Magnesium decreases aluminum accumulation and plays a role in protecting maize from aluminum-induced oxidative stress

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Running title: Mg alleviates Al toxicity in maize

Abstract

Background

Acidic soils with a pH_{water} below 5.5 occupy up to 40% of world's arable land. Aluminum (Al) toxicity and magnesium (Mg) deficiency often coexist in acidic soils, limiting crop production. In this study, we investigated a role of Mg in alleviation of Al stress in maize (*Zea mays*) by characterizing the growth responses, Al accumulation and the antioxidant properties of plants cultured hydroponically.

Methods

Physiological and molecular analyses were used to investigate the mechanisms of Mg governing the alleviation of Al-induced ROS production and root growth inhibition.

Results

Aluminum (50 μ M) decreased root growth and induced oxidative stress. Exogenously added millimolar concentrations of Mg significantly alleviated Al toxicity as evidenced by restoration of plant growth, suppression of Al uptake, and a decline in root H₂O₂ concentration. Furthermore, the addition of Mg to the Al treatment solution enhanced the activities and expression of genes encoding superoxide dismutase, catalase and peroxidase compared to the Al-only treatment.

Conclusions

The results indicate that Mg plays a role in alleviation of Al toxicity, reduction of Al accumulation and protection from Al-induced oxidative stress through activation of antioxidative enzymes.

Key words: Mg; Al toxicity; maize; H₂O₂; antioxidative enzymes

Introduction

Acid soils that limit crop production are found worldwide, occupying up to 40% of world's arable land, particularly in the tropics and subtropics (Kochian et al. 2004). To produce a better crop yield on acid soils, farmers are recommended to apply alkaline materials such as CaCO_3 to increase soil pH (Zheng, 2010). Although liming of acid soils can ameliorate soil acidity, this is neither an economic option for poor farmers nor an effective strategy for alleviating subsoil acidity (Whitten et al. 2000). Aluminum (Al) toxicity is a major constraint to plant growth and production in acidic soils (Rengel and Zhang, 2003). Overproduction of ROS (reactive oxygen species) in plants is an early event in Al toxicity; ROS formation can induce oxidative stress, leading to cell membrane peroxidation, structural damage in cells, chromosomal aberration and programmed cell death, and poor growth and development of plant roots. Compared with Al-sensitive plant cultivars, the Al-resistant cultivars have higher level of antioxidants, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione S-transferase (GST), resulting in lower accumulation of ROS in their roots (Darkó et al. 2004). Furthermore, overexpression of genes encoding antioxidants, such as tobacco GST gene (*parB*) and *Arabidopsis* POD gene (*AtPox*), conferred transgenic *Arabidopsis* resistance to Al-induced oxidative stress (Ezaki et al. 2000; Ezaki et al. 2001).

Magnesium (Mg) is an essential nutrient for plant growth. In addition to being the central atom of the chlorophyll molecule, Mg is an activator of a large number of enzymes, such as RNA polymerases, protein kinases, phosphatases and carboxylases (Bose et al. 2011). Due to low cation exchange capacity, ease of leaching and the competition with Al for plant uptake, Mg deficiency often occurs in acidic soils (Guo et al. 2016; Rengel et al. 2015). In plants, the bacterial cobalt resistance A (CorA) protein homologues are the best studied transporters associated with Mg uptake (Knoop et al. 2005; Rengel et al. 2015). There are 10 members in the Mg transporter family in *Arabidopsis* (*AtMRS2/AtMGT1*; Schock et al. 2000; Li et al. 2001) and 9 members in rice (*OsMRS2/OsMGT*; Saito et al. 2013). In *Arabidopsis*, *AtMGT1* is a high-affinity Mg^{2+} transporter, but its activity is inhibited by Al^{3+} (Li et al. 2001). However, overexpression of the *AtMGT1* gene in *Nicotiana benthamiana* conferred Al tolerance (Deng et al. 2006). Additionally, the mutant lines lacking *OsMGT1* with lower Mg content showed higher Al sensitivity than the wild type plants (Chen et al. 2012b), indicating that *OsMGT1*-mediated Mg uptake is required for rice Al resistance.

