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Essential trace metals in plant responses to heat stress

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1	Importance of essential trace metals in plant responses to heat stress
2	Sophie Hendrix ^{1,2,*} , Nathalie Verbruggen ³ , Ann Cuypers ² , Andreas J. Meyer ¹
3	
4	¹ Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Bonn, Germany
5	² Environmental Biology, Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium
6	³ Laboratory of Plant Physiology and Molecular Genetics, Université Libre de Bruxelles, Brussels, Belgium
7	
8	* Correspondence: <u>shendrix@uni-bonn.de</u>
9	
10	shendrix@uni-bonn.de
11	nathalie.verbruggen@ulb.be
12	ann.cuypers@uhasselt.be
13	andreas.meyer@uni-bonn.de
14	
15	
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23 Highlight

This work provides an overview of reciprocal interactions between trace metal homeostasis and heat stress responses in plants.

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27 Abstract

Essential trace metals function as structural components or cofactors in many proteins 28 29 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 30 concentrations of trace metals can also induce phytotoxicity, for example via an enhanced 31 32 production of reactive oxygen species. Besides their roles in plant growth under favourable 33 environmental conditions, trace metals also contribute to plant responses to biotic and abiotic 34 stresses. Heat is a stress factor that will become more prevalent due to progressing climate 35 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 36 37 develop strategies to optimise plant growth and quality under unfavourable temperatures. In this context, trace metals deserve particular attention as they contribute to defence responses 38 39 and are important determinants of plant nutritional value. Here, we provide an overview of 40 heat-induced effects on plant trace metal homeostasis and the involvement of trace metals and 41 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. 42

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

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Abbreviations: CPX: ciclopirox olamine; Fer-1: ferrostatin-1; GSH: glutathione; GRX: glutaredoxin; H_2O_2 : hydrogen peroxide; HSF: heat shock factor; HSP: heat shock protein; O_2^- : superoxide; ROS: reactive oxygen species; SOD: superoxide dismutase; UPR: unfolded protein response.

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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.

60

61 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.

68 Both micronutrient deficiency and toxicity can severely hamper plant growth. The negative 69 consequences of a micronutrient shortage on plant performance are related to the role of these 70 trace elements as structural components or cofactors in a wide variety of proteins. Zinc, for example, is essential for the activity of more than 300 enzymes belonging to all six major 71 72 Enzyme Commission (EC) classes (Clemens, 2019; Clemens et al., this issue). Zinc is a 73 structural component of proteins interacting with nucleic acids such as RNA polymerase and 74 many transcription factors. Furthermore, several enzymes involved in protein, carbohydrate 75 and lipid metabolism also rely on Zn for their structure or function (Sharma et al., 2013). The 76 importance of Fe and Cu as enzyme cofactors largely depends on their redox properties. They 77 are essential for cellular energy provision through their role in photosynthesis and respiration 78 and are crucial for the functioning of the respective electron transport chains (Clemens, 2019). 79 In biological systems, Fe can be found in multiple configurations in the form of mono- and di-80 iron centres, haem and iron-sulfur (Fe-S) clusters. Besides their role in electron transport 81 reactions, Fe metalloproteins are also involved in other processes including nucleotide 82 biosynthesis and repair and the biosynthesis of amino acids, proteins, cofactors and vitamins (Talib and Outten, 2021). Manganese is essential for photosynthesis, as it is a core component 83 84 of the metalloenzyme cluster of the oxygen-evolving complex in photosystem II (PSII) 85 (Schmidt et al., 2020). Furthermore, many enzymes depend on Mn as a cofactor. It should be 86 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 87 88 complex in PSII, oxalate oxidase and Mn superoxide dismutase (Mn-SOD) are the only plant 89 enzymes that exclusively require Mn (Alejandro et al., 2020). In contrast to Zn, Cu, Fe and 90 Mn, which are required for the structure and/or function of many different proteins, the 91 number of proteins relying on Mo and Ni is much smaller. Currently, urease is the only plant 92 enzyme known to depend on Ni in plants, whereas Mo is found in five types of 93 molybdoenzymes, requiring a pterin-based Mo cofactor: nitrate reductase, sulfite oxidase, 94 aldehyde oxidase xanthine dehydrogenase and amidoxime reducing component (Hänsch and 95 Mendel, 2005; Huang et al., this issue). In addition, it should not be neglected that 96 micronutrients are required for the growth and function of plant-associated microorganisms, 97 which in turn influence plant growth (Compant et al., 2019), but this is outside the scope of 98 the current review.

99 Whereas essential trace metals are indispensable for a plethora of physiological processes, 100 they become detrimental to plant growth and function when present in too high concentrations. Excessive Mn concentrations, for example, can interfere with the uptake and 101 translocation of other essential elements and disturb chlorophyll production and 102 103 photosynthesis (Alejandro et al., 2020). Phytotoxic Zn concentrations influence plant growth 104 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 105 106 groups in proteins, which alters their structure and enhances their degradation, ultimately triggering senescence. Furthermore, Zn has also been reported to activate lipoxygenase 107 108 enzymes, involved in lipid peroxidation reactions that negatively affect membrane stability 109 (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 110 their function in many cellular processes, it is also the reason for their participation in Fenton 111 112 and Haber-Weiss reactions which enhance the production of highly reactive hydroxyl radicals 113 (OH) from hydrogen peroxide (H_2O_2) . Although it is well known that reactive oxygen species 114 (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 115 Arnaud, 2019). To prevent toxicity, plants have developed strategies to fine-tune the delivery 116 117 of redox-active metals to specific metalloproteins while preventing uncontrolled reactivity by 118 keeping cellular free metal ion concentrations low. This is achieved by the chelation of metals 119 to ligands, chaperones and storage proteins as well as sequestration to vacuoles for storage 120 (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. 121 122 Interestingly, many enzymatic antioxidants depend on trace metals for their function (Ravet 123 and Pilon, 2013). For example, SODs catalyse the dismutation of superoxide (O_2^{-}) into O_2 and 124 H₂O₂ and are subdivided into Cu/Zn-SODs, Mn-SODs and Fe-SODs based on their metal 125 cofactor. These different SOD categories are also characterised by different subcellular 126 localisations (Dumanović et al., 2021). Furthermore, catalase and certain peroxidases such as 127 ascorbate peroxidase and guaiacol peroxidase, which mediate the reduction of H_2O_2 to H_2O_3 , 128 typically depend on an Fe-containing haem cofactor (Mhamdi et al., 2010).

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130 Plant responses to heat stress

131 Due to their sessile nature, the ability of plants to escape from unfavourable environmental 132 conditions is strongly limited in comparison to that of many other organisms. As such, they frequently fall prey to different biotic stresses such as pathogen attack and abiotic stresses like 133 drought, salinity, soil contamination with organic and inorganic compounds and unfavourable 134 135 temperatures (Choudhury et al., 2017; Huang et al., 2019). Since climate change is predicted 136 to entail the occurrence of climatologic extremes including high temperatures during summer, heat is a stress factor that will become increasingly prevalent in the future (Bita and Gerats, 137 138 2013; Zandalinas et al., 2021). This forms a severe threat to food security as the global 139 population continues to grow, while heat stress significantly reduces the yield of major crops 140 such as wheat, maize, rice and soybean (Wang et al., 2020). It has been estimated, for 141 example, that wheat and rice yield will decrease by 6% and 10%, respectively, for each 142 additional °C of temperature increase during the grain filling stage (Asseng et al., 2015; Khan 143 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 144 145 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 146 147 importance for the development of strategies to enhance plant growth and crop yield under 148 unfavourable temperature conditions. The detrimental impact of heat stress on plant growth 149 and crop productivity is a consequence of several effects at the cellular level including disturbances of membrane fluidity, cytoskeleton organisation, protein folding, transport 150 151 processes and enzymatic reactions and the induction of oxidative stress (Hayes et al., 2020; 152 Ashraf, 2021; Haider et al., 2021). High temperatures also damage thylakoid membranes, 153 which subsequently disturbs photosynthetic reactions and hence cellular energy provision, 154 essential for optimal plant growth and function (Hu et al., 2020). Especially pollen development is highly sensitive to heat, rendering plants particularly vulnerable to high 155 temperatures during the reproductive stage (Chaturvedi et al., 2021). Furthermore, seed 156 157 germination is also negatively impacted by heat stress (Haider et al., 2021). In order to 158 increase their tolerance to high temperatures – also termed 'thermotolerance' – plants activate 159 different molecular pathways ultimately aimed at preventing and restoring heat-induced 160 damage.

