves as a cofactor for ethylene

Table 2. Overview of research articles reporting effects of altered metal micronutrient availability on plant sensitivity to heat stress

Plant species	Micronutrient conditions	Effect	Reference
Cicer arietinum	Zn deficiency	Heat sensitivity ↑	Ullah *et al.* (2019)
Triticum aestivum	Zn deficiency	Heat sensitivity ↑	Peck and McDonald (2010)
Brassica chinensis	Foliar Zn spraying	Heat sensitivity ↓	W. Han *et al.* (2020)
Gossypium hirsutum	Foliar Zn spraying	Heat sensitivity ↓	Sarwar *et al.* (2019)
Triticum aestivum	Zn fertilizer application	Heat sensitivity ↓	Tao *et al.* (2018)
Zea mays	Cu and Zn treatment	Heat sensitivity ↓	Bonham-Smith *et al.* (1987)

↑ and ↓ symbols indicate increases and decreases, respectively. For detailed information on the specific temperature conditions and experimental set-up, readers are referred to the respective publications.

receptors (ETRs), which initiate the ethylene signalling cascade in response to stress conditions (Hoppen *et al.*, 2019). As shown by Huang *et al.* (2021), ethylene signalling positively affects basal thermotolerance in *A. thaliana*. Other important trace metal-dependent regulators of heat stress responses are zinc finger proteins, which constitute one of the largest transcription factor families in plants (G. Han *et al.*, 2020). A 'zinc finger' typically consists of two cysteines and/or histidines and one Zn ion, which is required for its structure and function. Among 112 predicted C2H2-type zinc finger proteins in tomato, many showed transcriptional up- or down-regulation upon heat exposure, suggesting their involvement in heat stress responses. Nevertheless, their role in plant stress responses is probably not limited to heat stress, as expression of the same genes was also affected by other abiotic stresses such as cold, salinity, and drought (Ming *et al.*, 2020). The involvement of zinc finger proteins in heat stress tolerance was also demonstrated in several other species. For example, constitutive overexpression of the gene encoding the zinc finger protein ZAT10 was shown to enhance the thermotolerance of *A. thaliana* (Mittler *et al.*, 2006). Similarly, heterologous expression of the wheat zinc finger protein gene *TaZnF* increased the tolerance of *A. thaliana* to heat (Agarwal and Khurana, 2018). On the other hand, *A. thaliana* knockout mutants of the zinc finger protein stress-associated protein 5 (SAP5) and its downstream transcriptional target multiprotein binding factor 1c (MBF1c) showed an increased sensitivity to heat stress (Kim *et al.*, 2015). Heterologous overexpression of the rice zinc finger protein gene *ZFP177* conferred tolerance to both heat and cold stress in tobacco plants, while increasing their sensitivity to salt and drought stress (Huang *et al.*, 2008). These data indicate that zinc finger proteins play crucial roles in plant responses to a wide variety of stress factors, but that their specific role depends on the stress factor. In *A. thaliana*, overexpression of the zinc finger protein SAP10 conferred tolerance to Ni, Mn, Zn, and heat stress, suggesting its involvement in signal transduction upon high temperature stress as well as exposure to excess trace metals (Dixit and Dhanker, 2011).