It has been shown that external application of Mg can alleviate plant toxicity of Al and heavy metals

(Rengel et al. 2015). Application of dolomite (Mg-Ca carbonate) ameliorates soil acidity and thus alleviates Al toxicity in many soil-plant systems (eg. Kasongo et al. 2012; Cristancho et al. 2014). In dicotyledons such as faba bean (Chen et al. 2015), rice bean (Yang et al. 2007) and soybean (Silva et al. 2001; Yang et al. 2007), micromolar concentrations of Mg alleviate Al toxicity via enhancing exudation of citrate from plant roots. On the other hand, millimolar concentrations of Mg were found to alleviate Al toxicity in monocot plants (eg. wheat and rice), which might be related to Mg competing with Al for binding sites on the plasma membrane (Chen et al. 2012b; Ryan et al. 1997; Watanabe and Okada 2005). Additionally, Pandey et al. (2013) found that the application of 0.25 mM MgSO₄ alleviated 0.5 mM AlCl₃-induced oxidative stress in rice, but no information was provided on the Mg-Al interactions in influencing expression of genes coding for antioxidative enzymes.

Maize (*Zea mays*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) are the three most important cereals that provide more than half of all calories consumed by humans worldwide (Awika 2011; Ma et al. 2014). In this study, we focus on the role of Mg in alleviation of oxidative stress under Al exposure in maize plants. We characterized cellular stress responses, including root elongation, Al uptake, gene expression, and activity of antioxidant enzymes. The results suggested that addition of micromolar concentrations of Mg could efficiently alleviate plant growth inhibition, reduce Al accumulation, alleviate Al-induced oxidative stress, and activate the antioxidant enzyme system under Al exposure.

Materials and Methods

Plant culture and treatments

Seeds of maize (*Zea mays* L. cv. YunRui-8) were obtained from the Food Crop Research Institute, Yunnan Academy of Agricultural Sciences (Kunming, Yunnan province, China). Undamaged seeds were selected and placed in a beaker, rinsed 5 times with deionized water, and soaked in deionized water in the dark at 25°C in a cultivation cabinet for 12 h. Phosphorus (P) could interact with Al to form Al(PO₄)₃; hence, the MGRL medium [3.0 mM KNO₃, 200 µM Ca(NO₃)₂, 1.5 mM MgSO₄, 67 µM Na₂EDTA, 8.6 µM FeSO₄, 10.3 µM MnSO₄, 30 µM H₃BO₃, 1.0 µM ZnSO₄, 24 nM (NH₄)₆MO₇O₂₄, 130 nM CoCl₂, and 1.0 µM CuSO₄] without P has been used widely for Al toxicity research (eg. Iuchi et al. 2007; Li et al. 2019; Yang et al. 2014). We therefore used the similar nutrient solution in the present study. For germination, the seeds were sown in polypropylene containers filled with 1/5 MGRL without Mg (pH 5.8). The 3-day-old uniform seedlings with the main seminal root about 5 cm long were transferred to

the treatment solutions (1/5 MGRL-Mg) containing 0, 0.01, 0.05, 0.15, 0.3, 2.0, or 10.0 mM MgSO₄ with or without 50 µM AlCl₃ (pH 4.2) for a short-term (24 h) or a long-term (7 day) treatment. The free activity of Al³⁺ was 22.98, 22.5, 20.79, 17.53, 14.34, 5.21 or 1.55 µM in 1/5 MGRL medium containing 0, 0.01, 0.05, 0.15, 0.3, 2.0 or 10 mM MgSO₄, respectively, as calculated by GEOCHEM-EZ software (www.plantmineralnutrition.net/Geochem/geochem%20home.htm).

