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Manganese Hyperaccumulators and their Hyperaccumulating and Tolerance Mechanisms: A Review of the Current State of Knowledge

Pan W-S¹, Wu K-K², Rajendran M², Li W-C³ and Wu C^{2*}

¹College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, PR China

²School of Metallurgy and Environment, Central South University, Changsha, PR China

³Department of Science and Environmental Studies, The Education University of Hong Kong, Tai Po, Hong Kong Special Administrative Region, PR China

*Corresponding author: Chuan Wu, School of Metallurgy and Environment, Central South University, Changsha, PR China

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Abstract

Manganese (Mn) is ubiquitous in the environment due to both geological and human activities. It is essential for plants, as for most other living organisms, but can also be toxic when it is present in excess. Some plant species, referred to as Mn hyperaccumulators, can accumulate over 10000 µg/g of Mn in their shoot tissues without showing any phytotoxicity. Approximately 24 Mn hyperaccumulators are currently known worldwide. However, ample data is available the Mn hyperaccumulator species and biological significance of Mn hyperaccumulation and tolerance mechanisms. To give new insights, this review highlights the current knowledge of Mn hyperaccumulation and tolerance mechanisms in hyperaccumulators, which include root uptake, xylem loading, transport, sequestration, and detoxification processes. Hyperaccumulators uptake Mn mainly accumulates as Mn²⁺ into the xylem, from which it is then transferred to the shoots. Foliar Mn²⁺ is mainly stored in vacuoles, the endoplasmic reticulum, and the Golgi. It is sequestered by organic ligands and some transporter proteins at a subcellular level in the root and shoot, which can allow the plants to exhibit great tolerance. From the in-depth examine the published literature; the main knowledge gap and future research are highlighted. In addition, Mn hyperaccumulator biomass disposal methods and applications also discussed.

Keywords: Hyperaccumulator; Manganese; Phytomining; Transporters; Root uptake; Manganese sequestration

Introduction

Environmental contamination by Manganese (Mn) has become a serious problem worldwide. Manganese is the fifth most abundant metal in nature [1] and is released by electroplating, the petrochemical and fertilizer industries and mining processes [2]. Mining is a main source of Mn contamination in soils [3]. Worldwide manganese ore production resulted in 17.7 million tonnes (mt) of contaminated metals in 2014, representing a production increase of 5.1% over the previous year. The main producers are South Africa with over 26.4% of the world production, China with 18.0%, Australia with 17.5% and Gabon with 11.3% of the total world production.

Manganese is an important micronutrient for plant growth and plays multiple roles in photosynthesis, nitrogen metabolism, hormone regulation, and the synthesis and breakdown of certain substances [4]. However, Mn accumulation in excess of the normal range of 20-500 mg/kg of the plant dry weight [5] can have negative effects on plants. Excess Mn inhibits seed germination [6] and plant growth [7], causes biomass loss [8], decreases the photosynthetic rate [9], inhibits root development [10] and hinders the adsorption of other nutrients [11]. Furthermore, Mn toxicity affect the human nervous system and cause parkinsonian syndrome [12].

The removal of excess Mn from contaminated sites by physical and chemical methods has been unsuccessful in restoring large areas of land due to the high cost, deterioration of soil quality and

other problems associated with these methods [13,14]. In contrast, phytoremediation is a promising technology for remediating heavy metal-contaminated sites by using hyperaccumulator plants [15,16]. The quality of hyperaccumulator species is judged by the following four characteristics: (1) Hyperaccumulation ability (100 times greater metals accumulation than normal plants); (2) Hypertolerance; (3) Bioaccumulation factor (BF)>1 and (4) Translocation factor (TF)>1 [17]. The first study of a hyperaccumulator plant described the extreme accumulation of nickel by shoots of *Sebertia acuminata* (new name is *Pycnanthus acuminata*) in New Caledonia [18]. Approximately 500 plant taxa capable of heavy metal accumulation have been identified worldwide [19], among which approximately 330 plant species are Ni hyperaccumulators, and 20 species are Mn hyperaccumulators [20,21]. In this review examines the current knowledge of Mn hyperaccumulators and their accumulation and tolerance mechanisms, which include root uptake, xylem loading, transport, distribution and detoxification mechanisms. In addition, this review provides information about Mn hyperaccumulator biomass disposal and applications.

Discover of Manganese Hyperaccumulators

Numerous studies have aimed on the identification of Mn hyperaccumulator plants based on tolerance, hyperaccumulation in highly Mn contaminated sites [22-25], hydroponics and pot experiments [22,24,26,27], and microprobe analysis [26,28,29]. A

Mn hyperaccumulator plant is defined as one that accumulates over 10000µg/g of Mn in its shoot tissues [30]. Approximately 24 Mn hyperaccumulator plants are currently known worldwide, including 8 species from Cleastaceae, 4 species from Polygonaceae, 3 species each from Myrtaceae and Proteaceae, and 1 species each from *Amaranthaceae*, *Araliaceae*, *Apocynaceae*, *Clusiaceae*, *Phytolacaceae* and *Theaceae* (Table 1). Jaffre [31-33] discovered the first Mn hyperaccumulator plants with Mn concentrations above 1.0% in the leaves of *Beaupreopsis paniculata*, *Macadamia neurophylla*, *Macadamia angustifolia* (*M. neurophylla* and *M. angustifolia* are now classified as *Viotia* species), *Maytenus fournieri*, *Macadamia sebertiana*, *Garcinia amplexicaulis* and *Eugenia clusioides*, all in New Caledonia. In addition, Brooks et al. [34] analysed 31 species in *Alyxia* from New Caledonia for their Mn content and found that *Alyxia rubricalis* showed the highest Mn uptake (1.15%) in dried leaves. Twenty years later, Bidwell et al. [28] identified the first native Australian Mn hyperaccumulator, *Gossia bidwillii* (formerly *Austromyrtus bidwillii*); Mn concentrations upto 19200µg/g and 26509µg/g were measured in the dried leaves of this rain forest tree. Similarly, Xue et al. [22] identified the first native Chinese Mn hyperaccumulator species (*Phytolacca acinosa*), which accumulate 19300µg/g Mn in shoot tissue. Despite, now *P. acinosa* is identified as *Phytolacca americana* L., *P. acinosa* Roxb based on the morphological characteristics [35]. Yang et al. [26] conducted a potted plant experiment to illustrate the Mn accumulation and tolerance ability of *Schima superba*. The results showed that *S. superba* accumulated 62412.3mg/kg Mn in the stem under 150mmol/l treatment, and when the high Mn supply 40mmol/L. The Mn concentration in shoot exceeded 10000mg/kg, which is suggested concentration for Mn hyperaccumulation. Fernando et al. [29] identified five new Mn hyperaccumulators, namely, *Maytenus cunninghamii*, *Gossia bamagensis*, *Gossia fragrantissima*, *Gossia sankowsiorum*, and *Gossia gonoclada*, in Australia. Between 2010 and 2016, additional new Mn hyperaccumulators, *P. pubescens* [36], *P. perfoliatum* [37], *Celosia argentea* [27], *Eucalyptus grandis* × *Eucalyptus urophylla* [38] and *Polygonum lapathifolium* [39], were discovered in China.