The role of trace metals in plant responses to increased temperatures is further supported by the fact that their availability significantly affects plant thermotolerance (Table 2). So far, studies investigating the effects of metal micronutrients on plant responses to heat stress have mainly focused on Zn. Ullah *et al.* (2019) showed that the sensitivity of *Cicer arietinum* to heat stress was reduced when plants were grown under Zn sufficiency rather than Zn deficiency. Sufficient Zn supply during heat stress improved plant growth and photosynthesis by positive effects on PSII efficiency, water relations, free proline levels, and antioxidative enzyme activities. In the same study, similar effects of Zn supply were observed in plants exposed to drought stress, indicating their pleiotropic character (Ullah *et al.*, 2019). Similarly, the positive effect of foliar Zn spraying on the thermotolerance of cotton plants grown on soil containing 1.6 ppm available Zn was associated with increased antioxidative enzyme activities, ascorbic acid, and total phenolic compound levels, chlorophyll contents, net photosynthetic rate, stomatal conductance, and water potential. It should be noted, however, that exogenous application of K and B had similar effects (Sarwar *et al.*, 2019). Foliar Zn spraying also positively affected the thermotolerance of *Brassica chinensis* grown in a Zn-deficient loamy clay soil by positive effects on SOD activity, chlorophyll content, and photosynthetic parameters. As the plants did not show any symptoms of nutrient deficiency before the heat stress treatment, the authors proposed that Zn demand increases during exposure to high temperatures (W. Han *et al.*, 2020). Furthermore, Zn fertilization mitigated the negative impact of high temperature stress on the grain yield and flour quality of wheat grown in loam soil with a diethylenetriamine pentaacetate-extractable Zn concentration of 0.85 mg kg^{-1} (Tao *et al.*, 2018).

Bonham-Smith *et al.* (1987) demonstrated that prior treatment with Zn as well as Cu reduced the sensitivity of maize seedlings to subsequent heat exposure. Interestingly, a similar effect was observed when plants were pre-treated with cadmium (Cd), a non-essential element which is already toxic at low concentrations. As such, it can be speculated that a treatment with excess levels of trace metals induces phytotoxicity, for example via increased ROS production, which triggers a defence response that 'primes' the plant and reduces its sensitivity to subsequent stress exposures. This priming response might comprise enhanced antioxidant production and activity, but could also involve other compounds, as considerable overlap exists between defence responses to heat and metal stress. Heat shock

proteins, for example, are known to play roles in plant defence against a wide variety of stress conditions including metal stress (Heckathorn *et al.*, 2004). Interestingly, the observed cross-tolerance between heat and metal stress functions in both directions, as heat shock is also able to mitigate negative effects of subsequent exposure to toxic metal concentrations. Indeed, heat was shown to attenuate Cu–induced cell death in rice (Chen *et al.*, 2008) and to protect against Cu phytotoxicity in specific Arabidopsis ecotypes. The latter response was related to heat-induced production of metallothioneins, which are small, cysteine-rich, metal-binding proteins involved in regulating metal homeostasis (Murphy and Taiz, 1995). Because of their metal-chelating function, metallothioneins prevent the catalysis of Fenton reactions and are also implicated in ROS scavenging. This role is supported by the fact that a type 1 metallothionein from the Cu-accumulating plant species *Elsholtzia haichowensis* was transcriptionally up-regulated in response to Cu stress as well as H_2O_2 and heat treatments (Xia *et al.*, 2012). Hence, the involvement of metallothioneins in heat stress tolerance might also be related to their ROS-scavenging properties.

Besides affecting plant responses to trace metal excess, heat stress was also shown to influence the effects of Fe deficiency in *A. thaliana*. Using an automated imaging and computation approach, Buckner *et al.* (2019) demonstrated that both Fe deficiency (induced by treatment with the Fe chelator ferrozine) and heat stress negatively influenced *A. thaliana* root growth. Surprisingly, plants subjected to a combination of Fe deficiency and heat stress showed a smaller root growth inhibition in comparison with plants exposed to the single stresses. This was related to an altered timing and persistence of expression of the cyclin gene *CYCB1;1*, a marker for cell entry into mitosis. Although the molecular mechanism underlying the antagonistic effects of heat stress and Fe deficiency on root growth was not determined, this work clearly indicates that plant responses to combined stresses can be non-intuitive (Buckner *et al.*, 2019). Hence, it is of crucial importance to consider the effects of stress combinations in future studies, as plants grown under field conditions are frequently exposed to combinations of stress factors. In this context, studying the combined effect of heat stress and trace metal deficiency and/or excess is particularly important as increased temperatures will become more prevalent due to climate change and nutrient availability in soils is often suboptimal.

