



Micronutrient homeostasis in plants for more sustainable agriculture and healthier human nutrition

Ana G L Assunção, Ismail Cakmak, Stephan Clemens, Manuel González-Guerrero, Adam Nawrocki, Sébastien Thomine

► To cite this version:

Ana G L Assunção, Ismail Cakmak, Stephan Clemens, Manuel González-Guerrero, Adam Nawrocki, et al.. Micronutrient homeostasis in plants for more sustainable agriculture and healthier human nutrition. *Journal of Experimental Botany*, 2022, 73 (6), pp.1789 - 1799. 10.1093/jxb/erac014 . hal-03866385

HAL Id: hal-03866385

<https://hal.science/hal-03866385>

Submitted on 22 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

REVIEW PAPER

Micronutrient homeostasis in plants for more sustainable agriculture and healthier human nutrition

Ana G.L. Assunção^{1,2, ID}, Ismail Cakmak^{3, ID}, Stephan Clemens^{4, ID}, Manuel González-Guerrero^{5, ID}, Adam Nawrocki⁶, and Sébastien Thomine^{7,*, ID}

¹ Department of Plant and Environmental Sciences, University of Copenhagen, 1871 Frederiksberg, Denmark

² CIBIO-InBIO, Research Centre in Biodiversity and Genetic Resources, University of Porto, 4485-661 Vairão, Portugal

³ Faculty of Engineering and Natural Sciences, Sabanci University, 34956 Istanbul, Turkey

⁴ Department of Plant Physiology and Faculty of Life Sciences: Food, Nutrition and Health, University of Bayreuth, 95440 Bayreuth, Germany

⁵ Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Universidad Politécnica de Madrid, 28223 Pozuelo de Alarcón (Madrid), Spain

⁶ PPC ADOB, 61-070 Poznań, Poland

⁷ Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, 91198 Gif-sur-Yvette, France

* Correspondence: sebastien.thomine@i2bc.paris-saclay.fr

Received 30 July 2021; Editorial decision 13 January 2022; Accepted 28 January 2022

Editor: Nathalie Verbruggen, Université Libre de Bruxelles, Belgium

Abstract

The provision of sustainable, sufficient, and nutritious food to the growing population is a major challenge for agriculture and the plant research community. In this respect, the mineral micronutrient content of food crops deserves particular attention. Micronutrient deficiencies in cultivated soils and plants are a global problem that adversely affects crop production and plant nutritional value, as well as human health and well-being. In this review, we call for awareness of the importance and relevance of micronutrients in crop production and quality. We stress the need for better micronutrient nutrition in human populations, not only in developing but also in developed nations, and describe strategies to identify and characterize new varieties with high micronutrient content. Furthermore, we explain how adequate nutrition of plants with micronutrients impacts metabolic functions and the capacity of plants to express tolerance mechanisms against abiotic and biotic constraints. Finally, we provide a brief overview and a critical discussion on current knowledge, future challenges, and specific technological needs for research on plant micronutrient homeostasis. Research in this area is expected to foster the sustainable development of nutritious and healthy food crops for human consumption.

Keywords: Essential metals, iron, micronutrients, nitrogen fixation, nutrition, photosynthesis, sustainability, zinc.

Introduction

Plants need a range of essential micronutrients, including (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn), for optimal growth (Marschner, 2012). Animals, including humans,

also require micronutrients to be in good health and for their well-being (Suttle, 2010; Marschner, 2012). Micronutrient deficiencies are a widespread and growing problem in both crop plants and human populations worldwide. Mild or hidden micronutrient deficiencies likely limit crop yields in much wider areas than those where obvious symptoms occur. Insufficient amounts and low bioavailability of micronutrients in plant-based diets represent a major reason for the high prevalence of micronutrient deficiencies in human populations. Generally, there is a geographical overlap between micronutrient deficiencies in cultivated soils and those in human populations (Welch *et al.*, 2014). This indicates a close connection between the micronutrient status of plants and the health of the population. However, compared with other plant mineral nutrients, there is a general lack of concern about micronutrients with respect to both yield sustainability and the nutritional quality of food crops.

The purpose of this review is to raise awareness about the importance of micronutrient supply to plants, not only to improve yield and correct micronutrient deficiencies in humans, but also to improve agricultural sustainability. In this review, using Europe as an example, we first focus on the problem of micronutrient deficiencies in human populations, and the improvement or diversification of food crops needed to alleviate this public health issue. Then, we highlight how knowledge on plant micronutrient homeostasis could be used to improve crop yield and sustainability. Finally, we provide an overview of the knowledge on the molecular and physiological mechanisms of micronutrient homeostasis in plants, focusing mostly on Fe and Zn, and of the specific challenges in this area to address the issues of human nutrition and agricultural sustainability.

Improving plant micronutrient content for human health

Micronutrient deficiencies: not only a problem of developing countries

At the population level, micronutrient deficiencies are a major threat to human health. About one-third of the human population is globally affected by micronutrient deficiencies (also known as ‘hidden hunger’) that result most commonly from low dietary intake, mainly in developing regions where populations rely on plant-based diets from micronutrient-poor cereal crops (Harding *et al.*, 2018; Van Der Straeten *et al.*, 2020). Low dietary intake of micronutrients is also widely reported in European populations, especially among elderly people and children, with significant adverse impacts on health. Micronutrients such as Zn, Fe, selenium (Se), and iodine (I) are required for several critical functions, including cognition, development, the immune response, thyroid function, maintaining antioxidant activity, and the mitigation of chronic diseases (Bailey *et al.*, 2015; Steinbrenner *et al.*, 2015; Read *et al.*, 2019; Kieliszek *et al.*, 2021).

Aging is an unavoidable biological process that is often associated with a decline in physical and mental health. However, age-related impairments in well-being can be greatly alleviated by dietary adjustments (Ruxton *et al.*, 2016). According to European dietary survey studies, the diets commonly consumed by elderly people in Europe are rich in saturated fat, but very low in mineral elements such as magnesium (Mg), Zn, and Cu (Ruxton *et al.*, 2016). A systematic compilation of several databases containing data on over 7200 older adults (≥ 60 years of age) in 13 Western countries [including 10 countries in the European Union (EU)] showed that micronutrient deficiencies are a critical nutritional problem among older adults. Among the eight micronutrients investigated, Zn, Se, Cu, and I deficiencies were prevalent in elderly people. Notably, 31% of women and 49% of men studied were found to consume less dietary Zn than the recommended intake (Vural *et al.*, 2020).

Children are highly vulnerable to micronutrient deficiencies (Van Der Straeten *et al.*, 2020; Vassilakou, 2021). For example, Fe deficiency was found to be prevalent among 12- to 36-month-old children in Germany, the Netherlands, and the UK (Akkermans *et al.*, 2016). Similarly, Fe deficiency was reported in 18-month-old children in Denmark and in children aged 1–11 years in Spain (Andersen *et al.*, 2019; López-Ruzafa *et al.*, 2021). Based on an extensive literature review, Eussen *et al.* (2015) evaluated the relevance of Fe deficiency in European children aged 6–36 months, and showed that it ranged between 3% and 48%, with children living in eastern Europe having a higher prevalence of Fe deficiency than those living in western Europe (Eussen *et al.*, 2015). However, this nutritional problem usually receives little attention or is often overlooked in Europe.

Additionally, the increase in the popularity of vegetarian and vegan diets worldwide will impact nutritional outcomes, especially in terms of micronutrients (Schüpbach *et al.*, 2017; Sebastiani *et al.*, 2019; Bakaloudi *et al.*, 2021). It is estimated that ~5% of the population of Europe and 19% in Asia (Hargreaves *et al.*, 2021) excludes animal products containing a high amount of bioavailable micronutrients in favor of plant products that are rich in antinutrient compounds (e.g. phytate), which reduce micronutrient bioavailability in the human body (Gibson *et al.*, 2018). In addition, the high alcohol consumption that is common in Europe significantly interferes with the intestinal absorption of micronutrients and contributes to Zn, Fe, and Se deficiencies in human populations (Barve *et al.*, 2017; Hillesund *et al.*, 2021).

