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Mechanism of zinc stress on magnesium deficiency in rice plants (*Oryza sativa* L.): Insights from magnesium isotopes

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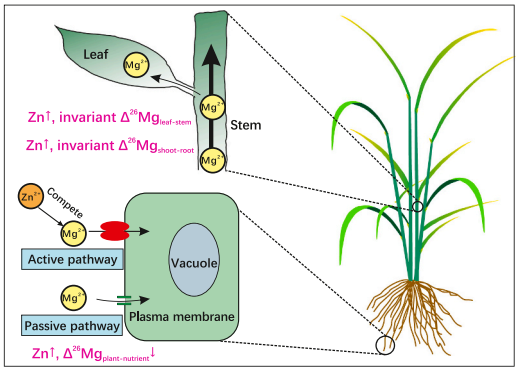
HIGHLIGHTS

- Increased Zn lowers Mg isotope fractionation between plants and nutrient solutions.
- Excess Zn weakens Mg acquisition by the active pathway during root uptake.
- Zn cannot competitively inhibit the movement of Mg from roots to aerial biomass.

GRAPHICAL ABSTRACT



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ABSTRACT

Magnesium (Mg) and zinc (Zn) are essential nutrients for plants. Mg deficiency often occurs in rice plants grown in Zn-polluted soil. However, the mechanism for this correlation is unclear. Here, we performed culture experiments on rice plants (*Oryza sativa* L.) and used Mg isotopes to investigate mechanisms of Zn stress on plant Mg deficiency. Our results show that excess Zn can significantly reduce the uptake of Mg in rice tissues. The root displays positive $\Delta^{26}Mg_{plant\text{-}nutrient}$ values ($\delta^{26}Mg_{plant}$-$\delta^{26}Mg_{nutrient}$; 1.90 ‰ to 2.06 ‰), which suggests that Mg enters the root cells mainly via Mg-specific transporters rather than non-selective diffusion. The decreased $\Delta^{26}Mg_{plant\text{-}nutrient}$ values with increasing Zn supply can be explained by the competition between Zn and Mg, both of which combine with same transporters in roots. In contrast, the shoots (stem and leaf) display much lower $\delta^{26}Mg$ values than roots, which suggests that the transport of Mg from roots to aerial biomass is mainly via free Mg ions, during which Zn cannot competitively inhibit the movement of Mg. Our study suggests that the Mg

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deficiency in rice plants can be caused by high Zn-levels in soils and highlights the necessity of soil Zn-remediation in solving Mg deficiency problems in rice plants.

1. Introduction

Magnesium (Mg) is an essential nutrient and plays a critical role in maintaining the physiological functions in plants, including photosynthetic carbon fixation (e.g., Lilley et al., 1974), chlorophyll synthesis (e.g., Knoop et al., 2005; Li et al., 2021), protein synthesis (e.g., Zheng et al., 1993), and enzyme activation (e.g., Marschner, 2011). Although Mg is relatively abundant in Earth's crust (with an abundance of ~2.33 % and ranking the 8th), Mg deficiency in plants is a widespread problem (e.g., Tan et al., 1991; Aitken et al., 1999; Guo et al., 2016), particularly in soils with long-term unbalanced crop fertilization (Guo et al., 2016) or in acidic soils with low cation exchange capacity (Tan et al., 1991; Aitken et al., 1999), posing significant threats to the yield and nutritional quality of crops. Therefore, improving our understanding on physiological processes during Mg homeostasis in plants is necessary to effectively implementing Mg-biofortification.

Zinc (Zn) is another essential nutrient in plants (e.g., Broadley et al., 2007; Wu et al., 2010) and can regulate redox reactions and protein synthesis during plant growth (Sinclair and Krämer, 2012). However, the Zn concentration in soils may rise as a result of expanded industrial and agricultural activities (e.g., mining, smelting, and use of fertilizers and pesticides) (Nriagu and Pacynat, 1988; Nriagu, 1996), which could limit plant growth (Horler et al., 1980; Sagardoy et al., 2009). More importantly, the excess amount of Zn could compete with other nutrients including Fe (e.g., Hanjagi and Singh, 2017; Kawakami and Bhullar, 2018; Vigani et al., 2018), affecting plant's capability of absorbing and utilizing them. For instance, the excess Zn has been reported to inhibit Fe-uptakes in roots of rice and affect Fe transport in stems due to the competition between Zn and Fe(II) which are likely combining with the same transporter-proteins (e.g., OsIRT1 in roots), resulting in the systematic variation of Fe isotope compositions in rice organs (Wu et al., 2022). Previous studies reported that cations including H^+, Mn^{2+}, Al^{3+}, NH_4^+, and K^+ can induce Mg deficiency in plants by interfering with root Mg^{2+} uptake (e.g., Lasa et al., 2000; Gransee and Führs, 2013; Kunhikrishnan et al., 2016; Xie et al., 2021). It is conceivable that excess Zn may also affect the acquisition of Mg during plant growth since Zn may share similar transporter-proteins with a variety of metal ions including Mg^{2+}, Zn^{2+} and Fe^{2+} (e.g., Shaul, 2002). However, this possibility has not been systematically studied.

