Role of magnesium in alleviation of aluminium toxicity in plants

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Abstract

Magnesium is pivotal for activating a large number of enzymes; hence, magnesium plays an important role in numerous physiological and biochemical processes affecting plant growth and development. Magnesium can also ameliorate aluminium phytotoxicity, but literature reports on the dynamics of magnesium homeostasis upon exposure to aluminium are rare. Herein existing knowledge on the magnesium transport mechanisms and homeostasis maintenance in plant cells is critically reviewed. Even though overexpression of magnesium transporters can alleviate aluminium toxicity in plants, the mechanisms governing such alleviation remain obscure. Possible magnesium-dependent mechanisms include (i) better carbon partitioning from shoots to roots; (ii) increased synthesis and exudation of organic acid anions; (iii) enhanced acid phosphatase activity; (iv) maintenance of proton-ATPase activity and cytoplasmic pH regulation; (v) protection against an aluminium-induced cytosolic calcium increase; and (vi) protection against reactive oxygen species. Future research should concentrate on assessing aluminium toxicity and tolerance in plants with overexpressed or antisense magnesium transporters to increase understanding of the aluminium–magnesium interaction.

Key words: Aluminium toxicity, cellular targets, long-distance transport, magnesium homeostasis, magnesium uptake.

Introduction

In plants, Mg2+ is an essential constituent of chlorophyll, an activator of >300 enzymes (e.g. RNA polymerases, ATPases, protein kinases, phosphatases, glutathione synthase, and carboxylases), and is involved in regulating ion transport and cation balance in plants. The physiological range of total Mg concentration in vegetative tissues is 1–4 g Mg per kg of dry weight, but variations among plants species and tissues are wide (Hailes et al., 1997; Reuter and Robinson, 1997). Mg2+ deficiency occurs particularly in plants growing in highly leached acid soils with low cation exchange capacity (Tan et al., 1991; vanPraag et al., 1997; Aitken et al., 1999). Mg2+ deficiency is induced in such soils because leaching removes Mg into deep layers, leaving the root zone poorly supplied with Mg.

The Mg2+ ion is unique among the major biological cations because it has the largest hydrated radius (0.428 nm), the smallest ionic radius (0.072 nm), and the highest charge density. Because it binds with water molecules 3–4 orders of magnitude more tightly than do other cations, Mg2+ often interacts with other ions and molecules while still maintaining its hydration sphere (Maguire and Cowan, 2002). As a result, Mg2+ binds relatively weakly to the negatively charged groups in the root cell wall, so the excess cations such as H+ and Al3+ present in acid soils can inhibit Mg2+ loading into the apoplasm and uptake across the plasma membrane (Marschner, 1991, 1995). The Al3+ and Mg2+ ions compete for membrane transporters (Rengel and Robinson, 1989; Rengel, 1990) and metal-binding sites on enzymes (e.g. Pécsváradi et al., 2009).
Both Al$^{3+}$ and Mg$^{2+}$ ions are hexahydrates, with the hydrated radius of Al$^{3+}$ (0.480 nm) and Mg$^{2+}$ (0.428 nm) being remarkably similar; hence, the Mg$^{2+}$ uptake system or the Mg$^{2+}$-binding sites on enzymes do not distinguish well between Al$^{3+}$ and Mg$^{2+}$ ions. Recent experimental evidence also suggests that plants offered a mix of Mg$^{2+}$ isotopes in nutrient solutions preferentially take up heavy isotope $^{26}$Mg (the daughter nuclei of $^{27}$Al) and store it in tissues (Black et al., 2008; Bolou-Bi et al., 2010); hence, pathways for both uptake and storage of $^{26}$Mg potentially provide molecular targets for Al$^{3+}$ toxicity in plants.

It has been well established that Al can enter the symplasm of root cells quite rapidly (Silva et al., 2000; Babourina and Rengel, 2009) and can be sequestered in the vacuole after 30 min (Taylor et al., 2000) (Fig. 1). Indeed, a putative plasma membrane-localized Al transporter, Nrat1 (Nramp aluminium transporter 1), has been identified recently in rice (Xia et al., 2010), but it remains unclear whether it is specific for Al$^{3+}$ or can transport other cations as well. The Al entry into the cytoplasm affects the homeostasis of various ions, such as H$^+$ (Babourina and Rengel, 2009; Bose et al., 2010b), Ca$^{2+}$ (Plieth et al., 1999; Rengel and Zhang, 2003), and K$^+$ (Bose et al., 2010a). However, there is no information on modulation of cytosolic free Mg$^{2+}$ activity upon Al$^{3+}$ exposure. To shed light on the Mg–Al interaction, available information on Mg$^{2+}$ uptake, cellular Mg$^{2+}$ distribution, and homeostasis regulation is reviewed first, followed by the critical appraisal of the potential mechanisms underpinning Mg alleviation of Al toxicity.