Towards crops with higher micronutrient content and bioavailability for food and feed

Several strategies can be proposed to achieve better micronutrient delivery through plant-based products: (i) major crops can be bred for higher micronutrient content and availability (Sayre *et al.*, 2011; Ludwig and Slamet-Loedin, 2019); (ii) orphan

or indigenous crop species or macroalgae can be screened for micronutrient content and availability, and introduced into the European diet (García-Casal *et al.*, 2007; Masuda *et al.*, 2015); (iii) plant species with outstanding micronutrient content, such as Zn-hyperaccumulating species, can be cultivated and used as an additive in other foods (Clemens, 2017). The first strategy would be most convenient from an agronomical and social point of view because it is based on crops that are already widely used, but so far this approach has resulted in only small improvements (Connorton and Balk, 2019; Kawakami and Bhullar, 2021). Nonetheless, knowledge is advancing on the regulatory networks of micronutrient homeostasis in plants, which could lead to the discovery of molecular switches that could have a significant impact on micronutrient accumulation. Once such molecular switches have been identified, existing genetic resources (e.g. germplasm collections, natural reserves of biodiversity) may be more efficiently screened for genetic variation that optimizes micronutrient use efficiency and accumulation. The second and third strategies, although probably very efficient, would require the domestication of novel species and changes of dietary habits in the population.

In working towards improving micronutrient bioavailability in the edible parts of crop plants, new challenges for research on micronutrient homeostasis in plants must be met. In particular, knowledge on the mechanisms of micronutrient distribution within the plant, the metal specificity of ligands and transporters, and metal speciation, especially in edible organs, is needed, as detailed below. Moreover, understanding how the chemical forms of micronutrients are altered by processing, such as sprouting, milling, and cooking, and how these changes affect their uptake by the digestive system is essential. To date, there is limited information about these aspects, and further research needs to be performed in close collaboration with nutrition scientists and physicians to determine accurately how crop varieties with improved micronutrient content and availability impact human health (Cakmak *et al.*, 2020).

Improving plant micronutrient status for yield and sustainability

Soil micronutrient deficiency: heterogeneous and not sufficiently documented

Soil micronutrient deficiencies significantly affect crop yield and quality for human consumption in many areas in Europe, with sharp regional differences (Sinclair and Edwards, 2008; Alloway, 2009). For example, Mn deficiency in soils and plants has been reported in the UK and the northern part of Europe (Hebberly *et al.*, 2005; Alejandro *et al.*, 2020), while in south European and Mediterranean countries, Zn and Fe deficiencies are common, although often without causing visible symptoms in plants (Abadía *et al.*, 2004; Rashid and Ryan, 2004; Moreno-Lora and Delgado, 2020). Soil micronutrient

deficiency is also widespread in dryland regions of Europe. In a survey study conducted in six European countries, Portugal, Spain, Italy, Hungary, Greece, and Malta, Moreno-Jiménez *et al.* (2021) showed that 60% and 28% of the investigated soils contained lower than adequate levels of Fe and Zn, respectively. Given that an important fraction of crop production takes place in dryland regions, improving the Fe and Zn nutritional status of crop plants growing in these regions is of great importance. Soil deficiency of phytoavailable Zn and Fe results in important impairments not only in crop productivity but also in crop nutritional quality.

To promote awareness about regional problems of micronutrient deficiencies and facilitate their targeted alleviation, it would be important to map, at a fine scale, the phytoavailable concentrations of mineral micronutrients in soils to identify areas where they limit crop production. Such knowledge would also allow precision agriculture with micronutrient fertilization treatments tailored to the particular conditions. In the context of agroecology, efficient intercropping systems, such as maize and bean, used for centuries in Central America, could be scientifically designed (Lopez-Ridaura *et al.*, 2021). Alternatively, micronutrient-rich green fertilizers could be applied to limit the use of synthetic fertilizers and pesticides. In contrast, soil-free urban or greenhouse intensive agriculture requires the fine tuning of plant micronutrient status through the use of fully controlled nutrient solutions (Eldridge *et al.*, 2020).

The fine mapping of micronutrient availability could be achieved through several complementary means: the mining of soil-related databases; the use of indicator plants, possibly through participative projects; and the design of devices to evaluate *in situ* crop micronutrient status using portable X-ray fluorescence equipment, or remotely, using drone-based hyperspectral analyses (Dobbels and Lorenz, 2019; Qu *et al.*, 2022). The European Soil Data Centre (ESDAC) is the center for soil-related data in Europe and runs a project responsible for a harmonized and regular survey of top soils across all EU member states, named the Land Use/Cover Area frame statistical Survey (LUCAS). This survey represents an attempt to build a consistent spatial database of the soil cover across the EU, based on standard sampling and analytical procedures. In addition to soil properties, the macronutrients, including phosphorus (P), nitrogen (N), and potassium (K), are analyzed. A report on a Cu survey has recently been published within the LUCAS project (Ballabio *et al.*, 2018). In the future, the LUCAS survey should also incorporate the analysis of phytoavailable concentrations of micronutrients such as Fe, Zn, B, Mn, and Se.

Correcting micronutrient deficiencies with fertilizers

In the past decades, key knowledge has been produced on the formulation of fertilizers to supply micronutrients to plants. Furthermore, innovative strategies have emerged, such as seed

priming or coating with micronutrients (Farooq *et al.*, 2012). Synthetic chelators that bind Fe or Zn with high affinity while making these metals available for uptake by plants, such as HBED [N,N'-di(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid monohydrochloride], are now used in the field (Abadía *et al.*, 2011). The agronomic effectiveness of the chelated micronutrients is much higher than that of the inorganic forms of the corresponding micronutrients when applied on calcareous soils (Gangloff *et al.*, 2002). At the global scale, only 6% of micronutrient fertilizers are applied as chelates, mostly in Europe, west Asia, and North America. Correction of micronutrient deficiencies in plants by using chelates is an expensive practice. According to Abadía *et al.* (2004), the cost of correcting Fe deficiency in plants grown in Mediterranean countries represents an important economic burden on farmers of about €80 million to €100 million per year (Abadía *et al.*, 2004). Therefore, the optimization of the mode of application of the chelated micronutrients is an important topic for studies. In this regard, foliar spray has many advantages as it minimizes the quantities applied, increases efficacy, and prevents loss of micronutrients by precipitation in soils. Foliar application of Zn is also more effective than soil application in increasing Zn concentrations in grains (Cakmak *et al.*, 2010a). Moreover, foliar spray can be applied locally as a precise treatment and in combination with other plant-protection products. Studies on the mechanisms of chelated metal uptake by the roots and the leaves may allow improvement of their efficiency and limit the costs to farmers.

Harnessing micronutrient homeostasis for sustainable yield and protein production

Global change calls for more sustainable and more productive agriculture, in which less N fertilizer is used and crops are more resilient to environmental constraints. Optimal micronutrient uptake and distribution are essential to achieve this goal, as photosynthesis, N nutrition, and resilience to abiotic and biotic stresses largely rely on sufficient mineral micronutrient supply (Fig. 1). For this to become a reality, it is essential to advance knowledge on the molecular and cellular pathways of micronutrients, from their acquisition from soils to their incorporation as cofactors in metalloproteins, and their regulation.

Mineral micronutrients are key to maintaining optimal photosynthesis and therefore crop productivity. Accordingly, many mutants identified in screens for defective photosynthesis are affected in genes encoding metal transport components (Schneider *et al.*, 2016; Eisenhut *et al.*, 2018; Zhang *et al.*, 2018; Schmidt *et al.*, 2020). The photosynthetic electron transport chain requires high levels of Fe, Mn, and Cu (Schmidt *et al.*, 2020). Photosystems are quantitatively among the most abundant enzymatic systems in ecosystems. Chloroplasts contain ~80% of total cellular Fe in a mesophyll cell. Up to 50% of the plant Cu is found in chloroplasts, bound mainly to plastocyanin, which is an essential electron carrier in the photosynthetic system (Schmidt *et al.*, 2020). To improve the supply of micronutrients to the photosystems, the mechanisms of their uptake into leaf cells and transport through the chloroplast envelope and thylakoid membranes need to be deciphered.

