Phosphorus Enhances Al Resistance in Al-resistant *Lespedeza bicolor* but not in Al-sensitive *L. cuneata* Under Relatively High Al Stress

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• *Background and Aims* Aluminium (Al) toxicity and phosphorus (P) deficiency often co-exist in acidic soils and limit crop production worldwide. *Lespedeza bicolor* is a leguminous forage species that grows very well in infertile, acidic soils. The objective of this study was to investigate the effects of Al and P interactions on growth of *Lespedeza* and the distributions of Al and P in two different Al-resistant species, and to explore whether P can ameliorate the toxic effect of Al in the two species.

• *Methods* Two species, *Lespedeza bicolor* and *L. cuneata*, were grown for 30 d with alternate Al and P treatments in a hydroponics system. Harvested roots were examined using a root-system scanner, and the contents of Al, P and other nutrient elements in the plants were determined using inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Haematoxylin staining was used to observe the distribution of Al in the roots of seedlings. After pre-culture with or without P application, organic acids in the exudates of roots exposed to Al were held in an anion-exchange resin, eluted with 2 M HCl and then analysed using high-performance liquid chromatography (HPLC).

• *Key Results Lespedeza bicolor* exhibited a stronger Al resistance than did *L. cuneata*; Al exclusion mechanisms may mainly be responsible for resistance. P application alleviated the toxic effect of Al on root growth in *L. bicolor*, while no obvious effects were observed in *L. cuneata*. Much less Al was accumulated in roots of *L. bicolor* than in *L. cuneata* after P application, and the P contents in both roots and shoots increased much more for *L. bicolor* than for *L. cuneata*. *Lespedeza bicolor* showed a higher P/Al ratio in roots and shoots than did *L. cuneata*. P application decreased the Al accumulation in root tips of *L. bicolor* but not in *L. cuneata*. The amount of Al-induced organic acid (citrate and malate) exudation from roots pre-cultured with P was much less than from roots without P application; no malate and citrate exudation was detected in *L. cuneata*.

• *Conclusions* P enhanced Al resistance in the Al-resistant *L. bicolor* species but not in the Al-sensitive *L. cuneata* under relatively high Al stress, although P in *L. cuneata* might also possess an alleviative potential. Enhancement of Al resistance by P in the resistant species might be associated with its more efficient P accumulation and translocation to shoots and greater Al exclusion from root tips after P application, but not with an increased exudation of organic acids from roots.

Key words: *Lespedeza bicolor*, *L. cuneata*, Al toxicity, Al resistance, root morphology, phosphorus.

INTRODUCTION

Normal plant growth in acidic soils can be limited by many chemicals or the interactions among them (Marschner, 1991). Aluminium (Al) toxicity and phosphorus (P) deficiency are recognized as the major constraints on crop productivity (Kochian *et al.*, 2004; Fukuda *et al.*, 2007). Although an improved crop production in acidic soils is possible with lime application, such application is neither economical for all farmers nor a thorough method for correcting subsoil acidity (Kochian *et al.*, 2005). Phosphate fertilizers are often applied in acid soil areas to decrease Al activity and increase crop production. However, further exploration of the mechanisms of Al–P interactions in plants is still needed. On the other hand, not all species or genotypes express the same traits for Al toxicity and P deficiency (Marschner, 1991). Hence, a reasonable alternative or supplemental approach is to select and breed plant genotypes having greater resistance to these stresses (Foy, 1988).

Genotypic differences in Al resistance have been found in many crops including wheat (Delhaize *et al.*, 1993; de Sousa, 1998; Kariuki *et al.*, 2007), triticale (Oettler *et al.*, 2000; Kim *et al.*, 2001), buckwheat (Yang *et al.*, 2005), barley (Zhao *et al.*, 2003), rice (Sivaguru and Paliwal, 1994) and rye (Aniol *et al.*, 1980; Gallego and Benito, 1997). *Lespedeza* is a considerably Al-resistant plant (Campbell *et al.*, 1991), but genetic differences in Al resistance still exist (Dong *et al.*, 2008). Previous studies have suggested that Al resistance mechanisms of *Lespedeza* are associated with the exudation of malic and citric acids under Al stress (Dong *et al.*, 2008). However, little information is available about Al and P interactions in different *Lespedeza* species.

Although many studies have been conducted on Al resistance and P efficiency of plants in acidic soils, there are few studies on Al and P interactions in plants (Liao *et al.*, 2006). McCormick and Borden (1972) found, using the molybdenum blue technique, that the Al–P interaction appeared to be associated with the cell wall and the outside of the cytoplasmic membrane of epidermal and cortical cells in

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barley. Tan and Keltjens (1990*a*) reported that an increase in the P concentration played a role in ameliorating Al phytotoxicity, possibly through improving root development and nutrient uptake. In addition, the toxic effect of Al on plant growth was alleviated with increasing P contents in roots. Al resistance in a resistant cultivar was associated with a higher Al immobilization ability in the presence of P in root tissues as compared with an Al-susceptible cultivar (Gaume *et al.*, 2001). As proposed by Zheng *et al.* (2005), Al resistance of buckwheat involves both the secretion of oxalic acid from root apices and the precipitation of Al by P in the root apoplast. Liao *et al.* (2006) provided evidence that P-efficient genotypes might be able to enhance Al resistance not only through direct Al–P interactions but also by indirect interactions associated with stimulated exudation of different Al-chelating organic acids in specific roots and root regions. Therefore, the relationship of Al and P is ubiquitous and may vary in different crops. However, for *Lespedeza* species, the effect of the interaction of P and Al in roots on alleviating Al toxicity may be another potential mechanism in addition to the mechanism of organic acid efflux. In the current study, root morphology, nutrient elements and organic acid exudation analyses were carried out to aid in the understanding of the the relationship of Al and P under hydroponic conditions in two *Lespedeza* species under Al stress.