Manganese Phytoavailable and Hyperaccumulation

Rhizosphere mobilization of Manganese

Manganese occurs in the soil in oxidation forms including Mn¹⁺, Mn²⁺, Mn³⁺, Mn⁴⁺, Mn⁶⁺ and Mn⁷⁺. Among them, the most phytoavailable form is Mn²⁺, and the most easily reducible forms are Mn³⁺ and Mn⁴⁺ [40]. Mn phytoavailability is influenced by pH, microorganisms, organic matter content, cation-exchange capacity and interactions with other metals (physiological and metabolic characteristics of the plant) [41]. A low soil pH (acid soil) can increase the redox potential [42] and phytoavailability of Mn [43-45]. At higher soil pH (>8.0), Mn²⁺ can auto-oxidize and will form MnO₂, Mn₂O₃ and Mn₃O₄, which are not available for plants [46,47]. For example, Xu et al. [48] reported that *P. americana* root achieved its maximum Mn adsorption at pH 5-6, while at pH values above 6, Mn adsorption decreased, probably because of the formation of hydroxide complexes. Most of Mn hyperaccumulator plants have been identified in areas with metal-rich and low-pH areas soils. For instance, the *Phytolacca* species were discovered in metal-rich and low-pH (4.8) soil in Xianglan, China, in manganese tailings [22,35]

and in the Roberts Mn mine in Cherokee County, South Carolina, USA [49]. Because, the hyperaccumulator plants rhizospheres have some influence on phytoavailability of heavy metals by release of exudates (carboxylates) and acidification of rhizosphere. For example, Godo and Reisenauer [50] reported that plant roots were released exudates (hydroxycarboxylase), which increased the phytoavailability of Mn in rhizosphere soil. Lambers et al. [45] showed that *P. americana* released protons into the rhizosphere in order to enhance the phosphate solubility, resulting in coincidental mobilization of Mn. Similarly, a comparative study showed that *P. americana* acidifies the rhizosphere soil and enhance Mn solubility more than *Acalypha rhomboidea* (non-accumulating) plant [51].

Moreover, rhizosphere microorganisms also have some great potential to reduce MnO₂ into phytoavailable Mn²⁺ by producing certain chelators and siderophores [52] that alter the soil pH and enhance chelation efficacy. For example, bacteria (*Bacillus manganica*) and fungi (*Mycogone*, *Tricholadium*) can reduce Mn⁴⁺ to Mn²⁺, which is the form required by plants. Meanwhile, some rhizosphere bacteria, namely, *Pseudomonas* and *Geobacter*, reduce oxidized Mn⁴⁺ to Mn²⁺, affecting its availability to the plants [41,53,54].

Manganese Uptake and Translocation from Roots-To-Shoots

Uptake and translocation of Mn from roots to shoots are dependent on several steps: (i) Root uptake, (ii) Xylem loading and (iii) Transport and distribution [46,55].

Root uptake

Bioavailable Mn²⁺ uptake by roots is generally exhibited as two phase processes, apoplastic binding phase and symplastic uptake [56]. Apoplastic binding is an initial, non-metabolic and rapid uptake phase [47,57]. In this phase, Mn²⁺ absorbed by root cells, which have a negatively charged cell wall that is strongly attractive to divalent cations such as Ca²⁺, Fe, Zn and K [58,59]. Interactions between Mn²⁺ and other divalent cations have been noted during the root uptake processes. For example, Peng et al. [60] studied the uptake of Mn and its interaction with Cd in *P. americana* and showed that the Cd addition can decreased the Mn uptake in the root and increased it in the stem. The Mn and Cd interaction in plant uptake indicated that *P. americana* might be use the same transport proteins for the uptake of these metal ions. Dou et al. [61] mentioned that the uptake and transport of Mn are closely related to those of Ca in *P. americana* because the ionic radius, charge and coordination chemistry properties of Ca and Mn are similar. Bidwell et al. [28] reported that the Mn hyperaccumulators *Alyxia* sp. and *Austromyrtus bidwillii* accumulate Mn at the expense of Ca and Mg. All of the above studies indicate that Mn transport pathway might be involved in the uptake of Ca, Cd and Mg. In addition, the low Fe/Mn ratio in *P. americana* noticed a high concentration of Mn uptake from soil [60]. Pedas et al. [62] reported that phosphate directly interferes with Mn uptake by causing alterations in membrane transporter. Xu et al. [58] also suggested that P and S probably played important roles in Mn absorption by *P. acinosa* a hyperaccumulator plant. A similar Mn uptake pattern seems to exist in Ni hyperaccumulators. For example, the Ni hyperaccumulators *Alyssum murale* and *Alyssum corsicum* accumulate the greatest Mn concentration upon receiving Ni/Mn treatment, but Ni uptake is inhibited by the addition of Mn. This

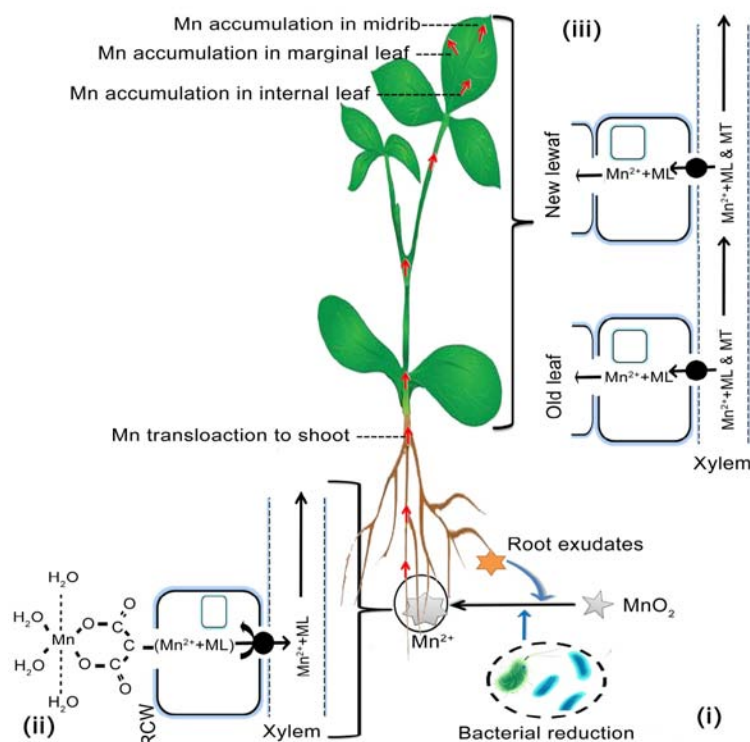


Figure 1: General mechanism of Mn hyperaccumulation in a hyperaccumulator, which includes (i) Mn solubilization in the rhizosphere; (ii) Mn root uptake and xylem loading; and (iii) Mn translocation from root to shoot. ML: Metal Ligands; MT: Metal Transporters; RCW: Root Cell Wall.

result suggests that Ni does not interfere with Mn uptake. Ghaderian et al. [63] showed that the Ni uptake of *Alyssum bracteatum* (a Ni hyperaccumulator) was inversely correlated with Mn, and likewise, Mn uptake was inversely correlated with Ni in the growth medium, suggesting competition between Ni and Mn for uptake at the roots.

