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1 **Importance of essential trace metals in plant responses to heat stress**

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22

23 **Highlight**

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

26

27 **Abstract**

28 Essential trace metals function as structural components or cofactors in many proteins
29 involved in a wide range of physiological processes in plants. Hence, trace metal deficiency
30 can significantly hamper plant growth and development. On the other hand, excess
31 concentrations of trace metals can also induce phytotoxicity, for example via an enhanced
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
36 security for future generations. Gaining insight into heat stress responses is essential to
37 develop strategies to optimise plant growth and quality under unfavourable temperatures. In
38 this context, trace metals deserve particular attention as they contribute to defence responses
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
42 future research on the interactions between heat stress and trace metals are discussed.

43

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

46

47 **Abbreviations:** CPX: ciclopirox olamine; Fer-1: ferostatin-1; GSH: glutathione; GRX:
48 glutaredoxin; H₂O₂: hydrogen peroxide; HSF: heat shock factor; HSP: heat shock protein; O₂⁻:
49 superoxide; ROS: reactive oxygen species; SOD: superoxide dismutase; UPR: unfolded
50 protein response.

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

60

61 **Essential trace metals**

62 Plants require relatively large quantities of nitrogen (N), phosphorus (P), sulfur (S), potassium
63 (K), calcium (Ca) and magnesium (Mg), which are hence classified as macronutrients (Kumar
64 *et al.*, 2021). In comparison, they only need trace amounts of micronutrients, which apart
65 from chloride (Cl) and boron (B) are all transition or post-transition metals: iron (Fe), zinc
66 (Zn), copper (Cu), manganese (Mn), molybdenum (Mo) and nickel (Ni) (Clemens, 2019).
67 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
71 example, is essential for the activity of more than 300 enzymes belonging to all six major
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
83 (Talib and Outten, 2021). Manganese is essential for photosynthesis, as it is a core component
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
87 divalent cations including Ca, Mg, cobalt (Co), Cu and Zn. Besides the oxygen-evolving
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
101 concentrations. Excessive Mn concentrations, for example, can interfere with the uptake and
102 translocation of other essential elements and disturb chlorophyll production and
103 photosynthesis (Alejandro *et al.*, 2020). Phytotoxic Zn concentrations influence plant growth
104 via interference with auxin biosynthesis and redistribution, water status, mineral nutrition,
105 photosynthesis and respiration. In addition, surplus Zn is able to bind unspecifically to thiol
106 groups in proteins, which alters their structure and enhances their degradation, ultimately
107 triggering senescence. Furthermore, Zn has also been reported to activate lipoxygenase
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
110 properties. Whereas the ability of these metals to donate and accept electrons is crucial for
111 their function in many cellular processes, it is also the reason for their participation in Fenton
112 and Haber-Weiss reactions which enhance the production of highly reactive hydroxyl radicals
113 ($\cdot\text{OH}$) 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
115 proteins when present in excess (Waszczak *et al.*, 2018; Kollist *et al.*, 2019; Smirnov and
116 Arnaud, 2019). To prevent toxicity, plants have developed strategies to fine-tune the delivery
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
121 system consisting of enzymatic and non-enzymatic components to tightly control ROS levels.
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 H_2O_2 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 ,
128 typically depend on an Fe-containing haem cofactor (Mhamdi *et al.*, 2010).

129

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
133 frequently fall prey to different biotic stresses such as pathogen attack and abiotic stresses like
134 drought, salinity, soil contamination with organic and inorganic compounds and unfavourable
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,
137 heat is a stress factor that will become increasingly prevalent in the future (Bita and Gerats,
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 $^{\circ}\text{C}$ 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

144 important source of micronutrients in the human diet and malnutrition due to micronutrient
145 deficiency (mainly Fe and Zn) affects a substantial part of the global population (Gupta *et al.*,
146 2021). Hence, increasing our knowledge on plant responses to heat stress is of crucial
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
150 disturbances of membrane fluidity, cytoskeleton organisation, protein folding, transport
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
155 development is highly sensitive to heat, rendering plants particularly vulnerable to high
156 temperatures during the reproductive stage (Chaturvedi *et al.*, 2021). Furthermore, seed
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.

