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# Soil metals, chloroplasts, and secure crop production: a review

Katalin Solymosi · Martine Bertrand

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**Abstract** An increasing number of soils of poor quality with unbalanced metal concentrations are used worldwide for crop cultivation. Even though plants are able to develop strategies to cope with metal stress, either metal deficiency or excess, this unbalance affects the whole plant. Chloroplasts are key organelles for organic matter synthesis and biomass production. Under metal stress, chloroplasts suffer severe alterations of their ultrastructure, associated with profound molecular and metabolic damages. These alterations are accompanied by unbalanced metal distribution in plants, in particular in edible crop organs. Toxic metals get either accumulated or there is a deficiency of nutrients, resulting in a weak nutritional value. Nonetheless, there is more and more knowledge on the functioning and regulation of metal transporters in plants. Such knowledge will allow growing crops with well-balanced metal concentrations in edible parts even on metal unbalanced soils. This review shows that almost all vital functions of chloroplasts, such as photosynthesis, CO<sub>2</sub> fixation, nitrogen and sulfur assimilation, and protein and nucleic acid metabolism, require metals. Therefore, the uptake of essential metals is necessary for the proper functioning of chloroplasts and, in turn, for crop productivity. We

describe nutrient uptake mechanisms of plants and processes that influence essential and non-essential metal concentrations in different plant organs. We present an overview of metal transporters in chloroplasts. Several questions still need to be elucidated about the uptake and the trafficking of essential and non-essential metals into and within chloroplasts. Similarly to transporters present in other cellular compartments, the carriers are often not metal-specific. Therefore, essential and non-essential metals may compete for carriers. As a result, unbalanced soil metal concentrations can be reflected in the plants and in the chloroplasts. Metal deficiency or excess causes reduced growth and decreased productivity of crops. It can lead to human malnutrition. Several complex physiological processes can be responsible for the reduced biomass observed in plants with unbalanced metal concentration. In this review, we have focused on the structural and functional alterations of chloroplasts under metal deficiency or excess. Interestingly, besides specific differences, our data indicate several similarities in the response of chloroplasts to metal deficiency or excess. Indeed, oxidative stress and several ultrastructural alterations, e.g., increase in the number and size of plastoglobuli, disorganized grana and disturbed thylakoids, and swelling of the intrathylakoidal space, are observed in both cases. This indicates that changes in chloroplast ion homeostasis rather than the specific effect of a metal are responsible for decreased plant productivity. Therefore, sustainable agriculture has to take into consideration solutions that enable undisturbed metal and ion homeostasis in chloroplasts of crop plants grown even in soils with unbalanced metal concentrations.

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## 1 Introduction

The growth of the world's population exerts a very strong pressure on food production. In order to satisfy the demand, more food must be produced, which necessitates the use of soils of poor quality, i.e., soils having unbalanced metal content (see, e.g., Adams et al. 2000; Wilson and Pyatt 2007; reviewed by Hagemeyer 2006; Wei and Zhou 2008). In addition, the gradual decline in the availability of freshwater for irrigation leads to the use of sewage sludge (e.g., El-Naim et al. 2004; Rattan et al. 2005; Antonious and Snyder 2007; Singh and Agrawal 2007; Jamali et al. 2009) or other wastewaters (e.g., Mapanda et al. 2005; Sahn et al. 2007; Arora et al. 2008; Nair et al. 2008).

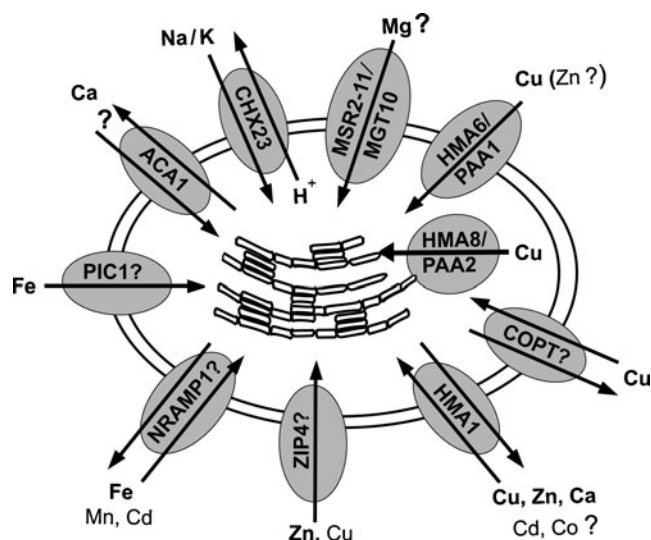
Numerous papers have reported the impact of metal unbalance on plant development and productivity and also on the strategies developed by plants to cope with these conditions. However, even if plants have the capacity to compensate the unbalance and to keep on growing to some extent in a polluted environment, this does not mean that

their nutritive quality is acceptable because their essential element content can be insufficient (see, e.g., reviews by Prasad 2004; Palmgren et al. 2008) or they may contain toxic metals (see, e.g., Muchuweti et al. 2006; reviewed by Dudka and Miller 1999).

Generally absorbed by roots, most of the metals are transported toward the leaves through the stem. They are distributed according to the demand of the different cell compartments (reviewed by Poirier et al. 2008). Among these compartments, the chloroplasts appear as key organelles because they are the producers of organic matter via photosynthesis. Plastids also harbor other vital biosynthetic functions, such as nitrogen (ammonia) and sulfur assimilation or the biosynthesis of fatty acids, (aromatic) amino acids, lipids, pigments (chlorophylls and carotenoids), purines, pyrimidines, and secondary metabolites (reviewed by Seddas et al. 2009). In consequence, these functions require an active solute exchange across the chloroplast envelope. Many reactions involved in these biosynthetic processes require metals, and therefore, metal import is organized through the plastid envelopes (Fig. 1, e.g., reviewed by Weber et al. 2005; Poirier et al. 2008; Spetea and Schoefs 2011). In the frame of the development of agriculture, and in particular of sustainable agriculture, the full understanding of metal transport and metabolism in plants becomes a prerequisite for improving plant productivity on soils with unbalanced metal contents. In this respect, the chloroplast should stand in the focus of the research because all food chains depend on the functioning of chloroplast metabolism. In this work, after having recalled the metal requirements of plants, the metal translocation to chloroplasts is briefly described. Then, the impacts of metal deficiency or excess are discussed in details. The last part of this work briefly reviews perspectives about how to secure crop production with balanced metals in edible parts of plants. All along this work, metals are designated using their symbols, without any charge, in order to avoid overloading the text by too many details.

## 2 Metal requirement of plants and their roles with emphasis on chloroplasts

Metals (italicized) and metalloids (asterisk) belong to essential (*K*, *Ca*, *Mg*, *Fe*, *B*\*, *Mn*, *Cu*, *Zn*, *Mo*, *Ni*) and non-essential elements (*Co*, *Na*, *Se*\*), the latter being beneficial for some plants only (*Co*: Oven et al. 2002; *Na*: reviewed by Subbarao et al. 2001; *Se*: reviewed by Sors et al. 2005). Besides the elements C, H, O, and N, those that are required in large quantities are called macronutrients (*P*, *S*, *K*, *Ca*, *Mg*), whereas those that are necessary in smaller amounts are named micronutrients (*Fe*, *B*\*, *Mn*, *Cu*, *Zn*, *Mo*, *Cl*, *Ni*; reviewed by Marschner 1995).



**Fig. 1** Scheme presenting the most important metal transporters located to the chloroplasts. The primary transported metals are indicated in **bold**. The *question marks* stand for uncertainties, i.e., the exact localization of the protein (when the chloroplast localization of the protein is only computer-predicted, a question mark is on the protein itself); the metals transported (the question mark stands after the symbol of the metal(s) in question); or in the direction of the transport (question mark between the *arrows*). ACA1/PEA1: Ca-ATPase 1 (Huang et al. 1993; White and Broadley 2003; Johnson et al. 2006); CHX23: Na<sup>+</sup>(K<sup>+</sup>)/H<sup>+</sup> antiporter 23 (Song et al. 2004a); MSR2-11/MGT10: magnesium transporter (Drummond et al. 2006; Mueller-Roeber and Arvidsson 2009); HMA6/PAA1 heavy metal transporter 6 (Shikanai et al. 2003; Abdel-Ghany et al. 2005; Puig et al. 2007a; Yruela 2009); HMA8/PAA2 heavy metal transporter 8 (Shikanai et al. 2003; Abdel-Ghany et al. 2005; Puig et al. 2007a; Burkhead et al. 2009; Yruela 2009); COPT: conserved copper transporter (Sancenon et al. 2003; Burkhead et al. 2009; Yruela 2009); HMA1: heavy metal transporter 1 (Seigneurin-Berny et al. 2006; Puig et al. 2007a; Burkhead et al. 2009; Kim et al. 2009; Yruela 2009); ZIP4: ZRT, IRT-like protein 4 (Grotz et al. 1998; Hall and Williams 2003; Grotz and Guerinot 2006); NRAMP1: natural resistance-associated macrophage protein 1 (Curie et al. 2000; Thomine et al. 2000; Grotz and Guerinot 2006; Puig et al. 2007a); PIC1: permease 1 (Duy et al. 2007; Puig and Penarrubia 2009)

In the chloroplasts, similarly to other cell compartments, the metals function as structural constituents of various molecules, essentially of proteins, or are used to modulate metabolic reactions or cellular processes. Table 1 indicates the metals required by plastids and some of their important functions specific to chloroplasts. In addition to these roles, the chloroplast can serve as a storage compartment for Ca (Seigneurin-Berny 2000). Alternatively, chloroplasts can be involved in the cellular detoxification (rather sequestration) of excess metals such as Fe, Cu, or Na (for details, see Section 5.1.2).