Far less is known regarding the molecular mechanism by which Mn binds to root cell wall during the process of uptake. For example, Kopittke et al. [64] showed that Mn binds to a carboxylic group of cowpea root cell wall, which was confirmed by using Mn K-edge XANES spectroscopy. This carboxylic group exists in multiple components of the root cell wall, including pectin, cellulose and lignin [65]. Similarly, Xu et al. [48] used FTIR spectral analysis to show that C-O, =C-O and -OH functional groups are the main binding sites for Mn on the root cell wall of *P. americana*. These study results indicate that Mn^{2+} can bind carboxylic and hydroxylic groups of the root cell wall in both hyperaccumulators and non-hyperaccumulator plants during the uptake process (Figure 1). Further detailed experiments are needed to reveal the specific compounds in the root cell walls that are responsible for Mn binding in hyperaccumulators.

Mn uptake also appears to involve metal transporters. For example, a group of Natural Resistance-Associated Macrophage Protein (NRAMP) transporters including OsNramp5 are involved in Mn uptake on the root surface of rice plants [66,67]; likewise, Cailliatte et al. [68] reported that the AtNramp1 (high-affinity) transporter is involved in Mn uptake by the roots of *Arabidopsis thaliana*. Takahashi and Sugiura [69] showed that transgenic tobacco transformed with tomato root protein (LeGlp1) with a metal binding site at its N-terminus specifically binds Mn with the recombinant

LeGlp1 protein. This binding of Mn to LeGlp1 strongly suggests the involvement of LeGlp1 in Mn uptake from the soil at the root plasma membranes, suggesting in turn that Mn uptake in hyperaccumulators might be associated with Mn-specific transporters such as the NRAMP and ZIP proteins. Liu et al. [27] reported that hyperaccumulative Mn uptake in *Celosia argentea* might be mediated by NRAMP or ZIP transporters.

Xylem loading transport and distribution

Manganese can reach the xylem through symplast pathway and occurs there both as free ions (Mn^{2+}) and in complex with organic acids. For example, Williams and Pittman [70] mentioned that in soybean and tomato, approximately 60% of the xylem Mn was in the form of Mn^{2+} and is complexed with citrate or malate. Duo et al. [71] showed that Mn is complexed with oxalic acid in *P. americana* root. Similarly, Mn is associated with citrate in the root of the non-hyperaccumulator cowpea [64]. These results indicate that Mn is present as Mn^{2+} and complexed with organic acids in the root xylem of both hyperaccumulators and non-hyperaccumulators (Figure 2). Far less information is available on membrane transporters in Mn loading in the xylem. For instance, it has been suggested that plasma membrane-localized NRAMP 1&5 [68] and IRT1 [72] transporters are involved in Mn transport in *Arabidopsis*. Likewise, ZmYS1 and HvYSL2 can transport Mn in maize and barley [73,74].

Manganese influx into the leaf symplast occurs across the plasma membrane of the leaf cell [75]. Studies on non-hyperaccumulators have reported that Mn is absorbed by leaf cell membrane transporters such as the IRT family and thereby transported to the cytosol [72]. The higher expression levels of NRAMP proteins and ZIP protein in

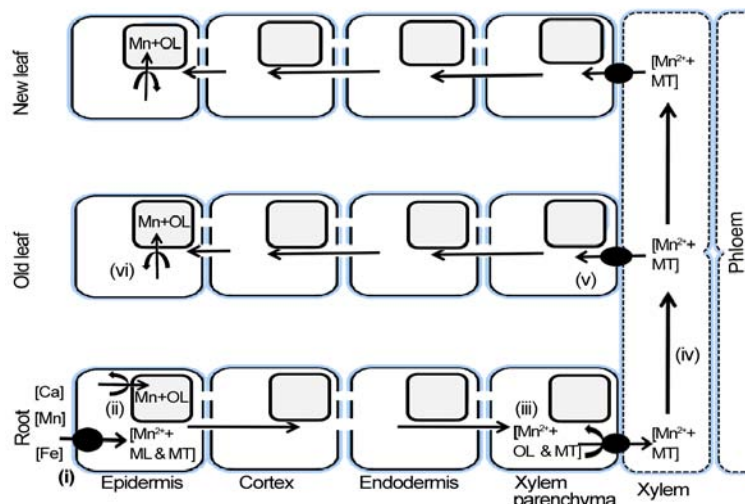


Figure 2: Hypothetical model for Mn tolerance processes in hyperaccumulator plants. (i) Root uptake, (ii) root sequestration, (iii) xylem loading and translocation to shoot, (iv) xylem transport, (v) transport and distribution in leaf, and (vi) foliar sequestration. ML: Metal Ligands; MT: Metal Transporters; OL: Organic Ligands; Mn influx and Mn efflux.

the cambium could transfer higher levels of Mn from the roots to the shoots [76]. In addition, Gustin et al. [77] reported that cation diffusion facilitator/metal tolerance protein (CDF/MTP) could transport Mn^{2+} into subcellular compartments or out of the cytoplasm. The Mn-CDF transporter was first identified in the hyperaccumulator *Stylosanthes hamata* [78]. Xie et al. [38] reported that high concentration of Mn accumulated in stem xylem vessel of *E. grandis* × *E. urophylla*. This result indicated that in Mn hyperaccumulator plants Mn mostly transported by xylem vessel and distributed to stem other parts. These studies suggested that IRT, NRAMP, ZIP and CDF/MTP transporters might be involved in Mn transport from root to the shoot in both hyperaccumulators and non-hyperaccumulators.

After the influx of Mn in leaf symplast, then distributed into various parts of the cells. Xu et al. [58] showed Mn uptake and distribution in the epidermis, cortex, endodermis and vascular bundles of the root cross section of *P. acinosa*. These results showed that Mn content decreased from the epidermis to the cortex and increased from the cortex to the vascular bundles via the endodermis, indicating the translocation of Mn from the cortex to the vascular bundles by an osmotic process. It is concluded that Mn transport exhibits some similarities to active transportation. Furthermore, Mn is transported to photosynthetically active leaves [79], showing that Mn is a most important plant nutrient for photosynthesis. Xie et al. [38] showed that high concentration of Mn distribution in leaf cross section, photosynthetic palisade and spongy tissue of *Eucalyptus grandis* × *E. urophylla*. Similarly, Fernando et al. [80] reported that Mn was evenly distributed in entire leaf cross section and photosynthetic tissues as well as in the palisade and spongy mesophyll of *Gossia*. Menon emphasized mesophyll cells is the major storage site of Mn in hyperaccumulator plants namely *P. americana* and *A. sciadophylloides*. In contrast, high Mn distribution has been observed in non-photosynthetic cells of *M. fournieri* [81,82]. Xu et al. [58] showed that a higher Mn concentration was found in the leaf margin than near the midrib regardless of the total Mn content of individual leaves of *P. acinosa*; consequently, Mn concentrations reduced from the palisade tissue to the spongy tissue in the mesophyll. To date,

no data are available to explain the why hyperaccumulators have different Mn transporters and Mn distribution patterns. Therefore, further detailed experiments are needed for a clear understanding of these processes in Mn hyperaccumulators.