**Target sites for Al$^{3+}$-induced inhibition of Mg$^{2+}$ uptake and long-distance transport**

**Competition for apoplastic binding sites**

Isotopic tracer studies using $^{25}$Mg$^{2+}$ demonstrated the existence of the apoplastic pathway for Mg$^{2+}$ ions in the cortex of mycorrhizal roots of Norway spruce (Kuhn et al., 2000). Further, entry of Mg$^{2+}$ into the endodermis was faster through the apoplastic than the symplasmic pathway (Kuhn et al., 2000). Given this importance of the apoplastic pathway for Mg uptake and transport, it should be borne in mind that in Al$^{3+}$ toxicity large amounts of Al (85–99.9% of total cellular Al) accumulate in the cell walls and intercellular root spaces (Reid et al. 1996; Taylor et al. 2000; Ma, 2007). More specifically, binding of Al$^{3+}$ to the negative charges on the pectin substances in the cell wall was observed (Blamey, 2001). Such binding of Al$^{3+}$ on the cell wall and precipitation of Al in the apoplast may decrease loading of Mg$^{2+}$ ions into the apoplast (Fig. 1) and movement via the apoplastic pathway.

**Mg$^{2+}$ uptake inhibition at the plasma membrane**

Because the endodermis is the major barrier to the apoplastic movement of Mg$^{2+}$ ions, further radial transport requires entry through the plasma membrane. The Mg$^{2+}$ ions may enter the cytoplasm through hyperpolarization-activated, depolarization-activated, cyclic nucleotide-activated, and voltage-independent cation channels (Fig. 2) (Ve´ry and Sentenac, 2002; Demidchik and Maathuis, 2007; Guo et al., 2010). However, the hyperpolarization-activated cation channels (Ding et al., 1993; Kiegle et al., 2000; Very and Davies, 2000) and depolarization-activated cation channels (Rengel et al., 1995; Pineros and Tester, 1997) are sensitive to Al$^{3+}$, with the inhibition by Al$^{3+}$ being higher in the former (87±7%) (Kiegle et al., 2000) than in the latter (only 44%) (Rengel and Zhang, 2003).

In addition to channels, it is evident that carrier proteins are also engaged in Mg$^{2+}$ transport. A 10-member AtMGT (Arabidopsis thaliana) magnesium transporter) gene family is known to encode proteins for Mg$^{2+}$ uptake and translocation in a variety of plant tissues. Green protein analysis of MRS2-10 (mitochondrial RNA splicing 2-10) AtMGT1 suggested its localization in the plasma membrane of the root tip cells of Arabidopsis (Fig. 2). AtMGT1 and AtMGT10 are high-affinity Mg$^{2+}$ transporters, but are found to be highly sensitive to Al$^{3+}$ inhibition (Li et al., 2001) (Fig. 1). However, overexpression of the AtMGT1 gene in Nicotiana benthamiana conferred Al$^{3+}$ tolerance (Deng et al., 2006). Further studies on overexpression of low- and high-affinity Mg$^{2+}$ transporters in other plant

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**Fig. 1.** Target sites of Al$^{3+}$ toxicity on Mg$^{2+}$ uptake and homeostasis. (A) Competition for apoplastic binding. (B) Inhibition of Mg$^{2+}$-permeable cation channels. (C) Inhibition of Mg$^{2+}$ transporters (AtMGT1 and AtMGT10). (D) Entry of Al ions into the cytoplasm (Nrat1 or other cation channels). (E) Competition for ATP binding. (F) Competition for anion [An$^-$] binding.
species are eagerly awaited to assess the potential usefulness of this strategy in enhancing Al³⁺ tolerance in crops.

The two low-affinity Mg²⁺ transporters [AtMGT7a (Mao et al., 2008) and AtMGT9 (Chen et al., 2009)] may also be involved in Mg²⁺ uptake and distribution in plants, but their sensitivity to aluminium ions remains to be elucidated. Another transporter from the same gene family, MRS2-5/MGT3, was found in either the plasma membrane fraction (Alexandersson et al., 2004) or the tonoplast fraction of Arabidopsis (Whiteman et al., 2008); hence, its potential role in Mg²⁺ uptake and distribution in plants remains unclear.

