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Higher Aluminum Tolerance of *Lespedeza bicolor* Relative to *Lespedeza cuneata* Is Associated with Saccharide Components of Root Tips

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Abstract: Aluminum (Al) toxicity is the primary factor limiting agricultural productivity in acid soils. The cell wall is mainly composed of saccharides and the first barrier for aluminum (Al) to enter plant root cells, but it is unknown whether and how root saccharide components are involved in regulating the Al tolerance of *Lespedeza* that is well adapted to acid soils. Here, we used Al-tolerant *Lespedeza bicolor* and Al-sensitive *Lespedeza cuneata* to examine the association of root saccharide components with *Lespedeza* Al tolerance through analyzing the saccharide changes of roots exposed to Al toxicity. Al-sensitive *Lespedeza* accumulated more Al and pectin but less hemicellulose in the root cell walls than Al-tolerant *Lespedeza*. Al treatment decreased the amounts of total sugar secreted from and within the roots of only Al-tolerant *Lespedeza*. Al treatment decreased the content of root monosaccharides including glucose and mannose in both *Lespedeza* species, but increased the xylose contents in only Al-sensitive *Lespedeza*. Taken together, less cell wall pectin rather than hemicellulose is responsible for less root Al accumulation, and Al-decreased root saccharide contents may enhance root organic-acid secretion to chelate toxic Al, both of which contribute to *Lespedeza* Al tolerance.

Keywords: Al tolerance; apoplast; cell wall; hemicellulose; *Lespedeza*; monosaccharide; pectin

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1. Introduction

Lespedeza plays important roles in land reclamation, forage production, and soil conservation. *Lespedeza* is also capable of improving soil fertility by rapidly increasing soil organic matter and nitrogen content by symbiotic N_2 fixation in agricultural production. It has been known since the early twentieth century that *Lespedeza* adapts well to acid infertile soils [1,2]. Aluminum (Al) toxicity is the primary factor limiting plant growth in acid soils [3]. It is critical to elucidate the mechanisms of *Lespedeza* in response to Al toxicity in order to find out new information on plant Al tolerance and to make full use of *Lespedeza* in acid soils. Plants generally detoxify Al by excluding toxic Al from roots and tolerating toxic Al within plants [4–6]. The secretion of organic acids from roots under Al toxicity is the main mechanism of Al exclusion in most plants [7–9]. Our previous study indicated that the secretion of malate and citrate from roots under Al toxicity was one of the Al-exclusion mechanisms in *Lespedeza* [10]. Furthermore, we found that efficient phosphorus use and ammonium supply were also involved in the Al tolerance of *Lespedeza* [11,12]. However, these mechanisms are still not enough to explain the extremely high Al tolerance of *Lespedeza*, because some Al-sensitive plants such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) can also secret organic acids from roots under Al toxicity [13,14].

The apoplast of plant roots represents the major site for plants to perceive and express Al toxicity [15,16]. The protection of the root apoplast is a prerequisite for plant Al tolerance [17]. The cell wall in the root apoplast plays an important role in the tolerance of plants to Al toxicity [18–23]. Most of Al entering the root apex is bound to the cell wall of roots [9,18,21,24]. The changes in the size and quantity of polysaccharides within the root cell wall regulate the stretching of the cell wall [9,25,26]. Aluminum causes the accumulation of cell wall polysaccharides and thus makes the cell wall thick and rigid, thereby inhibiting the growth of plant roots [27]. In addition, the cell wall polysaccharides play an important role in modulating the Al-binding capacity of the cell wall [8,20,21]. Aluminum inhibits xyloglucan endotransglucosylase that catalyzes the splitting of xyloglucan chains and the linking of the newly generated reducing end to the non-reducing end of another xyloglucan chain in the cell wall [23]. Therefore, the binding of Al in the cell wall can further change the mechanical and chemical properties of the cell wall. This supports that theory that Al inhibits root elongation initially through the interaction of Al with the apoplastic side of the cell wall–plasma-membrane–cytoskeleton continuum [6,18,19]. Although the importance of the root cell wall and apoplast for Al tolerance has been reported in some plants, as described above, it is still unknown whether they also play important roles in the Al tolerance of *Lespedeza*, a strongly Al-tolerant plant.

In this context, we used Al-tolerant and Al-sensitive *Lespedeza* and examined the Al injury site and distribution, root sugar secretion, and cell wall components when exposed to Al toxicity. The objective of this study was to address the possible role of the root cell wall in regulating *Lespedeza* Al tolerance, thereby providing new information on the explanation of Al tolerance mechanisms in this strongly Al-tolerant plant.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Lespedeza bicolor (Turcz. cv. Jiangxi) and *L. cuneata* (Dum.-Cours. G. Don cv. Zhejiang), which are, respectively, Al-tolerant and Al-sensitive, were used in this study [10,11]. The seed coat of *L. bicolor* is relatively hard and needed to be softened by 98% (m/m) sulfuric acid, but the seed coat of *L. cuneata* is soft and did not need such pretreatment. Seeds of *L. bicolor* were surface-softened in 98% (*m*/*m*) sulfuric acid for 20 min, washed with deionized water, soaked in deionized water overnight, and then germinated in a plastic Petri dish with filter paper saturated with deionized water, in darkness, at 25 °C for two days. Seeds of *L. cuneata* were soaked in deionized water for 2 h, and then germinated in a plastic Petri dish for two days according to the above-described method. Germinated seeds were transplanted to plastic net suspended on a plastic container (2.5 L) with 0.5 mM $CaCl_2$ solution (pH 4.5). The solution was renewed every day. Five-day-old seedlings were treated with different concentrations of Al ($AlCl_3 \cdot 6H_2O$) in 0.5 mM $CaCl_2$ solutions (pH 4.5) for subsequent experiments.