Manganese Tolerance Mechanisms

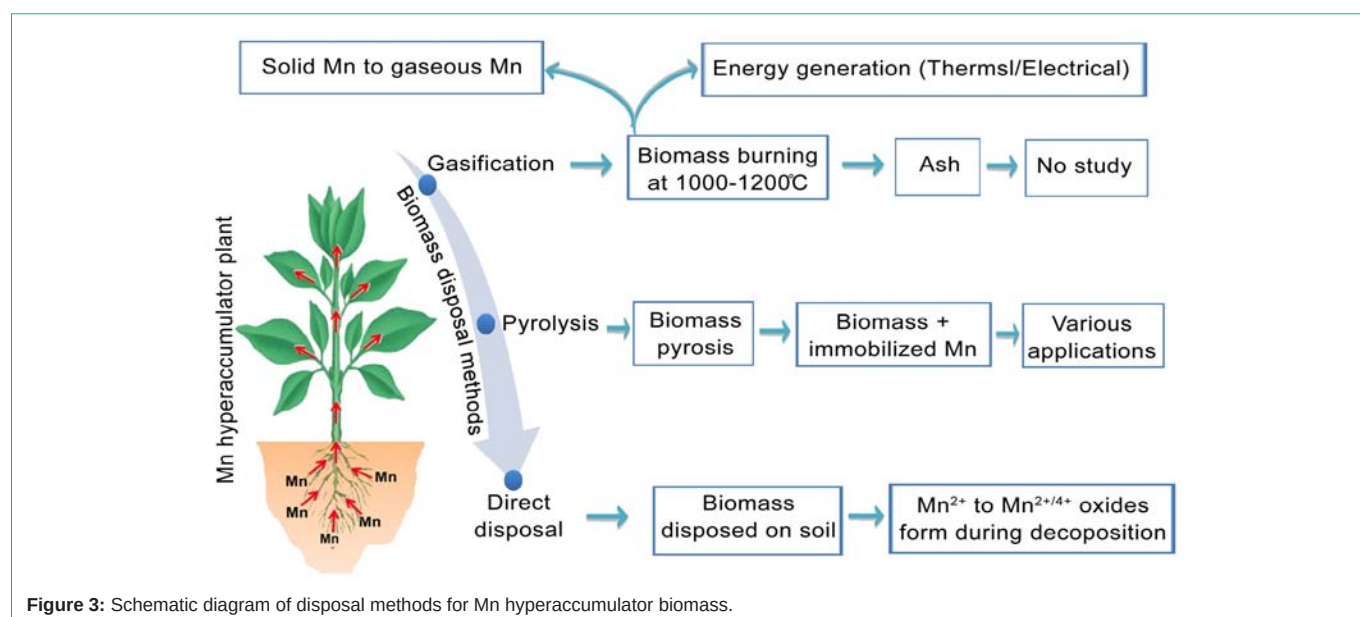
Hyperaccumulator plants have evolved several tolerance mechanisms to protect their normal growth and developments while under heavy metal ions stress [17]. Generally, Mn hyperaccumulators employ various tolerance mechanisms to detoxify Mn toxicity, such as translocation, sequestration and enhanced the production of antioxidants [44,55].

Root Sequestration Processes

Upon reaching the root apoplast, manganese is complexed by organic acids to alleviate its toxicity [56]. For example, oxalate and malate are favourable for Mn complexation in plant tissues and play a crucial role in Mn hyperaccumulation and detoxification in hyperaccumulator plants [71,83]. The total oxalic acid concentration was found to be higher than the Mn content in the root of *P. americana* (a Mn hyperaccumulator) [71]. This study indicated that oxalic acid could increase Mn tolerance and Mn hyperaccumulation in hyperaccumulators. In contrast, Kopittke et al. [64] estimated that 80% of the Mn^{2+} in roots could bind with citrate in cowpea (a non-hyperaccumulator). Similarly, Fernando et al. [83] showed that Mn is related with citrate rather than oxalate in Mn hyperaccumulators. Therefore, it may be concluded that both oxalate and citrate are involved in Mn sequestration in the root and can enhance Mn tolerance in both hyperaccumulators and non-hyperaccumulator plants (Figure 2).

Foliar Mn Sequestration

Foliar Mn in hyperaccumulators occurs mostly in soluble form (Mn^{2+}), complexed with organic ligands, and sequestered in vacuoles [28,83]. The sequestration of Mn in vacuoles is one of the important detoxification mechanisms in Mn hyperaccumulators



[71,84]. For example, Fernando et al. [82] reported that major Mn sequestration processes occurred primarily in the vacuoles of three Mn hyperaccumulator plants, namely, *Vriothia neurophylla*, *Maytenus founieri* and *Garcinia amplexicaulis*. Similarly, it has been reported that excess Mn accumulates in the leaves of *Gossia bidwillii* (a Mn hyperaccumulator) and is primarily sequestered in the vacuoles of palisade cells and photosynthetic tissues [85]. In contrast, Fernando et al. [86] found high Mn accumulation in non-photosynthetic cells of leaf epidermal tissues of *M. founieri*. Therefore, Mn hyperaccumulators can sequester excess of Mn in both non-photosynthetic tissues and photosynthetic tissues [41]. Further, the sequestration of foliar Mn by organic ligands at the subcellular level confers great tolerance on Mn hyperaccumulators [83,87]. Mn has been reported to be associated with oxalate in the freeze-dried leaves of *P. acinosa* [88]. Similarly, Mn-oxalate complexes were showed in the leaves of *P. americana* [89].

Antioxidative Defense System

Generally, the high concentration of Mn causes Reactive Oxygen Species (ROS) in plants [90] and affects the plant metabolism and growth [9]. Although, hyperaccumulators have effective antioxidative defense system to scavenging the heavy metals induced ROS. The antioxidative defense system comprising antioxidative enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POX), Ascorbate Peroxidase (APX) and nonenzymatic low molecular mass antioxidants. For example, a Mn hyperaccumulator *P. lapathifolium* can maintain high SOD, CAT and APX activities in both root and shoot tissues under the Mn toxicity condition, which implied that *P. lapathifolium* might have highly effective ROS scavenging mechanisms. Gao et al. [91] reported that Mn treatment increase the laccases activity and lignin content, but reduces SPOD activity and phenolics concentrations in apoplastic washing fluid of *P. americana* leaves. These results suggested that lignin formation by laccases and decreased POD activity are involved in the Mn tolerance mechanisms of *P. americana*. Zhao et al. [92] showed that *P. americana* have great ability to protect redox homeostatic disturbance and cellular

damages by enhancing their SOD and CAT activities under the Mn toxicity condition. Furthermore, the excess Mn decreased POX and APX activities these may reduce the ROS generation in *P. americana*. Weng et al. [93] observed that excess Mn increased the SOD, CAT and APX activities in *P. acinosa*. The increased SOD activity could be scavenged O₂⁻ and produced H₂O₂. This H₂O₂ can be scavenged by increased CAT and APX activities. These results suggested that increased SOD, CAT and APX activities could be reduced oxidative stress in *P. acinosa* under the excessive Mn concentration. Previous studies have been reported that a modulation of antioxidative enzymes (SOD, CAT, POX and APX) activities occurs in response to Mn induced oxidative stress. But no information is available on gene expression of these antioxidative enzymes isoforms. Indeed all the antioxidative enzymes have isoenzymes, which may be involved in regulatory mechanisms to heavy metals induced oxidative stress and play role in protecting all the organelles [94]. Therefore, it is most important to understand the role of isoenzymes in regulatory mechanism to excessive Mn toxicity in plants.

