Title: Magnesium Promotes Root Growth and Increases Aluminum Tolerance via Modulation of Nitric Oxide Production in *Arabidopsis*

Short running title: Mg promotes root growth and increases Al tolerance

Dongxu Li[1], Wenna Ma[1], Jian Wei[1], Yawen Mao[1,2], Zhongping Peng[1], Jiarong Zhang[1], Xiangying Kong[1], Qinqin Han[1], Wei Fan[4], Ye Yang[1], Jianghua Chen[2], L Wu[2], Zed Rengel[3], Qi Chen[1]*

[1] Faculty of Life Science and Technology, Kunming University of Science and Technology, Jingming South Road, Kunming, 650500, China

[2] Key Laboratory of Tropical Plant Resources and Sustainable Use, CAS Center for Excellence in Molecular Plant Sciences, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, 650023, China

[3] UWA School of Agriculture and Environment, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Perth WA 6009, Australia

[4] College of Resources and Environment, Yunnan Agricultural University, Kunming, 650201, China

Abstract

Aluminum (Al) toxicity and magnesium (Mg) deficiency often coexist in acidic soils. Nitric oxide (NO) is an important signaling molecule involved in diverse physiological processes and stress responses in plants. In this study, we investigated the role of NO and Mg availability in promotion of root growth and Al tolerance in Arabidopsis. The results showed that both Al toxicity- and Mg deficiency-induced NO production contributed to inhibition of primary root growth and the root cell cycle progression. Additionally, the NO production and root growth inhibition were aggravated in the absence of Mg under Al stress conditions. In contrast, Mg supply promoted root growth associated with decreasing NO production under Al stress and/or Mg deficiency conditions. Magnesium decreased the activities and expression of the genes related to NO biosynthesis enzymes in wild type Col-0, but not in the Mg transporter mutant seedlings (*mgt1*) in the presence or absence of Al toxicity. Accordingly, the *mgt1* mutant plants exhibited high Al accumulation, NO concentration and Al sensitivity in comparison with the Col-0 plants under Al stress. The NO-associated protein 1 mutant *noa1* and the nitrate reductase mutant *nia1nia2* with impaired NO production showed Al toxicity- and Mg deficiency-insensitive phenotypes, further confirming reduction of NO production was involved in Mg-mediated root growth promotion under Al toxicity and/or Mg deficiency conditions. Taken together, our results suggested that Mg-mediated enhancement of root growth and Al tolerance is associated with altering NO production in *Arabidopsis*.

Key words: Aluminum toxicity; Magnesium; Root growth; Nitric oxide; *Arabidopsis*

Introduction

Approximately 40% of the world's arable land contains acid soils (Kochian et al. 2004). Aluminum (Al) is an integral part of mineral soils and accounts for up to 7% by weight of the earth's crust. On acidic soils with a pH 5.5_{water} or below, Al is solubilized into Al^{3+}, which is highly phytotoxic and becomes a major limiting factor for plant growth and development (Ryan et al. 2011). Plant root growth inhibition is the first symptom of Al toxicity. Interference with cell elongation and apical meristematic zone cell division is involved in Al-induced root growth inhibition (Kochian 1995; Barcelo and Poschenrieder 2002).

Nitric oxide (NO) is a universal cellular signaling molecule participating in diverse physiological processes. NO synthase (NOS) is the main source for NO production in animals (Crawford 2006). Although the NOS activity could also be detected in plants (Liu et al., 2017; Zottini et al., 2007), the existence of NOS in plants remains controversial because the gene encoding NOS is still unknown. *Arabidopsis NO associated 1* (*AtNOA1*) was initially considered to encode a putative NOS (Guo et al., 2003). However, accumulating evidence indicated that AtNOA1 does not have an NOS activity, but a circular permuted GTPase activity that plays an important role in chloroplast functions (Flores-Perez et al. 2008; Moreau et al. 2008). Nevertheless, AtNOA1 is involved in NO synthesis (Zottini et al., 2007). On the other hand, nitrate reductase (NR) is one of the most important sources for NO synthesis in plants, being associated with nitrogen assimilation and NO production (Kaiser et al. 2002).

The NO signaling is involved in several stresses responses, including Al toxicity. For example, external SNP application (sodium nitroprusside, a NO donor) significantly ameliorated Al toxicity in *Hibiscus moscheutos* (Tian et al., 2007) and red kidney bean (Wang et al., 2010). However, in some plants such as rice bean (Zhou et al., 2012) and alfalfa (Chen et al. 2014), NO aggravated Al-mediated inhibition of root growth because the external application of tetramethylimidazoline-1-oxyl-3-oxide (cPTIO, a scavenger of NO), N^G nitro-L-Arg-methylester (L-NAME, an inhibitor of NOS) or tungstate (an inhibitor of NR) decreased NO production and promoted root elongation under Al stress. Similarly, we recently found that Al-mediated increase in NO production contributed to root growth elongation in *Arabidopsis* (Zhang et al., 2018). Additionally, the NO biosynthesis-related mutant lines of *noa1* (lacking *nitric oxide-associated1*) and *nia1nia2* (lacking *nitrate reductase1* and *2*) showed Al-insensitive phenotype with longer primary root length and lower Al accumulation. It is likely that the dual roles of NO in the Al response are dependent on its concentrations in different plant

tissues/organs.

Magnesium (Mg) is an essential nutrient in plants and animals. Magnesium deficiency often occurs in acidic soils due to low cation exchange capacity, ease of leaching and the competition with Al for plant uptake (Guo et al. 2016; Rengel et al. 2015). Several studies have shown that Mg supply is essential for plant Al resistance (Rengel et al. 2015). For example, millimolar concentrations of Mg reduced Al toxicity in monocot plants (eg. wheat and rice) by competing with Al for binding sites on the plasma membrane (Chen et al. 2012; Ryan et al. 1997; Watanabe and Okada 2005). In dicotyledons such as soybean (Silva et al. 2001), rice bean (Yang et al. 2007) and faba bean (Chen et al. 2015), micromolar concentrations of Mg significantly alleviated Al toxicity via increasing citrate exudation. Moreover, overexpression of Mg transporter genes was associated with Al resistance in yeast (MacDiarmid and Gardner 1998) and tobacco (Deng et al. 2006). However, the signaling pathway underlying Mg supply and Al toxicity in plant root growth remains elusive.

In this study, we investigated the role of NO signaling in Mg-mediated root growth promotion and Al tolerance in *Arabidopsis*. The results showed that both Mg deficiency- and Al toxicity-induced inhibition of primary root growth and cell cycle progression was associated with an increase in NO production in *Arabidopsis*. Application of Mg alleviated NO-mediated root growth inhibition and the cell cycle arrest under Al stress and/or Mg deficiency conditions. The application of Mg promoted root elongation through modulating the activities and expression of the genes encoding NO production-related enzymes in the presence or absence of Al toxicity. Additionally, genetic analysis using the Mg transporter mutant (*mgt1*) and NO biosynthesis-related mutants further confirmed that decreasing the NO production is involved in Mg-mediated promotion of root growth and Al tolerance in *Arabidopsis*.

Results

Magnesium supply increased root growth in the presence or absence of Al toxicity

Compared with the Mg deficiency treatment (0 μM Mg), the application of 10, 50, 100 or 300 μM Mg significantly promoted primary root length (by 22.3, 25.2, 21.4 and 21.2%, respectively) (Fig. 1a). In contrast, the highest concentration of Mg (2000 μM) decreased root growth by 34.3% (Fig. 1a). The root growth was inhibited by 31.8% after exposure to 50 μM $AlCl_3$ in the absence of Mg (Fig. 1b and c); in contrast, application of 10 to 300 μM Mg significantly increased primary root growth in the presence

of Al (Fig. 1b). Although 1000 and 2000 μM Mg significantly increased the relative root growth in comparison with the Al-only treatment (Fig. 1c), the total root length showed significant inhibition (Fig. 1b). These results indicated that optimal concentrations of Mg (eg. 10-300 μM) are essential for *Arabidopsis* primary root growth and alleviation of Al toxicity. Additionally, root growth did not show any significant difference between the treatments with $MgSO_4$ and $MgCl_2$, indicating that it was Mg^{2+}, rather than SO_4^{2-} or Cl^-, that alleviated Al-induced root growth inhibition. Given that 10-300 μM $MgCl_2$ or $MgSO_4$ were equally effective in alleviating Al toxicity, the 10 μM $MgCl_2$ was chosen for the following experiments.