2.2. Monitoring and Energy-Spectrum Analysis of Elements on Root Surface

We performed the monitoring and energy-spectrum analysis of elements on the root surface, as described previously [28]. Five-day-old seedlings were subjected to 0.5 mM $CaCl_2$ solution (pH 4.5) with or without 200 μM Al for 24 h. Root tips were taken from 1 to 5 mm behind the apex, stored in the refrigerator at −20 °C for 18 h, and then put into the freeze dryer (Thermo Savant, Hyannis, MA, USA) for 24 h. Subsequently, the dried complete root tips were fixed on the adhesive table with double-sided adhesive tape. After gold-plating with an ion-coating machine (Leica EM SCD 005, Sunnyvale, CA, USA; time 30 s, current 40 mA), the scanning pictures with a Philips field emission scanning electron microscope were obtained (Sirion 200, FEI, Amsterdam, The Netherlands). At the same time, the root surface energy spectrum was scanned with an X-ray energy-spectrum analyzer (Genesis, EDAX Inc., Berwyn, IL, USA).

2.3. Morin Staining and Fluorescence Microscopy

Morin was used to indicate the presence of Al in root tips and apoplast. Morin staining was performed as described previously [29]. Five-day-old seedlings were subjected to 0.5 mM $CaCl_2$ solution (pH 4.5) with or without 100 μM Al for 24 h. Root tips were cut, washed in 5 mM NH_4OAc buffer (pH 5.0) for 10 min, and then transferred to 100 μM morin solution plus 5 mM NH_4OAc (pH 5.0) for 1 h. Finally, these stained roots were washed 3 times in 5 mM NH_4OAc buffer (pH 5.0) for 10 min each time. The root tips were observed under a fluorescence microscope (excitation wavelength 420 nm, emission wavelength 510 nm, axivoert 40 CFL, Carl Zeiss, Jena, Oberkochen, Germany). The images were analyzed by Axio Vision-AC software.

2.4. Al Distribution in Apoplast and Symplast

The roots of five-day-old seedlings were exposed to 0.5 mM $CaCl_2$ solution (pH 4.5) with or without 50 μM Al for 24 h. Seedling roots were rinsed with ultrapure water, and the attached water in the root surface was blotted with filter paper. After that, 70 root tips (0–1.0 cm) were excised and placed in 50 mL polyethylene tubes. Fresh root tips were weighed and then refrigerated at 4 °C for fractionation testing. The method of continuous extraction of Al in apoplast and symplast was modified from a previous study [29]. The fractionation of Al was as follows: (1), root tips were sequentially washed 6 times on the oscillator for 60 min per cycle with 10 mL desorbing solution (5 mM $CaCl_2$ + 100 μM sodium citrate); (2), cell membranes were ruptured by freezing and sonication, where freezing duration was approximately 18 h at −20 °C and sonication was conducted with a sonicator with a 3 mm diameter head for 1 min at high power; (3), the same method as (1) and (2) was used 4 times to separate the root tip residues; (4), root tip residues were digested in 1.0 mL concentrated HNO_3 (trace metal grade) at 100 °C water bath for 1 h, and digested solutions were diluted to 10 mL with the ultrapure water. Al in wash and digest solutions was, respectively, apoplastic and symplastic Al, and analyzed spectro-fluorometrically using 8-hydroxyquinoline with butyl acetate extraction [30].

2.5. Determination of Total Sugar Secreted from and within Root Tips

Five-day-old seedlings were cultured for 30 d in the modified Hoagland's solution as described previously [11]. Then, the plants were exposed to a 0.5 mM $CaCl_2$ solution (pH 4.5) with or without 50 μM Al for 24 h. Thereafter, the original solution was filtered and evaporated to dryness at 40 °C on a rotary evaporator. The sample was fixed to 10 mL, centrifuged at 3000× *g* for 10 min, and the supernatant was used for the determination of total sugar secreted from root tips. In addition, the excised root apices (0–1.0 cm) were stored in the freezer at −20 °C for total sugar extraction. The frozen root tips were homogenized with 5% (*v*/*v*) $HClO_4$ in an ice bath, and then the combined material was centrifuged at 12,500× *g* for 10 min. These supernatants constituted the total sugar contents of the root apices. Total sugar in the supernatants was determined by anthrone colorimetry.