Role of Rhizosphere Microorganisms

The hyperaccumulators rhizosphere microorganisms could modify the heavy metal phytoavailability, accumulation and protect the plants from metal toxicity [95]. The rhizosphere of hyperaccumulator plant is consist of many heavy metal resistance endophytic bacteria [59], which significantly increases the uptake of heavy metals and root elongation. Endophytic bacteria producing phytohormones, siderophore, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase under the metal contaminated environment and these are promote the plant growth and increase heavy metal induced toxicity tolerance. A study isolated IAA and siderophore producing bacteria in leaves and ACC producing bacteria in root tissues of Mn hyperaccumulator (*P. americana*) under the Mn contaminated soils. These endophytic bacteria enhance the root growth (ranging from 6.4% to 18.3%) and above ground tissues (ranging from 19.3% to 70.2%) and Mn uptake (64%) in penisetum grown under Mn added soil [96]. This study concluded that endophytic bacteria

Table 1: List of manganese hyperaccumulator plants discovered worldwide.

Plant species	Plant family	Location	References
<i>Beaupreopsis paniculata</i>	Proteaceae	New Caledonia	Jaffre [33]
<i>Denhamia fournieri</i>	Celastraceae	New Caledonia	Jaffre [33]
<i>Macadamia neurophylla</i>	Proteaceae	New Caledonia	Jaffre [31]
<i>Eugenia clusioides</i>	Myrtaceae	New Caledonia	Jaffre [32]
<i>Garcinia amplexicaulis</i>	Clusiaceae	New Caledonia	Jaffre [32]
<i>Macadamia angustifolia</i>	Proteaceae	New Caledonia	Jaffre [32]
<i>Maytenus fournieri</i>	Celastraceae	New Caledonia	Jaffre [32,33]
<i>Macadamia sebertiana</i>	Celastraceae	New Caledonia	Jaffre [32]
<i>Alyxia rubricalis</i>	Apocynaceae	New Caledonia	Jaffre [33] & Brooks et al. [34]
<i>Gossia bidwillii</i>	Myrtaceae	Australia	Bidwell et al. [28]
<i>Phytolacca americana</i>	Phytolaccaceae	China	Xue et al. [22,35]
<i>Polygonum hydropiper</i>	Polygonaceae	China	Wang et al. [23]
<i>Schima superba</i>	Theaceae	China	Yang et al. [26]
<i>Chengiopanax sciadopylloide</i>	Araliaceae	Japan	Mizuno et al. 2008
<i>Maytenus cunninghamii</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Gossia bamagensis</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Gossia fragrantissima</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Gossia sankowsiorum</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Gossia gonoclada</i>	Celastraceae	Australia	Fernando et al. [29]
<i>Polygonum pubescens</i>	Polygonaceae	China	Hua et al. [36]
<i>Polygonum perfoliatum</i>	Polygonaceae	China	Liu et al. [37]
<i>Celosia argentea</i>	Amaranthaceae	China	Liu et al. [27]
<i>Eucalyptus grandis</i> x <i>Eucalyptus urophylla</i>	Myrtaceae	China	Xie et al. [38]
	Myrtaceae	China	Xie et al. [38]
<i>Polygonum lapathifolium</i>	Polygonaceae	China	Kehui et al. [39]

enhanced metal toxicity tolerance ability in plants by producing of ACC deaminase under the toxic metal stressed condition. Glick and Penrose and Glick et al. claimed that endophytic bacteria produced ACC deaminase can metabolize into-ketobutyric acid and ammonia then lowering the ethylene level in plants and promote plant growth under the heavy metal stress. Yuan et al. showed that number of endophytic bacterial community associated with *P. americana* in Mn polluted mining sites [97]. This is indicated that endophytic bacteria have Mn tolerance and play an important role in plant growth. This study has shown that endophytic bacteria are able to improve the Mn stress tolerance mechanisms in *P. americana*. However, less attention has been paid to the root environment of hyperaccumulator and endophytic bacteria, but more studies are needed to ascertain this. On the other hand numerous researchers have highlighted that, Arbuscular Mycorrhizal Fungi (AMF) can enhance the heavy metal tolerance ability in host plants [98]. In addition, AMF can enhance the heavy metal accumulation and biomass levels in hyperaccumulators. Miransari mentioned that, AMF could increase the growth of their host plants by enhance the water and nutrients uptake. Yuan et al. investigated the diversity of AMF species in the rhizosphere and root of *P. americana* grown under the high Mn contaminated mining area.

Conclusions and Scope of Future Research

Manganese hyperaccumulators have evolved highly efficient

physiological mechanisms for the uptake of Mn by their roots, followed by xylem loading, translocation to the shoots and detoxification. The Mn is mainly accumulated as Mn²⁺ and then loaded into the xylem, transferred to the shoot. During the process of Mn²⁺ uptake, Mn²⁺ can bind carboxylic and hydroxylic groups in the root cell wall of hyperaccumulators. Manganese uptake and transport are closely related to the uptake and transport of Ca and Fe. The presence of high levels of oxalate and citrate are involved in Mn sequestration in the roots. Foliar Mn²⁺ is mainly stored in vacuoles, the endoplasmic reticulum and the Golgi. It is sequestered by organic ligands and some transporter proteins at a subcellular level in shoots, which confers great tolerance on the plants. Pyrolysis is a suitable method for the disposal of Mn hyperaccumulator biomass. Phytomining is a novel technology for the recovery of Mn from plant biomass to be used for industrial applications.

This review has focused on the recent progress in understanding the mechanisms of hyperaccumulation and tolerance in hyperaccumulators. On the basis of the recent literature, a picture emerges of Mn uptake, transport and detoxification in hyperaccumulator plants. However, some aspects have not yet been fully understood, particularly at the molecular and genetic level. Moreover, the following research gap needs to be further explored.

- There is very less number of Mn hyperaccumulator plant

species have been identified. Therefore, more plant species need to be explored for Mn hyperaccumulation.

- Detailed research is required for understanding the underlying mechanism of Mn hyperaccumulation and their response to changing Mn levels in the environmental.
- There are no report is available on transporters role in uptake, translocation, distribution and detoxification in Mn hyperaccumulators. Hence, it is necessary for Mn hyperaccumulation.
- In some Mn hyperaccumulators, Mn deposited in non-photosynthetic tissues for the detoxification process, but no data are available to explain why different hyperaccumulators have different Mn distribution patterns. Therefore, further detailed experiments are needed for a clear understanding of these processes in Mn hyperaccumulators.
- More research is required to increase the understanding of the sequestration of Mn in root and leaf cells of Mn hyperaccumulators.
- The excessive Mn cause toxicity to plants by producing ROS, which affects plant growth and metabolism. However, the mechanism of antioxidative defines system in Mn hyperaccumulators are not well known. Some studies have been reported that modulation of antioxidative enzymes (SOD, CAT, POX and APX) activities occur in response to Mn-induced oxidative stress. But no information is available on gene expression of these antioxidative enzymes isoforms.

