Characterization of GmMATE13 in its contribution of citrate efflux and aluminum resistance in soybeans

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Citrate exudation mediated by a citrate transporter of the MATE protein family is critical for resisting aluminum (Al) toxicity in soybeans. However, the expression patterns of citrate transporter genes differ under Al stress. Thus, exploring the responsive pattern of GmMATEs in response to Al stress is of great importance to understand the Al resistance mechanism in soybeans. In the present study, the phylogenetic analysis, transcriptionally expressed pattern, and function of GmMATE13 were investigated. The results show that soybean GmMATE13 is highly homologous to known citrate transporter proteins from other plants. Under Al exposure, the transcript abundance of GmMATE13 was increased during a 24 h Al treatment period. The expression of GmMATE13 is specifically induced by Al exposure, but not by the status of Fe, Cu, Cd, or La. Moreover, it was also highly increased when soybean seedlings were grown on acidic soil with a high Al content. Subcellular localization showed that GmMATE13 was localized on the plasma membrane when it was transiently expressed in Arabidopsis protoplasts. Investigation of tissue localization of GmMATE13 expression by investigating GUS activity staining under control of the GmMATE13 promoter showed that it was mainly expressed in the central cylinder in the root tips of the soybean under Al-free conditions, yet extended to cortical and epidermis cells under Al stress. Finally, overexpressing GmMATE13 in soybean hairy roots enhanced Al resistance by increasing citrate efflux. Collectively, we conclude that GmMATE13 is a promising candidate to improve the resistance of soybean to Al toxicity in acidic soil.

KEYWORDS
aluminum resistance, resistance mechanism, multi-drug and toxic compound extrusion family, citrate transporter, Glycine max
Introduction

Unlike manganese, zinc, iron (Fe), copper, and molybdenum, aluminum (Al) is not essential for most creatures though it is the most abundant metal element in the earth’s crust (Rengel, 2004). Aluminum exists in toxic soluble ionic forms in acidic soil and inhibits the growth of plant roots, which decreases the absorption capability of plant roots for nutrients and water, ultimately leading to crop yield reduction (Kochian et al., 2005). Micromolar concentration of Al\(^{3+}\) can inhibit root elongation of many crop species within minutes of exposure (Kochian, 1995). The root tip is the most sensitive target of Al toxicity in plants, especially the transition zone located between the elongation zone of roots and the apical meristem (Sivaguru and Horst, 1998). Al can interact with the cell wall, plasma membrane, and symbions of root cells (Ma, 2000). Meanwhile, Al has a high binding affinity with oxygen-supplying compounds, such as phospholipids, nucleotides, carboxylic acids, proteins, DNA, RNA, and inorganic phosphates (Martin et al., 1988), which can damage the structure and function of root cells and inhibit root elongation.

Some plants have evolved multiple mechanisms to tolerate Al stress, helping plants grow normally in acidic soils. Of them, Al-induced organic acid secretion is one of the most important Al-induced organic acid secretion mechanisms (von Uexküll and Mutert, 1995; Yang et al., 2000; Kochian et al., 2004). The species of Al-induced resistance mechanisms (von Uexküll and Mutert, 1995; Yang et al., 2000; Kochian et al., 2004). The species of Al-induced organic acid secretion include citrate, oxalate, and malate, which form strong complexes with Al\(^{3+}\) to protect root cells. For instance, wheat (Triticum aestivum) can resist Al by secreting malate under Al stress (Delhaize et al., 1993). Maize (Zea Mays) (Pellet et al., 1995), Cassia Tora (Ma et al., 1997a), and soybeans (Glycine Max) (Yang et al., 2001) are resistant to Al by secreting citrate. Buckwheat (Fagopyrum esculentum Moench) (Ma et al., 1997b) and taro (Colocasia esculenta) (Ma and Miyasaka, 1998) resist Al by secreting oxalate. In some cases, there are two different organic acid anions secreted by plant roots to respond Al toxicity. For example, oilseed rape (Brassica Napus), maize, Avena sativa, Raphanus Sativus, and Secale cereale, simultaneously release malate and citrate, whereas the roots of Amaranthus hypochondriacus secrete oxalate and citrate under Al stress (Pellet et al., 2015; Fan et al., 2016).