Taken together, these data clearly indicate that practically all vital functions of chloroplasts require metals. This way, the uptake of essential metals is necessary for the proper functioning of this organelle and as a consequence for crop

productivity. On the other hand, plastids also play a role in essential metal (Ca, Cu, Fe) homeostasis.

### 3 Metal transport from soil to chloroplasts

Data about the total metal content of a soil give interesting information (e.g., the abundance of essential or non-essential elements), but scarcely help in predicting the metal contents and proportions found within a plant cultivated on it (Antonious and Snyder 2007; Wilson and Pyatt 2007). The main reasons for this difficulty are (1) the non-phytoavailability of some metal forms, (2) the active transport of solutes through plants, (3) the atmospheric deposition of metals on aboveground organs, (4) the competition between different metals for the uptake and transport within the plants, and (5) the possible presence of unbalanced metal concentrations already in the seeds before germination.

#### 3.1 Metals in soil

Metal phytoavailability results from complex interactions between the physicochemical properties of the soil (including, e.g., pH, presence of clay particles, complexation, organic matter, and moisture; e.g., Pinto et al. 2004; Basar 2009; reviewed by Fodor 2006) and those of the metal (chemical forms, stability, mobility, and solubility; Whiting et al. 2003; reviewed by Fodor 2006). For instance, metals are in general more soluble at low pH, and consequently, their phytoavailability increases. Therefore, soil acidification constitutes a real problem in case of pollution (see, for example, Poschenrieder et al. 2008 for aluminum). Note that acid soil represented in 2004 approximately 50% of the world's arable soils (Kochian et al. 2004).

Although acidification may increase the essential metal availability for plants, it leads to unbalanced metal concentrations in soils and can this way lead to the accumulation of toxic concentrations of either essential or non-essential elements in plants.

#### 3.2 Metal transport: from the roots to other plant organs

Plants and soil microbes possess different mechanisms that help in the acquisition of essential metals from the soil, including the lowering of the soil pH or excreting out different metal chelating agents (phytosiderophores or siderophores in plants and microbes, respectively; reviewed by Grusak et al. 1999; Schmidt 2003; Fodor 2006; Haydon and Cobbett 2007; Puig et al. 2007a; Kidd et al. 2009). Besides essential metal uptake, phytosiderophores may also be involved in toxic metal (e.g., Al, Cd, and Pb) uptake (Hill et al. 2002; reviewed by Kochian 1995; Fodor 2006).

**Table 1** Metabolic pathways of chloroplasts and their metal requirements (non-exhaustive list of enzymes or processes)

Chlorophyll synthesis and breakdown	
Co	Delta-aminolevulinic acid dehydratase (Shalygo et al., 1999)
Fe	Delta-aminolevulinic acid synthesis (Shalygo et al., 1999)
Mn	Porphobilinogenase (Shalygo et al., 1999)
Ni	
Fe	Chlorophyllide <i>a</i> oxygenase (Duy et al., 2007) Conversion of Mg-protoporphyrin monomethyl ester into protochlorophyllide (Myśliwa-Kurdziel and Strzałka, 2002) Pheophorbide <i>a</i> oxygenase (Duy et al., 2007)
Mg	Delta-aminolevulinic acid dehydratase (Beale, 1999) Mg-chelatase (Beale, 1999)
Haem biosynthesis	
Fe	Ferrochelatase (Cornah et al., 2002)
Photosynthetic light reactions	
Ca	Assembly of photosystem II (Raven et al., 1999)
Cu	Plastocyanin (Abdel-Ghany, 2009)
Fe	Cytochrome b6-f (Raven et al., 1999) Cytochrome c6 (Raven et al., 1999) Ferredoxin (Tognetti et al., 2007) NAD(P)H-plastoquinone oxidoreductase (Raven et al., 1999)
Mg	ATP synthase (Shaul, 2002, Kucera et al., 2008)
Mn	Water oxidase (Grace, 1990)
Zn	ATP-dependent Zn-metalloprotease FtsH (Bailey et al., 2001)
Carbon assimilation.	
Ca	Zn-protease degrading ribulose-1,5-bisphosphate carboxylase oxygenase (Bushnell et al., 1993)

**Table 1** (continued)

Mg	Fructose 1,6-bisphosphatase (Shaul, 2002) RuBisCO (Shaul, 2002, Kucera et al., 2008) Sedoheptulose 1,7-bisphosphatase (Shaul, 2002)
Mn	Malic enzyme (Takeuchi et al., 2000)
Zn	Carbonic anhydrase (Randall and Bouma, 1973) Zn-protease degrading ribulose biphosphate carboxylase oxygenase (Bushnell et al., 1993)
Antioxidant system	
Cu	Cu/Zn-superoxide dismutase (Grace, 1990) Metallochaperone for Cu/Zn superoxide dismutase (Puig et al., 2007a)
Fe	Ascorbate peroxidase (Raven et al., 1999) Fe-superoxide dismutase (Allen, 1995)
Mg	Glutathione synthetase (Shaul, 2002)
Mn	Mn-superoxide dismutase (Grace, 1990)
Zn	Cu/Zn-superoxide dismutase (Grace, 1990)
Sulfur metabolism	
Fe	Adenosine-5-phosphate reductase (Duy et al., 2007) Sirohydrochlorin ferrochelatae (Duy et al., 2007) Sulfite reductase (Duy et al., 2007)
Nitrogen metabolism	
Fe	Amidophosphoribosyltransferase (Duy et al., 2007) Glutamine-2-oxo-glutarate amido transferase (Duy et al., 2007) NADH-dependent glutamine-2-oxo-glutarate amido transferase (Duy et al., 2007) Nitrite reductase (Briat and Vert, 2004) Sirohydrochlorin ferrochelatae (Duy et al., 2007)
Mg	Glutamine synthetase (O'Neal and Joy, 1974)

**Table 1** (continued)

Basic metabolism	
Fe	Tic55 (Duy et al., 2007)
Zn	Acetyl-coenzyme A carboxylase carboxyl transferase (Sasaki et al., 1989) Chloroplast processing enzyme (VanderVere et al., 1995) Enzymes involved in RNA editing (Hänsch and Mendel, 2009) Metalloendopeptidase (Moberg et al., 2003) Stromal processing peptidase (Hänsch and Mendel, 2009) Zn-finger domains ( <i>e.g.</i> Sasaki et al., 1989)

Metals move into the root both via the apoplasm and symplasm pathways (reviewed by Grusak et al. 1999). At the endodermis, all ions to be transported must enter the stele via the symplastic route by metal transporters located in the plasma membranes of endodermis cells. However, apoplastic flow at sites of lateral root emergence may occur and the Casparian bands do not represent a perfect barrier, as is usually thought (Ranathunge et al. 2005). Once within the stele, the ions are unloaded into the xylem vessels. They are transported toward the shoot as chelates of organic ligands (reviewed by Grusak et al. 1999; Fodor 2006; Haydon and Cobbett 2007; Puig et al. 2007a; Yruela 2009). Once the solutes have reached an organ, for example a leaf, the chelates travel in general through the apoplasm to the target cell (reviewed by Leigh and Tomos 1993; Grusak et al. 1999).

### 3.3 Metals can also enter the plant through the aboveground organs

In industrial areas or along heavily used roads (Wu et al. 2009), metals such as Cd, Hg, or Pb can be present in the air and enter into the plant through its aerial parts such as leaves (*e.g.*, Nabulo et al. 2006; Sharma et al. 2008a, b), inflorescences (*e.g.*, Peris et al. 2007), and fruits (*e.g.*, Bi et al. 2006; Pandey and Pandey 2009). The metals can be absorbed through the cuticle and the epidermis during rainfalls or with dew formation. In these cases, the metals enter probably by stomata or ectodesmata and then move in the apoplasm of the organs (reviewed by Marschner 1995). Despite the fact that there are several data on metal deposition on crops (*e.g.*, palak: Sharma et al. 2008a, b; cabbage: Sharma et al. 2008b; maize: Bi et al. 2006; red spinach: Pandey and Pandey 2009; artichoke, lettuce, and

Swiss chard: Peris et al. 2007; spinach, amaranth, and eggplant: Pandey and Pandey 2009), there is almost no information about the mobilization and transport processes of airborne pollutants inside higher plants.

In general, a large proportion of the metals present in excess in the soil is retained in the roots (*e.g.*, Kawachi et al. 2009); however, in polluted areas, often considerable amounts of metals get transported to the shoots via the xylem. In addition, airborne pollutants may also increase metal concentration in aerial plant organs, and as a consequence, the probability of toxic or excess metals entering the chloroplasts increases.

### 3.4 Chloroplasts as metal targets

Despite the obvious roles of metals in plastid metabolism (Table 1), metal import mechanisms are not completely understood. Several transporters have been identified in plastid metal uptake and export (Fig. 1; reviewed by Weber et al. 2005; Spetea and Schoefs 2011). As the question marks indicate it in Fig. 1, the metal specificity, the direction of transport, and plastid localization are not yet completely determined for every metal transporter *in vivo*. Sometimes only the ion fluxes have been measured, but the protein has not been characterized in detail, as in the case of a thylakoid-located  $\text{Ca}^{2+}/\text{H}^{+}$  antiporter directing Ca influx into the lumen (Ettinger et al. 1999, not shown in Fig. 1). Note that the non-physiological ions such as Cd can be transported into the plastids using transporters of other essential metals (Fig. 1).