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References

1. Bjorklund G, Chartrand MS, Aaseth J. Manganese exposure and neurotoxic effects in children Environ Res. 2017; 155: 380-384.
2. Veliz GD, Mora S, Go mez P, Dossi MT, Montie J, Arriagada C, et al. Behavioral effects of manganese injected in the rat sub stantianigra are potentiated by dicumarol, DT-diaphoras inhibitor. Pharmacol Biochem. 2004; 7: 245-251.
3. Singh A, Zeng DH, Chen FS. Heavy metal concentrations in redeveloping soil of mine spoil under plantations of certain native woody species in dry tropical environment, India. J Environ Sci. 2005; 17: 168-174.
4. Garcia CG, Puente LSDL. The absorption of manganese (III) in oat plants. Plant Soil. 1977; 47: 229-237.
5. Lei YB, Chen K, Tian XR, Korpelainen H, Li CY. Effect of Mn toxicity on morphological and physiological changes in two *Populus cathayana* populations originating from different habitats. Trees. 2007; 21: 569-580.
6. Leiva N, Calderon C, Castro S. Differential effect of manganese on the germination of *Triglochin striata* (Juncaginaceae) and *Cotula coronopifolia* (Asteraceae) in Laguna de Carrizal Bajo wetland, Atacama Region, Chile. Gayana Bot. 2016; 73: 161-165.
7. Lee TJ, Luitel BP, Kang WH. Growth and physiological response to manganese toxicity in Chinese cabbage (*Brassica rapa* L. ssp. *campestris*). Horticult Environ Biotech. 2011; 52: 252-258.
8. Kosiada T. The effects of manganese and other factors on the formation of spots on leaves of barley (*Hordeum vulgare* L.). Fresenius Environ Bul. 2013; 22: 1132-1138.
9. Nable RO, Houtz RL, Cheniae GM. Early inhibition of photosynthesis during development of Mn toxicity in tobacco. Plant Physiol. 1988; 86: 1136-1142.
10. Lidon F. Rice plant structural changes by addition of excess manganese. J Plant Nutri. 2002; 25: 287-296.
11. Paschke MW, Valdecantos A, Redente EF. Manganese toxicity thresholds for restoration grass species. Environ Pollut. 2005; 135: 313-322.
12. Kwakye GF, Paoliello MMB, Mukhopadhyay S, Bowman AB, Aschner M. Manganese-induced parkinsonism and Parkinson's disease: shared and distinguishable features. Inter J Env Res Pub Heal. 2015; 12: 7519-7540.
13. Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I, et al. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnol. 1995; 13: 468-474.
14. Memon AR, Aktoprakligil D, Özdemir A, Vertii AM. Heavy metal accumulation and detoxification mechanisms in plants. Turk J Bot. 2001; 25: 111-121.
15. Fellet G, Marchiol L, Perosa D, Zerbi G. The application of phytoremediation technology in a soil contaminated by pyrite cinders. Ecol Eng. 2007; 31: 207-214.
16. Bouwman LA, Bloem J, Romkens PFAM, Japenga J. EDGA amendment of slightly heavy metal loaded soil affects heavy metal solubility, crop growth and microbivorous nematodes but not bacteria and herbivorous nematodes. Soil Biol Bioche. 2005; 37: 271-278.
17. Sun YB, Zhou QX, Wang L, Liu WT. Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator. J Hazard Mater. 2009; 161: 808-814.
18. Jaffre T, Brooks RR, Lee J, Reeves RD. *Sebertia acuminata*: a hyperaccumulator of nickel from New Caledonia. Sci. 1976; 193: 579-580.
19. Kramer U. Metal hyperaccumulation in plants. Annu Rev Plant Biol. 2010; 61: 517-534.
20. Reeves RD. Tropical hyperaccumulators of metals and their potential for phytoextraction. Plant Soil. 2003; 249: 57-65.
21. Moosavi AA, Ronaghi A. Influence of foliar and soil applications of iron and manganese on soybean dry matter yield and iron-manganese relationship in a Calcareous soil. Aust J Crop Sci. 2011; 5: 1550-1556.
22. Xue SG, Chen YX, Reeves RD, Baker AJM, Lin Q, Fernando DR. Manganese uptake and accumulation by the hyperaccumulator plant *Phytolacca acinosa* Roxb. (*Phytolaccaceae*). Environ Pollut. 2004; 131: 393-399.
23. Wang H, Tang SM, Liao XJ, Cao Q, Yang A, Wang TZ. A new manganese-hyperaccumulator: *Polygonum hydropiper* L. Ecol Environ. 2007; 16: 830-834.
24. Min Y, Boqing T, Meizhen T, Aoyama I. Accumulation and uptake of manganese in a hyperaccumulator *Phytolacca americana*. Miner Eng. 2007; 20: 188-190.
25. Deng H, Li MS, Chen YX. Accumulating characteristics of manganese by *Polygonum pubescens* Blume. J Guangxi Norm Univ Nat Sci Ed. 2010; 28: 58-62.
26. Yang SX, Deng H, Li MS. Manganese uptake and accumulation in a woody hyperaccumulator, *Schima Superba*. Plant Soil Environ. 2008; 54: 441-446.
27. Liu J, Shang L, Zhang X, Zhu Y, Yu K. Mn accumulation and tolerance in *Celosia argentea* Linn.: A new Mn-hyperaccumulating plant species. J Hazard Mater. 2014; 267: 136-141.
28. Bidwell SD, Woodrow IE, Batianoff GN, Sommer Knudsen J. Hyperaccumulation of manganese in the rainforest tree *Austromyrtus bidwillii* (*Myrtaceae*) from Queensland, Australia. Funct Plant Biol. 2002; 29: 899-905.
29. Fernando DR, Guymer G, Reeves RD, Woodrow IE, Baker AJ, Batianoff GN. Foliar Mn accumulation in eastern Australian herbarium specimens: prospecting for 'new' Mn hyperaccumulators and potential applications in taxonomy. Annals of Bot. 2009; 103: 931-939.
30. Baker AJM, Brooks RR. Terrestrial higher plants which hyperaccumulate metal elements: a review of their distribution, ecology, and phytochemistry. Biorecovery. 1989; 1: 81-126.