2.6. Cell Wall Extraction

Five-day-old seedlings were exposed to a 0.5 mM $CaCl_2$ solution (pH 4.5) with or without 50 μM Al (pH 4.5) for 24 h. Root tips (0–2.0 cm) were washed with ultrapure water and the excess liquid was blotted with filter paper. After weighing, the samples were temporarily stored at −20 °C for cell wall extraction. The method of cell wall extraction was modified from a previous study [31]. The frozen root tips were homogenized with a mortar in an ice bath. The homogenate was subsequently rinsed twice with 10 × volume of 80% (*v*/*v*) ice ethanol, transferred into a centrifugal tube, and kept in ice bath for 2 h. The homogenate was then centrifuged at 12,500× *g* for 10 min, and the supernatant was decanted. The same amount of 80% (*v*/*v*) ice ethanol was added, and the above step was repeated once. After that, the precipitate was washed sequentially with 10 mL ultrapure water, 10 mL a methanol:chloroform mixture (1:1 [*v*/*v*]), and 10 mL phenol: acetic acid:water mixture (2:1:1 [*v*/*v*/*v*]), and this step was repeated once. Finally, the insoluble material

was resuspended in water with α-amylase (10 μg mL^{-1}) for 3 h at 37 °C, and was washed twice with 20 mL ultrapure water. Centrifugation was repeated after each wash. After frozen-drying, this material was stored at 4 °C for further use.

2.7. Measurement of Cell Wall Polysaccharides

Cell wall polysaccharides were measured according to the previous report [20]. Extracted cell walls were fractionated into three fractions: pectin, hemicellulose 1 (HC1), and hemicellulose 2 (HC2). To remove pectin, the pellet was resuspended in 5.0 mL of 0.5% (*m*/*v*) ammonium oxalate containing 0.1% (*m*/*v*) $NaBH_4$ (pH 4.0) for 1 h in a boiling water bath. This material was centrifuged and washed twice with 0.5% (*m*/*v*) ammonium oxalate. These combined supernatants were the pectin fraction. The resulting pellet was extracted three times with 5.0 mL 4% (*m*/*v*) KOH containing 0.1% (*m*/*v*) $NaBH_4$ (pH 4.0) on a water bath shaker (25 °C) for a total time of 24 h. Then, similar extraction with 24% (*m*/*v*) KOH containing 0.1% (*m*/*v*) $NaBH_4$ (pH 4) was repeated. The collected supernatants from the 4% and 24% KOH extraction thus yielded the HC1 and HC2 fractions, respectively.

The pectin extract was hydrolyzed for 1 h in a boiling water bath under the action of 3 M sulfuric acid. HC1 and HC2 extract was hydrolyzed for 1 h in a boiling water bath. Polysaccharide content in each fraction was determined by the phenol−sulfuric acid method [32]. Glucose was used as a calibration standard. All treatments consisted of at least three repetitions within each experiment.

2.8. Pretreatment and Determination of Monosaccharide in Root Tips

Root tips (0–1.0 cm) of 5-day-old seedlings were homogenized in 0.5 mL 5% $HClO_4$ (*v*/*v*) in an ice bath. The homogenate was then rinsed with 0.5 mL 5% (*v*/*v*) $HClO_4$ and transferred into a 1.5 mL Eppendorf tube. Subsequently, the homogenate was centrifuged at 12,500× *g* for 10 min, filtered by 0.45 μm membrane, and used to determine the monosaccharide content of root tips.

The concentration of monosaccharide in the solution was determined by high performance liquid chromatography (HPLC; LC-10AT VP, Shimadzu, Tokyo, Japan) with gradient elution and post-column derivatization fluorescence detection using a Shim-pack ISA-07 column (4.0 mm i.d. × 25 cm). The mobile phase included A solution of 0.1 mM boric acid (adjusted to pH 8.0 with KOH) and B solution of 0.4 M boric acid (adjusted to pH 9.0 with KOH). The mobile phase was eluted from 100% (*v*/*v*) of A solution to 100% (*v*/*v*) of B solution in a linear gradient of 2% (*v*/*v*)/min. The total flow rate was 0.6 mL min^{-1}, and the column temperature was 65 °C. The post-column derivatization reagent was 1% (*m*/*v*) L-arginine containing 3% (*m*/*v*) boric acid. The flow rate was 0.5 mL min^{-1}, and reaction temperature was 150 °C. The wavelengths for fluorescence detection were 320 nm (excitation) and 430 nm (emission).

2.9. Statistical Analysis

All experiments and observations were repeated three times. One-way ANOVA was used and a least significant difference (Tukey) test was applied for multiple means' comparisons at a significance level of $p < 0.05$. For the statistical analyses, the software SPSS (version 13.0, Chicago, IL, USA) was used.

3. Results

3.1. Aluminum-Induced Element Exclusion from Lespedeza Roots

Al exposure induced irregular curve and crack formation in the meristematic zone of two species (Figure 1A,B). The energy-spectrum analysis of elements showed that Al, potassium (K), calcium (Ca), and iron (Fe) were detected on the root surface of Al-sensitive *L. cuneata* (Figure 1D,F), whereas only Al was detected on that of Al-tolerant *L. bicolor* (Figure 1C,E).