Taken together, the available data indicate a clear relationship between trace metal levels and heat stress responses in plants. As metal micronutrients play crucial roles in many physiological processes and defence mechanisms via their function as cofactors in a plethora of proteins, their effects on thermotolerance are likely to be pleiotropic. However, in-depth knowledge of the involvement of trace metals in plant responses to heat stress is currently very scarce and a large amount of work remains to be done in this field. Three molecular studies illustrating the relationship between trace metal–related processes and responses to increased temperature are discussed in more detail

in the subsequent sections of this review and are summarized in Fig. 2.

Heat-induced ferroptosis-like cell death in plants

An example of a trace metal-mediated, heat-induced process in plants is ferroptosis-like cell death. The term ferroptosis was first introduced by Dixon *et al.* (2012) to describe a regulated cell death type occurring in mammalian tumour cells exposed to erastin, an inhibitor of the cystine/glutamate antiporter system X_c^-. Ferroptotic cell death is characterized by its dependence on ROS, intracellular Fe, and peroxidation of membrane lipids, causing the accumulation of lipid hydroperoxides. In mammalian cells, the decreased cystine uptake upon erastin exposure ultimately leads to a depletion of cellular levels of the antioxidative metabolite glutathione (GSH). This consequently diminishes the activity of glutathione peroxidase 4 (Gpx4), which reduces lipid hydroperoxides using GSH as an electron donor. As a consequence, lipid peroxidation products accumulate, ultimately triggering cell death (Feng and Stockwell, 2018). The dependence of ferroptotic cell death on Fe is related to its ability to promote lipid peroxidation via two mechanisms. First, the Fenton reaction of Fe^{2+} with H_2O_2 leads to the formation of hydroxyl radicals, which are highly reactive towards polyunsaturated fatty acids in lipid membranes. In addition, Fe can also enhance lipid peroxidation via its role as a cofactor for lipoxygenases (Thi Tuyet Le *et al.*, 2019; Distéfano *et al.*, 2021). These proteins catalyse enzymatic lipid peroxidation and functionally depend on a non-haem Fe in their catalytic domain (Porta and Rocha–Sosa, 2002). As a consequence of its dependence on Fe and lipid peroxidation, ferroptosis in mammalian cells is typically inhibited by intracellular Fe chelators such as ciclopiroxolamine (CPX) and deferoxamine, as well as lipophilic antioxidants such as ferrostatin-1 (Fer-1) and liproxstatin (Feng and Stockwell, 2018).