The multidrug and toxic compound extrusion (MATE) family is a secondary transporter family, mainly involved in transporting secondary metabolites, such as alkaloids (Shoji et al., 2009), flavonoids (Debeaujon et al., 2001), and anthocyanins (Pérez-Diaz et al., 2014). The members of this family are demonstrated to be implicated in detoxification of toxic compounds and heavy metals (Diener et al., 2001), modulation of disease resistance (Sun et al., 2011), outflow of plant hormones (abscisic acid) (Zhang et al., 2014), Fe transport (Durrett et al., 2007), and toleration of Al toxicity (Furukawa et al., 2007). The matrix transport is mediated by MATE transporters coupled with the electrochemical gradient of transmembrane cations, such as H\(^+\) or Na\(^+\) ions (Kuroda and Tsuchiya, 2009; Shoji, 2014). The MATE transporter was revealed to have a unique topology of 12 transmembrane (TM) helices, which is different from any other known transporters (He et al., 2010).
From the soybean genome, there were 117 genes encoding MATE transporters identified. Based on sequence similarity with another 14 MATE transporters from other plant species, eight soybean MATE proteins in subgroup C4-3 were predicted as citrate transporters to be involved in Al detoxification or Fe translocation in soybeans (Liu et al., 2016). GmMATE75, GmMATE79, and GmMATE87 were identified as the plasma-membrane-localized citrate transporters, and overexpression of them in soybean hairy roots and Arabidopsis driven by 35S promoter can increase citrate efflux, decrease Al accumulation, and alleviate root elongation inhibition. However, the three GmMATEs are quite different in expression pattern and tissue-localization, therefore, playing different roles in Al-induced citrate efflux and protection of the roots from Al toxicity (Zhou et al., 2019). Al treatment extended the expression of GmMATE75 and GmMATE79 from the central cylinder to cortical and epidermis cells in soybean transgenic hairy roots, but the expression of GmMATE87 was restricted to the central cylinder irrespective of Al treatment. Obviously, more GmMATEs deserved to be characterized in terms of its role in Al resistance, emphasizing the link between expression pattern and biological function.

Material and methods

Plant materials and growth conditions

Seeds of Al-resistant soybean variety Jiyu 70 were sterilized with 1.0% sodium hypochlorite and planted in a mixture of soil and vermiculite at a ratio of 3:1. The seeds were cultured under 25°C dark for about 3 days until germination, transplanting, and nutrient solution was replaced every 2 days. Incubating the seedlings for 1 week in nutrient solution (pH 4.5) was required, and nutrient solution was replaced every 2 days. For the time course experiment, 0.5 mM CaCl2 solution plus 30 µM AlCl3 (pH 4.5) in a 0.5 mM CaCl2 solution at pH 4.5. After 7 days cultivation, roots were washed overnight with 0.5 mM CaCl2 solution at pH 4.5.

For the time course experiment, 0.5 mM CaCl2 solution plus 30 µM AlCl3 (pH 4.5) were treated for 0, 4, 6, 12, and 24 h, respectively, and root tip 0-1 cm was taken. For the other metal stresses, 0.5 mM CaCl2 with pH 4.5 (including 10 µM La3+, 25 µM Cd2+, 30 µM Al3+, or 1 µM Cu2+) for 4 h were used, excising root tip 0-1 cm, respectively. In the Fe deficiency assay, soybean seedlings were cultured in nutrient solution (Horst et al., 1992) without Fe (pH 4.5) for 5 days, and 0-1 cm of root tip was excised by a scalpel.

The sequence analysis and gene cloning of GmMATE13

Sequences of GmMATE13 (Glyma.02G181800.1) were searched from NCBI (https://www.ncbi.nlm.nih.gov). Software MEGA5 was used to generate a phylogenetic tree of GmMATE13 and other identified citrate transporters. The cDNA template was prepared with the root apices of Jiyu 70 after 4 h Al treatment. The sequences of GmMATE13 were cloned by reverse transcription PCR. The sequence was aligned in DNASTAR.

Quantitative real-time PCR (qRT-PCR)

The reaction system and protocol of qRT-PCR were described in previous literature (Zhou et al., 2019). Gene-specific primers are shown on Table S1. The internal standard is GmTubulin (GenBank ID: 100811275). The relative GmMATE13 transcriptional expression level was calculated by the 2-ΔΔCt method as described (Livak and Schmittgen, 2001).