MATERIALS AND METHODS

Plant materials and growth conditions

Lespedeza bicolor (Turcz. 'Jiangxi') seeds were collected from Jiangxi Province, China. Seeds were surface softened in 98 % H_2SO_4 for 20 min, washed with tap water, then distilled water, and soaked in distilled water overnight and germinated in a plastic Petri dish with filter paper saturated with distilled water in darkness at 25 °C for 48 h. *Lespedeza cuneata* [(Dum.-Cours.) G. Don 'Zhejiang'] seeds were collected from Zhejiang Province, China. Seeds (the seed coat is very soft and does not need sulfuric acid treatment for germination) were soaked in distilled water for 2 h, and then germinated according to the above-described method. After 2 d, seedlings with roots 0·5 cm long were transplanted into a plastic net suspended on a plastic container (2·5 L) filled with 0·5 mM $CaCl_2$ solution (pH 4·5). The solution was renewed every day. After 3 d, uniform seedlings were selected to evaluate Al resistance or to measure the Al content in root tips (0–1·0 cm). The remaining seedlings were transplanted into aerated nutrient solution containing 0 P (−P) or 1·0 mM P (+P) in a 1·5 L container (14 seedlings per pot) and cultivated for 7 d. The nutrient solution was modified from Hoagland solution and consisted of KNO_3 (5·0 mM), $Ca(NO_3)_2$ (5·0 mMN), $MgSO_4$ (2·0 mM), $NH_4H_2PO_4$ (1·0 mM), Na-FeEDTA (20 μM), HBO_3 (3 μM), $MnCl_2$ (0·5 μM), $CuSO_4$ (0·2 μM), $ZnSO_4$ (0·4 μM) and $(NH_4)_6Mo_7O_{24}$ (1 μM). NH_4Cl (1·0 mM) was substituted for $NH_4H_2PO_4$ so that the concentration of P was zero. The solution was adjusted to pH 4·5 with 1 M HCl and renewed every 3 d. All the experiments were conducted in an environmentally controlled growth room with a 14 h/25 °C day and 10 h/20 °C night regime, a light intensity of 150 μmol photon m^{-2} s^{-1} and a relative humidity of 65 %.

Assessment of Al resistance and determination of Al in the root tips

Al resistance in *Lespedeza* was examined by measuring the elongation of primary roots of 5-d-old seedlings grown in 0·5 mM $CaCl_2$ solution (pH 4·5) containing 0, 25, 50, 100 or 200 μM $AlCl_3$. Root length was measured with a ruler before and after Al treatment (24 h). Relative root elongation was defined as the percentage of root elongation under Al treatment compared with that of the Al-free control.

The Al content of root tips in samples was determined using a method modified from that of Osawa and Matsumoto (2001). Roots (0–1·0 cm) exposed to various Al concentrations for 24 h were excised and placed in an Eppendorf tube (1·5 mL, ten tips per tube) containing 1 mL of 2 M HCl. The tubes were left to stand for at least 24 h with occasional shaking. Al contents in the solution were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (IRIS-Advantage, Thermo Elemental, MA, USA) after appropriate dilution.

Alternating Al and P treatment

Intermittent Al treatment was adopted to avoid interactions between Al and other nutrients such as Al–P precipitation. After 1 week in the nutrient solution (−P/ + P), the seedlings were exposed to 0·5 mM $CaCl_2$ (pH 4·5) with or without 50 μM $AlCl_3$ for 1 d and then grown in nutrient solution containing 0 P (−P) or 1·0 mM P (+P) on alternate days. After 30 d of alternating treatment, the *Lespedeza* seedlings were harvested and separated into shoots and roots.

Morphological analysis of roots

The roots harvested after the alternating experiment were scanned by a root-system scanner, and several parameters including root length, root surface area, root volume, root diameter and the amount of root tips were measured and analysed using the WinRHIZO image analysis system (WIN MAC, Regent Instruments Inc., Quebec, Canada, http://www.regentinstruments.com/).

Mineral contents in plants

After the alternating treatment with Al and P, roots and shoots were dried in an oven for 2 d (70 °C) and ground to a fine powder as necessary. Samples were digested with concentrated HNO_3 (heavy-metal grade). Mineral (Al, Ca, Mg, P, K, Mn and Fe) contents were then determined by ICP-AES, after appropriate dilution with 0·1 M HNO_3.

Haematoxylin staining test

Haematoxylin is a histochemical indicator used to show the presence of Al. Haematoxylin stain was prepared as described by Polle *et al.* (1978). It consisted of 0·2 % haematoxylin and 0·02 % KIO_3, the dissolution of which was aided by adding a drop of 0·1 M NaOH. Intact seedlings at 4–6 d old were grown in a solution containing 0·5 mM $CaCl_2$ with or without 1·0 mM P at pH 4·5 for 24 h, then treated in 0·5 mM $CaCl_2$ with or without 50 μM Al at pH 4·5 for 3 h. At the end of the treatments, roots were rinsed with distilled water and placed in approx. 60 mL of haematoxylin stain for 40 min with occasional shaking at room temperature. The roots were then placed in the aerated distilled water for 10 min to remove excess stain. The root tips were observed under a light stereoscopic microscope (Olympus-SZX7, Japan) and images were taken with a digital camera. At the same time, Al in the root tips was extracted and determined using the same methods as described above.

Collection of root exudates and determination of organic acids

After 30 d culture in the nutrient solution with or without P as described above, root exudates of the two *Lespedeza* species were collected. Roots were transplanted to a 0·5 mM $CaCl_2$ solution (pH 4·5) overnight before collection and then exposed to a 0·5 mM $CaCl_2$ solution with or without 50 μM $AlCl_3$ (pH 4·5). After 24 h, root exudates were collected and passed through a cation-exchange column (16 × 14 mm) filled with 5·0 g of Amberlite IR-120B resin (H form), followed by an anion-exchange column (16 × 14 mm) filled with 2·0 g of AG 1-X8 resin (100–200 mesh, formate form) in a cold room. The organic acids retained on the anion-exchange resin were eluted by 2 M HCl, and the eluate was concentrated to dryness using a rotary evaporator (40 °C). After the residue was redissolved in 1·0 mL of ultrapure water, the concentration of organic acids was analysed by high-performance liquid chromatography (HPLC; LC-10AT VP, Shimadzu, Tokyo, Japan) using a Shim-pack SCR-102H column (8·0 mm i.d. × 30 cm) according to Ma *et al.* (2002).