Mg has three stable isotopes (^{24}Mg, ^{25}Mg and ^{26}Mg), which have almost identical chemical properties. Therefore, Mg homeostasis generally has little effect on Mg isotope fractionation. However, previous studies have shown that subtle Mg isotope fractionations may occur during physiological processes (e.g., Mg uptake and transport), thus Mg isotope ratios have great potential in identifying and differentiating these processes, as different physiological processes trigger varied Mg isotope fractionations (e.g., Wiggenhauser et al., 2022; Black et al., 2006; Black et al., 2008; Bolou-Bi et al., 2010; Pokharel et al., 2018; Wrobel et al., 2020; Wang et al., 2020). Positive shifts in $\delta^{26}Mg$ values have been reported between plant tissues and the source of their nutrients (i.e., soil and hydroponic nutrient solutions) (e.g., Black et al., 2008; Bolou-Bi et al., 2010; Pokharel et al., 2018; Wrobel et al., 2020; Wang et al., 2020; Tipper et al., 2010; Kimmig et al., 2018; Bolou-Bi et al., 2012; Opfergelt et al., 2014), suggesting that plants preferentially take up isotopically heavy Mg from the source of their nutrients. In addition, ground tissues (e.g., grain, stem, and foliage) tend to have lower $\delta^{26}Mg$ values than roots, suggesting the preferential translocation of isotopically light Mg in plant bodies (e.g., Black et al., 2008; Bolou-Bi et al., 2010; Wang et al., 2020; Gao et al., 2018). Yet, Mg isotopic studies regarding Zn stress on Mg deficiency in plants are lacking.

In this study, rice plants (*Oryza sativa* L.) were cultured in hydroponic solutions with varied Zn concentrations. Mg isotope compositions are measured to understand how the uptake and translocation of Mg respond to excess Zn supply during rice growth. These Mg isotope fractionations provide constraints on biological processes during Mg uptake and internal translocation.

2. Methods and materials

2.1. Experimental design

Rice plants (*Oryza sativa* L. cv. *Youyou 128*) were cultivated hydroponically in a growth chamber under controlled conditions using Kimura-B nutrient (KBN) solutions containing ~370 μM $(NH_4)_2SO_4$, ~550 μM $MgSO_4•7H_2O$, ~90 μM K_2SO_4, ~180 μM KNO_3, ~370 μM $Ca(NO_3)_2•4H_2O$, ~180 μM KH_2PO_4, ~100 μM NaCl, ~50 μM Fe-EDTA, ~1 μM $CuSO_4•5H_2O$, ~5 μM $MnSO_4•H_2O$, ~10 μM H_3BO_3, ~0.5 μM $Na_2MoO_4•2H_2O$, and ~0.2 μM $CoSO_4•7H_2O$ (Wu et al., 2022). The day and night temperatures were set to 25 °C and 20 °C, respectively, and the photoperiod and light intensity were set as 14 h and 400 μmol m^{-2} s^{-1}, respectively. The relative humidity of the growth chamber was approximately 70–95 %.

Rice seeds were sterilized with ~10 % H_2O_2 (v/v) for ~10 min followed by rinsing in ultrapure water (~18.2 MΩ•cm) for ~10 min. After being soaked in sterile deionized water for ~1 day at ~37 °C, they were germinated at ~25 °C for ~3 days on moist filter paper placed in Petri dishes. After germination, seedlings were grown in deionized water for ~7 days and subsequently cultivated in half-strength KBN solution until the fifth leaf appeared. The plants were then divided and transferred into containers with KBN solution. A previous study demonstrated that Zn phytotoxicity occurs when external Zn^{2+} concentration reached 56 μM (Wang et al., 2022). As such, the Zn concentrations in the nutrient solutions were set to ~1 μM (CK, the control experiment), ~10 μM (T1), ~50 μM (T2), and ~ 100 μM (T3, possible with Zn phytotoxicity) by adding $ZnSO_4•7H_2O$ to study the effect of Zn stress on Mg uptake. The pH value of the original KBN solution was adjusted to ~5.6 with sodium bicarbonate. The Mg speciation in the KBN nutrient solution was calculated using MINTEQ 3.0 (Gustafsson, 2011), and the results are shown in Table S1. The hydroponic containers had the same specifications, and the nutrient solution was replaced every three days. A cap was placed over each container to minimize water loss by evaporation.