Plant growth measurements

After treatments, the plants were harvested for root length and biomass measurements. The root length was determined by measuring the length of the longest root of 20 plants randomly selected from each pot. For biomass measurement, shoots and roots were separated, rinsed with deionized water, dried to a constant value at 65°C (48 h), and weighed.

Hematoxylin and morin staining of roots

Morin and hematoxylin are two Al-sensitive histochemical indicators widely used to indicate the presence of Al. After treatments, roots were rinsed for 10 min in each of three lots of distilled water. Then, the root tips (2- 3 cm) were excised and transferred into new tubes containing 0.2% (w/v) hematoxylin and 0.02% (w/v) KIO₃ for 1 h, or 100 µM morin hydrate (Sigma-Aldrich) for 15 min under dark. After rinsing for 10 min in distilled water 3-5 times to remove residual dye from the root surface, the root tips were observed and photographed under a stereomicroscope (Olympus, SZ61 equipped with a Nikon D600 digital SLR camera) for hematoxylin staining, or a fluorescence microscope (Olympus, BX60 equipped with a DP70 CCD camera) for morin staining (excitation at 440 nm and emission at 510 nm).

Measurement of magnesium and aluminum concentration

Quantitative assessments of Al and Mg concentration in tissues were done using inductively coupled plasma-atomic emission spectroscopy (ICP-AES, model PS-1000, Lowell, MA, USA) as we described recently (Li et al. 2019).

H₂O₂ detection

H₂O₂ was measured by the titanium sulfate method and a Micro Hydrogen Peroxide (H₂O₂) Assay Kit (BC3595, Solarbio Life Sciences, China).

Measurements of SOD, POD and CAT activities

The frozen roots (0.5 g) were homogenized in 1 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 0.2 mM EDTA-Na₂, 0.1 mM ascorbic acid and 1% w/v polyvinylpolypyrrolidone (PVPP) in an ice bath using a mortar and pestle. The homogenate was centrifuged for 20 min at 12,000 g at 4°C, and the supernatant was used for enzyme analysis. SOD activity was measured according to the published method (Giannopolitis and Ries, 1977). POD activity was measured according to the method of Maehly and Chance (Maehly and Chance, 1954), and CAT activity was assayed by monitoring the consumption of H₂O₂ at 240 nm for 2 min (Aebi, 1984). The protein content was measured by the Bradford method (Bradford, 1976).

Real time RT-PCR analysis

Total RNA was isolated from seedlings using Trizol reagent (Invitrogen, USA). Reverse transcription was performed using a PrimeScript RT reagent kit with gDNA eraser (Takara, Dalian, China) according to the manufacturer's instructions. The expression levels of genes were analyzed using SYBR Premix Ex Taq II (TaKaRa, Da Lian, China). PCR was performed using a Bio-Rad CFX-96 real-time PCR system. *Actin* was used as an internal control. All primers used for RT-PCR (listed in Table 1) were based on the literature (Li et al. 2016; Maschietto et al. 2016; Shi et al. 2018).

Table 1. PCR primers used for Real-Time PCR analysis

Gene	Sequence ID		Primer (5'-3')
<i>Actin</i>	J01238.1	forward	CACCTTCTACAACGAGCTCC
		reverse	CAGTCAGGATCTTCATGAGG
<i>ZmMGT1</i>	NM_001156163.1	forward	CAGCTGATCCAGTTTGAATTG
		reverse	GAGGGCATATACATTCTCTAC
<i>ZmMGT2</i>	NM_001358546.1	forward	GTTGGAGATGTTACTGGAAGCT
		reverse	GGTGAAGTGAATTCATCGGTGCT

