MANGANESE AS ESSENTIAL AND TOXIC ELEMENT FOR PLANTS: TRANSPORT, ACCUMULATION AND RESISTANCE MECHANISMS

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ABSTRACT

Manganese is an essential element for plants, intervening in several metabolic processes, mainly in photosynthesis and as an enzyme antioxidant-cofactor. Nevertheless, an excess of this micronutrient is toxic for plants. Mn phytotoxicity is manifested in a reduction of biomass and photosynthesis, and biochemical disorders such as oxidative stress. Some studies on Mn toxicity and Mn translocation from soil to plant cells in Mn^{2+} form have demonstrated their importance under low pH and redox potential conditions in the soil. When Mn is inside the cells, mechanisms that can tolerate this toxicity are also observed, being important the compartmentalization of this metal in different organelles of shoot and leaf plant cells. A key role of antioxidative systems in plants in relation to high Mn amounts has also been reported as a defense mechanism. The purpose of this review is to show the role of Mn as an essential micronutrient and as a toxic element to higher plants as well as to their transport and tolerance mechanisms. The forms and dynamics of this element in soils and the importance of the acidity for this dynamic and availability for plants are also given.

Keywords: Manganese, Mn toxicity, resistance mechanisms

INTRODUCTION

Manganese (Mn) is an essential micronutrient in most organisms. In plants, it participates in the structure of photosynthetic proteins and enzymes. Its deficit is dangerous for chloroplasts because it affects the water-splitting system of photosystem II (PSII), which provides the necessary electrons for photosynthesis (Buchanan, 2000). However, its excess seems also to be particularly damaging to the photosynthetic apparatus (Mukhopadhyay and Sharma, 1991). Thus, Mn has two roles in the plant metabolic processes: as an essential micronutrient and as a toxic element when it is in excess (Kochian *et al*., 2004; Ducic and Polle, 2005). Mn toxicity is favored in acid soils (Pendias and Pendias, 1992). With decreasing pH, the amount of exchangeable manganese – mainly Mn^{2+} form – increases in the soil solution. This Mn form is available for

plants and can be readily transported into the root cells and translocated to the shoots, where it is finally accumulated (Marschner, 1995). In contrast, other forms of Mn predominate at higher pH values, such as Mn (III) and Mn (IV), which are not available and cannot be accumulated in plants (Rengel, 2000).

Excessive Mn concentrations in plant tissues can alter various processes, such as enzyme activity, absorption, translocation and utilization of other mineral elements (Ca, Mg, Fe and P), causing oxidative stress (Ducic and Polle, 2005; Lei *et al*., 2007). The threshold of Mn injury as well as the tolerance to an excess of this metal is highly dependent on the plant species and cultivars or genotypes within a species (Foy *et al*., 1988, Horst, 1988).

The purpose of this review is to illustrate the most current understanding about Mn role as an essential micronutrient and as a toxic element to higher plants, the long distance and cellular transport in plants as well as the mechanisms or strategies involved for to resist an overload of this metal. The forms and dynamics of this element in soils and the importance of the acidity for this dynamic and availability to plants are also given.

MANGANESE FORMS AND DYNAMICS IN SOILS

Manganese biogeochemistry in soils is complex, because it is present in several oxidation states (0, II, III, IV, VI and VII), while in biological systems it occurs preferably as II, III and IV. Divalent manganese (Mn II) is the most soluble species of Mn in soil, whereas the solubility of Mn III and Mn IV are very low (Guest *et al*., 2002). Mn oxides can form co-precipitates with iron (Fe) oxides, exhibiting amphoteric behavior. In addition, Mn interacts both with cations and anions in oxidation-reduction reactions involving Mn. These reactions are influenced by a variety of physical, chemical and microbiological processes (Bradl, 2004).

Both pH and redox conditions influence Mn bioavailability in soils (Marschner, 1995; Porter *et al*., 2004). In most acid soils at low pH (<5.5) and an increased redox potential of Mn, oxides can be easily reduced in the soil exchange sites (Kogelmann and Sharpe, 2006), increasing the concentration of soluble Mn^{2+} (Watmough *et al*., 2007), which is the predominant Mn form in the soil solution (Adriano, 2001) and the most available Mn form for plants (Marschner, 1995). At higher soil pH (up to pH 8), chemical Mn^{2+} auto-oxidation is favored over MnO_2, Mn_2O_3, Mn_3O_4 and even Mn_2O_7, which are not normally available to plants (Ducic and Polle, 2005; Humpries *et al*., 2007; Gherardi and Rengel, 2004). Furthermore, high pH allows Mn adsorption into soil particles, decreasing their availability (Fageria *et al*., 2002). Nevertheless, some reports have suggested that an excess of available Mn is produced under reduced soil conditions, even at high soil pH values (Hue, 1988). A reducing environment can be produced when there is an excess of water, poor drainage or applications of organic material (Hue, 1988; El-Jaoual and Cox, 1998). Different organic molecules can dissolve solid Mn oxides through transfer of electrons, transforming them into an available Mn form for plants (Laha and Luthy, 1990). Soil acidification is also accentuated by abundant pluviometry during winter, causing the main cations to leak from the soil (Mora *et al*., 2006). On the other hand, lime application is a key factor in decreasing soluble Mn in acid soils with a high Mn content, given that it can increase soil pH (Hue and Mai, 2002).