Metal transporters located in the chloroplast belong to the same metal transporter families found in other cell compartments. For instance, the protein HMA8/PAA2, located in the thylakoid membrane and transporting Cu into the thylakoid lumen, has homologues in the chloroplast envelope (HMA6/PAA1) in the tonoplast (*e.g.*, Morel et al.

2009) and in the plasma membrane (reviewed by Palmgren and Harper 1999; Yruela 2009).

Although some data are available about plastidial metal transporters, several questions still need to be elucidated about the uptake and the trafficking of metals into and within chloroplasts. However, it is clear that similarly to transporters present in other cellular compartments, often the carriers are not metal-specific; therefore, different metals may compete for them.

### 3.5 Cross talk between different metals

Nearly all studies on metal excess or deficiency have dealt with the effect of single elements, but such studies usually do not reflect a real situation in agriculture, or the critical response levels in nature. Actually, whenever a land becomes contaminated with metals, often two or more elements are in excess simultaneously and may compete for transporters (reviewed by Manthey et al. 1994). For instance, P<sub>1B</sub>-type heavy metal ATPases are involved in the transport of a range of essential and potentially toxic metals (i.e., Cu, Zn, Cd, Pb) across cell membranes or other membranes (reviewed by Yruela 2009). This way, Zn competes with its toxic analogue, Cd, for these transporters (Fig. 1). The consequence of such metal competition can be the deficiency of essential elements in chloroplasts. Therefore, the addition of the deficient metal often alleviates the toxic effects of the metal in excess (e.g., Ca in the case of excess Al: Guo et al. 2006; excess Cd: Wang and Song 2009; or excess Ni: Ouzounidou et al. 2006; Fe in the case of excess Cd: Siedlecka and Krupa 1996; Solti et al. 2008; Zn in the case of excess Cd: Zhao et al. 2005; Aravind and Prasad 2004; Podar et al. 2004). Interestingly, sometimes, a small amount of non-essential metal can be beneficial for the plant due to improvement of ion balance; for example, a low Cr level improves Fe/Mn ratio in Fe-deficient bean chloroplasts (Poschenrieder et al. 1991).

Cross talk between two or more essential elements also happens. For instance, Zn in high excess causes Cu and Fe deficiency (sugar beet: Sagardoy et al. 2009; rapeseed: Wang et al. 2009) and interference with P, Mg, K, and Mn uptake (reviewed by Broadley et al. 2007). Ni excess can trigger decreased micronutrient levels (Ca, Mg, Fe, K; wheat: Ouzounidou et al. 2006), Fe deficiency (cabbage: Pandey and Sharma 2002), and K deficiency (pea: Gabbrielli et al. 1999) and hinder the transport of K and Zn (cucumber: Varga et al. 1999). On the other hand, Mg and Ca excess inhibits Ni uptake (reviewed by Chen et al. 2009). Excess Cu induces chlorosis (i.e., yellowing of the leaves due to decreased chlorophyll content), probably through a competitive interaction with Fe and/or Mn uptake and/or transportation (reviewed by Yruela 2009), but competitive interactions of Cu with Mg, Mn, Ni, and Ca have been also observed (reviewed by Barón et al. 1995; Chen et al. 2009).

It is clear from many studies that unbalanced soil metal concentrations can be reflected also in the plants. Either some essential elements are lacking and/or metals are in excess because the essential or non-essential metal(s) present in excess outcompete(s) the (other) essential metals from the uptake and transport mechanisms.

## 4 Impact of metal deficiency

In several agricultural fields, nutrient deficiency limits crop production (reviewed by Guerinot 2000). The metals, which are most likely to give rise to deficiency problems in plants, are Cu, Fe, Mn, Mo, and Zn (reviewed by Alloway 1995). When the deficiency is very strong, plants might be unable to compensate it and their use as human food might contribute to malnutrition. Below, we briefly discuss symptoms of metal deficiency on the metabolic, microscopic, and macroscopic levels with emphasis on chloroplasts.

### 4.1 Molecular and metabolic alterations in chloroplasts

Optimal concentrations of essential metals are needed for normal plastid ultrastructure, homeostasis, and functioning (for details, see Table 1 and Section 2). Chl biosynthesis requires several metals (Table 1); therefore, decreased Chl content is a general symptom of essential metal deficiency (e.g., Thoirion et al. 1997; Misra et al. 2006; Duy et al. 2007; Mahmoudi et al. 2007; Timperio et al. 2007; reviewed by Myśliwa-Kurdziel and Strzałka 2002). Electron transport efficiency (e.g., Droppa et al. 1984; Baszyński et al. 1978; reviewed by Maksymiec 1997) and CO<sub>2</sub> fixation (e.g., Srivastava et al. 1997; Siedlecka and Krupa 1996; reviewed by Myśliwa-Kurdziel et al. 2002) also decrease as several enzymes or proteins involved in these processes require essential metals (Table 1). At the same time, oxidative stress is increased (e.g., under Zn, Mn, Fe, Cu, B, Mg, and K deficiency; Yu et al. 1999; reviewed by Cakmak 2000), which can be also explained partially by the fact that important components of reactive oxygen-scavenging systems of plastids are metalloenzymes (e.g., Cu-, Zn-, Fe-containing superoxide dismutases). Lipids are also often affected by metal deficiency (e.g., Zn: reviewed by Cakmak 2000; Cu: reviewed by Barón et al. 1995).

Disturbances in stomatal conductance and water status are often reported for metal-deficient plants (e.g., Zn: Khan et al. 2004). Stomata opening requires ionic currents and special ion balance, including K, Ca, and Mg (reviewed by Shaul 2002; Maathuis 2009). As a consequence, deficiency or unbalanced K, Ca, and Mg concentrations lead to disturbances in the respiration and the transpiration (water homeostasis and mineral nutrition) of plants. Reduced CO<sub>2</sub>



uptake resulting from stomata closing inhibits the dark phase of photosynthesis and, this way, indirectly decreases crop productivity. All these observations are consistent with data of Table 1 and indicate profound alterations in plastid functions upon metal deficiency.

#### 4.2 Ultrastructural alterations of chloroplasts

Metal-deficient conditions trigger ultrastructural changes in plastids (Table 2). The organelles are sometimes less abundant and smaller than in non-deficient plants. They have essentially disorganized thylakoid membranes, dismantled grana, and swollen intrathylakoidal space. An increase in the number and the size of plastoglobuli (spherical bodies that contain lipids, carotenoids, plastoquinone, and proteins) is also often observed.

Interestingly, the profound ultrastructural alterations observed in chloroplasts under different metal deficiencies are quite similar. This indicates that any disturbance in plastid ion homeostasis has drastic consequences on chloroplast structure and function and, this way, also on plant productivity.

#### 4.3 Alterations on the level of the entire plant

Nutrient deficiency symptoms are usually expressed as reduced growth and altered physiology. When Cu, Fe, or Zn deficiency occurs, the root architecture is modified in order to allow an increase in the nutrient uptake and retention (reviewed by Wintz et al. 2002; Schmidt 2003; Puig et al. 2007a; Walker and Connolly 2008; Pilon et al. 2009). The interactions within the rhizosphere may also be altered (e.g., Rengel 1997; reviewed by Kidd et al. 2009). Another symptom is chlorosis accompanied by reduced photosynthetic activity leading to decreased biomass (e.g., Cu deficiency: reviewed by Maksymiec 1997; Fe deficiency: Timperio et al. 2007; Mn deficiency: Henriques 2004; Zn deficiency: Chen et al. 2008). Metal deficiency response of plants involves (1) increased expression and regulation of the uptake systems, (2) prioritization of the use of metals in essential versus dispensable pathways by mobilizing them within the plant, and (3) substitution of metalloproteins with similar or overlapping functions (reviewed by Puig et al. 2007a).

## 5 Impact of metal excess

Atmospheric deposition, urban–industrial activities, and agricultural practices (e.g., use of agrochemical products and addition of organic amendments) are the main anthropogenic sources of heavy metals in agricultural soils (e.g., Mico et al. 2006; Wei and Yang 2010). Representing a serious threat to mankind, the effect of metals present in

excess on whole plants and chloroplasts has been widely studied and will be summarized below.

### 5.1 Molecular and metabolic alterations in chloroplasts

In the case of metal excess, a decrease in photosynthetic activity is often observed and other metabolic pathways are also altered. Metals trigger not only an oxidative burst but also the synthesis of specific molecules able to trap them. When toxic metals cannot be completely removed, inactivated, sequestered, or detoxified, they impact various molecules and prevent them from functioning properly (Fig. 2). Metal excess may also induce changes in membrane rigidity, permeability, and stability (i.e., Pb: Stefanov et al. 1995, Hg: reviewed by Boening 2000). The following section describes these events in detail.

#### 5.1.1 Metal-induced oxidative burst

When present in excess, metals are directly involved in the formation of toxic reactive oxygen species causing oxidative stress. To protect themselves, cells try to maintain metal levels in a very narrow range between deficiency and toxicity (reviewed by Wintz et al. 2002). When they do not succeed, the following processes can be observed.