In a recent study, Distéfano *et al.* (2017) demonstrated that the cell death induced in *A. thaliana* root hairs upon exposure to a temperature of 55 °C for 10 min was characterized by many of the same hallmarks as ferroptosis in mammalian systems, including increases in cellular ROS levels, GSH depletion, and the occurrence of lipid peroxidation. Interestingly, this heat-induced cell death was also inhibited by Fer-1 and CPX, and was hence termed 'ferroptosis-like' cell death. Pre-treatment with these ferroptosis inhibitors also enhanced the survival rate of *A. thaliana* seedlings exposed to 43 °C for 1 h, suggesting that ferroptotic cell death takes place under more environmentally realistic heat stress scenarios as well. In contrast, Fer-1 and CPX pre-treatment did not inhibit cell death induced by a more severe heat stress of 77 °C or other stress factors such as H_2O_2 and salt treatment. Nevertheless, stress-induced ferroptosis-like cell death in plants is not limited to heat stress, but was also reported to occur during avirulent *Magnaporthe oryzae* infections in rice plants (Dangol *et al.*, 2019).
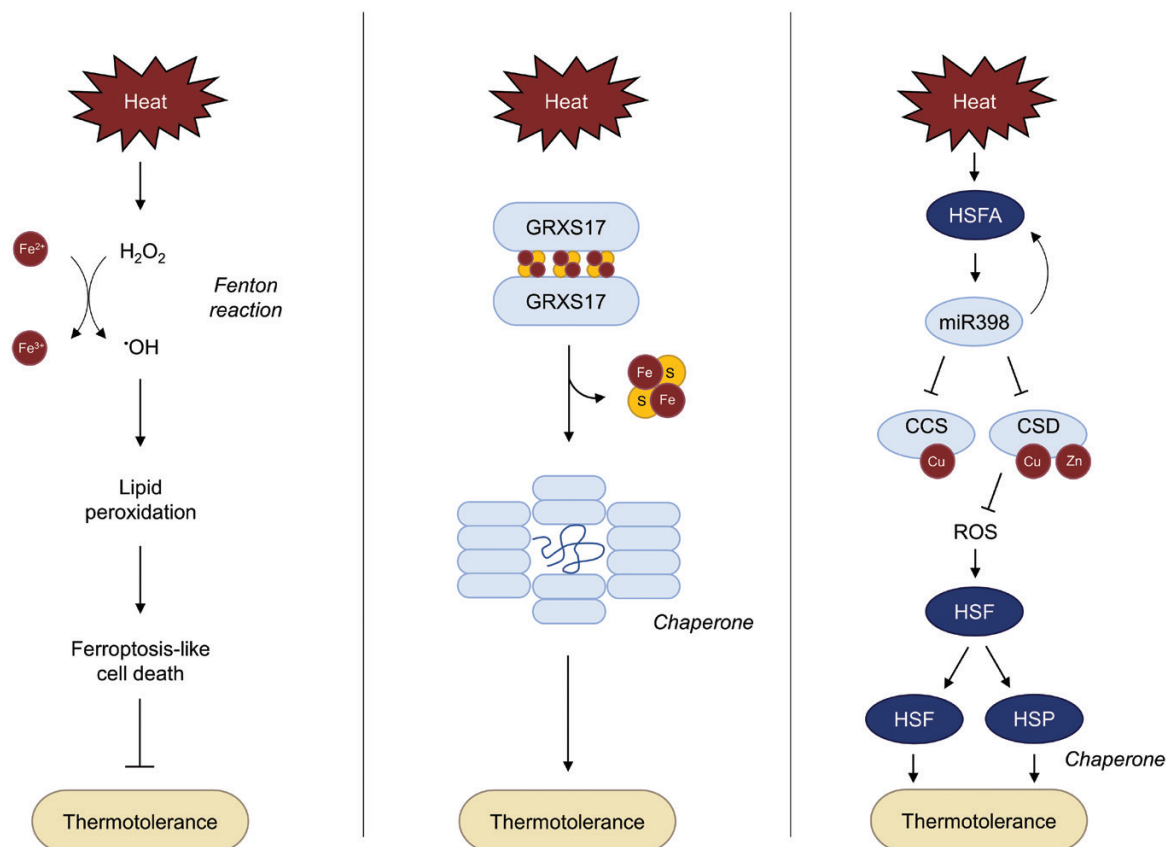
Fig. 2. Overview of heat-induced effects on trace metal-related processes and their impact on thermotolerance at the molecular level. Left: in the presence of iron (Fe), hydrogen peroxide (H_2O_2) generated in response to heat stress can be converted to hydroxyl radicals ($\cdot OH$) via the Fenton reaction. These radicals are highly reactive towards polyunsaturated fatty acids in lipid membranes and hence induce lipid peroxidation, ultimately causing ferroptosis-like cell death, which reduces the survival rate of heat-stressed plants. Middle: in response to increased temperatures, glutaredoxin S17 (GRXS17) loses its iron–sulfur (Fe–S) clusters and oligomerizes via the formation of intermolecular disulfide bridges and non-covalent interactions, activating its holdase function. The association of GRXS17 with a different set of proteins in response to heat stress suggests its involvement in their protection against heat-induced damage, thereby governing thermotolerance. Right: heat stress induces the expression of miRNA398 (*MIR398*) via direct binding of heat shock factor A1b (HSFA1b) and HSFA7b transcription factors to its promoter. This subsequently decreases transcript levels of the copper (Cu) chaperone *CCS* and copper–zinc (Zn) superoxide dismutase (*CSD*). Via a positive feedback loop, miR398 promotes the expression of *HSFA7b* and other HSF genes. Changes in the cellular redox state as a consequence of reduced CSD activity might be sensed by HSFs, which then regulate the expression of other HSFs and HSPs, ultimately contributing to thermotolerance.