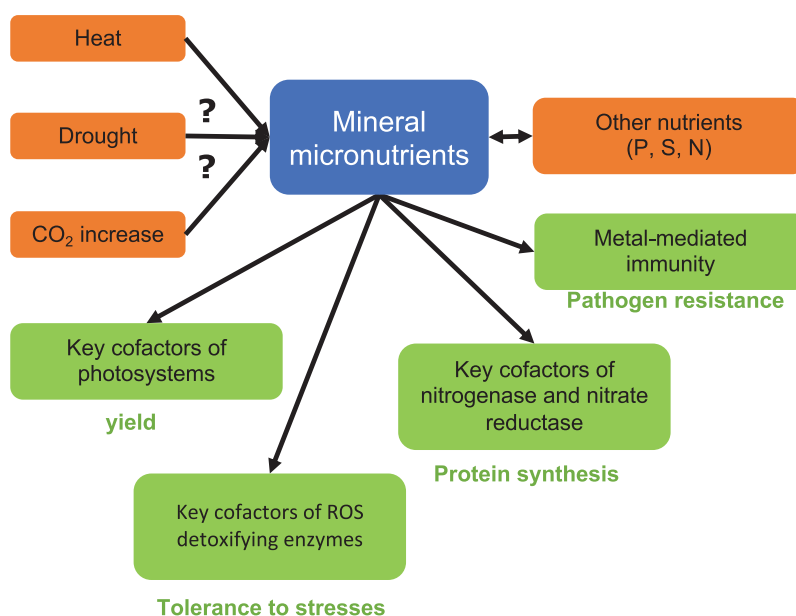


Fig. 1. Micronutrient interactions in the face of global change. How micronutrition is influenced by heat, drought, and atmospheric CO₂ concentration, all of which are expected to increase during climate change, needs to be investigated. The mechanisms underlying the interactions of micronutrients with each other and with macronutrients need to be deciphered. In turn, micronutrients act as key cofactors in processes that help mitigate the impact of global change, such as photosynthesis, nitrogen assimilation, reactive oxygen species detoxification, and immunity.

Key enzymes for N fixation and assimilation rely on Fe and Mo for their activity: nitrogenase, which is required for atmospheric N₂ fixation, and nitrate reductase, which catalyzes the first step in the assimilation of N into proteins, both require Fe- and Mo-containing cofactors. Accordingly, Fe deficiency specifically decreases nitrate reductase activity with no change in glutamine synthetase activity (Borlotti *et al.*, 2012) and affects nodule initiation and development (Brear *et al.*, 2013). Owing to their high content of nitrogenase, which requires 38 atoms of Fe, and leghemoglobin, a heme protein, nodules represent a major sink accounting for ~35% of the total Fe in a legume (Burton *et al.*, 1998; Brear *et al.*, 2013; Burén *et al.*, 2020). To improve N fixation, the mechanisms responsible for the distribution of the metal cofactors of enzymes involved in N fixation and assimilation need to be determined.

To maintain a sustainable agricultural yield, it is also essential to harness the functions of micronutrients in tolerance to biotic and abiotic constraints. This includes understanding the roles of micronutrients in plant resilience to abiotic stresses, through their antioxidant role, for example, and understanding the competition for micronutrients between the host and invading pathogens and how it is regulated or manipulated during the plant immune response. With global change, crops are expected to be increasingly affected by abiotic stresses such as heat, drought, high light, and radiation. Whether metal micronutrients, which are key players in redox reactions, can mitigate or aggravate the effect of global change, and how nutrition with Zn, Mn, Fe, and B could mitigate oxidative stress, needs to be explored (Fig. 1). On the other hand, the atmospheric CO₂ concentration is increasing steadily, and tentative evidence indicates that this is causing a decrease in the micronutrient nutritional value of plant products (Dong *et al.*, 2018). Knowledge on micronutrient homeostasis may also be useful to fight plant diseases. There is growing evidence that micronutrients are involved in plant immunity against pathogens (Aznar *et al.*, 2015; Verbon *et al.*, 2017; Cesco *et al.*, 2020; Escudero *et al.*, 2022; Morina *et al.*, 2021). In addition, micronutrients, especially B, Cu, and Mn, are important for the structural stability and mechanical resistance of the cell wall, which is the first barrier against pathogens (Krzesłowska, 2011; Malinovsky *et al.*, 2014). However, the underlying mechanisms need to be better understood to allow the inclusion of micronutrient nutritional supply as a support for crop protection, which may open a new venue of business for micronutrient fertilizer companies. Furthermore, better knowledge of the roles of micronutrients in defense responses may allow mitigation of the overuse of exogenous Cu to protect plants against fungal infection that has led to persistent pollution in vineyards, for example (Provenzano *et al.*, 2010).

Overview of current knowledge and future challenges for research on plant mineral micronutrient homeostasis

Plants need micronutrients for specific biochemical processes such as photosynthesis or N assimilation, in addition to more

general processes such as protein synthesis and function, DNA replication, respiration, or reactive oxygen species detoxification (Marschner, 2012). To ensure a sufficient micronutrient supply in plants, a highly specialized and regulated network is in place to optimize uptake from soil, distribution throughout the plant, delivery to sink organs, and allocation to specific metalloproteins. This is particularly challenging considering the prevalent metal micronutrient deficiencies in soils described above, and the toxic effects of concentrations slightly above physiological levels.

So far, research on the molecular mechanisms of plant micronutrient acquisition and use efficiency has been strongly focused on the two main plant models, *Arabidopsis thaliana* and rice. In the future, information from these models needs to be extended to other important crops.

Micronutrient uptake

For most micronutrients, the molecular mechanisms of uptake by plants have been elucidated in the past decades (Olsen and Palmgren, 2014; Connorton *et al.*, 2017; Alejandro *et al.*, 2020; Stanton *et al.*, 2021). The main metal micronutrient transporters have been identified, as has the role of rhizosphere acidification in metal acquisition. In the past few years, the importance of root exudates in the mobilization of essential micronutrients in dicots has been uncovered. While it was already known that cereals acquire Fe by secreting phytosiderophores into the rhizosphere, the central role of secreted coumarins in the mobilization of Fe in the vicinity of growing roots has been highlighted in several dicot species (Sisó-Terraza *et al.*, 2016; Tsai and Schmidt, 2017), with possible implications for the acquisition of other micronutrients, as well as shaping the bacterial communities associated with the roots (Stringlis *et al.*, 2018). In addition, recent studies have reported the existence of specific mechanisms for metal uptake from arbuscular mycorrhizal fungi, which are of particular relevance in natural ecosystems and organic agrosystems (Senovilla *et al.*, 2020). Root mycorrhizal colonization contributes up to 50% of plant Zn uptake (Watts-Williams *et al.*, 2015). Moreover, decreasing root mycorrhizal colonization results in massive increases in cadmium (Cd) accumulation in wheat (Yazici *et al.*, 2021). Particular attention should thus be given to the abundance and activity of mycorrhizal fungi in agricultural soils, to manage micronutrient nutrition and limit Cd accumulation in food crops (Ma *et al.*, 2021).

Micronutrient distribution within the plant

Within the plant, micronutrients are delivered through the sap to sink organs, typically the youngest leaves and seeds. This is done by transporters moving these nutrients across membranes, and by small organic ligands that maintain their solubility in solution and prevent mis-metallation (Olsen and Palmgren, 2014; Connorton *et al.*, 2017). Whereas many genes involved in micronutrient mobilization and uptake

from the soil have been identified, for most micronutrients, the mechanisms for loading into the xylem and the phloem, as well as the ligands in conducting tissues, are still mostly unknown. The transporters localized in the nodes and tiller buds play fundamental roles in the distribution of micronutrients within plants, especially in the delivery of micronutrients to the younger leaves and seeds, and should be targeted for more research (Durbak *et al.*, 2014; Shao *et al.*, 2018; Mu *et al.*, 2021). The mechanisms of micronutrient distribution to the edible organs are still only partially understood (Andresen *et al.*, 2018). Improving the micronutrient content in the edible parts of crop plants will require better knowledge of the mechanisms of micronutrient distribution to the organs and tissues in order to be able to breed or engineer plants for targeted micronutrient allocation.