Approximately 5 mL of nutrient solutions in the CK and T3 experiments were sampled on the 24th, 25th, and 26th day. Rice plants were sampled on the 30th day during the tillering stage. Approximately 10–20 plants were collected from each container and then dissected into roots, stems, and leaves with ceramic scissors. After collection, plant tissues were rinsed with ultrapure water (~18.2 MΩ•cm) to remove impurities and then dried in an oven at ~105 °C for ~1.5 h and at ~75 °C for another ~48 h. The dry mass of plant tissues was weighed and recorded. The same plant tissues from each container were combined and cut into small pieces (~2 mm) with ceramic scissors prior to chemical analyses.

2.2. Element concentration analysis

Acids (including HNO_3, HCl, and HF) used in this study were of BV-III grade and were distilled using a DST-1000 sub-boiling distillation system (Savillex, USA). Ultrapure water (18.2 MΩ·cm) was obtained using a Milli-Q® Element system (Millipore Reference A+, USA). H_2O_2 (trace analysis grade) was purchased from Thermo Scientific™ (USA). The digestion of plant tissues was conducted in a class-1000 clean laboratory. Approximately 3–300 mg of sample was first digested using a

microwave digestion system (Milestone, Italy) in ~10 mL of concentrated acid mixture (HNO_3/HF = 7/3, *v*/v). Following the complete digestion, the digests were evaporated to dryness in Teflon beakers (Savillex, USA) on a hotplate and then treated with ~7 mL of solution containing HNO_3 and H_2O_2 (HNO_3/H_2O_2 = 5/2, v/v). The nutrient solution was evaporated to dryness and directly treated with ~3.5 mL of HNO_3 and H_2O_2 mixture (HNO_3/H_2O_2 = 5/2, v/v) to remove any organic matter. Subsequently, each sample solution was split into two aliquots for analyzing elemental concentrations and for column chemistry to separate pure Mg.

One aliquot of digested samples was analyzed for Mg concentrations using an inductively- coupled-plasma optical-emission-spectrometer (ICP-OES, Perkin Elmer, USA) and for Zn concentrations by an inductively-coupled-plasma mass-spectrometer (ICP-MS, Perkin Elmer, USA) at the Guangdong Institute of Eco-environmental Sciences & Technology, Guangdong, China. The detection limits are 40 ng/L for Mg using ICP-OES and 0.3 ng/L for Zn using ICP-MS. Replicate measurements of standard reference material 1570a (spinach leaves) yielded a relative error less than ±1 % for Mg and ± 5 % for Zn compared with certified values.

2.3. Mg purification and isotope analysis

Mg isotope ratios were analyzed for plant tissues and nutrient solution at the Institute of Geochemistry, Chinese Academy of Sciences. Detailed procedures for column chemistry and isotopic measurements have been previously reported (Gao et al., 2019). Briefly, sample solutions containing ~20 μg of Mg were loaded onto ~2.3 mL of 200–400 mesh AG50W-X8 resin (Bio-Rad, USA) and eluted with 1 M HNO_3. Matrix elements were removed using ~23 mL of 1 M HNO_3, and then Mg was eluted by ~15 mL of 1 M HNO_3. The geological reference material BHVO-2 was processed together with samples during each purification session. The total procedural blank was <10 ng, which represented <0.1 % of the Mg loaded on the column. Mg isotope ratios were determined on a Neptune Plus Multi-Collector Inductively Coupled Plasma Mass Spectrometry (Thermo Finnigan, USA) under wet plasma mode and using the standard-sample bracketing technique. The GSB Mg solution was routinely used as an in-house standard. Each sample was measured at least three times to achieve the desired precision. The Mg isotope composition of a sample was expressed as the deviation of the $^{x}Mg/^{24}Mg$ ratio from that of the DSM3 standard as follows:

$$\delta^{x}Mg\ (‰) = \left[\frac{\left({}^{x}Mg/{}^{24}Mg\right)_{sample}}{\left({}^{x}Mg/{}^{24}Mg\right)_{DSM3}} - 1\right] \times 1000, \quad (1)$$

where *x* refers to mass of 25 or 26. Two standard deviations (2SD) were reported in tables and shown in plots. The $\delta^{25}Mg$ and $\delta^{26}Mg$ values of GSB relative to DSM3 are −1.04 ± 0.02 ‰ and −2.03 ± 0.04 ‰ (2SD, *n* = 225), respectively, and USGS standard BHVO-2 yielded an average $\delta^{26}Mg$ value of −0.23 ± 0.06 ‰ (2SD, *n* = 3). They are consistent with previously published results within analytical uncertainty (Teng et al., 2015; Gao et al., 2019).