Magnesium-mediated root growth promotion and Al toxicity alleviation were associated with decreasing NO production

Nitric oxide (NO) is a ubiquitous signaling molecule in regulating root growth. We therefore tested the role of NO in root elongation in *Arabidopsis* stressed by various Al concentrations with or without 10 μM $MgCl_2$. Compared with the Mg deficiency (-Mg) treatment, the root elongation was increased by 24%, but NO concentration was decreased 16%, after application of 10 μM $MgCl_2$ in the treatment solution (Fig. 2a, b and c). The root growth inhibition (Fig. 2a, Fig. S1a) and NO production (Fig. 2b and c; Fig. S1b) were significantly increased with an increase in Al concentration in the treatment medium under Mg deficiency conditions. However, the toxic effects of 10-100 μM Al on root growth inhibition associated with increased NO production were significantly alleviated by the application of 10 μM Mg (Fig. 2a-c; Fig. S1a and b). However, the treatment of 10 μM Mg failed to alleviate root growth inhibition and NO production associated with the 200-500 μM Al treatments, likely due to high concentrations of Al causing too severe a toxicity effect (Fig. 2a-c). Additionally, the primary root elongation exhibited a significant negative linear relationship with NO concentration in *Arabidopsis* stressed with Al in the presence or absence of Mg (Fig. 2d). Moreover, the interaction between Mg and Al in the regulation of root growth (Fig. 2a) and NO production (Fig. 2c) was significant ($p<0.0001$). These results indicated that (i) both Mg-deficiency and Al-induced root growth inhibition might be related to increased NO production, and (ii) Mg-mediated root growth promotion and Al toxicity alleviation are associated with decreasing NO production.

Decreasing NO production alleviated Al-induced root growth inhibition in a Mg-independent

manner

The role of NO in Mg deficiency- and Al-induced root growth inhibition was further confirmed after the seedlings were treated by Al in the presence or absence of Mg, SNP (sodium nitroprusside, a NO donor), tungstate (Na_2WO_4, a nitrate reductase inhibitor), L-NAME (N^G nitro-L-Arg-methylester, a nitric oxide synthase inhibitor) and cPTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, a NO scavenger). The results showed that the NO fluorescent intensity (Fig. 3a and Fig. S2) and root growth inhibition (Fig. 3b) were significantly increased by the Mg deficiency, Al toxicity, SNP or Al+SNP treatments. However, the application of Mg significantly decreased NO production associated with Mg deficiency, Al toxicity (Fig. 3a and Fig. S2) and root growth inhibition (Fig. 3b). Moreover, the addition of tungstate, L-NAME and cPTIO significantly promoted root elongation in the presence or absence of Al in an Mg-independent manner (Fig. 3b), which coincided with a decrease in NO production (Fig. 3a, Fig. S2). The primary root growth inhibition and relative NO fluorescent intensity exhibited a significant negative linear relationship (Fig. 3c). These results indicated that Mg supply increased Al tolerance via decreasing NO production.

Disturbance of cell division was involved in inhibition of root growth mediated by Mg deficiency and/or Al toxicity

The reduction in root growth can be caused by disturbing a division potential of meristematic cells in plant roots. Therefore, we analyzed the cell cycle progression using transgenic lines expressing CYCB1;1::GUS, allowing the visualization of cells in the G2-M phase of the cell cycle. The Mg deficiency (-Mg) and Al (+Al) toxicity treatments obviously decreased the GUS activity in CYCB1;1::GUS (Fig. 4b and c), which coincided with the inhibition of primary root elongation (Fig. 4a). Additionally, the application of SNP further decreased the primary root growth and GUS activities (Fig. 4a-c). On the contrary, the application of $MgCl_2$ or cPTIO significantly increased the GUS staining (Fig. 4b) and activity (Fig. 4c) in the roots of CYCB1;1::GUS seedlings in the presence or absence of Al. Moreover, the relative root growth showed a significant linear correlation with GUS activities in roots of CYCB1;1::GUS seedlings (Fig. 4d). These results indicated that Mg supply promoted root growth, at least partly through decreasing NO-mediated interference with the mitotic potential of stem cells under Mg deficiency and/or Al toxicity conditions.

Aluminum decreased the expression of genes encoding Mg transporters

There are 10 Mg transporter genes in *Arabidopsis* genome. Among them, *AtMGT1*, *AtMGT7* and *AtMGT9* show increased expression in roots and are involved in Mg uptake (Chen et al. 2012; Gebert et al. 2009; Li et al. 2001). The expression of *AtMGT1*, *AtMGT7* and *AtMGT9* was significantly decreased by Al toxicity and Mg deficiency, whereas the application of Mg increased expression of all of these three genes regardless of the presence or absence of Al (Fig. 5).

The *mgt1* mutant with decreased Mg accumulation showed increased Al sensitivity and NO production

AtMGT1 is a high-affinity Mg transporter associated with Mg assimilation and resistance to Al toxicity (Deng et al. 2006); therefore, the mutant line lacking *AtMGT1* and having Mg deficiency phenotype (Fig. S3) was used to further investigate the role of NO in Mg-mediated root growth promotion and Al toxicity alleviation in *Arabidopsis*. The application of Mg to the root medium significantly increased fluorescent intensity of the Mg-selective fluorescent dye (Magnesium™ Green, AM; Fig. 6a) and total Mg concentration (Fig. 4Sa), whereas Al application decreased Mg accumulation in the Col-0 roots. In contrast, Mg concentration was lower in the *mgt1* mutant than Col-0 roots, even when Mg was supplied in the medium (Fig. 6a, Fig. S4a), indicating that AtMGT1 plays an important role in Mg uptake.

The application of Mg significantly decreased morin fluorescence intensity and Al concentration in Col-0, but not in the *mgt1* mutant roots (Fig. 6b, Fig. S4b). In the absence of Al, the root elongation of *mgt1* mutant seedlings was decreased by 17.2% in comparison with the wild-type Col-0 (Fig. 6c and Fig. S5). Furthermore, the *mgt1* mutant seedlings were more susceptible to Al toxicity than the wild-type seedlings (Fig. 6c and Fig. S5), eg. the relative root growth of *mgt1* was decreased by 15.4% in comparison with wild type (Col-0) plants under Al stress (Fig. 6c and Fig. S5). Moreover, application of Mg failed to increase Al tolerance in *mgt1* seedlings. Similarly, the NO production in roots was significantly increased in *mgt1* after Al treatment, this increase being greater than in the Col-0 plants (Fig. 6d and Fig. S6). An exogenous addition of Mg did not reduce NO production in the *mgt1* roots in the presence or absence of Al. These results indicated that AtMGT1-mediated Mg uptake was essential for root growth promotion and Al toxicity alleviation associated with decreased NO production in *Arabidopsis*.

Modulating gene expression and activities of enzymes related to NO biosynthesis was involved in Mg-mediated enhancement of root growth and Al tolerance

Having ascertained that NO is involved in Mg-mediated root growth promotion and Al alleviation, we then analyzed the expression of genes encoding the NO-associated enzymes, including NO associated 1 (*AtNOA1*) and nitrate reductase (*AtNIA1* and *AtNIA2*) in roots of Col-0 and *mgt1* (Fig. 7a-c). The results showed that either Al toxicity or Mg deficiency induced the expression of *AtNOA1*, *AtNIA1* and *AtNIA2*, whereas application of Mg reduced the expression of these three genes in roots of Col-0, but not in *mgt1* regardless of the presence or absence of Al toxicity. Additionally, the expression of these genes was higher in *mgt1* than the wild type Col-0 under Al stress.

We then examined the activities of the nitrate reductase (NR) and NO synthase like (NOS-like) enzymes in Col-0 and *mgt1* plants (Fig. 7d and e). The activities of these two enzymes were induced by Mg deficiency or Al toxicity in roots of both Col-0 and *mgt1*, but more so in the latter, which coincided with the changes in NO concentrations. The application of Mg significantly decreased the Al toxicity- and Mg deficiency-induced activities of NR and NOS in the Col-0 roots, but not in the *mgt1* mutant seedling roots.

The root growth was examined in the NR-null-deficiency double mutant *nia1,2* and the NOA1-deficient mutant *noa1*, both of which show lower cellular NO concentrations than the Col-0 plants (Desikan et al., 2002; Guo, 2006). The results showed that the *noa1* and *nia1nia2* mutant lines were insensitive to Al toxicity or Mg deficiency (Fig. 7f). Additionally, the application of Mg increased root elongation about 21% in Al-treated Col-0, whereas the relative root growth did not show significant difference between the Al and Al+Mg treatments in *noa1* and *nia1nia2*. These results further confirmed that Mg supply increased root growth and Al tolerance by modulating the NO-synthesis related enzymes that mediate NO production in *Arabidopsis*.

Discussion

Magnesium is an important divalent cation, influencing more than 300 enzymes in plant cells (Bose et al. 2011; Chen et al. 2015). Due to easy leaching, Mg deficiency often occurs in acidic soils and may become a constraint for plant growth and crop production (Rengel and Zhang 2003). Plant responses to Mg deficiency include an enhancement of antioxidant enzymes (Cakmak and Kirkby 2008; Cakmak

and Marschner 1992; Hermans et al. 2010) and inhibition of sucrose phloem loading (Abdel-Basset et al. 2010). Additionally, Mg supply enhances root growth and development through regulating auxin, ROS, NO and Ca^{2+} signals (Liu et al. 2017; Niu et al. 2014; Niu et al. 2015). In the present study, we observed that either Mg deficiency or Al toxicity induced NO burst in *Arabidopsis* roots (Fig. 2b and c; Fig. S1b), indicating that activation of NO production might be a conserved mechanism governing root growth inhibition caused by Mg deficiency or Al toxicity. With an application of relatively low Mg concentration (eg. 10 μM) under Al toxicity and/or Mg deficiency conditions, the NO concentration was significantly decreased and root growth inhibition was alleviated (Fig. 1; Fig. 2a-c; Fig. S1). The primary root growth exhibited a significantly negative linear correlation with NO concentration in *Arabidopsis* stressed by Al in the presence or absence of Mg (Fig. 2d). Furthermore, the interaction between Mg and Al significantly ($p<0.0001$) influenced root growth and NO production (Figs. 1 and 2). These results indicated that both Mg-deficiency- and Al-induced root growth inhibitions might be related to increased NO production; nevertheless, the application of Mg significantly enhanced Al resistance by interfering with the NO signaling in *Arabidopsis*.