Currently, our knowledge on how plants "feel the heat" is far from complete (Vu et al., 2019). 161 162 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 163 in thermosensing (Jung et al., 2016; Lamers et al., 2020; Murcia et al., 2021). Phytochromes 164 165 undergo conformational changes in response to light, with red light causing a shift to the 166 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 167 reversal from Pfr to Pr is higher at warmer temperatures (Hayes et al., 2020). A recent study 168 169 identified EARLY FLOWERING 3 (ELF3) as another temperature sensor in plants. This 170 protein is a component of the circadian clock evening complex that functions as a 171 transcriptional repressor. The circadian clock is believed to control about one third of the 172 transcriptome in plants (Covington et al., 2008). In response to warm temperatures, ELF3 173 forms 'speckles' (i.e. liquid droplets) in the nucleus through its prion-like domain and is 174 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 175 176 the mRNA hairpin structure of PHYTOCHROME INTERACTING FACTOR 7 (PIF7, a bHLH 177 transcription factor interacting with phytohchromes and regulating plant growth), enhancing are 178 its translation. These thermosensing mechanisms mainly involved in 179 thermomorphogenesis, a process during which plants alter their morphology to avoid 180 exposure to potentially harmful temperatures. This includes changes in leaf shape, root growth and induction of flowering (Casal and Balasubramanian, 2019; Hayes et al., 2020). 181

182 Thermomorphogenesis takes place at temperatures that exceed the optimum for growth, but 183 are still within the physiological range. However, plants also need mechanisms to sense and 184 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 185 alterations in membrane fluidity have been proposed to play key roles (Hayes et al., 2021). 186 Signalling pathways operating in heat-stressed plants can be triggered by activation of Ca^{2+} 187 channels at the plasma membrane. In Arabidopsis thaliana, heat induces an increase in cyclic 188 AMP (cAMP) levels, which triggers Ca^{2+} influx into the cytosol via cyclic nucleotide-gated 189

190 channel 6 (CNGC6) (Gao et al., 2012). Although the exact underlying molecular mechanism 191 for heat-induced cAMP accumulation is still unclear, activation of a membrane-associated 192 adenylyl cyclase by increased membrane fluidity has been proposed to play a role (Hayes et al., 2020). Other Ca^{2+} channels besides CNGC6 are likely involved in Ca^{2+} influx as well, but 193 have not been identified so far (Hayes *et al.*, 2020). The elevated cytosolic Ca^{2+} 194 concentrations can subsequently recruit annexin 1 (ANN1) to membranes where it could 195 either form a Ca^{2+} channel itself or activate other Ca^{2+} channels to further enhance Ca^{2+} 196 influx, required to trigger multiple downstream signalling pathways (Wang et al., 2015). 197 198 Besides Ca²⁺, also ROS play key roles as signalling molecules in heat stress responses. Heat induces the activation of respiratory burst oxidase homolog D (RBOHD), a plasma 199 200 membrane-localised NADPH oxidase that catalyses the formation of O_2^{-1} in the apoplast. The 201 produced O_2^- is subsequently converted to H_2O_2 , which can enter cells and activate multiple 202 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 203 intertwined, as RBOHD activation depends on Ca²⁺ binding to its EF hand motifs as well as 204 phosphorylation by calcium-dependent protein kinases (CDPKs) (Ogasawara et al., 2008; 205 Steinhorst and Kudla, 2013). Lipid signals, which also play crucial roles in plant responses to 206 heat stress, depend on ROS and Ca2+ as well. In heat-stressed guard cells, H2O2 oxidises 207 cysteine residues in the C2 domain of phospholipase $D\delta$ (PLD δ), which subsequently 208 promotes Ca^{2+} binding to this enzyme, resulting in microtubule depolymerisation, which in 209 turn influences stomatal movement (Zhang et al., 2017; Song et al., 2020). Furthermore, 210 211 PLD δ catalyses the production of phosphatidic acid (PA), which functions as a signalling 212 molecule through its interaction with different cytosolic target proteins. Other lipid signals 213 such as phosphatidylinositol-4,5-bisphosphate (PIP₂) and D-myoinositol-1,4,5-trisphosphate 214 (IP₃) are also involved in heat stress responses (Hayes *et al.*, 2020). Downstream pathways triggered by heat-induced Ca²⁺, ROS and lipid signals ultimately induce transcriptional 215 responses, many of which are the result of activation of heat shock factors (HSFs) (Hayes et 216 217 al., 2020). These form a class of conserved transcription factors regulating the expression of a 218 broad array of stress-inducible genes including those encoding heat shock proteins (HSPs). 219 The latter function as molecular chaperones that promote correct protein folding and prevent 220 aggregation of misfolded proteins. Among plant HSFs, those of the A1-type are considered 221 master regulators of the heat stress response, as they control the expression of many other 222 HSFs as well as DREB2A, another key transcription factor regulating plant responses to heat 223 stress (Andrási et al., 2020).

224 Heat-induced accumulation of unfolded and misfolded proteins in the endoplasmic reticulum 225 (ER) lumen due to overloading of the protein quality control system (*i.e.* ER stress) triggers 226 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) 227 228 transcription factor and inositol-requiring enzyme 1 (IRE1), respectively. Upon activation, 229 IRE1 mediates alternative splicing of the mRNA encoding the bZIP60 transcription factor, 230 allowing translocation of bZIP60 from the ER membrane to the nucleus. Both bZIP28 and 231 bZIP60 induce the transcription of a plethora of genes to enhance protein folding capacity and 232 suppress translation with the ultimate aim of restoring protein homeostasis (Liu and Howell, 233 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 236 HSP genes in Arabidopsis induces thermomemory and enables a strong induction of these 237 genes in response to repeated heat stress (Lämke et al., 2016). In addition, also nucleosome remodelling and microRNAs (miRNAs) are involved in heat stress memory (Haider et al., 238 239 2021). The ability of plants to memorise previous heat stress episodes contributes to the 240 maintenance of 'acquired thermotolerance' over time. This allows plants to survive otherwise 241 lethal temperatures after a period of acclimation to a sub-lethal temperature (Sharma et al., 242 2019). Although most of this memory disappears several days after the heat stress ends, some 243 aspects of epigenetic heat stress memory can be passed on to the next generation (Haider et 244 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 245 246 plant heat stress responses, readers are referred to Haider et al. (2021) and Hayes et al. 247 (2020).

Although many of the molecular mechanisms underlying plant heat stress responses have been characterised, knowledge on the involvement of essential trace metals is currently scarce. To gain more insight into this topic, the next sections of this review summarise how heat stress influences plant uptake and translocation of trace metals and how trace metals take part in plant responses to heat stress.

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254 Heat stress influences plant metal micronutrient levels

255 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 256 257 context have used a broad range of experimental approaches, ranging from tightly controlled 258 set-ups with plant exposure to specific, pre-determined temperature conditions (Giri et al., 259 2017) to observational studies comparing nutrient levels between plants grown in a greenhouse during different seasons (Darawsheh et al., 2006). Whereas in some studies, 260 261 analyses were performed on plants grown in climate chambers (Giri et al., 2017) or 262 greenhouses (Darawsheh et al., 2006; Dias and Lidon, 2009), others employed field-grown 263 plants (Impa et al., 2019). It should be noted that although studies in greenhouses and field 264 conditions can yield interesting insights into plant nutrient homeostasis, they do not allow 265 completely separating temperature-induced effects from those of other variables such as light 266 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 on 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 279 abundance and activity of mycorrhizal fungi (León-Sánchez et al., 2020). It should be taken 280 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 281 small temperature increases (Menzel et al., 1987; Darawsheh et al., 2006; León-Sánchez et 282 283 al., 2020). On the other hand, a positive impact of increased temperatures on root 284 development could also enhance the ability of plants to take up nutrients from the soil 285 (Viciedo et al., 2021). Besides influencing the uptake of nutrients in roots, high temperatures 286 can alter their translocation to various plant organs. Darawsheh et al. (2006), for example, reported that during winter the extractable Zn concentration in the top part of the stem of 287 288 tomato plants was significantly higher as compared to that in the bottom part of the stem. The 289 opposite was observed during summer, indicating a strong seasonal impact on Zn 290 translocation (Darawsheh et al., 2006). However, heat might also promote mineral uptake and 291 translocation when plants increase their transpiration rate to promote leaf cooling (Martins et 292 al., 2014).