Metal ions block the electron flow in photosystem II (PSII), leading to the formation of excited triplet chlorophyll ( $^3\text{Chl}^*$ ) whose lifetime is long enough to react with a molecule of oxygen with triplet electronic configuration. During the interaction, chlorophyll returns to the ground state and oxygen in turn is changed into highly reactive singlet oxygen. Oxidative stress leads to an unbalance in the regeneration and removal of reactive oxygen species, including singlet oxygen ( $^1\text{O}_2$ ), superoxide radical ( $\text{O}_2^{\cdot-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and the most damaging and reactive hydroxyl radical ( $\text{OH}^{\cdot}$ ) which can lead to further lipid peroxidation and can damage membranes, proteins, and nucleic acids, leading to altered membrane fluidity, loss of enzyme functioning, and genomic damage (reviewed by Kucera et al. 2008). Induction of free radicals and reactive oxygen species by metals is very well documented and might be responsible for membrane injuries and some of the ultrastructural changes observed under metal stress (e.g., Babu et al. 2001; Zhang et al. 2005; Rodríguez-Serrano et al. 2009; reviewed by Sharma and Dietz 2009). The photooxidation risk is even increased by the inhibition of some photoprotective mechanisms such as the xanthophyll cycle (Bertrand et al. 2001).

Some metals such as Fe (Gallego et al. 1996) and Cu (Drazkiewicz et al. 2004; reviewed by Yruela 2009) induce reactive oxygen species directly, while others are thought to induce them only indirectly (Cd: Gallego et al. 1996; Pál et al. 2007; Cr: Panda et al. 2003; Panda 2007; Hg: Cho and

**Table 2** Examples of alterations in chloroplast structure of crop plants caused by deficiency in essential metals

Metal	Species	Chloroplast alterations (reference)
Ca	Maize ( <i>Zea mays</i> )	Disruption of the envelope membrane, lower grana, increased number and size of plastoglobuli (Hall et al., 1972)
Cu	Oat ( <i>Avena sativa</i> )	Disintegration of thylakoid membranes, swollen intrathylakoidal space (Baszyński et al., 1978)
Fe	Apple ( <i>Malus domestica</i> )	Disorganized (dispersed) grana, reduced starch content, increased number of plastoglobuli (Ji et al., 1984)
	Maize ( <i>Zea mays</i> )	Swollen intrathylakoidal space, disorganized thylakoids, no grana (Thoiron et al., 1997)
	Pecan ( <i>Carya illinoensis</i> )	Reduced internal membrane system, almost no grana, increased starch content, increased number of plastoglobuli (Henriques, 2003)
	Sugar beet ( <i>Beta vulgaris</i> )	Small plastids, undeveloped, disorganized grana, clusters of peripheral vesicles (Platt-Aloia et al., 1983)
	Tobacco ( <i>Nicotiana tabacum</i> )	Unstacked and diffuse thylakoids, increased starch content, unusual vesicles (Briat et al., 1999)
	K	Bean ( <i>Phaseolus vulgaris</i> )
Maize ( <i>Zea mays</i> )		No significant alterations, membranes lacking contrast (Hall et al., 1972)
Mg	Bean ( <i>Phaseolus vulgaris</i> )	Disorganized grana (Thomson and Weier, 1962)
	Maize ( <i>Zea mays</i> )	Reduced inner membranes, increased number and size of plastoglobuli (Hall et al., 1972)

Park 2000; Mn: Lidon and Teixeira 2000a, b; Na: Mitsuya et al. 2000; Yamane et al. 2009; reviewed by Wang et al. 2003; Miyake et al. 2006; Ni: reviewed by Chen et al. 2009; Zn: Panda et al. 2003; reviewed by Sharma and Dietz 2009; Kawachi et al. 2009; Fig. 2).

Both metal deficiency (see Section 4.1) and metal excess trigger lipid peroxidation (e.g., Al: Giannakoula et al. 2008; Cd: Gallego et al. 1996; Sandalio et al. 2001; Djebali et al. 2005; Skórzyńska-Polit and Krupa 2006; Hg: Cho and Park 2000; excess Cu: Babu et al. 2001; excess Fe: Gallego et al.

**Table 2** (continued)

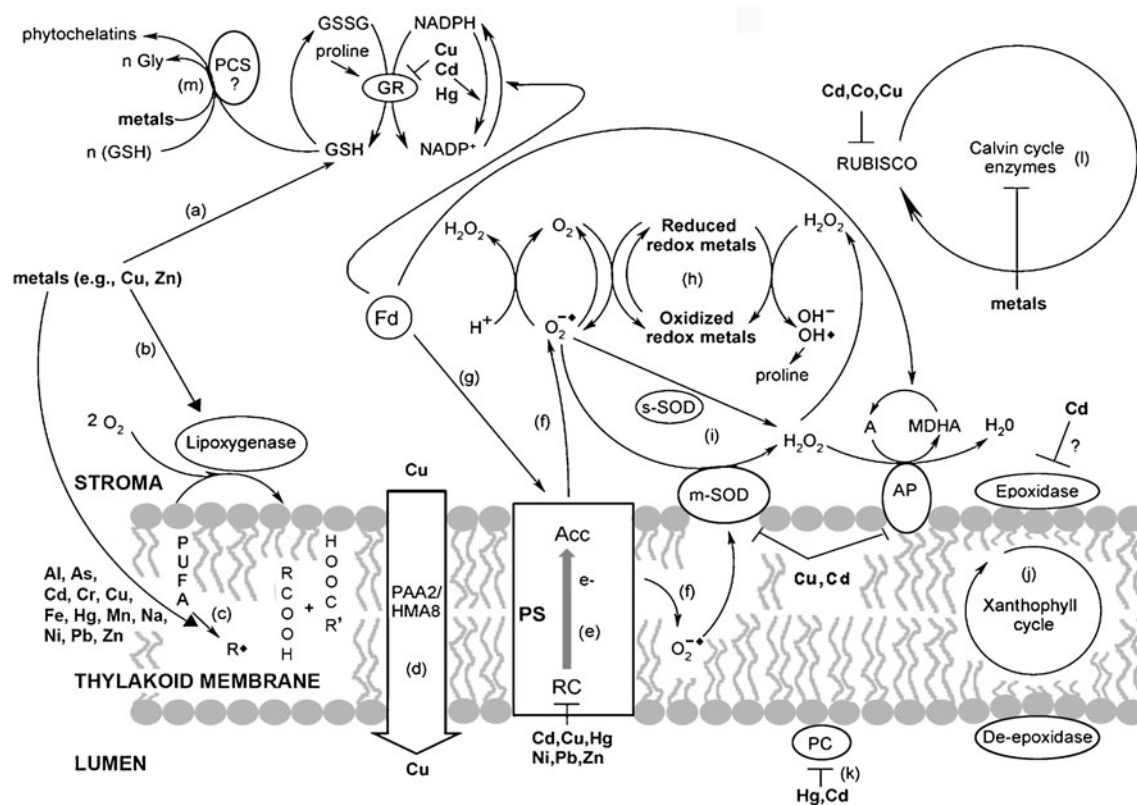
Mn	Lemon ( <i>Citrus volkameriana</i> )	Reduced chloroplast size and starch size, few, slightly swollen intrathylakoidal spaces (Papadakis et al., 2007)
	Pecan ( <i>Carya illinoensis</i> )	Decrease in chloroplast number, no apparent change in the thylakoids (Henriques, 2003, 2004)
	Soybean ( <i>Glycine max</i> )	Disruption of thylakoids and swollen intrathylakoidal space, strong decrease in starch content, increased number of plastoglobuli (Weiland et al., 1975, Izaguirre-Mayoral and Sinclair, 2005)
	Spinach ( <i>Spinacia oleracea</i> )	Disruption of thylakoids, swollen intrathylakoidal space in grana, appearance of vesicles corresponding to swollen stromal thylakoids (Mercer et al., 1962, Possingham et al., 1964)
Zn	Bean ( <i>Phaseolus vulgaris</i> )	Disturbed grana development (Thomson and Weier, 1962)
	Rice ( <i>Oryza sativa</i> )	Swollen chloroplasts, disruption of the envelope membrane, swollen intrathylakoidal space, decreased number of grana, disorganization of the thylakoids, increased number of plastoglobuli (Chen et al., 2008)
	Sugar beet ( <i>Beta vulgaris</i> )	Premature senescence: disruption of thylakoids, unstacking of grana, degeneration of stromal components (Henriques, 2001)

1996; Babu et al. 2001; excess Zn: Wang et al. 2009; reviewed by Poirier et al. 2008) and production of reactive oxygen species; therefore, we can conclude that metal unbalance is responsible for oxidative stress. This is not so surprising if we also consider that besides the direct effect of metals, several enzymes involved in the detoxification of reactive oxygen species contain different metals (e.g., Cu, Fe, and Zn in superoxide dismutases; Table 1) and their functioning is also disturbed under conditions with unbalanced metal concentrations.