GmMATE13 subcellular localization

The CDS of GmMATE13 was constructed into the pENSG-N-GFP vector with a CaMV 35S promoter. The GFP control and GFP-GmMATE13 fusion proteins were transiently transformed into Arabidopsis protoplasts. The transformed protoplast cells were stained by marker (Cell Mask™ Orange plasma membrane stain, C10045, USA). GFP fluorescence was observed by confocal microscopy (Zeiss, LSM 900 with Airyscan 2, Germany).

Overexpressing GmMATE13 in soybean hairy roots

The CDS of GmMATE13 was constructed in the pCAMBIA3301 vector with CaMV 35S as a promoter and nanoluciferase as the label and then diverted into the Agrobacterium strain K599. The Jiyu 62 cotyledons were transformed with strain K599 according to Subramanian et al. (2005). The luciferase activity in soybean was confirmed via luminometer (Centro LB960X33, Bert-hold, Germany) with substrate Coelenterazine (Prolume Ltd., Pinctop, USA) to verify the success rate of transformants. The luciferase value ≥5000 of hairy roots was identified as a successful transformation (GmMATE13-OE) and then selected for next experiment. Hairy roots transformed by only K599 were the wild-type (WT) control. Then, the WT and GmMATE13-OE hairy roots were cultured in the 0.5 mM CaCl2 solution plus 0 or 30 µM AlCl3 (pH 4.5) in a container. After 4 h, collecting the root exudates to measure citrate efflux via the enzymatic method according to Delhaize et al. (1993). Meanwhile, excising 0-1 cm root apices to measure Al content, which were extracted by 2 M HCl and detected via inductively coupled plasma mass spectrometry (1CP-MS) (Agilent Technologies 7500C, USA). Callose of soybean hairy roots (0-3 cm) was extracted and then evaluated according to a previous study...
Briefly, the roots were fixed in ethanol and homogenized in NaOH (1 M), incubated in a water bath at 80°C for 30 min and then centrifuged. The supernatant (200 µL) was mixed with 0.1% aniline blue solution (400 µL), HCl (1 M, 210 µL), glycine buffer (1 M, pH 9.5, 590 µL) in a water bath at 50°C for 20 min. The callose content was determined using fluorescence spectrophotometry (excitation wavelength 400 nm; emission wavelength 500 nm) with laminarin as a standard.

### Tissue-localization of the expression of GmMATE13

The promoter sequence of GmMATE13 was cloned from Jiyu70, Jiyu62, Williams82 genomic DNA. The cloned sequences (1500 bp) did not differ at all and then were constructed into pCAMBIA3301 vector with GUS as the label and diverted into the Agrobacterium strain K599. The cotyledon transformation is the same as the previous step. With exposure to 0.5 mM CaCl2 solution plus 0 or 30 µM AlCl3 at pH 4.5, the same growing roots were dipped in a mixture X-gluc solution to stain and photographed with microscope. Using the microtome to dissect slices of the root apex to 10 µm, they put them on the glass slides, which were observed via microscope (Zeiss 2012 Observer A1, Göttingen, Germany).

### Transcriptional expression analysis of GmMATE13

For analysis of the tissue location of expression of GmMATE13 in different types of soil, Jiyu70, Jiyu62, Williams82 genomic DNA. The cloned sequences did not differ at all and then were constructed into pCAMBIA3301 vector with GUS as the label and diverted into the Agrobacterium strain K599. The cotyledon transformation is the same as the previous step. With exposure to 0.5 mM CaCl2 solution plus 0 or 30 µM AlCl3 at pH 4.5, the same growing roots were dipped in a mixture X-gluc solution to stain and photographed with microscope. Using the microtome to dissect slices of the root apex to 10 µm, they put them on the glass slides, which were observed via microscope (Zeiss 2012 Observer A1, Göttingen, Germany).

### Statistical analysis

All of the data were analyzed by IBM SPSS, and P<0.05 represented statistically significant.

### Results

#### Phylogenetic analysis of GmMATE13

The GmMATE13 amino acid sequence was highly conserved with other citrate transporters and contained 12 transmembrane domains named TM1-TM12. There was a loop of 73 amino acids between TM2 and TM3. There was a large variation in the N-terminal among these citrate transporters (Figure 1A).