The balance between cell elongation and cell division determines root elongation and growth (Beemster and Baskin 1998). The primary target of Al toxicity is plant root apex. Interference with cell elongation and division is involved in inhibition of root growth under Al stress (Kochian 1995; Barcelo and Poschenrieder 2002; Rounds and Larsen 2008; Ruíz-Herrera and López-Bucio 2013; Zhang et al. 2018). It is generally accepted that NO regulates root growth and development under normal and stress conditions. NO showed a similar action with auxin on root growth and development, promoting at low but inhibiting at high concentrations (Stohr and Stremlau 2006), which might be dependent on the sensitivity to the changes in NO concentration in different plant species and tissues/organs. For example, while NO production was decreased by Al in roots of *Hibiscus moscheutos* (Tian et al. 2007) or red kidney bean (Wang et al. 2010), SNP application significantly enhanced root elongation in these two plant species in the presence of Al toxicity. By contrast, exogenous NO aggravated Al-mediated inhibition of root elongation in rice bean (Zhou et al. 2012) and alfalfa (Chen et al. 2014), which might be attributed to Al-induced overaccumulation of NO in roots. In *Arabidopsis*, overproduction of NO interfered with the cell cycle and quiescent center activities and was involved in root growth inhibition induced by methyl 3-(4-hydroxyphenyl) propionate, Cd toxicity or Al toxicity (Liu et al. 2016; Yuan and Huang 2016; Zhang et al., 2018). Comparable results were obtained in the study presented here.

The roots of *Arabidopsis* showed high sensitivity to NO production given that decreasing NO production increased root growth under Al stress and/or Mg deficiency (Fig. 3). Moreover, the root growth inhibition was related to a decrease in GUS staining and activity in roots of CYCB1;1::GUS seedlings under Al toxicity and/or Mg deficiency (Fig. 4a-c). Application of SNP further exacerbated this decreasing effect, whereas the opposite was observed for the treatment with cPTIO or Mg. In the presence of Mg, an Al- or SNP-induced decreases in root elongation and GUS activity were significantly alleviated (Fig. 4a-c). Additionally, the relative root elongation and GUS activity showed a significant positive linear relationship (Fig. 4d), indicating that Al toxicity-induced root growth inhibition is, at least partly, related to the NO-mediated arresting of cell cycle and disturbance of cell division. Moreover, Mg supply is essential for alleviation of Al toxicity by lessening NO-mediated disturbance of cell division.

Nitrate reductase (NR) and nitric oxide synthase (NOS) are the two key enzymes in NO synthesis. Overproduction of NO under Mg deficiency might be brought about by regulating the activities of the NOS-like and NR enzymes. For example, it has been reported that Mg deficiency induced NO production in pig endothelial cells (Howard et al. 1995) and rat plasma (Rock et al. 1995) by increasing NOS activity. In *Arabidopsis*, Mg-deficiency-induced NO production was associated with activating the NR and NOS-like enzymes (Liu et al. 2017). Similarly, we also found that either Al toxicity or Mg deficiency caused an increase in NO production (Fig. 2b and c, Fig. 6D, Fig. S2 and Fig. S6) by enhancing the NR and NOS-like activities in wild type Col-0 seedlings (Fig. 7d and e). Moreover, RT-PCR showed that Al toxicity or Mg deficiency induced the expression of the NO-biosynthesis related genes, including *AtNOA1*, *AtNIR1* and *AtNIR2* (Fig. 7a-c). Genetic analysis using Mg transporter mutant (*mgt1*) seedlings further confirmed that Mg mediated root growth promotion and alleviated Al toxicity via lessening of NO overproduction brought about by modulating the expression of genes encoding the NO-biosynthesis related enzymes (Fig. 7a-c). For example, compared with the wild type Col-0, the application of Mg to the Al-stressed *mgt1* roots failed to (i) reduce NO production (Fig. 6 d), (ii) alleviate root growth inhibition (Fig. 6c), (iii) decrease activities of the NR and NOS-like enzymes (Fig. 7d and e), and (iv) decrease the expression of *AtNOA1*, *AtNIA1* and *AtNIA2* (Fig. 7a-c).

The expression of *AtMGT1*, *AtMGT7* and *AtMGT9*, which are mainly expressed in roots (Chen et al. 2012; Gebert et al. 2009; Li et al. 2001), was decreased by the Al treatments (Fig. 5), indicating that inhibition of Mg uptake by Al might be attributed, at least partly, to decreasing the expression of these

genes. *AtMGT1* is dominantly expressed in the root cells, and is responsible for Mg uptake from soils (Guo et al., 2015). Under normal growth conditions, the *mgt1* plants showed the yellow-leaf phenotype (Fig. S3a), and Mg foliar application turned yellow leaves to green again (data not shown), confirming that *AtMGT1* is essential for root Mg uptake. Additionally, involvement of the MGT1 transporter family-mediated Mg uptake in alleviation of Al toxicity was shown in other plant species. For example, expression of *OsMGT1* was involved in Al tolerance in rice (Chen et al. 2012) and overexpression of *Arabidopsis AtMGT1* enhanced Al tolerance in tobacco (Deng et al. 2006). In the present study, we found that the *mgt1* had lower Mg (Fig. 6a and Fig. S4a) and higher Al concentrations in roots (Fig. 6b and Fig. S4b) and showed higher Al sensitivity than wild-type Col-0 (Fig. 6c), which coincided with higher expression of the NO-biosynthesis related genes (Fig. 7a-c), higher activities of the NR and NOS-like enzymes (Fig. 7 d and e), and higher NO production in comparison with Col-0 roots (Fig. 6d). These results indicated that AtMGT1-mediated Mg uptake is essential for Al resistance in *Arabidopsis*.

The data obtained in this study suggested that Mg deficiency or Al toxicity induced primary root inhibition, at least partly by NO-mediated interference with the root cell cycle progression in *Arabidopsis* (Fig. 8). Nevertheless, Mg supply was essential for enhancement of Al tolerance via modulating NO biosynthesis.

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana Columbia ecotype (Col-0) was used throughout. The transgenic line CYCB1;1::GUS and the mutant lines of *nia1nia2* and *noa1* were used in this study. The homozygous seeds of *mgt1* (SALK100361C) mutant line was obtained from ABRC (Arabidopsis Biological Resource Center). The seeds were surface-sterilized in 75% (v/v) ethanol for 5 min and 8% (w/v) sodium hypochlorite for 15 min, washed five times in distilled water, and sown on plates with 1/6 Murashige and Skoog (MS) agar medium [containing 1.0% (w/v) sucrose and 0.8% (w/v) agar, pH 5.7]. After incubation at 4°C in the dark for 2-3 days, the plates were positioned vertically in a growth chamber at 22°C under 16 h light/8 h dark conditions.

Treatments

After 6 days of growth, the seedlings were transferred onto $^1/_5$ MGRL medium (Fujiwara et al. 1992),

supplemented with 0-2000 μM Mg, 0 or 50 μM Al, and various chemicals we use regularly (eg. Kan et al. 2016), including 10 μM SNP, 10 μM tungstate, 100 μM L-NAME and/or 100 μM cPTIO. All chemical reagents were obtained from Sigma-Aldrich. The plants were incubated under the conditions specified above for 4 days.

Primary root length measurement

The root elongation was measured as we described previously (Zhang et al., 2018). Briefly, 6-day-old seedlings about 1 cm long were transferred to Petri dishes containing agar-solidified $^1/_5$ MGRL medium (pH 4.5) with or without the chemicals as described above. After grown vertically for an additional 4 d, the seedlings were photographed using a digital camera, and primary root length was analyzed by ImageJ.

Measurements of nitric oxide production

After treatments, the roots were placed in 2.5 μM DAF-2DA solution (4,5-diaminofluorescein diacetate, a fluorescent probe for NO; excitation 491 nm and emission 531 nm) at 37°C for 20 minutes. The roots were then washed 3-5 times in distilled water; the NO accumulated in the *Arabidopsis* roots was measured using a Leica fluorescence microscope (DMI3000B). The ImageJ program was used for quantification of fluorescence intensity.

The measurement of NO concentration in *Arabidopsis* roots was performed as previously described (Kan et al., 2016). After several rinses in deionized water, roots were gently blotted and weighed, followed by homogenization in 50 mM acetic acid solution (pH 3.6) containing 4% (w/v) zinc acetate. After centrifugation at 9,500 *g* at 4°C for 15 minutes, NO concentration was measured using an NO detection kit (Beyotime Institute of Biotechnology, China).

GUS staining and quantitative GUS assay

GUS staining analysis was performed as previously described (Jefferson et al. 1987). Roots were incubated in GUS staining solution containing 2 mM potassium ferricyanide, 1 mg/mL 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, 10 mM ethylenediamine tetraacetic acid (EDTA), 0.1% (v/v) Triton X-100 in 100 mM Na phosphate buffer (pH 7.0) at 37°C overnight. After rinsing in deionized water, roots were viewed under a microscope (Nikon, Optiphot-2) and photographed.