Statistical analysis

Tukey's test was applied to test differences among treatments at $P < 0.05$ using the SPSS 13·0 statistical package.

RESULTS

Effects of Al on root growth and Al content

In a dose–response experiment, root elongation of *L. bicolor* was inhibited by 52, 75, 87 and 92 % after exposure to 25, 50, 100 and 200 μM Al for 24 h, while that of *L. cuneata* was inhibited by 78, 89, 93 and 97 %, respectively (Fig. 1A). These results showed that *L. bicolor* had a higher degree of Al resistance than *L. cuneata*. The Al content in root tips (0–1·0 cm) was lower in *L. bicolor* than in *L. cuneata* at each Al concentration tested (Fig. 1B), which indicated

that Al exclusion mechanisms may mainly work in *Lespedeza*.

Root morphology and fresh weight under Al and P interaction

The root morphology of the two *Lespedeza* species was determined following a 30 d alternating treatment with 50 μM Al and P solutions. The root morphologies of the two *Lespedeza* species were similar under −P and +P treatments in Al-free solution, whereas, after Al treatments, obvious differences were seen between the two *Lespedeza* species (Fig. 2). The root growth of *L. cuneata*, especially that of the lateral root, was obviously inhibited by Al treatment compared with that of *L. bicolor* for both −P and +P treatments.
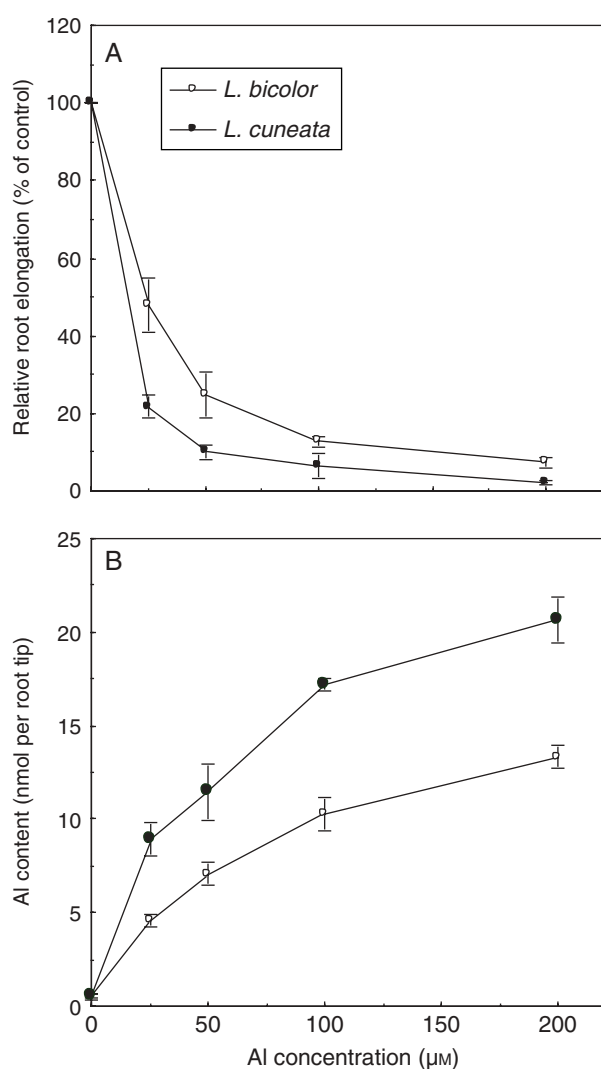

FIG. 1. Effects of different Al concentrations on (A) root elongation and (B) Al contents in root tips of *L. bicolor* and *L. cuneata*. Relative root elongation was calculated from the root elongation of 5-d-old seedlings during 24 h exposure to 0·5 mM $CaCl_2$ solution, containing 0, 25, 50, 100 or 200 μM Al (pH 4·5). Root tips (0–1·0 cm) were excised (ten tips per sample, $n = 3$) and the Al content was determined by ICP-AES. Data are means ± s.d. ($n = 10$ for root elongation and $n = 3$ for Al contents in root tips).
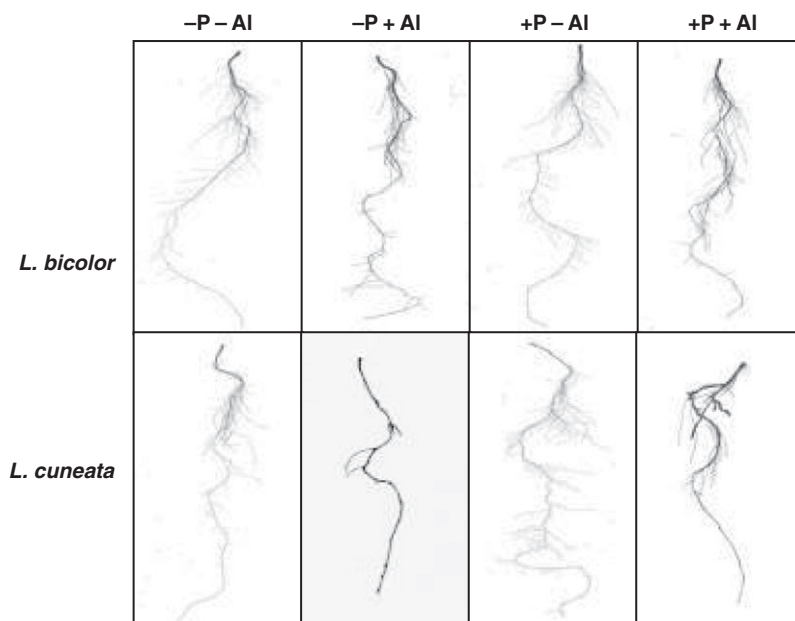
F_IG. 2. Scanning charts of *L. bicolor* and *L. cuneata* after the 30 d alternating Al and P treatments. Seedlings were exposed to 0·5 mM CaCl₂ (pH 4·5) with or without 50 μM AlCl₃ every other day and grown in nutrient solution containing 0 P (−P) or 1·0 mM P (+P) on alternate days. After 30 d of alternating treatment, the harvested roots were scanned by a root-system scanner.