MEGA5.0 software was used for phylogenetic analysis of MATE proteins from different species, and the results are shown in Figure 1B. GmMATE13 has the highest sequence homology with EcMATE3, which is shown to function as a citrate transporter involved in Al resistance in plants (Sawaki et al., 2013). ZmMATE1, ZmMATE2 (Maron et al., 2010; Maron et al., 2013), GmMATE87 (Zhou et al., 2019), AtMATE (Liu et al., 2009; Liu et al., 2012), BoMATE (Wu et al., 2014), and ScFRDL2 (Yokosho et al., 2010) also had high homology with GmMATE13, and these proteins are proved to be involved in Al resistance in plants. The homology of GmMATE13 with ZmMATE1, ZmMATE2, AtMATE, BoMATE, and ScFRDL2 was 52.43%, 53.17%, 60.19%, 59.20%, and 56.32%, respectively. The homology of the GmMATE13 protein sequence with GmMATE79, GmMATE87, GmMATE47, and GmMATE75 was 50.36%, 56.19%, 51.75%, and 49.64%, respectively.

#### Transcriptional expression pattern of GmMATE13

GmMATE13 was expressed in the root tips of both Al-resistant soybean variety Jiyu 70 and Al-sensitive soybean variety Jiyu 62, but the relative expression level was different between the two varieties. The expression level of Jiyu 70 was higher than Jiyu 62 along with Al. With the extension of treatment time, the expression level of GmMATE13 increased gradually in the root tips of Jiyu 62, remained stable from 4 to 12 h, and suddenly increased at 24 h. By contrast, in the root tips of Jiyu 70, with the extension of Al treatment time, the expression level of GmMATE13 increased first and then decreased and reached the maximum at 8 h (Figure 2A).

To determine whether the induction of GmMATE13 expression was Al-specific, we tested the effect of other metals on GmMATE13 expression in the root apices. As shown in Figure 2B, GmMATE13 expression was induced by Al3+ but not other metals (Cd2+, La3+, and Cu2+). Moreover, after 10 days culturing, there was no significant difference in the expression level of GmMATE13 between the +Fe treatment and the -Fe treatment (Figure 2C), indicating that the expression level of GmMATE13 did not respond to the Fe deficiency.

#### Subcellular localization of GmMATE13

To examine the subcellular localization of GmMATE13, we introduced plasmid-containing GFP or GFP-GmMATE13 fusion protein into the Arabidopsis protoplast, and the GFP fluorescence was observed to study the location of GmMATE13. While the GFP control was expressed in both the cytoplasm and plasma membrane, GFP-GmMATE13 was expressed on the plasma membrane, which was overlapped with the plasma...
membrane marker staining, indicating that GmMATE13 was a plasma membrane-localized protein. The location of GmMATE13 was the same as other identified citrate transporters, such as GmMATE75 (Zhou et al., 2019).

Overexpressing GmMATE13 in soybean hairy roots

To investigate the biological function of GmMATE13, GmMATE13 was expressed in soybean hairy roots under the control of the CaMV35S promoter. GmMATE13-OE hairy roots increased the transcript levels of GmMATE13 and citrate efflux under either -Al or +Al treatment (Figures 3A, B). The Al content of hairy root tips of GmMATE13-OE treated with Al was also lower than that of WT (Figure 3C). GmMATE13-OE roots showed lighter hematoxylin staining after 4 h of Al treatment compared with WT roots, which was consistent with higher citrate efflux and lower Al content in root tips (Figure 3D). Overexpression of GmMATE13 decreased callose concentration in soybean root apices compared with WT (Figure 3E). Al treatment increased the callose concentration in WT and GmMATE13-OE; however, the callose content of GmMATE13-OE remained significantly lower than that of WT, which is consistent with the phenotype of Al resistance in GmMATE13-OE soybean hairy roots.

β-Glucuronidase (GUS) staining

To study the tissue-localization of the expression of GmMATE13, the promoter region of GmMATE13 fused with the GUS gene was introduced into soybean hairy roots. In the absence of Al treatment, GUS staining of pGmMATE13::GUS transformation was confined to the central cylindrical region of the root apex (Figures 4A, B). Its GUS staining got heavier and extended from the central cylinder to cortical and epidermis cells after 4 h Al treatment (Figures 4C, D), which was similar to other pGmMATEs::GUS (Zhou et al., 2019). Further observation showed that the expression location of GmMATE13 was different from the other three genes; namely, it was only expressed in the root tip of soybean hairy roots but had almost no expression in other parts of the hairy roots, indicating that GmMATE13 may have specificity and high efficiency for soybean Al resistance.