161 Currently, our knowledge on how plants “feel the heat” is far from complete (Vu *et al.*, 2019).
162 Nevertheless, several players involved in the perception of high temperature have been
163 identified. For example, certain light sensors such as phytochromes are known to play a role
164 in thermosensing (Jung *et al.*, 2016; Lamers *et al.*, 2020; Murcia *et al.*, 2021). Phytochromes
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
167 these light sensors in temperature sensing is explained by the fact that the rate of spontaneous
168 reversal from Pfr to Pr is higher at warmer temperatures (Hayes *et al.*, 2020). A recent study
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
175 plant thermosensing, as Chung *et al.* (2020) demonstrated that increased temperatures alter
176 the mRNA hairpin structure of *PHYTOCHROME INTERACTING FACTOR 7* (*PIF7*, a bHLH
177 transcription factor interacting with phytochromes and regulating plant growth), enhancing
178 its translation. These thermosensing mechanisms are 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
181 growth and induction of flowering (Casal and Balasubramanian, 2019; Hayes *et al.*, 2020).

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
185 unclear how plants sense such extreme temperatures, accumulation of unfolded proteins and
186 alterations in membrane fluidity have been proposed to play key roles (Hayes *et al.*, 2021).
187 Signalling pathways operating in heat-stressed plants can be triggered by activation of Ca^{2+}
188 channels at the plasma membrane. In *Arabidopsis thaliana*, heat induces an increase in cyclic
189 AMP (cAMP) levels, which triggers Ca^{2+} influx into the cytosol via cyclic nucleotide-gated

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.*,
193 2020). Other Ca²⁺ channels besides CNGC6 are likely involved in Ca²⁺ influx as well, but
194 have not been identified so far (Hayes *et al.*, 2020). The elevated cytosolic Ca²⁺
195 concentrations can subsequently recruit annexin 1 (ANN1) to membranes where it could
196 either form a Ca²⁺ channel itself or activate other Ca²⁺ channels to further enhance Ca²⁺
197 influx, required to trigger multiple downstream signalling pathways (Wang *et al.*, 2015).
198 Besides Ca²⁺, also ROS play key roles as signalling molecules in heat stress responses. Heat
199 induces the activation of respiratory burst oxidase homolog D (RBOHD), a plasma
200 membrane-localised NADPH oxidase that catalyses the formation of O₂⁻ in the apoplast. The
201 produced O₂⁻ is subsequently converted to H₂O₂, which can enter cells and activate multiple
202 signalling pathways such as those mediated by mitogen-activated protein kinases (MAPKs)
203 (Miller *et al.*, 2009; Haider *et al.*, 2021). Interestingly, ROS and Ca²⁺ signals are closely
204 intertwined, as RBOHD activation depends on Ca²⁺ binding to its EF hand motifs as well as
205 phosphorylation by calcium-dependent protein kinases (CDPKs) (Ogasawara *et al.*, 2008;
206 Steinhorst and Kudla, 2013). Lipid signals, which also play crucial roles in plant responses to
207 heat stress, depend on ROS and Ca²⁺ as well. In heat-stressed guard cells, H₂O₂ oxidises
208 cysteine residues in the C2 domain of phospholipase Dδ (PLDδ), which subsequently
209 promotes Ca²⁺ binding to this enzyme, resulting in microtubule depolymerisation, which in
210 turn influences stomatal movement (Zhang *et al.*, 2017; Song *et al.*, 2020). Furthermore,
211 PLDδ 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
215 triggered by heat-induced Ca²⁺, ROS and lipid signals ultimately induce transcriptional
216 responses, many of which are the result of activation of heat shock factors (HSFs) (Hayes *et al.*,
217 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
227 plant UPR consists of two arms that depend on the basic leucine zipper 28 (bZIP28)
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).

234 Besides transcriptional responses, heat stress is also known to induce epigenetic alterations. It
235 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
238 remodelling and microRNAs (miRNAs) are involved in heat stress memory (Haider *et al.*,
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
245 increased temperatures is provided in Fig. 1. For a detailed overview of recent insights into
246 plant heat stress responses, readers are referred to Haider *et al.* (2021) and Hayes *et al.*
247 (2020).

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

253

254 **Heat stress influences plant metal micronutrient levels**

255 Research has demonstrated that increased temperatures affect Cu, Fe, Mn and Zn
256 concentrations in a wide variety of plant species (Table 1). The studies performed in this
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
260 greenhouse during different seasons (Darawsheh *et al.*, 2006). Whereas in some studies,
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.

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

274 León-Sánchez *et al.* (2020) proposed that the desiccating effect of heat could induce drying of
275 the fertile top soil, thereby lowering nutrient availability. In addition, increased temperatures
276 might cause roots to grow into deeper soil layers, which contain more water but are less
277 fertile. Finally, the authors suggest that heat-induced disturbances of photosynthesis can result
278 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
281 consequence of so-called ‘growth dilution’ when plant growth is favoured in response to
282 small temperature increases (Menzel *et al.*, 1987; Darawsheh *et al.*, 2006; León-Sánchez *et al.*,
283 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,
287 reported that during winter the extractable Zn concentration in the top part of the stem of
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 al.*,
292 2014).