### 5.1.2 Synthesis of molecules trapping metals

Chloroplasts have been reported not only as metabolic units that require metals in well-defined amounts for their proper

functioning but also as possible compartments for the sequestration of excess metals. The storage of essential metals in chloroplasts in non-toxic form contributes to homeostasis and provides a reservoir for future requirements. For instance, excess Fe is stored as ferritin (Izaguirre-Mayoral and Sinclair 2005, 2009; reviewed by Briat et al. 1999). Interestingly, in transgenic plants over-expressing ferritin and grown under normal Fe supply, ultrastructural symptoms of Fe-deficient chloroplasts were observed, indicating that ferritin is important in storing iron in a metabolically inactive form (reviewed by Briat et al. 1999) and thus preventing oxidative damage (Ravet et al. 2009; reviewed by Arosio and Levi 2002). Similarly, the role of a plastocyanin isoform as a Cu sink under excess Cu has been reported recently (reviewed by Abdel-Ghany



**Fig. 2** Scheme summarizing most important chloroplast processes and components impacted by metal excess with emphasis on oxidative stress and defense reactions. **a** Depletion of sulfhydryl groups by metals on reduced glutathione (*GSH*) regenerated by glutathione reductase (*GR*) from its oxidized form (*GSSG*). **b** Activation of lipoygenase by metals. **c** Peroxidation of polyunsaturated fatty acids (*PUFA*) to free radicals ( $R^{\bullet}$ ) and saturated fatty acids ( $RCOOH+R'COOH$ ) in membranous phospholipids. **d** Cu transporter *PAA2/HMA8* directing Cu transport toward the lumen. **e** Electron transfer from the reaction center (*RC*) to the acceptor (*Acc*) of *PSI* or *PSII*. **f** Univalent oxygen reduction by *PSII*. **g** Electron transfer through ferredoxin (*Fd*) and  $NADP^{+}$  (specific to *PSI*). **h** Fenton and Haber–Weiss reactions: one-electron oxidoreductions performed by redox metals leading to hydroxyl radicals ( $OH^{\bullet}$ ). **i** Spontaneous and/or superoxide dismutase (*SOD*) catalyzed disproportionation of superoxides ( $O_2^{\bullet-}$ ). Superoxide radicals generated by *PSI* and *PSII* are dismutated by the membrane-bound *m-SOD*, and the formed hydrogen peroxide ( $H_2O_2$ ) is reduced to water by the thylakoid-bound ascorbate peroxidase (*AP*). Monodehydroascorbate (*MDHA*) is reduced back to ascorbate (*A*) by the photoreduced ferredoxin (*Fd*).  $O_2^{\bullet-}$  radicals escaped from the thylakoid system are scavenged by a stromal *s-SOD*. **j** Xanthophyll cycle associated to the enzymes epoxidase and de-epoxidase, preventing superoxide formation at high light intensity. **k** Inactivation of plastocyanin (*PC*) by non-essential metals after its release from the membrane or the release of its Cu. **l** Inactivation of Calvin cycle enzymes. **m** Chelation of metals by phytochelatin either imported from the cytoplasm or synthesized through the activity of a putative phytochelatin synthetase (*PCS*). → activation by metals; ⊥ inhibition by metals

2009). Besides its function as an electron carrier (for which a small amount of plastocyanin is enough), plastocyanin seems to have a role in buffering excess Cu within the plastids and to contribute this way to intracellular tolerance of excess Cu. Concerning excess  $Na^{+}$ , recent studies on mutants lacking *CHX23* (an envelope located  $Na^{+}(K^{+})/H^{+}$  ATPase) suggest that plastidial sequestration of excess  $Na^{+}$  may be important in salt tolerance mechanisms in plants and can prevent phytotoxic effects of this element in the cytosol (Song et al. 2004a). These data outline the importance of plastids also in metal tolerance of plants grown in polluted areas and show that plants may need to synthesize specific proteins to store excess metals in a metabolically inactive form in the chloroplasts.

Phytochelatin also have an essential function in trapping metals present in excess. They are metal-complexing

peptides with a high proportion of Cys residues, a general structure of  $(\gamma-Glu-Cys)_{2-11}-Gly$ , and are generally known to function in the vacuolar sequestration of excess metals (reviewed by Cobbett 2000; Hall 2002). The occurrence of phytochelatin in the chloroplasts has been also reported under Cd stress; however, their origin and exact role still remains unclear (reviewed by Kucera et al. 2008). On the other hand, in transgenic plants expressing phytochelatin synthase in chloroplasts, the accumulation and tolerance of Cd was not changed, but As sensitivity of the plants increased (Picault et al. 2006).

Heat-shock proteins and chaperones may also be involved and related to plastid sequestration of metals and to the metal tolerance of plastids. Examples include the plant homologue of CCS, the yeast chaperone to Cu/Zn superoxide dismutase, and some predicted *Arabidopsis*

homologues of yeast ATX1 Cu chaperones (reviewed by Wintz and Vulpe 2002; Puig et al. 2007a). The encoded proteins possess a potential chloroplast-targeting sequence, suggesting that they are chloroplast proteins and most likely involved in the maturation of the chloroplast Cu/Zn superoxide dismutase apoprotein, in the delivery of Cu to this protein, or in chloroplast transport mechanisms. The ATX1 family of Cu chaperones specifically delivers Cu to heavy metal P-type ATPases (Puig et al. 2007b), such as HMA1 located in the chloroplast envelope which can remove excess metals from plastids (Kawachi et al. 2009; Kim et al. 2009). This way, this chaperone may be involved in the detoxification of plastids or in the maintenance of plastid ion homeostasis. In addition, the CUTA protein, which binds Cu and localizes to the intermembrane space of chloroplast envelopes, has been proposed as a candidate for a Cu metallochaperone (reviewed by Puig et al. 2007a).

Plastids possess a variety of mechanisms to cope with metals in excess; however, in the case of heavily polluted areas and plants, these strategies may also be not satisfactory and plastid metabolism can be altered.

### 5.1.3 Molecules impacted by metals

When present in excess, metals may interact directly or indirectly with plastid lipids, pigments, proteins, and important cofactors required for photosynthesis. They may replace another functional metal within enzymes or in key molecules such as chlorophyll. In this part, we try to summarize these interactions of excess metals.

**Lipids** The observed changes in membrane structure might at least be partially due to membrane lipid alterations in metal exposed plants (reviewed by Devi and Prasad 2006). Excess metals influence the lipid composition, the saturation, or even the chain length of the fatty acids of membrane lipids (e.g., Cd—pepper: Jemal et al. 2000; tomato: Djebali et al. 2005; Ben Ammar et al. 2007, maize: Pál et al. 2007; Cu—spinach: Maksymiec et al. 1992; runner bean: Maksymiec et al. 1994; Na—spinach: Stefanov et al. 1995; Pb—spinach: Stefanov et al. 1995). The excess of several non-essential metals (e.g., Cd: Skórzyńska et al. 1991; Skórzyńska and Baszyński 1993; Skórzyńska-Polit and Baszyński 1997; Skórzyńska-Polit et al. 1998; Jemal et al. 2000; Nouairi et al. 2006; Na: and Pb: Stefanov et al. 1995), but also excess micronutrients (e.g., Cu: Maksymiec et al. 1992; Quartacci et al. 2000; Mn: Lidon et al. 2004), decrease the monogalactosyldiacylglycerol (MGDG) content. As MGDG and digalactosyldiacylglycerol ratios influence membrane curvature, the changes in their ratios can explain the observed alterations in grana stacking and/or grana-to-stroma thylakoid ratios under metal stress (Table 3). Similarly, the swollen intrathylakoi-

dal space has been related to decreased MGDG levels caused by increased galactolipase activity (Skórzyńska et al. 1991).

MGDG is not only an important structural element of the thylakoid membranes but is also required for the proper functioning of photosynthesis (PSII complexes); therefore, changes in this lipid fraction may have a detrimental effect on thylakoid functions and the total photosynthetic efficiency of plants. Similarly, the metal-induced changes in fatty acid desaturation influence the membrane fluidity (Quartacci et al. 2000), which in turn leads to altered membrane physiological functions and, particularly, influences the ionic permeability of the thylakoid membranes (reviewed by Devi and Prasad 2006). Metal-induced lipid peroxidation can lead to the release of pigment–protein complexes, oxygen evolving complex, and plastocyanin, blocking further electron transport (reviewed by Siedlecka and Krupa 1996, 1999; Pál et al. 2006).

Lipids and, as a consequence, all membrane-related processes of chloroplasts are sensitive to metal excess, and their alterations may be reflected also at the ultrastructural level as disturbed chloroplast structure.

**Pigments** The inhibitory effect of excess metals on chlorophyll and carotenoid biosynthesis (reviewed by Myśliwa-Kurdziel and Strzałka 2002; Bertrand and Poirier 2005; Schoefs 2005) and on photosynthesis (reviewed by Myśliwa-Kurdziel et al. 2004; Sárvári 2005; Fodor 2006; Briat et al. 2007) are reviewed elsewhere in more detail.

Cr(VI), Fe(III), and Hg(II) have been shown to directly inhibit one of the key enzymes of chlorophyll biosynthesis, NADPH:protochlorophyllide oxidoreductase in vitro (Hg: Lenti et al. 2002; Solymosi et al. 2004; Cr and Fe: Myśliwa-Kurdziel and Strzałka 2005). Cd also inhibits chlorophyll biosynthesis directly through ALA dehydratase and NADPH:protochlorophyllide reductase in vitro (Stobart et al. 1985; Böddi et al. 1995; Myśliwa-Kurdziel and Strzałka 2005).

Loss of chlorophyll (e.g., chlorophyll *b* in the case of Zn and Cd stress: Ebbs and Uchil 2008) and decreased levels of carotenoids (e.g., Cd: Drazkiewicz and Baszyński 2005; excess Cu: Baszyński et al. 1988; reviewed by Myśliwa-Kurdziel and Strzałka 2002) are often reported under metal stress and can lead to impaired photosynthetic activities. Some authors suggest that metals interfere with carotenoid biosynthesis on the level of phytoene or in terpenoid biosynthesis prior to the formation of geranyl–geranyl pyrophosphate (reviewed by Barón et al. 1995), the latter being also needed for the phytol chain in chlorophyll molecules.

