Physiological Functions of

Mineral Micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl)

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Micronutrients are involved in all metabolic and cellular functions. Plants differ in their need for micronutrients, and we will focus here only on those elements that are generally accepted as essential for all higher plants: boron (B), chloride (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn). In this review, we focus on the major functions of mineral micronutrients, mostly in cases where they were shown as constituents of proteins, making a selection and highlighting some functions in more detail.

Introduction

Micronutrients are elements that are essential for plant growth but are required inmuch smaller amounts (Table 1) than those of the primary nutrients such as nitrogen, phosphorus, sulfur, and potassium. This list may grow as more protein structures are elucidated. All organisms have to acquire appropriate amounts of each micronutrient that requires a metal homeostasis network involving mobilization, uptake and distribution within the plant, intracellular trafficking, and storage. Several essential metal ions are redox-active that is the basis for their occurrence as catalytically active cofactors in many metalloenzymes. Other metals (like zinc) fulfill in addition to their catalytic role a structural role in stabilizing proteins.

Micronutrients are involved in virtually all metabolic and cellular functions, like energy metabolism, primary and secondary metabolism, cell protection, gene regulation, hormone perception, signal transduction, and reproduction among others. Historically, their physiological role was first described on the basis of deficiency symptoms. In this review, we focus on the major functions of mineral micronutrients, concentrating on cases where the micronutrient is a constituent of a particular protein.

Table 1

Micronutrients in plants.

Element	Symbol	Absorbed by plant	Concentration in plant [µg g^{-1} dry weight][a]	Protein complexed with the micronutrient (or other effects)
Boron	(B)	H_3BO_3	3–100	Rhamnogalacturonan II
Chlorine	(Cl)	Cl^-	20 000[b]	Oxygen evolving complex Seismonastic movement
Copper	(Cu)	Cu^{2+}	1–20	Ascorbate oxidase Polyphenol oxidase Cu–Zn superoxide dismutase Cytochrome *c* oxidase Plastocyanin Cu-metallothionein Ethylene receptor Mo-cofactor biosynthesis
Iron	(Fe)	Fe^{3+}, Fe^{2+}	50–150	
	Fe–S-cluster			Aconitase Succinate dehydrogenase NADH-Q oxidoreductase Thioredoxin reductase Xanthine dehydrogenase Aldehyde oxidase Ferredoxin
	Heme			Cytochromes Catalase, Peroxidase Cytochrome *c* oxidase Nitrate reductase Nitrite reductase Cytochrome P450 Leg hemoglobin
	Non-heme			Fe-superoxide dismutase Lipoxygenase Alternative oxidase Ferritin
Manganese	(Mn)	Mn^{2+}	10–100	Mn-superoxide dismutase PEP-carboxykinase Allantoate amidohydrolase Malic enzyme Isocitrate lyase PEP carboxylase
Molybdenum	(Mo)	MoO_4^{2-}	0.1–1	Nitrate reductase Sulfite oxidase Aldehyde oxidase Xanthine dehydrogenase
Nickel	(Ni)	Ni^+	15–22	Urease Ni-chaperone
Zinc	(Zn)	Zn^{2+}	15–50	SPP Carbonic anhydrase Cu–Zn superoxide dismutase Alcohol dehydrogenase Peptide deformylase α-Mannosidase Matrix metalloproteinase

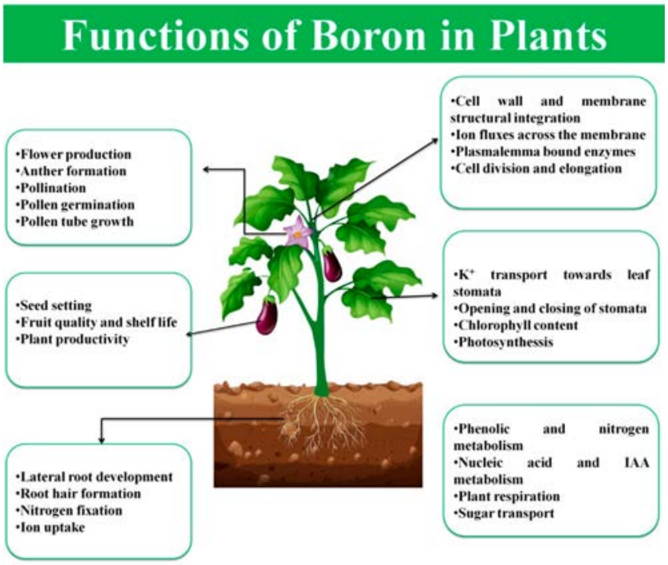
[a] The concentration of micronutrients in plants can vary widely depending on the species, genotype, organ, tissue, and growth condition. Therefore ranges are given.
[b] Requirement for optimal growth: 200–400 µg g^{-1} dry weight.