2.4. Calculation of Mg mass and isotope composition of plant tissues

The mass of Mg in each plant tissue (M_{tissue}) was calculated by multiplying the Mg concentration by the dry weight:

$$M_{tissue} = C_{tissue} \times W_{tissue}, \quad (2)$$

where C_{tissue} and W_{tissue} are Mg concentration and dry weight of tissue of interest, respectively. The Mg isotope compositions of shoots ($\delta^{56}Mg_{shoot}$) and whole plants ($\delta^{56}Mg_{whole}$) were calculated based on the mass balance equation as follows:

$$\delta^{26}Mg_{shoot\ or\ whole} = \frac{\sum \delta^{26}Mg_{tissue} \times M_{tissue}}{\sum M_{tissue}}, \quad (3)$$

where $\delta^{26}Mg_{tissue}$ is $\delta^{26}Mg$ value of tissue. The shoot includes stem and leaf. The whole plant includes all tissues. The differences in the $\delta^{26}Mg$ value between the bulk plant and nutrient solution ($\Delta^{26}Mg_{plant\text{-}solution}$) and between the shoot and root ($\Delta^{26}Mg_{shoot\text{-}root}$) were calculated as follows:

$$\Delta^{26}Mg_{plant-solution} = \delta^{26}Mg_{plant} - \delta^{26}Mg_{solution}, \quad (4)$$

$$\Delta^{26}Mg_{shoot-root} = \delta^{26}Mg_{shoot} - \delta^{26}Mg_{root}. \quad (5)$$

The standard errors of these mean values are propagated from standard errors of dry weight of tissues and $\delta^{26}Mg$ values using the Monte Carlo method after 2 million simulations.

2.5. Statistical analysis

Group differences were assessed by one-way ANOVA with Duncan's post hoc test using SPSS 23.0 (IBM, IL, USA). Statistical significance (p) was set at <0.05 (two-tailed).

3. Result and discussion

3.1. Excess Zn supply limits rice plant growth

Data of dry masses and Zn concentrations on the same samples have been published in Wu et al. (2022). Analytical results of Mg concentrations and Mg isotope compositions in this study are summarized in Table 1. Statistically-insignificant differences in dry mass can be observed among bulk plant and individual tissues (shoot, root, stem, and leaf) among the CK, T1, and T2 groups (Fig. S1), despite the T3 group showing slightly low mass. This provides strong evidence that excess Zn supply could limit plant growth.

3.2. Excess Zn supply limits uptake of Mg by rice plants

Zn and Mg concentrations of bulk plants and tissues from individual organs (including shoot, root, stem, and leaf) show opposite trends with increasing Zn concentrations in the nutrient solutions (Fig. 1a and c), i.e., Zn concentrations increased but Mg concentrations decreased from experiments CK, T1, T2 to T3. The biomasses of Mg and Zn in bulk plants and individual tissues were calculated by multiplying Mg and Zn concentrations with their dry weight using Eq. (2). The increase of Zn biomasses and decrease of Mg biomasses can be noted for bulk plants and individual tissues from experiments CK, T1, T2 to T3 as shown in Fig. 1b and d. These results suggest that excess Zn supply could limit the uptake of Mg by rice plants.

3.3. Mg isotope fractionation during uptake of Mg by rice plant

The source of Mg in the nutrient solution ($MgSO_4 \bullet 7H_2O$) has a $\delta^{26}Mg$ value of −4.69 ± 0.04 ‰ (2SD, *n* = 4) (Table 1). Throughout the experiment, the deviations in $\delta^{26}Mg$ value of the nutrient solution from that of the $MgSO_4 \bullet 7H_2O$ salt (reported as $\Delta^{26}Mg_{nutrient\text{-}salt}$) ranged from −0.03 ± 0.06 ‰ (2SD, *n* = 3) to 0.02 ± 0.05 ‰ (2SD, n = 3) for group CK and from −0.04 ± 0.08 ‰ (2SD, n = 3) to 0.00 ± 0.08 ‰ (2SD, n = 3) for group T3 (Fig. S2). However, all tissues show higher $\delta^{26}Mg$ values than the nutrient solution (Table 1), consistent with previous observations that plants preferentially take up isotopically heavy Mg from the growth medium (e.g., Black et al., 2008; Pokharel et al., 2018; Wang et al., 2020).