Besides the direct inhibition of pigment biosynthetic processes of plastids, metals may induce directly or indirectly the function loss or the oxidative breakdown of

**Table 3** Examples of alterations in chloroplast structure of crops caused by excess of metals

Metal	Species	Chloroplast alterations (Reference)
Al (ne)	Wheatgrass ( <i>Thinopyrum bessarabicum</i> )	Swelling and distension of the chloroplast, grana arranged in bow-like lines (Moustakas et al., 1996, 1997)
Cd (ne)	Common bean ( <i>Phaseolus vulgaris</i> )	Sometimes disruption of the envelopes, disordered position and stacking of thylakoids, increased number and size of plastoglobuli (Barceló et al., 1988)
	Oilseed rape ( <i>Brassica napus</i> )	Reduced number of chloroplasts, slightly reduced grana size, slightly reduced number of plastoglobuli, but no striking ultrastructural effect (Baryla et al., 2001, Carrier et al., 2003)
	Pea ( <i>Pisum sativum</i> )	Reduced number of chloroplasts, senescence (disorganization of grana, increased starch content, increased number and size of plastoglobuli) (McCarthy et al., 2001, Sandalio et al., 2001)
	Tomato ( <i>Lycopersicon esculentum</i> )	- Swollen intrathylakoidal space, senescence (disorganization of grana, increased number and size of plastoglobuli) (Baszyński et al., 1980) - Disturbed chloroplast shape, swollen intrathylakoidal space (Djebali et al., 2005)
	Wheat ( <i>Triticum aestivum</i> )	Senescence (disturbed thylakoid shape, swollen intrathylakoidal space), regularly spotted bodies (Ouzounidou et al., 1997)
Cr (III) (ne)	Common bean ( <i>Phaseolus vulgaris</i> )	No ultrastructural effect (Poschenrieder et al., 1991)
Cr (VI) (ne)	Common bean ( <i>Phaseolus vulgaris</i> )	Slightly distorted (rounded) chloroplast shape, decrease in grana thylakoids, swollen intrathylakoidal space (Vázquez et al., 1987)
Cu	Durum wheat ( <i>Triticum durum</i> )	- Swollen intrathylakoidal space, regularly spotted bodies (Ciscato et al., 1997) - Swollen intrathylakoidal space (Quartacci et al., 2000)

**Table 3** (continued)

	Oregano ( <i>Origanum vulgare</i> )	Reduced number and size of chloroplasts, dilated envelope, no starch, increased size of plastoglobuli (Panou-Filotheou et al., 2001)
	Runner bean ( <i>Phaseolus coccineus</i> )	Senescence (disorganization of chloroplast structure, disturbances in stroma lamellae) (Maksymiec et al., 1995)
	Soybean ( <i>Glycine max</i> )	Smaller, but more numerous chloroplasts with rounded shape, denser structured internal membranes (increased grana size and stacking) (Bernal et al., 2006)
	Spinach ( <i>Spinacia oleracea</i> )	Degradation of grana stacks and stroma lamellae, slightly swollen intrathylakoidal space, increased number and size of plastoglobuli (Baszyński et al., 1988)
	Wheat ( <i>Triticum aestivum</i> )	Reduced number and size of chloroplasts, less developed internal membrane system, impaired grana formation, decreased starch content, decreased number of plastoglobuli (Eleftheriou and Karataglis, 1989)
Fe	Soybean ( <i>Glycine max</i> )	Appearance of ferritin (Izaguirre-Mayoral and Sinclair, 2005)
Mn	Lemon ( <i>Citrus volkameriana</i> )	More rounded chloroplasts with slightly increased size, reduced number of grana, increased starch content, increased number and size of plastoglobuli, increased depositions of dark-materials in thylakoids (Papadakis et al., 2007)
	Maple ( <i>Acer saccharum</i> )	Discrete electron-dense areas in chloroplast thylakoid membranes, increased starch content (McQuattie and Schier, 2000)
	Rice ( <i>Oryza sativa</i> )	Swollen intrathylakoidal space, increased number and size of plastoglobuli (Lidon and Teixeira, 2000a)
Na (ne)	Alfalfa ( <i>Medicago sativa</i> )	Swollen intrathylakoidal space, increased number of plastoglobuli in cotyledonal chloroplasts (Baranova et al., 2007)

**Table 3** (continued)

	Maize ( <i>Zea mays</i> )	Swollen intrathylakoidal space, increased number and size of plastoglobuli (Hasan et al., 2005)
	Pea ( <i>Pisum sativum</i> )	Increased number of plastoglobuli (Hernandez et al., 1995)
	Rice ( <i>Oryza sativa</i> )	Swollen intrathylakoidal space, with increasing concentrations: swelling of chloroplasts, disruption of the envelope membrane, increased number and size of plastoglobuli (Rahman et al., 2000, Mitsuya et al., 2003, Yamane et al., 2003, 2004, 2008, 2009)
	Sweet potato ( <i>Ipomoea batatas</i> )	Swollen intrathylakoidal space, degradation of thylakoid membranes (Mitsuya et al., 2000)
	Tomato ( <i>Lycopersicon esculentum</i> )	- Genotype-specific differences in chloroplast shape (more rounded or flatter) and number, in starch and plastoglobuli content, less organized internal membrane system (Sam et al., 2003)  - no grana and thylakoids (Khavari-Nejad and Mostofi, 1998)
	Wheat ( <i>Triticum aestivum</i> )	Swollen intrathylakoidal space, accumulation of starch (Salama et al., 1994)
Ni	Cabbage ( <i>Brassica oleracea</i> )	Reduced number of grana, alterations in size and shape of thylakoids, swollen intrathylakoidal space, increased starch content, increased size of plastoglobuli (Molas, 1997, 2002)
Pb (ne)	Barley ( <i>Hordeum vulgare</i> )	Fewer, but more elongated grana, increased starch content, increased number and size of plastoglobuli, dark deposits at grana margins, regularly spotted bodies (Woźny et al., 1995)
	Pea ( <i>Pisum sativum</i> )	Sometimes disruption of the envelopes, dark deposits at the grana margins (Sabnis et al., 1969)
	Soybean ( <i>Glycine max</i> )	Disruption of the envelope, reduced lamellar system, disorganization of thylakoids (Weryszko-Chmielewska and Chwil, 2005)
<sup>99</sup> Tc (ne)	Common bean ( <i>Phaseolus vulgaris</i> )	Swollen chloroplasts, disintegration of the thylakoid system, decreased starch content (Vázquez et al., 1990)



**Table 3** (continued)

Zn	Pea ( <i>Pisum sativum</i> )	Disintegration of stromal thylakoids, with increasing concentrations: reduced number of grana, swollen intrathylakoidal space, disappearance of starch, increased number and size of plastoglobuli (Doncheva et al., 2001)
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*ne* non-essential elements

pigments. For example, Hg has been shown to induce the loss of the central Mg from a chlorophyll precursor, resulting in non-functional pigments (Solymosi et al. 2004). Similarly, several metals (e.g., Cd, Hg, Pb, Cu, Zn, and Ni) can replace Mg in chlorophylls, resulting in photosynthetically inactive molecules (Küpper et al. 1996, 1998, 2002, 2003). A primary site of Cu inhibition was identified on the antenna chlorophyll *a* molecules of PSII (Lidon et al. 1993). Without antenna chlorophylls, photosynthesis is blocked.

Clearly, several data indicate that excess of either essential or non-essential metals can interact with photosynthetic pigments (chlorophylls and carotenoids) and inhibit their biosyntheses, which might be a reason for the observed decline in photosynthetic functions and pigments. However, most of these studies used very high (and this way often non-physiological and non-natural, agronomically not relevant) concentrations of the metals and/or did not work with intact plants but with different *in vitro* preparations. Therefore, the direct effect of excess metals is only theoretically proved *in planta*, but probably, other indirect interactions and impacts (e.g., changes of ion and water homeostasis, competition with essential metals) must also be considered and may be more probable *in vivo*.

**Proteins** One metal in excess may substitute another one in metalloproteins. For instance, Hg can substitute Cu in plastocyanin (cf. Radmer and Kok 1974), Co replaces Mg in ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) or Zn in transcription factors (reviewed by Poirier et al. 2008), and Cd replaces Ca in photosystem II reaction center, causing inhibition of PSII photoactivation (Skórzyńska and Baszyński 1993; Faller et al. 2005; reviewed by Kucera et al. 2008). Cd can replace Zn and Ca in metalloenzymes (reviewed by Clemens et al. 2009). The substitution of Mn by Zn or Cd leads to the inactivation of the oxygen evolving complex, and as a consequence, electron donation to PSII is inhibited (e.g., Baszyński et al. 1980; reviewed by Bertrand and Poirier 2005; Pál et al. 2006; Kucera et al. 2008).

