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Aluminium alleviates manganese toxicity to rice by decreasing root symplastic Mn uptake and reducing availability to shoots of Mn stored in roots

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• **Background and Aims** Manganese (Mn) and aluminium (Al) phytotoxicities occur mainly in acid soils. In some plant species, Al alleviates Mn toxicity, but the mechanisms underlying this effect are obscure.
• **Methods** Rice (*Oryza sativa*) seedlings (11 d old) were grown in nutrient solution containing different concentrations of Mn^{2+} and Al^{3+} in short-term (24 h) and long-term (3 weeks) treatments. Measurements were taken of root symplastic sap, root Mn plaques, cell membrane electrical surface potential and Mn activity, root morphology and plant growth.
• **Key Results** In the 3-week treatment, addition of Al resulted in increased root and shoot dry weight for plants under toxic levels of Mn. This was associated with decreased Mn concentration in the shoots and increased Mn concentration in the roots. In the 24-h treatment, addition of Al resulted in decreased Mn accumulation in the root symplasts and in the shoots. This was attributed to higher cell membrane surface electrical potential and lower Mn^{2+} activity at the cell membrane surface. The increased Mn accumulation in roots from the 3-week treatment was attributed to the formation of Mn plaques, which were probably related to the Al-induced increase in root aerenchyma.
• **Conclusions** The results show that Al alleviated Mn toxicity in rice, and this could be attributed to decreased shoot Mn accumulation resulting from an Al-induced decrease in root symplastic Mn uptake. The decrease in root symplastic Mn uptake resulted from an Al-induced change in cell membrane potential. In addition, Al increased Mn plaques in the roots and changed the binding properties of the cell wall, resulting in accumulation of non-available Mn in roots.

Key words: Aluminium, manganese toxicity, rice, *Oryza sativa*, Gouy–Chapman–Stern model, manganese plaques, plasma membrane surface electrical potential, root aerenchyma, root symplast, acid soils.

INTRODUCTION

Manganese (Mn) is an essential element for plants and is involved in regulating several metabolic processes, such as photosynthesis and antioxidant activity (Marschner, 1995*a*). Nevertheless, an excess of Mn is toxic for most plants (Millaleo *et al*., 2010). Manganese toxicity is one of the major factors restricting plant growth on poorly drained and acidic soils with high concentrations of readily available Mn^{2+} (Marschner, 1995*b*). In plants, Mn tends to accumulate predominantly in shoots rather than roots (Lidon, 2001; Page and Feller, 2005; Page *et al*., 2006). Therefore, in addition to decreased growth rate, symptoms of Mn toxicity, such as chlorosis and necrotic brown spots, are commonly visible on leaves (Moroni *et al*., 2003; Rosas *et al*., 2007; Mora *et al*., 2009; Führs *et al*., 2010; Li *et al*., 2011). The threshold of Mn toxicity and tolerance varies among different plant species and among cultivars and genotypes within a species (Foy *et al*., 1988; Horst, 1988). In general, rice (*Oryza sativa*) plants are considered to be tolerant to Mn toxicity (Lidon, 2001). Compared with other grasses, rice plants can accumulate 5–10 times more Mn in the leaves and show milder symptoms of toxicity (Foy *et al*., 1978). Although Mn toxicity rarely occurs in flooded or paddy rice, this is not the case for upland rice grown on aerobic soils. Acid upland soils may contain toxic levels of both Mn and aluminium (Al) (Nelson, 1983), and as a consequence Al and Mn toxicities may limit the yields of upland rice (Ponnamperuma, 1975).

Aluminium toxicity is generally considered to be the primary limiting factor for plant growth in acid soils (Kochian *et al*., 2004). Low concentrations (<10 μM) of Al can inhibit root elongation and cause structural and functional damage to roots (Kochian, 1995). Many plants predominately accumulate Al in the roots, while some plants, such as buckwheat (*Fagopyrum esculentum*) (Ma *et al*., 1998), tea (*Camellia* spp.) (Chenery, 1955; Zeng *et al*., 2013) and plants in the Melastomataceae (e.g. *Melastoma malabathricum*) (Watanabe *et al*., 2005), accumulate high concentrations of Al in either roots or leaves.

High concentrations of Al and Mn can co-occur in soils, restricting crop production in acid and high-Mn soils. In previous studies, Australian soils were classified into seven categories covering the range of Al and Mn combinations (Dolling *et al*., 2001; Yang *et al*., 2009). Most agricultural soils were

designated as Class 2, with a low concentration of available Al and a high Mn concentration. Heavy rainfall can change the concentrations of available Al and Mn, and consequently, change Class 2 soils to Class 3, with moderate Al and very high Mn concentrations (Yang *et al*., 2009). Although high concentrations of Mn and Al can co-occur in acid soil, the tolerances of plants/cultivars to Al and Mn do not necessarily coincide (Nelson, 1983). This raises questions as to how plants adapt to both Mn and Al toxicity and how Mn interacts with Al in plants.

Previous reports have indicated that Al can reduce Mn accumulation in barley (*Hordeum vulgare*), atriplex (*Atriplex hastata*) (Rees and Sidrak, 1961), corn (*Zea mays*) (Clark, 1977), wheat (*Triticum aestivum*) (Blair and Taylor, 1997), cowpea (*Vigna unguiculata*) (Taylor *et al*., 1998) and soybean (*Glycine max*) (Yang *et al*., 2009). Alleviation of the effects of Al on Mn toxicity has been reported for atriplex (Rees and Sidrak, 1961) and soybean (Yang *et al*., 2009), and a dose-dependent effect of Al on Mn toxicity has been demonstrated in wheat and cowpea (Blair and Taylor, 1997; Taylor *et al*., 1998). One explanation for this alleviation is that Al may have an antagonistic effect on Mn uptake by plant roots. However, the exact mechanism is still poorly understood. The results of Kopittke *et al*. (2011*a*), based on the Gouy–Chapman–Stern (GCS) model, revealed that the effects of cations on Mn nutrition were related to the electrical potential (ψ^o_0) of the root cell membrane (CM) surface. In their study, when cowpea was cultured in solutions containing different Mn concentrations, the root tissue Mn concentration was closely related to the activity of Mn^{2+} at the outer surface of the CM. This result provided new clues about the interaction between Al and Mn.