The total Mn content in soils is variable. Sparks (1995) reported small amounts of Mn in soils, fluctuating from 20 to 10,000 mg kg^{-1} soil, whereas other authors have registered total Mn contents between 450 and ~ 4,000 mg Mn kg^{-1} soil (Adriano, 2001). In addition, the total Mn soil content was from 15 to 17 mg kg^{-1} in acid soils without liming (pH about 4.4) (Hue and Mai, 2002). In liming acid soils, the interchangeable Mn concentration varied from 14 to 96 mg kg^{-1} soil in one year, with higher concentrations under high moisture and temperature conditions (Conyers *et al*., 1997). In Chilean volcanic soils, so-called Andisols, Mn concentrations fluctuate between 4.5 and 80 mg kg^{-1} depending on the agronomic management. Moreover, the Mn amount is higher in pasture soils (up to 400 mg kg^{-1}) mainly in winter (Data from Laboratorio de Análisis de Suelo y Planta, Universidad de La Frontera, Temuco, Chile).

Environmental conditions also affect Mn soil contents. The highest concentrations of soluble and exchangeable Mn are found after hot, dry summers and under warm waterlogged conditions in acid soils. This is probably due to the inhibition of Mn-oxidizing organisms, thereby allowing the chemical reduction of Mn oxides in these soils (Sparrow and Uren, 1987; Conyers *et al*., 1997).

Manganese dynamics in the rhizosphere

Rhizosphere, which is the narrow zone of soil immediately surrounding the root system, is of great importance for mineral plant nutrition. In this zone, both the mobilization and immobilization of nutrients occur (Marschner, 1995). As shown in Figure 1, a mobilization of Mn^{2+} is produced by the rhizosphere acidification due to the release of H^+ or low molecular weight organic acids (LMWOA) from plants (Rengel and Marschner, 2005). Organic acids released in anion forms from roots can chelate Mn^{2+} released from the MnOx (Mn oxides) (Ryan *et al.,* 2001). Neumann and Römheld (2001) reported that mobilization of micronutrients (including Mn) into the rhizosphere is due mainly by its acidification and complexation with the organic acids (citrate) in various plant species. It has been reported that organic amendments (chip compost and pine bark) applied to melon plants released organic compounds such as arabinose and malic acid that can dissolve MnO_x (Tsuji *et al*., 2006). Soil microorganisms can also help Mn mobilization and immobilization, depending on soil conditions (Marschner, 1995). In aerated soils, microorganisms may mobilize Mn through MnOx reduction favored by H^+ root excretion. In contrast, Mn-oxidizing bacteria can decrease Mn availability in aerated and calcareous soils or in poorly aerated and/or submerged soils. Another key factor in the Mn dynamics in soil is organic matter (OM). Given that OM is negatively charged, it has a great Mn adsorption capacity, forming Mn complexes which decrease the amount of exchangeable Mn. However, the Mn adsorbed by OM can be exchanged by the H^+ released from the roots (Bradl, 2004).

MANGANESE TRANSPORT AND ACCUMULATION IN PLANTS

As mentioned above, reduced Mn (Mn^{2+}) form is the only available metal form for plants. It can be taken up via an active transport system in epidermal root cells and transported as divalent cation Mn^{2+} into the plants (Marschner, 1995; Gherardi and Rengel, 2003; Pittman, 2005). Manganese uptake by roots is characterized as a biphasic process. The initial and rapid uptake phase is reversible and non-metabolic, with Mn^{2+} and Ca^{2+} or

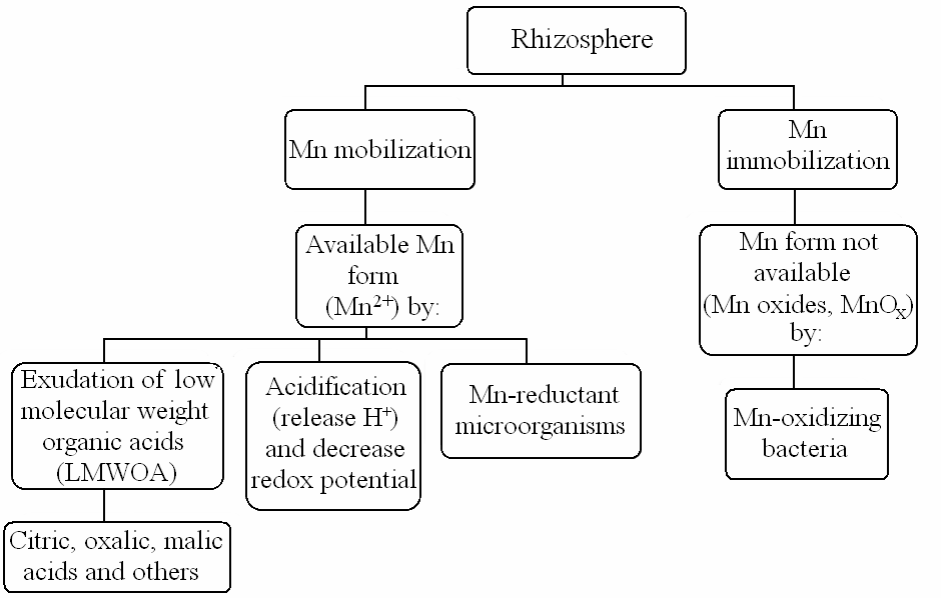

Figure 1. The role of soil rhizosphere in the mobilization and immobilization of Mn in soils.