The root length, root surface area, root volume and the quantities of root tips in *L. cuneata* significantly decreased under Al stress, while *L. bicolor* did not exhibit significant inhibition of root growth due to Al treatment except for a decline in root length and root surface area under the −P condition, which was in accordance with the images in Fig. 2 (Fig. 3). Once P was applied, the root length and root surface area did not decrease for *L. bicolor* under Al stress while this effect was not evident in *L. cuneata*. Thus, it seemed that P increased the Al resistance of *L. bicolor* but not in *L. cuneata* judging from the root morphological parameters.

Under −P conditions the shoot mass of Al-resistant species was not influenced by Al stress, while it increased significantly under Al stress after P application. However, for Al-sensitive species, the shoot mass changed little under Al stress whether P was applied or not (Fig. 4A), i.e. the effect of Al and P interaction on shoot mass of *L. cuneata* was insignificant. Neither Al nor P addition had significant effects on the root mass of the two species (Fig. 4B).

By calculating from the data of Figs 3 and 4, it could be shown that both the separate negative effects of Al ([(+Al) − (−Al)]/(−Al) × 100) and the separate positive effects of P ([(+P) − (−P)]/(−P) × 100) on root morphological parameters and fresh weight of *L. cuneata* were more obvious than for *L. bicolor* (Table 1).

Al and P contents in plants

The Al content in shoots was much lower than that in roots (Fig. 5). Al contents in the roots of the two species increased after P was suppleid, but the extent of the increase was much greater for *L. cuneata* than for *L. bicolor* (Fig. 5).

P contents in shoots and roots of *L. bicolor* were significantly higher than those in *L. cuneata* after P was supplied, either with or without Al (Fig. 5). In the presence of P, the P content in roots of *L. cuneata* increased with Al exposure, but that in shoots decreased, indicating that P transport was constrained from roots to shoots. However, for the resistant species, although the P contents in roots and shoots were decreased when Al was supplied in the presence of P, the decrease of P in shoots was less than that in *L. cuneata*.

P application led to the augmentation of the P/Al ratio in roots and shoots of the Al-resistant species, while that of the Al-sensitive species was much smaller (Fig. 6).

Ca, Mg, K, Mn and Fe contents in plant tissues

In the presence of Al, the contents of Ca and Mn in shoots of *L. bicolor* were reduced but those of Mg and Fe were not (Table 2). Both under P starvation and when P was supplied, interestingly, although the contents of Ca and Mn in shoots of *L. cuneata* decreased under Al stress, in roots they increased due to Al (Table 2). This result indicated that the transport of nutrients (especially Ca and Mn) to shoots in *L. cuneata* was restrained by Al stress. The K content of the two species increased with supply of P whether under Al stress or not.

Effects of supply of P on Al uptake

To investigate further whether the different responses of the two *Lespedeza* species to P being supplied are associated with Al uptake, the haematoxylin staining method was used in a short-term experiment. The root tips or medial segment of *L. bicolor* pre-treated with P was
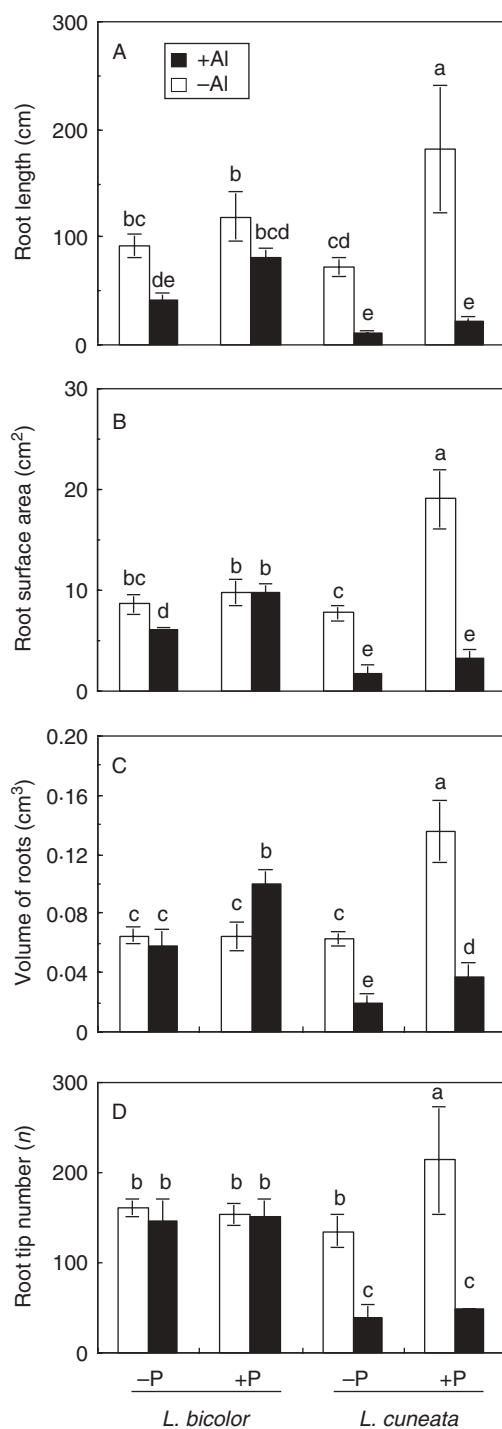
FIG. 3. Root parameters of the two *Lespedeza* species from root scanning after 30 d alternating Al and P treatments. Results without Al treatment (−) and with Al treatment (+) are shown. Vertical bars represent ± s.d. (*n* = 3). Significant differences between mean values are indicated by different letters at the *P* < 0·05 level (Tukey's test).

stained much less than that not pre-treated with P, but this was not the case for *L. cuneata* (Fig. 7A).