Interestingly, several studies have addressed the impact of increased temperatures in the root 293 294 zone only. Du and Tachibana (1994) investigated the effects of supra-optimal root zone temperatures on cucumber plants via the use of a heated nutrient solution. Their results 295 296 showed that Fe and Mn concentrations in the leaves decreased with increasing root 297 temperatures. Similar results were observed for most of the other micro- and macronutrients 298 studied, suggesting a general effect of root temperature on nutrient uptake capacity (Du and 299 Tachibana, 1994). Tan et al. (2002) showed that growth-related increases in root and shoot 300 concentrations of Cu, Fe, Mn and Zn were more pronounced in Lactuca sativa plants grown 301 in a nutrient solution with a temperature of 20°C as compared to those grown in nutrient 302 solutions with a higher temperature. These responses are likely related to temperature-303 dependent alterations in root morphology, as a supra-optimal temperature of the nutrient 304 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 305 306 involvement of root temperature-mediated alterations in enzyme activities and/or 307 phytohormone signalling on mineral levels in roots and shoots cannot be excluded (Tan et al., 308 2002).

309 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 310 al. (2017) demonstrated that exposure of tomato plants to a temperature of 42° C for 6 days 311 312 significantly decreased the rate of Fe uptake in roots. This effect coincided with lower root 313 levels of the iron reductase LeFRO1, which is one of the two main proteins responsible for Fe 314 reduction (Giri et al., 2017). In Strategy I (i.e. non-graminaceous) plants, reduction of Fe at 315 the root surface is required for its uptake (Kobayashi and Nishizawa, 2012). As knowledge on 316 heat-induced effects on specific proteins involved in trace metal homeostasis is currently very 317 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 318 319 regulators of trace metal homeostasis occurs under heat stress conditions. As recently 320 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 321 322 alternative protein products with different functions or activities. Interestingly, alternative 323 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).

326 Even though the underlying mechanisms have not been fully elucidated, it is clear that heat 327 stress influences plant uptake and translocation of trace metals. The fact that plant levels of 328 other micronutrients and macronutrients are often affected by increased temperatures in a 329 similar manner, suggests that heat causes a general impact on the plant nutrient uptake 330 capacity (Menzel et al., 1987; Maboko et al., 2013; Cabral et al., 2016; Giri et al., 2017; 331 León-Sánchez et al., 2020; Viciedo et al., 2021). Heat-induced alterations of root morphology 332 due to an increased soil temperature likely play an important role in this process. However, 333 more targeted heat-induced effects on specific nutrient uptake and transport systems at the 334 transcriptional, translational or post-translational level cannot be excluded. This topic 335 deserves further attention in future research, as altered mineral contents can affect the 336 development of plants as well as their nutritional quality (Martins et al., 2014). As such, the 337 more frequent occurrence of heat waves due to climate change could have a negative impact 338 on both crop yield and quality. Therefore, increasing our knowledge on how heat stress 339 affects plant uptake and translocation of specific nutrients can aid in the development of 340 strategies to safeguard food security for future generations.

341

342 Metal micronutrients influence plant responses to heat stress

343 Metal micronutrients are highly likely to play important roles in plant responses to heat stress 344 due to their function as cofactors for a wide variety of proteins involved in physiological 345 processes and defence responses against stressful conditions. These might include SOD 346 enzymes involved in antioxidative defence, as increased ROS production is a well-known 347 consequence of plant exposure to heat stress. Shiraya et al. (2015) reported that MSD1, a Mndependent SOD of rice plants, plays a key role in their thermotolerance. Rice MSD1 has been 348 349 proposed to be targeted to the plastid stroma from the Golgi apparatus via the secretory 350 pathway, effectively leading to a dual Golgi/plastid localisation. While knockdown mutants of 351 *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 352 353 constitutive high expression of Golgi/plastid-type MSD1 improves the detoxification of O_2^{-1} 354 together with increased formation of H_2O_2 , which in turn may induce changes in expression of 355 several other antioxidant genes. In addition, MSD1 is proposed to control the redox state in 356 the endomembrane system, leading to the normal programmed formation of protein bodies 357 (Shiraya et al., 2015). Metal micronutrients might also contribute to plant heat stress 358 responses via their involvement in phytohormone signalling. For example, Cu serves as a 359 cofactor for ethylene receptors (ETRs), which initiate the ethylene signalling cascade in 360 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 361 362 trace metal-dependent regulators of heat stress responses are zinc finger proteins, which 363 constitute one of the largest transcription factor families in plants (Han et al., 2020a). A 'zinc 364 finger' typically consists of two cysteines and/or histidines and one Zn ion, which is required 365 for its structure and function. Among 112 predicted C2H2-type zinc finger proteins in tomato, 366 many showed transcriptional up- or downregulations upon heat exposure, suggesting their 367 involvement in heat stress responses. Nevertheless, their role in plant stress responses is likely

368 not limited to heat stress, as expression of the same genes was also affected by other abiotic 369 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, 370 371 constitutive overexpression of the gene encoding the zinc finger protein ZAT10 was shown to 372 enhance the thermotolerance of A. thaliana (Mittler et al., 2006). Similarly, heterologous 373 expression of the wheat zinc finger protein TaZnF increased the tolerance of A. thaliana to 374 heat (Agarwal and Khurana, 2018). On the other hand, A. thaliana knockout mutants of the 375 zinc finger protein stress-associated protein5 (SAP5) and its downstream transcriptional target 376 multiprotein binding factor1c (MBF1c) showed an increased sensitivity to heat stress (Kim et 377 al., 2015). Heterologous overexpression of the rice zinc finger protein ZFP177 conferred 378 tolerance to both heat and cold stress in tobacco plants, while increasing their sensitivity to 379 salt and drought stress (Huang et al., 2008). These data indicate that zinc finger proteins play 380 crucial roles in plant responses to a wide variety of stress factors, but that their specific role 381 depends on the stress factor. In A. thaliana, overexpression of the zinc finger protein SAP10 382 conferred tolerance to Ni, Mn and Zn and heat stress, suggesting its involvement in signal 383 transduction upon high temperature stress as well as exposure to excess trace metals (Dixit and Dhanker, 2011). 384

385 The role of trace metals in plant responses to increased temperatures is further supported by 386 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 387 mainly focussed on Zn. Ullah et al. (2019) showed that the sensitivity of Cicer arietinum to 388 389 heat stress was reduced when plants were grown under Zn sufficiency rather than Zn 390 deficiency. Sufficient Zn supply during heat stress improved plant growth and photosynthesis 391 by positive effects on PSII efficiency, water relations, free proline levels and antioxidative 392 enzyme activities. In the same study, similar effects of Zn supply were observed in plants 393 exposed to drought stress, indicating their pleiotropic character (Ullah *et al.*, 2019). Similarly, 394 the positive effect of foliar Zn spraying on the thermotolerance of cotton plants grown on soil 395 containing 1.6 ppm available Zn was associated with increased antioxidative enzyme 396 activities, ascorbic acid and total phenolic compound levels, chlorophyll contents, net 397 photosynthetic rate, stomatal conductance and water potential. It should be noted, however, 398 that exogenous application of K and B had similar effects (Sarwar et al., 2019). Foliar Zn 399 spraying also positively affected the thermotolerance of *Brassica chinensis* grown in a Zn-400 deficient loamy clay soil by positive effects on SOD activity, chlorophyll content and 401 photosynthetic parameters. As the plants did not show any symptoms of nutrient deficiency 402 before the heat stress treatment, the authors proposed that Zn demand increases during 403 exposure to high temperatures (Han et al., 2020b). Furthermore, Zn fertilization mitigated the 404 negative impact of high temperature stress on the grain yield and flour quality of wheat grown 405 in loam soil with a diethylenetriamine pentaacetate-extractable Zn concentration of 0.85 mg kg⁻¹ (Tao *et al.*, 2018). 406

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 414 415 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 416 including metal stress (Heckathorn et al., 2004). Interestingly, the observed cross-tolerance 417 418 between heat and metal stress functions in both directions, as heat shock is also able to 419 mitigate negative effects of subsequent exposure to toxic metal concentrations. Indeed, heat 420 was shown to attenuate Cu-induced cell death in rice (Chen et al., 2008) and to protect against 421 Cu phytotoxicity in specific Arabidopsis ecotypes. The latter response was related to heat-422 induced production of metallothioneins, which are small, cysteine-rich, metal-binding 423 proteins involved in regulating metal homeostasis (Murphy and Taiz, 1995). Because of their 424 metal-chelating function, metallothioneins prevent the catalysis of Fenton reactions and are 425 also implied in ROS scavenging. This role is supported by the fact that a type 1 426 metallothionein from the Cu-accumulating plant species Elsholtzia haichowensis was 427 transcriptionally upregulated in response to Cu stress as well as H_2O_2 and heat treatments (Xia 428 et al., 2012). Hence, the involvement of metallothioneins in heat stress tolerance might also 429 be related to their ROS-scavenging properties.