Moreover, the process of metal recycling and reuse in the transition from vegetative to reproductive growth remains largely unaddressed. The study of metal remobilization from senescing leaves appears to be a promising area to modulate metal accumulation in seeds (Mari *et al.*, 2020). Modulating leaf senescence and nutrient recycling by autophagy in wheat or *Arabidopsis* leaves leads to change in the contents of both protein and mineral micronutrients in seeds (Uauy *et al.*, 2006; Chen *et al.*, 2019; Pottier *et al.*, 2019).

Another critical process that calls for further investigation is the mechanism of mineral micronutrient loading into seeds. At the moment, only fragmentary information on micronutrient entry into seeds is available. Metal-pumping ATPases have been shown to play an important role in releasing Zn from the mother tissues before its entry into the seed (Olsen *et al.*, 2016). The secretion of ascorbate is used to reduce Fe and allow its uptake by the embryo (Grillet *et al.*, 2014). Developing this research will require detailed characterization of the dynamics of micronutrient distribution in plant organs and tissues using elemental imaging approaches as well as metal isotopes as tracers (Sheraz *et al.*, 2021).

Micronutrient supply to endosymbionts

Plant endosymbionts, particularly those associated with N fixation, are major metal sinks for which specific metal-delivery systems have evolved (Tejada-Jiménez *et al.*, 2017; Brear *et al.*, 2020; Escudero *et al.*, 2020). To improve N nutrition through endosymbiotic bacteria, the genes involved in transport pathways that supply micronutrients to the nitrogenase of symbiotic bacteria, enclosed in symbiosomes in the nodules of legume species, or of endophytic bacteria that colonize the extracellular space in other species, including wheat and other cereals, need to be identified. Moreover, the mechanisms controlling micronutrient allocation to two opposite sinks (leaves and nodules) remain largely unknown, with important physiological implications on how legumes dynamically distribute essential limiting cofactors for photosynthesis and for N fixation.

Micronutrient interactions

Besides their importance for N metabolism, micronutrients also strongly interact with each other and with several macronutrients. In fact, deficiency symptoms are often related to nutritional imbalances rather than a lack of a single micronutrient per se. There are important interactions between micronutrient homeostasis and other mineral nutrients, such as inorganic phosphate (Pi) with Zn and Fe (Briat *et al.*, 2015; Dong *et al.*, 2017; Hanikenne *et al.*, 2021). High Pi has an inhibitory effect on root Zn uptake (Watts-Williams *et al.*, 2015; Ova *et al.*, 2015). On the other hand, Zn deficiency increases Pi uptake (Kisko *et al.*, 2018). Zn and Fe root uptake, as well as their root-to-shoot transport and remobilization, are positively affected by N fertilization (Kutman *et al.*, 2011). In line with this, the concentrations and localizations of Zn, Fe, and proteins in seeds are closely correlated (Cakmak *et al.*, 2010b). In contrast, Mo uptake is severely affected by sulfur (S) fertilization, resulting in Mo deficiency (Shinmachi *et al.*, 2010; Maillard *et al.*, 2016a, b). To determine the molecular bases of these interactions, the metalloproteins involved and their biological roles need to be elucidated.

A major risk encountered in biofortification strategies targeting mineral micronutrients is enhancement of the accumulation of chemically similar toxic trace elements (Kawakami and Bhullar, 2021). In rice, enhancing the expression of a transporter involved in Cd sequestration in root vacuoles, or knocking out a gene involved in Cd uptake in roots, have proved to be efficient strategies to prevent Cd accumulation in grains (Ueno *et al.*, 2010; Ishikawa *et al.*, 2012). Because of their chemical similarities, micronutrient metals compete with non-essential metals for transport and binding to ligands. Addressing these fundamental scientific questions requires in-depth biochemical knowledge of the substrate specificities and relative affinities of proteins and transporters for micronutrients and toxic elements with similar chemical properties (e.g. Zn and Cd). The determinants for metal specificity, particularly among divalent metals, need to be better defined to promote the exclusion of non-biogenic metals (Pottier *et al.*, 2015).

Micronutrient speciation

The major metal ligands in plants, such as citrate or nicotianamine, and their role in micronutrient transport between cells and organs have been established (Clemens, 2019). Enhanced accumulation of the metal-binding molecule nicotianamine through the activation of the gene encoding nicotianamine synthase allowed an increase in the content of bioavailable Fe and Zn in rice grains (Lee *et al.*, 2009, 2011). Nevertheless, improving micronutrient content in the edible parts of crop plants and optimizing metal distribution for photosynthesis and N metabolism requires a better knowledge of the mechanisms of micronutrient speciation and subcellular

distribution. The analysis of micronutrient speciation, including the determination of the metalloproteome and metallome for each essential metal, using analytic and spectroscopic methods is crucial to achieve this goal (Flis *et al.*, 2016). The control of metal speciation requires knowledge on the mechanisms that drive micronutrient allocation to distinct cell compartments, as speciation varies among the different cellular compartments. For example, Fe is often stored as an insoluble complex with phytate in the vacuole, whereas it is stored as bioavailable ferritin in the plastids (Mari *et al.*, 2020).

Regulation of metal homeostasis

Progress has also been made in understanding the regulation of micronutrient acquisition: transcription factors (TFs) controlling plant responses to Fe, Zn, and Cu deficiency have been identified (Assunção *et al.*, 2010; Bernal *et al.*, 2012; Kobayashi and Nishizawa, 2012). These findings highlight the importance of the transcriptional control of genes involved in micronutrient transport and distribution in the plant response to micronutrient deficiencies. In addition, more information is emerging on the mechanisms through which plants perceive micronutrient status, and on micronutrient status signaling at the systemic plant level, as has recently been reported for Zn and Fe (Dubeaux *et al.*, 2018; Grillet *et al.*, 2018; Sinclair *et al.*, 2018; Lilay *et al.*, 2021). In the case of Fe, a complex network involving over 10 bHLH TFs has been uncovered (Gao and Dubos, 2021). Interestingly, the stability of several of these TFs is controlled by Fe-dependent E3 ligases (Rodríguez-Celma *et al.*, 2019). A recent publication indicates that phosphorylation of one of these bHLH TFs is responsible for recognition by E3 ligases, raising the possibility that some kinases mediate the Fe response (Kim *et al.*, 2019). For Zn, F-group bZIP TFs have been identified as the central regulators of the deficiency response. Under Zn deficiency, they transcriptionally activate Zn transporters and ligand-producing enzymes, which increase the plant's Zn uptake and distribution capacity as an adaptation to low Zn availability (Assunção *et al.*, 2010). The F-bZIP TF itself acts as a sensor of cellular Zn status by directly binding to Zn ions (Lilay *et al.*, 2021). In the case of Cu, the Arabidopsis SPL7 TF plays a pivotal role in the response to Cu deficiency through the transcriptional activation of genes involved in Cu uptake and mobilization. The TF also mediates miRNA-dependent down-regulation of Cu metalloprotein transcripts, as a mechanism to spare Cu under conditions of deficiency (Yamasaki *et al.*, 2009; Bernal *et al.*, 2012; Garcia-Molina *et al.*, 2014).

Advancing knowledge on the regulatory networks that control micronutrient acquisition at the cellular and plant levels will help to improve micronutrient use efficiency and micronutrient content in crops. Moreover, the long-distance signaling mechanisms that allow adjustment of the uptake and redistribution of micronutrients according to the need of the sink organs should also be elucidated. This is important to optimize micronutrient use efficiency and also to improve plant responses to micronutrient fertilizers.