The GCS model combines electrostatic theory (Gouy–Chapman theory) with an ion-binding model (Stern model) to calculate ψ^o_0. This could be a useful and powerful tool to explain the Mn–Al interaction in plants. To better understand the effects of Al on Mn toxicity, we explored how Al affected Mn toxicity using Al- and Mn-tolerant rice plants. Our results differ from those reported previously; in particular, the effects of Al on root Mn accumulation in rice completely contrast with those in other plant species.

MATERIALS AND METHODS

Plant material and growth conditions

We used Wuyunjing7, an Al-tolerant cultivar of rice (*Oryza sativa* var. *japonica*) (Zhao *et al*., 2009) in these experiments. The rice seeds were surface-sterilized in 1 % (v:v) sodium hypochlorite solution for 30 min, washed thoroughly with deionized water, and then soaked in deionized water overnight. The seeds were germinated in an incubator at 30 °C for 24 h, and then transferred to a nylon net floating on a 6·5-L plastic pot contained 0·5 mM $CaCl_2$ (pH 4·5). The solution was renewed every 2 d. After 3 days of growth, the seedlings were transferred to light conditions and grown in 0·5 mM $CaCl_2$ (pH 4·5) for a further 2 d. Then, uniform seedlings (5 d old) were transferred to 1·5 L of half-strength Kimura B nutrient solution (pH 4·5) and grown for 6 d. The half-strength Kimura B nutrient solution contained 0·18 mM $(NH_4)_2SO_4$, 0·27 mM $MgSO_4{\cdot}7H_2O$, 0·09 mM KNO_3, 0·18 mM Ca $(NO_3)_2{\cdot}4H_2O$, 0·09 mM KH_2PO_4, 6·7 μM $MnCl_2{\cdot}4H_2O$, 9·4 μM H_3BO_3, 0·01 μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0·15 μM $ZnSO_4{\cdot}7H_2O$, 0·16 μM $CuSO_4{\cdot}5H_2O$ and 10 μM EDTA-Fe. The solution was renewed every 2 d without aeration. Finally, 11-d-old seedlings were used in experiments with different Al and Mn treatments. The seedlings were grown in a growth room under a 14:10-h light:-dark photoperiod with 28 ± 1:25 ± 1 °C, day:night temperatures. The relative humidity was controlled at 65 ± 1 % and the light level was controlled at 375 μmol photon m^{-2} s^{-1}.

For all Al–Mn interaction experiments, four seedlings were grown in each 1·5 L of nutrient solution. To identify a suitable Al concentration for experiments on the Al–Mn interaction, 11-d-old seedlings were grown in half-strength Kimura B nutrient solutions containing 0, 200, 500 or 1000 μM Al for 3 weeks. Based on the results of this preliminary experiment, 200 μM Al was chosen for further experiments. For the Al–Mn interaction experiments, 11-d-old seedlings were grown in half-strength Kimura B nutrient solution containing 6·7, 200, 500 or 1000 μM Mn in the presence or absence of 200 μM Al for 24 h (short term) or 3 weeks (long term). These solutions were renewed every 2 d without aeration. At the end of the long-term experiments, we measured rice growth, Mn concentration, root Mn plaques, root aerenchyma, root diameter and root volume. At the end of the short-term experiments, we determined Mn concentrations in the root symplast, whole roots and whole shoots. The Al and Mn were supplied as $AlCl_3$ and $MnCl_2$, respectively. The initial pH of each solution was adjusted to 4·5 using 1 M NaOH or HCl. All experiments were conducted with three replicates, and each experiment was performed at least twice.

Extraction of root symplastic sap

The roots were washed with deionized water and gently blotted dry, and then cut into pieces. The pieces were placed in an ice-cool filter unit. The root symplastic sap was collected by centrifugation according to Klug *et al*. (2010) and Xia *et al*. (2010). Briefly, roots that were freshly cut from the shoot base of four seedlings were placed in a filter unit with a pore size of 0·45 μm (Ultrafree-MC, Millipore, Billerica, MA, USA). The nutrient solution adhering to the surface of the roots was removed by centrifugation at 60 *g* for 2 min. Apoplastic solutions were removed by centrifugation at 3000 *g* for 15 min at 4 °C. The roots were then frozen at −80 °C overnight. The root symplastic sap solution was obtained by thawing the samples at room temperature and then centrifuging them at 21 600 *g* for 10 min. The residues (i.e. whole root) were washed three times with deionized water and then oven-dried at 75 °C to constant weight. The Mn concentrations in root symplastic sap and whole roots were determined by inductively coupled plasma mass spectrometry (ICP-MS) using an Agilent 7700x instrument (Agilent, Santa Clara, CA, USA), as described below.

Extraction and observation of root Mn plaques

Roots were washed twice with deionized water to remove any extraneous precipitate, and then cut from each plant at the shoot base. Root Mn plaques on the root surfaces were extracted using the dithionite citrate bicarbonate (DCB) technique (Taylor and Crowder, 1983; Crowder and Coltman, 1993),

which was modified as follows: all root segments from each plant were agitated in a solution containing 40 mL of 0·3 M $Na_2C_6H_5O_7 \bullet 3H_2O$ and 5 mL of 1 M $NaHCO_3$ for 3 h. At the start of the experiment, 3 g of $Na_2S_2O_4$ was added to the solution. The extractant was then decanted into a 100-mL volumetric flask and the roots were washed twice with deionized water, which was also decanted into the 100-mL volumetric flask. The resulting solution was made up to 100 mL with deionized water. We prepared DCB control solutions without roots as described above, but substituting an equimolar concentration of Na_2SO_3 for $Na_2S_2O_4$ (with respect to Na). After roots had been extracted, they were oven-dried at 75 °C to constant weight. The dry masses of roots and shoots were measured and recorded. The Mn concentrations of Mn plaques were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Optima 8000, Perkin Elmer) after appropriate dilution. The Mn concentrations of roots after extraction were determined as described below.

