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Title

The binding of Al in different polysaccharides alleviates Al toxicity to root apices

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Introduction

Aluminum toxicity is a worldwide problem in acid soils. The prompt symptom of Al toxicity is the inhibition of cell as well as root elongation. Root apex is generally surrounded by a number of root border cells and mucilage which are assumed to function as a protective layer to root apex to the environmental stresses (Horst et al., 1982; Hawes et al., 1998; Yu et al., 2009). However, there are still arguments regarding the roles of mucilage (Li et al., 2000). Different binding effects of polysaccharides were observed by the isolation of Al-polysaccharide complex in root mucilage and the comparison of root Al uptake in solution with exogenous pectin and dextran, as well as by the comparison of root growth and Al uptake with and without mucilage under mist culture, in order to distinguish the roles of mucilage polysaccharides in protecting root apices from Al toxicity.

Materials and methods

Seed germination

Seeds of pea (*Pisum sativum* L. cv. Zhongwan No. 5) were germinated under mist culture as described previously (Yu et al., 2006) at 24°C in 20 L plastic tanks with 30 s mist produced every 5 min for 36 h for mist culture and 48 h for solution culture. Calcium (0.5 mM CaCl₂, pH 4.0) was added to the seed-soaking solution and the mist-producing ultra-pure water to avoid damage to membranes and cell walls.

Root growth with/without mucilage under mist culture

Seeds were exposed to 0 and 0.5 mM AlCl₃ (pH 4.0, 0.5 mM CaCl₂) mist for 24 h after the radicles had begun to emerge. The seedlings from one tank were separated into two parts: +mucilage (with mucilage intact) and –mucilage (mucilage was removed by 1 min agitation by a magnetic stirrer every 8 h). Root length was determined using a ruler after exposure to Al for 24 h. The roots were then rinsed extensively in ultra-pure water to remove mucilage, border cells and surface Al. Root apices (0–5 mm and 5–10mm) were cut with a razor blade and immersed in 2 M HCl for 48 h and Al content was determined spectrometrically with chromazurol S, cetrimethylammonium bromide (CTMAB) at 635nm. The reaction system was 1:12.5 ethanol water solution including 0.4mM CTMAB, 4mM EDTA-Zn, 66 μM chromazurol S, and 0.57 M methenamine.

Size exclusion chromatography of Al-polysaccharides from root mucilage

Seeds were exposed to 0 and 4.0 mM AlCl₃ (pH 4.0, 0.5 mM CaCl₂) mist for 48 h after the radicles had begun to emerge. The mucilage and border cells were removed by 1 min agitation by a Vortex. The mucilage was condensed by poly ethylene glycol 6000 and freeze-desiccated after border cells were filtrated. The mucilage dissolved in 1mL distilled water was loaded on SephacrylTM S-200 column (1.6cm×40cm) and eluted by 0.5 M NaCl at flow rate of 2.8 ml/10min. The elution fraction was collected every 10 min to determine the content of sugar and Al. Al content was determined spectrometrically as previously discussed. Sugar content was determined by phenol-sulfuric acid method expressed as glucose equivalents. The approximate molecular weights of material eluting in each of the peaks were determined by comparing with Dextran standards of known size of 500 KD, 70 KD, 40 KD and glucose (0.18 KD) indicated by fraction numbers of the peaks 12, 13, 14, 22.

Exogenous polysaccharide treatment

The germinated seedlings were transplanted to 20 μM AlCl_3 (pH 4.5, 0.5 mM CaCl_2) for 24 h with 0 (CK), 0.1 mg/ml pectin, 0.4mg/ml pectin, 0.1 mg/ml dextran T-40, 0.4mg/ml pectin dextran T-40. Al content was determined in root apices (0-5 mm and 5-10 mm) as discussed above.

Statistical analysis

Duncan's multiple range test was applied to test differences among the treatments at $p < 5\%$ using Statistical Analysis Systems (SAS 6.12) software.