Specific approaches required to decipher mineral micronutrient homeostasis

All these breakthroughs have been facilitated by advances in micronutrient visualization and quantification. In the past decades, ionomic screens based on atomic absorption and emission spectroscopies as well as mass spectrometry have been used to quantify elements in large collections of plants at a tissue/organ level. These approaches have proved useful for identifying genes involved in micronutrient homeostasis in model species (Huang and Salt, 2016; Campos *et al.*, 2017; Yang *et al.*, 2018). Spatial resolution has been achieved using synchrotron X-ray fluorescence (sXRF) or micro-particle induced X-ray emission (micro-PIXE) to map micronutrient localization in the nanomolar to micromolar range. Improved synchrotron lines with cryo-facilities have become the gold standard in terms of sensitivity, resolution, and minimal preparation artefacts. When associated with X-ray absorption spectroscopy, synchrotron-based approaches also provide information about metal micronutrient speciation together with the localization data (Escudero *et al.*, 2020) and can be used to analyze the efficiency of micronutrient fertilization (Ajiboye *et al.*, 2015). Other imaging techniques based on mass spectrometry, such as secondary ion mass spectrometry (SIMS), laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS), or matrix-assisted laser desorption/ionization (MALDI) imaging, have been used to reveal micronutrient localization (Moore *et al.*, 2018; Detterbeck *et al.*, 2020). They provide isotopic information allowing dynamic analysis after isotopic labeling and, in some cases, allow *in situ* analysis of metal complexes. Finally, metal-sensitive fluorescent probes, such as Förster resonance energy transfer (FRET) sensors, dynamically monitor labile micronutrient pools *in vivo* (Lanquar *et al.*, 2014). In addition, analytical chemistry methods combining chromatography with mass spectrometry and elemental analysis, such as size-exclusion chromatography (SEC)-ICP-MS, have identified metal complexes with small molecules or with proteins (Cakmak *et al.*, 2010a; Flis *et al.*, 2016; Küpper *et al.*, 2019). Ultimately, the development of these approaches should allow the determination of the complete spectrum of micronutrient metal complexes with small molecules (metallome), and the full complement of metalloproteins (metalloproteome) for any given biological sample. This knowledge is essential to fully understand the role of micronutrients in living cells and to improve their bioavailability to ultimately address micronutrient deficiencies in human populations.

Conclusion

Mineral micronutrient uptake and use efficiency in plants are still underutilized traits in crop breeding programs, even though they have the potential to improve both the sustainability of crop production and the quality of food and feed. To alleviate micronutrient deficiency problems in humans and in livestock, supplementation and fortification methods are most commonly

recommended. However, these methods are unsustainable because they need continued financing, monitoring, and dedicated logistic services. Breeding food plants for higher mineral micronutrient contents appears to be a more sustainable solution to reduce the global prevalence of micronutrient deficiencies, which are expected to increase as the populations of developed countries move toward more plant-based diets. Moreover, breeding for mineral micronutrient use efficiency will also allow sustainable crop production, which is required to face global change. In this context, there is a need to encourage research and innovation in this field to ensure the well-being of human populations and the competitiveness of the agri-food industry. Understanding the molecular and physiological mechanisms of micronutrient homeostasis, including the regulation of the acquisition, transport, and distribution of micronutrients in food crops, is essential to identify sustainable solutions to micronutrient deficiencies in human populations.

Conflict of interest

The authors have no conflicts to declare.

Funding

The authors are supported by an Independent Research Fund Denmark (DFF) grant (no. 9041-00182B) to AA, ERC-StG-2013-335284 to MGG, HarvestPlus-HarvestZinc Project (<https://www.harvestzinc.org/>) to IC, ANR (Agence Nationale de la Recherche) projects 17-CE20-0008 (MOBIFER) and 19-CE13-0007 (PHLOWZ) to ST, and German Research Foundation (DFG) grant CL 152/11 and Federal Ministry of Education and Research (BMBF) grant 031B0840 to SC. The ST lab benefits from the support of Saclay Plant Sciences-SPS (ANR-17-EUR-0007). This article/publication is based upon work from COST Action CA19116 PLANTMETALS, supported by COST (European Cooperation in Science and Technology). www.cost.eu. This review was written on the basis of a report from the Focus Group 'Improving micronutrient uptake and use efficiency' in the context of the CropBooster-P project (EU Horizon 2020 Research and Innovation grant agreement 81769).

References

- Abadía J, Álvarez-Fernández A, Rombolaà AD, Sanz M, Tagliavini M, Abadía A. 2004. Technologies for the diagnosis and remediation of Fe deficiency. *Soil Science and Plant Nutrition* **50**, 965–971.
- Abadía J, Vázquez S, Rellán-Álvarez R, El-Jendoubi H, Abadía A, Álvarez-Fernández A, López-Millán AF. 2011. Towards a knowledge-based correction of iron chlorosis. *Plant Physiology and Biochemistry* **49**, 471–482.
- Ajiboye B, Cakmak I, Paterson D, de Jonge MD, Howard DL, Stacey SP, Torun AA, Aydin N, McLaughlin MJ. 2015. X-ray fluorescence microscopy of zinc localization in wheat grains biofortified through foliar zinc applications at different growth stages under field conditions. *Plant and Soil* **392**, 357–370.
- Akkermans MD, van der Horst-Graat JM, Eussen SRBM, van Goudoever JB, Brus F. 2016. Iron and vitamin D deficiency in healthy young children in Western Europe despite current nutritional recommendations. *Journal of Pediatric Gastroenterology and Nutrition* **62**, 635–642.
- Alejandro S, Höller S, Meier B, Peiter E. 2020. Manganese in plants: from acquisition to subcellular allocation. *Frontiers in Plant Science* **11**, 300.
- Alloway BJ. 2009. Soil factors associated with zinc deficiency in crops and humans. *Environmental Geochemistry and Health* **31**, 537–548.
- Andersen ATN, Husby S, Kyhl HB, Sandberg MB, Sander SD, Mølgaard C. 2019. Iron deficiency in healthy 18-month-old Danish children is associated with no oral iron supplementation in infancy and prolonged exclusive breast-feeding. *British Journal of Nutrition* **122**, 1409–1416.
- Andresen E, Peiter E, Küpper H. 2018. Trace metal metabolism in plants. *Journal of Experimental Botany* **69**, 909–954.
- Assunção AGL, Herrero E, Lin Y-F, et al. 2010. *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. *Proceedings of the National Academy of Sciences, USA* **107**, 10296–10301.
- Aznar A, Chen NWG, Thomine S, Dellagi A. 2015. Immunity to plant pathogens and iron homeostasis. *Plant Science* **240**, 90–97.
- Bailey RL, West KP Jr, Black RE. 2015. The epidemiology of global micronutrient deficiencies. *Annals of Nutrition and Metabolism* **66**, 22–33.
- Bakaloudi DR, Halloran A, Rippin HL, Oikonomidou AC, Dardavesis TI, Williams J, Wickramasinghe K, Breda J, Chourdakis M. 2021. Intake and adequacy of the vegan diet. A systematic review of the evidence. *Clinical Nutrition* **40**, 3503–3521.
- Ballabio C, Panagos P, Lugato E, Huang J-H, Orgiazzi A, Jones A, Fernández-Ugalde O, Borrelli P, Montanarella L. 2018. Copper distribution in European topsoils: an assessment based on LUCAS soil survey. *Science of The Total Environment* **636**, 282–298.
- Barve S, Chen S-Y, Kirpich I, Watson WH, McClain C. 2017. Development, prevention, and treatment of alcohol-induced organ injury: the role of nutrition. *Alcohol Research: Current Reviews* **38**, 289–302.
- Bernal M, Casero D, Singh V, et al. 2012. Transcriptome sequencing identifies *SPL7*-regulated copper acquisition genes *FRO4/FRO5* and the copper dependence of iron homeostasis in *Arabidopsis*. *The Plant Cell* **24**, 738–61.
- Borlotti A, Vigani G, Zocchi G. 2012. Iron deficiency affects nitrogen metabolism in cucumber (*Cucumis sativus* L.) plants. *BMC Plant Biology* **12**, 189.
- Breare EM, Bedon F, Gavrin A, Kryvoruchko IS, Torres-Jerez I, Udvardi MK, Day DA, Smith PMC. 2020. GmVTL1a is an iron transporter on the symbiosome membrane of soybean with an important role in nitrogen fixation. *New Phytologist* **228**, 667–681.
- Breare EM, Day DA, Smith PMC. 2013. Iron: an essential micronutrient for the legume-rhizobium symbiosis. *Frontiers in Plant Science* **4**, 359.
- Briat J-F, Rouached H, Tissot N, Gaymard F, Dubos C. 2015. Integration of P, S, Fe, and Zn nutrition signals in *Arabidopsis thaliana*: potential involvement of PHOSPHATE STARVATION RESPONSE 1 (PHR1). *Frontiers in Plant Science* **6**, 290.
- Burén S, Jiménez-Vicente E, Echavarri-Erasun C, Rubio LM. 2020. Biosynthesis of nitrogenase cofactors. *Chemical Reviews* **120**, 4921–4968.
- Burton JW, Harlow C, Theil EC. 1998. Evidence for reutilization of nodule iron in soybean seed development. *Journal of Plant Nutrition* **21**, 913–927.
- Cakmak I, Kalayci M, Kaya Y, et al. 2010a. Biofortification and localization of zinc in wheat grain. *Journal of Agricultural and Food Chemistry* **58**, 9092–102.
- Cakmak I, Marzorati M, Van den Abbeele P, Hora K, Holwerda HT, Yazici MA, Savasli E, Neri J, Du Laing G. 2020. Fate and bioaccessibility of iodine in food prepared from agronomically biofortified wheat and rice and impact of cofertilization with zinc and selenium. *Journal of Agricultural and Food Chemistry* **68**, 1525–1535.
- Cakmak I, Pfeiffer WH, McClafferty B. 2010b. Biofortification of durum wheat with zinc and iron. *Cereal Chemistry Journal* **87**, 10–20.
- Campos ACA, Kruijer W, Alexander R, Akkers RC, Danku J, Salt DE, Aarts MG. 2017. Natural variation in *Arabidopsis thaliana* reveals shoot