430 Besides affecting plant responses to trace metal excess, heat stress was also shown to 431 influence the effects of Fe deficiency in A. thaliana. Using an automated imaging and 432 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 433 434 root growth. Surprisingly, plants subjected to a combination of Fe deficiency and heat stress 435 showed a smaller root growth inhibition in comparison to plants exposed to the single 436 stresses. This was related to an altered timing and persistence of expression of the cyclin 437 *CYCB1:1*, a marker for cell entry into mitosis. Although the molecular mechanism underlying 438 the antagonistic effects of heat stress and Fe deficiency on root growth was not determined, 439 this work clearly indicates that plant responses to combined stresses can be non-intuitive 440 (Buckner et al., 2019). Hence, it is of crucial importance to consider the effects of stress 441 combinations in future studies, as plants grown under field conditions are frequently exposed 442 to combinations of stress factors. In this context, studying the combined effect of heat stress 443 and trace metal deficiency and/or excess is particularly important as increased temperatures 444 will become more prevalent due to climate change and nutrient availability in soils is often 445 sub-optimal.

446 Taken together, the available data indicate a clear relationship between trace metal levels and 447 heat stress responses in plants. As metal micronutrients play crucial roles in many 448 physiological processes and defence mechanisms via their function as cofactors in a plethora 449 of proteins, their effects on thermotolerance are likely pleiotropic. However, in-depth 450 knowledge on the involvement of trace metals in plant responses to heat stress is currently 451 very scarce and a large amount of work remains to be done in this field. Three molecular 452 studies illustrating the relationship between trace metal-related processes and responses to 453 increased temperature are discussed in more detail in the subsequent sections of this review 454 and are summarised in Fig. 2.

455

457 An example of a trace metal-mediated, heat-induced process in plants is ferroptosis-like cell 458 death. The term ferroptosis was first introduced by Dixon et al. (2012) to describe a regulated 459 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 characterised by its 460 dependence on ROS, intracellular Fe and peroxidation of membrane lipids, causing the 461 462 accumulation of lipid hydroperoxides. In mammalian cells, the decreased cystine uptake upon 463 erastin exposure ultimately leads to a depletion of cellular levels of the antioxidative 464 metabolite glutathione (GSH). This consequently diminishes the activity of glutathione 465 peroxidase 4 (Gpx4), which reduces lipid hydroperoxides using GSH as an electron donor. As 466 a consequence, lipid peroxidation products accumulate, ultimately triggering cell death (Feng 467 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_2 468 leads to the formation of hydroxyl radicals, which are highly reactive towards poly-469 470 unsaturated fatty acids in lipid membranes. In addition, Fe can also enhance lipid peroxidation 471 via its role as a cofactor for lipoxygenases (Thi Tuyet Le et al., 2019; Distéfano et al., 2021). 472 These proteins catalyse enzymatic lipid peroxidation and functionally depend on a non-haem 473 Fe in their catalytic domain (Porta and Rocha-Sosa, 2002). As a consequence of its 474 dependence on Fe and lipid peroxidation, ferroptosis in mammalian cells is typically inhibited 475 by intracellular Fe chelators such as ciclopirox olamine (CPX) and deferoxamine as well as 476 lipophilic antioxidants such as ferrostatin-1 (Fer-1) and liproxstatin (Feng and Stockwell, 477 2018).

478 In a recent study, Distéfano et al. (2017) demonstrated that the cell death induced in A. 479 thaliana root hairs upon exposure to a temperature of 55°C for 10 min was characterised by 480 many of the same hallmarks as ferroptosis in mammalian systems, including increases in 481 cellular ROS levels, GSH depletion and the occurrence of lipid peroxidation. Interestingly, 482 this heat-induced cell death was also inhibited by Fer-1 and CPX and was hence termed 483 "ferroptosis-like" cell death. Pre-treatment with these ferroptosis inhibitors also enhanced the 484 survival rate of A. thaliana seedlings exposed to 43°C for 1 h, suggesting that ferroptotic cell 485 death takes place under more environmentally realistic heat stress scenarios as well. In 486 contrast, Fer-1 and CPX pre-treatment did not inhibit cell death induced by a more severe heat 487 stress of 77°C or other stress factors such as H_2O_2 and salt treatment. Nevertheless, stress-488 induced ferroptosis-like cell death in plants is not limited to heat stress, but was also reported 489 to occur during avirulent Magnaporthe oryzae infections in rice plants (Dangol et al., 2019).

490 Interestingly, heat-induced ferroptosis was also observed in photosynthetic cyanobacteria, 491 suggesting the involvement of chloroplasts (Aguilera et al., 2019, Preprint). This hypothesis is 492 further supported by the fact that the death of A. thaliana seedlings upon heat stress was less 493 pronounced when the plants were further grown under dark instead of light conditions after 494 the heat shock (Distéfano et al., 2017). The involvement of chloroplasts in plant ferroptosis-495 like cell death could be related to several inherent characteristics of these organelles. 496 Chloroplasts are major subcellular sources of ROS and thylakoid membranes harbour the 497 largest level of lipid unsaturation of any membrane, which renders them particularly 498 vulnerable to lipid peroxidation (Asada, 2006; Routaboul et al., 2012). In addition, 499 chloroplasts represent the largest Fe sink in most plant cells and contain up to 80% of total Fe 500 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. 501 502 Nevertheless, the presence of large amounts of Fe also entails a risk for oxidative damage 503 (Kroh and Pilon, 2020). Therefore, chloroplasts are equipped with multiple Fe transporters, 504 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 505 store large numbers of Fe atoms in their central cavity (Ravet et al., 2009). The importance of 506 507 a strict regulation of free Fe levels in heat-exposed plants is illustrated by the observation that 508 several ferritin-encoding genes in Pyrus pyrifolia displayed a rapid transcriptional 509 upregulation 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 510 effect was likely related to an enhanced membrane stability, as heat-induced increases in the 511 512 levels of malondialdehyde (*i.e.* a marker of lipid peroxidation) were less pronounced in the 513 transgenic plants as compared to non-transformed plants (Zhao et al., 2016). Similarly, expression levels of the wheat ferritin gene TaFER-5B were increased in response to heat 514 stress and overexpression of this gene resulted in an enhanced thermotolerance of wheat 515 plants. Heterologous expression TaFER-5B in A. thaliana was able to rescue the heat-516 517 sensitive phenotype of the *fer1-3-4* triple mutant, which contains mutations in the three major 518 leaf ferritin genes. However, it should be mentioned that transgenic A. thaliana lines 519 harbouring the wheat TaFER-5B also displayed an enhanced tolerance to drought and H_2O_2 520 treatment (Zang et al., 2017). This emphasises that the role of ferritin in stress tolerance is not 521 limited to heat stress but that it likely contributes to a more general response to prevent the Fe-dependent formation of highly reactive hydroxyl radicals under a wide variety of stress 522 523 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 characterisation 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.