<i>ZmMGT8</i>	NM_001148636.1	forward	CTAGAGCTGAGGTTGATGAT
		reverse	GCATCATTGCAGTAATCCAC
<i>ZmMGT9</i>	XM_008676457.3	forward	GAGCTGAGGTTGATGATCCAT
		reverse	AACCCATGGCTATGATGTAC
<i>CAT 1</i>	NM_001254879.2	forward	GTGAATGCACCAAAATGTGC
		reverse	TGATGCACTTCTCACGACAG
<i>CAT 2</i>	NM_001111840.2	forward	AACATCGACAACCTTCTTC
		reverse	TCGGAGTAGTAGATACCA
<i>POD 1</i>	FJ797426.1	forward	ACAACAGCTACTACCACAACA
		reverse	CCTTCTCCGTGAGCAGTT
<i>POD 3</i>	NM_001138821.1	forward	AACGTCTACTACAAGAACCT
		reverse	TGTAGGGTCCCTGATGAG
<i>Cytosolic SOD</i>	NM_001320832.1	forward	CGTGTTGCTTGTGGGATCATTGGA
		reverse	TCGGTGGCTACAGGTGCATAATGA
<i>MnSOD</i>	NM_001138523.1	forward	TTGTGTACCTGCTGGACCAAGTGT
		reverse	ACTACGAGCAGCAGAAAGTGGAGT

169

170 *Statistical analysis*

171 Experiments contained at least three replicates, and the data are expressed as means and S.D. SPSS 12.0
172 for Windows (SPSS, Chicago, IL, USA) software package was used to conduct the least significant
173 difference (LSD) test to determine statistical significance at $p < 0.05$.

174

175 **Results**

176 *Magnesium increases root growth and plant biomass under Al stress*

177 The most significant symptom of Al toxicity is the inhibition of root growth. Therefore, we firstly
178 analyzed the effect of Mg on Al-induced root growth inhibition in maize in a short (24 h) and long-term
179 (7 d) experiment. The results showed that the growth of maize primary roots was inhibited approximately
180 66% and 89% by the treatment with 50 μ M Al for 24 h and 7 d, respectively, in comparison with the
181 plants grown under control conditions (1/5 MGRL with 0.3 mM MgSO₄, pH 4.2; Fig. 1A and B).
182 However, exogenous application of Mg recovered root growth of the Al-treated seedlings, and the most

optimal alleviation effect was observed at 0.3 and 2.0 mM Mg (Fig. 1A and B).

We further analyzed the biomass of maize plants under various treatments. The results showed that Al stress did not significantly affect shoot dry weight after 7-day treatments (Fig. 2A). However, the 50 μ M Al treatment significantly decreased root dry weight and the root/shoot ratio, but these parameters were restored by the application of 2.0 and 10.0 mM Mg in the Al treatment solution (Fig. 2B and C).

Effects of Mg on Al accumulation in maize root

As shown in Fig. 3A, the fluorescence intensity of morin was barely detectable in root tips not exposed to Al, whereas the root apex exhibited severe damage and strong Al accumulation after the plants were exposed to 50 μ M Al for 24 h. Staining with hematoxylin also revealed substantial accumulation of Al in roots treated with 50 μ M Al (Fig. 3B). The application of 2.0 mM Mg in the Al treatment solution lessened the staining of morin (Fig. 3A) and hematoxylin (Fig. 3B) and significantly decreased Al concentration in roots (Fig. 3C), but Mg concentration in roots (Fig. 3D) was significantly increased under Al stress.

Magnesium restored Al-induced inhibition of the expression of Mg transporter genes

Given the MGT1 transporter family-mediated Mg uptake is involved in alleviation of Al toxicity in *Arabidopsis* and rice (Deng et al. 2006; Chen et al. 2012b), several *AtMGT1* and *OsMGT1* homologous genes, namely *ZmMGT1*, *ZmMGT2*, *ZmMGT8*, and *ZmMGT9* were identified in maize genome (Fig. 4A) and the expression of these genes was analyzed by RT-PCR (Fig. 4B). The results showed that the expression of all these four genes was reduced by the application of Al; in contrast, Mg supply promoted the expression of these genes to even a higher level than that of the control treatment (0.3 mM Mg without Al) (Fig. 4B).