Expression of GmMATE13 in soybean grown on soils with different pH

In order to analyze the expression patterns of GmMATE13, the soybeans were grown on different soils characteristic of varied pH, exchangeable Al and Fe (Table S2). As shown in Figure 5, GmMATE13 is mainly expressed in roots and only weakly in stems and leaves. The expression level of GmMATE13 is the lowest in the soybeans grown on soils with high pH, low Al, and low Fe conditions and highest in roots on soils of low pH, high Al, and low Fe soils. In addition to Fe and Al, the expression of GmMATE13 might also be affected by other soil factors.

Discussion

Al-induced citrate efflux is one of the most important Al-resistance mechanisms in soybeans (Yang et al., 2000; Yang
FIGURE 2
Transcriptional expression pattern analysis of GmMATE13. (A) Time course of GmMATE13 expression patterns in root tips under aluminum stress. After 7 days, the seedlings (Jiyu70 and Jiyu62) were transplanted to 0.5 mM CaCl2 solution plus 30 µM AlCl3 at pH 4.5. The 0-1 cm root tips were excised after 0, 2, 4, 8, 12, and 24 h Al exposure to investigate the expression of GmMATE13. (B) Effect of different metals on the expression of GmMATE13 in root apices. Seedlings were treated to 30 µM Al, 1 µM Cu, 10 µM La, or 25 µM Cd for 4 h, excising the 0-1 cm root tips. (C) Transcriptional expression of GmMATE13 with -Fe solution. The seedlings were cultivated in nutrient solution with or without Fe-EDTA for 10-day culture, excising the 0-1 cm root apices. Data are represented as means ± SD (n=3). Error bars with different letters represented significantly different by Tukey’s test (P<.05).
FIGURE 3
Characteristics of Al resistance in soybean hairy roots overexpressing GmMATE13. (A) Transcriptional expression of GmMATE13 in soybean hairy roots of GmMATE13-OE. (B) Citrate efflux from GmMATE13-OE soybean hairy roots. (C) Al accumulation in the root apices of GmMATE13-OE hairy roots. (D) The hematoxylin staining in WT and GmMATE13-OE soybean hairy roots under Al stress. (E) Callose content in the hair roots of WT and GmMATE13-OE without or with 30 µM AlCl₃ treatment for 4 h. Values represent the means ± SD (n=3). Different letters indicate statistically significant difference by Tukey’s test (P<.05).

FIGURE 4
Tissue-level localization of GmMATE13 expression. Gus staining observed the activation of GmMATE13 promoter in intact root or its cross-section slices. The promoter of GmMATE13 without Al stress is shown in (A, B) The promoter of GmMATE13 with Al stress was shown in (C, D).
et al., 2001; Silva et al., 2001). Bioinformatics analysis shows that GmMATE13 proteins belong to the MATE family and are citrate transporters with 12 transmembrane domains and a large loop structure between the second and third domains but with high specificity in the N-terminal sequence (Figure 1A). GmMATE13 has high homology with AtMATE, VuMATE, and LjMATE (Figure 1B), all located on the plasma membrane (Figure 6), which are transporters associated with both Al and Fe (Liu et al., 2012; Liu et al., 2013; Takanashi et al., 2013).

Analysis of the temporal expression pattern of GmMATE13 showed that GmMATE13 was induced by Al, and the expression level distinguished between Al-tolerant soybean variety Jiyu 70 and Al-sensitive soybean variety Jiyu 62. The expression of GmMATE13 showed a trend of first increase and then decrease in Jiyu 70, whereas in Jiyu 62, increasing first, after stabilizing for a period of time, and then increasing again (Figure 2A). For a specific experiment, the expression of GmMATE13 was downregulated by La^{3+}, Cu^{2+}, and Cd^{2+} (Figure 2B), which was a slight difference from other GmMATEs (Zhou et al., 2019). The expression level of GmMATE13 was not induced by Fe deficiency (Figure 2C), indicating that GmMATE13 might not be involved in the Fe transport pathway in soybeans. Previous studies have found that the function of the citrate transporter is not only involved in the Al resistance of plants, but also in the Fe translocation (Liu et al., 2012; Liu et al., 2013; Takanashi et al., 2013; Liu et al., 2016). HvAACT1 expression is mainly involved in Fe transport in the root and the Al-resistance pathway when the expression of HvAACT1 migrates to the epidermis (Fujii et al., 2012).