Interestingly, as the Zn supply increased, the $\delta^{26}Mg$ values of plant tissues decreased from −2.53 ± 0.06 ‰ (2SD, n = 3) to −2.62 ± 0.01 ‰ (2SD, n = 4) in roots, from −2.42 ± 0.03 ‰ (2SD, n = 4) to −2.60 ±

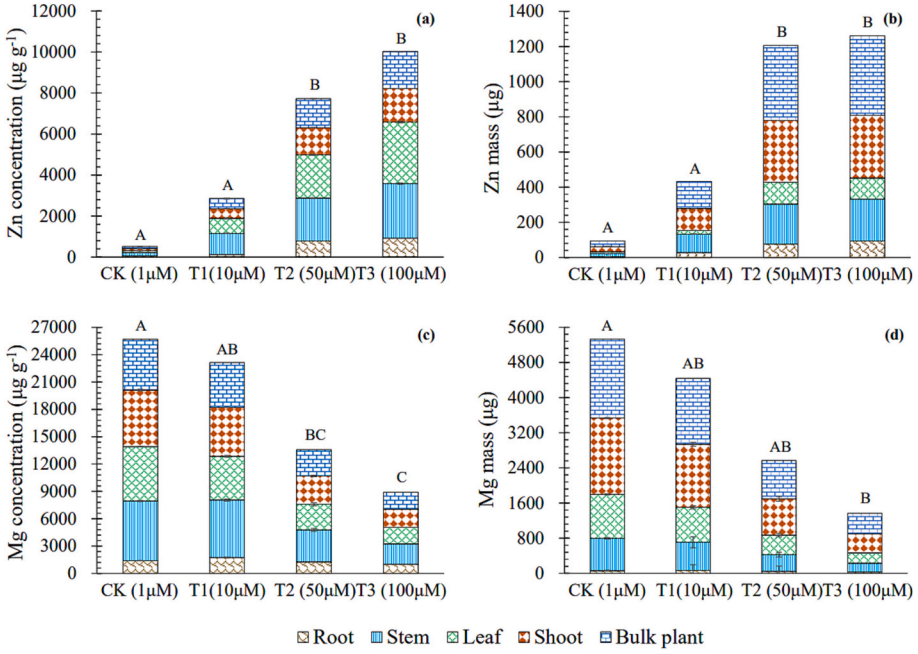

Fig. 1. Concentrations and biomasses of Zn (a-b) and Mg (c-d) in roots, stems, leaves, shoots, and bulk plants. Samples from 10 to 20 plants were combined and analyzed for Mg and Zn concentrations, and 2SDs were calculated from three repeated measurements of each sample. The different uppercase letters (in A, B, and C) represent significant differences among the four treatments (one-way ANOVA $p < 0.05$).

0.01 ‰ (2SD, n = 3) in stems, and from −2.80 ± 0.04 ‰ (2SD, n = 3) to −2.97 ± 0.09 ‰ (2SD, n = 3) in leaves (Fig. 2). This suggests that the elevated Zn stress leads to smaller Mg isotope fractionation between growth medium and plants. These changes are unlikely to be caused by variations in Mg speciation and Mg concentration in the nutrient solution, as modeling results show that most Mg is present as $Mg(H_2O)_6^{2+}$ complexes (93.8–94.5 %; Table S1), and the Mg concentrations of all nutrient solutions are consistently around 550 μM. In addition, the lack of significant changes in the δ^{26}Mg value of nutrient solution over three days before the solution was replaced (Fig. S2) cannot explain large variances in δ^{26}Mg values of plant tissues.