**Inhibition of long-distance Mg²⁺ transport**

Apart from Mg²⁺ uptake from the external environment, the remobilization of Mg²⁺ within the plant may play a major role in mitigation of Al³⁺-induced Mg²⁺ transport inhibition. Like many nutrient ions, when Mg²⁺ is abundant, it is stored in the vacuoles and is released when needed (Stelzer et al., 1990). In addition, when Mg²⁺ is present in the environment in suboptimal amounts, plants have the capacity to mobilize Mg²⁺ from the old tissues (Laing et al., 2000). This process involves the release of Mg²⁺ from the bound and stored states in the vacuoles and its transport into the vascular tissue for distribution to the rest of the plant. In this regard, the amount of Mg²⁺ available in the cytosol for xylem loading was suggested to be regulated by AtMHX (Arabidopsis thaliana magnesium–proton exchanger) localized in the vacuoles of xylem parenchyma cells (Shaul, 2002). Expression analysis also confirmed that AtMHX is involved in the regulation of long-distance metal transport and metal homeostasis (David-Assael et al., 2006). Considering that overexpression of AtMHX resulted in reduction in plant size through inhibition of cell expansion (Berezn et al., 2008a, b), a negative regulator of AtMHX is essential to maintain normal cell growth and long-distance transport of Mg²⁺ ions. Interestingly, AtMHX expression is not regulated by Mg²⁺ concentration in the growth media, as shown for plants grown at a high Mg²⁺ concentration of 2.5 mM (David-Assael et al., 2006), and in Mg²⁺ depletion experiments (Hermans et al., 2010). However, AtMHX expression was induced by auxin and abscisic acid (ABA) accumulation in vascular tissues (David-Assael et al., 2006), a scenario that can also be induced by Al³⁺ toxicity via inhibition of the basipetal transport of auxin to the root meristematic zone (Kollmeier et al., 2000). Thus, Al³⁺ toxicity may affect the long-distance transport of Mg²⁺ ions.

Characterization of Arabidopsis AtCNGC10 (A. thaliana cyclic nucleotide gated channel) antisense plants demonstrated that (i) AtCNGC10 was localized to the plasma membrane (Borsics et al., 2007) and (ii) was involved in long-distance Mg²⁺ transport (Guo et al., 2010). Further, AtCNGC10 antisense plants accumulate starch in leaves (Borsics et al., 2007), which is similar to Mg²⁺-deficient plants (Hermans et al., 2004). Hence, it would be worth testing whether manipulation of the AtCNGC10 channel may result in increased Mg²⁺ uptake and alleviation of Al³⁺ toxicity.

**Effect of Al³⁺ on intracellular Mg²⁺ distribution**

**Cytosol**

The concentration of Mg²⁺ in the cytosol of leaf cells is assumed to be in the range of 2–10 mM (Leigh and Wyn Jones, 1986). A maximum total Mg²⁺ concentration of 100 mM has been measured in leaf endodermal cells (Stelzer et al., 1990). However, the concentration of free Mg²⁺ in the cytosol should be much lower (usually <2 mM) because Mg²⁺ is chelated by ATP and can be stored in organelles (Fig. 2). Indeed, Yazaki et al. (1988) measured a free Mg²⁺ concentration of 0.4 mM in the cytoplasm of Mg-sufficient mung bean root tips compared with 3.9 mM total concentration in the same tissue. They reported that ~90% of

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**Fig. 2.** Schematic diagram of the regulation of Mg²⁺ uptake and homeostasis inside plant cells. (A) Cation channels (depolarization-activated, hyperpolarization-activated, cyclic nucleotide-gated, and non-selective cation channels). (B) Arabidopsis thaliana magnesium transporter family (AtMGT1, 7a, 9, and 10). (C) Arabidopsis thaliana magnesium–proton exchanger (AtMHX). (D) Slow activating vacuolar (SV) channels. (E) AtMGT5. (F) AtMGt10. (G) Fast-activating chloroplast channels (FACCs).
cytoplasmic ATP was complexed with Mg$^{2+}$. In our laboratory, intracellular free Mg$^{2+}$ concentrations in the range of 0.8–1.4 mM were measured in the epidermal root cells of Arabidopsis genotypes using an Mg$^{2+}$-selective fluorescence dye (Magnesium Green$^6$) (JB, OB, and ZR, unpublished results). Therefore, a low concentration of free Mg$^{2+}$ in the cytoplasm is relatively tightly regulated. Rapid entry of aluminium into the cytosol and its seven orders of magnitude stronger binding affinity for ATP at low cytosolic pH compared with Mg$^{2+}$ (Piña and Cervantes, 1996) might alter the cytosolic free Mg$^{2+}$ concentration, which in turn may affect Mg$^{2+}$-dependent metabolic processes inside the cell (Fig. 2). Surprisingly, there is no information available in the literature about Al$^{3+}$-induced changes in the cytosolic concentration of free Mg$^{2+}$.

Vacuole

The vacuolar system occupies most of the volume of differentiated plant cells and is involved in turgor regulation, signal transduction, defence mechanisms, pH maintenance, and detoxification of metabolic compounds; in addition, vacuoles play a major role in ion homeostasis (Marschner, 1995; Andreev, 2001).