Interestingly, heat-induced ferroptosis was also observed in photosynthetic cyanobacteria, suggesting the involvement of chloroplasts (Aguilera *et al.*, 2022). This hypothesis is further supported by the fact that the death of *A. thaliana* seedlings upon heat stress was less pronounced when the plants were further grown under dark instead of light conditions after the heat shock (Distéfano *et al.*, 2017). The involvement of chloroplasts in plant ferroptosis–like cell death could be related to several inherent characteristics of these organelles. Chloroplasts are major subcellular sources of ROS, and thylakoid membranes harbour the largest level of lipid unsaturation of any membrane, which renders them particularly vulnerable to lipid peroxidation (Asada, 2006; Routaboul *et al.*, 2012). In addition, chloroplasts represent the largest Fe sink in most plant cells and contain up to 80% of total Fe in leaves. This large Fe pool is essential for proper functioning of photosynthesis, as several

proteins involved in photosynthetic electron transfer reactions depend on Fe as a cofactor. Nevertheless, the presence of large amounts of Fe also entails a risk for oxidative damage (Kroh and Pilon, 2020). Therefore, chloroplasts are equipped with multiple Fe transporters, enabling them to fine-tune their Fe levels in response to developmental and environmental cues. In addition, these organelles contain ferritins, which are multimeric proteins able to store large numbers of Fe atoms in their central cavity (Ravet *et al.*, 2009). The importance of a strict regulation of free Fe levels in heat-exposed plants is illustrated by the observation that several ferritin-encoding genes in *Pyrus pyrifolia* displayed a rapid transcriptional up-regulation upon heat stress (Xi *et al.*, 2011). Moreover, heterologous expression of the *Vigna cylindrica FER* gene was shown to improve the thermotolerance of wheat plants. This effect was probably related to an enhanced membrane stability, as heat–induced

increases in the levels of malondialdehyde (i.e. a marker of lipid peroxidation) were less pronounced in the transgenic plants as compared with non-transformed plants (Zhao *et al.*, 2016). Similarly, expression levels of the wheat ferritin gene *TaFER-5B* were increased in response to heat stress, and overexpression of this gene resulted in an enhanced thermotolerance of wheat plants. Heterologous expression of *TaFER-5B* in *A. thaliana* was able to rescue the heat-sensitive phenotype of the *fer1-3-4* triple mutant, which contains mutations in the three major leaf ferritin genes. However, it should be mentioned that transgenic *A. thaliana* lines harbouring the wheat *TaFER-5B* also displayed an enhanced tolerance to drought and H_2O_2 treatment (Zang *et al.*, 2017). This emphasizes that the role of ferritin in stress tolerance is not limited to heat stress but that it probably contributes to a more general response to prevent the Fe-dependent formation of highly reactive hydroxyl radicals under a wide variety of stress conditions associated with an oxidative imbalance.

Although ferroptosis has been shown to play a key role in plant responses to heat stress, this research field is still in its infancy and the mechanisms regulating plant ferroptosis are still largely unknown. Future characterization of the mechanisms underlying heat-induced ferroptosis might aid in the development of strategies to prevent this form of heat-induced cell death and thereby improve crop production under climate change conditions.

Glutaredoxins contribute to plant thermotolerance

Other Fe-related proteins involved in plant tolerance to heat stress are glutaredoxins (GRXs). GRXs constitute a group of small, ubiquitous thiol oxidoreductases and are part of the thioredoxin superfamily. They are further subdivided into different classes based on the amino acid sequence found in their active site. The Arabidopsis genome encodes 50 GRXs belonging to five different classes. In addition to the role of GRXs in reducing glutathionylated proteins, several class I, and probably all class II GRXs have the ability to incorporate Fe–S clusters, and are hence also involved in regulating Fe homeostasis (Couturier *et al.*, 2015; Wu *et al.*, 2017). Due to small structural differences, Fe–S clusters in class II GRXs are more labile as compared with those in class I GRXs. As such, class II GRXs are generally able to accept and transfer Fe–S clusters to target proteins, whereas class I GRXs are not (Martins *et al.*, 2020).