Mg in nutrient solution must pass through the cell wall before reaching the plasma membrane of root cells (Marschner, 2011). During the process, Mg can be either transported via Mg-specific transporters (e.g., Li et al., 2001; Gebert et al., 2009), or via non-selective cation channels in the plasma membrane (e.g., Hermans et al., 2013; Tang and Luan, 2020). In the former case, the pectins in cell walls consist of

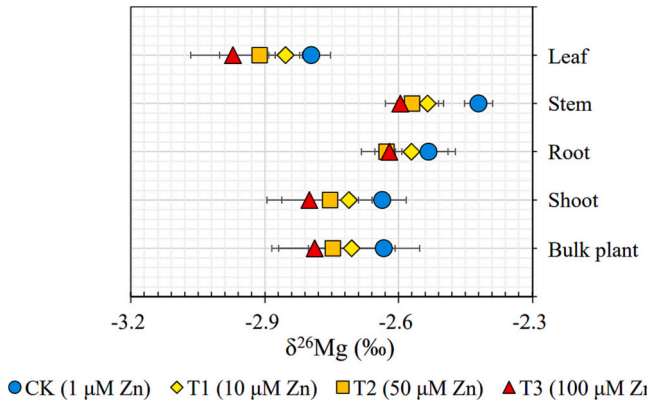

Fig. 2. Mg isotope compositions of roots, stems, leaves, shoots, and bulk plants. Samples from 10 to 20 plants were combined and analyzed for Mg isotope composition, and 2SDs were calculated from three repeated measurements of each sample.

polygalacturonic acid, of which the carboxylic groups ($R\text{-}COO^-$) act as cation exchangers in the cell wall continuum of roots. Both laboratory experiments and theoretical calculations have suggested that binding with organic molecules induces an enrichment of heavy Mg isotopes in Mg-organic complexes (e.g., Black et al., 2007; Bolou-Bi et al., 2010; Moynier and Fujii, 2017; Pokharel et al., 2018), as the Mg—O bond strength in water complexes may be weaker than that in organic complexes because stronger bonds concentrate heavy isotopes relative to weaker bonds (Urey, 1947; Schauble, 2004). This process is an active transport pathway that is energy-consuming and requires Mg to be bound to membrane proteins. This process is usually unidirectional, as revealed by isotope labeling experiments (Kuhn et al., 2000; Tanoi et al., 2014), and will preserve this enrichment of ^{26}Mg in plants. In the latter case, Mg migrates passively mainly as a free $Mg(H_2O)_6^{2+}$ complex (Marschner, 2011), and cross-membrane transport of free $Mg(H_2O)_6^{2+}$ complex may produce limited Mg isotope fractionation (e.g., Pokharel et al., 2017; Pokharel et al., 2018) due to its large mass, as observed for the negligible Mg isotope fractionation during diffusion in water (Richter et al., 2006). Thus, the degree of Mg isotope fractionation during root uptake depends on the relative contribution of these two pathways.

Wu et al. (2022) discovered that the Fe isotope fractionation between whole plant and nutrient solution ($\Delta^{56}Fe_{plant\text{-}nutrient}$) decreases with increasing Zn supply, suggesting that excess Zn inhibits Fe acquisition in rice due to the competition between Zn^{2+} and Fe^{2+}, both of which can combine with OsIRT1 (Fe^{2+} transporter) in roots. The decreasing $\Delta^{26}Mg_{plant\text{-}nutrient}$ values with increasing Zn mass can be explained by a similar mechanism (Fig. 3a). Previous studies have shown that Mg uptake by roots in higher plants is mediated by MGT family members (Li et al., 2001; Chen et al., 2012). Although it is possible that binding with different organic molecules induces various degrees of ^{26}Mg enrichment in Mg-organic complexes (e.g., Black et al., 2007; Bolou-Bi et al., 2010; Moynier and Fujii, 2017; Pokharel et al., 2018), this fractionation is not expected to be affected by Zn. Later, ^{63}Ni tracer experiments showed that MGT family members might also transport other divalent cations, including Ni, Co, Fe, Mn and Cu (Li et al., 2001), and potentially Zn. *MAGNESIUM/PROTON EXCHANGER 1* (*MHX1*) encodes a vacuolar