The Mg$^{2+}$ concentration in vacuoles was estimated at 3–7 mM in barley (Dietz et al., 1992), 8.3 mM in beet roots (Perez et al., 2008), and 13 mM in mesophyll and parenchyma cells of spruce needles (Picea abies L) (Stelzer et al., 1990) (Fig. 2). These high Mg$^{2+}$ vacuolar concentrations do not necessarily represent free Mg$^{2+}$ because a large proportion of vacuolar Mg$^{2+}$ is bound to anionic ligands (e.g. sulphate and phosphate) (Shaul, 2002). Indeed, Stelzer et al. (1990) observed large variations in Mg$^{2+}$ concentration in vacuoles of endodermal cells (from 20 mM to >120 mM, with an average of 64 mM). These variations were accompanied by variations in the major charge-balancing anions (sulphate and phosphate). Sequestration of aluminium in vacuoles has been confirmed in maize root tip cells (Vázquez et al., 1999) and single cells of Chara corallina (Taylor et al., 2000), presumably as aluminium-anion complex (e.g. Al−P$^−$). Such sequestration of aluminium may increase the free Mg$^{2+}$ concentration in the vacuole (Fig. 2). No information could be found in the literature about changes in the vacuolar concentration of free Mg$^{2+}$ under Al$^{3+}$ stress in plant cells.

The tonoplast potential is estimated at ~10 mV to −20 mV (cytosol negative) (Walker et al., 1996). Therefore, when the vacuolar Mg$^{2+}$ concentrations are higher than or equal to the cytosolic concentrations, Mg$^{2+}$ influx into the vacuole requires active transport. A cation exchanger AtMHX is localized to the tonoplast and is encoded by a single gene (Fig. 2). It can exchange protons with Mg$^{2+}$, Zn$^{2+}$, and Fe$^{2+}$ (Shaul et al., 1999). An additional role for this transporter was suggested: it might regulate H$^+$ homeostasis in plant cells (Berezin et al., 2008). It is well established that aluminium entry into the cytoplasm affects the H$^+$ homeostasis (Babourina and Rengel, 2009; Bose et al., 2010a). Thus, aluminium may affect the normal functioning of AtMHX, but there is no published experimental attempt to decipher the role of aluminium in AtMHX expression. Similarly, SV (slow activating vacuolar) channels are permeable to Mg$^{2+}$ (Allen and Sanders, 1996; Pottosin et al., 1997; Perez et al., 2008b), but their conductance in the presence of aluminium ions needs to be characterized.

Mitochondria

A recent study on Rhodotorula glutinis demonstrated that the Al-resistant yeast strain had 2.5- to 3-fold more mitochondria than the wild-type strain (Tani et al., 2008). Plant cells also have numerous mitochondria; however, no correlation has yet been established between abundance of mitochondria and Al resistance. This research might be an important component of characterizing the physiology of Al resistance.

The Mg$^{2+}$ ions are essential for normal functioning of mitochondria because Mg$^{2+}$ deficiency often results in mitochondrial disintegration (Marinos, 1963), reactive oxygen species (ROS) production, and photooxidative damage in many plant species (see Cakmak and Kirkby, 2008, for references). Al$^{3+}$ toxicity may also provoke similar mitochondrial dysfunction (Yamamoto et al., 2002) and ROS production in many plant species (Darko et al., 2004; Tamás et al., 2004; Boburina et al., 2006; Jones et al., 2006; Tahara et al., 2008) presumably by (i) causing Mg$^{2+}$ deficiency inside the mitochondria or (ii) substituting Mg$^{2+}$ for Al$^{3+}$ in Mg$^{2+}$-dependent enzymes (Mailloux et al., 2006; Rezabal et al., 2006). Thus, mitochondrial Mg$^{2+}$ transporters could be the target site for Al$^{3+}$ toxicity.

The yeast MRS2 and Arabidopsis AtMRS2-6/AtMGT5 transporters may regulate Mg$^{2+}$ transport (Bui et al., 1999; Schock et al., 2000; Li et al., 2008) (Fig. 2). MRS2 and AtMRS2 genes are distantly related to the CorA group of bacterial Mg$^{2+}$ transporters (Kehres et al., 1998; Smith and Maguire, 1998). The yeast MRS2 protein is localized to the inner membrane of mitochondria and is involved in the maintenance of Mg$^{2+}$ homeostasis in this organelle (Bui et al., 1999; GREGAN et al., 2001; Kolisek et al., 2003). A disruption in the yeast MRS2 gene led to a significant decrease in Mg$^{2+}$ content in mitochondria (Bui et al., 1999). AtMGT5 might also facilitate Mg$^{2+}$ influx and efflux across a mitochondrial membrane in Arabidopsis; importantly, unlike AtMGT1 and AtMGT10, AtMGT5 is not sensitive to aluminium (Li et al., 2008). Thus, overexpression of
AtMGT5 may help plants to maintain normal functioning of mitochondria under Al3+ stress.