other cations being freely exchanged in the rhizosphere. In this phase, Mn^{2+} appears to be adsorbed by the negatively charged cell wall constituents of the root-cell apoplastic spaces (Humphries *et al.*, 2007; Clarkson, 1988). The second phase is slow, with Mn^{2+} being less readily exchanged. Its uptake into the symplast is dependent on plant metabolism (Maas and Moore, 1968), although the exact mechanisms are not clear (Humphries *et al.*, 2007). It has been shown that in transgenic tobacco transformed with a tomato root protein with a metal binding side at its N-terminus (LeGlp1), Mn binds to this protein. This strongly suggests the involvement of LeGlp1 in Mn uptake from the soil (Takahashi and Sugiura, 2001). Kinetic measurements have demonstrated 100 to 1,000 times higher rates of Mn transport than the estimated plant requirement for this element (Clarkson, 1988). These transport rates are explained by the high capacity of ion carriers and channels in the Mn ion transportation through the plasma membrane at a speed of several hundred to several million ions per second per protein molecule (Humphries *et al.*, 2007). According to these authors, Mn distribution from root cells within the whole plant involves primary transport in the xylem, transference from the xylem to the phloem and re-translocation into the phloem. Xylem transport from roots to the above-ground parts of plants is performed by the transpiration stream, whereas phloem transport is more selective, taking place from sources to sinks (Marschner, 1995). Nonetheless, a low mobility in phloem has been reported for Mn, and its redistribution may depend on the plant species and stages of development (Herren and Feller, 1994). In fact, it has been reported that Mn transport from roots to grains is frequently insufficient at

a mature stage of wheat. The relatively poor Mn mobility in the phloem emphasizes the importance of xylem in the transport of this element, even in wheat grain discharge (Rengel, 2001).

Manganese generally tends to accumulate predominantly in the plant shoots than in the roots, as demonstrated in Mn labeling experiments with ^{54}Mn at an early stage of wheat (*Triticum aestivum* cv. Arina) development, where a fast Mn transport from roots to shoots was visualized in the xylem and was essentially immobile in the phloem (Page and Feller, 2005). Similar effects on Mn translocation have been shown by the same technique in young (28 days) white lupine plants (*Lupinus albus*) (Page *et al.,* 2006). Nevertheless, Mn was present in a large amount in the root system, hypocotyls and stem in older lupine plants, immediately after the labeling phase (day 0). Seven days later (day 7) almost all ^{54}Mn had moved to the youngest fully expanded leaves and only a small fraction to the other leaves. Mn accumulation was observed in the periphery of the oldest leaves. These authors reported that Mn was rapidly released from the roots into the xylem, reaching photosynthetically active leaves via the transpiration stream. Furthermore, the low mobility of this element via phloem in the shoot may be due to a restricted loading of soluble Mn into the phloem or by insolubilization in the leaves, although the issue remains to be clarified (Page *et al.,* 2006). Page and Feller (2005) emphasized that little is known about the mechanisms involved in the loading of Mn into the phloem and the chemical transport forms.

In addition to the long distance transport of Mn, short distance transport mechanisms are important for the translocation of this metal into the cell and cell organelles. These mechanisms involve Mn translocation throughout the plasma membrane and the biomembranes of organelles (Ducic and Polle, 2005; Pittman, 2005). Possible mechanisms of homeostasis and Mn transport, based on studies performed in yeast (*Saccharomyces cerevisiae*) cells and in *Arabidopsis thaliana* plants, have been discussed (Delhaize *et al.,* 2007; Reddi *et al*., 2009). They pointed out that transport proteins play an important role for the maintenance of adequate Mn concentrations in the cytoplasm. Moreover, a variety of metal transporter family proteins with a broad-specificity such as Fe^{2+} and Ca^{2+} transporters have also the ability to transport Mn into the plant cells (Pittman, 2005). Migocka and Klobus (2007) demonstrated the activation of an antiport system in *Cucumis sativus* with different affinities for Pb, Mn, Ni and Cd in the root plasma membrane. This antiport system participates as part of the general defense mechanism activated under heavy metal stress. Using tonoplast-enriched vesicles, Shigaki *et al*., (2003) suggested that a Cd/H antiporter might also be involved in Mn accumulation in vacuoles.

In Table 1 we have summarized some of the metal transporter proteins, their families, cellular localization and the transported metals. Transporter proteins are described according to their localization. In the plasma membrane, four Mn^{2+} uptake transporters are identified: AtIRT1, Nramp, AtYSL (Ducic and Polle, 2005; Pittman, 2005) and PHO84 (Ducic and Polle, 2005). IRT1 can transport Mn when expressed in yeast (Korshunova *et al*., 1999). Nramp is also considered a metal transporter protein that can transport Mn away from other cations. This protein may be localized in the tonoplast rather than in the plasma membranes (Thomine *et al*., 2003). AtYSL belongs to the yellow stripe-like (YSL) proteins, also involved in metal-complex transport in the plasma

membrane (Roberts *et al*., 2004). This complex can be formed by nicotianamine (NA), which is a strong chelator of metals including Mn^{2+} (Pittman, 2005). PHO84 is a transporter protein identified in *S. cerevisiae* and it has a high-affinity phosphate uptake (Mitsukawa *et al*., 1997). Luk *et al*. (2003) reported a new form in Mn transport as $MnHPO_4$. However, there is no evidence for Mn^{2+} or $MnHPO_4$ accumulation by a plant phosphate transporter (Pittman, 2005).