For quantitative GUS assay, approximately 100 mg roots were homogenized in ice-cold potassium phosphate buffer (50 mM, pH 7.8) supplemented with 0.1 mM ascorbic acid, 1% (w/v) PVPP (polyvinylpolypyrrolidone) and 0.2 mM EDTA-Na_2. After centrifugation at 12,000 *g* at 4°C for 20 min, the supernatant was used for quantitative GUS assays as previously described (Jefferson et al. 1987) using 4-methyl-umbelliferyl-β-D-glucuronide (Sigma-Aldrich) as a substrate. The fluorescence was detected by an Infinite M200 Pro microplate reader (Tecan, Research Triangle Park, NC, USA). The protein concentrations were analyzed using Bradford method (Bradford 1976).

Magnesium™ Green and morin staining

The intracellular Mg concentration in root tips was measured using a high-affinity Mg-selective fluorescent dye {Magnesium™ Green, AM; glycine, N-[2-(carboxymethoxy)-4-[[(20,70-dichloro-30,60-dihydroxy-3-oxospiro[isobenzofuran-1(3H),9'-[9H] xanthen]-5-yl)carbonyl]amino]phenyl]-N-carboxymethyl-, acetoxymethyl ester} (Molecular Probes, Invitrogen, Carlsbad, CA, USA; excitation 506 nm and emission 531 nm), which can permeate across the cell membrane and emit fluorescence upon binding to Mg; we have previously used Magnesium™ Green AM dye to measure cytosolic Mg concentrations in *Arabidopsis* roots under Al stress (Bose et al. 2013). A stock solution of Magnesium™ Green AM dye was made by adding 50 μL of DMSO (dimethyl sulfoxide) to one vial of the dye (50 μg). Roots were immersed in this solution for 30 min in the dark.

Aluminum accumulation in *Arabidopsis* roots was detected using the morin (excitation 440 nm and emission 510 nm) staining method. After treatments, roots were washed with deionized water and then incubated in 100 μM morin hydrate (Sigma-Aldrich) for 15 min in the dark. After 3-5 washes in deionized water to remove residual dye from the root surface, observations were performed on a fluorescence microscope (Leica, DMI3000B).

Magnesium and aluminum quantification

After treatments, roots were sampled, washed several times in deionized water and weighed. These samples were then ashed at 550°C for 12 h and dissolved in 1 mL of 2 mM HCl overnight. After dilution to 5 mL with deionized water, concentrations of Mg and Al were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, model PS-1000, Leeman Labs., Lowell, MA,

USA).

Measurement of nitric oxide synthase and nitrate reductase activity

After treatments, roots were homogenized in extraction solution containing 50 mM Hepes-KOH (pH 7.5), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μM flavin adenine dinucleotide (FAD), 1 mM dithiothreitol (DTT), 10 mM $MgCl_2$, and 5% (v/v) glycerol. The homogenates were centrifuged at 13,000 *g* at 4°C for 20 min. The Bradford method was used to measure protein content (Bradford 1976). The activity of nitrate reductase was measured according to the method described by Kan et al. (2016). The activity of NO synthase-like enzyme was determined using an NOS Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis

RNAiso Plus (Takara, Dalian, China) was used to isolate the total RNA using approximately 100 mg of root tissue according to the manufacturer's instructions. Quantitative reverse transcription was performed using a PrimeScript RT reagent kit with gDNA eraser (Takara, Dalian, China). PCR was performed using a Bio-Rad CFX-96 real-time PCR system. The specific primers of the detected genes were used as shown in supplemental Table 1.

Statistical analysis

All experiments were repeated at least three times. For relative root growth, at least 20 roots were analyzed for each treatment. JMP 13.2 (SAS Institute, Cary, NC) with one-way or two-way ANOVA was used for statistical tests followed by Tukey HSD. Bars marked with the same letter do not differ from each other at the significance level of $p<0.05$ according to Tukey HSD.

Author Contributions

Qi Chen conceived and designed the study, analyzed the data, interpreted the results, wrote and revised the manuscript. Dongxu Li performed most of the experiments, analyzed the data and wrote the manuscript. Wenna Ma, Jian Wei, Yawen Mao, Zhongping Peng, Jiarong Zhang, Xiangying Kong and Qinqin Han provided technical assistance. Wei Fan and Ye Yang analyzed the data and commented on the writing of the manuscript. Zed Rengel and Jianghua Chen analyzed the data and revised the

manuscript. All authors discussed the results and commented on the manuscript.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 31660595 and 31360340), Science and Technology Project of Yunnan province (2017FB063 and 2015FB121). Zed Rengel was supported by Australian Research Council (DP160104434).

Disclosure

The authors declare that they do not have a conflict of interest.

References

Abdel-Basset, R., Ozuka, S., Demiral, T., Furuichi, T., Sawatani, I., Baskin, T.I., Matsumoto, H., Yamamoto, Y. (2010) Aluminium reduces sugar uptake in tobacco cell cultures: a potential cause of inhibited elongation but not of toxicity. *J Exp Bot.* 61: 1597-1610.

Barcelo, J., Poschenrieder, C. (2002) Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. *Environ Exp Bot.* 48: 75-92.

Beemster, G.T., Baskin, T.I. (1998) Analysis of cell division and elongation underlying the developmental acceleration of root growth in *Arabidopsis thaliana*. *Plant Physiol.* 116: 1515-1526.

Bose, J., Babourina, O., Rengel, Z. (2011) Role of magnesium in alleviation of aluminium toxicity in plants. *J Exp Bot.* 62: 2251-2264.

Bose, J, Babourina, O, Shabala, S, Rengel, Z. (2013) Low-pH and aluminum resistance in *Arabidopsis* correlates with high cytosolic magnesium content and increased magnesium uptake by plant roots. *Plant Cell Physiol.* 54: 1093-1104.

Cakmak, I,, Kirkby, E.A. (2008) Role of magnesium in carbon partitioning and alleviating photooxidative damage. *Physiol Plant.* 133: 692-704.

Cakmak, I., Marschner, H. (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. *Plant Physiol.* 98: 1222-1227.

Chen, M., Cui, W.T., Zhu, K.K., Xie, Y.J., Zhang, C.H., Shen, W.B. (2014) Hydrogen-rich water alleviates aluminum-induced inhibition of root elongation in alfalfa via decreasing nitric oxide production. *J Hazard Mater.* 267: 40-47.

Chen, Q., Kan, Q., Wang, P., Yu, W., Yu, Y., Zhao, Y., Li, K.Z., Chen, L.M. (2015) Phosphorylation and interaction with the 14-3-3 protein of the plasma membrane H^+-ATPase are involved in the regulation of magnesium-mediated increases in aluminum-induced citrate exudation in broad bean (*Vicia faba*. L). *Plant Cell Physiol.* 56: 1144-1153.

Chen, Z.C., Yamaji, N., Motoyama, R., Nagamura, Y., Ma, J.F. (2012) Up-regulation of a magnesium transporter gene OsMGT1 is required for conferring aluminum tolerance in rice. *Plant Physiol.*

159: 1624-1633.
Crawford, N.M. (2006) Mechanisms for nitric oxide synthesis in plants. *J Exp Bot.* 57: 471-478.
Deng, W., Luo, K.M., Li, D.M., Zheng, X.L., Wei, X.Y., Smith, W., Thammina, C., Lu, L.T., Li, Y., Pei, Y. (2006) Overexpression of an *Arabidopsis* magnesium transport gene, AtMGT1, in *Nicotiana benthamiana* confers Al tolerance. *J Exp Bot.* 57: 4235-4243.
Desikan, R., Griffiths, R., Hancock, J., Neill, S. (2002). A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. *P Natl Acad Sci USA*. 99(25), 16314-16318.
Flores-Perez, U., Sauret-Gueto, S., Gas, E., Jarvis, P., Rodriguez-Concepcion, M. (2008) A mutant impaired in the production of plastome-encoded proteins uncovers a mechanism for the homeostasis of isoprenoid biosynthetic enzymes in *Arabidopsis* plastids. *Plant Cell* 20: 1303-1315.
Fujiwara, T., Hirai, M.Y., Chino, M., Komeda, Y., Naito, S. (1992) Effects of sulfur nutrition on expression of the soybean seed storage protein genes in transgenic petunia. *Plant Physiol.* 99: 263-268.
Gebert, M., Meschenmoser, K., Svidova, S., Weghuber, J., Schweyen, R., Eifler, K., Lenz, H., Weyand, K., Knoop, V. (2009) A root-expressed magnesium transporter of the MRS2/MGT gene family in *Arabidopsis thaliana* allows for growth in low-Mg^{2+} environments. *Plant Cell.* 21: 4018-4030.
Guo, F.Q., Okamoto, M., Crawford, N.M. (2003). Identification of a plant nitric oxide synthase gene involved in hormonal signaling. *Science*. 302(5642), 100-103.
Guo, W., Nazim, H., Liang, Z., Yang, D. (2016) Magnesium deficiency in plants: An urgent problem. *Crop J.* 4: 83-91.
Hermans, C., Vuylsteke, M., Coppens, F., Cristescu, S.M., Harren, F.J.M., Inze, D., Verbruggen, N. (2010) Systems analysis of the responses to long-term magnesium deficiency and restoration in *Arabidopsis thaliana*. *New Phytol.* 187: 132-144.
Howard, A.B., Alexander, R.W., Taylor, W.R. (1995) Effects of magnesium on nitric oxide synthase activity in endothelial cells. *Am. J. Physiol.* 269: C612-618.
Jefferson, R.A., Kavanagh, T.A., Bevan, M.W. (1987) GUS fusions: beta‐glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *Embo J.* 6: 3901-3907.
Kaiser, W.M., Weiner, H., Kandlbinder, A., Tsai, C.B., Rockel, P., Sonoda, M., Planchet, E. (2002) Modulation of nitrate reductase: some new insights, an unusual case and a potentially important side reaction. *J Exp Bot.* 53: 875-882.
Kan, Q., Wu. W.W., Yu, W.Q., Zhang, J.R., Xu, J., Rengel, Z., Chen, L.M., Cui, X.M., Chen, Q. (2016) Nitrate reductase-mediated NO production enhances Cd accumulation in *Panax notoginseng* roots by affecting root cell wall properties. *J Plant Physiol*. 193, 64-70.
Kochian, L,V, (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. *Annual Annu Rev Plant Biol.* 46: 237-260.
Kochian, L.V., Hoekenga, O.A., Pineros, M.A. (2004) How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu Rev Plant Biol.* 55: 459-493.
Li, L., Tutone, A.F., Drummond, R.S., Gardner, R.C., Luan, S. (2001) A novel family of magnesium transport genes in *Arabidopsis*. *Plant Cell.* 13: 2761-2775.
Liu, M., Liu, X.X., He, X.L., Liu, L.J., Wu, H., Tang, C.X., Zhang, Y.S., Jin, C.W. (2017) Ethylene