**Chloroplast and endoplasmic reticulum**

Because Mg2+ is the coordinating metal ion in the chlorophyll molecule, the chloroplasts require a significant amount of internal Mg2+ (Demmig and Gimmel, 1979; Huber and Maury, 1980). Indeed, Schroppelmeier and Kaiser (1988) measured a total Mg2+ concentration of ~13–18 mM in spinach chloroplasts, whereas Potiris and Heldt (1976) reported a stroma Mg2+ concentration in the order of 5 mM (Fig. 2). Pottosin and Schonknecht (1996) calculated (by considering the relative volumes of stroma and lumen) that the Mg2+ concentration in the lumen of spinach chloroplasts needs to be in the range of 30–50 mM to support the observed 2 mM increase in stromal Mg2+ concentration during illumination (Fig. 2). A putative Mg2+ transporter AtMRS2-11 (AtMGT10) protein was found to be localized in chloroplasts, implicating its involvement in Mg2+ uptake (Drummond et al., 2001). Given that AtMGT10 is sensitive to aluminium (Li et al., 2001), Mg2+ uptake via AtMGT10 might be impeded under Al3+ stress. The Mg2+ ions, along with Na+, K+, and Ca2+ ions, may enter through the fast-activating chloroplast channel (FACC) that was identified in the chloroplast envelope of pea. Pharmacological analysis showed that Gd3+ completely blocks this channel (Pottosin et al., 2005). Further characterization of the FACC in the presence of aluminium ions may provide further insights into the transport of Mg2+ and other cations into chloroplasts under Al3+ stress.

Other subcellular organelles, such as the Golgi apparatus or the endoplasmic reticulum, also require Mg2+ for normal functioning, but no reports could be found on Mg2+ concentration in these organelles. Recently, Gebert et al. (2009) identified an Arabidopsis root-expressed MRS2-7 gene localized in the endoplasmic reticulum, which may be involved in Mg2+ homeostasis between the cytosol and the endoplasmic reticulum. Overexpression of MRS2-7 (MGT7) resulted in the Mg2+-responsive phenotype that showed better root and shoot growth than the wild type when grown in Mg2+-deficient medium (50 μM). Further, an MRS2-7/MGT7 knockout mutant showed an increase in sensitivity to Al3+ and enhanced tolerance upon gene overexpression (e-mail communication with Michael Gebert, IZMB-Institut für Zelluläre und Molekulare Botanik, Universität Bonn). This suggests that maintaining Mg2+ transport across the endoplasmic reticulum is pivotal for Al3+ tolerance in plants.

The regulation of Mg2+ homeostasis in other subcellular organelles (e.g. the Golgi apparatus) and potential implications for the severity of aluminium stress remains to be characterized.

**Role of Mg2+ in alleviating Al3+ toxicity**

Al3+ toxicity studies often used simple nutrient solutions (with Ca2+ alone) in order to avoid potential complications with aluminium speciation. However, these simple nutrient solutions did not allow plants to take up Mg2+ in the presence of Al3+ ions, thus depleting internal Mg2+ reserves and eventually causing Mg2+ deficiency in plants in long-term experiments. Recently, it has been shown that 5-week-old Arabidopsis plants demonstrated remarkable changes in the transcriptome within 1 week of Mg2+ starvation (Hermans et al., 2010).

Adding Mg2+ to the external medium at high concentrations (usually millimolar) alleviated Al3+ toxicity in many plant species (Kinraide, 2003; Kinraide et al., 2004). The proposed mechanisms for Al3+ toxicity alleviation by Mg2+ ions include (i) increased ionic strength of the solutions (Noble and Sumner, 1988); (ii) reduction in Al3+ saturation at the apoplastic exchange sites (Grauer and Horst, 1992); and (iii) decreased Al3+ activity at the root cell plasma membrane surface (Kinraide, 2003; Kinraide et al., 2004). However, the actual mechanisms underlying root growth improvement by Mg2+ in the presence of constant Al3+ activity (Lazof and Holland, 1999; Silva et al., 2001b, c) are poorly understood, as illustrated by recent reports about micromolar concentrations of Mg2+ causing non-electrostatic alleviation of Al3+ toxicity (Silva et al., 2001a; Yang et al., 2007).

Up-regulation of Mg2+ transporter genes, particularly those for high-affinity Mg2+ transporters, can contribute to amelioration of Al3+ toxicity (Zhang et al., 2007). For example, overexpression of the Arabidopsis plasma membrane high-affinity Mg2+ transporter gene (AtMGT1) in N. benthamiana increased Al3+ tolerance (Deng et al., 2006) by enhancing Mg2+ uptake from Mg2+-deficient medium. Similarly, overexpression of Mg2+ transporter genes in yeast conferred Al3+ tolerance by alleviating Al3+-induced Mg2+ deficiency (MacDiarmid and Gardner, 1998). In our laboratory, a higher Mg2+ uptake and higher free Mg2+ concentration were observed in the cytoplasm of Al-tolerant Arabidopsis genotypes (Col-0 and alr104) compared with Al-sensitive genotypes (als5 and als3) when exposed to 50 μM AlCl3 at pH 4.2 (J Bose et al., unpublished results).

The above-mentioned studies did not provide sufficient evidence about the role of enhanced Mg2+ uptake in alleviation of Al3+ toxicity. A recent comparative transcriptome analysis using DNA microarrays involving Al-tolerant and Al-sensitive soybean genotypes revealed that Mg2+ ameliorated Al3+ toxicity in the Al-sensitive genotype by specifically controlling expression levels (both up- and down-regulation) of multiple genes necessary for Al tolerance (Duressa et al., 2010). Therefore, the following sections focus on potential links between enhanced Mg2+ nutrition and alleviation of Al3+ toxicity through multiple pathways (Fig. 3).