Some transport proteins have been related to Mn^{2+} transport and accumulation into the intracellular compartments, such as the vacuole. It has been suggested that a metal transporter (specifically antiporter CAX2, calcium exchanger 2) originally identified as a Ca^{2+} transporter (which can also transport Cd^{2+}) located in the cytosol. It has also the ability to transport Mn to the vacuole in tobacco plants (*Nicotiana tabacum*) and yeast (Hirschi *et al*., 2000; Pittman, 2005) (Table 1). ATP-binding cassette (ABC) protein transporters are considered to be involved in detoxification processes (Martinoia *et al*., 2002). Studies on cyanobacteria also suggested the putative role of these proteins in Mn^{2+} transport (Bartsevich and Pakrasi, 1996).

Another protein, considered indirectly as a metal transport protein is the ShMTP1, which is able to sequester metal ions within cells or efflux them out of the cells (Delhaize *et al*., 2003). Therefore, these authors considered it a metal-tolerant protein. Ducic and Polle, (2005) and Pittman (2005) highlighted that, despite the available information about Mn transport across membranes in plant cells, the Mn transport and efflux strategies into the mitochondria, chloroplasts and Golgi are not completely understood. Nonetheless, Mills *et al*. (2008) have recently identified a Ca-ATPase that also transports Mn^{2+} into Golgi apparatus (Table 1).

MANGANESE AS AN ESSENTIAL ELEMENT IN PLANT METABOLISM

The main Mn role in photosynthesis is its involvement in the water-splitting system of photosystem II (PSII), which provides electrons necessary for photosynthetic electron transport. In water photolysis, a group of four Mn atoms (Mn cluster) is associated with the oxygen evolving complex (OEC) bound to the reaction center protein (D1) of PSII (Goussias *et al*., 2002). The Mn cluster in PSII accumulates four positive charges, which oxidize two water molecules, releasing one O_2 molecule and four protons. Therefore, this metal cluster is considered a catalyst compound of water oxidation (Zouni *et al*., 2001), where Mn ions are close to a redox-active tyrosine residue (Z and D) (Goussias *et al*., 2002).

Manganese also plays a role in ATP synthesis (Pfeffer *et al*., 1986), in RuBP carboxylase reactions (Houtz *et al*., 1988) and the biosynthesis of fatty acids, acyl lipids and proteins (Ness and Woolhouse, 1980). In addition, Mn plays a primary role in the activation and as cofactor of various enzymes in plants (~35) (Burnell, 1988), such as: Mn-superoxide dismutase, Mn-catalase, pyruvate carboxylase and phospho-enolpyruvate carboxykinase (Ducic and Polle, 2005). Manganese is also essential for the biosynthesis of chlorophyll (through the activation of specific enzymes), aromatic amino acids (tyrosine), secondary products, like lignin and flavonoids (Lidon *et al*., 2004). It also participates in the biosynthetic pathway of isoprenoids (Lidon *et al*., 2004) and assimilation of nitrate (Ducic and Polle, 2005). Hence, Mn is involved in metabolic processes such as respiration, photosynthesis, synthesis of aminoacids and hormone activation (indol acetic acid, IAA) throughout the IAA-oxidases (Burnell, 1988).

Table 1. Some transporter proteins implicated in Mn^{2+} and other cations transport and their cellular localization. (Summarized from Ducic and Polle, 2005; Pittman, 2005 and Mills *et al*., 2008). Abbreviations as follows: At= *Arabidopsis thaliana*; Sh= *Stylosanthes hamata*).

Transporter proteins	**Protein cellular localization and protein family transporters**	**Transported ions**
AtIRT1	Plasma membrane protein (ZIP, zinc-regulated transporter/iron regulated transported (ZRT/IRT1) related protein) family transporter	Mn^{2+} and Fe^{2+}, Zn^{2+} and Cd^{2+} under Fe-deficiency conditions.
AtECA1	Endoplasmic reticulum (ER) Ca^{2+}- and Mn^{2+}-transporting P-type ATPase	Ca^{2+} and Mn^{2+}
AtCAX2	Vacuolar cation/H^+ antiporter CAX (the cation exchanger)	Mn^{2+}, Ca^{2+} and Cd^{2+}
AtNramp3	Vacuolar Nramp transporter. Also, possible plasma membrane localization (Nramp?)	Mn^{2+}, Fe^{2+} and Cd^{2+} in Fe-deficiency conditions
ShMTP1	Vacuolar-localized cation diffusion facilitator (CDF) family transporter	Related to the *Stylosanthes hamata* Mn^{2+} transporter
ABC	Vacuolar- localized ATP binding cassette transporter families	Related to the cyanobacterium *Synechocystis* Mn^{2+} transporter
AtOPT3	Probably located in the plasma membrane (AtOPT3?). It is an oligopeptide transporter-like protein (OPT).	Possible transport of Cu^{2+} and Fe^{2+} and Mn^{2+}
AtYSL	Probably located in the plasma membrane. Yellow stripe-like transporter with equivalent function to rice OsYSL2, a Mn^{2+}-nicotianamine (NA) and Fe^{2+}-NA transporter	Mn^{2+} and Fe^{2+}
AtECA3	Ca^{2+} transporters (Ca-ATPases) in Golgi.	Mn^{2+} and Ca^{2+}
PHO84	Probably located in the plasma membrane. It is $MnHPO_4$ transporter.	Mn binding to phosphate.