Results

*Root elongation and Al uptake of pea (*Pisum sativum*) under mist culture with/without mucilage*

Al content in root apices obviously increased after the removal of root mucilage, and was significantly higher in 0-5mm root apices when roots of pea were cultured in mist with 0.5 mM Al (Fig. 1 A). The elongation rate of root decreased significantly after the removal of mucilage accordingly (Fig. 1 B).

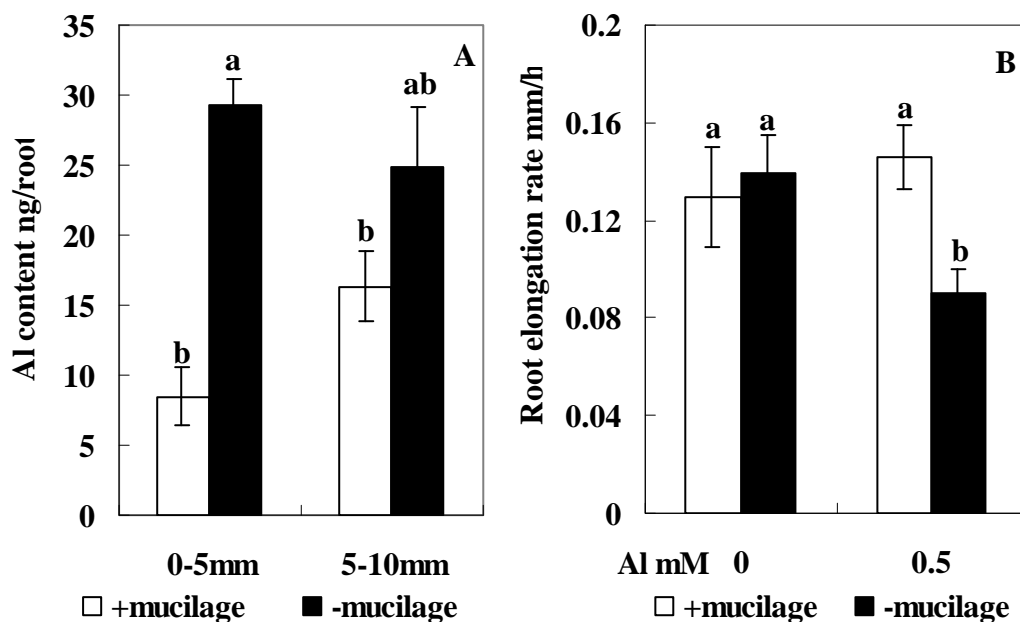


Fig. 1 The effects of mucilage on root Al uptake (A) and elongation (B) under Al mist exposure for 24h
Note: Different letter indicates significant differences at $p < 5\%$ and the error bar=mean \pm SE (n=3 for Al content, n=20 for root elongation rate).

The isolation of Al-polysaccharides from root mucilage

Root mucilage of pea was obtained from mist culture and removed border cells. The size exclusion chromatography of mucilage polysaccharides using SephacrylTM S-200 showed two major peaks of sugar with the fraction number of 5 and 14 at CK while they advanced to number 3 and 13 after Al exposure. From the fraction numbers the peaks 12, 13, 14, 22 of dextran standards of known size of 500 KD, 70 KD, 40 KD and glucose (0.18 KD), the

polysaccharides from mucilage were isolated to one fraction with molecular weight far more than 500 KD and one fraction with molecular weight of 40 KD at CK and 70 KD after Al exposure (Fig. 2 A). The sizes of both the polysaccharides with low and high molecular weight were enlarged by Al exposure in comparison with CK.

Al content from the elution solution showed similar curves as sugar two peaks overlapping with sugar. More Al was found in the fraction with high molecular weight although its sugar content was lower than the fraction with high molecular weight (Fig. 2B).