**Better carbon partitioning from shoots to roots**

It is well established that proper Mg2+ nutrition is pivotal for root growth via carbohydrate partitioning from shoots to roots. Mg2+-deficient plants exhibit reduced root growth and concomitant sucrose accumulation in source leaves,
sugesting impairment of sucrose transport from shoots to roots in the early stages of Mg deficiency (Fisher and Bremer, 1993; Cakmak et al., 1994a, b; Mehne-Jakobs, 1995; Fisher et al., 1998; Hermans and Verbruggen, 2005; Ding et al., 2006; Cakmak and Kirkby, 2008). This sugar accumulation, rather than poor Mg availability, has been suggested as the primary reason for a decrease in the chlorophyll content and plant growth reduction (Hermans et al., 2004; Hermans and Verbruggen, 2005). It has recently been found that reduced carbon allocation in A. thaliana affects the youngest leaves more than roots, which is in line with earlier findings that the growth reduction under Mg deficiency was higher in young leaves than in roots (Hermans and Verbruggen, 2005; Hermans et al., 2006).

Increased organic acid synthesis and exudation

Exudation of organic acid anions is the best-described Al exclusion mechanism in a range of plant species (Ryan et al., 2001; Kochian et al., 2004, 2005). Depending on the plant species, Al activates exudation of various organic acid anions, such as malate, citrate, oxalate, pyruvate, and/or succinate (Larsen et al., 1998; Ryan et al., 2001; Kochian et al., 2005), through organic-anion-permeable plasma membrane channels (Pinos et al., 1999; Zhang et al., 2001; Sasaki et al., 2004). For example, malate is released through aluminium-activated malate transporters in wheat (TaALMT1) (Sasaki et al., 2004, 2006; Delhaise et al., 2007), A. thaliana (AtALMT1) (Hoeckenga et al., 2006), and Secale cereale (ScALMT1) (Fontecheva et al., 2007; Collins et al., 2008). Citrate efflux occurs through multidrug and toxin efflux (MATE) transporters in A. thaliana (AtMATE) (Liu et al., 2009), wheat (Ryan et al., 2009), barley (HvMATE1) (Furukawa et al., 2007), and sorghum (ShMATE) (Magalhaes et al., 2007).

Upon Al3+ exposure, organic acid anion exudation may occur immediately (classified as pattern I) or after a time delay (classified as pattern II) (Ma et al., 2001; Pinoers et al., 2002). However, long-term efflux of organic acid anions requires continuous synthesis of organic acids inside the root cells (Basu et al., 1994). In this regard, cytoplasmic Mg2+ activity is critical for activation of many enzymes involved in organic acid synthesis and metabolism (Fig. 3).

In fact, Mg acts as a cofactor for the activation of citrate synthase (Boulton and Ratledge, 1980), malate synthase (Goodwin and Sutter, 2009). Maintenance of H+-ATPase activity and cytoplasmic pH regulation

A difference in H+ activity (ΔpH) between the cytoplasm and the apoplas is the major driving force for translocation of ions across the plasma membrane. Under no stress, the pH is 7.3–7.6 in the cytoplasm, 4.5–5.9 in vacuoles, ~7 in mitochondria, 7.2–7.8 in chloroplasts, and ~5.5 in the apoplasm (Kurkdjian and Guern, 1989). Thus, the cytoplasm is less acidic when compared with vacuoles and the apoplasm (Fig. 2). This pH difference is regulated by proton pumps (H+-ATPase and H+-PPase) located in the plasma membrane and the tonoplast, respectively, driving H+ from the cytoplasm to either the apoplasm or the vacuole (Marty, 1999). Hence, disturbance in H+-ATPase activity by Al3+ toxicity (Matsu moto, 1988; Matsumoto et al., 1992; Sasaki et al., 1995; Ahn et al., 2001, 2002).
would affect cytoplasmic pH regulation (Babourina and Rengel, 2009; Bose et al., 2010b).

The Mg$^{2+}$ activity inside the cytoplasm is directly involved in the regulation of H$^+$-ATPase activity (Figs 1, 3) (Brooker and Slayman, 1983; Costa and de Meis, 1996). An elevated cytosolic concentration of free Mg$^{2+}$ (up to 10 mM) is essential for H$^+$-ATPase activity at pH 6.0 in maize roots as shown using the plasma membrane vesicles (Costa and de Meis, 1996). A recent study using rice bean (Vigna umbellata) roots found that Al$^{3+}$ stress, in the absence of Mg$^{2+}$ in the growth medium, inhibited H$^+$-ATPase by 37%; however, addition of a micromolar (10 μM) concentration of Mg$^{2+}$ in the external medium restored plasma membrane H$^+$-ATPase activity, even though Al$^{3+}$ activity was maintained constant (Yang et al., 2007).