To detect Mn plaques by microscopy, roots were harvested after treatments and cut into 5-mm pieces, and then quick-frozen and freeze-dried. After coating with gold, root segments were observed under a scanning electron microscope (SEM-3000N, Hitachi, Tokyo, Japan) and analysed by energy-dispersive X-ray spectrometry (EDX) (EDX-250, Horiba, Kyoto, Japan).

Observation of aerenchyma and measurement of root diameter and volume

Newly formed adventitious roots, ~30 mm long, were collected after the long-term experiment. Fresh samples were cut into 5-mm pieces using a razor blade to observe aerenchyma by SEM using the method of David and Olga (2000). Then, the root samples were viewed and photographed under an SEM (3000N, Hitachi).

To measure root diameter and volume, whole roots were washed with deionized water and then scanned using a root scanner (Perfection V700 Photo, Seiko Epson, Nagano, Japan). Root diameter and volume were calculated using the WinRHIZO analysis system (Regent, Quebec, Canada).

Computation of CM surface electrical potential and Mn activity at CM surface

Values for the CM surface electrical potential (ψ^o_0) and Mn^{2+} activity at the CM surface ($\{Mn^{2+}\}^o_0$) were calculated using a computer program for the GCS model (Kinraide *et al*., 1998; Kopittke *et al*., 2014). This computer program was a kind gift from Professor T. B. Kinraide and Dr Peng Wang. Briefly, the GCS model consists of the Gouy–Chapman portion and the Stern portion. The Gouy–Chapman portion of the model refers to electrostatic theory pertaining to charged surfaces in contact with solutions. As regards the Stern portion, the CM was considered to contain negatively charged (R^-) and neutral (P^0) sites to which ions (I^Z) may bind. A converging calculation between the Gouy–Chapman and Stern portions allows the calculation of ψ^o_0 (Kinraide and Wang, 2010; Tatulian, 1999). The ψ^o_0 values calculated from the GCS model allow the calculation of ion I^Z at the CM surface ($\{I^Z\}^o_0$) by the Nernst equation ($\{I^Z\}^o_0 = \{I^Z\}_b \exp[-ZF\psi^o_0/(RT)]$), where F, R and T are the Faraday constant, the gas constant and temperature, respectively (Wang *et al*., 2011). For details of the calculation of ψ^o_0, refer to Kinraide (1994) and Kinraide *et al*. (1998).

Rice harvest and Mn analysis

At the end of the long-term experiment, the roots were rinsed briefly with deionized water and blotted dry. The roots and shoots were then harvested separately. The longest root and the longest shoot on each seedling were measured with a ruler. The roots and shoots were oven-dried at 75 °C to constant weight and then weighed and ground. Then, ground samples (100–500 mg) were digested with 5 mL of 5:1 (v:v) concentrated nitric acid:perchloric acid. After complete digestion, the samples were diluted to 50 mL using deionized water and the Mn concentrations were determined by ICP-AES (Optima 8000, Perkin Elmer). At the end of the short-term experiment, roots were harvested and their symplastic sap was collected, and then the roots were digested in 0·5 mL of ultra-trace concentrated nitric acid. After appropriate dilution, the Mn concentrations in the samples were determined by ICP-MS using an Agilent 7700x instrument.

Statistical analysis

All data were subjected to one-way analysis of variance after testing for normality and homogeneity. Multiple comparisons among treatments were performed using Tukey's multiple range test at the 0·05 probability level. All these analyses were performed using SAS 9.1 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Rice growth

We conducted a preliminary Al toxicity experiment to identify a suitable Al concentration for research on the Mn–Al interaction. As shown in Supplementary Data Table S1, there was no significant difference in the root dry weights among all Al concentrations, but Al significantly inhibited root elongation at treatment concentrations of 500 and 1000 μM Al but not with the treatment concentration of 200 μM Al. The shoot dry weight and shoot height were lower in plants treated with 1000 μM Al than in control plants (0 μM Al), and shoot dry weight was significantly decreased by 500 μM Al. However, 200 μM Al had little effect on shoot height and shoot dry weight. Therefore, 200 μM Al (18·8 μM $\{Al^{3+}\}^o_0$) was used in the following Mn–Al interaction experiments.

In the absence of Al, 500 and 1000 μM Mn significantly decreased root length relative to that of plants grown with 6·7 μM Mn (Fig. 1A). However, there was no significant difference in root length among all Mn concentrations in the presence of Al (Fig. 1A). Shoot height and dry weights of roots and shoots significantly decreased with increasing Mn concentrations in the absence of Al (Fig. 1B–D). However, addition of Al markedly alleviated this inhibition, because all of these parameters were increased by Al addition at 500 and 1000 μM Mn (Fig. 1B–D).