It has been proved that H_{2}S acts on the downstream of NO and mediates Al-induced citrate efflux, attaching resistance to Al toxicity in plants (Wang et al., 2019). The effect of other soil factors on GmMATE13 gene expression needs to be studied in the future. In the four types of soil, GmMATE13 was mainly expressed in roots, not in stems, and only weakly in leaves, and the highest expression level was found in the soil with low Fe, high Al, and low pH, indicating that GmMATE13 showed a response to high Al in highly acid soil and played a key role in detoxifying Al from soybeans (Figure 5).

Overexpression of GmMATE13 under the control of the CaMV 35S promoter in soybean hair roots increased citrate
secretion irrespective of being treated with Al or not, and reduced Al content in root tips when exposed to Al (Figure 3). Al-induced callose deposition is a sensitive indicator of Al toxicity and has been used as a convenient and rapid screening parameter for Al injury in addition to root elongation measurement (Horst et al., 1997; Yang et al., 2000; Zhang et al., 2015; Wang et al., 2021). Overexpression of GmMATE13 decreased callose concentration in soybean root apices compared with WT (Figure 3). Overexpression of SbMATE and ZmMATE1 in Arabidopsis thaliana (Magalhaes et al., 2007; Maron et al., 2010) and HvAACT1 overexpression in tobacco (Furukawa et al., 2007) showed significantly enhanced citrate secretion and Al resistance. The function of GmMATE13 was shown to increase Al resistance by heterogeneous expression in Arabidopsis by high Al concentration treatment (400 µM) (Wang et al., 2019). Multiple genes, such as AtMATE1, AtALMT1, AtALS3, and AtSPO1, are involved in the Al-resistance responses in Arabidopsis (Hoekenga et al., 2006; Liu et al., 2009; Liu et al., 2014; Huang, 2021). The functional and structural characteristics of GmMATE13 are consistent and resemble those reported for other citrate-permeable MATEs in barley (Furukawa et al., 2007), sorghum (Magalhaes et al., 2007), maize (Maron et al., 2010), and Arabidopsis (Liu et al., 2009).

The physiological functions of plant MATEs reported so far include xenobiotic secretion, accumulation of secondary metabolites such as alkaloids and flavonoids, Fe translocation, Al detoxification, and plant hormone signal transduction, suggesting that MATE transporters are involved in a series of biological events during plant development (Diner et al., 2001; Durrett et al., 2007; Furukawa et al., 2007; Magalhaes et al., 2007; Thompson et al., 2010; Takanashi et al., 2014). Through the tissue-level localization analysis, Zhou et al. (2019) revealed that the expression of GmMATEs extended from the central cylinder to cortical and epidermis cells after 4 h Al exposure, and so did GmMATE13 (Figures 4B, D). Interestingly, it only expressed in the root tip of soybean (Figures 4A, C), indicating that GmMATE13 may have specificity and high efficiency for soybean Al resistance.

In conclusion, GmMATE13 was identified in soybean as a plasma-membrane localized citrate transporter. The transcriptional expression of GmMATE13 was induced by Al stress, and different expression patterns were observed between Al-sensitive Jiyu 62 and Al-resistant Jiyu 70. The overexpression of GmMATE13 in hairy roots increased the Al resistance of soybeans. There was a high expression of GmMATE13 in root tips of soybeans grown on acidic soil, indicating that GmMATE13 play a key role in the soybean Al resistance signaling pathway. The regulation mechanism deserved further investigation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

JY designed the entire experiment. ZW, YL, WC, LG, YH, and QZ performed the major experiments. XM and ZY helped in data analysis and some useful advice in experiment design. JY
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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.1027560/full#supplementary-material
Magnesium ameliorates aluminum rhizotoxicity in soybean by increasing citric acid production and exudation by roots.

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