In Arabidopsis, GRXS17 is involved in temperature-dependent post-embryonic growth and development (Cheng *et al.*, 2011; Martins *et al.*, 2020). This class II GRX is localized in the nucleus and cytosol, and contains three GRX domains with a CGFS motif in their active site that coordinate three Fe–S clusters in a GSH-dependent manner. When exposed to H_2O_2, the reconstituted holo-form of GRXS17 was shown to lose its Fe–S clusters, indicating its sensitivity to oxidation. Interestingly, simultaneous treatment with H_2O_2 and an increased temperature of 35 °C caused an acceleration of the

Fe–S cluster loss from the protein, whereas heat treatment alone did not affect the stability of the cluster. Together, these data indicate that heat potentiates the effect of oxidation on Fe–S cluster stability. Upon loss of its Fe–S clusters, GRXS17 oligomerizes via the formation of intermolecular disulfide bridges and non-covalent interactions, activating its holdase activity (Martins *et al.*, 2020). Further evidence for the role of GRXS17 was provided by the fact that a *grxs17* knockout mutant displayed a significantly reduced viability in comparison with wild-type plants after 8 d recovery from a 6 d exposure to 35 °C (Martins *et al.*, 2020). Furthermore, *grxs17* knockout mutants and RNAi lines displayed an enhanced sensitivity to a restrictive temperature of 28 °C, as indicated by an inhibition of primary root growth and a pin-like shoot phenotype. Also when grown at a milder temperature of 25 °C, *GRXS17* loss-of-function plants showed severe growth defects including leaf curling, leafy shoots, and malformed ovules (Cheng *et al.*, 2011). In contrast, an enhanced sensitivity of the mutant was not observed under short- and long-term acquired thermotolerance regimes, indicating the involvement of GRXS17 in responses to specific heat stress scenarios only (Martins *et al.*, 2020). The authors demonstrated that under these conditions, GRXS17 protects both shoot and root apical meristems and that this effect depends on cysteine residues in its active site. Furthermore, they showed that GRXS17 interacts with different sets of proteins under control and heat stress conditions, suggesting its involvement in protecting proteins against the negative consequences of moderate heat stress via a redox-dependent chaperone activity (Martins *et al.*, 2020).

The involvement of GRXs in thermotolerance has also been observed in yeast, where a *grx3 grx4* double knockout mutant showed an enhanced sensitivity to heat shock. Interestingly, the survival rate of this mutant in response to heat stress as well as oxidative stress was improved by heterologous expression of the *A. thaliana GRXS17* (Wu *et al.*, 2012). Overexpression of *AtGRXS17* also conferred heat and cold tolerance in tomato plants. Using green fluorescent protein (GFP) fusion proteins, the authors demonstrated that GRXS17 migrated from the cytosol into the nucleus during these stress conditions (Wu *et al.*, 2012; Hu *et al.*, 2015). Furthermore, transcript levels of HSF- and HSP-encoding genes were significantly increased in *GRXS17*-ovexpressing plants in comparison with wild-type plants upon heat stress (Wu *et al.*, 2012). Hence, GRXS17 and potentially other GRXs might be interesting targets in the search for strategies to enhance plant resistance to elevated temperatures, and their protective properties in heat-exposed plants should be further investigated in future studies.

Copper-related MIR398 plays a key role in plant thermotolerance

Besides Fe-related processes, mechanisms involved in the regulation of Cu homeostasis have also been suggested to play a