Hg ions can directly interact with some sites in the photosynthetic electron transport chain situated in D1 and D2 proteins and with the Mn cluster in the oxygen-evolving complex (reviewed by Romanowska 2002). Al inhibits ATPase and hexokinase activity in chloroplasts (reviewed by Romanowska 2002). Each of these effects could be responsible individually or in combination for Al toxicity to photosynthesis, i.e., the decreased photosynthetic electron transport associated with photosystem I (PSI) and the reduced CO<sub>2</sub> assimilation rates. Excess Cu also inhibits several polypeptides of PSII and PSI (e.g., Lidon and Henriques 1993; Maksymiec et al. 1994).

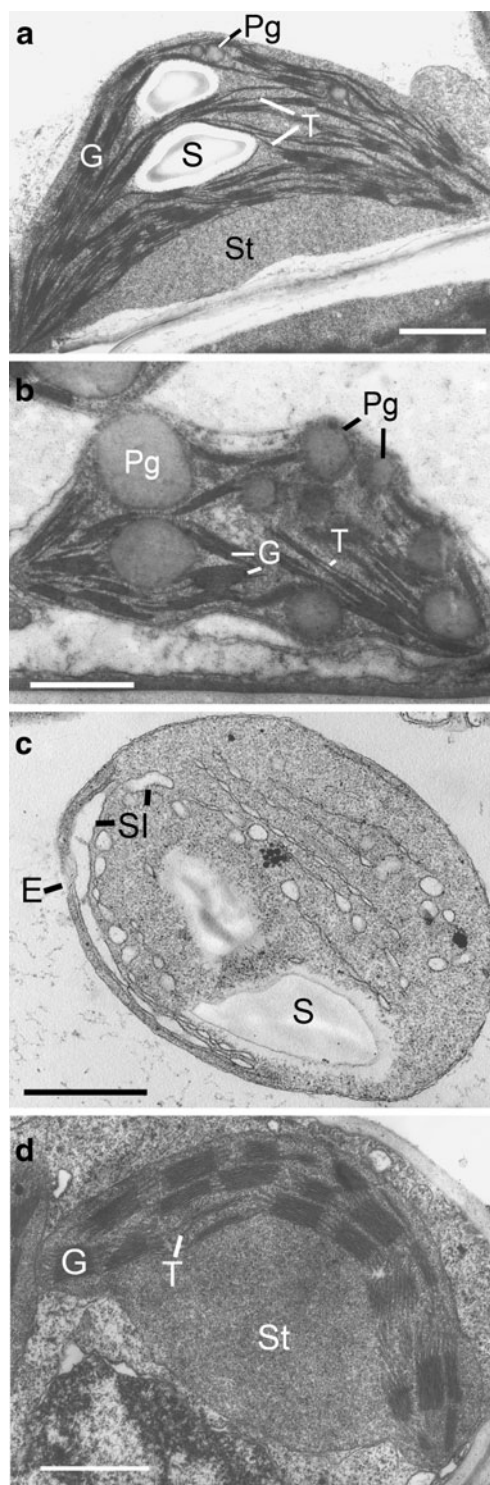
One of the most often reported direct toxic effects of metals on plastid proteins is attributed to the ability of some metals to bind to sulfhydryl, histidyl, and carboxyl groups of proteins, inducing therefore conformational changes, resulting often in inactivation or disturbed function (Cd, Pb, Hg: reviewed by Vallee and Ulmer 1972; Hg: Lenti et al. 2002; Solymosi et al. 2004; Cu: reviewed by Maksymiec 1997; Yruela 2009).

Metal-sensitive plant enzymes of CO<sub>2</sub> fixation include RuBisCO (e.g., inhibited by Cd: Siedlecka and Krupa 1996, 1999; Cu excess: Lidon and Henriques 1991, 1993; Maksymiec et al. 1994), phosphoenolpyruvate carboxylase (Cd: reviewed by Siedlecka and Krupa 1996, 1999; Pál et al. 2006; Mg, Mn, Co, Fe, Cd: Nguyen et al. 1988), glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, and nitrate reductase from nitrogen assimilation (reviewed by Romanowska 2002; Myśliwa-Kurziel et al. 2002, 2004; Kucera et al. 2008).

There is sufficient experimental evidence to explain the strong impact and inhibitory effect of metals on chloroplast enzymes and proteins which can result in decreased physiological functions and productivity.

**Other molecules required for photosynthesis** The observed decreased photosynthetic activity and disturbed plastid metabolism may be related to the direct oxidation of NADPH by non-essential metals (e.g., Cd: Böddi et al. 1995; reviewed by Pál et al. 2006; Hg: Lenti et al. 2002; Solymosi et al. 2004, 2006). Excess of non-essential metals

**Fig. 3** Some ultrastructural alterations of chloroplasts under metal excess. **a** Control chloroplast from *Spirodela polyrhiza* fronds. **b** Chloroplast showing symptoms of senescence (containing numerous, large plastoglobuli, fewer stroma thylakoids than the control) from *S. polyrhiza* fronds treated for 8 days with 50  $\mu\text{M}$   $\text{Na}_2\text{CrO}_4$ . **c** Developing chloroplast from wheat (*Triticum aestivum* L.) leaf treated for 12 h with 600 mM NaCl/KCl mixture (1:1 molar ratio). Note the presence of swollen intrathylakoidal space (sometimes appearing as vesicles) and disrupted envelope. **d** Chloroplast with altered shape (swelling), increased stroma region and bended, bow-like thylakoid arrangement from *Phragmites australis* leaf treated for 10 days with 15.7  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . **a, b** Courtesy of Áron Keresztes. **d** Courtesy of Károly Bóka. For more details, see Appenroth et al. (2003) (**a, b**), Abdelkader et al. (2007); the figure was prepared with Jeol JEM 1011 TEM at 75 kV (**c**) and Hakmaoui et al. (2007) (**d**). *E* envelope (indicated only in case of rupture), *G* grana, *Pg* plastoglobuli, *S* starch, *SI* swollen intrathylakoidal space, *St* stroma, *T* thylakoid (stromal thylakoid). The bar indicates 1  $\mu\text{m}$



has also been reported to cause high ATP content and also to change gene expression through DNA hypomethylation and DNA damage (reviewed by Poirier et al. 2008). This way, their carcinogenic effect has also been reported (e.g., Monteiro et al. 2009). Hg-induced inhibition of photosynthesis occurs probably by inducing a severe loss of adenylate pool and decreasing this way the rate of cyclic and non-cyclic photophosphorylation. Hg also decreases PSII-associated reactions,  $\text{O}_2$  evolution and  $\text{CO}_2$  fixation, probably due to the retardation of all ATP-dependent processes (reviewed by Romanowska 2002).

Considering the above-described profound functional, molecular, and metabolic alterations of chloroplasts caused by excess of metals, it is not surprising that the structure of the plastids of metal-stressed plants is also often seriously impacted.

## 5.2 Ultrastructural alterations of chloroplasts

It is rather easy to measure the total metal content in a whole plant or in an organ, but it is rather difficult to quantify metals in organelles such as plastids, as Neuhaus and Wagner (2000), Ramos et al. (2002), or Johnson et al. (2006) did it. Electron microscopy gives information about the integrity of chloroplasts. In the following section, we briefly describe the ultrastructural alterations induced by metal excess at the plastid level.

In the leaves of plants grown in experimental conditions with excess metals, in general, the number of chloroplasts decreases (Table 3; reviewed by Kucera et al. 2008). Structural damages are also frequently observed (Fig. 3). The most common symptoms are the swelling of the organelle, disruption of the envelope, distortion of thylakoids leading to loss of the parallel arrangement of the thylakoid membranes, disorganization of grana, reduction

of the thylakoid surface area, and swelling of the intrathylakoidal space (Table 3; reviewed by Barceló and Poschenrieder 2006). There are contradictory data about the changes in the starch content upon metal excess, but often it also increases.

In some cases, different steps of plastid ultrastructural alterations were distinguished as a function of increasing

concentrations and/or length of metal exposure (e.g., Na: Rahman et al. 2000; Zn: Doncheva et al. 2001). Most studies, however, have been conducted under laboratory conditions (e.g., hydroponic cultures, etc.). In this respect, one of the most natural and most interesting examples is the study of Eleftheriou and Karataglis (1989) in which the ultrastructure of wheat plants grown in “naked spots” of naturally Cu-contaminated soils was studied.

Interestingly, when comparing the alterations of chloroplast ultrastructure in the case of metal deficiency (Table 2) or metal excess (Table 3), we can clearly conclude that with the exception of few specific symptoms, they are very similar. Consequently, unbalanced metal distribution within chloroplasts, more than a specific metal deficiency or excess, is reflected by disorganized thylakoids. This means that typical chloroplasts without any structural alteration could be good indicators of optimal metal distribution within the organelles, although the alterations observed under metal stress resemble those observed under other abiotic or biotic stresses.

### 5.3 Alterations on the level of the entire plant

The sustainability of current land use in agroecosystems can be assessed with respect to metal accumulation in soils by balancing their input and output fluxes. Many studies point to the fact that polluted agricultural lands may produce metal-contaminated crops (e.g., Keller et al. 2002; Muchuweti et al. 2006; reviewed by Witter 1996). The study of Muchuweti et al. (2006) highlights well the potential risks involved in the cultivation and consumption of vegetables on plots irrigated with sewage sludge by pointing out that metal contents in crops may be much higher than the permissible levels established by the Commission Regulation (EU, no. 466/2001). Similarly, crops with higher metal levels than those established by the European Union (EU) and the Food and Agriculture Organization (FAO) of the United Nations (Table 4) are not rarely found when plants are cultivated on contaminated soils or areas with strong atmospheric metal deposition

**Table 4** Permissible maximal levels (mg/kg wet weight) of regulated metals established by the joint FAO/WHO Expert Committee on Food Additives (1999) and the Commission Regulation (EU, no. 466/2001) for crops

Metal	FAO/WHO	EU
Cd	1	0.05–0.2
Cu	40	20
Hg	1	–
Pb	1	0.1–0.3
Zn	60	50

(e.g., Peris et al. 2007; Sharma et al. 2008a, b; Baize et al. 2009; Mansour et al. 2009a, b; reviewed by Baize 2009).