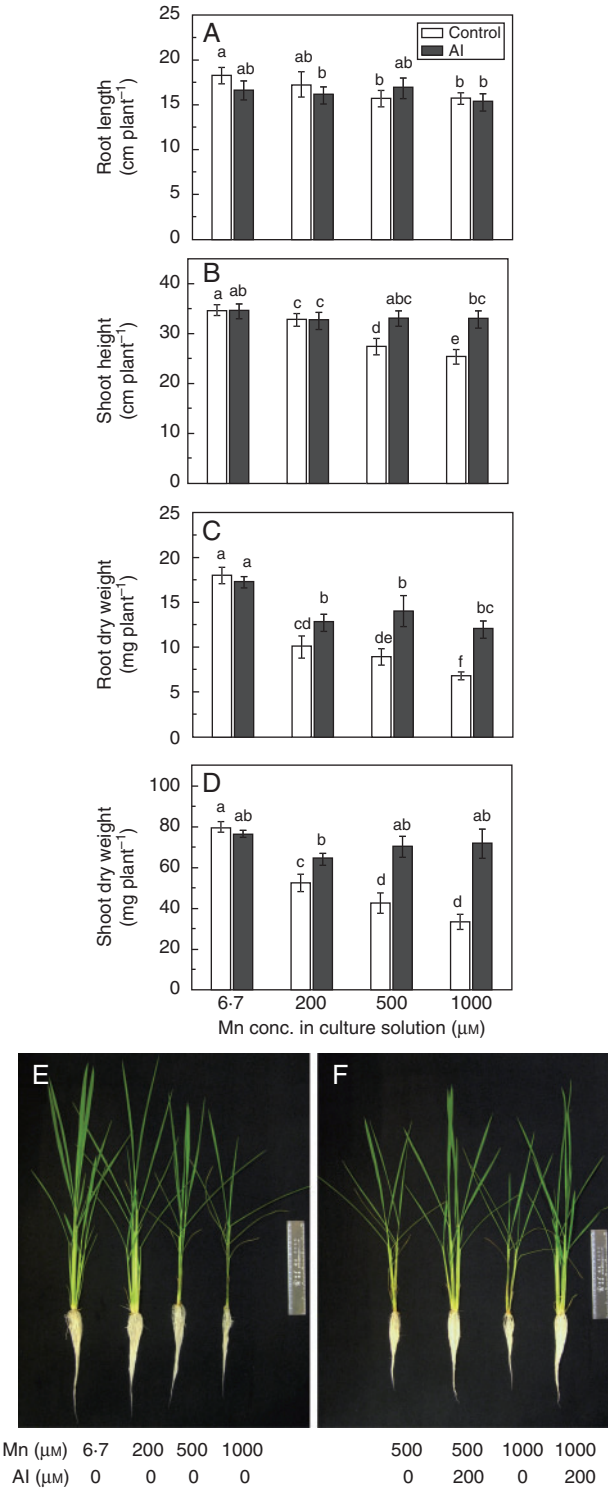

FIG. 1. Effects of Al on root length (A), shoot height (B), root dry weight (C) and shoot dry weight (D) of rice under Mn toxicity in a long-term experiment. (E) Toxicity symptoms of rice under Mn toxicity. (F) Ameliorating effect of Al on rice Mn toxicity. Rice seedlings (11 d old) were grown in nutrient solution with 6·7, 200, 500 or 1000 μM Mn in the presence or absence of 200 μM Al for 3 weeks. Values are mean ± s.e. ($n = 3$ replicates, each comprising four seedlings). Different letters in A–D show significant differences among treatments ($P < 0{\cdot}05$; Tukey's test).

These results indicated that Al addition alleviated the toxic effects of Mn on rice growth. The photographs of the seedlings in the various treatments illustrate the alleviating effects of Al on Mn toxicity (Fig. 1E, F). Rice growth was inhibited by high Mn concentrations and old leaves were nearly wilting (Fig. 1E), but addition of Al markedly improved growth of rice and the old leaves at 500 and 1000 μM Mn (Fig. 1F).

Rice Mn accumulation

After the long-term Al–Mn treatments (3 weeks), both root and shoot Mn accumulation significantly increased with increasing Mn concentrations, regardless of whether Al was present or not (Fig. 2A, B). At 6·7 μM Mn, addition of Al did not significantly affect Mn accumulation in roots and shoots (Fig. 2A, B). At 200, 500 and 1000 μM Mn, Al significantly decreased Mn accumulation in shoots (Fig. 2B) but increased Mn accumulation in roots (Fig. 2A).

After the short-term Al–Mn treatments (24 h), Mn concentration in the root symplast, whole roots and whole shoots also gradually increased with increasing Mn in all treatments (Fig. 3A–C). At 6·7 μM Mn, addition of Al did not significantly affect the Mn concentrations in the root symplast (Fig. 3A), whole roots (Fig. 3B) or whole shoots (Fig. 3C). At 200, 500 and 1000 μM Mn, addition of Al significantly reduced the Mn concentration in the root symplast (Fig. 3A) and whole shoots (Fig. 3C), consistent with the results of the long-term experiment (Fig. 2B). In roots, the Mn concentrations were significantly reduced by addition of Al at 1000 μM Mn, but not at 200 or 500 μM Mn (Fig. 3B). These results suggested that Al inhibited Mn uptake into the root symplast, resulting in less Mn being transported to shoots.

Cell membrane surface electrical potential and Mn activity in the bulk solution and at the cell membrane surface

At the same Al concentration, the value of ψ^{o}_{0} gradually became more positive with increasing Mn concentration (Table 1). Addition of Al drastically changed the ψ^{o}_{0} values from negative to positive (Table 1). The values of $\{Mn^{2+}\}^{o}_{0}$ and Mn^{2+} activity in bulk solution ($\{Mn^{2+}\}_{b}$) increased with increasing Mn supply, while addition of Al drastically decreased $\{Mn^{2+}\}^{o}_{0}$ but barely affected $\{Mn^{2+}\}_{b}$ (Table 1).

Root Mn plaques

We detected root Mn plaques using EDX. The EDX spectra showed that Mn signals had two peaks, one around 0·5 keV and the other is around 6·0 keV (Fig. 4F–H). Since the first one could not easily be differentiated from other element peaks, the second peak was usually used for Mn analysis (Batty *et al.*, 2002). This peak was absent from all Mn treatments in the absence of Al (Fig. 4A–D) and from the 6·7 μM Mn treatment in the presence of Al (Fig. 4E). However, Mn signals intensified when Al was added to solutions containing 200, 500 or 1000 μM Mn (Fig. 4F–H). The Mn plaques in roots were quantified using the DCB extraction technique. The Mn concentration in the DCB extract from roots and in roots after DCB extraction