ionome, biomass, and gene expression changes as biomarkers for zinc deficiency tolerance. *Journal of Experimental Botany* **68**, 3643–3656.

Cesco S, Tolotti A, Nadalini S, et al. 2020. *Plasmopara viticola* infection affects mineral elements allocation and distribution in *Vitis vinifera* leaves. *Scientific Reports* **10**, 18759.

Chen Q, Soulay F, Saudemont B, Elmayan T, Marmagne A, Masclaux-Daubresse C. 2019. Overexpression of *ATG8* in *Arabidopsis* stimulates autophagic activity and increases nitrogen remobilization efficiency and grain filling. *Plant and Cell Physiology* **60**, 343–352.

Clemens S. 2017. How metal hyperaccumulating plants can advance Zn biofortification. *Plant and Soil* **411**, 111–120.

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

Connorton JM, Balk J. 2019. Iron biofortification of staple crops: lessons and challenges in plant genetics. *Plant and Cell Physiology* **60**, 1447–1456.

Connorton JM, Balk J, Rodríguez-Celma J. 2017. Iron homeostasis in plants – a brief overview. *Metallomics* **9**, 813–823.

Detterbeck A, Pongrac P, Persson DP, et al. 2020. Temporal and spatial patterns of zinc and iron accumulation during barley (*Hordeum vulgare* L.) grain development. *Journal of Agricultural and Food Chemistry* **68**, 12229–12240.

Dobbels AA, Lorenz AJ. 2019. Soybean iron deficiency chlorosis high-throughput phenotyping using an unmanned aircraft system. *Plant Methods* **15**, 97.

Dong J, Gruda N, Lam SK, Li X, Duan Z. 2018. Effects of elevated CO₂ on nutritional quality of vegetables: a review. *Frontiers in Plant Science* **9**, 924.

Dong J, Piñeros MA, Li X, Yang H, Liu Y, Murphy AS, Kochian LV, Liu D. 2017. An *Arabidopsis* ABC transporter mediates phosphate deficiency-induced remodeling of root architecture by modulating iron homeostasis in roots. *Molecular Plant* **10**, 244–259.

Dubeaux G, Neveu J, Zelazny E, Vert G. 2018. Metal sensing by the IRT1 transporter-receptor orchestrates its own degradation and plant metal nutrition. *Molecular Cell* **69**, 953–964.e5.

Durbak AR, Phillips KA, Pike S, O'Neill MA, Mares J, Gallavotti A, Malcomber ST, Gassmann W, McSteen P. 2014. Transport of boron by the *tassel-less1* aquaporin is critical for vegetative and reproductive development in maize. *The Plant Cell* **26**, 2978–95.

Eisenhut M, Hoecker N, Schmidt SB, et al. 2018. The plastid envelope CHLOROPLAST MANGANESE TRANSPORTER1 is essential for manganese homeostasis in *Arabidopsis*. *Molecular Plant* **11**, 955–969.

Eldridge BM, Manzoni LR, Graham CA, Rodgers B, Farmer JR, Dodd AN. 2020. Getting to the roots of aeroponic indoor farming. *New Phytologist* **228**, 1183–1192.

Escudero V, Abreu I, Tejada-Jiménez M, et al. 2020. *Medicago truncatula* Ferroportin2 mediates iron import into nodule symbiosomes. *New Phytologist* **228**, 194–209.

Escudero V, Ferreira Sánchez D, Abreu I, Sopena-Torres S, Makarovskiy-Saavedra N, Bernal M, Krämer U, Grolimund D, González-Guerrero M, Jordá L. 2022. *Arabidopsis thaliana* Zn²⁺-efflux ATPases HMA2 and HMA4 are required for resistance to the necrotrophic fungus *Plectosphaerella cucumerina* BMM. *Journal of Experimental Botany* **73**, 339–350.

Eussen S, Alles M, Uijtershout L, Brus F, van der Horst-Graat J. 2015. Iron intake and status of children aged 6–36 months in Europe: a systematic review. *Annals of Nutrition and Metabolism* **66**, 80–92.

Farooq M, Wahid A, Siddique KHM. 2012. Micronutrient application through seed treatments: a review. *Journal of Soil Science and Plant Nutrition* **12**, 125–142.

Flis P, Ouerdane L, Grillet L, Curie C, Mari S, Lobinski R. 2016. Inventory of metal complexes circulating in plant fluids: a reliable method based on HPLC coupled with dual elemental and high-resolution molecular mass spectrometric detection. *New Phytologist* **211**, 1129–1141.

Gangloff WJ, Westfall DG, Peterson GA, Mortvedt JJ. 2002. Relative availability coefficients of organic and inorganic Zn fertilizers. *Journal of Plant Nutrition* **25**, 259–273.

Gao F, Dubos C. 2021. Transcriptional integration of plant responses to iron availability. *Journal of Experimental Botany* **72**, 2056–2070.

García-Casal MN, Pereira AC, Leets I, Ramírez J, Quiroga MF. 2007. High iron content and bioavailability in humans from four species of marine algae. *The Journal of Nutrition* **137**, 2691–5.

García-Molina A, Xing S, Huijser P. 2014. A conserved KIN17 curved DNA-binding domain protein assembles with SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE7 to adapt *Arabidopsis* growth and development to limiting copper availability. *Plant Physiology* **164**, 828–40.

Gibson RS, Raboy V, King JC. 2018. Implications of phytate in plant-based foods for iron and zinc bioavailability, setting dietary requirements, and formulating programs and policies. *Nutrition Reviews* **76**, 793–804.

Grillet L, Lan P, Li W, Mokkapat G, Schmidt W. 2018. IRON MAN is a ubiquitous family of peptides that control iron transport in plants. *Nature Plants* **4**, 953–963.

Grillet L, Ouerdane L, Flis P, Hoang MTT, Isaure M-P, Lobinski R, Curie C, Mari S. 2014. Ascorbate efflux as a new strategy for iron reduction and transport in plants. *Journal of Biological Chemistry* **289**, 2515–2525.

Hanikenne M, Esteves SM, Fanara S, Rouached H. 2021. Coordinated homeostasis of essential mineral nutrients: a focus on iron. *Journal of Experimental Botany* **72**, 2136–2153.

Harding KL, Aguayo VM, Webb P. 2018. Hidden hunger in South Asia: a review of recent trends and persistent challenges. *Public Health Nutrition* **21**, 785–795.

Hargreaves SM, Raposo A, Saraiva A, Zandonadi RP. 2021. Vegetarian diet: an overview through the perspective of quality of life domains. *International Journal of Environmental Research and Public Health* **18**, 4067.

Hebborn CA, Peadar P, Schjoerring JK, Knudsen L, Husted S. 2005. Genotypic differences in manganese efficiency: field experiments with winter barley (*Hordeum vulgare* L.). *Plant and Soil* **272**, 233–244.

Hillesund ER, Øverby NC, Valen EL, Engeset D. 2021. Alcohol consumption among students and its relationship with nutritional intake: a cross-sectional study. *Public Health Nutrition* **24**, 2877–2888.