The Al content was much lower with +P + Al than with −P + Al in *L. bicolor*, but not in *L. cuneata* (Fig. 7B). In addition, the Al content of root tips was significantly
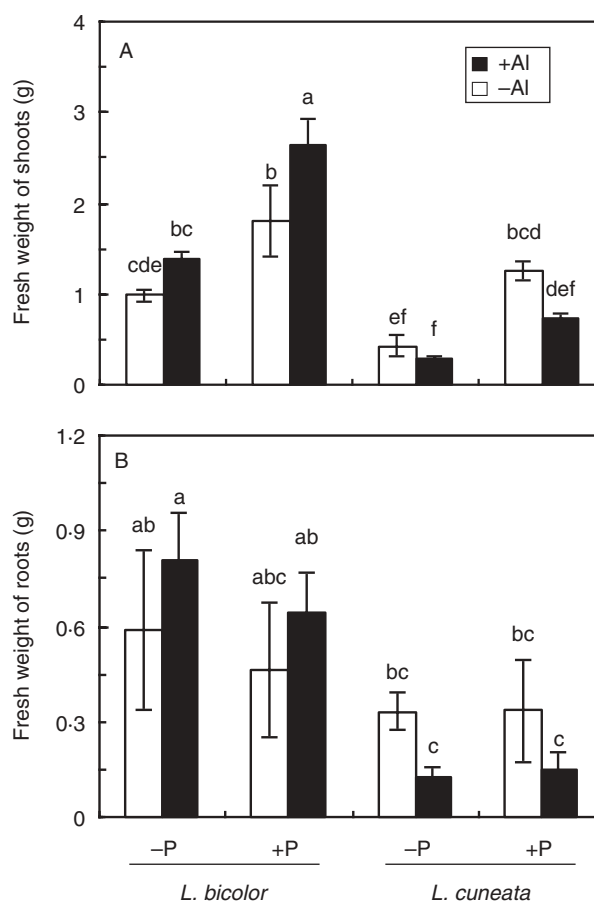

FIG. 4. Effects of the 30 d alternating Al and P treatments on the fresh weight of the two *Lespedeza* species. Results without Al (−) treatment and with Al (+) treatment are shown. Vertical bars represent ± s.d. (*n* = 3). Significant differences between mean values are indicated by different letters at the *P* < 0·05 level (Tukey's test).

lower in *L. bicolor* than in *L. cuneata* (Fig. 7B). This result was consistent with the images in Fig. 7A.

Organic acid exudation affected by supply of P

Roots of *L. cuneata* did not release any organic acids with or without Al exposure. No organic acid anions were detected in root exudates of *L. bicolor* without Al treatment. However, both citrate and malate were secreted from roots of *L. bicolor* under Al stress. The Al-induced exudation of organic acids (citrate and malate) under conditions where P was supplied was less than that under P deficiency (Fig. 8).

DISCUSSION

Responses of Lespedeza *to the Al and P interaction*

Al interferes with a wide range of physical and cellular processes in plants (Kochian *et al.*, 2005). The major symptom of Al toxicity observed in plants is the inhibition of root growth (Delhaize and Ryan, 1995). This extensive root damage results in limited water and mineral nutrient

TABLE 1 *Calculation of the effects of Al and P on root morphological parameters (length, surface area, volume and root tip numbers) and fresh weight of shoots and roots after 30 d of alternating treatment with Al and P*

	Effects of Al (%)*		Effects of P (%)[†]	
	−P	+P	−Al	+Al
Root length				
L. bicolor	−55	−32	30	98
L. cuneata	−85	−88	151	103
Root surface area				
L. bicolor	−30	1	13	62
L. cuneata	−77	−83	147	85
Volume of roots				
L. bicolor	−12	54	0	74
L. cuneata	−68	−72	116	88
Root tip number				
L. bicolor	−9	−1	−5	3
L. cuneata	−72	−77	58	27
Shoot fresh weight				
L. bicolor	42	46	84	89
L. cuneata	−36	−42	193	168
Root fresh weight				
L. bicolor	38	39	−21	−20
L. cuneata	−62	−55	0	19

Note: negative data represent negative effects, and vice versa.
* Effects of Al (%) =[(+Al) − (−Al)]/(−Al) × 100.
[†] Effects of P (%) =[(+P) − (−P)]/ (−P) × 100.

uptake (Barcelo and Poschenrieder, 2002). Relative root elongation of *L. bicolor* and *L. cuneata* was 25 and 11 %, respectively, under 50 μM Al for 24 h (Fig. 1A), and nutrient uptake including P, Ca and Mn was significantly reduced after 30 d of Al–P interactions (Fig. 5; Table 2). Thus 50 μM Al was a relative high stress for the *Lespedeza* especially for *L. cuneata*.

Ciamporova (2002) indicated that the architecture of the root system can be modified with long-term Al application. As reported by others (e.g. Foy *et al.*, 1978), the detrimental effects of Al are clearly reflected in root development. In this experiment with alternating Al and P treatments, Al–P interactions were partly exhibited as effects on the growth pattern (root morphology and fresh weight, Table 1). Four of the root morphology parameters (length, surface area, volume and root tip numbers) of *L. cuneata* were inhibited by Al more seriously than those of *L. bicolor* (Figs 2 and 3). P application reduced the negative effect of Al on root morphology parameters in *L. bicolor* which was the embodiment of P alleviation on Al toxicity, while in *L. cuneata* the alleviative effects of P were not significant (Table 1). Compared with the increase in shoot fresh weight caused by Al in *L. bicolor* especially after P supply, the effect of Al on the fresh weight of *L. cuneata* was negative (Fig. 4; Table 1). This result was consistent with growth stimulation with Al application (Osaki *et al.*, 1997; Kidd and Proctor, 2000).