and nitric oxide interact to regulate the magnesium deficiency-induced root hair development in *Arabidopsis*. *New Phytol.* 213: 1242-1256.

Liu, Y.Y., Wang, R.L., Zhang, P., Chen, Q., Luo, Q., Zhu, Y.Y., Xu, J. (2016) The nitrification inhibitor methyl 3-(4-hydroxyphenyl) propionate modulates root development by interfering with auxin signaling via the NO/ROS pathway. *Plant Physiol.* 171: 1686-1703.

MacDiarmid, C.W., Gardner, R.C. (1998) Overexpression of the *Saccharomyces cerevisiae* magnesium transport system confers resistance to aluminum ion. *J Biol Chem.* 273: 1727-1732.

Moreau, M., Lee, G.I., Wang, Y., Crane, B.R., Klessig, D.F. (2008) AtNOS/AtNOA1 is a functional *Arabidopsis thaliana* cGTPase and not a nitric-oxide synthase. *J Biol Chem.* 283: 32957-32967.

Niu, Y., Jin, G., Zhang, Y.S. (2014) Root development under control of magnesium availability. *Plant Signal Behav.*9: e29720.

Niu, Y.F., Jin, G.L., Li, X., Tang, C.X., Zhang, Y.S., Liang, Y.C., Yu, J.Q. (2015) Phosphorus and magnesium interactively modulate the elongation and directional growth of primary roots in *Arabidopsis thaliana* (L.) Heynh. *J Exp Bot.* 66: 3841-3854.

Rengel, Z., Bose, J., Chen, Q., Tripathi, B.N. (2015) Magnesium alleviates plant toxicity of aluminium and heavy metals. *Crop Pasture Sci* 66: 1298-1307.

Rengel, Z., Zhang, W.H. (2003) Role of dynamics of intracellular calcium in aluminium toxicity syndrome. *New Phytol.* 159: 295-314.

Rock, E., Astier, C., Lab, C., Malpuech, C., Nowacki, W., Gueux, E., Mazur, A., Rayssiguier, Y. (1995) Magnesium deficiency in rats induces a rise in plasma nitric oxide. *Magnesium Res.* 8: 237-242.

Rounds, M.A., Larsen, P.B. (2008) Aluminum-dependent root-growth inhibition in *Arabidopsis* results from AtATR-regulated cell-cycle arrest. *Cur Biol.* 18: 1495-1500.

Ruiz-Herrera, L.F., Lopez-Bucio, J. (2013) Aluminum induces low phosphate adaptive responses and modulates primary and lateral root growth by differentially affecting auxin signaling in *Arabidopsis* seedlings. *Plant Soil.* 371: 593-609.

Ryan, P.R., Reid, R.J., Smith, F.A. (1997) Direct evaluation of the Ca^{2+}-displacement hypothesis for Al toxicity. *Plant Physiol.* 113: 1351-1357.

Ryan, P.R., Tyerman, S.D., Sasaki, T., Furuichi, T., Yamamoto, Y., Zhang, W.H., Delhaize, E. (2011) The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils. *J Exp Bot.* 62: 9-20.

Silva, I.R., Smyth, T.J., Israel, D.W., Raper, C.D., Rufty, T.W. (2001) Magnesium is more efficient than calcium in alleviating aluminum rhizotoxicity in soybean and its ameliorative effect is not explained by the Gouy-Chapman-Stern model. *Plant Cell physiol.* 42: 538-545.

Stohr, C., Stremlau, S. (2006) Formation and possible roles of nitric oxide in plant roots. *J Exp Bot.* 57: 463-470.

Tian, Q.Y., Sun, D.H., Zhao, M.G., Zhang, W.H. (2007) Inhibition of nitric oxide synthase (NOS) underlies aluminum-induced inhibition of root elongation in Hibiscus moscheutos. *New Phytol.* 174: 322-331.

Wang, H.H., Huang, J.J., Bi, Y.R. (2010) Nitrate reductase-dependent nitric oxide production is involved in aluminum tolerance in red kidney bean roots. *Plant Sci.* 179: 281-288.

Watanabe, T., Okada, K. (2005) Interactive effects of Al, Ca and other cations on root elongation of rice cultivars under low pH. *Ann Bot.* 95: 379-385.

548 Yang, .JL, You, J.F., Li, Y.Y., Wu, P., Zheng, S.J. (2007) Magnesium enhances aluminum-induced
549 citrate secretion in rice bean roots (*Vigna umbellata*) by restoring plasma membrane
550 H^+-ATPase activity. *Plant Cell physiol.* 48: 66-73.
551 Yuan, H.M., Huang, X. (2016) Inhibition of root meristem growth by cadmium involves nitric
552 oxide-mediated repression of auxin accumulation and signalling in *Arabidopsis*. *Plant Cell*
553 *Environ.* 39: 120-135.
554 Zhang, Y., Guo, J., Chen, M., Li, L., Wang, L., Huang, C.F. (2018) The cell cycle checkpoint regulator
555 ATR is required for internal aluminum toxicity-mediated root growth inhibition in
556 *Arabidopsis*. *Front Plant Sci.* 9: 118.
557 Zhou, Y., Xu, X.Y., Chen, L.Q., Yang, J.L., Zheng, S.J. (2012) Nitric oxide exacerbates Al-induced
558 inhibition of root elongation in rice bean by affecting cell wall and plasma membrane
559 properties. *Phytochemistry* 76: 46-51.
560 Zottini, M., Costa, A., De Michele, R., Ruzzene, M., Carimi, F., Lo Schiavo, F.. (2007). Salicylic acid
561 activates nitric oxide synthesis in *Arabidopsis*. *J Exp Bot*. 58(6), 1397-1405.
562
563 **Figure Legends**
564 **Fig. 1** Effect of Mg and Al on primary root elongation in Col-0 *Arabidopsis*. Effect of different
565 concentrations of $MgCl_2$ or $MgSO_4$ on root length (a), primary root length (b), and relative root growth
566 (c) in the presence of 0 or 50 µM $AlCl_3$. Six-day-old Col-0 seedlings were transferred to solutions
567 containing 0, 10, 50, 300, 1000, 2000 µM $MgCl_2$ or $MgSO_4$ with 0 or 50 µM $AlCl_3$ for 4 days. Each
568 experiment was repeated at least three times, with 20 seedlings per treatment. Values are means ± S.D.
569 (n=20). Different letters indicate significantly different values ($p \leq 0.05$) using Tukey HSD test. ***, $p <$
570 0.0001; Mgf, Mg forms; Mgc, Mg concentration; Alc: Al concentration; Mgc*Alc: interaction Mg x Al;
571 ns, no significant difference.
572
573 **Fig. 2** Effects of Mg (0 or 10 µM $MgCl_2$) on root length (a) and NO production (b and c) in the
574 presence of 0-500 µM $AlCl_3$. The linear relationship between root elongation and NO concentration in
575 the presence of 0-500 µM $AlCl_3$ with or without 10 µM $MgCl_2$ (d). Six-day-old Col-0 seedlings were
576 transferred to medium containing 0-500 µM $AlCl_3$ with or without 10 µM $MgCl_2$ for 4 days. Values are
577 means ± S.D. The experiments were performed at least three times. For (a), at least 20 independent
578 seedlings per treatment per trial; for (b) and (c), n=6. In (b), the NO-specific fluorescent probe
579 DAF-2DA (4,5-diaminofluorescein diacetate) was used. Bar = 100 µm. Different letters indicate
580 significantly different values ($p \leq 0.05$) using Tukey HSD test. ***, $p < 0.0001$; Mgc, Mg concentration;
581 Alc: Al concentration; Mgc*Alc: interaction Mg x Al; ns, no significant difference.
582
583 **Fig. 3** Effect of Mg, SNP, L-NAME, tungstate and cPTIO on NO production (a) and root length (b) in