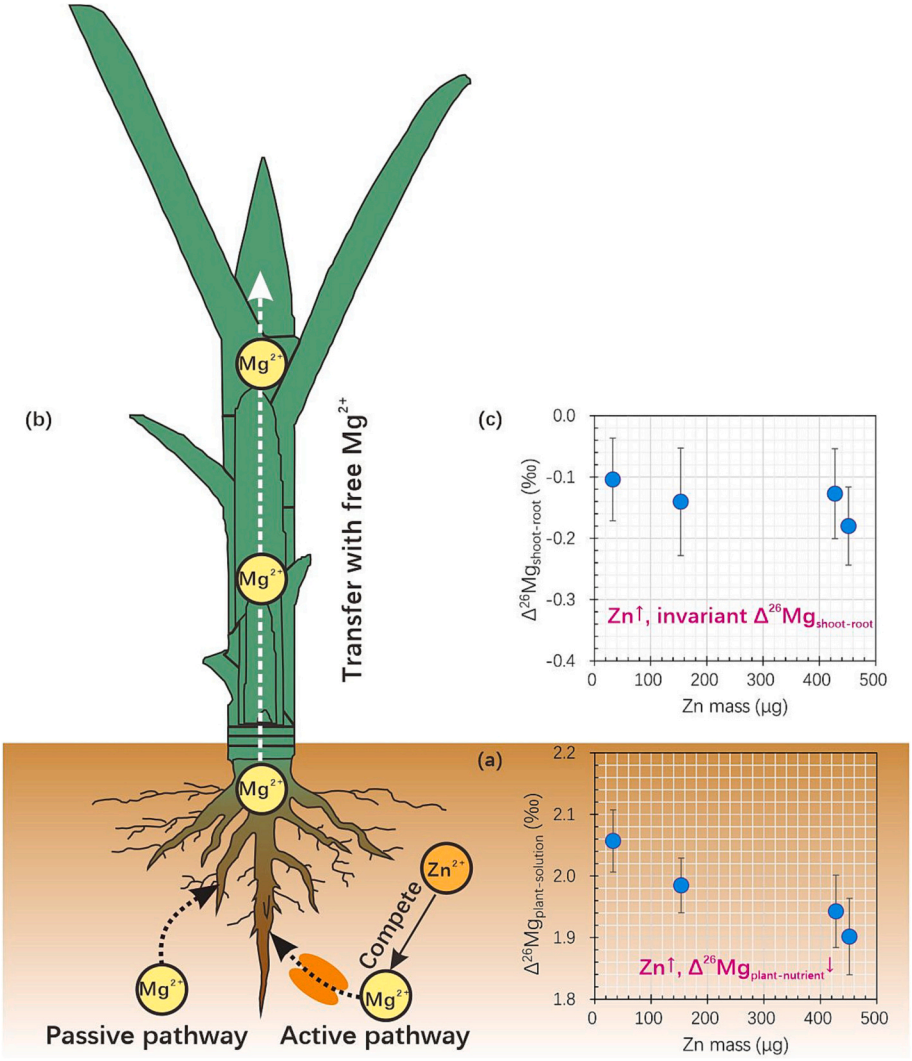

Fig. 3. Zn biomass vs. $\Delta^{26}Mg_{plant\text{-}nutrient}$ (a) and $\Delta^{26}Mg_{shoot\text{-}root}$ (c). (b) Scheme of Mg uptake and transport regulated by excess Zn in rice.

transporter that is present in roots and exchanges protons with both Mg^{2+} and Zn^{2+} ions (Shaul, 2002). Thus, Zn^{2+} and Mg^{2+} may compete for transporter proteins (e.g., MGT and MHX1), weakening Mg acquisition by the active pathway but enhancing Mg acquisition by the passive pathway (Fig. 3b), thereby leading to decreases in both $\Delta^{26}Mg_{plant\text{-}nutrient}$ value and Mg biomass with increasing Zn supply.

3.4. Mg isotope fractionation during transport from roots to shoots

Once within the root, Mg moves radially toward the root center where stem vessels are localized for transport to the leaves (Hermans et al., 2013). Our results showed that the root-shoot translocation process can slightly fractionate Mg isotopes ($\Delta^{26}Mg_{shoot\text{-}root} = -0.10$ ‰ to -0.18 ‰; Fig. 2), leading to an enrichment of isotopically light Mg in stems and leaves relative to roots. Bolou-Bi et al. (2010) ascribed the negative fractionation to two different Mg pools in roots: ionic Mg favoring light isotopes and Mg bound to organic ligands (e.g., ATP and proteins) favoring heavy isotopes. Previous studies indicated that Mg in both pools migrates to shoots, while free ionic Mg is more readily transported from roots to shoots (e.g., Todd, 1961; Kirkby and Mengel, 1976; Bradfield, 1976; Schell, 1997). This may explain the negative $\Delta^{26}Mg_{shoot\text{-}root}$ values observed in our study.