Early reports have established that increasing the P supply could alleviate the toxic effects of Al (Tan and Keltjens, 1990*a, b*). Zheng *et al.* (2005) concluded that the immobilization of Al with P present in roots was
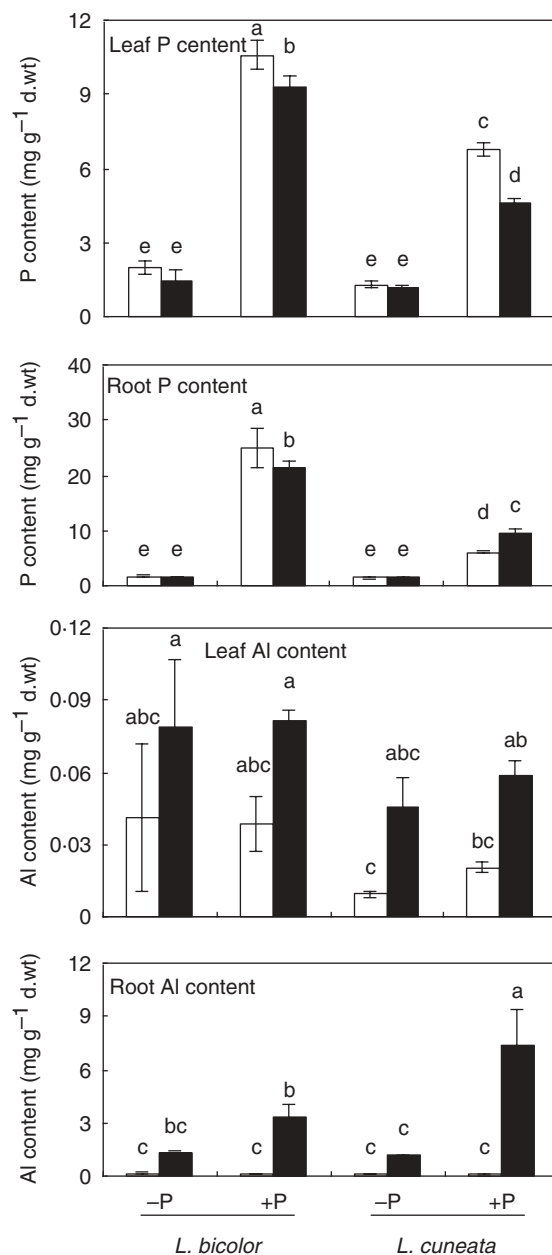

FIG. 5. Al and P distribution in *L. bicolor* and *L. cuneata*. After 7 d culture in nutrient solution (−P/ + P), the seedlings were exposed to 0·5 mM CaCl$_2$ (pH 4·5) with or without 50 μM AlCl$_3$ for 1 d and then grown in nutrient solution containing 0 P (−) or 1·0 mM P (+P) every other day. After 30 d alternating treatment, the *Lespedeza* seedlings were harvested. The contents of Al and P were determined by ICP-AES. Results without Al treatment (open bars) and with Al treatment (solid bars) are shown. Values shown are means ± s.d. (*n* = 3). Significant differences between mean values are indicated by different letters at the *P* < 0·05 level (Tukey's test).

associated with high Al resistance in buckwheat. Foy *et al.* (1978) suggested that Al absorbed by root surfaces or that in intercellular free spaces may immobilize the P present in the root tissue or external substrates. The effect of Al on maize growth decreased with increasing P content in roots (Gaume *et al.*, 2001). P addition
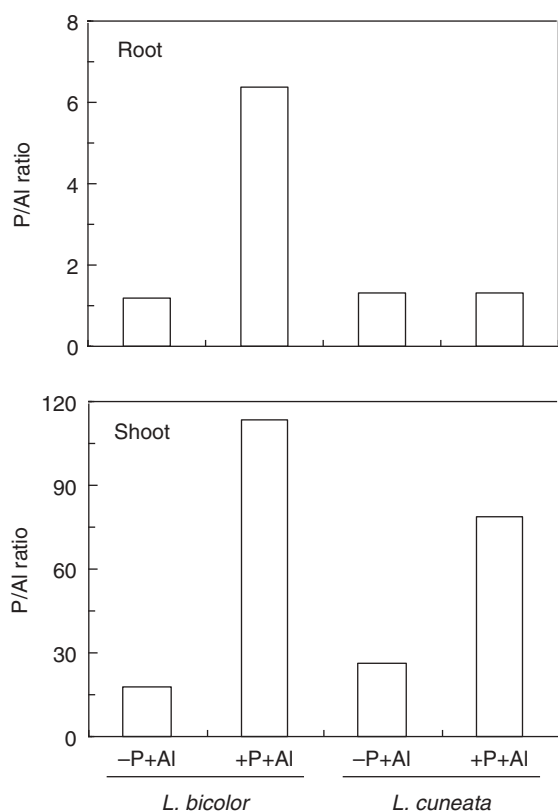
FIG. 6. P/Al ratios in the roots and shoots of the two species after 30 d alternating Al and P treatments.