31. Jaffre T. Accumulation du manganese par les *Protocacccs* dc Nouvelle Caldonie. Complex Rendus de l' Academic des Sciences, Paris, Seriel. 1979; 289: 425-428.
32. Jaffre T. Etude Ecologique du Peuplement Vegetal des Sols Derives de Roches Ultrabasiqes en Nouvelle Caldonie. ORSTOM, Paris. 1980; 273.
33. Jaffre T. Accumulation du manganese par les cspcccs associees aux terrains ultrabasiqes de Nouvelle Caldonie. Complex Rendus de l' Academic des Sciences, Paris, Serie D. 1977; 284: 1573-1575.
34. Brooks RR, Trow JM, Vcillon JM, Jaffre T. Studies on manganese accumulating *Alyxia* from New Caledonia. Taxon. 1981; 30: 420-423.
35. Xue SG, Jun W, Zhou X, Liu H, Chen Y. A critical reappraisal of *Phytolacca acinosa* Roxb. (Phytolaccaceae) - A manganese-hyperaccumulating plant. Acta Ecologica. Sinica. 2010; 30: 335-338.
36. Hua D, Shun LM, Xu CY, Ping LY, Ming YF. A new discovered manganese hyperaccumulator - *Polygonum pubescens* Blume. Fresenius Environ Bull. 2010; 19: 94-99.
37. Liu P, Tang X, Gong C, Xu GD. Manganese tolerance and accumulation in six Mn hyperaccumulators or accumulators. Plant Soil. 2010; 335: 385-395.
38. Xie Q, Li Z, Yang L, Lv J, Jobe TO, Wang Q. A newly identified passive hyperaccumulator *Eucalyptus grandis* × *E. urophylla* under Manganese Stress. PLoS ONE. 2015; 10: e0136606.
39. Kehui L, Yu F, Chen M, Zhou Z, Chen C, Li MS, et al. A newly found manganese hyperaccumulator-*Polygonum lapathifolium* Linn. Inter J Phytoremedi. 2016; 18: 348-353.
40. Marschner P. Marschner's Mineral Nutrition of Higher Plants. Boston, MA: Academic Press 2012.
41. Socha AL, Gueriot ML. Mn-euvering manganese: the role of transporter gene family members in manganese uptake and mobilization in plants. Frontiers in Plant Sci, Plant Nutr. 2014; 5: 106.
42. Kogelmann W, Sharpe W. Soil acidity and manganese in declining and nondeclining sugar maple stands in Pennsylvania. J Environ Qual. 2006; 35: 433-441.
43. Marschner H. Mineral Nutrition of Higher Plants. Academic Press, San Diego. 1995; 889.
44. Boobar MMA, Goodarzi F. Comparative evaluation of oxidative stress status and manganese availability in plants growing on manganese mine. Ecotox Environ Safety. 2008; 71: 692-699.
45. Lambers H, Hayes PE, Laliberte E, Oliveira RS, Turner BL. Leaf manganese accumulation and phosphorus-acquisition efficiency. Trends Plant Sci. 2015; 20: 83-90.
46. Ducic T, Polle A. Transport and detoxification of manganese and copper in plants. Braz J Plant Physiol. 2005; 17: 103-112.
47. Humphries J, Stangoulis J, Graham R. Manganese. In: A. Barker, D. Pilbeam (eds). Handbook of Plant Nutrition, Taylor and Francis, USA. 2007; 351-366.
48. Xu X, Yang J, Zhao X, Zhang X, Li R. Molecular binding mechanisms of manganese to the root cell wall of *Phytolacca americana* L. using multiple spectroscopic techniques. J Hazard Mater. 2015; 296: 185-191.
49. Pollard AJ. Heavy metal tolerance and accumulation in plants of the southeastern United States. Castanea. 2016; 81: 257-269.
50. Godo GH, Reisenauer HM. Plant effects on soil manganese availability. Soil Sci Soc Amer J. 1980; 44: 993-995.
51. DeGroote KV, Grace L, McCartha A, Pollard J. Interactions of the manganese hyperaccumulator *Phytolacca americana* L. with soil pH and phosphate. Ecol Res. 2017.
52. Lovley DR, Ueki T, Zhang T, Malvankar NS, Shrestha PM, Flanagan KA. Geobacter: the microbe electric's physiology, ecology, and practical applications. Adv Microb Physiol. 2011; 59: 1-100.
53. Vega NWO. A review on beneficial effects of rhizosphere bacteria on soil nutrient availability and plant nutrient uptake. Rev Fac Nal Agr Medellin. 2007; 60: 3621-3643.
54. Carmichael SK, Brauer SL. Microbial diversity and manganese cycling: a review of manganese-oxidizing microbial cave communities. Life in extreme environment, De Gruyter, Boston, MA, PP. 2015; 137-160.
55. Mou D, Yao Y, Yang Y, Zhang Y, Tian C, Achal V. Plant high tolerance to excess manganese related with root growth, manganese distribution and antioxidative enzyme activity in three grape cultivars. Ecotoxi Environ Safety. 2011; 74: 776-786.
56. Millaleo R, Díaz MR, Ivanov AG, Mora ML, Alberdi M. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. J Soil Sci Plant Nutr. 2010; 10: 470-481.
57. Clarkson DT. The uptake and translocation of manganese by plant roots. In Graham RD, Hannam RJ, Uren NC, (eds.), Manganese in soils and plants. Kluwer Academic Publishers, the Netherlands. 1988; 101-111.
58. Xu XH, Yun S, Xu CY, Xue S, Wu B, Huang Y. An investigation of cellular distribution of manganese in hyperaccumulator plant *Phytolacca acinosa* Roxb: using SRXRF analysis. J Environ Sci. 2006; 18: 746-775.
59. White PJ, Bowen HC, Demidchik V, Nichols C, Davies JM. Genes for calcium-permeable channels in the plasma membrane of plant root cells. Biochem Biophys Acta. 2002; 1564: 299-309.
60. Peng K, Luo C, You W, Lian C, Li X, Shen Z. Manganese uptake and interactions with cadmium in the hyperaccumulator-*Phytolacca americana* L. J Hazard Mater. 2008; 154: 674-681.
61. Dou C, Fu X, Chen X, Shi J, Chen Y. Accumulation and interaction of calcium and manganese in *Phytolacca americana*. Plant Sci. 2009; 177: 601-606.
62. Pedas P, Husted S, Skytte K, Schjoerring JK. Elevated phosphorus impedes manganese acquisition by barley plants. Front Plant Sci. 2011; 2: 37.
63. Ghaderian SM, Ghasemi R, Hajhashemi F. Interaction of nickel and manganese in uptake, translocation and accumulation by the nickel-hyperaccumulator plant, *Alyssum bracteatum* (Brassicaceae). Austr J Bot. 2015; 63: 47-55.
64. Kopitke PM, Lombi E, McKenna BA, Wang P, Donner E, Webb RI, et al. Distribution and speciation of Mn in hydrated roots of cowpea at levels inhibiting root growth. Physiol Plant. 2013; 147: 453-464.
65. Kopitke PM, Menzies NW, Wang P, McKenna BA, Wehr JB, Lombi E, et al. The rhizotoxicity of metal cations is related to their strength of binding to hard ligands. Environ Toxicol Chem. 2014; 33: 268-277.
66. Sasaki A, Yamaji N, Yokosho K, Ma JF. Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell. 2012; 24: 2155-2167.
67. Ishimaru Y, Takahashi R, Bashir K, Shimo H, Senoura T, Sugimoto K. Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. Sci Rep. 2012; 2:286.
68. Cailliatte R, Schikora A, Briat JF, Mari S, Curie C. High-affinity manganese uptake by the metal transporter NRAMP1 is essential for *Arabidopsis* growth in low manganese conditions. Plant Cell. 2010; 22: 904-917.
69. Takahashi M, Sugiura M. Strategies for uptake of a soil micronutrient, manganese, by plant roots. RIKEN Rev. 2001; 35: 76-77.
70. Williams LE, Pittman JK. Dissecting pathways involved in manganese homeostasis and stress in higher plant cells. R. Hell and R.R. Mendel (eds.), Cell Biology of Metals and Nutrients, Plant Cell Monographs. 2010: 17.
71. Dou CM, Fu XP, Chen XC, Shi JY, Chen YX. Accumulation and detoxification of manganese in hyperaccumulator *Phytolacca Americana*. Plant Biol. 2009; 11: 664-670.
72. Pedas P, Ytting CK, Fuglsang AT, Jahn TP, Schjoerring JK, Husted S. Manganese efficiency in barley: identification and characterization of the metal ion transporter HvIRT1. Plant Physiol. 2008; 148: 455-466.
73. Murata Y, Harada E, Sugase K, Namba K, Horikawa M, Ma JF. Specific transporter for iron (III)-phytosiderophore complex involved in iron uptake by barley roots. Pure Appl Chem. 2008; 80: 2689-2697.