Magnesium decreased Al-induced H₂O₂ production

H₂O₂, a major ROS in plants, is an important indicator of oxidative stress. As shown in Fig. 5, the H₂O₂ concentration was significantly increased by Al, whereas the application of 0.05, 0.3, 2.0, and 10.0 mM Mg significantly diminished H₂O₂ production by, respectively, 18%, 60%, 55%, and 21% compared with the Al-only treatment.

Magnesium up-regulated activity of antioxidant enzymes under Al stress

The activities of antioxidant enzymes SOD, POD and CAT were determined (Fig. 6). As the result of Al stress, the activities of the SOD and CAT increased by 15% and 59%, respectively, whereas the activity of POD was decreased by 47% (Fig. 6A). The addition of Mg in the Al treatment solution not only further stimulated the activities of SOD and CAT, but also restored POD activity to the control level, compared with the Al-only treatment. Additionally, the expression of several genes, such as *cytosolic SOD*, *MnSOD*, *POD1*, *POD3*, *CAT1*, and *CAT2* was also enhanced by Mg application (Fig. 6B), which fitted well with the changes in antioxidant enzyme activities (Fig. 6A). These results suggested that Mg efficiently increased the gene expression and activity of the antioxidant enzymes under Al stress.

Discussion

Aluminum toxicity is a major factor limiting plant growth and crop yield in acidic soils. It has been shown to induce oxidative stress and root cell death, thereby leading to significant reductions in water and nutrient uptake (Chen et al. 2011). In the present study, we elucidated amelioration of Al toxicity in maize roots by Mg. The results showed that Al-induced plant growth inhibition (Fig. 1 and Fig. 2), Al accumulation (Fig. 3) and oxidative stress (Fig. 5) were significantly ameliorated by exogenous application of Mg. Moreover, Mg significantly increased activity and gene expression of antioxidant enzymes (Fig. 6) as well the expression of Mg transporters (Fig. 4) under Al stress.

Uptake and translocation of Mg is mainly mediated by the homologues of bacterial Mg transporters in plants (bacterial cobalt resistance A, CoA; Knoop et al. 2005; Chen and Ma, 2013). Ten and 9 members of the MGT transporter family are found in *Arabidopsis* and rice, respectively. Among them, only *AtMGT6* (Mao et al., 2014) in *Arabidopsis* roots and *OsMGT1* in rice roots (Zhang et al., 2019) are induced by Mg deficiency, indicating their role in Mg uptake in response to low Mg status. Although Al toxicity and Mg deficiency did not affect the expression of *AtMGT1* in *Arabidopsis*, the *AtMGT1*-mediated Mg uptake was highly sensitive to Al toxicity (Li et al. 2001). In contrast to *Arabidopsis*, *OsMGT1* was upregulated by Al toxicity (Chen et al., 2012b) and Mg deficiency (Zhang et al., 2019). Additionally, the upregulation of *OsMGT1* increased Mg uptake in rice plants under Al stress. In comparison with the wild type plants, the rice mutant lines lacking *OsMGT1* and having low Mg concentration in tissues (Chen et al. 2012b) showed increased Al sensitivity, whereas transgenic tobacco plants overexpressing

Arabidopsis AtMGT1 and having high tissue Mg concentration (Deng et al. 2006) exhibited increased Al resistance. These results indicated that MGT1-mediated Mg uptake is essential for plant Al resistance.