529

530 *Glutaredoxins contribute to plant thermotolerance*

531 Other Fe-related proteins involved in plant tolerance to heat stress are glutaredoxins (GRXs). 532 Glutaredoxins 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 533 534 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 535 536 glutathionylated proteins, several class I, and likely all class II GRXs have the ability to 537 incorporate Fe-S clusters, and are hence also involved in regulating Fe homeostasis (Couturier 538 et al., 2015; Wu et al., 2017). Due to small structural differences, Fe-S clusters in class II 539 GRXs are more labile as compared to 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 540 not (Martins et al., 2020). 541

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 localised 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 547 548 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 549 550 that heat potentiates the effect of oxidation on Fe-S cluster stability. Upon loss of its Fe-S 551 clusters, GRXS17 oligomerises via the formation of intermolecular disulfide bridges and non-552 covalent interactions, activating its holdase activity (Martins et al., 2020). Further evidence 553 for the role of GRXS17 was provided by the fact that a grxs17 knockout mutant displayed a 554 significantly reduced viability in comparison to wild-type plants after 8 days recovery from a 6-day exposure to 35°C (Martins et al., 2020). Furthermore, grxs17 knockout mutants and 555 556 RNAi lines displayed an enhanced sensitivity to a restrictive temperature of 28°C, as 557 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 558 growth defects including leaf curling, leafy shoots and malformed ovules (Cheng et al., 2011). 559 560 In contrast, an enhanced sensitivity of the mutant was not observed under short- and long-561 term acquired thermotolerance regimes, indicating the involvement of GRXS17 in responses 562 to specific heat stress scenarios only (Martins et al., 2020). The authors demonstrated that 563 under these conditions, GRXS17 protects both shoot and root apical meristems and that this 564 effect depends on cysteine residues in its active site. Furthermore, they showed that GRXS17 565 interacts with different sets of proteins under control and heat stress conditions, suggesting its 566 involvement in protecting proteins against the negative consequences of moderate heat stress via a redox-dependent chaperone activity (Martins et al., 2020). 567

568 The involvement of GRXs in thermotolerance has also been observed in yeast, where a grx3569 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 570 571 by heterologous expression of the A. thaliana GRXS17 (Wu et al., 2012). Overexpression of 572 AtGRXS17 also conferred heat and cold tolerance in tomato plants. Using GFP fusion 573 proteins, the authors demonstrated that GRXS17 migrated from the cytosol into the nucleus 574 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 575 576 in comparison to wild-type plants upon heat stress (Wu et al., 2012). Hence, GRXS17 and 577 potentially other GRXs might be interesting targets in the search for strategies to enhance 578 plant resistance to elevated temperatures and their protective properties in heat-exposed plants 579 should be further investigated in future studies.

580

581 *Copper-related MIR398 plays a key role in plant thermotolerance*

Besides Fe-related processes, also mechanisms involved in the regulation of Cu homeostasis 582 583 have been suggested to play a role in plant thermotolerance. Guan et al. (2013) reported that heat stress rapidly induces the expression of microRNA398 (MIR398) in A. thaliana, which 584 585 subsequently reduces transcript levels of the Cu chaperone CCS and the SOD-encoding genes 586 *CSD1* and *CSD2*. This mechanism likely contributes to thermotolerance, as transgenic plants 587 that express MIR398-resistant forms of CCS, CSD1 and CSD2 display an enhanced sensitivity 588 to heat stress in comparison to their counterparts which express the normal coding sequences 589 of these genes. Furthermore, knockout mutants of these genes were characterised by an 590 increased tolerance to heat stress. Chromatin immunoprecipitation assays revealed that 591 HSFA1b and HSFA7b bind directly to the promoter region of *MIR398* in response to heat 592 stress. Via a feedback loop, MIR398 can in turn positively affect the expression of HSFA7b as 593 well as other HSF-encoding genes. The authors proposed that the reduced activity of CSDs in 594 response to the MIR398-mediated reduction of their transcript levels causes the accumulation 595 of ROS. The subsequent alteration of the cellular redox state can then be either directly or 596 indirectly sensed by specific HSFs to regulate the expression of other HSFs and HSPs (Guan 597 et al., 2013). A recent study by Li et al. (2020) provided more insight into the regulation of 598 MIR398. They showed that the *cis*-natural antisense transcripts of MIR398b/c genes repress 599 the processing of their pre-miRNAs. Furthermore, they demonstrated that these natural 600 antisense transcripts were activated in response to MIR398b and MIR398c overexpression, 601 thereby constituting a regulatory feedback loop that attenuates thermotolerance (Li et al., 602 2020). As a heat-induced upregulation of MIR398 was also observed in maize, manipulation 603 of MIR398 and/or its target genes could provide an interesting strategy to improve the heat 604 tolerance of economically important crop species (Guan et al., 2013).

605

606 Conclusion and perspectives

607 Micronutrients play key roles in a wide range of physiological processes and stress responses. 608 It has been shown that heat stress affects micronutrient uptake in plants and that plants with 609 altered micronutrient levels display an altered thermosensitivity. The involvement of metal 610 micronutrients in plant responses to heat stress seems to be strongly intertwined with their 611 role in redox homeostasis (Fig. 2). Although several enzymes containing trace metals have 612 been associated with temperature responses, in-depth knowledge on the underlying 613 mechanisms is largely lacking. Nevertheless, it is of crucial importance to further elucidate 614 how micronutrients influence plant tolerance to elevated temperatures, as heat stress becomes 615 more prevalent as a consequence of global warming and nutrient levels and bioavailability in 616 agricultural soils are often sub-optimal.

617 In this context, many questions remain to be answered in the future. Several studies revealed 618 that heat stress influences trace metal uptake and translocation (Table 1). The fact that heat 619 stress-induced effects on micronutrient and macronutrient concentrations often follow a 620 similar pattern suggests a general impact on plant nutrient uptake capacity, for example as a 621 consequence of changes in root morphology or enzyme activity. Nevertheless, it cannot be 622 excluded that heat influences plant concentrations of specific trace metals by affecting 623 proteins involved in their homeostasis at the transcriptional, translational and/or post-624 translational level. The use of a multi-omics approach in future studies will help address this 625 question. Furthermore, it would be of particular interest to investigate the contribution of 626 alternative splicing to trace metal homeostasis during heat stress. To enable distinguishing the 627 effects of temperature from those of other environmental variables, plant growth and heat 628 stress treatments should be conducted under highly controlled conditions. The importance of 629 Zn in plant thermotolerance was highlighted by several studies investigating heat stress 630 responses under Zn-deficient conditions or upon treatment with additional Zn. Similar studies 631 with other trace metals are needed to further unravel their role in plant heat stress responses. 632 Moreover, it would be highly interesting to compare the thermotolerance of trace metal 633 hyperaccumulating plants with that of their non-hyperaccumulating relatives. As trace metal 634 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.

646

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653 Author contributions

654 SH, NV, AC, and AJM: conceptualization; writing – revision and editing; SH: data collection, 655 writing – original draft. All authors have read and approved the final version of the 656 manuscript.

657

658 Conflict of interest

The authors declare no conflict of interest.

References

Abd El-Naby S, Esmail A, Baiea M, Amin O, Abdelkhalek Ahmed Mohamed A. 2020. Mitigation of heat stress effects by using shade net on Washington Navel orange trees grown in Al-Nubaria region, Egypt. Acta Scientiarum Polonorum Hortorum Cultus **19**, 15-24.

Agarwal P, Khurana P. 2018. Characterization of a novel zinc finger transcription factor (TaZnF) from wheat conferring heat stress tolerance in *Arabidopsis*. Cell Stress Chaperones **23**, 253-267.

Aguilera A, Berdun F, Bartoli C, Steelheart C, Alegre M, Salerno G, Pagnussat G, Martin MV. 2019. Heat stress induces ferroptosis in a photosynthetic prokaryote. bioRxiv, 828293. doi: 10.1101/828293

Alejandro S, Höller S, Meier B, Peiter E. 2020. Manganese in plants: from acquisition to subcellular allocation. Frontiers in Plant Science 11, 300.

Andrási N, Pettkó-Szandtner A, Szabados L. 2020. Diversity of plant heat shock factors: regulation, interactions, and functions. Journal of Experimental Botany 72, 1558-1575.

Asada K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiology **141**, 391-396.

Ashraf M. 2021. Thermotolerance in plants: Potential physio-biochemical and molecular markers for crop improvement. Environmental and Experimental Botany 186, 104454.

Asseng S, Ewert F, Martre P, *et al.* 2015. Rising temperatures reduce global wheat production. Nature Climate Change 5, 143-147.

Bita CE, Gerats T. 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Frontiers in Plant Science **4**, 273.

Bonham-Smith PC, Kapoor M, Bewley JD. 1987. Establishment of thermotolerance in maize by exposure to stresses other than a heat shock does not require heat shock protein synthesis. Plant Physiology **85**, 575-580.

Buckner E, Madison I, Chou H, Matthiadis A, Melvin CE, Sozzani R, Williams C, Long TA. 2019. Automated imaging, tracking, and analytics pipeline for differentiating environmental effects on root meristematic cell division. Frontiers in Plant Science 10, 1487.