Huang X-Y, Salt DE. 2016. Plant ionomics: from elemental profiling to environmental adaptation. *Molecular Plant* **9**, 787–97.

Ishikawa S, Ishimaru Y, Igura M, Kuramata M, Abe T, Senoura T, Hase Y, Arai T, Nishizawa NK, Nakanishi H. 2012. Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. *Proceedings of the National Academy of Sciences, USA* **109**, 19166–19171.

Kawakami Y, Bhullar NK. 2021. Delineating the future of iron biofortification studies in rice: challenges and future perspectives. *Journal of Experimental Botany* **72**, 2099–2113.

Kieliszek M, Bano I, Zare H. 2021. A comprehensive review on selenium and its effects on human health and distribution in Middle Eastern countries. *Biological Trace Element Research* **200**, 971–987.

Kim SA, LaCroix IS, Gerber SA, Guerinot ML. 2019. The iron deficiency response in *Arabidopsis thaliana* requires the phosphorylated transcription factor URI. *Proceedings of the National Academy of Sciences, USA* **116**, 24933–24942.

Kisko M, Bouain N, Safi A, et al. 2018. LPCAT1 controls phosphate homeostasis in a zinc-dependent manner. *eLife* **7**, e32077.

Kobayashi T, Nishizawa NK. 2012. Iron uptake, translocation, and regulation in higher plants. *Annual Review of Plant Biology* **63**, 131–52.

Krzyszowska M. 2011. The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. *Acta Physiologiae Plantarum* **33**, 35–51.

Küpper H, Bokhari SNH, Jaime-Pérez N, Lyubenova L, Ashraf N, Andresen E. 2019. Ultratrace metal speciation analysis by coupling of sector-field ICP-MS to high-resolution size exclusion and reversed-phase liquid chromatography. *Analytical Chemistry* **91**, 10961–10969.

- Kutman UB, Yildiz B, Cakmak I. 2011. Effect of nitrogen on uptake, remobilization and partitioning of zinc and iron throughout the development of durum wheat. *Plant and Soil* **342**, 149–164.
- Lanquar V, Grossmann G, Vinkenborg JL, Merckx M, Thomine S, Frommer WB. 2014. Dynamic imaging of cytosolic zinc in *Arabidopsis* roots combining FRET sensors and RootChip technology. *New Phytologist* **202**, 198–208.
- Lee S, Jeon US, Lee SJ, *et al.* 2009. Iron fortification of rice seeds through activation of the nicotianamine synthase gene. *Proceedings of the National Academy of Sciences, USA* **106**, 22014–22019.
- Lee S, Persson DP, Hansen TH, *et al.* 2011. Bio-available zinc in rice seeds is increased by activation tagging of nicotianamine synthase: activation and overexpression of OsNAS2. *Plant Biotechnology Journal* **9**, 865–73.
- Lilay GH, Persson DP, Castro PH, Liao F, Alexander RD, Aarts MGM, Assunção AGL. 2021. *Arabidopsis* bZIP19 and bZIP23 act as zinc sensors to control plant zinc status. *Nature Plants* **7**, 137–143.
- Lopez-Ridaura S, Barba-Escoto L, Reyna-Ramirez CA, Sum C, Palacios-Rojas N, Gerard B. 2021. Maize intercropping in the milpa system. Diversity, extent and importance for nutritional security in the Western Highlands of Guatemala. *Scientific Reports* **11**, 3696.
- López-Ruzafa E, Vázquez-López MA, Galera-Martínez R, Lendínez-Molinós F, Gómez-Bueno S, Martín-González M. 2021. Prevalence and associated factors of iron deficiency in Spanish children aged 1 to 11 years. *European Journal of Pediatrics* **180**, 2773–2780.
- Ludwig Y, Slamet-Loedin IH. 2019. Genetic biofortification to enrich rice and wheat grain iron: from genes to product. *Frontiers in Plant Science* **10**, 833.
- Ma X, Geng Q, Zhang H, Bian C, Chen HYH, Jiang D, Xu X. 2021. Global negative effects of nutrient enrichment on arbuscular mycorrhizal fungi, plant diversity and ecosystem multifunctionality. *New Phytologist* **229**, 2957–2969.
- Maillard A, Etienne P, Diquélou S, Trouverie J, Billard V, Yvin J-C, Ourry A. 2016a. Nutrient deficiencies modify the ionic composition of plant tissues: a focus on cross-talk between molybdenum and other nutrients in *Brassica napus*. *Journal of Experimental Botany* **67**, 5631–5641.
- Maillard A, Sorin E, Etienne P, *et al.* 2016b. Non-specific root transport of nutrient gives access to an early nutritional indicator: the case of sulfate and molybdate. *PLoS One* **11**, e0166910.
- Malinovsky FG, Fangel JU, Willats WGT. 2014. The role of the cell wall in plant immunity. *Frontiers in Plant Science* **5**, 178.
- Mari S, Bailly C, Thomine S. 2020. Handing off iron to the next generation: how does it get into seeds and what for? *Biochemical Journal* **477**, 259–274.
- Marschner P, ed. 2012. *Marschner's mineral nutrition of higher plants*, 3rd ed. London: Elsevier/Academic Press.
- Masuda T, Yamamoto A, Toyohara H. 2015. The iron content and ferritin contribution in fresh, dried, and toasted nori, *Pyropia yezoensis*. *Bioscience, Biotechnology, and Biochemistry* **79**, 74–81.
- Moore KL, Rodríguez-Ramiro I, Jones ER, Jones EJ, Rodríguez-Celma J, Halsey K, Domoney C, Shewry PR, Fairweather-Tait S, Balk J. 2018. The stage of seed development influences iron bioavailability in pea (*Pisum sativum* L.). *Scientific Reports* **8**, 6865.
- Moreno-Jiménez E, Orgiazzi A, Jones A, Saiz H, Aceña-Heras S, Plaza C. 2021. Aridity and geochemical drivers of soil micronutrient and contaminant availability in European drylands. *European Journal of Soil Science* **73**, e13163.
- Moreno-Lora A, Delgado A. 2020. Factors determining Zn availability and uptake by plants in soils developed under Mediterranean climate. *Geoderma* **376**, 114509.
- Morina F, Mijovilovich A, Koloniuk I, Pěnčík A, Grúz J, Novák O, Küpper H. 2021. Interactions between zinc and *Phomopsis longicolla* infection in roots of *Glycine max*. *Journal of Experimental Botany* **72**, 3320–3336.
- Mu S, Yamaji N, Sasaki A, *et al.* 2021. A transporter for delivering zinc to the developing tiller bud and panicle in rice. *The Plant Journal* **105**, 786–799.
- Olsen LI, Hansen TH, Larue C, *et al.* 2016. Mother-plant-mediated pumping of zinc into the developing seed. *Nature Plants* **2**, 16036.
- Olsen LI, Palmgren MG. 2014. Many rivers to cross: the journey of zinc from soil to seed. *Frontiers in Plant Science* **5**, 30.
- Ova EA, Kutman UB, Ozturk L, Cakmak I. 2015. High phosphorus supply reduced zinc concentration of wheat in native soil but not in auto-claved soil or nutrient solution. *Plant and Soil* **393**, 147–162.
- Pottier M, Dumont J, Masclaux-Daubresse C, Thomine S. 2019. Autophagy is essential for optimal translocation of iron to seeds in *Arabidopsis*. *Journal of Experimental Botany* **70**, 859–869.
- Pottier M, Oomen R, Picco C, Giraudat J, Scholz-Starke J, Richaud P, Carpaneto A, Thomine S. 2015. Identification of mutations allowing Natural Resistance Associated Macrophage Proteins (NRAMP) to discriminate against cadmium. *The Plant Journal* **83**, 625–637.
- Provenzano MR, El Bilali H, Simeone V, Baser N, Mondelli D, Cesari G. 2010. Copper contents in grapes and wines from a Mediterranean organic vineyard. *Food Chemistry* **122**, 1338–1343.
- Qu M, Guang X, Liu H, Zhao Y, Huang B. 2022. Additional sampling using *in situ* portable X-ray fluorescence (PXRF) for rapid and high-precision investigation of soil heavy metals at a regional scale. *Environmental Pollution* **292**, 118324.
- Rashid A, Ryan J. 2004. Micronutrient constraints to crop production in soils with Mediterranean-type characteristics: a review. *Journal of Plant Nutrition* **27**, 959–975.
- Read SA, Obeid S, Ahlenstiel C, Ahlenstiel G. 2019. The role of zinc in antiviral immunity. *Advances in Nutrition* **10**, 696–710.
- Rodríguez-Celma J, Chou H, Kobayashi T, Long TA, Balk J. 2019. Hemerythrin E3 ubiquitin ligases as negative regulators of iron homeostasis in plants. *Frontiers in Plant Science* **10**, 98.
- Ruxton CHS, Derbyshire E, Toribio-Mateas M. 2016. Role of fatty acids and micronutrients in healthy ageing: a systematic review of randomised controlled trials set in the context of European dietary surveys of older adults. *Journal of Human Nutrition and Dietetics* **29**, 308–324.
- Sayre R, Beeching JR, Cahoon EB, *et al.* 2011. The BioCassava plus program: biofortification of cassava for Sub-Saharan Africa. *Annual Review of Plant Biology* **62**, 251–72.
- Schmidt SB, Eisenhut M, Schneider A. 2020. Chloroplast transition metal regulation for efficient photosynthesis. *Trends in Plant Science* **25**, 817–828.
- Schneider A, Steinberger I, Herdean A, *et al.* 2016. The evolutionarily conserved protein PHOTOSYNTHESIS AFFECTED MUTANT71 is required for efficient manganese uptake at the thylakoid membrane in *Arabidopsis*. *The Plant Cell* **28**, 892–910.
- Schüpbach R, Wegmüller R, Berguerand C, Bui M, Herter-Aeberli I. 2017. Micronutrient status and intake in omnivores, vegetarians and vegans in Switzerland. *European Journal of Nutrition* **56**, 283–293.
- Sebastiani G, Herranz Barbero A, Borrás-Novell C, Alsina Casanova M, Aldecoa-Bilbao V, Andreu-Fernández V, Pascual Tutusaus M, Ferrero Martínez S, Gómez Roig M, García-Algar O. 2019. The effects of vegetarian and vegan diet during pregnancy on the health of mothers and offspring. *Nutrients* **11**, 557.
- Senovilla M, Abreu I, Escudero V, Cano C, Bago A, Imperial J, González-Guerrero M. 2020. MtCOPT2 is a Cu⁺ transporter specifically expressed in *Medicago truncatula* mycorrhizal roots. *Mycorrhiza* **30**, 781–788.
- Shao JF, Yamaji N, Liu XW, Yokosho K, Shen RF, Ma JF. 2018. Preferential distribution of boron to developing tissues is mediated by the intrinsic protein OsNIP3. *Plant Physiology* **176**, 1739–1750.
- Sheraz S, Wan Y, Venter E, Verma SK, Xiong Q, Waites J, Connorton JM, Shewry PR, Moore KL, Balk J. 2021. Subcellular dynamics studies of iron reveal how tissue-specific distribution patterns are established in developing wheat grains. *New Phytologist* **231**, 1644–1657.