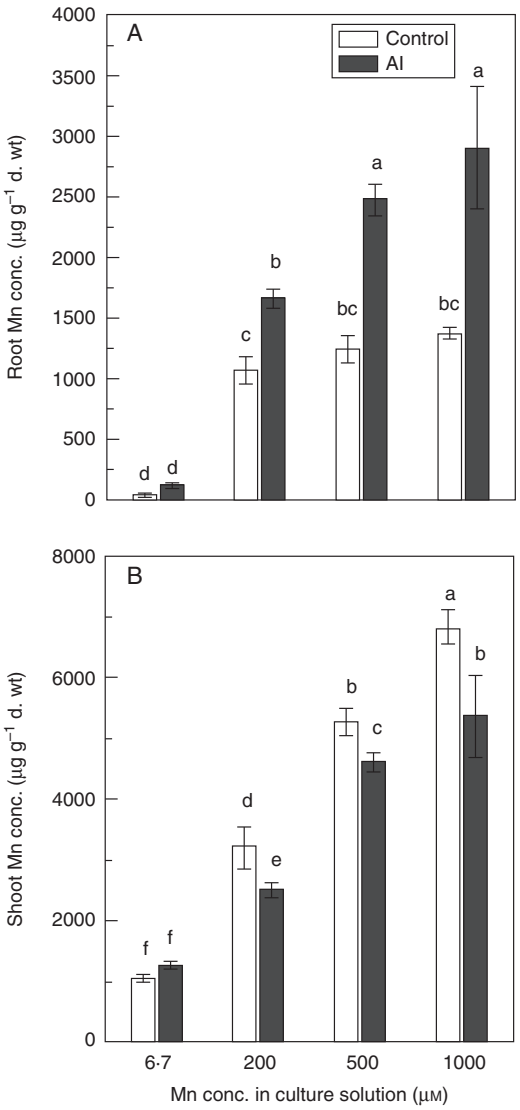

FIG. 2. Effects of Al on Mn accumulation in roots (A) and shoots (B) of rice under Mn toxicity in a long-term experiment. Rice seedlings (11 d old) were grown in nutrient solution with 6·7, 200, 500 or 1000 μM Mn in the presence or absence of 200 μM Al for 3 weeks. Values are mean ± s.e. ($n = 3$ replicates, each comprising four seedlings). Different letters show significant differences among treatments ($P < 0.05$; Tukey's test).

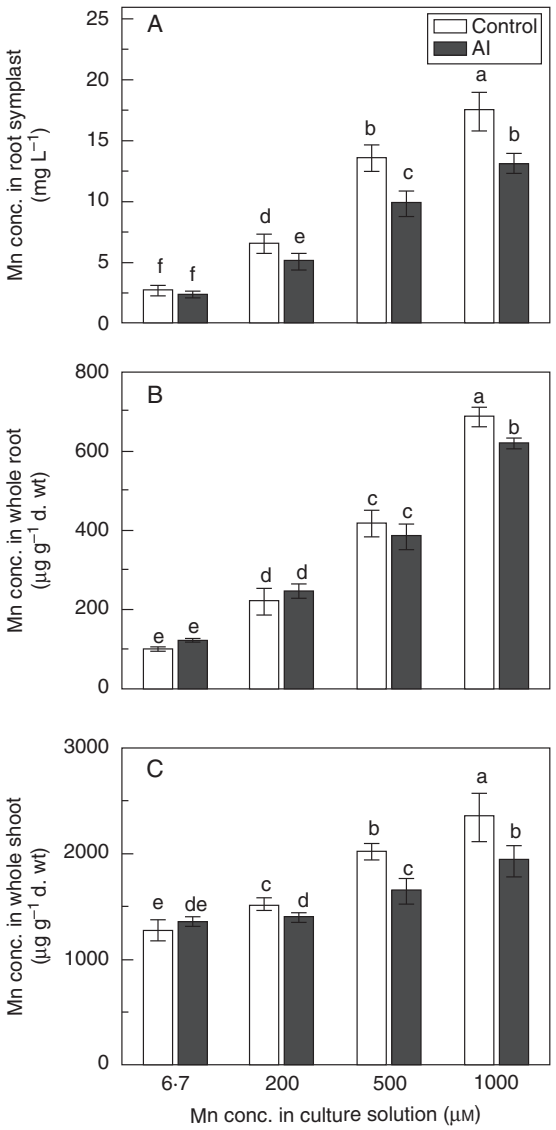

FIG. 3. Concentrations of Mn in root symplast (A), whole root (B) and whole shoot (C) after short-term treatment. Rice seedlings (11 d old) were grown in nutrient solution with 6·7, 200, 500 or 1000 μM Mn in the presence or absence of 200 μM Al for 24 h. Values are mean ± s.e. ($n = 3$ replicates, each comprising four seedlings). Different letters show significant differences among treatments ($P < 0.05$, Tukey's test).

TABLE 1. *Calculated surface electrical potential at outer surface of the plasma membrane and Mn activity in bulk solution and at the cell membrane surface*

Al (μM)	Mn (μM)	ψ^o_0 (mV)	$\{Mn^{2+}\}_b$ (μM)	$\{Mn^{2+}\}_0{}^o$ (μM)
0	6·7	–30·4	5·1	55·0
200	6·7	7·9	5·1	2·8
0	200	–26·7	152·0	1212·9
200	200	8·2	150·0	79·0
0	500	–22·8	370·6	2183·2
200	500	8·6	365·6	187·6
0	1000	–18·5	715·8	3010·0
200	1000	9·1	707·3	349·2

ψ^o_0, surface electrical potential at outer surface of plasma membrane; $\{Mn^{2+}\}_b$, Mn activity in bulk solution; $\{Mn^{2+}\}^o_0$, Mn activity at cell membrane surface.

All pH values were adjusted to 4·5 at the start of experiments. All solutions were 1/2 Kimura B nutrient solution containing various concentrations of Al and Mn.