Enhanced acid phosphatase activity

A release of phosphorus (Pi) from roots or from chemical compounds in the rhizosphere has the potential to be an Al resistance mechanism in plants because phosphate ions strongly bind to Al$^{3+}$ to form non-toxic Al–phosphate complexes either in the apoplastic, on the root surface, or in the rhizosphere (cf. Taylor, 1991). Al-induced Pi release was observed in Al-tolerant genotypes of sugar beet (Sylvia, 1990), wheat (Pellet et al., 1996), and maize (Pellet et al., 1995). Sylvia (1990) hypothesized that an Al-resistant cultivar of sugar beet exuded Pi in a metabolically dependent manner in the presence of Al$^{3+}$. However, the exact mechanism responsible for Pi release is unknown.

Acid phosphatase plays a vital role in Pi release at the rhizoplane and in the rhizosphere; hence, it has long been proposed as an Al resistance mechanism (Marschner, 1991). The Al-induced acid phosphatase activity in the root cells (Huttová et al., 2002; Zelinová et al., 2009) appears to be Al concentration dependent. Huttová et al. (2002) observed a steep increase in the cellular acid phosphatase activity when an Al-sensitive barley genotype was treated with Al. In contrast, an Al-resistant genotype showed a sustained (up to 2 d) increase in phosphatase activity when exposed to Al. Higher acid phosphatase activity in the Al-resistant compared with the Al-sensitive cultivars could be due to the ability of the resistant cultivar to maintain a relatively high Mg$^{2+}$ concentration inside the root tissues (unfortunately, Mg tissue concentrations were not measured in that study). However, Mg$^{2+}$ ions play a major role in the modulation of acid phosphatase activity (Fig. 3) (Gabbrielli et al., 1989). Either a high concentration of Mg-EDTA (2.5 mM Mg, 5 mM EDTA) or a high Mg$^{2+}$/Ca$^{2+}$ ratio stimulated acid phosphatase activity in cucumber, radish, rocket salad, and Alyssum species (Gabbrielli et al., 1989; Tabaldi et al., 2008).

Protection against an Al-induced cytosolic Ca$^{2+}$ rise

Free cytosolic Ca$^{2+}$ activities in plant cells are usually maintained in the 100–200 nM range (Bush, 1995; Webb et al., 1996) to prevent Ca$^{2+}$ cytotoxicity by the formation of insoluble calcium precipitates with inorganic phosphates (Webb et al., 1996). In many plant species, Al$^{3+}$ toxicity caused an increase in cytosolic Ca$^{2+}$ activity (Fig. 1) within minutes of Al$^{3+}$ exposure, with such an increase being higher in the Al-sensitive than the Al-resistant genotypes of the same species (Jones et al., 1998; Zhang and Rengel, 1999; Ma et al., 2002; Rengel and Zhang, 2003). This cytosolic Ca$^{2+}$ rise would play a major role in expression of Al$^{3+}$ toxicity because the cell responsive elements may stop responding to transient rises in cytosolic Ca$^{2+}$ caused by a variety of signals under non-stress conditions (Rengel and Zhang, 2003). For example, an increase in cytosolic Ca$^{2+}$ caused closure of plasmodesmata (Holdaway-Clarke et al., 2000) and inhibited plasmodesmata-mediated cell to cell transport in Al-sensitive wheat roots (Sivaguru et al., 2000). In fact, a good correlation was observed between the Al-induced increase in cytosolic Ca$^{2+}$ (within 30 min) and root growth inhibition in wheat genotypes (Zhang and Rengel, 1999), leading to the hypothesis that disruption of Ca$^{2+}$ homeostasis may be the primary cause of Al$^{3+}$ toxicity (Rengel and Zhang, 2003).

The above studies did not take into account the role of Mg$^{2+}$ in cytosolic Ca$^{2+}$ homeostasis. In yeast cells, removal of Mg$^{2+}$ from the external medium elicited an increase in the cytosolic Ca$^{2+}$ concentration (Wiesenberger et al., 2007). Moreover, Mg$^{2+}$ modulates Ca$^{2+}$ release from internal organelles and alters the activity of many plasma membrane and vacuolar Ca$^{2+}$-permeable channels (Fig. 3) (Bruggemann et al., 1999; Murphy, 2000; Wiesenberger et al., 2007). Thus, it is hypothesized that enhanced Mg$^{2+}$ nutrition under Al$^{3+}$ stress may prevent Al-induced cytosolic Ca$^{2+}$ spikes.