74. Araki R, Murata J, Murata Y. A novel barley yellow stripe 1-like transporter (HvYSL2) localized to the root endodermis transports metal-phytosiderophore complexes. *Plant Cell Physiol.* 2011; 52: 1931-1940.
75. Pinto E, Isabel MPL, Ferreira VO. Cation transporters/channels in plants: Tools for nutrient biofortification. *J Plant Physiol.* 2015; 179: 64-82.
76. Yao Y, Xiao X, Ou Y, Wu X, Xu G. Root transcriptome analysis on the grape genotypes with contrast translocation pattern of excess manganese from root to shoot. *Plant Soil.* 2015; 387: 49-67.
77. Gustin JL, Zanis MJ, Salt DE. Structure and evolution of the plant cation diffusion facilitator family of ion transporters. *BMC Evol Biol.* 2011; 11: 76.
78. Delhaize E, Gruber BD, Pittman JK, White RG, Leung H, Miao Y. A role for the AtMTP11 gene of *Arabidopsis* in manganese transport and tolerance. *Plant J.* 2007; 51: 198-210.
79. Page V, Weisskopf L, Feller U. Heavy metals in white lupin: uptake, root-to-shoot transfer and redistribution within the plant. *New Phytol.* 2006; 171: 329-341.
80. Fernando DR, Marshall AT, Forster PI, Hoebee SE, Siegle R. Multiple metal accumulation within a manganese-specific genus. *American J Bot.* 2013; 100: 690-700.
81. Donchevaa SPC, Stoyanovaa ZI, Georgievaa K, Velichkovac M, Barceló J. Silicon amelioration of manganese toxicity in Mn-sensitive and Mn-tolerant maize varieties. *Environ Exper Bot.* 2009; 65: 189-197.
82. Fernando DR, Woodrow IE, Baker AJ, Marshall AT. Plant homeostasis of foliar manganese sinks: specific variation in hyperaccumulators. *Planta.* 2012; 236: 1459-1470.
83. Fernando DR, Mizuno T, Woodrow IE, Baker AJM, Collins RN. Characterization of foliar manganese (Mn) in Mn (hyper) accumulators using X-ray absorption spectroscopy. *New Phytol.* 2010; 188: 1014-1027.
84. Fernando DR, Batianoff GN, Baker AJ, Woodrow IE. In vivo localization of manganese in the hyperaccumulator *Gossia bidwillii* (Benth.) N. Snow & Guymer (Myrtaceae) by cryo-SEM/EDAX. *Plant Cell Environ.* 2006; 29: 1012-1020.
85. Fernando DR, Bakkaus EJ, Perrier N, Baker AJM, Woodrow IE, Batianoff GN, et al. Manganese accumulation in the leaf mesophyll of four tree species: A PIXE/EDAX localization study. *New Phytol.* 2006; 171: 751-758.
86. Fernando DR, Woodrow IE, Jaffre T, Dumontet V, Marshall AT, Baker AJ. Foliar manganese accumulation by *Maytenus founieri* (Celastraceae) in its native New Caledonian habitats: Populational variation and localization by X-ray microanalysis. *New Phytol.* 2008; 177: 178-185.
87. Blamey F, Pax C, Maria C, Soriano M, Cheng M, Tang C, et al. Synchrotron-Based Techniques Shed Light on Mechanisms of Plant Sensitivity and Tolerance to High Manganese in the Root Environment. *Plant Physiol.* 2015; 169: 2006-2020.
88. Xu XH, Shi JY, Chen XC, Chen YX, Hu TD. Chemical forms of manganese in the leaves of manganese hyperaccumulator *Phytolacca acinosa* Roxb. (Phytolaccaceae). *Plant Soil.* 2009; 318: 197-204.
89. Dou C, Fu X, Chen X, Shi J, Chen Y. Accumulation and interaction of calcium and manganese in *Phytolacca americana*. *Plant Sci.* 2009; 177: 601-606.
90. Fecht-Christoffers MM, Maier P, Horst WJ. Apoplastic peroxidases and ascorbate are involved in manganese toxicity and tolerance of *Vigna unguiculata*. *Physiol. Plant.* 2003; 117: 237-244.
91. Gao L, Kejian Peng, Yahua C, Guiping Wang, Zhenguo Shen. Roles of apoplastic peroxidases, laccases, and lignifications in the manganese tolerance of hyperaccumulator *Phytolacca Americana*. *Acta Physiol Plant.* 2012; 34: 151-159.
92. Zhao H, Liangqi Wu, Tuanyao Chai, Yuxiu Zhang, Jinjuan Tan, Shengwen M. The effects of copper, manganese and zinc on plant growth and elemental accumulation in the manganese-hyperaccumulator *Phytolacca americana*. *Journal of Plant Physiology.* 2012; 169: 1243-1252.
93. Weng XY, Zhao LL, Zheng CJ, Zhu JW. Characteristics of the hyperaccumulator plant *Phytolacca acinosa* (Phytolaccaceae) in response to excess manganese. *J Plant Nutr.* 2013; 36: 1355-1365.
94. Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 2002; 7: 405-410.
95. Gill SS, Naser A Anjum, Iqbal Ahmad, P Thangavel, G Sridevi, M Pacheco, Armando C. et al. Metal Hyperaccumulation and Tolerance in *Alyssum*, *Arabidopsis* and *Thlaspi*: An Overview. N.A. Anjum et al. (eds.), the Plant Family *Brassicaceae*: Contribution towards Phytoremediation, Environmental Pollution 21, # Springer Science+Business Media B.V. 2012.
96. Brooks RR. Plants that hyperaccumulate heavy metals. CAB International, Wallingford. 1997; 88-105.
97. MCQuattie CJ, Schier GA. Response of sugar maple (*Acer saccharum*) seedlings to manganese. *Canad J Forest Res.* 2011; 30: 456-467.
98. National Department of Mineral Production (DNPM). Annual summary. 2015.