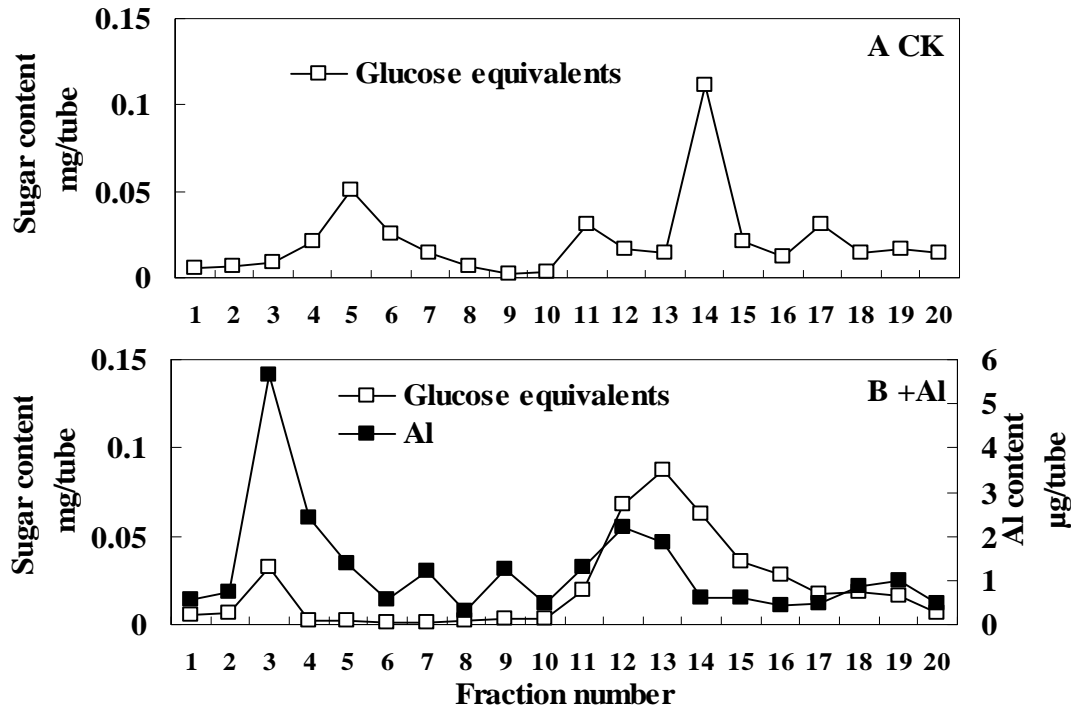


Fig.2 The separation of polysaccharides in mucilage from root of pea with/without Al treatment by Sephacryl™ S-200 size exclusion chromatography

The effects of exogenous pectin and dextran on root Al uptake

Root apices were treated by 20 μM AlCl_3 (pH 4.5, 0.5 mM CaCl_2) for 24 h with different concentration of pectin and dextran in the Al solution. It was found that Al content in 0-5mm and 5-10mm root apices was significantly decreased by the inclusion of pectin at a dose dependent manner. Al content in 0-5mm and 5-10mm root apices was also significantly lower at 0.1 mg/mL dextran compared to CK, however, no dose dependent manner was observed. Al content in root apices was still higher in treatments with dextran compared to pectin.

Discussion

Mist culture was adopted and roles of root mucilage and border cells in protection of root apices from Al toxicity were proved by the removal of mucilage or not. This is not in agreement with reports in *Zea mays* that mucilage did not prevent root from Al injury although it bound Al strongly (Li et al., 2000). One explanation was that all mucilage and border cells were kept intact in root apices of pea under mist culture, while only part of mucilage and border cells was left in root apices of *Zea mays* under solution culture. The second explanation was that mucilage and border cells differed in different plant species, e.g.