There are 12 Mg transporter genes in the maize genome. Among them, *ZmMGT1* and *ZmMGT2* showed, respectively, 68% and 67% identity to *AtMGT1*, and *ZmMGT8* and *ZmMGT9* had, respectively, 55% and 55% identity to *OsMGT1* (Fig. 4A). We found that the expression of *AtMGT1* and *OsMGT1* homologues, including *ZmMGT1*, *ZmMGT2*, *ZmMGT8*, and *ZmMGT9*, was decreased by Al toxicity after the 24-h treatment (Fig. 4B). The difference in the expression of MGT genes in *Arabidopsis* (Li et al. 2001), rice (Chen et al., 2012b) and maize (Fig. 4B) might be due to different plant species responding differently to Al stress. Additionally, the results presented here indicated that inhibition of Mg uptake by Al might be attributed, at least partly, to decreased expression of the Mg transporter genes in maize. However, the application of Mg significantly alleviated Al-mediated inhibition of the expression of these genes (Fig. 4B). The functions of these Mg transporter genes in Mg uptake and Al toxicity warrant further research.

Magnesium ion (Mg^{2+}) has the smallest ionic radius (0.072 nm) and the largest hydrated radius among the major biological cations (Bose et al. 2011; Rengel et al. 2015). The hydrated radii of Al^{3+} (0.480 nm) and Mg^{2+} (0.476 nm) are similar. Therefore, Al^{3+} and Mg^{2+} could compete for the membrane transporters (Rengel et al. 2015). For example, Mg blocks non-selective slow vacuole (SV) channels and non-selective cation channels (NSCC) that are associated with Al transport to the vacuole and passive uptake by roots, respectively (Perez et al. 2008; Wherrett et al. 2005). Relatively low (micromolar) concentrations of Mg significantly alleviated Al-induced root growth inhibition in legumes, including soybean (Silva et al. 2001), rice bean (Yang et al. 2007) and faba bean (Chen et al. 2015) through enhancement of Al-induced citrate exudation. However, relatively high concentrations (millimolar) of Mg were needed to alleviate Al toxicity in Poaceae species, eg. application of 3 mM and 0.45 mM Mg alleviated Al toxicity in wheat (Ryan et al. 1997) and maize (Watanabe and Okada, 2005), respectively. Similarly, we found exogenous application of Mg efficiently ameliorated Al-induced root elongation and plant growth in this study (Fig. 1 and 2). In addition, 2 mM Mg significantly reduced Al accumulation in maize roots, further confirming millimolar concentrations of Mg could effectively alleviate Al toxicity in Poaceae species. The alleviation of Al toxicity and reduction of Al accumulation in maize by millimolar concentrations of Mg might be due to 1) a decrease in extracellular free Al^{3+} activity and 2) blocking of Al uptake by competition for transporters. The differences in concentrations of Mg (eg.

millimolar vs micromolar) needed for alleviation of Al toxicity in monots vs dicots might be due to differential cell wall chemistry, Mg uptake efficiency and cellular Mg concentration (White et al. 2018).

The balance between generation and scavenging of ROS (such as superoxide anion and H₂O₂) was disturbed under a range of environmental stresses, including Al toxicity (Mittler, 2002; Richards et al. 1998). Overproduction of ROS contributes to Al toxicity through causing cell membrane peroxidation, structural damage in cells, chromosomal aberration, and programmed cell death (Yi et al. 2010; Yoko et al. 2001). To alleviate such oxidative stress, plants have evolved mechanisms to scavenge ROS via the up-regulation of genes encoding antioxidant enzymes and thereby enhance the activities of antioxidant enzymes, such as SOD, POD and CAT (Chen et al. 2012a). For example, Al exposure induced an efficient antioxidant system in Al-tolerant maize, but not in the Al-sensitive genotype (Giannakoula et al. 2010). Overexpression of a peroxidase gene from *Arabidopsis* elevated Al tolerance in transgenic tobacco plants (Wu et al. 2017). In addition, enhanced activities of the antioxidant enzymes due to external application of salicylic acid, nitric oxide, melatonin, and Mg were involved in alleviation of Al toxicity in *Cassia tora* (Wang and Yang, 2005; Wang et al. 2004), soybean (Zhang et al. 2017) and rice (Pandey et al. 2013). In the present study, we also found that the application of Mg caused a significant decrease in H₂O₂ accumulation (Fig. 5) that had been induced by Al toxicity. Furthermore, higher activities and gene expression levels of SOD, POD and CAT were observed after the application of Mg in the Al-treatment solutions (Fig. 6).