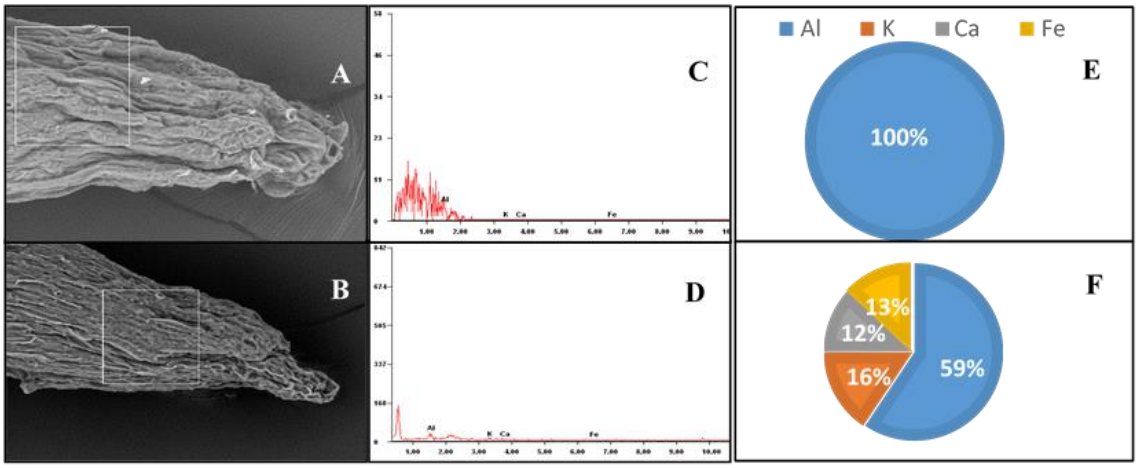

Figure 1. Scanning images, energy-spectrum analysis, and element percentage in the meristematic zone of two *Lespedeza* species. (**A**,**C**,**E**), *L. bicolor*; (**B**,**D**,**F**), *L. cuneata*. Seedlings (five-day-old) of two *Lespedeza* species were subjected to 0.5 mM $CaCl_2$ solutions (pH 4.5) with 200 μM Al for 24 h. Scanning images (**A**,**B**) were observed by the scanning electron microscopy microscope. The image magnification was 750 under 20 kV voltage. All pictures were taken under the same conditions. Energy-spectrum analysis (**C**,**D**) was carried out using energy-dispersive X-ray spectroscopy, and element percentages (**E**,**F**) were obtained from the energy-spectrum analysis.

3.2. Aluminum Distribution in Apoplast and Symplast of Roots

In order to explore the Al distribution in the *Lespedeza* root apex, morin, as a fluorochrome, was used to localize Al in roots. The Al accumulation in the root tips of Al-tolerant *L. bicolor* was much less than that of Al-sensitive *L. cuneata* (Figure 2). When exposed to Al toxicity, Al-sensitive *L. cuneata* accumulated more Al in both apoplast and symplast of root tips than did Al-tolerant *L. bicolor* (Figure 3). About 38%, 15%, and 47% of the accumulated Al were distributed in the apoplastic, symplastic, and residual fractions, respectively.

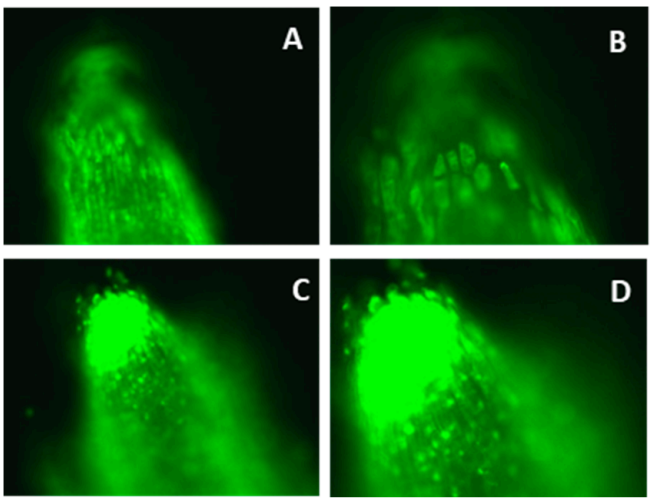

Figure 2. Aluminum distribution in the roots of two *Lespedeza* species using morin staining. (**A**,**B**), *L. bicolor*; (**C**,**D**), *L. cuneata*. (**A**,**C**), ×200; (**B**,**D**), ×320. The roots were exposed to a 0.5 mM $CaCl_2$ solution (pH 4.5) with 100 μM Al for 1 h, and then stained with 100 μM morin. The sections were taken from the root zone between 0 and 3 mm behind the root apex of *Lespedeza* seedlings.