293 Interestingly, several studies have addressed the impact of increased temperatures in the root
294 zone only. Du and Tachibana (1994) investigated the effects of supra-optimal root zone
295 temperatures on cucumber plants via the use of a heated nutrient solution. Their results
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
305 proposed that this could in turn affect root nutrient uptake dynamics. Nevertheless, the
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
310 effects on proteins involved in their uptake, assimilation and metabolism. For example, Giri *et al.*
311 (2017) demonstrated that exposure of tomato plants to a temperature of 42°C for 6 days
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
318 particularly interesting to investigate whether alternative splicing of mRNAs encoding
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
321 splicing of primary transcripts of many genes, resulting in their degradation or translation to
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

324 (Dong *et al.*, 2018) and Fe deficiency led to alternative splicing of mRNAs encoding proteins
325 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; Viciado *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 Mn-
348 dependent SOD of rice plants, plays a key role in their thermotolerance. Rice MSD1 has been
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
352 grain quality than wild-type plants when grown under heat stress. The authors infer that
353 constitutive high expression of Golgi/plastid-type *MSD1* improves the detoxification of O₂⁻
354 together with increased formation of H₂O₂, 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),
361 ethylene signalling positively affects basal thermotolerance in *A. thaliana*. Other important
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
370 proteins in heat stress tolerance was also demonstrated in several other species. For example,
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
384 and Dhanker, 2011).

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,
387 studies investigating the effects of metal micronutrients on plant responses to heat stress have
388 mainly focussed on Zn. Ullah *et al.* (2019) showed that the sensitivity of *Cicer arietinum* to
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
406 kg⁻¹ (Tao *et al.*, 2018).

407 Bonham-Smith *et al.* (1987) demonstrated that prior treatment with Zn as well as Cu reduced
408 the sensitivity of maize seedlings to subsequent heat exposure. Interestingly, a similar effect
409 was observed when plants were pre-treated with cadmium (Cd), a non-essential element
410 which is already toxic at low concentrations. As such, it can be speculated that a treatment
411 with excess levels of trace metals induces phytotoxicity, for example via increased ROS
412 production, which triggers a defence response that 'primes' the plant and reduces its
413 sensitivity to subsequent stress exposures. This priming response might comprise enhanced

414 antioxidant production and activity, but could also involve other compounds, as considerable
415 overlap exists between defence responses to heat and metal stress. Heat shock proteins, for
416 example, are known to play roles in plant defence against a wide variety of stress conditions
417 including metal stress (Heckathorn *et al.*, 2004). Interestingly, the observed cross-tolerance
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 H₂O₂ 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
433 by treatment with the Fe chelator ferrozine) and heat stress negatively influenced *A. thaliana*
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

456 *Heat-induced ferroptosis-like cell death in plants*

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
460 cystine/glutamate antiporter system X_c⁻. Ferroptotic cell death is characterised by its
461 dependence on ROS, intracellular Fe and peroxidation of membrane lipids, causing the
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
468 promote lipid peroxidation via two mechanisms. First, the Fenton reaction of Fe²⁺ with H₂O₂
469 leads to the formation of hydroxyl radicals, which are highly reactive towards poly-
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 H₂O₂ 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
501 proteins involved in photosynthetic electron transfer reactions depend on Fe as a cofactor.
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
505 cues. In addition, these organelles contain ferritins, which are multimeric proteins able to
506 store large numbers of Fe atoms in their central cavity (Ravet *et al.*, 2009). The importance of
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
510 *Vigna cylindrica* *FER* gene was shown to improve the thermotolerance of wheat plants. This
511 effect was likely related to an enhanced membrane stability, as heat-induced increases in the
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,
514 expression levels of the wheat ferritin gene *TaFER-5B* were increased in response to heat
515 stress and overexpression of this gene resulted in an enhanced thermotolerance of wheat
516 plants. Heterologous expression *TaFER-5B* in *A. thaliana* was able to rescue the heat-
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 H₂O₂
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
522 Fe-dependent formation of highly reactive hydroxyl radicals under a wide variety of stress
523 conditions associated with an oxidative imbalance.