As a cofactor of superoxide dismutase (SOD), manganese participates in the plant's defense against oxidative stress, produced by elevated levels of activated forms of oxygen and free radicals (reactive oxygen species, ROS), which are harmful to plants. It has been proposed that Mn can act as a scavenger of superoxide (O_2^-) and hydrogen peroxide (H_2O_2). However, this mechanism is still unclear (Ducic and Polle, 2005).

Manganese SOD (MnSOD) belongs to the group of metal-containing SOD enzymes which are classified according to their metal cofactor: iron SOD (Fe-SOD), localized in the chloroplast; copper-zinc SOD (Cu/Zn-SOD), located in the chloroplast, cytosol, and possibly in the extracellular space. Manganese SOD is found mainly in mitochondria (Clemens *et al*., 2002) and peroxisomes (Alscher *et al*., 2002). MnSOD may play an important role in the adaptive responses of plant cells under environmental stresses such as salt stress, enhancing their tolerance. This tolerance has been demonstrated in transgenic plants of *Arabidopsis,* where Mn-SOD was overexpressed (Wang *et al*., 2004). Similarly, in tomato transgenic plants which overexpressed MnSOD, an improvement in tolerance to NaCl stress in seedlings was concomitant with an improvement in seed germination and root development (Wang *et al*., 2007).

MANGANESE PHYTOTOXICITY AND INJURY SYMPTOMS

As an essential micronutrient, low Mn levels are absolutely necessary for normal nutrition and development of plants. Normal Mn contents of leaves differ greatly between species (30-500 mg kg^{-1} Mn dry mass, Clarkson, 1988). Nonetheless, when it is present in excessive amounts, it is extremely toxic to plant cells (Migocka and Klobus, 2007). The injury extent of Mn toxicity is approximately proportionate to the concentration of accumulated Mn excess. However, there is considerable inter- and intra- specific variation among Mn levels that induce toxicity as well as the symptoms of this toxicity in plant species (Foy *et al*., 1988).

In addition to a decrease in growth rate, symptoms of Mn toxicity such as chlorosis in leaves (intervenial and marginal) and necrotic leaf spots are very common and have been reported in the whole plants of canola (Moroni *et al*., 2003), clover (Rosas *et al*., 2007), ryegrass (Mora *et al*., 2009) as well as in leaves of barley and cowpea (Demirevska-Kepova *et al*., 2004; Führs *et al*., 2008) (Table 2). Necrotic brown spots and chlorotic leaves are frequently reliable indicators of the severity of Mn toxicity in plants (Wissemeier and Horst, 1991). The intervenial chlorosis due to Mn toxicity can have an appearance similar to that observed under Fe deficiency (Sarkar *et al*., 2004). Moreover, the Mn toxicity is intensified when other available elements such as Ca, Mg, K, Fe and Si are in a low quantity (Abou *et al*., 2002). However, a decrease in productivity by Mn toxicity without the appearance of leaf visual symptoms is sometimes observed (Miner and Sims, 1983). It is important to know that all these symptoms induced by Mn toxicity are preceded by an alteration of the photosynthetic apparatus and the photosynthetic performance of plants.

Other studies have shown that in rice (*Oryza sativa* cv. Safari) exposed to Mn excess in a nutrient solution, Mn was predominantly accumulated in leaves compared with roots (Lidon, 2001), whereas in *Sinapis alba* Mn mostly accumulated in the shoots (Farasova and Beinrohr, 1998).

Table 2. Symptoms of Mn toxicity and Mn concentrations in organs of some plant species subjected to toxic Mn concentrations according references from the last decade (earlier references in the text). Mn treatments were performed in nutrient solutions with $MnCl_2$ or $MnSO_4$.

Species	**Mn treatment**	**Mn concentrations in different plant organs**	**Symptoms of Mn toxicity**	**References**
Rice (*Oryza sativa* L.)	583 µM	Shoots: 2020 µg g^{-1} dw	Decrease of shoot growth rate.	Lidon and Texeira (2000a)
Barley (*Hordeum vulgare* L.)	1830 µM 18300 µM	Leaves: 656 mg g^{-1} dw Leaves:1615 mg g^{-1} dw	Dark-brown necrotic spots, individually or in groups.	Demirevska-Kepova *et al.* (2004)
Ryegrass (*Lolium perenne* L.)	355 µM	Shoot: 2357 mg kg^{-1} Roots: 2408 mg kg^{-1}	Chlorotic leaves. Decrease of dry weight in roots.	Rosas *et al.* (2007)
Ryegrass (*L*. perenne L.)	150 µM	Shoot: 902 mg kg^{-1} Roots: 1342 mg kg^{-1}	Dry weight reduction. Dry weight reduction.	Mora *et al*. (2009)
Clover (*Trifolium repens* L.)	355 µM	Shoot: 2050 mg kg^{-1} Root: 7481 mg kg^{-1}	Reddish borders on leaves. Decrease of dry weight.	Rosas *et al*. (2007)
Soybean (*Glycine max* L.)	200 µM	Leaves: 806 mg kg^{-1} Roots: 502 mg kg^{-1}	Chlorotic leaves. No visual symptoms, increase in root diameter.	Lavres Jr *et al.* (2009)
Cowpea (*Vigna unguiculata* L.)	50 µM	Leaves: ~ 25 µmol g^{-1} dw	Brown spots on leaves.	Führs *et al.* (2008, 2009)
Canola (*Brassica napus* L.)	200 µM	Shoot: ~ 3500 µg g^{-1} dw	Necrotic leaf spots, chlorosis in leaf margin.	Moroni *et al*. (2003)
Juncus effuses L. (wetland plant)	500 µM	176 mg kg^{-1}	Reduction in plant dry biomass and height, no phytotoxic visual symptoms.	Najeeb *et al*. (2009)
Populus cathayana	1000 µM	Leaves: 713 mg kg^{-1} dw	Decrease in shoot height, total biomass, and total leaf area.	Lei *et al.* (2007)