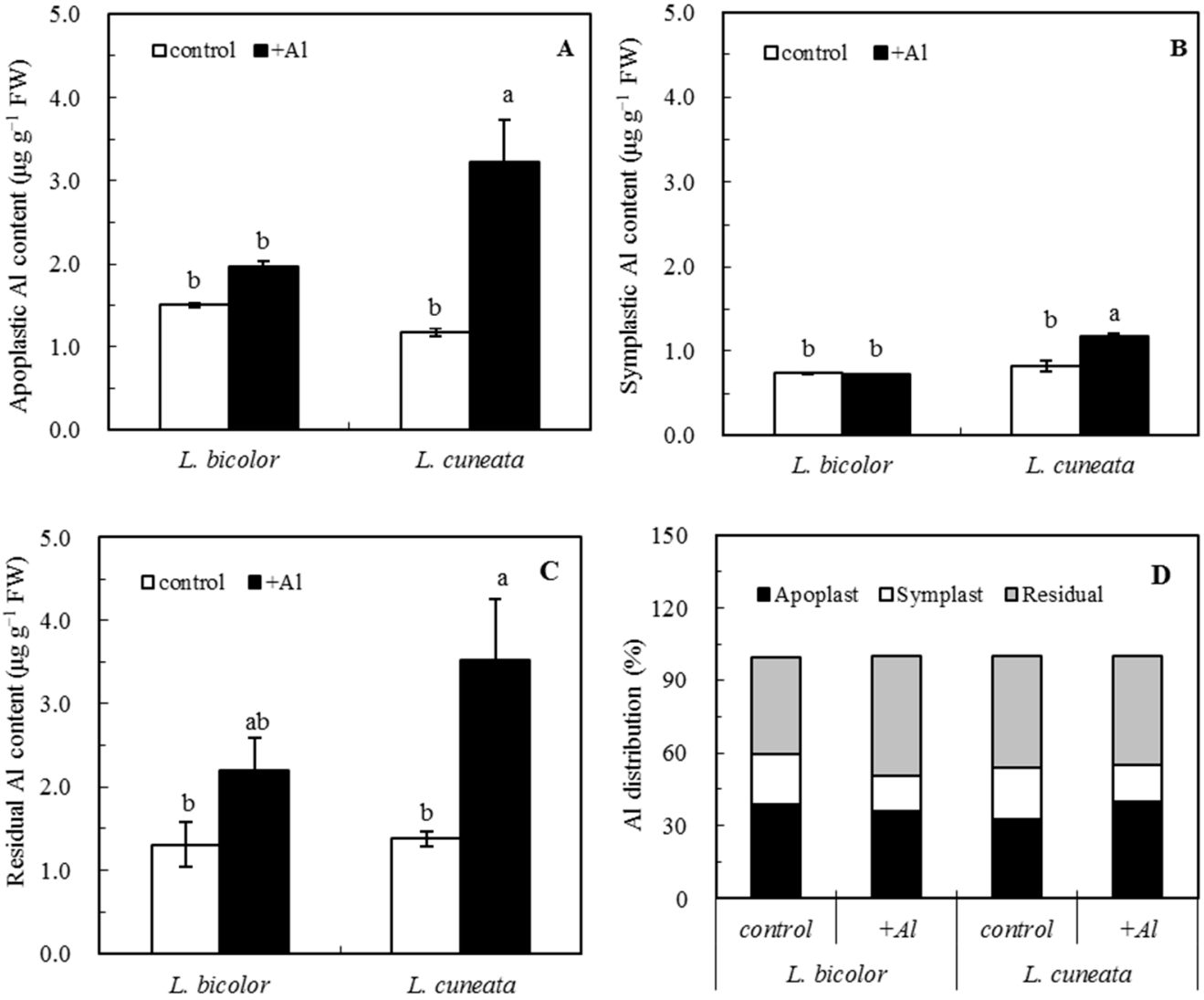

Figure 3. Distribution of Al in the root apices of two *Lespedeza* species. (**A**) apoplastic Al, (**B**) symplastic Al, (**C**) residual Al, and (**D**) percentage Al distribution in different fractions. Five-day-old seedlings were exposed to 0.5 mM $CaCl_2$ solutions (pH 4.5) containing 0 (control) or 50 μM Al (+Al) for 24 h. The apoplastic, symplastic, and residual Al contents of root apices were analyzed. Significant differences between mean values are indicated by different letters at the $p < 0.05$ level (Tukey test). Error bars represent ±SD ($n = 3$).

3.3. Total Sugar Secreted from and within Root Tips

Aluminum toxicity reduced the amounts of total sugar secreted from and within the roots of only Al-tolerant *L. bicolor* (Figure 4). In addition, Al-tolerant *L. bicolor* secreted much more total sugar than Al-sensitive *L. cuneata* regardless of Al toxicity (Figure 4A). The contents of total sugar within the root tips of Al-tolerant *L. Bicolor* were higher than those of Al-sensitive *L. cuneata* under control treatment, but this was reversed under Al treatment (Figure 4B).