524 Although ferroptosis has been shown to play a key role in plant responses to heat stress, this
525 research field is still in its infancy and the mechanisms regulating plant ferroptosis are still
526 largely unknown. Future characterisation of the mechanisms underlying heat-induced
527 ferroptosis might aid in the development of strategies to prevent this form of heat-induced cell
528 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
533 thioredoxin superfamily. They are further subdivided into different classes based on the amino
534 acid sequence found in their active site. The *Arabidopsis* genome encodes 50 GRXs
535 belonging to five different classes. In addition to the role of GRXs in reducing
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
540 generally able to accept and transfer Fe-S clusters to target proteins, whereas class I GRXs are
541 not (Martins *et al.*, 2020).

542 In *Arabidopsis*, GRXS17 is involved in temperature-dependent post-embryonic growth and
543 development (Cheng *et al.*, 2011; Martins *et al.* 2020). This class II GRX is localised in the
544 nucleus and cytosol and contains three GRX domains with a CGFS motif in their active site
545 that coordinate three Fe-S clusters in a GSH-dependent manner. When exposed to H₂O₂, the
546 reconstituted holo-form of GRXS17 was shown to lose its Fe-S clusters, indicating its

547 sensitivity to oxidation. Interestingly, simultaneous treatment with H₂O₂ and an increased
548 temperature of 35°C caused an acceleration of the Fe-S cluster loss from the protein, whereas
549 heat treatment alone did not affect the stability of the cluster. Together, these data indicate
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
555 6-day exposure to 35°C (Martins *et al.*, 2020). Furthermore, *grxs17* knockout mutants and
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
558 grown at a milder temperature of 25°C, *GRXS17* loss-of-function plants showed severe
559 growth defects including leaf curling, leafy shoots and malformed ovules (Cheng *et al.*, 2011).
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
567 via a redox-dependent chaperone activity (Martins *et al.*, 2020).

568 The involvement of GRXs in thermotolerance has also been observed in yeast, where a *grx3*
569 *grx4* double knockout mutant showed an enhanced sensitivity to heat shock. Interestingly, the
570 survival rate of this mutant in response to heat stress as well as oxidative stress was improved
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
575 of *HSF* and *HSP*-encoding genes were significantly increased in *GRXS17*-overexpressing plants
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*

582 Besides Fe-related processes, also mechanisms involved in the regulation of Cu homeostasis
583 have been suggested to play a role in plant thermotolerance. Guan *et al.* (2013) reported that
584 heat stress rapidly induces the expression of microRNA398 (*MIR398*) in *A. thaliana*, which
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

635 are required to unravel whether combined exposure to both stress types has cumulative effects
636 on the accumulation of ROS and unfolded proteins and how this influences signalling
637 pathways and downstream responses. Based on the significant overlap between plant
638 responses to heat and metal toxicity, cross-tolerance to both stressors also deserves further
639 attention in future studies.

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

646

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652

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**

659 The authors declare no conflict of interest.

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

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

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

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

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

Figure legends

Fig. 1. Simplified overview of heat-induced signalling responses in plants. Calcium (Ca^{2+}), reactive oxygen species (ROS) and lipid signals are key players in plant responses to heat stress. Upon heat stress perception (via largely unidentified mechanisms), cytosolic Ca^{2+} influx is mediated by cyclic nucleotide-gated channel 6 (CNGC6) and other Ca^{2+} transporters. Activation of CNGC6 is suggested to be triggered by 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 Ca^{2+} 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 Ca^{2+} binding. This enzyme generates phosphatidic acid (PA), which together with PIP_2 and IP_3 , constitutes an important lipid signal involved in plant heat stress signalling. Different signals trigger a mitogen-activated protein kinase (MAPK) cascade, which ultimately causes activation of heat shock factors (HSFs). These transcription factors regulate the expression of other HSFs as well as heat shock proteins (HSPs) that function as protein chaperones. Accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum (ER), also known as “ER stress”, triggers the unfolded protein response (UPR) mediated by bZIP28 and bZIP60 transcription factors. In addition to transcriptional changes, epigenetic modifications are also implied in plant responses to heat stress. Organelles except the nucleus and the 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 (H_2O_2) generated in response to heat stress can be converted to hydroxyl radicals ($\cdot\text{OH}$) via the Fenton reaction. These radicals are highly reactive towards poly-unsaturated fatty acids in lipid membranes and hence induce lipid peroxidation, ultimately causing ferroptosis-like cell death, which reduces the survival rate of heat-stressed plants. Middle: In response to increased temperatures, glutaredoxin S17 (GRXS17) loses its iron-sulfur (Fe-S) clusters and 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