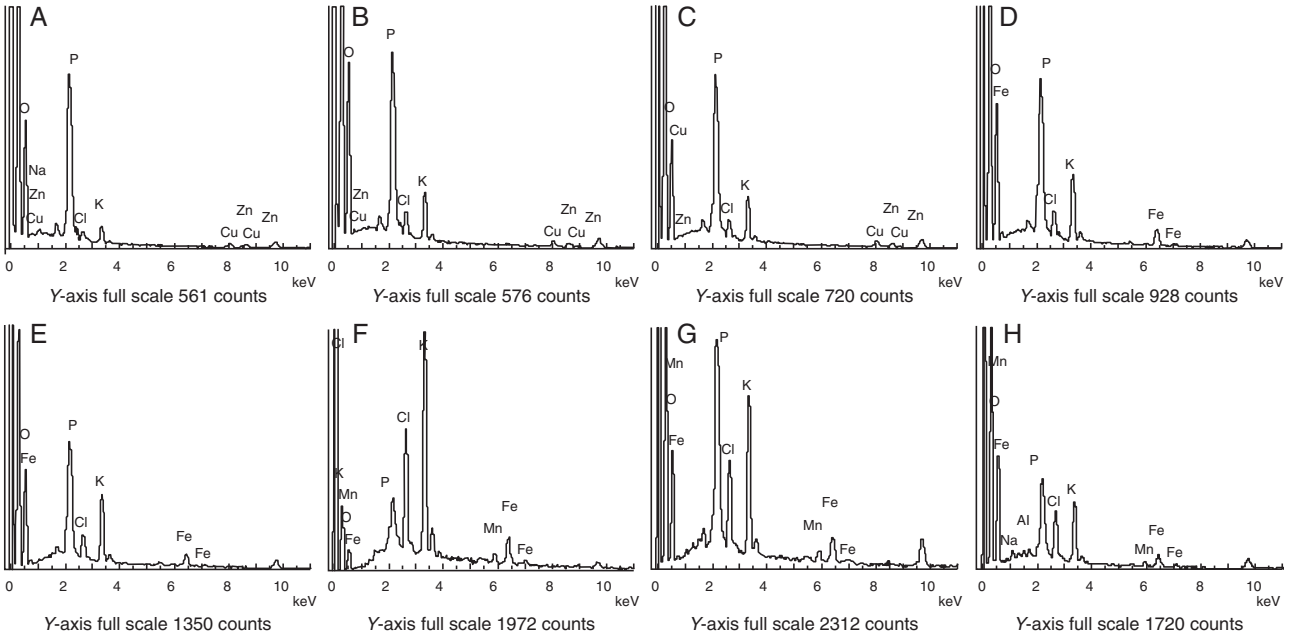

FIG. 4. Energy-dispersive X-ray analysis (EDX) of precipitates formed in roots of rice in hydroponic culture supplemented with Mn and Al or Mn only in a long-term experiment. The *y*-axis shows the counts, which refer to the number of X-rays received and processed by the detector. The counts principally represent the intensity of the elements in the surface of root cell. Rice seedlings (11 d old) were grown in nutrient solution with 6·7 (A, E), 200 (B, F), 500 (C, G) or 1000 (D, H) μM Mn in the absence (A–D) or presence (E–H) of 200 μM Al for 3 weeks.

gradually increased with increasing Mn supply (Fig. 5A, B). Addition of Al significantly increased the DCB-extracted Mn concentrations in roots (Fig. 5A), consistent with the EDX results (Fig. 4). Root Mn concentrations after DCB extraction were higher in the presence of Al than in its absence. These results indicated that Mn plaques in roots were enhanced by Al addition under Mn toxicity.

Root aerenchyma, diameter and volume

We observed roots using SEM to identify changes in root aerenchyma. There were fewer gas spaces in roots in the absence of Al (Fig. 6A–C) than in the presence of Al (Fig. 6D–F). Moreover, Mn toxicity significantly decreased the average diameter and volume of roots, while addition of Al increased these parameters (Fig. 7). These results indicated that Al facilitated the formation of more root aerenchyma and thicker, larger roots under Mn toxicity.

DISCUSSION

In preliminary experiments, we identified 200 μM Al as an appropriate concentration to determine the effects of Al on Mn toxicity in rice, because of its weak toxicity to rice growth. Shen *et al.* (2006) reported that the Al concentration range in solutions of acid soils from southern China was 100–200 μM. These soils also contain high concentrations of total Mn (763 mg kg^{-1} soil) and exchangeable Mn (196 mg kg^{-1} soil) (Chinese National Soil Survey Office, 1998). Given that the amount of exchangeable Mn varies depending on the total Mn content and waterlogging, it was reasonable to use 200 μM Al to interact with 6·7, 200, 500 and 1000 μM Mn in these Al–Mn interaction experiments.

Our results show that Al alleviated Mn toxicity to rice seedlings, consistent with previous reports for barley and atriplex (Rees and Sidrak, 1961), corn (Clark, 1977), wheat (Blair and Taylor, 1997), cowpea (Taylor *et al*., 1998) and soybean (Yang *et al*., 2009). Our data show that Al addition decreased Mn accumulation in the root symplast, whole roots and shoots in a 24-h treatment and in shoots in a 3-week treatment, which resulted in alleviation of Mn toxicity. Rice grew much better at 500 and 1000 μM Mn supply in the presence of Al than plants grown at 200 μM Mn supply in the absence of Al in the long-term treatment (Fig. 1D). However, shoot Mn accumulation in the former was higher than in the latter (Fig. 2B). This might be involved in the effects of Al on Mn internal detoxication, such as Mn distribution between apoplast and symplast or old and new leaves. These processes could be regulated by OsYLS6 and OsNRAMP3 under different Mn environments (Sasaki *et al*., 2011; Yamaji *et al*., 2013). This is a topic needing further investigation.

In previous studies it has been postulated that antagonistic effects of Al on Mn, or non-specific competition between Al and Mn, may be responsible for reduced Mn accumulation in plants. However, these ideas have not been tested using detailed and deep analyses. In this study we used the GCS model, which is based on classical electrostatic theory (Gouy–Chapman theory) (Kinraide, 1994; Kinraide *et al*., 1998), to explain how Al reduced Mn accumulation in rice plants. We observed that Al decreased Mn accumulation in the root symplast and the whole root in a short-term Al treatment, resulting in less Mn being transported to the shoots.