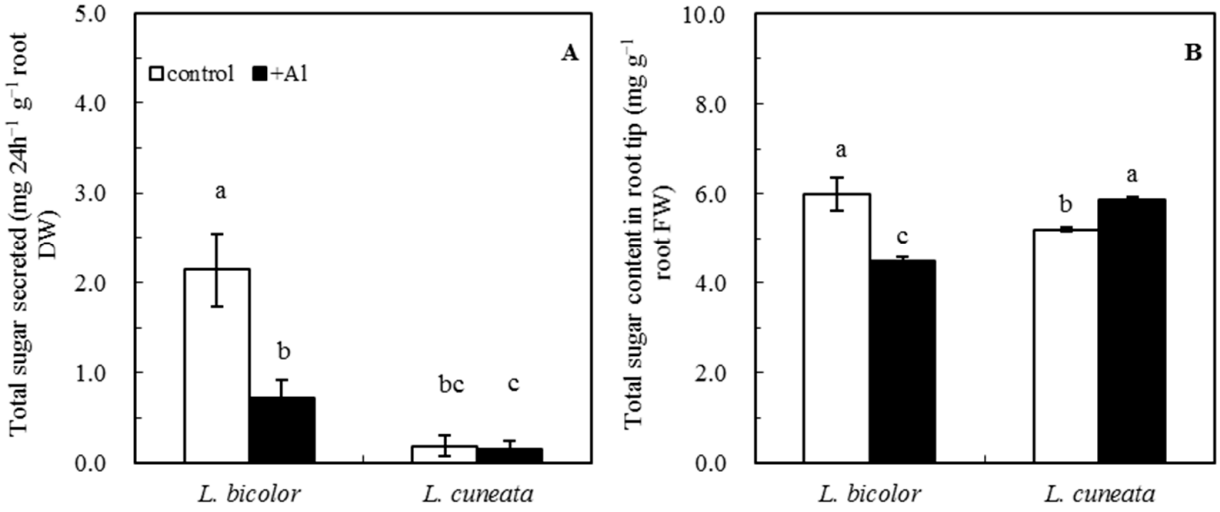

Figure 4. Effects of Al treatments on the contents of total sugar secreted from and within root tips of two *Lespedeza* species. (**A**) total sugar secreted from roots and (**B**) total sugar within root apices. *Lespedeza* seedlings were exposed to 0.5 mM $CaCl_2$ solutions (pH 4.5) with or without 50 μM $AlCl_3$ for 24 h. Thereafter, the contents of total sugar in the culture solution and root tips (0–1.0 cm) were both determined by anthrone colorimetry. Significant differences between mean values are indicated by different letters at the $p < 0.05$ level (Tukey test). Values shown are means ± SD ($n = 3$).

3.4. Polysaccharides of Root Cell Walls

As the Al bound in the apoplast can cause changes in the cell wall polysaccharides, the cell wall fractions of root tips were isolated and analyzed to determine whether cell wall composition was changed by Al toxicity in two *Lespedeza* species (Figure 5). Al treatment did not affect the pectin contents of root tips of two *Lespedeza* species, whereas the contents of pectin in the root tips of Al-sensitive *L. cuneata* were significantly higher than those of Al-tolerant *L. bicolor* (Figure 5A). Al treatment increased the contents of HC1 and HC2 in root tips of Al-tolerant *L. bicolor* but not in Al-sensitive *L. cuneata*, and those contents were higher in Al-tolerant *L. bicolor* than in Al-sensitive *L. cuneata* under Al toxicity (Figure 5B,C).

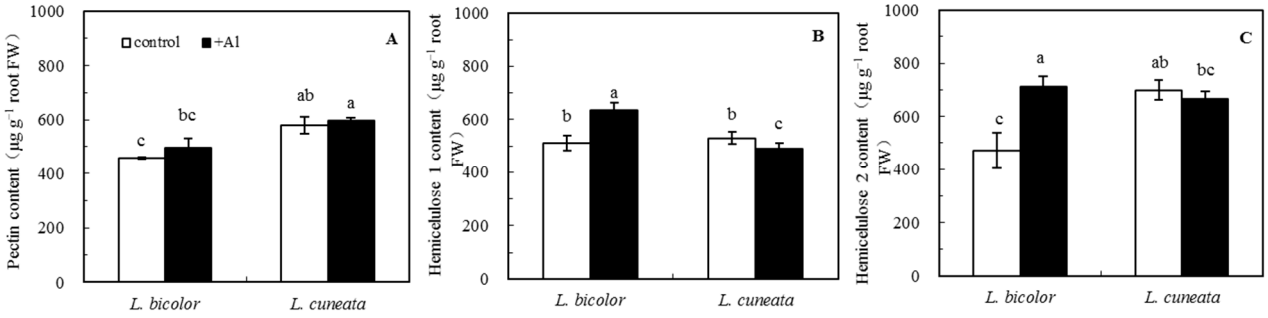

Figure 5. Effect of Al treatments on the contents of polysaccharides in root tips of two *Lespedeza* species. (**A**) pectin, (**B**) HC1, and (**C**) HC2. *Lespedeza* seedlings were exposed to 0.5 mM $CaCl_2$ solutions (pH 4.5) with or without 50 μM Al for 24 h. Root apices (0–1 cm) were cut and cell wall polysaccharides were fractionated into pectin, HC1, and HC2 for glucose-content measurement. Significant differences between mean values are indicated by different letters at the $p < 0.05$ level (Tukey test). Error bars represent ±SD ($n = 3$).

3.5. Monosaccharides of Root Tips

The monosaccharides of root apices were mainly composed of glucose and mannose (Figure 6). The contents of glucose and mannose of roots were significantly decreased under Al toxicity, and were higher in Al-sensitive *L. cuneata* than in Al-tolerant *L. bicolor* (Figure 6A,B). There was no significant difference in the ribose content between the control

and Al treatment, and between the two species (Figure 6C). Al treatment substantially increased the contents of xylose in root tips of only Al-sensitive *L. cuneata* being higher than those of Al-tolerant *L. bicolor* under Al toxicity (Figure 6D).