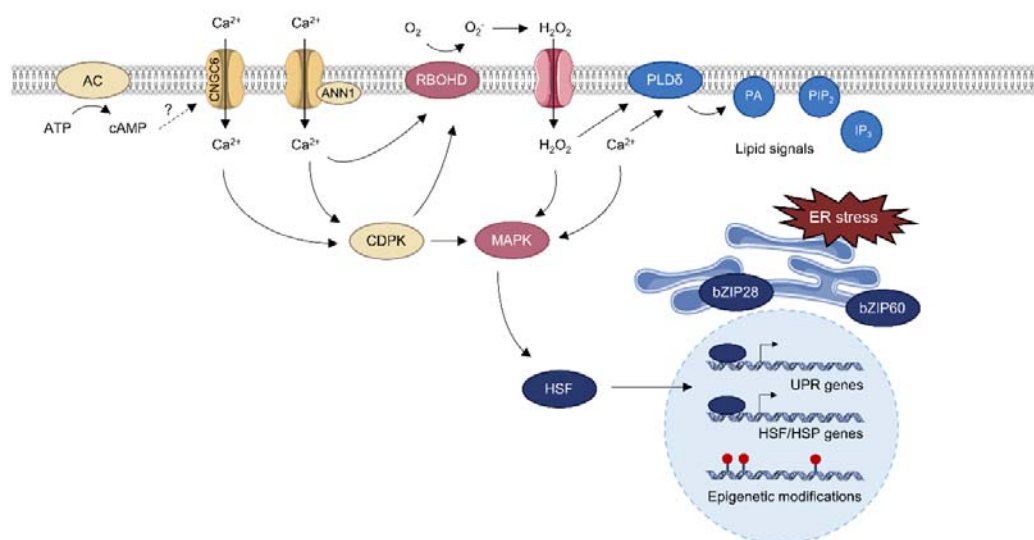


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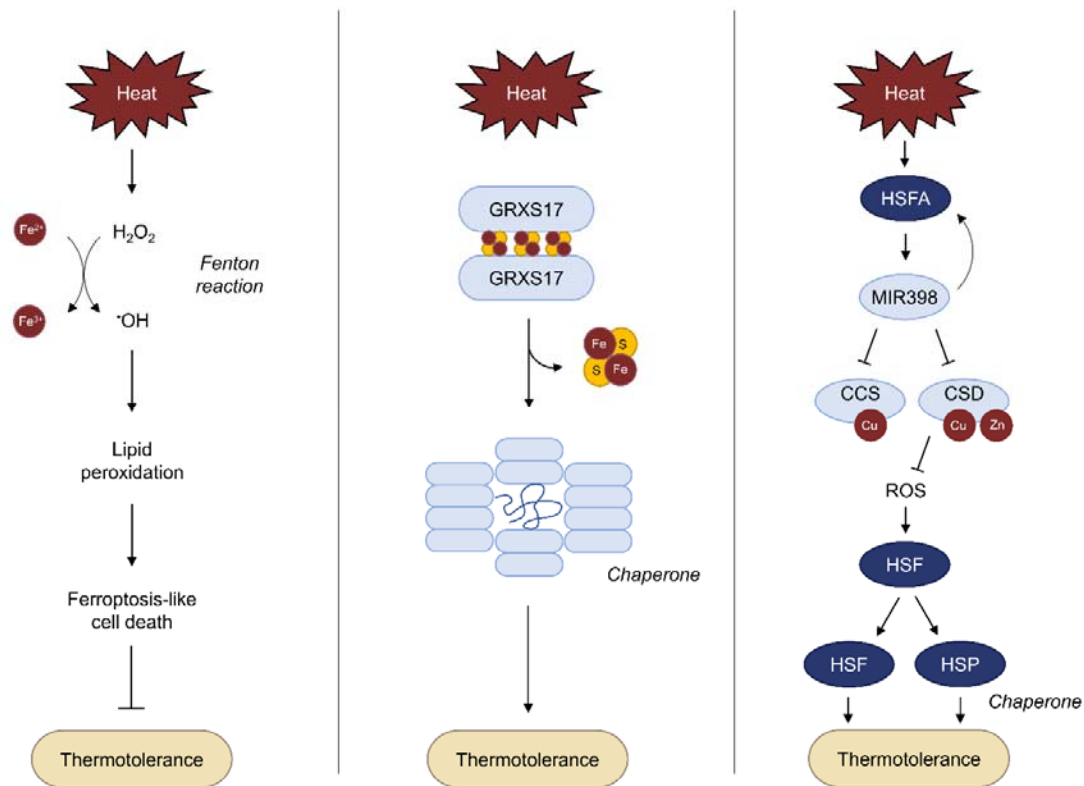


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