In general, one third of the micronutrients are translocated to the aboveground organs while two thirds are retained in the roots (e.g., Kawachi et al. 2009; Zhang et al. 2009). When added in excess, most of the surplus metal(s) also accumulates in the roots (e.g., Cd: Jarvis et al. 1976; Obata and Umebayashi 1993; Zhang et al. 2008). Therefore, the accumulation of toxic metals is the most dangerous in the case of root crops (e.g., carrot, radish, sugar beet), but can reach considerable levels also by atmospheric deposition in aboveground organs (for details, see Section 3.3).

Several visible symptoms have been observed in plants grown in metal-polluted areas or in the presence of excess metals. Leaf expansion is inhibited, leaf tissues can become deformed, and chlorosis often occurs (e.g., Cd: Ebbs and Uchil 2008; Ben Ghnaya et al. 2009; Cr: Vázquez et al. 1987; Cu Ciscato et al. 1997; Panou-Filothéou et al. 2001; Mn: González and Lynch 1999; reviewed by Barceló and Poschenrieder 2006; Pb: Woźny et al. 1995; Zn: Ebbs and Uchil 2008; Wang et al. 2009). The decreased growth and biomass of metal-stressed plants can be related to several processes, including disturbances in water and nutritional status or respiration of the plants linked to changes in stomata number, differentiation, and functioning (e.g., Vázquez et al. 1987; Moustakas et al. 1996; Molas 1997).

Taken together, the above results summarized in Section 5 further outline the complicated interrelations and the difficulties in interpreting the effects of metals on the molecular and cellular levels. The careful comparison of the ultrastructural effects induced by essential metal deficiency (Table 2) and excess of metals (Table 3) indicates that besides several similar structural alterations, there are, however, few specific differences too. Altogether, plastids and their ultrastructure are relatively sensitive indicators of metal unbalance within plants. Although it has to be mentioned that several other biotic or abiotic stresses may also cause similar ultrastructural alterations, the discussion of these phenomena is beyond the scope of this review.

## 6 Conclusions and perspectives

For plants and also for chloroplasts, the physiological range between deficiency and toxicity of essential metals is narrow (reviewed by Maret and Sandstead 2006). However, more and more reports exist on crop cultivation on soils with unbalanced metal concentrations where, possibly, toxic non-essential elements can also be present.

Plastids possess some mechanisms to cope with metal deficiency or excess, such as the production of specific molecules trapping or chaperoning metals, exporting some

metals present in excess, and/or sequestering and storing them in metabolically inactive forms, like for instance the iron-storing ferritin. However, the data reviewed in this work indicate that plastids are highly sensitive to metal stress and their function is often impaired when the metal ion homeostasis of the plant is disturbed. Both essential metal deficiency and excess of metals induce profound molecular, structural, and metabolic alterations that lead to decreased physiological function and, as a consequence, to decreased biomass and productivity. Most vital functions and reactions of chloroplasts require essential metals or are inhibited by metal excess. Therefore, a better understanding of chloroplast metabolism, chloroplast metal uptake, and homeostasis under metal stress is necessary because the optimal functioning of these organelles is essential for high crop productivity. In parallel to laboratory experiments, field assays have to be performed in well-defined conditions (e.g., metal content and pH in soil, crop species) and with precise prediction/objectives. In such experiments, the data must be considered on the level of the entire organism because plants surely possess several mechanisms to secure physiological ion homeostasis in chloroplasts.

Crops that are cultivated in polluted environments with excess metals have two basically different strategies: avoidance or tolerance, i.e., either they do not absorb potentially toxic elements or they take them up and tolerate them.

In the first case, all transporters at the root periphery and at the endodermis have to be able to prevent the uptake and transport of non-desired elements. Passive apoplasmic transport can occur until the endodermis, and at sites of lateral root formation, metals may enter the central cylinder, so the roots are slightly contaminated. Therefore, root crops should preferably not be cultivated in soils with unbalanced metal concentrations. However, occasionally, fortification of border cells occurs and might be responsible for exclusion. Several other exclusion mechanisms exist that involve down-regulation of genes linked to nutrient uptake or protein level modification of channels and transporters that can be observed under excess of essential metals (e.g., Cu: reviewed by Burkhead et al. 2009; Zn: Kawachi et al. 2009; K and Ca and other macronutrients: reviewed by Amtmann and Blatt 2009). On the other hand, there are only few examples for reduced uptake mechanisms for non-essential metals, but these include the synthesis of cysteine-rich membrane proteins in the plasma membrane that mediate Cd tolerance by reducing Cd uptake (Song et al. 2004b; Kuramata et al. 2009). Also, concerning Cd, the ABC transporter AtPDR8 was shown to mediate the extrusion of Cd out of the plasma membrane of root epidermal cells (Kim et al. 2007). Crop plants with such metal avoidance mechanisms are ideal for a secure crop production.

The interactions between soil, plant roots, and different soil microbes play important roles in regulating metal

movement from soil to edible parts of crops and may represent this way an important tool to increase the uptake of required essential elements and/or to decrease the uptake of toxic metals by the plants in soils with unbalanced metal concentrations (e.g., Islam et al. 2007; Ma et al. 2009; reviewed by Burleigh and Bechmann 2002; Hall 2002; Aguilar-Aguilar et al. 2009; Lemanceau et al. 2009; Wenzel 2009). Similarly, the way of irrigation and of course fertilization may also influence metal uptake by plants and their distribution between shoots and roots (Singh and Sinha 2005; Gupta and Sinha 2006; Nair et al. 2008); therefore, it should be considered in sustainable agricultural practice.

When potentially toxic metals enter plants, they get accumulated in variable amounts, preferentially in the roots or in the shoots (Ogundiran and Osibanjo 2008), and plants have to tolerate them somehow. At the cellular level, the toxicity of metals can be reduced, for instance, by retention on the cell wall (e.g., Zhang et al. 2009), sequestration in the vacuole (reviewed by Haydon and Cobbett 2007), or by storage in inactivated forms on specific proteins, amino acids, or peptides. However, if high levels of potentially toxic metals accumulate in edible parts of crops, they constitute a threat to mankind (Sinha et al. 2006). Therefore, knowledge about the molecular events by which metals are taken up by cells or get detoxified could provide potential targets to reduce metal toxicity or to regulate their distribution (e.g., Cailliatte et al. 2009). Detoxification of Cd can be achieved by members of the heavy metal-transporting P-type ATPase (HMA) subfamily (reviewed by Williams and Mills 2005). For instance, HMA4 is a Zn/Cd transporter that confers plant tolerance to Cd by loading it into the xylem, thus increasing translocation to the shoot where it might have less damaging effects for the plants (Verret et al. 2004; Hanikenne et al. 2008). The translocation of metals in plants can also be controlled by producing transgenic plants. For instance, Hanikenne et al. (2008) constructed a transgenic plant of *Arabidopsis thaliana* with altered metal partitioning between roots and shoots. Future research should address the possibility of modifying the tissue- or organ-specific expression and/or ion selectivity of metal transporters, for example, to enhance the sequestration of non-essential elements in root vacuoles if the edible part of the plant is on the shoot (reviewed by Palmgren et al. 2008). An alternative to reduce toxicity in plants might be to express non-essential element export systems at the root level.

When metal-unbalanced lands may be used for crop production, genetically modified crops could be cultivated on them. The genetic modification in such organisms should be chosen in such a way that edible parts contain balanced metal concentrations. It means that the edible parts should be devoid of toxic elements and should contain

enough essential nutrients. To fight against malnutrition due to essential element deficiency in edible parts of crops, biofortification has been suggested (reviewed by Nestel et al. 2006; Yang et al. 2007; Palmgren et al. 2008; White and Broadley 2009). The aim is to produce crops enriched in Fe, Zn, Cu, Ca, Mg, Se, and I, but also in “promoter” substances, such as ascorbate (vitamin C), beta-carotene, cysteine-rich polypeptides, and certain organic and amino acids, which stimulate the absorption of essential mineral elements by the gut (reviewed by White and Broadley 2009). The seeds of legumes generally have far higher concentrations of Fe and Zn than seeds of cereals (reviewed by White and Broadley 2009). This highlights the importance of the choice of plant species in strategies designed to increase the delivery of mineral elements to vulnerable populations. Another knowledge to consider is the fact that some elements such as Se and Mg are transported readily in the phloem while others such as Fe, Zn, Cu, and I are not, and Ca has little phloem mobility (reviewed by White and Broadley 2009). Thus, phloem-fed tissues such as fruits, seeds, and tubers are often poor sources of Fe, Zn, Cu, I, and Ca, while leafy vegetables are rich sources of these elements. Zn- or Fe-enriched cereal grains would potentially generate major health benefits. Moreover, adequate Zn content is known to enhance crop productivity (reviewed by Cakmak 2008). Finally, it is worth mentioning that once in place, the biofortified crop system is highly sustainable (reviewed by Nestel et al. 2006).

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