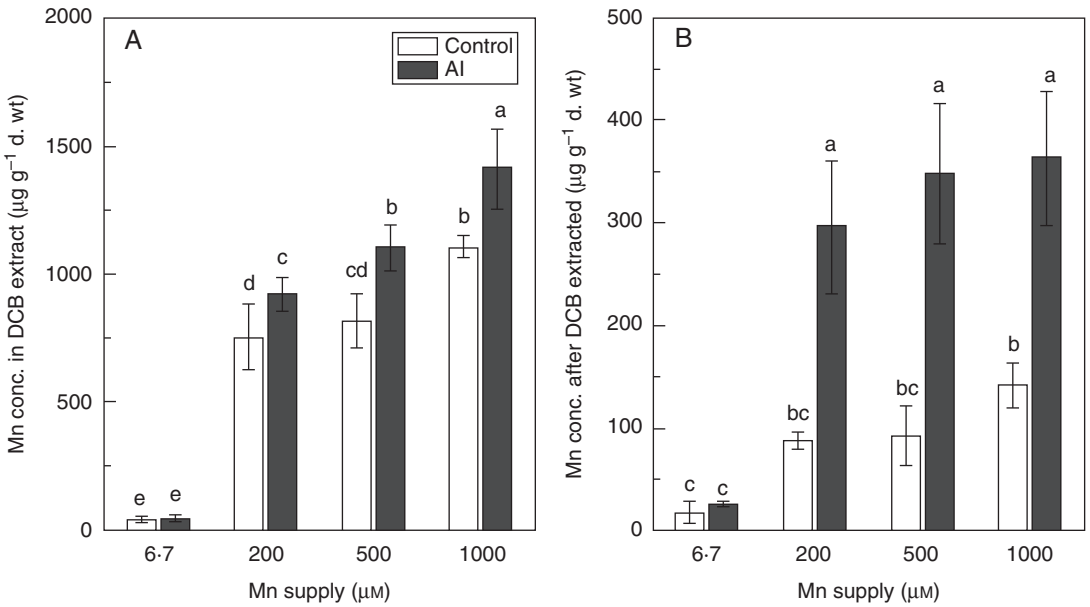

FIG. 5. Manganese concentrations in dithionite citrate bicarbonate extract (A) and root (B) after long-term treatment with Mn and Al or Mn only. Rice seedlings (11 d old) were grown in nutrient solution with 6·7, 200, 500 or 1000 μM Mn in the presence or absence of 200 μM Al for 3 weeks. Values are mean ± s.e. ($n = 3$ replicates, each comprising four seedlings). Different letters show significant differences among treatments ($P < 0.05$, Tukey's test).

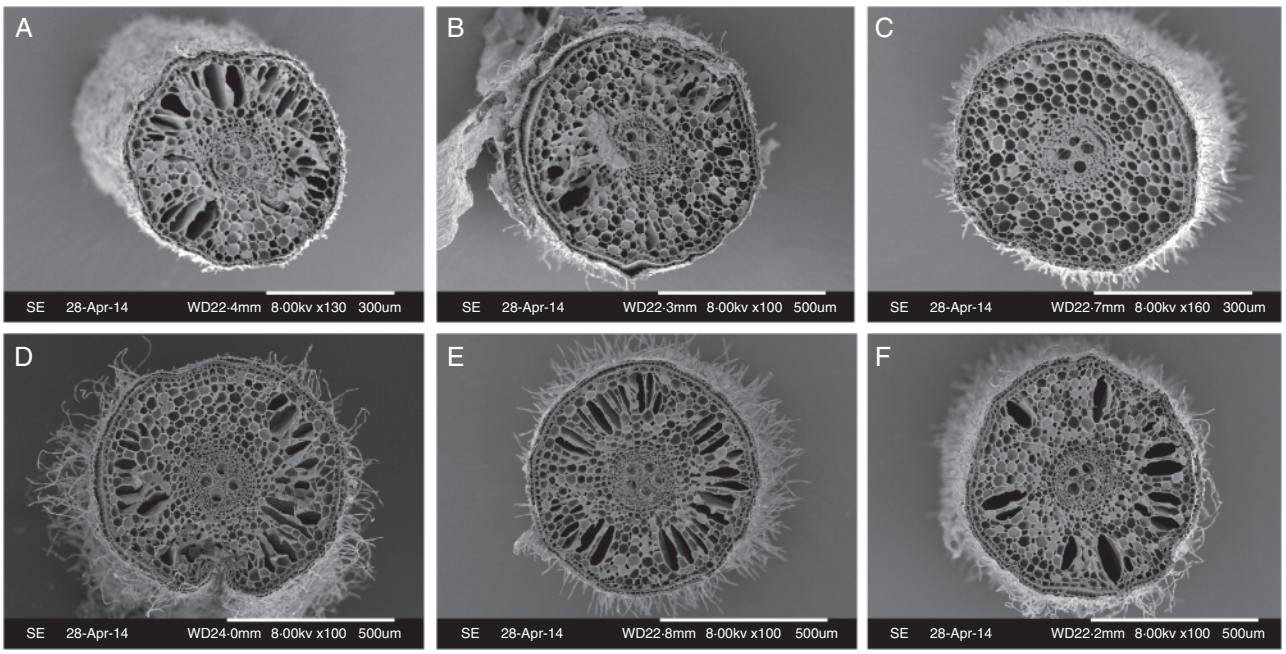

FIG. 6. Effects of Al and Mn supply on aerenchyma formation in long-term experiment. Rice seedlings (11 d old) were grown in nutrient solution containing 6·7 (A, D), 500 (B, E) or 1000 (C, F) μM Mn without (A–C) or with (D–F) 200 μM Al for 3 weeks. After the 3-week treatment, newly formed adventitious roots were cut transversely 10 mm behind the root tip.

Generally, Al is considered to be toxic for plant growth, especially root growth. However, Al has been shown to alleviate H^+ (Kinraide, *et al*., 1992; Kinraide, 1993) and Cu^{2+} toxicity (Wang *et al*., 2008; Kopittke *et al*., 2011*b*). In our previous reports we showed that Al enhanced the growth of *Lespedeza bicolor* (Chen *et al*., 2010) and rice (Zhao *et al*., 2013) in the presence of ammonium, which can be phytotoxic at high concentrations (Britto and Kronzucker, 2002). It has been suggested that the alleviation of Cu^{2+} and H^+ toxicity resulted from the Al-induced decrease in the negativity of ψ^o_0, which corresponded to reduced Cu^{2+} or H^+ activity at the CM surface (Kinraide, 1993; Kopittke *et al*., 2011*b*; Wang *et al*., 2012). In the present study we calculated that Al drastically increased the positivity of ψ^o_0, resulting in lower $\{Mn^{2+}\}^o_0$ (Table 1).