Surprisingly, our results show that within error bars, the $\Delta^{26}Mg_{shoot\text{-}root}$ value varied slightly (less than ~0.09 ‰) with increasing Zn supply (Fig. 3c). This suggests that increasing Zn supply has a limited effect on Mg isotope fractionation during the translocation of Mg from root to ground tissues, implying that the upward translocation of Mg may not be affected by Zn stress. To test this hypothesis, the transport efficiencies of Mg from root to stem ($\varphi2/\varphi1$) and from stem to leaf ($\varphi3/\varphi2$) were determined based on a mass balance box model using the biomasses, Mg concentrations, and Mg isotope compositions of rice tissues (a detailed model calculation process is presented in Text S1 of the SI). The modeling results showed that both the $\varphi2/\varphi1$ and $\varphi3/\varphi2$ ratios remained unchanged with increasing Zn supply (Fig. 4), suggesting that the addition of Zn did not affect the Mg transport efficiency from roots to stems or from stems to leaves. This is consistent with the idea that Mg^{2+} may be transported to the aerial biomass mainly by a transpiration stream moving through the xylem vessels (Hermans et al., 2013), during which Zn^{2+} cannot competitively inhibit the movement of free Mg^{2+} from the roots to the aerial parts (Fig. 3c).

4. Conclusion and environmental implication

The Mg deficiency in cereal grains becomes an increasingly severe problem on a global scale (e.g., Cakmak and Yazici, 2010; Rosanoff et al., 2012), and Mg biofortification is a promising approach to address human Mg deficiency. Mg deficiency may occur in plants growing in highly leached acid soils with low cation concentration and exchange capacity (Tan et al., 1991; Aitken et al., 1999), and the deficiency of Mg in plant may be induced by the presence of excess cations that may

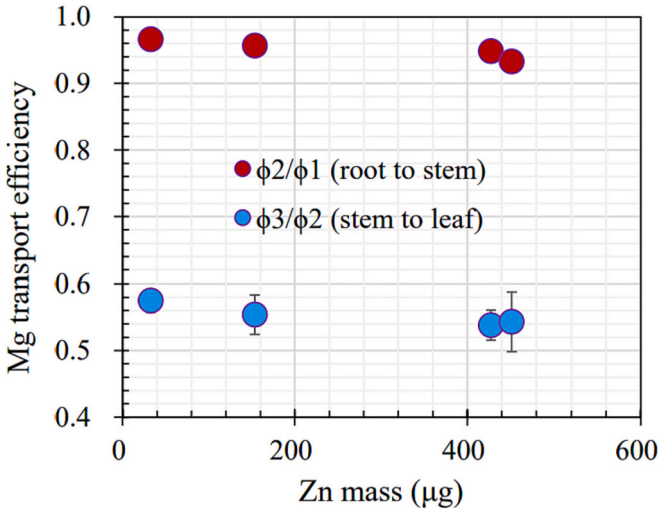

Fig. 4. Zn biomass vs. Mg transport efficiency. Error bars are two times the standard deviation (2SD).

compete with Mg^{2+} for binding with negatively-charged sites in root apoplasm (e.g., Liu and Huettl, 1991; Marschner, 2011).

To the best of our knowledge, the current study is the first to investigate the mechanism of Zn stress on Mg deficiency in rice plants based on Mg isotope compositions. This study shows that excess Zn supply can limit the uptake of Mg by roots of rice plants. The Mg isotope fractionations indicate that the Mg-uptake by rice roots from nutrient solutions is mainly facilitated by active Mg-specific transporters, with which Zn may also combine. Thus, the excess amount of Zn may weaken the capability of Mg-uptake by rice roots. In contrast, $Mg(H_2O)_6^{2+}$ complexes are mostly transported from roots to aerial biomass when there is little competition between Zn and Mg (Fig. 3). However, these mechanisms shall be verified and refined at molecular level by studying transporter genes in our future studies.

It has been reported that Mg concentrations in rice have undergone a clear decline over the past 60 years, most probably due to the dilution of Mg associated with marked increases in grain yields as well as imbalanced mineral fertilization without considering crop demand for Mg (Guo et al., 2016). In highly leached and Zn-contaminated soils with low Mg/Zn ratio, our study suggests that measures, including optimizing soil pH, providing balanced fertilization, and avoiding excessive Zn application, should be taken at the root level to minimize the negative effects of excess Zn on Mg-uptake.

Our results also demonstrate that stable Mg isotope signatures of plants can provide new insights into Mg-uptake and translocation pathways. Once precise Mg isotope fractionation factors involved in active or passive pathways are determined, the relative contribution from them may be quantified. This is important information for breeding rice crops with high Mg-uptake efficiency.

CRediT authorship contribution statement

Yucong Fu: Writing – original draft, Formal analysis. **Ting Gao:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Qiqi Wu:** Writing – review & editing, Methodology, Formal analysis. **Meng Qi:** Writing – review & editing, Methodology, Formal analysis. **Zhengrong Wang:** Writing – review & editing, Formal analysis. **Chengshuai Liu:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

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