Boron

The unusual nature of boron chemistry suggests the possibility of a wide variety of biological functions for the micronutrient; however, the exact metabolic functions are not finally understood. Boron is involved in numerous important processes, including protein synthesis, transport of sugars, respiration, RNA and carbohydrate metabolism, and the metabolism of plant hormones (indole acetic acid). Moreover, functions of boron are related to cell wall synthesis, lignification, and cell wall structure by cross-linking of cell wall polysaccharides as well as the structural integrity of biomembranes. It increases the transport of chlorine and phosphorus as a result of plasmalemma ATPase induction.

More than 90% of the boron in plants is found in cell walls, and rhamnogalacturonan II was shown to bind boron. Because the wall-associated kinase in the plasmamembrane has an extracellular matrix connection with the pectin molecule, the membrane cell wall connection is finally also boron-dependent. Boron was found to promote the structural integrity of biomembranes and the formation of lipid rafts].

Since all these functions are fundamental to meristematic tissues, boron deficiency is predominantly damaging actively growing organs such as shoot and root tips so that the whole plant may be stunted (rosetting). Flower retention, pollen formation, pollen tube growth or germination, nitrogen fixation, and nitrate assimilation are also affected by boron.



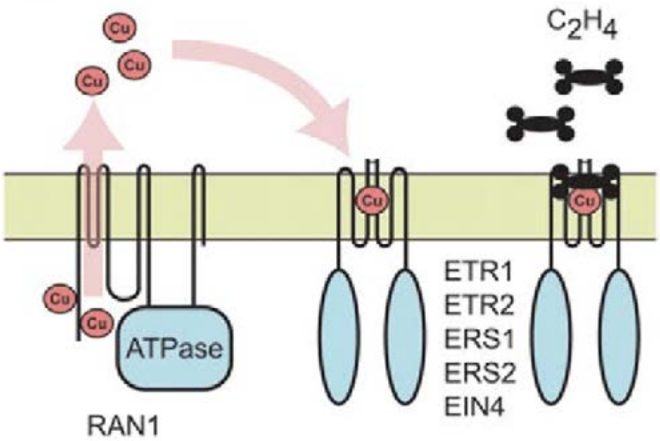
Chlorine

Chlorine is known to exist in more than 130 organic compounds in plants [7]. Most soils contain sufficient levels of chlorine. However, chlorine deficiencies have been described in sandy soils in high rainfall areas or could be created artificially in experiments to prove its requirement as a micronutrient for higher plants. Because chloride is a mobile anion in plants, most of its functions are related to electrical charge balance.

In the chloroplast, chloride is a structural constituent of photosystem II in the oxygen evolving complex as one of the three important cofactors. Proton-pumping ATPase at the tonoplast is specifically stimulated by chloride . The overall chlorine concentration in the whole plant is too low to be an effective osmo-regulator; however, chlorine is accumulated in certain tissues or single cells (e.g. guard cells). Opening and closing of the guard cells is mediated by the flux of potassium and anions such as malate and chloride. Therefore, chlorine indirectly affects plant growth by stomatal regulation. Reduction of leaf surface area, wilting of the plant, and restricted, highly branched root systems are the main chlorine-deficiency symptoms. On the contrary, seismonastic leaf movement of Mimosa pudica is directly chlorine-dependent. The 'osmotic motor' for the leaf movement is powered by a plasma membrane proton ATPase, which drives KCl and water fluxes. The movement results from different volume and turgor changes in the two oppositely positioned parts in the specialized motor leaf organs called pulvinus.

Copper

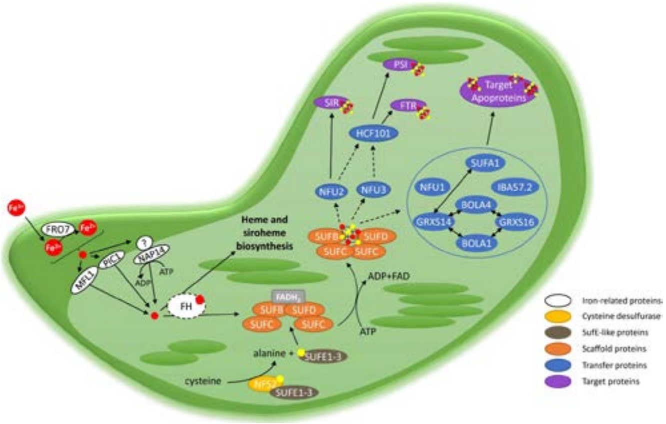
Copper is of utmost importance for life. Copper is essential for photosynthesis and mitochondrial respiration, for carbon and nitrogen metabolism, for oxidative stress protection, and is required for cell wall synthesis. Under physiological conditions, copper exists in the two oxidation states Cu^{1+} and Cu^{2+} and can interchange between these forms (monovalent copper is unstable). This allows copper to function as a reducing or oxidizing agent in biochemical reactions.