Cabral C, Ravnskov S, Tringovska I, Wollenweber B. 2016. Arbuscular mycorrhizal fungi modify nutrient allocation and composition in wheat (*Triticum aestivum* L.) subjected to heat-stress. Plant and Soil **408**, 385-399.

Casal JJ, Balasubramanian S. 2019. Thermomorphogenesis. Annual Review or Plant Biology **70**, 321-346.

Chaturvedi P, Wiese AJ, Ghatak A, Záveská Drábková L, Weckwerth W, Honys D. 2021. Heat stress response mechanisms in pollen development. New Phytologist 231, 571-585.

Chen P-Y, Lee K-T, Chi W-C, Hirt H, Chang C-C, Huang H-J. 2008. Possible involvement of MAP kinase pathways in acquired metal-tolerance induced by heat in plants. Planta 228, 499.

Cheng N-H, Liu J-Z, Liu X, *et al.* 2011. *Arabidopsis* monothiol glutaredoxin, AtGRXS17, is critical for temperature-dependent postembryonic growth and development via modulating auxin response. The Journal of Biological Chemistry **286**, 20398-20406.

Choudhury FK, Rivero RM, Blumwald E, Mittler R. 2017. Reactive oxygen species, abiotic stress and stress combination. The Plant Journal 90, 856-867.

Choukri H, Hejjaoui K, El-Baouchi A, El haddad N, Smouni A, Maalouf F, Thavarajah D, Kumar S. 2020. Heat and drought stress impact on phenology, grain yield, and nutritional quality of lentil (*Lens culinaris* Medikus). Frontiers in Nutrition **7**, 596307.

Chung BYW, Balcerowicz M, Di Antonio M, Jaeger KE, Geng F, Franaszek K, Marriott P, Brierley I, Firth AE, Wigge PA. 2020. An RNA thermoswitch regulates daytime growth in *Arabidopsis*. Nature Plants 6, 522-532.

Clemens S. 2019. Metal ligands in micronutrient acquisition and homeostasis. Plant, Cell & Environment 42, 2902-2912.

Compant S, Samad A, Faist H, Sessitsch A. 2019. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. Journal of Advanced Research 19, 29-37.

Couturier J, Przybyla-Toscano J, Roret T, Didierjean C, Rouhier N. 2015. The roles of glutaredoxins ligating Fe-S clusters: Sensing, transfer or repair functions? Biochimica et Biophysica Acta 1853, 1513-1527.

Covington MF, Maloof JN, Straume M, Kay SA, Harmer SL. 2008. Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. Genome Biology **9**, R130.

Dangol S, Chen Y, Hwang BK, Jwa NS. 2019. Iron- and reactive oxygen species-dependent ferroptotic cell death in rice-magnaporthe oryzae interactions. The Plant Cell **31**, 189-209.

Darawsheh MK, Zerva G, Bouranis DL. 2006. Season-dependent fruit loading: effect on nutrient homeostasis of tomato plants. Journal of Plant Nutrition 29, 505-519.

Deng Y, Srivastava R, Quilichini TD, Dong H, Bao Y, Horner HT, Howell SH. 2016. IRE1, a component of the unfolded protein response signaling pathway, protects pollen development in *Arabidopsis* from heat stress. The Plant Journal **88**, 193-204.

Depaepe T, Hendrix S, van Rensburg HCJ, Van den Ende W, Cuypers A, Van Der Straeten D. 2021. At the crossroads of survival and death: the reactive oxygen species-ethylene-sugar triad and the unfolded protein response. Trends in Plant Science **26**, 338-351.

Dias AS, Lidon FC. 2009. II. Heat stress in Triticum: kinetics of Cu and Zn accumulation. Brazilian Journal of Plant Physiology **21**, 135-142.

Dias AS, Lidon FC, Ramalho JC. 2009. IV. Heat stress in Triticum: kinetics of Fe and Mn accumulation. Brazilian Journal of Plant Physiology **21**, 153-164.

Distéfano AM, López GA, Setzes N, Marchetti F, Cainzos M, Cascallares M, Zabaleta E, Pagnussat GC. 2021. Ferroptosis in plants: triggers, proposed mechanisms, and the role of iron in modulating cell death. Journal of Experimental Botany 72, 2125-2135.

Distéfano AM, Martin MV, Córdoba JP, et al. 2017. Heat stress induces ferroptosis-like cell death in plants. Journal of Cell Biology 216, 463-476.

Dixit AR, Dhanker OP. 2011. A novel stress-associated protein 'AtSAP10' from *Arabidopsis thaliana* confers tolerance to nickel, manganese, zinc, and high temperature stress. PLoS ONE **6**, e20921.

Dixon SJ, Lemberg KM, Lamprecht MR, et al. 2012. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 149, 1060-1072.

Dong C, He F, Berkowitz O, *et al.* 2018. Alternative splicing plays a critical role in maintaining mineral nutrient homeostasis in rice (*Oryza sativa*). The Plant Cell **30**, 2267-2285.

Du YC, Tachibana S. 1994. Effect of supraoptimal root temperature on the growth, root respiration and sugar content of cucumber plants. Scientia Horticulturae **58**, 289-301.

Dumanović J, Nepovimova E, Natić M, Kuča K, Jaćević V. 2021. The significance of reactive oxygen species and antioxidant defense system in plants: a concise overview. Frontiers in Plant Science **11**, 552969.

Feng H, Stockwell BR. 2018. Unsolved mysteries: How does lipid peroxidation cause ferroptosis? PLoS Biology 16, e2006203.

Gao F, Han X, Wu J, Zheng S, Shang Z, Sun D, Zhou R, Li B. 2012. A heat-activated calcium-permeable channel – Arabidopsis cyclic nucleotide-gated ion channel 6 – is involved in heat shock responses. The Plant Journal **70**, 1056-1069.

Giri A, Heckathorn S, Mishra S, Krause C. 2017. Heat stress decreases levels of nutrientuptake and -assimilation proteins in tomato roots. Plants 6, 6.

Guan Q, Lu X, Zeng H, Zhang Y, Zhu J. 2013. Heat stress induction of miR398 triggers a regulatory loop that is critical for thermotolerance in *Arabidopsis*. The Plant Journal **74**, 840-851.

Gupta PK, Balyan HS, Sharma S, Kumar R. 2021. Biofortification and bioavailability of Zn, Fe and Se in wheat: present status and future prospects. Theoretical and Applied Genetics **134**, 1-35.

Haider S, Iqbal J, Naseer S, Yaseen T, Shaukat M, Bibi H, Ahmad Y, Daud H, Abbasi NL, Mahmood T. 2021. Molecular mechanisms of plant tolerance to heat stress: current landscape and future perspectives. Plant Cell Reports. doi: https://doi.org/10.1007/s00299-021-02696-3.

Han G, Lu C, Guo J, Qiao Z, Sui N, Qiu N, Wang B. 2020a. C2H2 zinc finger proteins: master regulators of abiotic stress responses in plants. Frontiers in Plant Science 11, 115.

Han W, Huang L, Owojori OJ. 2020b. Foliar application of zinc alleviates the heat stress of pakchoi (*Brassica chinensis* L.). Journal of Plant Nutrition **43**, 194-213.

Hänsch R, Mendel RR. 2005. Sulfite oxidation in plant peroxisomes. Photosynthesis Research 86, 337-343.

Hayes S, Schachtschabel J, Mishkind M, Munnik T, Arisz SA. 2020. Hot topic: Thermosensing in plants. Plant, Cell & Environment 44, 2018-2033.

Heckathorn SA, Mueller JK, Laguidice S, Zhu B, Barrett T, Blair B, Dong Y. 2004. Chloroplast small heat-shock proteins protect photosynthesis during heavy metal stress. American Journal of Botany 91, 1312-1318.

Hoppen C, Müller L, Hänsch S, Uzun B, Milić D, Meyer AJ, Weidtkamp-Peters S, Groth G. 2019. Soluble and membrane-bound protein carrier mediate direct copper transport to the ethylene receptor family. Scientific Reports 9, 10715.

Hu S, Ding Y, Zhu C. 2020. Sensitivity and responses of chloroplasts to heat stress in plants. Frontiers in Plant Science 11, 375.

Hu Y, Wu Q, Sprague SA, *et al.* 2015. Tomato expressing *Arabidopsis* glutaredoxin gene *AtGRXS17* confers tolerance to chilling stress via modulating cold responsive components. Horticulture Research 2, 15051.