Protection from oxidative damage

Formation of ROS in response to Al$^{3+}$ has been observed in many studies (Darko et al., 2004; Tamás et al., 2004; Babourina et al., 2006; Jones et al., 2006; Tahara et al., 2008), even though Al$^{3+}$ is not a transition metal and therefore cannot catalyse redox reactions (Fig. 1). However, Al$^{3+}$ in combination with iron caused peroxidation of lipids in the plasma membrane of soybean (Cakmak and Horst, 1991) and rice roots (Meriga et al., 2004), and in cultured tobacco cells (Ono et al., 1995; Yamamoto et al., 1997). Further, Al$^{3+}$ induced the expression of several genes encoding antioxidant enzymes, such as glutathione S-transferase, peroxidase, and superoxide dismutase in A. thaliana (Richards et al., 1998; Ezaki et al., 2000), after a few hours of Al$^{3+}$ exposure, which indicated that increased production of ROS under Al$^{3+}$ toxicity may be an indirect effect. A number of hypotheses have been proposed for Al$^{3+}$-induced rapid production of ROS, including dysfunction of mitochondria (Yamamoto et al., 2002), formation of aluminium superoxide semi-reduced radicals (Exley, 2004), and activation of oxidizing enzymes (Šimonícová et al., 2004a, b). Although Mg$^{2+}$ deficiency also induced ROS production (Fig. 1) and photooxidative damage in many
plant species (see Cakmak and Kirkby, 2008, for references), surprisingly the relative contribution of Al-induced Mg\(^{2+}\) deficiency to ROS production has received no attention in Al\(^{3+}\) toxicity studies so far.

Concluding remarks and future research directions

Mg\(^{2+}\) ions have vital functions in many biochemical and physiological pathways. Some of the Mg-dependent functions, such as carbohydrate allocation from shoots to roots, organic acid synthesis and exudation, acid phosphatase and H\(^{+}\)-ATPase activity, and cytosolic pH and Ca\(^{2+}\) homeostasis, can be important in increasing Al resistance in plants.

One possible approach for engineering Al\(^{3+}\)-resistant plant genotypes could rely on improved Mg\(^{2+}\) transport and/or accumulation. Broadley et al. (2008) demonstrated a 2.3-fold variation in shoot magnesium concentrations in various genotypes of Brassica oleracea, and reported that variations in Mg\(^{2+}\) tissue concentrations are highly heritable. It is therefore suggested that breeding for enhanced Mg\(^{2+}\) nutrition under Al\(^{3+}\) toxicity may be both possible and desirable.

Overexpression of the MRS2-7 (MGT7) gene in A. thaliana resulted in the Mg\(^{2+}\)-responsive phenotype with a better shoot and root growth than the wild type (Col-0) when they were grown in Mg\(^{2+}\)-deficient medium (Gebert et al., 2009). Similarly, overexpression of the Arabidopsis plasma membrane high-affinity Mg\(^{2+}\) transporter (AtMGT1 gene) in N. benthamiana increased Al\(^{3+}\) resistance (Deng et al., 2006) by enhancing Mg\(^{2+}\) uptake from Mg\(^{2+}\)-deficient medium. In addition to overexpression of Mg\(^{2+}\) transporters, other approaches can be used for increasing the Mg\(^{2+}\) concentration in cells and tissues. For example, yeasts treated with pulsed electric fields demonstrated increased Mg\(^{2+}\) accumulation (Pankiewicz and Jamroz, 2010). Although it might not work at the whole-plant level, this technique can be used for \textit{in vitro} studies on plant cell culture or suspension.

Arabidopsis AtCNGC10 is localized in the plasma membrane (Borsics et al., 2007) and involved in Mg\(^{2+}\) uptake and long-distance transport (Guo et al., 2010). Thus, manipulation of the AtCNGC10 channel may result in increased Mg\(^{2+}\) uptake or remobilization and alleviation of Al\(^{3+}\) toxicity.

In addition to the already discovered and characterized Mg\(^{2+}\) transport systems in living organisms, it is anticipated that new Mg\(^{2+}\) transporters will be identified (Quamme, 2010). However, studies on mammalian Mg\(^{2+}\) transporters suggest potential difficulties. Although the human channel Mrs2 and SLC41 family of Mg\(^{2+}\) transporters share some similarities with Mg\(^{2+}\) transporters found in prokaryotes, yeasts, and plants, several newly identified mammalian Mg\(^{2+}\) transporters are not represented in prokaryotic and yeast genomes (Quamme, 2010). Therefore, mammalian Mg\(^{2+}\) transporters have evolved more recently. This indicates that \textit{in silico} screening for new Mg\(^{2+}\) transporters in plants, based on already described transporters in other living organisms, might not be very successful. In addition, there are no obvious sequence similarities between already characterized mammalian families of Mg\(^{2+}\) transporters. The only common GMN motif in the first transmembrane domain is found in bacterial CorA, yeast Mrs2 and Alr1, the plant Mrs2/MGT family, and mammalian Mrs2, whereas other Mg\(^{2+}\) transporters do not contain this motif (Quamme, 2010).

Further studies should include a reciprocal effect of Al\(^{3+}\) and Mg\(^{2+}\) transport across the plasma membrane and endomembranes, and Mg\(^{2+}\) effects on organic acid synthesis and transport across the plasma membrane and the tonoplast. Plants with overexpressed or antisense Mg\(^{2+}\) transporters should be assessed for Al\(^{3+}\) resistance, which will lead to better understanding of Al\(^{3+}\) toxicity and resistance.

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