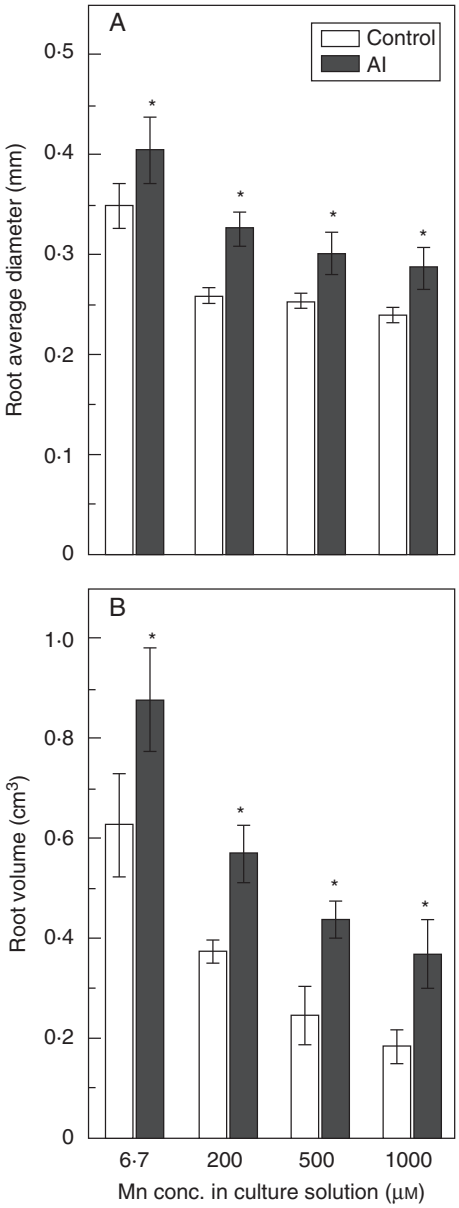

FIG. 7. Root average diameter (A) and volume (B) after long-term treatment with Mn plus Al or Mn only. Rice seedlings (11 d old) were grown in nutrient solution containing 6·7, 200, 500 or 1000 μM Mn without or with 200 μM Al for 3 weeks. Values are mean ± s.e. ($n = 12$ replicates). Asterisks above columns indicate statistically significant differences between control and Al treatment ($P < 0·05$; independent samples *t*-test).

This may have contributed to the decreased Mn concentrations in the root symplast in the short-term Al treatment (Fig. 3A) and consequently less Mn being transported to the shoots (Fig. 3C). The roots are the main site of Al toxicity, while the symptoms of Mn toxicity are mainly observed in the shoots (stunted growth, and chlorosis and necrotic lesions in the leaves) (Kochian *et al*., 2004). Thus, the toxicity of Mn to plants generally results from excess Mn accumulation in the leaves (Foy *et al*., 1978, 1988; Wissemeier *et al*., 1992; Migocka and Klobus, 2007). In rice, the roots are much less sensitive to Mn toxicity than are the shoots (Nelson, 1983; Lidon, 2001). Given the delay in the translocation of Mn from the roots to the shoots, Al may have repressed root Mn uptake in the short-term experiment. This would prevent excess Mn accumulation in shoots, thereby alleviating Mn toxicity.

We observed that Al increased Mn accumulation in roots after the 3-week Al and Mn treatments (Fig. 2A). This result contrasts with those reported for other plant species (Rees and Sidrak, 1961; Clark, 1977; Blair and Taylor, 1997; Taylor *et al*., 1998; Yang *et al*., 2009). It is difficult to explain this based on the decreased Mn accumulation in the root symplast and whole roots in the short-term Al–Mn treatments (Fig. 3). There was an increase in Mn plaques in the Al treatments (Figs 4 and 5A), consistent with the Al-induced increase in Mn accumulation in roots. Because Mn plaques carry variable charge, they have greater capacity for adsorbing cation and P. In line with this, we also detected P, K and Na in Mn plaques (Batty *et al*., 2002; Chen *et al*., 2006). The development of root structure (aerenchyma, and root diameter and volume) was also improved by Al under Mn toxicity (Figs 6 and 7). The improved root structure may have played a role in the increased Mn accumulation in roots in the long-term Al treatment. A similar effect was reported for silicon (Horiguchi and Morita, 1987). That is, silicon promoted the development of root and root aerenchyma and improved the oxidizing power of the root, allowing more Mn to be oxidized on the root surface (Horiguchi and Morita, 1987; Luiz *et al*., 2010). Our results also showed that root Mn concentrations after DCB extraction were higher in Al–Mn treatments than in Mn-only treatments (Fig. 5B), suggesting that the increased Mn accumulation in roots could be attributed not only to the increase in Mn plaques, but also to increased Mn binding in the roots. Several previous studies have reported that Al increased cell wall pectin and hemicellulose (Chang *et al*., 1999; Tabuchi and Matsumoto, 2001; Tabuchi *et al*., 2004; Yang *et al*., 2008), which are primary Al-binding components (Kochian *et al*., 2005; Horst *et al*., 2010). We also observed that Al increased the amounts of these cell wall components in rice (Wang *et al*., 2015), suggesting that Al-induced increase in pectin and hemicellulose might result in increased Mn binding in the roots. Therefore, increases in both Mn plaques and Mn binding could lead to more unavailable Mn being accumulated in the roots. Together with the Al-induced decrease in root symplastic Mn uptake, these changes resulted in less Mn being transported to shoots, thereby alleviating Mn toxicity in rice.

In conclusion, our results show that Al alleviated Mn toxicity in rice. This was attributed to decreased shoot Mn accumulation resulting from an Al-induced decrease in root symplastic Mn uptake. The decrease in root symplastic Mn uptake resulted from an Al-induced change in cell membrane potential. Also, Al increased Mn plaques in the roots and changed the binding properties of the cell wall, resulting in accumulation of unavailable Mn in roots. To our knowledge, this is the first report that Al can increase Mn accumulation in roots.

SUPPLEMENTARY DATA

Supplementary data are available at www.aob.oxfordjournals.org and consist of Table S1: effects of aluminium at different concentrations on rice growth in the 3-week (long-term) experiment.

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