REVIEW PAPER

Essential trace metals in plant responses to heat stress

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Received 22 August 2021; Editorial decision 16 November 2021; Accepted 17 November 2021

Editor: Zsuzsanna Kolbert, University of Szeged, Hungary

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 PSI (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 PSI, 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 (Hänisch 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 (H₂O₂). 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 (O₂²⁻) into O₂ and H₂O₂, 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 H₂O₂ to H₂O, 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., 2020).
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 (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 Ca2+ channels at the plasma membrane. In Arabidopsis thaliana, heat induces an increase in cAMP levels, which triggers Ca2+ 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 adenyl cyclase by increased membrane fluidity has been proposed to play a role (Hayes et al., 2021). Other Ca2+ channels besides CNGC6 are probably involved in Ca2+ influx as well, but have not been identified so far (Hayes et al., 2021). The elevated cytosolic Ca2+ concentrations can subsequently recruit annexin 1 (ANN1) to membranes where it could either form a Ca2+ channel itself or activate other Ca2+ channels to further enhance Ca2+ influx, required to trigger multiple downstream signalling pathways (Wang et al., 2015). As well as Ca2+, 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 O2− in the apoplast. The O2− produced is subsequently converted to H2O2, 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 Ca2+ signals are closely intertwined, as RBOHD activation depends on Ca2+ 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 Ca2+ as well. In heat-stressed guard cells, H2O2 oxidizes cysteine residues in the C2 domain of phospholipase Dδ (PLDδ), which subsequently promotes Ca2+ 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 (PIP₂) and α-myoinositol-1,4,5-trisphosphate (IP₃) are also involved in heat stress responses (Hayes et al., 2021). Downstream pathways triggered by heat-induced Ca²⁺, 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 (András 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 sub-lethal 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\textsuperscript{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\textsuperscript{2+} influx is mediated by cyclic nucleotide-gated channel 6 (CNGC6) and other Ca\textsuperscript{2+} transporters. Activation of CNGC6 is suggested to be triggered by cAMP, generated by adenylyl cyclase (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\textsuperscript{2+} channel itself or enhance the activity of other Ca\textsuperscript{2+} channels. Activation of respiratory burst oxidase homologue D (RBOHD) causes apoplastic generation of superoxide (\textit{O}_2^{-}), which is converted to hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) that can subsequently enter the cell and trigger downstream signalling pathways. RBOHD is activated by Ca\textsuperscript{2+} binding to its EF-hand motifs and phosphorylation by calcium-dependent protein kinases (CDPKs). In addition, H\textsubscript{2}O\textsubscript{2} oxidizes cysteines in the C2 domain of phospholipase D\textsubscript{δ} (PLD\textsubscript{δ}), promoting Ca\textsuperscript{2+} binding. This enzyme generates phosphatidic acid (PA) which, together with PIP\textsubscript{2} and IP\textsubscript{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

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

† 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$_2$O$_2$, 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...
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 diethylenetriaminepentaacetate-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

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

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Micronutrient conditions</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cicer arietinum</td>
<td>Zn deficiency</td>
<td>Heat sensitivity †</td>
<td>Ullah et al. (2019)</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>Zn deficiency</td>
<td>Heat sensitivity †</td>
<td>Peck and McDonald (2010)</td>
</tr>
<tr>
<td>Brassica chinensis</td>
<td>Foliar Zn spraying</td>
<td>Heat sensitivity †</td>
<td>Sarwar et al. (2019)</td>
</tr>
<tr>
<td>Gossypium hirsutum</td>
<td>Foliar Zn spraying</td>
<td>Heat sensitivity †</td>
<td>W. Han et al. (2020)</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>Zn fertilizer application</td>
<td>Heat sensitivity †</td>
<td>Tao et al. (2018)</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Cu and Zn treatment</td>
<td>Heat sensitivity †</td>
<td>Bonham-Smith et al. (1987)</td>
</tr>
</tbody>
</table>

† 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.
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 of Fenton reactions and are also implicated in ROS scavenging.

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 underpinning 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 thermostolerance 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 antipporter system Xc−. 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 cysteine uptake upon erastin exposure ultimately leads to a depletion of cellular levels of the antioxidant 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 Fe2+ with H2O2 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 H2O2 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).
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 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
Interestingly, simultaneous treatment with H$_2$O$_2$ and an inhibitor of the enzyme that loses its Fe–S clusters, indicating its sensitivity to oxidation. 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 hollase activity (Martins et al., 2020). Further evidence for the role of GRXS17 was provided by the fact that a gxs17 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, gxs17 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 gxs3 gxs4 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 stress conditions (Wu et al., 2012; Hu et al., 2015). Furthermore, transcripts encoding GRXS17 were significantly increased in GRXS17-overexpressing 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.

**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$_2$O$_2$, the reconstituted holo-form of GRXS17 was shown to lose its Fe–S clusters, indicating its sensitivity to oxidation. Interestingly, simultaneous treatment with H$_2$O$_2$ 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 hollase activity (Martins et al., 2020). Further evidence for the role of GRXS17 was provided by the fact that a gxs17 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, gxs17 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).

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**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 HSE47b 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.

**Funding**

This work was supported by a Humboldt Research Fellowship from the Alexander von Humboldt Foundation to SH. Additional funding came from Research Foundation - Flanders (FWO) project G0C7518N.
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