584 the presence or absence of Al. (c), the linear relationship between relative root growth and NO
585 fluorescence intensity. Six-day-old Col-0 seedlings were transferred to medium containing 50 μM Mg
586 $AlCl_3$ with or without 10 μM $MgCl_2$, 10 μM SNP (an NO donor), 100 μM L-NAME (an NOS inhibitor),
587 10 μM tungstate (an NR inhibitor), or 100 μM cPTIO (an NO scavenger) for 4 days. Values are means
588 ± S.D. (n=20). Different letters indicate significantly different values ($p \leq 0.05$).
589
590 **Fig. 4** Exogenous application of Mg alleviated Al-induced root growth inhibition associated with
591 enhancement of the meristematic cell division potential in CYCB1;1::GUS transgenic seedlings. (a),
592 Effect of Mg, SNP or cPTIO on relative root growth in CYCB1;1::GUS transgenic seedlings under Al
593 stress. (b), Images of GUS staining. (c), Quantitative GUS activity of CYCB1;1::GUS roots.
594 Six-day-old CYCB1;1::GUS transgenic seedlings were exposed to 0 or 50 μM $AlCl_3$ with or without 10
595 μM $MgCl_2$, 10 μM SNP (an NO donor) or 100 μM cPTIO (an NO scavenger) for 4 days. (d), The linear
596 relationships between relative root growth and relative GUS activity. Bar = 100 μm. For (a), n=20-25;
597 for (c), n=6. The values are means ± S.D. Different letters indicate significantly different values
598 ($p \leq 0.05$).
599
600 **Fig. 5** Expression profiles of *AtMGT1*, *AtMGT7* and *AtMGT9* in wild type (Col-0) roots exposed to 0 or
601 50 μM $AlCl_3$ with or without 10 μM $MgCl_2$ for the 4-day treatment. Values are means ± S.D. (n=3).
602 Different letters indicate significantly different values ($p \leq 0.05$).
603
604 **Fig. 6** Mg (a) and Al (b) accumulation, root length (c) and NO concentration (d) in wild type (Col-0)
605 and *mgt1* roots exposed to 0 or 50 μM $AlCl_3$ with or without 10 μM $MgCl_2$ for the 4-day treatment. For
606 (a) and (b), the treated plant roots were washed in deionized water, and the intracellular Mg or Al
607 concentrations were examined using Magnesium™ Green AM or morin fluorescent probes,
608 respectively. For (c, n=20) and (d, n=6-8), values are means ± S.D. Different letters indicate
609 significantly different values ($p \leq 0.05$) using Tukey HSD test. ***: $p < 0.0001$; Alc: Al concentration;
610 Mgc: Mg concentration; genotype*Mgc: interaction genotype x Mg supply; genotype*Alc: genotype x
611 Al supply; Mg*Al: interaction Mg x Al; genotype*Mg*Al: interaction genotype x Mg supply x Al
612 supply; ns, no significant difference.
613
614 **Fig. 7** Mg-mediated alleviation of Al toxicity and lessening of NO production was associated with

615 decreasing activities and expression of genes encoding the NO-biosynthesis related enzymes. (a) and
616 (b), effect of Mg on the activities of the NR and NOS-like enzymes. (c), (d) and (e), relative expression
617 of *AtNOA1*, *AtNIA1* and *AtNIA2* in Col-0 and *mgt1* roots in the presence or absence of Al or Mg. (f),
618 relative root growth in Col-0, *noa1* and *nia1nia2* in the presence or absence of 50 μM $AlCl_3$ and/or 10
619 μM $MgCl_2$. Values are means ± S.D. For (a) - (e), n=3. For (f), n=20. Different letters indicate
620 significantly different values ($p \leq 0.05$).
621
622 **Fig. 8** A proposed mechanism of Mg supply promoting root growth and enhancing Al resistance in
623 *Arabidopsis*. Magnesium deficiency occurs often in acidic soils due to low cation exchange capacity
624 and ease of leaching. Additionally, Al could inhibit Mg uptake (1) and cause Mg deficiency.
625 Magnesium deficiency (2) and Al toxicity (3) induced NO overproduction via increasing activities and
626 expression of the genes related to the NO biosynthesis enzymes. The overproduction of NO arrested the
627 root meristematic cells in the G2-M phase of the cell cycle (4), which was associated with root growth
628 inhibition (5). Magnesium supply (6) decreased the production of NO, and therefore alleviated cell
629 cycle arresting and promoted root growth under Al stress. Arrows indicate positive effects. Capped
630 lines indicate negative effects.
631
632 **Supplemental Data**
633 **Table S1** List of the primers and their uses
634 **Fig. S1** Effects of Mg (0 or 10 μM $MgCl_2$) on relative root growth (a) and NO relative fluorescent
635 intensity (b) in the presence of 0-500 μM $AlCl_3$.
636 **Fig. S2** The NO production in *Arabidopsis* Col-0 roots exposed to 50 μM $AlCl_3$ with or without 10 μM
637 $MgCl_2$, 10 μM SNP (an NO donor), 100 μM cPTIO (an NO scavenger), 100 μM L-NAME (an NOS
638 inhibitor) or 10 μM tungstate (an NR inhibitor) for 4 days detected using the NO-specific fluorescent
639 probe DAF-2DA (4,5-diaminofluorescein diacetate).
640 **Fig. S3** Phenotype (a) and expression of *AtMGT1* (b) in wild-type (Col-0) and the homozygous mutant
641 *mgt1*.
642 **Fig. S4** Mg (a) and Al (b) concentration in Col-0 and *mgt1* roots exposed to 0 or 50 μM $AlCl_3$ with or
643 without 10 μM $MgCl_2$ for 4 days. Values are means ± S.D (n=3-6).
644 **Fig. S5** Relative root growth of Col-0 and *mgt1* exposed to 0 or 50 μM $AlCl_3$ with or without 10 μM

645 $MgCl_2$ for the 4-day treatment.

646 **Fig. S6** Application of Mg did not decrease Al-induced NO production in *mgt1* roots.

647

648

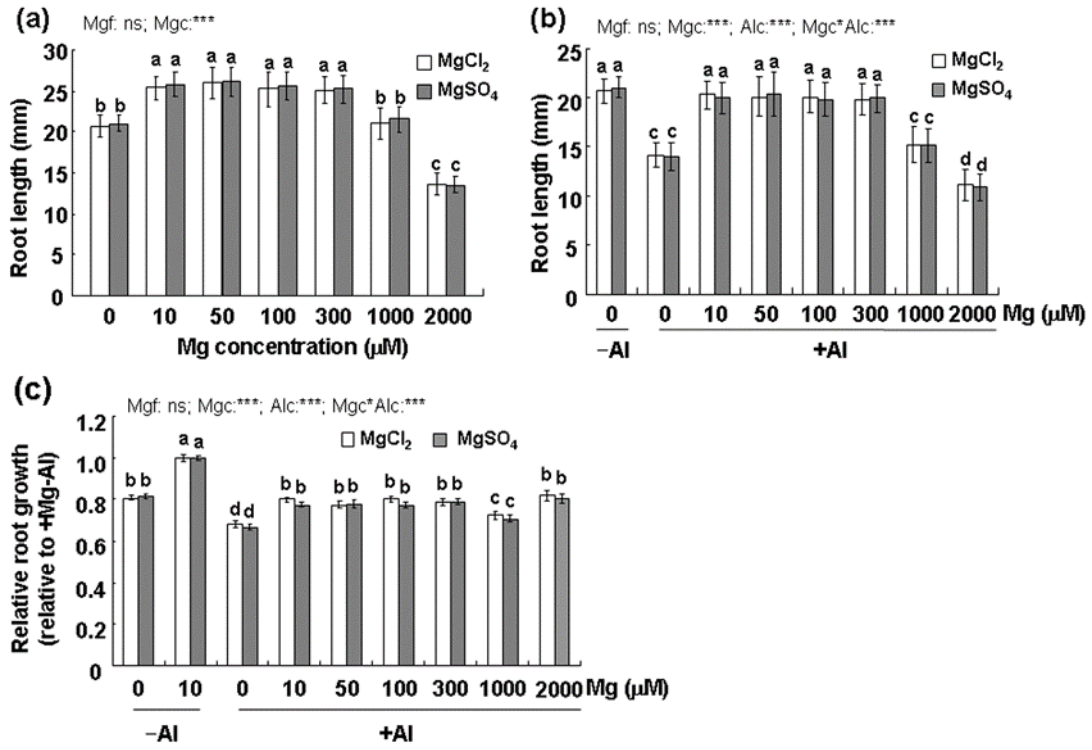

(a)
Mgf: ns; Mgc:***
Root length (mm)
MgCl2
MgSO4
Mg concentration (μM)
(b)
Mgf: ns; Mgc:***; Alc:***; Mgc*Alc:***
Root length (mm)
Mg (μM)
−Al
+Al
(c)
Mgf: ns; Mgc:***; Alc:***; Mgc*Alc:***
Relative root growth (relative to +Mg-Al)
Mg (μM)
−Al
+Al