The data presented here showed that application of millimolar concentrations of Mg effectively alleviated Al-induced root elongation inhibition, Al accumulation and oxidative stress in maize plants. Additionally, increased expression of Mg transporter genes as well as enhancement of the gene expression and activity of antioxidant enzymes play a role in Mg alleviation of Al-induced oxidative stress in maize plants.

Conflicts of Interest

The authors declare no conflicts of interest.

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Figure captions

Fig. 1 Root elongation of maize seedlings exposed to 0 or 50 μM Al with 0 to 10.0 mM Mg for 24 h (A) or 7 d (B). The 3-day-old uniform seedlings were transferred to the treatment solution (1/5 MGRL without Mg) containing 0 (control), 0.01, 0.05, 0.15, 0.3, 2.0 or 10.0 mM MgSO_4 with 50 μM AlCl_3 (pH 4.2) for the short-term (24 h) or the long-term (7 day) treatment. Data are presented as means $\pm$ standard deviation (S.D, n=6). Different letters indicate significantly different values ($p<0.05$) using LSD test.

Fig. 2 Effect of external application of Mg on maize shoot dry weight (A), root dry weight (B) and shoot/root ratio (C) under Al stress. Three-day-old seedlings were treated by 0 or 50 μM Al with 0 to 10.0 mM Mg for 7 d (pH 4.2). The treatment solution was renewed every second day. The experiment was repeated three times, each treatment containing 12 independent biological samples. Values are means $\pm$ S.D. (n=3). Different letters indicate significantly different values ($p<0.05$) using LSD test.

Fig. 3 Effect of Mg on Al accumulation in maize roots. Morin (A) and hematoxylin (B) staining were used for detecting Al accumulation in maize root tips. C and D, quantitative analysis of Al and Mg in maize roots. The 3-day-old seedlings were treated by 0 or 50 μM Al with or without 2 mM Mg for 24 h. The plants grown in the nutrient solution with the standard Mg concentration (0.3 mM) and without Al were used as the control (Con). White (A) and black (B) scale bars represent 500 μm . For C and D, values are means $\pm$ S.D. (n=3). Different letters indicate significantly different values ($p<0.05$) using LSD test.

Fig. 4 Expression levels of genes encoding putative Mg transporters in maize roots. A, Evolutionary relationships of the amino acid sequence of AtMGT1 and OsMGT1 and its homologues in maize. The evolutionary history was inferred using the Neighbor-Joining method of MEGA7 program. B, Relative expression of *ZmMGT1*, *ZmMGT2*, *ZmMGT8*, and *ZmMGT9* in maize roots. The 3-day-old seedlings were treated by 50 μM Al with or without 2 mM Mg for 24 h. The plants grown in the nutrient solution with the standard Mg concentration (0.3 mM) and without Al were used as the control (Con). Values are means $\pm$ S.D. (n=3). Different letters indicate significantly different values ($p<0.05$) using LSD test.

Fig. 5 H₂O₂ concentration in roots of maize seedlings under Al stress with variable concentrations of Mg in the rooting medium (0, 0.05, 0.3, 2.0, and 10.0 mM Mg). Values are means $\pm$ S.D. (n=3). Different letters indicate significantly different values ($p < 0.05$) using LSD test.

Fig. 6 Effect of external application of Mg on the activity (A) and gene expression (B) of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in maize roots under Al stress. Three-day-old maize seedlings were treated by 0 or 50 μ M Al supplemented with 0, 0.05, 0.3, 2.0, or 10.0 mM Mg for 24 h. Values are means $\pm$ S.D. (n=3-6). Different letters indicate significantly different values ($p < 0.05$) using LSD test.

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