Despite the importance of Mn excess in the photosynthetic performance of plants, only a few studies about this issue are available. A reduction in photosynthesis, in chlorophyll *a* and *b* contents and their biosynthesis, as well as a reduction in carotenoids is frequently found in plants and also in algae under Mn excess (Macfie and Taylor, 1992; Hauck *et al*., 2003). In rice cultivated at different Mn concentrations (from 2.3 to 583 µM), a significant decrease in chlorophyll *a* content has been reported at the highest Mn concentration (Lidon and Teixeira, 2000a). Nable *et al*. (1988) reported an early inhibition of photosynthesis concomitant with a high Mn accumulation in the leaves in *Nicotiana tabacum* cultivated in nutrient solutions with a Mn excess (1,000 µM). The authors concluded that the inhibition of photosynthesis is an early indicator for Mn toxicity in tobacco leaves. Lidon *et al*., (2004) also observed a decline in net photosynthesis (µmol CO_2 $m^{-2}s^{-1}$) and photosynthetic capacity (µmol O_2 $m^{-2}s^{-1}$) in rice plants subjected to 9.1 and 36.4 µM Mn treatment, respectively, with no changes in the levels of the ratio between variable (Fv) and maximum (Fm) chlorophyll fluorescence (Fv/Fm). However, an increase in the photochemical quenching and the quantum yield of non-cyclic electron transport was found up to 36.4 µM Mn. Since Mn is accumulated in thylakoids, this element may interfere with thylakoid stacking, decreasing net photosynthesis (Lidon and Teixeira, 2000a).

Similarly to Mn toxicity, Mn deficiency also depressed leaf photosynthetic capacity in plants of *Carya illinoinensis*, primarily by reducing the number of PSII units per unit leaf area, but these PSII units maintained abilities similar to those of the control plants (Henriques, 2003). Increased Mn concentrations (0 – 1,000 and 10,000 µM Mn) in wheat plants inhibited the biosynthesis of chlorophyll and carotenoids, inducing a decrease in photosynthetic electron transport rates and therefore a decrease in the rate of photosynthesis (Macfie and Taylor, 1992). Higher Mn concentrations are also involved in a shortening of root and shoot and in a decreased chlorophyll concentration in the Mn accumulator plant, *Alyssum murale* (Abou *et al*., 2002). Recently, Amao and Ohashi (2008) suggested that high Mn amounts in spinach leaves inhibited the activity of oxygen evolved complex of PSII.

Manganese toxicity and oxidative stress

Mn toxicity can also trigger oxidative stress in plant cells (Demirevska-Kepova *et al*., 2004). As a toxic metal, Mn can cause metabolic alterations and macromolecular damage that disrupt the cell homeostasis (Hegedüs *et al*., 2001; Polle, 2001). According to Lynch and St. Clair (2004), Mn toxicity in plants generates reactive oxygen species (ROS), mainly $OH^{\cdot}$, the most reactive oxidant and harmful species in cells (Lidon and Henriques, 1993). With respect to the oxidative stress responses to Mn excess, Lidon and Teixeira (2000b) reported that at the first growth stages of rice, two kinetic phases can be distinguished: in the first one, there is an increase in Mn accumulation in the thylakoid lamellae (McCain and Markley, 1989), which inhibits electron leakage from the Hill and Mehler reactions, limiting ROS formation. In the second kinetic phase, higher Mn amounts inhibit the non-cyclic photophosphorylation process, promoting an increase in ROS production that parallels an injury increase in the thylakoid peroxidase system (Lidon and Teixeira, 2000b). These authors concluded that Mn excess increases the disorganization in chloroplast lamellae,

but elevated activity of superoxide dismutase (SOD) still limits cell damage.

It has also been reported that in *Cucumis sativus* plants both Mn excess and optimum light intensity determine an enhancement in oxidative stress by increased Mn content in the tissues concomitant with an inhibition of plant growth (Shi *et al*., 2006). Investigations performed by González *et al*., (1998) showed that lipid peroxidation was not induced by Mn-toxicity stress in the mature leaves of *Phaseolus vulgaris*, although other studies have shown that lipid peroxidation occurred in isolated chloroplast of wheat (Panda *et al*., 1986). However, González *et al*. (1998) mentioned that this damage process could be related to the development stage of leaves, with the damage being more intense in immature than in mature leaves.