Fig. 1

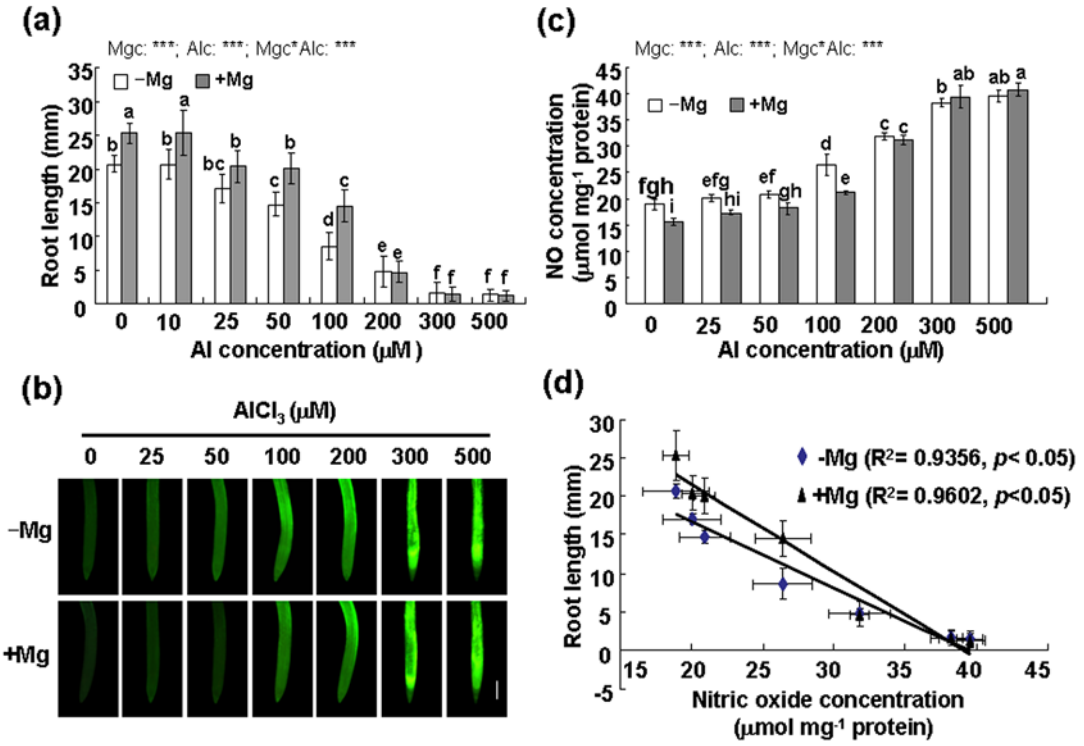

(a)
Mgc: ***; Alc: ***; Mgc*Alc: ***
−Mg
+Mg
Root length (mm)
Al concentration (μM)
(b)
AlCl3 (μM)
0 25 50 100 200 300 500
−Mg
+Mg
(c)
Mgc: ***; Alc: ***; Mgc*Alc: ***
−Mg
+Mg
NO concentration (μmol mg-1 protein)
Al concentration (μM)
(d)
-Mg (R2= 0.9356, p< 0.05)
+Mg (R2= 0.9602, p<0.05)
Root length (mm)
Nitric oxide concentration (μmol mg-1 protein)

Fig. 2

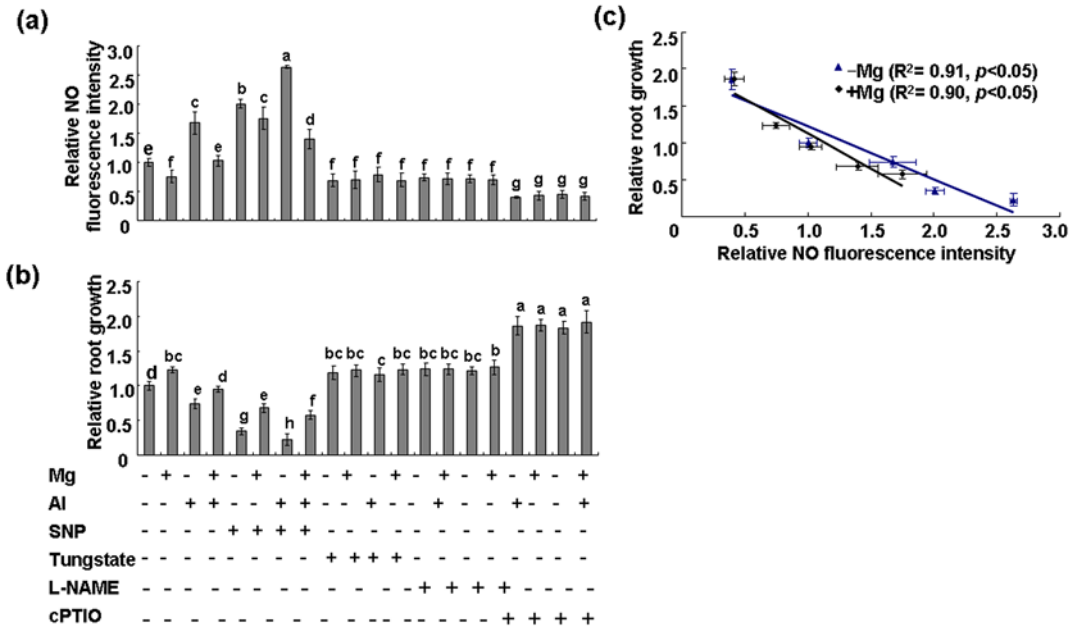
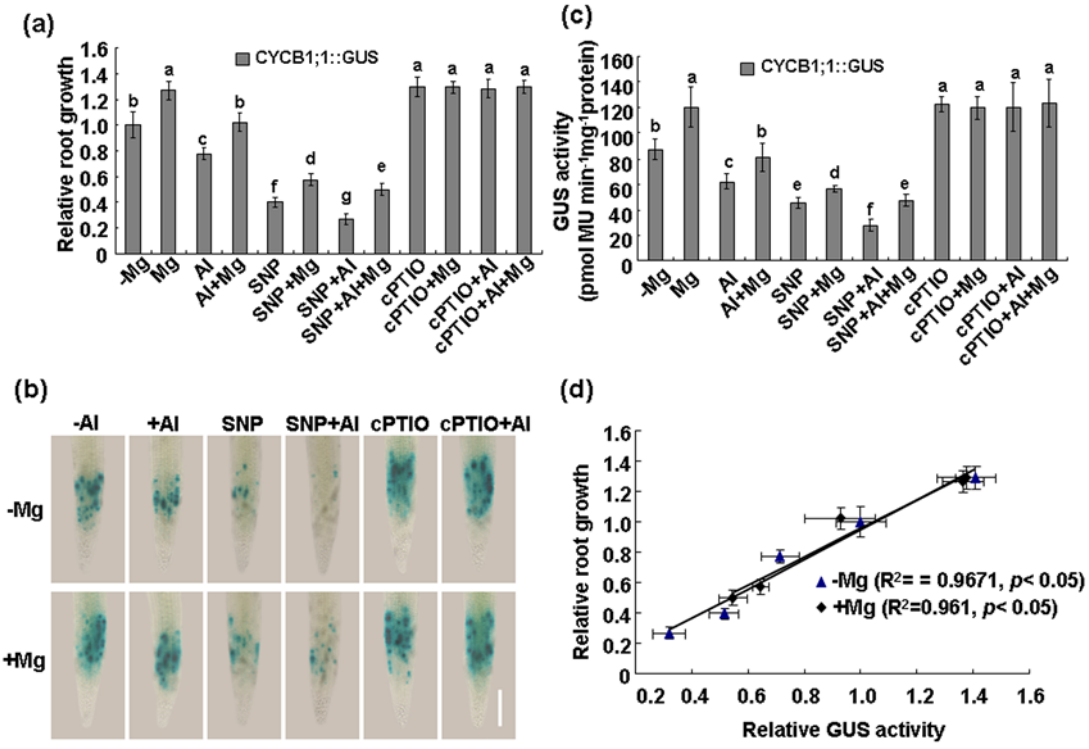

(a)
Relative NO fluorescence intensity
(b)
Relative root growth
Mg
Al
SNP
Tungstate
L-NAME
cPTIO
(c)
Relative root growth
Relative NO fluorescence intensity
–Mg (R²= 0.91, p<0.05)
+Mg (R²= 0.90, p<0.05)