role in plant thermotolerance. Guan *et al.* (2013) reported that heat stress rapidly induces the expression of miRNA398 (*MIR398*) in *A. thaliana*, which subsequently reduces transcript levels of the Cu chaperone gene *CCS* and the SOD-encoding genes *CSD1* and *CSD2*. This mechanism probably contributes to thermotolerance, as transgenic plants that express miR398-resistant forms of *CCS*, *CSD1*, and *CSD2* display an enhanced sensitivity to heat stress in comparison with their counterparts which express the normal coding sequences of these genes. Furthermore, knockout mutants of these genes were characterized by an increased tolerance to heat stress. ChIP assays revealed that HSFA1b and HSFA7b bind directly to the promoter region of *MIR398* in response to heat stress. Via a feedback loop, miR398 can in turn positively affect the expression of *HSFA7b* as well as other HSF-encoding genes. The authors proposed that the reduced activity of CSDs in response to the miR398-mediated reduction of their transcript levels causes the accumulation of ROS. The subsequent alteration of the cellular redox state can then be either directly or indirectly sensed by specific HSFs to regulate the expression of other HSFs and HSPs (Guan *et al.*, 2013). A recent study by Li *et al.* (2020) provided more insight into the regulation of miR398. They showed that the *cis*-natural antisense transcripts of *MIR398b/c* genes repress the processing of their pre-miRNAs. Furthermore, they demonstrated that these natural antisense transcripts were activated in response to *MIR398b* and *MIR398c* overexpression, thereby constituting a regulatory feedback loop that attenuates thermotolerance (Li *et al.*, 2020). As a heat-induced up-regulation of *MIR398* was also observed in maize, manipulation of *MIR398* and/or its target genes could provide an interesting strategy to improve the heat tolerance of economically important crop species (Guan *et al.*, 2013).

Conclusion and perspectives

Micronutrients play key roles in a wide range of physiological processes and stress responses. It has been shown that heat stress affects micronutrient uptake in plants and that plants with altered micronutrient levels display an altered thermosensitivity. The involvement of metal micronutrients in plant responses to heat stress seems to be strongly intertwined with their role in redox homeostasis (Fig. 2). Although several enzymes containing trace metals have been associated with temperature responses, in-depth knowledge of the underlying mechanisms is largely lacking. Nevertheless, it is of crucial importance to further elucidate how micronutrients influence plant tolerance to elevated temperatures, as heat stress becomes more prevalent as a consequence of global warming, and nutrient levels and bioavailability in agricultural soils are often suboptimal.

In this context, many questions remain to be answered in the future. Several studies revealed that heat stress influences trace metal uptake and translocation (Table 1). The fact that heat stress-induced effects on micronutrient and macronutrient concentrations often follow a similar pattern suggests

a general impact on plant nutrient uptake capacity, for example as a consequence of changes in root morphology or enzyme activity. Nevertheless, it cannot be excluded that heat influences plant concentrations of specific trace metals by affecting proteins involved in their homeostasis at the transcriptional, translational, and/or post-translational level. The use of a multi-omics approach in future studies will help address this question. Furthermore, it would be of particular interest to investigate the contribution of alternative splicing to trace metal homeostasis during heat stress. To enable distinguishing the effects of temperature from those of other environmental variables, plant growth and heat stress treatments should be conducted under highly controlled conditions. The importance of Zn in plant thermotolerance was highlighted by several studies investigating heat stress responses under Zn-deficient conditions or upon treatment with additional Zn. Similar studies with other trace metals are needed to further unravel their role in plant heat stress responses. Moreover, it would be highly interesting to compare the thermotolerance of trace metal-hyperaccumulating plants with that of their non-hyperaccumulating relatives. As trace metal excess and heat stress both induce oxidative stress and protein misfolding, additional studies are required to unravel whether combined exposure to both stress types has cumulative effects on the accumulation of ROS and unfolded proteins and how this influences signalling pathways and downstream responses. Based on the significant overlap between plant responses to heat and metal toxicity, cross-tolerance to both stressors also deserves further attention in future studies.

A better understanding of how micronutrients affect plant responses to heat stress might allow for the development of strategies to improve plant thermotolerance through interfering with micronutrient availability, uptake, and/or homeostasis. Furthermore, unravelling the mechanisms underlying heat-induced alterations in trace metal uptake in plants is crucial, as these micronutrients are not only essential for plant growth and development but are also major determinants of nutritional quality.

Author contributions

SH, NV, AC, and AJM: conceptualization, writing—revision and editing; SH: data collection, writing—original draft. All authors have read and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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