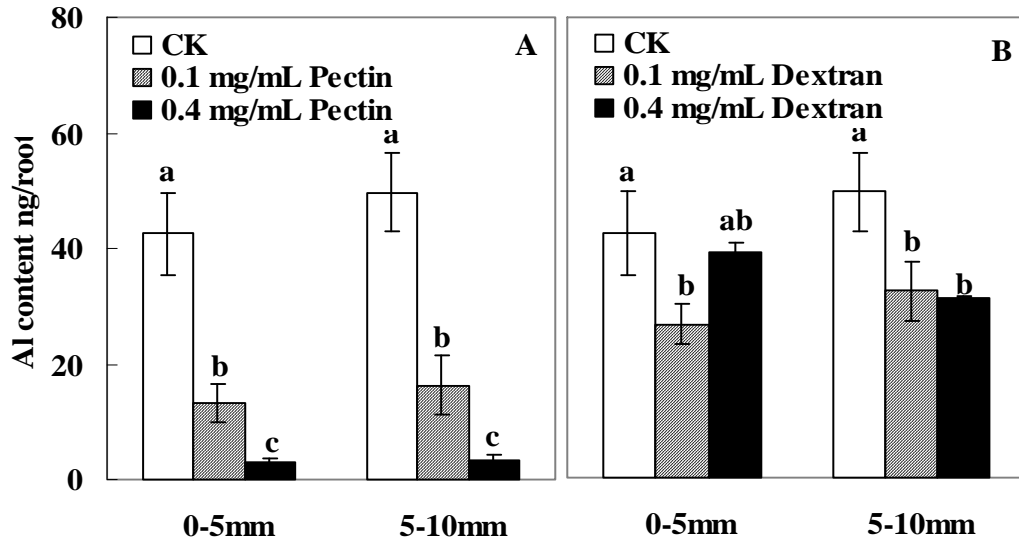


Fig. 3 Effects of exogenous pectin on Al content of root apices of pea under Al exposure

Note: Different letter indicates significant differences at $p < 5\%$ and the error bar=mean \pm SD (n=3).

the number of border cells was found to be over 10,000 per root in pea (Yu et al., 2006) while only 2700 found in *Zea mays* (Hawes and Pueppke, 1986). Size exclusion chromatography indicated Al was mainly bound in polysaccharide fractions with high molecular weight thus disclosing that polysaccharide with higher molecular weight might have a higher capacity for binding Al. The binding of Al in polysaccharides enlarged the molecular weight of the fractions obviously indicated that Al binding two or more chains of polysaccharides into a higher molecular weight complex. This complex inhibited the mobility of Al thus decreasing Al content in root apices both in mist culture and solution culture. Polysaccharides in mucilage might be acidic, alkaline, and neutral and have different molecular weight. Pectin that had higher molecular weight as well as more Al binding sites effectively decreased the Al uptake in root apices at a dose dependent manner in solution culture. However, dextran with lower molecular weight (Mr=40 KD) was less effective. This might be due to the lower molecular weight as well as fewer Al binding sites. The different effects of pectin and dextran indicated that polysaccharides originating from cell wall pectin might have advantages in binding Al, thus immobilized Al in pectin, and in its decompositions. This was in accordance with our results that root border cells enhanced aluminum resistance of root of pea (*Pisum sativum*) grown in mist culture (Yu et al., 2009). These results indicated that Al could be immobilized in the pectin of root border cells, and thus that root apices were effectively protected by exogenous pectin as well as the mixture of border cells and mucilage.

Acknowledgements

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Main References

- Hawes MC, Brigham LA, Woo HH, Zhu Y, Wen F. Function of root border cells in plant health: pioneers in the rhizosphere. *Annu Rev Phytopathol*, 1998, 36: 311–327 [\[Abstract\]](#)
- Hawes MC, Pueppke SG. Sloughed peripheral root cap cells: yield from different species and callus formation from single cells. *Amer J Bot* 1986, 73: 1466-1473 [\[Abstract\]](#)
- Horst WJ, Wagner A, Marschner H. Mucilage protects root meristems from aluminium injury. *Z. Pflanzenphysiol* 1982, 105: 435–444
- Li XF, Ma JF, Hiradate S, Matsumoto H. Mucilage strongly binds aluminum but does not prevent roots from aluminum injury in *Zea mays*. *Physiol Plant* 2000, 108: 152–160 [\[Abstract\]](#)
- Yu M, Feng YM, Goldbach HE. Mist culture for mass harvesting of root border cells: aluminum effects. *J Plant Nutr Soil Sci* 2006, 169: 670–674 [\[Abstract\]](#)
- Yu M, Shen RF, Liu JY, Chen RF, Xu MM, Yang Y, Xiao HD, Wang HZ, Wang HY, Wang CQ. The role of root border cells in aluminum resistance of pea (*Pisum sativum*) grown in mist culture *J Plant Nutr Soil Sci*, 2009 (in press DOI: 10.1002/jpln.200800039)