Fig. 3



698

699 **Fig. 4**

700

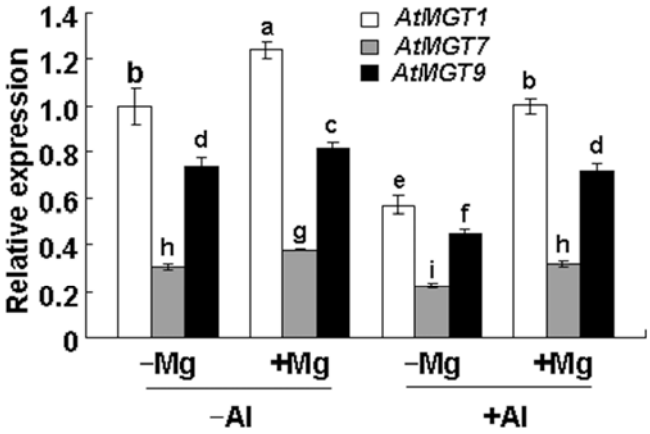

Relative expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
AtMGT1
AtMGT7
AtMGT9
a
b
c
d
e
f
g
h
i
b
d
h
−Mg
+Mg
−Mg
+Mg
−Al
+Al

Fig. 5

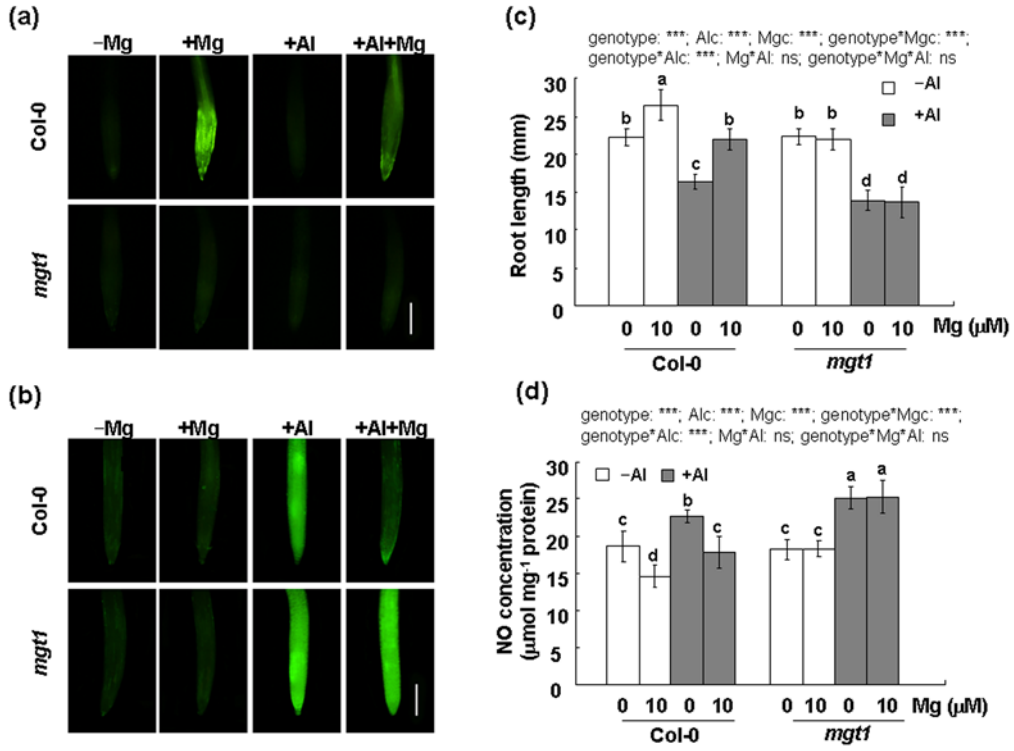
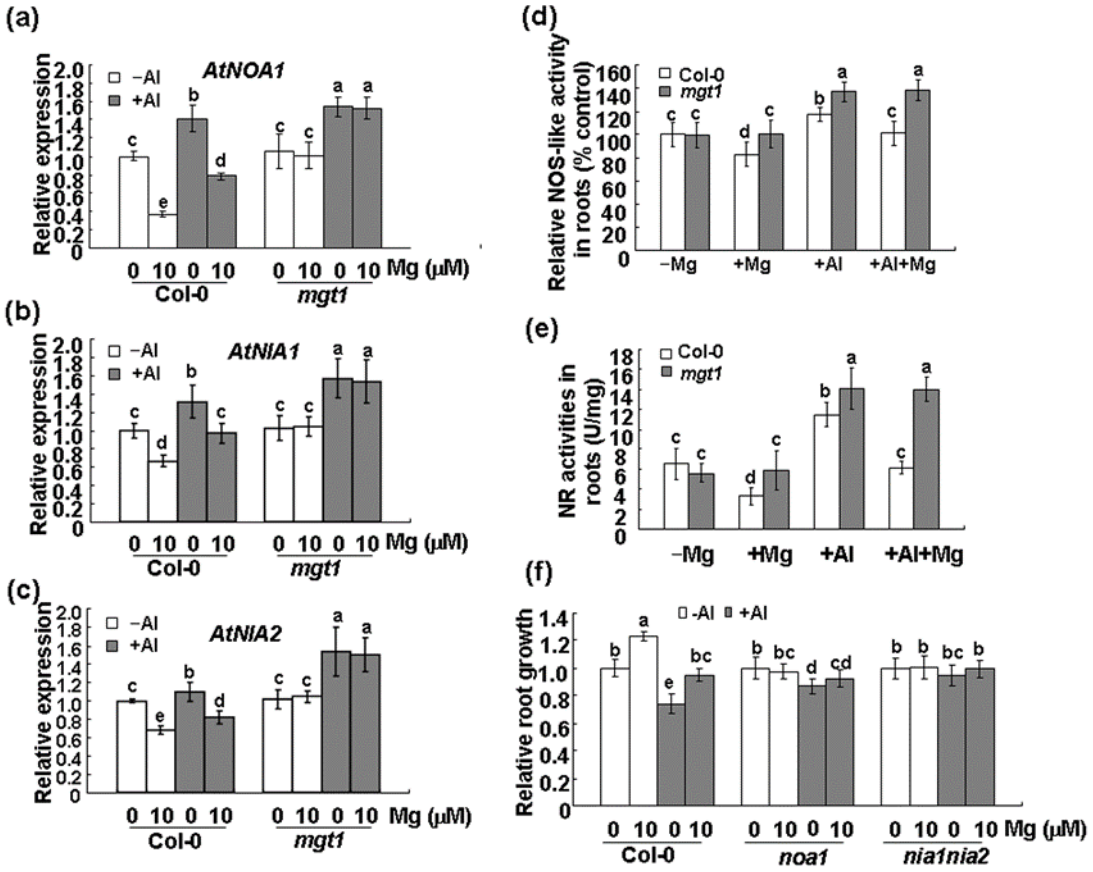

(a)
−Mg
+Mg
+Al
+Al+Mg
Col-0
mgt1
(b)
−Mg
+Mg
+Al
+Al+Mg
Col-0
mgt1
(c)
genotype: ***; Alc: ***; Mgc: ***; genotype*Mgc: ***;
genotype*Alc: ***; Mg*Al: ns; genotype*Mg*Al: ns
−Al
+Al
Root length (mm)
0 10 0 10 0 10 0 10 Mg (μM)
Col-0
mgt1
(d)
genotype: ***; Alc: ***; Mgc: ***; genotype*Mgc: ***;
genotype*Alc: ***; Mg*Al: ns; genotype*Mg*Al: ns
−Al
+Al
NO concentration (μmol mg-1 protein)
0 10 0 10 0 10 0 10 Mg (μM)
Col-0
mgt1

Fig. 6



747

748 **Fig. 7**

749

750

751

752

753

754

755

756

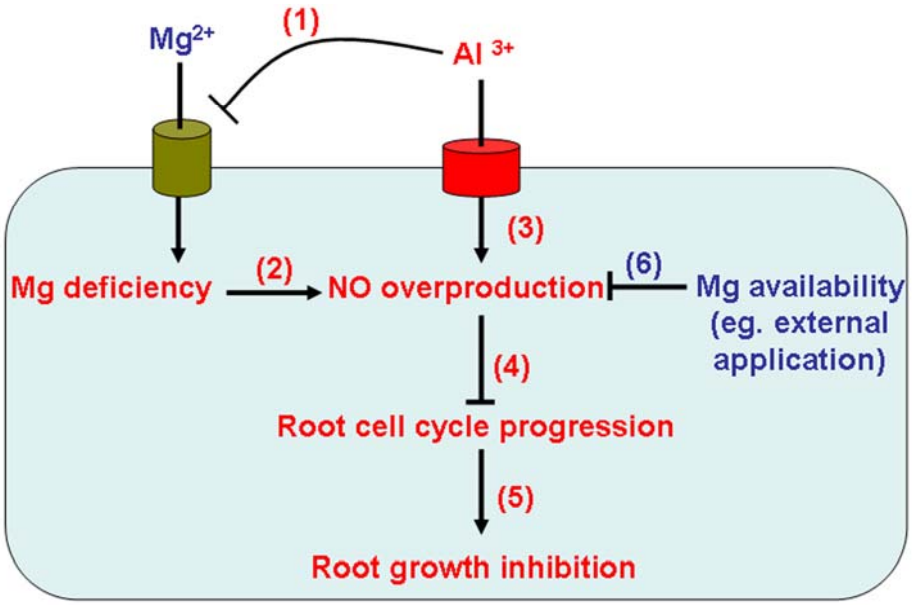

Mg2+
(1)
Al 3+
(3)
Mg deficiency
(2)
NO overproduction
(6)
Mg availability
(eg. external
application)
(4)
Root cell cycle progression
(5)
Root growth inhibition

Fig. 8