Huang H, Ullah F, Zhou D-X, Yi M, Zhao Y. 2019. Mechanisms of ROS regulation of plant development and stress responses. Frontiers in Plant Science 10, 800.

Huang J, Wang MM, Jiang Y, Bao YM, Huang X, Sun H, Xu DQ, Lan HX, Zhang HS. 2008. Expression analysis of rice A20/AN1-type zinc finger genes and characterization of ZFP177 that contributes to temperature stress tolerance. Gene **420**, 135-144.

Huang J, Zhao X, Bürger M, Wang Y, Chory J. 2021. Two interacting ethylene response factors regulate heat stress response. The Plant Cell **33**, 338-357.

Impa SM, Perumal R, Bean SR, John Sunoj VS, Jagadish SVK. 2019. Water deficit and heat stress induced alterations in grain physico-chemical characteristics and micronutrient composition in field grown grain sorghum. Journal of Cereal Science **86**, 124-131.

John S, Olas JJ, Mueller-Roeber B. Regulation of alternative splicing in response to temperature variation in plants. Journal of Experimental Botany 72, 6150-6163.

Jung JH, Barbosa AD, Hutin S, et al. 2020. A prion-like domain in ELF3 functions as a thermosensor in *Arabidopsis*. Nature 585, 256-260.

Jung JH, Domijan M, Klose C, *et al.* 2016. Phytochromes function as thermosensors in *Arabidopsis*. Science **354**, 886-889.Kaur H, Garg N. 2021. Zinc toxicity in plants: a review. Planta **253**, 129.

Khan A, Ahmad M, Ahmed M, Iftikhar Hussain M. 2020. Rising atmospheric temperature impact on wheat and thermotolerance strategies. Plants 10, 43.

Kim GD, Cho YH, Yoo SD. 2015. Regulatory functions of evolutionarily conserved AN1/A20-like Zinc finger family proteins in *Arabidopsis* stress responses under high temperature. Biochemical and Biophysical Research Communications **457**, 213-220.

Kobayashi T, Nishizawa NK. 2012. Iron uptake, translocation, and regulation in higher plants. Annual Review of Plant Biology **63**, 131-152.

Kollist H, Zandalinas SI, Sengupta S, Nuhkat M, Kangasjärvi J, Mittler R. 2019. Rapid responses to abiotic stress: Priming the landscape for the signal transduction network. Trends in Plant Science 24, 25-37.

Kroh GE, Pilon M. 2020. Regulation of iron homeostasis and use in chloroplasts. International Journal of Molecular Sciences **21**, 3395.

Kumar S, Kumar S, Mohapatra T. 2021. interaction between macro and micro-nutrients in plants. Frontiers in Plant Science **12**, 665583.

Lamers J, van der Meer T, Testerink C. 2020. How plants sense and respond to stressful environments. Plant Physiology 182, 1624-1635.

Lämke J, Brzezinka K, Altmann S, Bäurle I. 2016. A hit-and-run heat shock factor governs sustained histone methylation and transcriptional stress memory. EMBO Journal **35**, 162-175.

León-Sánchez L, Nicolás E, Prieto I, Nortes P, Maestre FT, Querejeta JI. 2020. Altered leaf elemental composition with climate change is linked to reductions in photosynthesis, growth and survival in a semi-arid shrubland. Journal of Ecology **108**, 47-60.

Li W, Lin W-D, Ray P, Lan P, Schmidt W. 2013. Genome-wide detection of conditionsensitive alternative splicing in Arabidopsis roots. Plant Physiology 162, 1750-1763.

Li Y, Li X, Yang J, He Y. 2020. Natural antisense transcripts of *MIR398* genes suppress microR398 processing and attenuate plant thermotolerance. Nature Communications 11, 5351.

Liu J-X, Howell SH. 2016. Managing the protein folding demands in the endoplasmic reticulum of plants. New Phytologist **211**, 418-428.

Maboko MM, Bertling I, Plooy CPD. 2013. Effect of arbuscular mycorrhiza and temperature control on plant growth, yield, and mineral content of tomato plants grown hydroponically. HortScience 48, 1470.

Martins L, Knuesting J, Bariat L, *et al.* 2020. Redox modification of the iron-sulfur glutaredoxin grxs17 activates holdase activity and protects plants from heat stress. Plant Physiology **184**, 676-692.

Martins LD, Tomaz MA, Lidon FC, DaMatta FM, Ramalho JC. 2014. Combined effects of elevated [CO₂] and high temperature on leaf mineral balance in *Coffea* spp. plants. Climatic Change 126, 365-379.

Menzel CM, Simpson DR, Winks CW. 1987. Effect of temperature on growth, flowering and nutrient uptake of three passionfruit cultivars under low irradiance. Scientia Horticulturae **31**, 259-268.

Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G. 2010. Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. Journal of Experimental Botany **61**, 4197-4220.

Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R. 2009. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. Science Signaling 2, ra45.

Ming N, Ma N, Jiao B, Lv W, Meng Q. 2020. Genome wide identification of C2H2-type zinc finger proteins of tomato and expression analysis under different abiotic stresses. Plant Molecular Biology Reporter **38**, 75-94.

Mittler R, Kim Y, Song L, Coutu J, Coutu A, Ciftci-Yilmaz S, Lee H, Stevenson B, Zhu JK. 2006. Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress. FEBS Letters **580**, 6537-6542.

Murcia G, Enderle B, Hiltbrunner A, Casal JJ. 2021. Phytochrome B and PCH1 protein dynamics store night temperature information. The Plant Journal **105**, 22-33.

Murphy A, Taiz L. 1995. Comparison of metallothionein gene expression and nonprotein thiols in ten *arabidopsis* ecotypes (correlation with copper tolerance). Plant Physiology **109**, 945-954.

Ogasawara Y, Kaya H, Hiraoka G, *et al*. 2008. Synergistic activation of the *Arabidopsis* NADPH oxidase AtrobhD by Ca²⁺ and phosphorylation. Journal of Biological Chemistry **283**, 8885-8892.

Peck AW, McDonald GK. 2010. Adequate zinc nutrition alleviates the adverse effects of heat stress in bread wheat. Plant and Soil **337**, 355-374.

Porta H, Rocha-Sosa M. 2002. Plant lipoxygenases. Physiological and molecular features. Plant Physiology **130**, 15-21.

Ravet K, Pilon M. 2013. Copper and iron homeostasis in plants: the challenges of oxidative stress. Antioxidants & Redox Signaling **19**, 919-932.

Ravet K, Touraine B, Boucherez J, Briat JF, Gaymard F, Cellier F. 2009. Ferritins control interaction between iron homeostasis and oxidative stress in *Arabidopsis*. The Plant Journal **57**, 400-412.

Rivero RM, Sánchez E, Ruiz JM, Romero L. 2003. Influence of temperature on biomass, iron metabolism and some related bioindicators in tomato and watermelon plants. Journal of Plant Physiology **160**, 1065-1071.

Routaboul JM, Skidmore C, Wallis JG, Browse J. 2012. *Arabidopsis* mutants reveal that short- and long-term thermotolerance have different requirements for trienoic fatty acids. Journal of Experimental Botany **63**, 1435-1443.

Sarwar M, Saleem MF, Ullah N, Ali S, Rizwan M, Shahid MR, Alyemeni MN, Alamri SA, Ahmad P. 2019. Role of mineral nutrition in alleviation of heat stress in cotton plants grown in glasshouse and field conditions. Scientific Reports 9, 13022.

Schmidt SB, Eisenhut M, Schneider A. 2020. Chloroplast transition metal regulation for efficient photosynthesis. Trends in Plant Science 25, 817-828.

Sharma A, Patni B, Shankhdhar D, Shankhdhar SC. 2013. Zinc - an indispensable micronutrient. Physiology and Molecular Biology of Plants **19**, 11-20.

Sharma M, Banday ZZ, Shukla BN, Laxmi A. 2019. Glucose-regulated HLP1 acts as a key molecule in governing thermomemory. Plant Physiology **180**, 1081-1100.

Shiraya T, Mori T, Maruyama T, Sasaki M, Takamatsu T, Oikawa K, Itoh K, Kaneko K, Ichikawa H, Mitsui T. 2015. Golgi/plastid-type manganese superoxide dismutase involved in heat-stress tolerance during grain filling of rice. Plant Biotechnology Journal 13, 1251-1263.

Smirnoff N, Arnaud D. 2019. Hydrogen peroxide metabolism and functions in plants. New Phytologist **221**, 1197-1214.