The geographical origin of the species and climatic conditions also affect the degree of Mn toxicity in plant species or populations as shown in two populations of *Populus cathayana*, coming from a wet and dry climate cultivated in an acid solution with increasing Mn concentrations (Lei *et al*., 2007). The results showed that the wet climate population accumulated more Mn in plant tissues especially in leaves, decreasing their growth, chlorophyll contents, and activities of antioxidant enzymes than the dry climate population.

TOLERANCE MECHANISMS TO MANGANESE TOXICITY

The ability of plant for to grow and survive in a metal-contaminated environment, commonly called resistance, can be achieved through different mechanisms: avoidance and/or tolerance. The former involves a protective role that prevents the metal ions from entering the cytoplasm of plant cells (Blamey *et al*., 1986; Marschner, 1991). The latter strategy (tolerance) implies a detoxification of metal ions after they have crossed the plasma cell membrane or internal organelle biomembranes (Macfie *et al*., 1994). However, as shown in the following paragraphs, the differentiation of these mechanisms in the pertinent literature is very confusing.

The sequestering of Mn in the apoplast is considered an avoidance mechanism. However, some researchers have included this feature as a tolerance mechanism. For example, Horst *et al*., (1999) suggested that tolerance to Mn excess in *Vigna unguiculata* is performed by the reduction of Mn^{2+} activity in the apoplast throughout complexation by organic acids. In this species, symptoms such as brown leaf cell spots are also identified as oxidized Mn, and phenolic compounds present in the cell walls are considered a Mn tolerance mechanism (Wissemeier and Horst, 1992). On the other hand, the Mn^{2+} oxidation by peroxidases in the cell walls of roots is considered by Marschner (1991) as an avoidance mechanism, although the existence of such a mechanism was not considered in the study of Horiguchi (1987). In this study, it is suggested that oxidized Mn deposition in plant tissues corresponds to a tolerance mechanism to Mn toxicity, with *Cucumis sativus* being more tolerant to high Mn deposition in tissues than melon (*Cucumis melo)*. Blamey *et al*., (1986) reported the accumulation and secretion of Mn^{2+} in and around the trichomes of sunflower plants (*Helianthus annuus*) as a Mn tolerance mechanism. Another strategy that plants use to prevent the toxic effects of heavy metals as well as of Mn can be the efflux from the cell. In this process, the Mn cell is delivered into the Golgi apparatus and finally exported from the cell via secretory pathway vesicles that carry the metal to the cell surface (Ducic and Polle, 2005).

Summarizing the available literature regarding tolerance mechanisms to Mn, it appears that the main Mn tolerance mechanism is the sequestration by organic compounds in metabolically less active cells or organelles. The vacuole is considered the biggest and most important compartment, because it can store many toxic compounds (Pittman, 2005). Hence, an increase in phenolic compounds was found in the hydrophyte (*Trapa natans*) leaves exposed to high Mn levels (130 µM) (Baldisserotto *et al*., 2004). These compounds chelate Mn inside the vacuole, segregating the metal ion in the protoplasm and thus reducing the damage (Davis *et al*., 2001). A similar key role has recently been assigned to oxalic acid in Mn internal sequestration by chelating specifically the Mn excess in vacuoles of Mn hyperaccumulator plants (*Phytolacca americana*) (Dou *et al*., 2008). Furthermore, it has been observed that a Mn excess can accumulate dark material in the vacuoles, probably for deposition of Mn oxides or an increase in polyphenol oxidase activity in *Citrus volkameriana* plants (Papadakis *et al*., 2007). Similarly, studies about the effect of Mn excess in varieties of the conifer *Pseudotsuga menziesii* showed dark deposits of Mn-complexes in plant root vacuoles, which were associated with phosphate, establishing "free" Mn^{2+} to form insoluble complexes, giving a greater tolerance (Ducic and Polle, 2007). Additionally, these studies showed that both, root elongation and biomass production were inhibited by Mn treatment above 2,500 µM, mainly in *P. menziesii* var. *glauca*, confirming that it is a species with lower tolerance than *P. menziesii* var. *viridis.*

Another strategy to confer Mn tolerance inside plant cells is associated with several metal transporter proteins identified in the Mn transport mechanisms described in section 4. Metal transporter proteins located in the tonoplast (CAX2 in tobacco plants, ShMTP1 in *Arabidopsis* plants) conferred greater tolerance on elevated Mn^{2+} levels due to the internal sequestering of this element (Hirschi *et al*., 2000; Delhaize *et al*., 2003). Other transporter proteins (ECA1) can maintain low cytosolic Mn, since it moves into the endoplasmic reticulum (Wu *et al*., 2002). In this research, ECA1 was able to reduce cytosolic Mn^{2+}, preventing an interference with the internal distribution of other ions (Mg^{2+}, Fe^{2+} or Ca^{2+}).

Unlike other metal stresses, the accumulation of Mn excess does not have a single cell target. Depending on the plant species, different organelles can serve as stores for this accumulation (Lidon *et al*., 2004). Under high Mn levels apart from vacuoles, chloroplasts are important sinks of this metal in *Citrus volkmeriana*. This feature, together with the larger size of this organelle, is considered to be an adaptive response of this plant to Mn excess (Papadakis *et al*., 2007). In general, cultivated plants like rice are considered tolerant to Mn toxicity (Lidon, 2001) because their leaf tissues can accumulate from 5 to 10 times more Mn than other grasses (Foy *et al*., 1978). The Mn tolerance mechanism in this species included the inhibition of apoplastic influx from the cortex toward the stele and symplastic Mn assimilation in the shoot protoplast, where the chloroplast is the main target (Lidon, 2001).