Effects of P on the exudation of organic acids by roots under Al stress

Previous results indicated that secretion of malate and citrate from roots was related to the high Al resistance in *L. bicolor*, and P deficiency did not induce the exudation of organic acids in the two *Lespedeza* species (Dong *et al.*, 2008). Further results demonstrated that P application significantly reduced the amount of Al-induced organic acid (citrate and malate) exudation in the Al-resistant species under Al stress (Fig. 8), indicating that the decreased exudation of organic acids due to P application could be due to the amelioration of Al toxicity in the resistant species. This result was in accordance with the suggestion by Gaume *et al.* (2001) that when P was supplied it led to a decrease in organic acid exudation compared with Al exposure without P being supplied in maize. In addition, Dong *et al.* (2004) provided evidence for root Al and P interactions in that organic acid exudation was differentially induced by Al toxicity and P deficiency in soybean plants, and specific induction of oxalate and malate with P deficiency and citrate exudation with Al stress. Jemo *et al.* (2007) concluded that the Al-resistant cowpea was better adapted to acidic Al-toxic and P-deficient soils than Al-sensitive cowpea because both malate and citrate exudations were more enhanced by the combined Al and P deficiency. These findings indicated that the type and amount of organic acid release depended on the plant species and external factors, including P deficiency and/or Al stress.

significantly increased Al tolerance in four soybean genotypes differing in P efficiency (Liao *et al.*, 2006). However, from the analysis of the root parameters, the present study suggested that P application could alleviate Al stress in the Al-resistant *Lespedeza* species to some extent, but not in the Al-sensitive species (Figs 2 and 3). There are several ways in which P might ameliorate the toxic effect of Al in the resistant species, and the main possibilities are discussed below.

Possible mechanisms of P alleviation of Al toxicity in L. bicolor

Al toxicity and P deficiency often co-exist in acidic soils, so the Al-resistant species, native to the acidic soil might be responsible for mechanisms by which P is used efficiently and there is simultaneous Al detoxification (Foy *et al.*, 1978). Zheng *et al.* (2005) proposed that Al-resistant buckwheat species had evolved mechanisms to use P efficiently and for there to be simultaneous Al detoxification in acidic

TABLE 2 *Effects of 30 d of alternating Al treatment on nutrient contents in shoots and roots of the two* Lespedezas *(dry weight)*

		L. bicolor				L. cuneata			
Treatment	Plant part	$-P - Al$	$-P + Al$	$+P - Al$	$+P + Al$	$-P - Al$	$-P + Al$	$+P - Al$	$+P + Al$
Ca (mg g^{-1})	Shoot	18·2[b]	12·2[de]	24·7[a]	14·0[cd]	16·6[bc]	10·9[e]	23·7[a]	10·5[e]
	Roots	4·92[bc]	2·93[c]	5·27[ab]	3·95[bc]	2·97[c]	4·04[bc]	4·69[bc]	7·08[a]
Mg (mg g^{-1})	Shoot	2·97[a]	2·25[ab]	2·37[ab]	2·23[b]	2·79[ab]	2·17[b]	2·29[ab]	2·34[ab]
	Roots	1·29[bc]	1·11[cd]	3·51[a]	3·02[a]	0·937[c]	1·03[bc]	1·03[bc]	1·51[b]
K (mg g^{-1})	Shoot	18·1[ab]	14·2[cd]	17·6[ab]	19·3[a]	11·2[e]	12·5[de]	15·7[bc]	16·9[abc]
	Roots	19·9[c]	17·8[c]	30·9[a]	25·9[b]	11·2[d]	9·3[d]	32·7[a]	17·8[c]
Mn (mg kg^{-1})	Shoot	69·1[b]	46·1[c]	85·5[a]	53·3[c]	25·9[d]	12·6[e]	27·1[d]	12·2[e]
	Roots	23·8[ab]	21·1[b]	26·7[a]	24·8[ab]	12·8[d]	17·1[c]	13·1[d]	21·0[b]
Fe (mg kg^{-1})	Shoot	158[ab]	113[bc]	117[bc]	89·0[c]	201[a]	155[ab]	124[bc]	115[bc]
	Roots	591[ab]	322[c]	698[a]	619[ab]	440[bc]	531[abc]	492[bc]	699[a]

Significant differences between mean values are indicated by different letters at the $P < 0.05$ level (Tukey's test). Values shown are means ± s.d. ($n = 3$).
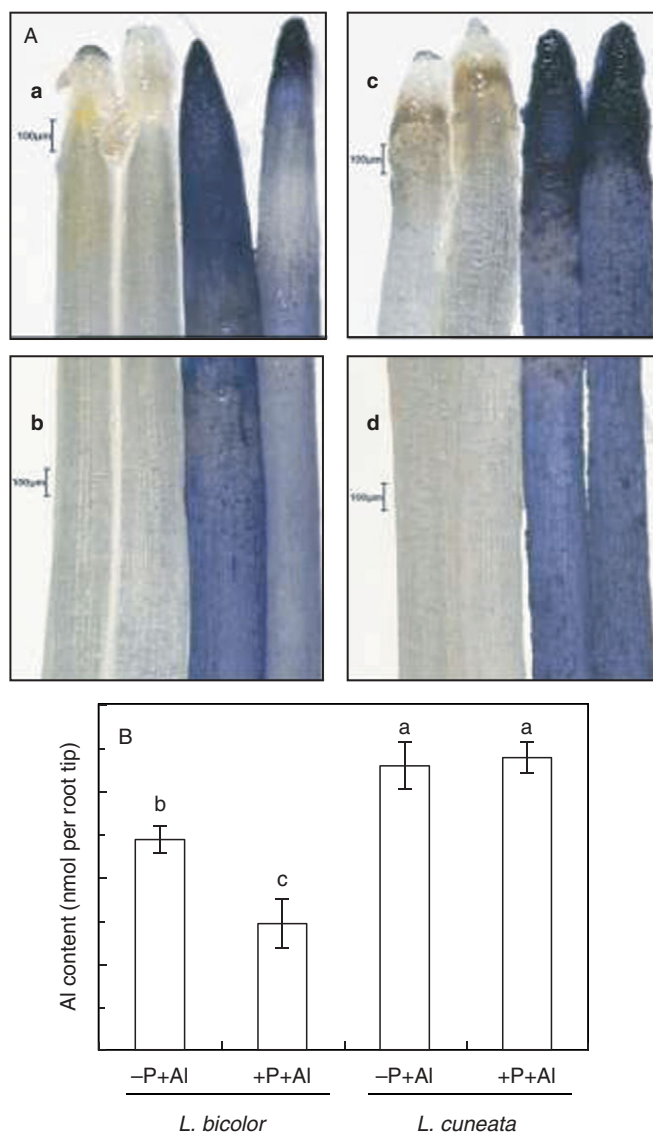
FIG. 7. Al contents in two *Lespedeza* species detected by the haematoxylin staining method (A), and extraction and determination by ICP-AES (B). Al distribution in root tips or the medial segment of *L. bicolor* (a, b) and *L. cuneata* (c, d) detected by the haematoxylin staining method (scale bars = 100 μm). Roots from left to right in each figure are $-P - Al$, $+P - Al$, $-P + Al$, $+P + Al$. Four- to 6-d-old intact seedlings were grown in a solution containing 0·5 mM $CaCl_2$ with or without 1·0 mM P at pH 4·5 for 24 h, then treated in 0·5 mM $CaCl_2$ with or without 50 μM Al at pH 4·5 for 3 h. Thereafter, the root was stained with haematoxylin and observed under a microscope. The image magnification was $25 \times$. At the same time, Al in the root tips was extracted and determined with the same methods described above. Data are means $\pm$ s.d. ($n = 3$). Significant differences between mean values are indicated by different letters at the $P < 0.05$ level (Tukey's test).