Copper has a particularly high affinity to dioxygen molecules that explains why copper is the catalytic metal in many oxidases. More than half of the copper in plants is found in chloroplasts and participates in photosynthetic reactions. Hence, copper deficiency becomes first visible in young leaves and reproductive organs, later consequences are stunted growth of the whole plant and pale green leaves that wither easily. Interestingly, copper metabolism is intimately linked to iron metabolism. Finally, copper is also part of the ethylene receptor and is involved in molybdenum cofactor biosynthesis.

Iron

Like copper, iron is also of great importance for life. As redox-active metal it is involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis (ethylene, gibberellic acid, jasmonic acid), production and scavenging of reactive oxygen species, osmoprotection, and pathogen defense. Up to 80% of the cellular iron is found in the chloroplasts that is consistent with its major function in photosynthesis. Depending on the type of iron ligand, three groups of iron-containing proteins can be defined: (1) proteins with iron–sulfur clusters (Fe–S), (2) heme-containing proteins, and (3) other iron proteins.



Fe–S proteins: Fe–S clusters have pivotal functions in electron transfer, they constitute part of substrate binding sites in enzymes, they form iron storage moieties, they are involved in transcriptional or translational regulation, they can control protein structure in the vicinity of the cluster, and finally they have been shown to be involved in disulfide reduction and sulfur donation (e.g. thioredoxins). Hence, Fe–S proteins serve functions as enzymes, as electron carriers (e.g. ferredoxin), and as regulator proteins (e.g. aconitase).

Heme proteins: The well-known hemoproteins are the photosynthetic and respiratory cytochromes, involved in electron transfer, and the globins that bind oxygen. Other examples include the oxidative enzymes catalase, peroxidase, and NADPH oxidase, involved in the production and/or scavenging of free radicals, and the very large group of cytochrome P450 enzymes. In plants and microbes, these latter catalyze mono-oxygenation reactions in biosynthetic pathways, such as for sterols and many secondary metabolites, whereas in animals their major role is in the detoxification of xenobiotics. Further, globins like leg-hemoglobin are involved in oxygen binding and transport.

Other iron proteins: These proteins (that are sometime also grouped as non-heme proteins) bind iron ions directly, i.e. neither as heme nor in the Fe–S form. Among these proteins, ferritins are most prominent. Ferritins are plastidic iron storage proteins and control the interaction between iron homeostasis and oxidative stress in Arabidopsis. Ferritins occur mostly in nongreen plastids like etioplasts and amyloplasts but not in mature chloroplasts.

Manganese

Manganese is essential for plant metabolism and development and occurs in oxidation states II, III, and IV in approximately 35 enzymes of a plant cell [25]. Manganese can fulfill two functions in proteins: (1) it serves as catalytically active metal, or (2) it exerts an activating role on enzymes. Examples for the catalytic role are manganese-containing superoxide dismutase protecting the cell from damaging effects of free radicals, the oxalate oxidase, and the manganese-containing water splitting system of photosystem II [26]. Examples for the manganese- activated enzymes are malic enzyme, isocitrate dehydrogenase, PEP carboxykinase, and phenylalanin ammonia lyase.

Among the rather large group of manganese- activated enzymes, the role of manganese is less specific as in many cases it can be replaced by magnesium.

Proteins belonging to this group are involved in the shikimic acid pathway and subsequent pathways leading to the formation of aromatic amino acids, lignins, flavonoids, and the phytohormone indole acetic acid. Manganese activation was seen in enzymes of nitrogen metabolism (glutamin synthetase, arginase), gibberellic acid biosynthesis, RNA polymerase activation, and fatty acid biosynthesis.

Molybdenum

Only a handful of plant proteins are known to contain molybdenum. These proteins, however, are very important as they are involved in nitrogen assimilation, sulfur metabolism, phytohormone biosynthesis, and stress reactions. Nitrate reductase is the key-enzyme for nitrate assimilation while nitrogenase is found in nitrogen fixing bacteria inside nodules of symbiotically growing species. The last step of abscisic acid biosynthesis is catalyzed by the molybdenum-enzyme aldehyde oxidase, and sulfite oxidase protects the plant against toxic levels of sulfite (acid rain!). Hence a defect in molybdenum-metbolism leads to the pleiotropic loss of these enzyme activities with lethal consequences for the organism.

In all organisms, molybdenum has to be complexed by a pterin compound thereby forming the molybdenum cofactor in order to gain biological activity. Molybdenum metabolism is intimately linked to iron and copper metabolism at several crosspoints (Figure 1). Another crosstalk was discovered between molybdenum and copper metabolism as copper was found to be essential for the formation of a molybdenum cofactor intermediate.