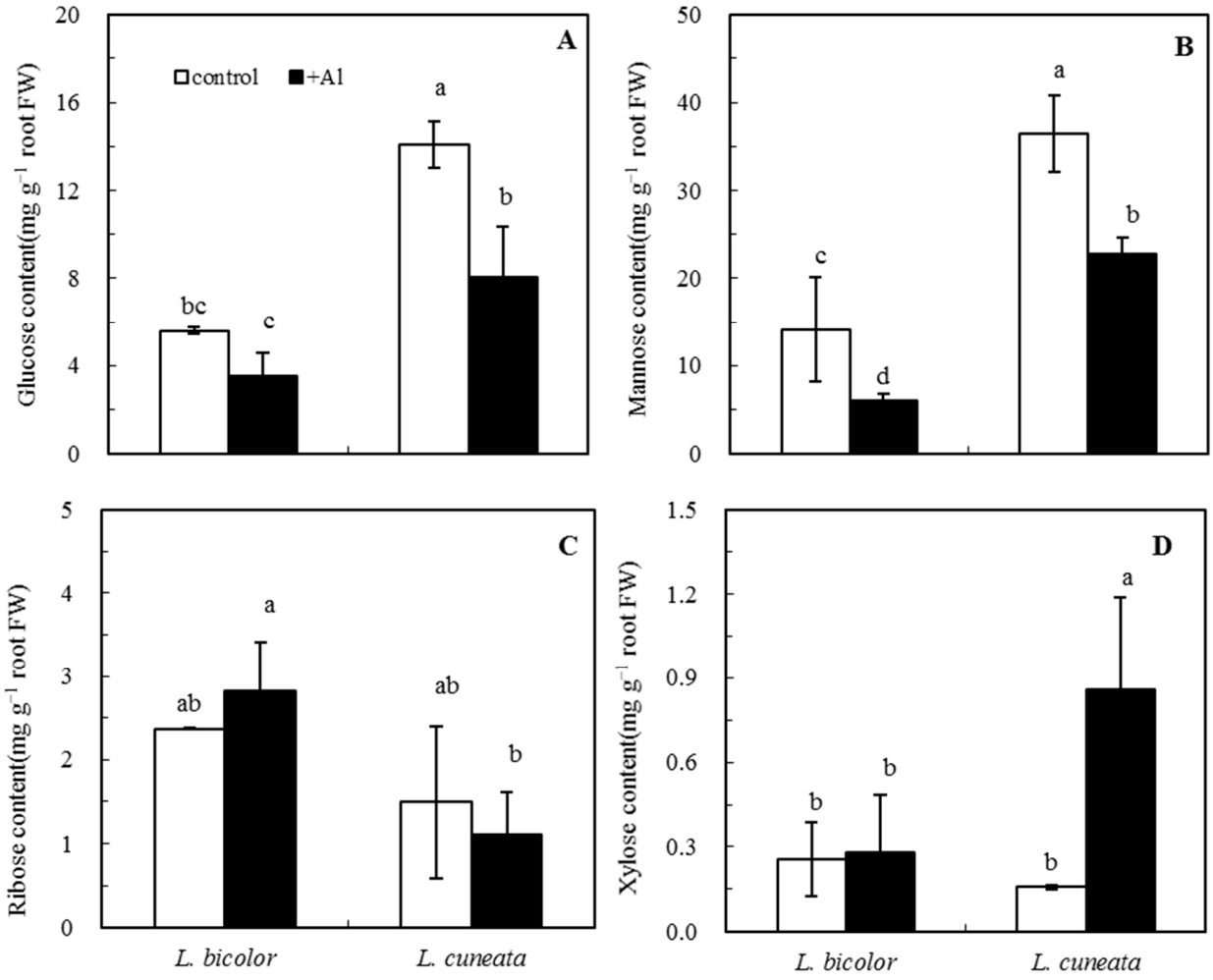

Figure 6. Effects of Al treatments on the contents of monosaccharides in root apices of two *Lespedeza* species. (**A**) glucose, (**B**) mannose, (**C**) ribose, and (**D**), xylose. Five-day-old seedlings were exposed to 0.5 mM $CaCl_2$ solutions (pH 4.5) with or without 50 μM Al for 24 h. Significant differences between mean values are indicated by different letters at the $p < 0.05$ level (Tukey test). Values shown are means ± SD ($n = 3$).

4. Discussion

Root growth inhibition is the most dramatic visual symptom of Al toxicity in various plants, and the location of Al toxicity is mainly at the root tips [11,19,33–36]. Our previous research suggests that the occurrence of Al toxicity to *Lespedeza* is mainly in the 0–5 mm root tips [11,37]. Here, the scanning images further revealed that Al toxicity affected the morphology of root tips including the irregular curve and crack formation, and the morin staining results further suggested that Al was accumulated much more in the root tips of the Al-sensitive *Lespedeza* than in those of the Al-tolerant one. The analysis of energy spectrum elements showed that the destroyed cell structure of root tips of the Al-sensitive *Lespedeza* due to Al toxicity resulted in the release of several nutrients such as K, Ca, and Fe from the cytosol to the root surface. However, this loss of nutrients did not occur in the root tips of the Al-tolerant *Lespedeza*. We infer that Al toxicity may result in much bigger damage to root cell integrity and cause more nutrient leakage from roots in Al-sensitive *Lespedeza* compared with Al-tolerant *Lespedeza*. These results suggest that root tips play a decisive

role in the expression of Al toxicity in *Lespedeza*, which is in accordance with previous reports on other plants [6,19,38]. In the root tips, the apoplast accumulated more Al than did the symplast, and the Al-sensitive *Lespedeza* had much more apoplastic Al than did the Al-tolerant one. This is also in accordance with previous reports on other plants [19,23]. Although the apoplast occupies 5% or less of the total tissue volume in higher plants, more than 70% of Al is accumulated in the cell wall of root apoplast [18,23,37,39]. The protection of the root-tip apoplast represents an important way by which Al-tolerant *Lespedeza* adapts to Al toxicity.