soils. Liao *et al.* (2006) also provided evidence for root Al and P interactions whereby the taproot growth of two P-efficient genotypic soybeans (more Al tolerant) was less affected by Al toxicity than that of two P-inefficient genotypes. Therefore, plants that perform well in low-P acidic soils should possess root systems that are both Al tolerant and P efficient. The present results showed that Al-resistant species had higher P contents than did Al-sensitive species after P was supplied whether Al was present or not, and in Al-sensitive species the decrease in P in shoots was much greater under Al stress compared with that of Al-resistant species (Fig. 5). Therefore, Al might inhibit P transport from roots to shoots in Al-sensitive species.

On the other hand, when P was supplied this resulted in much greater Al accumulation in roots of Al-sensitive species than in Al-resistant species (Fig. 5), and this result could explain the difference in Al damage to the two species. In addition, it was found that P application to Al-resistant species resulted in the augmentation of the P/Al ratio in the roots and shoots, while the increase of the P/Al ratio in Al-sensitive species was much smaller (Fig. 6), indicating that the Al-resistant species possessed a mechanism for constraining Al uptake and facilitating the entry of P. The higher Al resistance in *L. bicolor* appeared to be associated with higher P and lower Al accumulation in roots. The finding of reduced Al accumulation in root tips of Al-resistant species when P is supplied
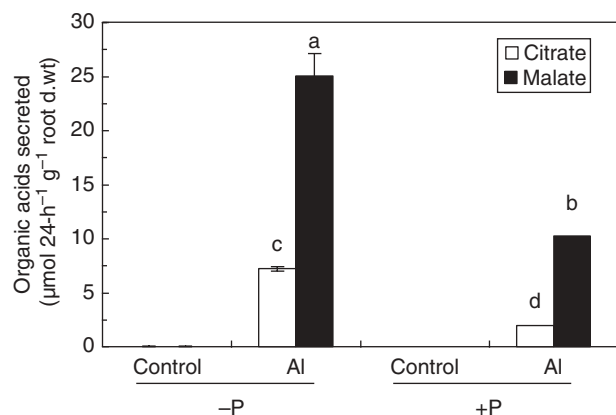
FIG. 8. Effects of P supply on Al-induced citrate and malate efflux from roots of *L. bicolor*. Plants were pre-cultured in nutrient solution containing 0 or 1·0 mM P for 30 d and then exposed to 0·5 mM CaCl₂ solution containing 0 or 50 μM Al. Root exudates were collected after 24 h exposure and organic acids were analysed by HPLC. The pH of all experiments was controlled at 4·5. Values shown are means ± s.d. ($n = 3$). Significant differences between mean values are indicated by different letters at the $P <$ 0·05 level (Tukey's test).

(Fig. 7) led to the conclusion that P reduced the Al accumulation in root tips of *L. bicolor* but not in *L. cuneata*, indicating that Al-resistant species might have greater ability to exclude Al from root tips after P application.

Why P application had no alleviative effect on the Al toxicity in L. cuneata

Acid soils often present a series of stresses to plants including Al toxicity and P deficiency that commonly occur together. The reasons why some plants grow better in these soils might be due to a combination of mechanisms that help overcome each of these stresses separately, or it might be due to a single mechanism that manages to ameliorate both of them. The present study demonstrated, by analysing the root parameters, that P application could not ameliorate Al stress in the Al-sensitive *Lespedeza* species (Figs 2–4). However, the positive effects of P on root morphology parameters and fresh weight of shoots and roots were higher for *L. cuneata* than for *L. bicolor* (Table 1), i.e. the root growth of *L. cuneata* had a greater response to P application, which hinted that the potential P ameliorative effects on Al stress in *L. cuneata* may be greater, though this was not shown under the present experimental conditions. The root growth of *L. cuneata* was greatly constrained under this Al stress (Figs 1 and 3). It suffered significantly more damage to its root tissues than did the more resistant species *L. bicolor*. This could be a possible reason why P application did not alleviate the Al toxicity in the Al-sensitive species. In acid soils, the effects of P could only be exerted when Al stress is firstly overcome for the Al-sensitive species.

In conclusion, Al resistance of the resistant species, *L. bicolor*, increased with application of P but that of the Al-sensitive species *L. cuneata* did not under this relatively high Al stress. Enhancement of Al resistance with the presence of P in the resistant species might be associated with

its more efficient accumulation of P and translocation to the shoot; the greater Al exclusion from root tips after P application was also responsible for the alleviative effects of P, but not with the exudation of organic acids in roots. To clarify further why P enhances Al resistance in Al-resistant *Lespedeza* but not in Al-sensitive *Lespedeza*, more studies are needed with a wider range of Al levels.

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