- Shinmachi F, Buchner P, Stroud JL, Parmar S, Zhao F-J, McGrath SP, Hawkesford MJ.** 2010. Influence of sulfur deficiency on the expression of specific sulfate transporters and the distribution of sulfur, selenium, and molybdenum in wheat. *Plant Physiology* **153**, 327–36.
- Sinclair AH, Edwards AC.** 2008. Micronutrient deficiency problems in agricultural crops in Europe. In: Alloway BJ, ed. *Micronutrient deficiencies in global crop production*. Dordrecht: Springer Netherlands, 225–244.
- Sinclair SA, Senger T, Talke IN, Cobbett CS, Haydon MJ, Krämer U.** 2018. Systemic upregulation of MTP2- and HMA2-mediated Zn partitioning to the shoot supplements local Zn deficiency responses. *The Plant Cell* **30**, 2463–2479.
- Sisó-Terraza P, Luis-Villarroya A, Fourcroy P, Briat J-F, Abadía A, Gaymard F, Abadía J, Álvarez-Fernández A.** 2016. Accumulation and secretion of coumarinolignans and other coumarins in *Arabidopsis thaliana* roots in response to iron deficiency at high pH. *Frontiers in Plant Science* **7**, 1711.
- Stanton C, Sanders D, Krämer U, Podar D.** 2021. Zinc in plants: integrating homeostasis and biofortification. *Molecular Plant* **15**, 65–85.
- Steinbrenner H, Al-Quraishy S, Dkhil MA, Wunderlich F, Sies H.** 2015. Dietary selenium in adjuvant therapy of viral and bacterial infections. *Advances in Nutrition* **6**, 73–82.
- Stringlis IA, Yu K, Feussner K, de Jonge R, Van Bentum S, Van Verk MC, Berendsen RL, Bakker PAHM, Feussner I, Pieterse CMJ.** 2018. MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proceedings of the National Academy of Sciences, USA* **115**, E5213–E5222.
- Suttle N, ed. 2010. *Mineral nutrition of livestock*. Wallingford: CABI.
- Tejada-Jiménez M, Gil-Díez P, León-Mediavilla J, Wen J, Mysore KS, Imperial J, González-Guerrero M.** 2017. *Medicago truncatula* Molybdate Transporter type 1 (MtMOT1.3) is a plasma membrane molybdenum transporter required for nitrogenase activity in root nodules under molybdenum deficiency. *New Phytologist* **216**, 1223–1235.
- Tsai HH, Schmidt W.** 2017. Mobilization of iron by plant-borne coumarins. *Trends in Plant Science* **22**, 538–548.
- Uauy C, Distelfeld A, Fahima T, Blechl A, Dubcovsky J.** 2006. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* **314**, 1298–301.
- Ueno D, Yamaji N, Kono I, Huang CF, Ando T, Yano M, Ma JF.** 2010. Gene limiting cadmium accumulation in rice. *Proceedings of the National Academy of Sciences, USA* **107**, 16500–16505.
- Van Der Straeten D, Bhullar NK, De Steur H, et al.** 2020. Multiplying the efficiency and impact of biofortification through metabolic engineering. *Nature Communications* **11**, 5203.
- Vassilakou T.** 2021. Childhood malnutrition: time for action. *Children* **8**, 103.
- Verbon EH, Trapet PL, Stringlis IA, Kruijs S, Bakker PAHM, Pieterse CMJ.** 2017. Iron and immunity. *Annual Review of Phytopathology* **55**, 355–375.
- Vural Z, Avery A, Kalogiros DI, Coneyworth LJ, Welham SJM.** 2020. Trace mineral intake and deficiencies in older adults living in the community and institutions: a systematic review. *Nutrients* **12**, 1072.
- Watts-Williams SJ, Smith FA, McLaughlin MJ, Patti AF, Cavagnaro TR.** 2015. How important is the mycorrhizal pathway for plant Zn uptake? *Plant and Soil* **390**, 157–166.
- Welch RM, Graham RD, Cakmak I.** 2014. Linking agricultural production practices to improving human nutrition and health. In: ICN2: Second International Conference on Nutrition. Rome, Italy, 19–21 November 2014. *Proceedings*. Rome: Food and Agriculture Organization of the United Nations.
- Yamasaki H, Hayashi M, Fukazawa M, Kobayashi Y, Shikanai T.** 2009. *SQUAMOSA* promoter binding protein-like7 is a central regulator for copper homeostasis in *Arabidopsis*. *The Plant Cell* **21**, 347–61.
- Yang M, Lu K, Zhao F-J, et al.** 2018. Genome-wide association studies reveal the genetic basis of ionomic variation in rice. *The Plant Cell* **30**, 2720–2740.
- Yazici MA, Asif M, Tutus Y, Ortas I, Ozturk L, Lambers H, Cakmak I.** 2021. Reduced root mycorrhizal colonization as affected by phosphorus fertilization is responsible for high cadmium accumulation in wheat. *Plant and Soil* **468**, 19–35.
- Zhang B, Zhang C, Liu C, et al.** 2018. Inner envelope CHLOROPLAST MANGANESE TRANSPORTER 1 supports manganese homeostasis and phototrophic growth in *Arabidopsis*. *Molecular Plant* **11**, 943–954.