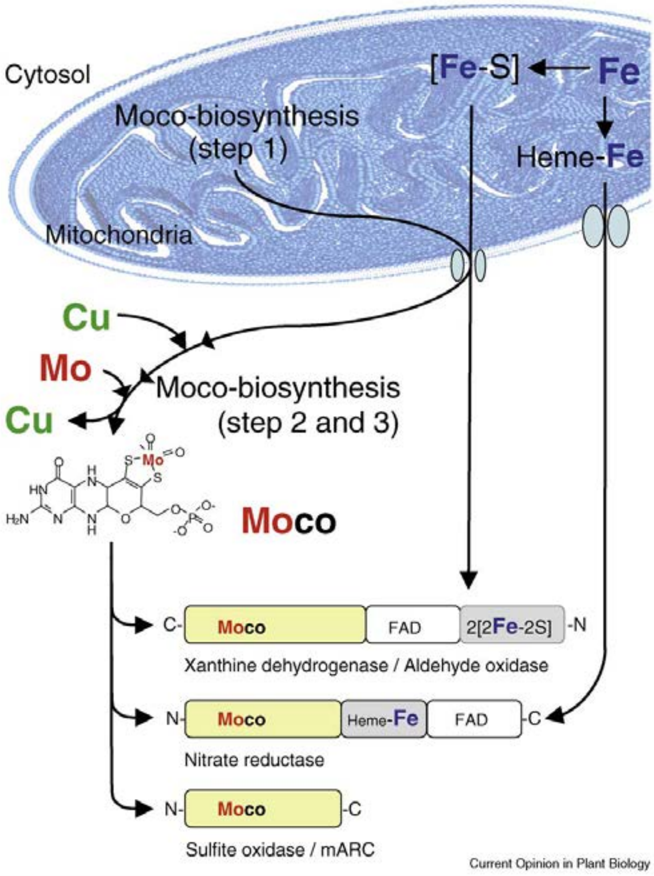


Figure 1 Schematic representation of the metabolic link between the three micronutrients molybdenum, iron, and copper as it is found in molybdenum metabolism. In order to become biologically active, molybdenum has to be complexed by a pterin compound thereby forming the molybdenum cofactor (Moco).

Nickel

Nickel is essential in numerous prokaryotic enzymes like dehydrogenases, hydrogenases, and methylreductases but is barely used as cofactor in eukaryotes. Among plants, it occurs not only in oxidation states II, but also in states I and III. A deficiency symptom in plants is the accumulation of toxic urea that could be explained with the complete loss of urease activity within the cell. Plant urease hydrolyzes its substrate to carbon dioxide and ammonia. An additional Ni^{2+}-binding protein could be identified in soybean that acts as Ni-metallochaperone essential for urease activity. It seems to be possible that a few more Ni-dependent enzymes will be identified in plants in the future.

Zinc

Zinc is important as a component of enzymes for protein synthesis and energy production and maintains the structural integrity of biomembranes. More than 1200 proteins are predicted to contain, bind, or transport Zn^{2+}, including – among others – a large numbers of zinc-finger containing proteins and transcription factors, oxidoreductases, and hydrolytic enzymes such as metalloproteases. Zinc plays also an important role in seed development, and zinc-deficient plants show a delayed maturity.

Most of the zinc enzymes are involved in regulation of DNA-transcription, RNA-processing, and translation. In chloroplasts, zinc-dependent enzymes fulfill several major functions. The stromal processing peptidase SPP is zinc-dependent in analogy to the mitochondrial system. Moreover inside the chloroplasts proteolytic activities are dependent on zinc. Zinc deficiency also reduces net photosynthesis in plants by disturbing the activity of carbonic anhydrase.

In addition to chloroplasts and mitochondria, also the cytoplasm, lysosome, and the apoplastic space are compartments with zinc-dependent hydrolytic activities: different nucleases and aminopeptidases, peptide deformylases, the 26S-proteasome, the a-mannosidase, and matrix-metalloproteinases associated with the extracellular matrix. Further, Zinc was found to be involved in signal transduction via mitogen-activated protein kinases.

Conclusion

Essential micronutrients were found as constituents in over 1500 proteins where they fulfill catalytic, (co-)activating, and/or structural functions.

The largest group (>1200) is formed by zinc-proteins (with transcription factors as major subgroup). Proteins containing iron, copper, or manganese make up groups in the range of 50–150 members each, while molybdenum and nickel proteins can be counted on one hand each. Boron and chlorine are very important, but proteins or compounds that were unambiguously shown to contain these micronutrients are very rare and mostly elusive.