As the first barrier for all ions entering the cell, the cell wall plays a major role in regulating the normal growth and development of plants under Al stress [5,9,23]. The apoplast is mainly composed of cell walls which contain large amounts of saccharides, so the responses of cell wall saccharides to Al toxicity were further examined in this study. Al accumulation in roots is closely positively correlated with the pectin contents of root tips of maize and faba bean [19]. Our study showed that the Al-sensitive *Lespedeza* accumulated more Al and pectin in the cell walls of root tips than did the Al-tolerant one, which is consistent with previous studies in other plants [20,26]. The experimental evidence has proved that the modification of the root cell wall might contribute to Al tolerance [8,18–21,23,26,27,40]. Al accumulation in the root apoplast could modify the mechanical components and chemical properties of the cell walls [6,19–21]. In our study, Al treatment did not affect the cell wall pectin contents of root tips in either *Lespedeza* species. However, previous studies showed that Al treatment induced a significant increase in the pectin contents of cell walls of tea and rice roots [23,41]. In addition, the enhancement of HC1 and HC2 contents of root tips by Al toxicity was found in the Al-tolerant *Lespedeza* but not in the Al-sensitive. However, previous studies showed that Al addition also increased the HC contents of root tips of Al-sensitive species [20,26,41]. These inconsistencies may be attributed to the specific properties of cell walls of *Lespedeza* differing from other plant species [8,20,21,23].

The fundamental constituents of pectin are homogalacturonan (HGA) and rhamnogalacturonan I (RG I), while the major parts of HCs are xyloglucans and mixed-linkage β-(1/3),(1/4)-D-glucans [21,33]. The increase in the HCs was attributed to the increases in arabinose, xylose, and glucose [26]. Our results showed that the contents of the root monosaccharides including glucose and mannose were decreased in Al-tolerant *Lespedeza* by Al treatment. This suggested that Al might promote the synthesis of monosaccharides into polysaccharides such as HCs, thereby increasing the contents of HCs of root tips of Al-tolerant *Lespedeza*. For the Al-sensitive *Lespedeza*, Al treatment decreased the contents of the root monosaccharides including glucose and mannose but increased the root xylose (xyloglucan monomer) contents, which may be the reason for the lack of effect of Al treatment on the contents of HCs of root tips of Al-sensitive *Lespedeza*.

Subjected to Al toxicity, plants might activate multiple mechanisms of Al tolerance to maintain their normal growth. Our previous study showed that Al-tolerant *Lespedeza* secreted large amounts of organic acids to alleviate Al toxicity while Al-sensitive *Lespedeza* did not [10]. In the present study, Al-tolerant *Lespedeza* secreted more sugars than Al-sensitive *Lespedeza*, indicating that sugar secretion could also provide some donors to alleviate the effect of Al toxicity. Meanwhile Al treatment decreased the amounts of sugars secreted from Al-tolerant *Lespedeza* but not from Al-sensitive *Lespedeza*, which proved that Al exposure might hasten the intracellular physiological process and transform sugars into more organic acids in Al-tolerant species. In addition, Al decreased the amounts of sugars in the roots of Al-tolerant *Lespedeza* but increased those of Al-sensitive *Lespedeza*. Based these results, we infer that Al-tolerant *Lespedeza* might transform more sugars into organic acids that can chelate toxic Al^{3+} and alleviate Al phytotoxicity, but Al-sensitive *Lespedeza* could not. Consequently, Al-tolerant *Lespedeza* secrets much more organic acid to chelate toxic Al in the rhizosphere and is more tolerant to Al than Al-sensitive *Lespedeza* [10,11]. The specific mechanisms for the transformation of sugars into organic acids under Al toxicity in *Lespedeza* will be an interesting research topic in the future.

5. Conclusions

Less accumulation of pectin rather than hemicellulose in the cell walls of root tips is responsible for the less Al accumulation in root tips and the high Al tolerance of Al-tolerant *L. bicolor* relative to Al-sensitive *L. cuneata*. Furthermore, the decreased amounts of sugars secreted from and within the roots by Al toxicity may contribute to the increased secretion of organic acids from the roots of Al-tolerant *L. bicolor*, thereby increasing *Lespedeza* Al tolerance through chelating toxic Al in the rhizosphere. These findings update the physiological mechanisms of *Lespedeza* Al tolerance and supply the reference for understanding Al tolerance mechanisms in other plants. In the future, it would be worth uncovering the molecular mechanism of saccharide metabolism related to *Lespedeza* Al tolerance.

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