Song P, Jia Q, Chen L, *et al.* 2020. Involvement of *Arabidopsis* phospholipase D δ in regulation of ROS-mediated microtubule organization and stomatal movement upon heat shock. Journal of Experimental Botany **71**, 6555-6570.

Steinhorst L, Kudla J. 2013. Calcium and reactive oxygen species rule the waves of signaling. Plant Physiology 163, 471-485.

Talib EA, Outten CE. 2021. Iron-sulfur cluster biogenesis, trafficking, and signaling: Roles for CGFS glutaredoxins and BolA proteins. BBA – Molecular Cell Research **1868**, 118847.

Tan LP, He J, Lee SK. 2002. Effects of root-zone temperature on the root development and nutrient uptake of *Lactuca sativa* L. "Panama" grown in an aeroponic system in the tropics. Journal of Plant Nutrition **25**, 297-314.

Tao Z-q, Wang D-m, Chang X-h, Wang Y-j, Yang Y-s, Zhao G-c. 2018. Effects of zinc fertilizer and short-term high temperature stress on wheat grain production and wheat flour proteins. Journal of Integrative Agriculture 17, 1979-1990.

Thi Tuyet Le C, Brumbarova T, Bauer P. 2019. The interplay of ROS and iron signaling in plants. In: Panda SK, Yamamoto YY, eds. *Redox Homeostasis in Plants: From Signalling to Stress Tolerance*. Cham: Springer International Publishing, 43-66.

Ullah A, Romdhane L, Rehman A, Farooq M. 2019. Adequate zinc nutrition improves the tolerance against drought and heat stresses in chickpea. Plant Physiology and Biochemistry 143, 11-18.

Viciedo DO, Prado RD, Martinez CA, Habermann E, Branco RBF, Piccolo MD, Hurtado AC, Calzada KP, Tenesaca LFL. 2021. Water stress and warming impact nutrient use efficiency of Mombasa grass (*Megathyrsus maximus*) in tropical conditions. Journal of Agronomy and Crop Science 207, 128-138.

Vu LD, Gevaert K, De Smet I. 2019. Feeling the heat: Searching for plant thermosensors. Trends in Plant Science 24, 210-219.

Wang X, Ma X, Wang H, Li B, Clark G, Guo Y, Roux S, Sun D, Tang W. 2015. Proteomic study of microsomal proteins reveals a key role for *Arabidopsis* annexin 1 in mediating heat stress-induced increase in intracellular calcium levels. Molecular & Cellular Proteomics 14, 686-694.

Wang X, Zhao C, Müller C, Wang C, Ciais P, Janssens I, Peñuelas J, Asseng S, Li T, Elliott J, Huang Y, Li L, Piao S. 2020. Emergent constraint on crop yield response to warmer temperature from field experiments. Nature Sustainability 3, 908-916.

Waszczak C, Carmody M, Kangasjärvi J. 2018. Reactive oxygen species in plant signaling. Annual Review of Plant Biology **29**, 209-236.

Wu Q, Lin J, Liu JZ, *et al.* 2012. Ectopic expression of *Arabidopsis* glutaredoxin *AtGRXS17* enhances thermotolerance in tomato. Plant Biotechnology Journal **10**, 945-955.

Wu Q, Yang J, Cheng N, Hirschi KD, White FF, Park S. 2017. Glutaredoxins in plant development, abiotic stress response, and iron homeostasis: From model organisms to crops. Environmental and Experimental Botany 139, 91-98.

Xi L, Xu K, Qiao Y, Qu S, Zhang Z, Dai W. 2011. Differential expression of ferritin genes in response to abiotic stresses and hormones in pear (*Pyrus pyrifolia*). Molecular Biology Reports **38**, 4405-4413.

Xia Y, Lv Y, Yuan Y, Wang G, Chen Y, Zhang H, Shen Z. 2012. Cloning and characterization of a type 1 metallothionein gene from the copper-tolerant plant *Elsholtzia haichowensis*. Acta Physiologiae Plantarum **34**, 1819-1826.

Yeasmin R, Bonser SP, Motoki S, Nishihara E. 2019. Arbuscular mycorrhiza influences growth and nutrient uptake of asparagus (*Asparagus officinalis* L.) under heat stress. HortScience 54, 846-850.

Zandalinas SI, Fritschi FB, Mittler R. 2021. Global warming, climate change, and environmental pollution: recipe for a multifactorial stress combination disaster. Trends in Plant Science 26, 588-599.

Zang X, Geng X, Wang F, Liu Z, Zhang L, Zhao Y, Tian X, Ni Z, Yao Y, Xin M, Hu Z, Sun Q, Peng H. 2017. Overexpression of wheat ferritin gene *TaFER-5B* enhances tolerance to heat stress and other abiotic stresses associated with the ROS scavenging. BMC Plant Biology **17**, 14.

Zhang Q, Qu Y, Wang Q, Song P, Wang P, Jia Q, Guo J. 2017. *Arabidopsis* phospholipase D alpha 1-derived phosphatidic acid regulates microtubule organization and cell development under microtubule-interacting drugs treatment. Journal of Plant Research **130**, 193-202.

Zhao Y, Shui X, Wang X, Hou Q, Li B, Ni Z, Sun Q, Liang R. 2016. Ectopic expression ofthe Vigna eylindrica ferritin gene enhanced heat tolerance in transgenic wheat (TriticumaestivumL.).Euphytica209,23-30.

Tables

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 \uparrow	Martins et al., 2014
Different shrub species	Leaf	Overall Cu, Fe and Zn \downarrow	Léon-Sánchez et al., 2020
Lens culinaris Medikus	Grains	Fe and Zn ↓	Choukri et al., 2020
Megathyrsus maximus	Leaf	Cu, Fe, Mn and Zn \uparrow	Viciedo et al., 2021
Passiflora edulis f. edulis X P. edulis f. flavicarpa	Shoot	Cu, Fe, Mn and Zn \downarrow	Menzel et al., 1987
Solanum lycopersicum	Root and leaf	Total and free Fe \downarrow	Rivero et al., 2003
Solanum lycopersicum	Stem, root and leaf	Zn translocation \downarrow	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 \downarrow	Maboko et al., 2013
Solanum lycopersicum	Root	Fe uptake rate ↓	Giri et al., 2017
Sorghum bicolor	Grains	Cu, Fe, Mn and Zn \downarrow	Impa et al., 2019
Triticum aestivum	Tillers	Cu, Fe, Mn and Zn \downarrow	Cabral <i>et al.</i> , 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

 \uparrow and \downarrow 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.

Plant species	Micronutrient conditions	Effect	Reference
Cicer arietinum	Zn deficiency	Heat sensitivity ↑	Ullah et al., 2019
Triticum aestivum	Zn deficiency	Heat sensitivity ↑	Peck et al., 2010
Brassica chinensis	Foliar Zn spraying	Heat sensitivity \downarrow	Han et al., 2020b
Gossypium hirsutum	Foliar Zn spraying	Heat sensitivity \downarrow	Sarwar et al., 2019
Triticum aestivum	Zn fertilizer application	Heat sensitivity \downarrow	Tao <i>et al.</i> , 2018
Zea mays	Cu and Zn treatment	Heat sensitivity \downarrow	Bonham-Smith et al., 1987

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

 $\uparrow \text{ and } \downarrow \text{ symbols indicate increases and decreases, respectively. For detailed information on the specific temperature conditions and experimental set-up, readers are referred to the respective publications.$

Figure legends

Fig. 1. Simplified overview of heat-induced signalling responses in plants. Calcium (Ca²⁺), 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 Ca²⁺ influx is mediated by cyclic nucleotide-gated channel 6 (CNCG6) and other Ca^{2+} transporters. Activation of CNGC6 is suggested to be triggered by cyclic AMP (cAMP), generated by adenylyl cyclase. 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 homolog 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 Ca²⁺ binding to its EF hand motifs and phosphorylation by calcium-dependent protein kinases (CDPKs). In addition, H_2O_2 oxidises cysteines in the C2 domain of phospholipase D δ (PLD δ), promoting Ca²⁺ binding. This enzyme generates phosphatidic acid (PA), which together with PIP₂ and IP₃, 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 endoplasmic reticulum are not shown.

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 (H₂O₂) generated in response to heat stress can be converted to hydroxyl radicals ('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 ironsulfur (Fe-S) clusters and oligomerises 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 micro-RNA 398 (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 HSFs. 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.

Figures



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