The distribution of Mn excess in both roots and shoots is dependent on plant species and genotype. Early research associated Mn tolerance in some plants with a greater retention of Mn excess in the roots, as mentioned by Andrew and Hegarty (1969) in regard to tropical and temperate legume species. The root retention of heavy metals has been attributed to the formation of metal complexes in roots (Foy *et al*., 1978). Metals with high electro-negativity

accumulate in roots in larger amounts than metals with low electro-negativity. In the latter instance, Mn and Zn metals can easily be translocated to the tops (Chino, 1981).

In proteomics studies, a comparison of Mn-sensitive and Mn-tolerant cultivars of cowpea (*Vigna unguiculata*.) has shown relevant features of leaf apoplast in the expression of Mn toxicity: formation of brown spots, induction of callose formation and an enhanced release of phenols and peroxidases into the apoplast (Fecht-Christoffers *et al*., 2003; Fecht-Christoffers *et al*., 2006). Specific proteins involved in the regulation processes, such as CO_2 fixation, stabilization of the Mn cluster of the photosystem II, pathogenesis-response reactions and protein degradation, were affected at low or high Mn levels, mainly in the Mn-sensitive cowpea cultivar (Führs *et al*., 2008). Chloroplastic proteins, which are important for CO_2 fixation and photosynthesis, were of lower abundance upon Mn-induced stress, suggesting the scavenging of metabolic energy for a specific stress response. Führs *et al*., (2008) concluded that a coordinated interplay of apoplastic and symplastic reactions seems to be important during the Mn-stress response in plants.

To alleviate metal toxicity in plants, the antioxidant systems are also considered an important tolerance mechanism. The antioxidant systems include antioxidant enzyme "scavengers" such as superoxide dismutase (SOD), catalase (CAT), peroxidases (phenol peroxidase, POX, ascorbate peroxidase, APX, guaiacol peroxidase, GPX) and the non-enzymatic antioxidant molecules: ascorbate, α-tocopherol, carotenoids, flavonoids and glutathione (Foyer and Noctor, 2003; Apel and Hirt, 2004). These constitute other mechanisms against ROS, produced by Mn toxicity. Higher activities of antioxidant enzymes are found in response to a Mn excess in woody plants (Lei *et al*., 2007), in herbs such as white clover (*Trifolium repens*.) and in ryegrass (*Lolium perenne*), which suggest a lower oxidative stress (Rosas *et al*., 2007; Mora *et al*., 2009). Another plant tolerance mechanism to metal toxicity is associated with lower metal uptake and translocation to other organs (Hall, 2002). The exudation of organic acid anions (carboxylates) to the rhizosphere may minimize the absorption by roots of such metals as aluminium and nickel (Ma *et al*., 2001; Yang *et al*., 1997). There are few reports regarding the release of organic acid anions by roots in the event of Mn toxicity (González and Lynch, 1999). Recently, Mora *et al*. (2009) reported that the root exudates of oxalate and citrate may decrease Mn availability in the rhizosphere, enhancing their Mn tolerance in ryegrass subjected to Mn toxicity.

Other nutrient applications can help minimize the effects of Mn toxicity. Thus, it has been reported that silica (Si) addition significantly decreases lipid peroxidation caused by an Mn excess decreasing the symptoms of Mn phytotoxicity and improving plant growth in some plants (Iwasaki *et al*., 2002; Shi *et al*., 2005). There is also an association between Mn toxicity and the decrease in Ca concentration in barley plants (*Hordeum vulgare*), indicating a competition and a specific interaction during the absorption and/or translocation of these elements (Alam *et al*., 2001). Another study, in which Ca is applied to reduce the Mn toxicity, showed that Ca additions inhibited Mn translocation from roots to shoots in barley plants, but did not affect the Mn absorption in roots (Alam *et al*., 2006). This suggested that Ca could avoid Mn accumulation in shoots, protecting the photosynthetic apparatus from the dangerous effect of an

excess of Mn. In barley, Mn toxicity could also be repressed by high K contents, which inhibit both the absorption and the translocation of Mn (Alam *et al*., 2005).

CONCLUSIONS AND PERSPECTIVES

Manganese is considered an essential micronutrient for the metabolic process in plants. Nevertheless, both deficiency and excess alter these processes. Acid soils make excessive Mn amounts toxic for the plants. Mn toxicity is a world-wide problem in areas with acid soils. This toxicity alters physiological, biochemical and molecular processes at the cell level. It is crucial to know the limitations of these soils for the purpose of soil-plant interaction management, especially in relation to the presence and Mn excess. Thus, the knowledge of Mn uptake, translocation, accumulation and resistance mechanisms in crop plants under Mn excess and toxicity is of great importance to crop improvement.

Most molecular and physiological approaches to Mn transport inside plant cells have recently been analyzed, as these are useful tools for understanding resistance mechanisms. However, the question as to which is the best candidate gene related to Mn toxicity in acid soil continues to elude plant scientists due to Mn toxicity in plants being a complex trait and involving multiple physiological and biochemical mechanisms and a wide array of genes. The understanding of these mechanisms will contribute to improving the yield and quality of cultivated plants in acid soils. Future efforts for developing Mn-tolerant plants should take all these aspects into account.

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