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Pattern of aluminium-induced efflux of organic acid anions in root tips differs between *Cryptomeria japonica* and *Pinus thunbergii* seedlings

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ABSTRACT

Differential resistance of forest trees to aluminium (Al) may alter carbon flow to the soil. One of the well-known physiological mechanisms of the resistance to Al includes internal detoxification and exclusion of Al via root organic acid anions (OA) exudation. The objective of this study was to clarify whether Japanese plantation tree species such as *Cryptomeria japonica* grown in acidic soils and *Pinus thunbergii* in alkalized soils have similar functions. We hypothesized that roots of *C. japonica* exude more amounts of OA than those in *P. thunbergii*. Seedlings of *C. japonica* and *P. thunbergii* were incubated with Al solutions for 24 h under controlled conditions. Different patterns of OA exudation were observed between two species. Oxalates and citrate exuded by both species in the presence of Al. Malate was exuded only by *C. japonica* in the presence of Al. The amount of exuded oxalate was higher in *P. thunbergii* than in *C. japonica*, whereas with citrate it was the opposite. We conclude that OA exudations in these species might serve for the exclusion of Al but we cannot conclusively support the hypothesis that OA play a critical role in the resistance mechanism against Al.

KEYWORDS: aluminium, citrate, *Cryptomeria japonica*, organic acid anion, oxalate, soil acidity

1. INTRODUCTION

High aluminum (Al) levels in soils can disrupt nutrient relations and alter carbon (C) flow to the forest soils (e.g., Vanguelova et al. 2007). One of the well-known resistance mechanisms to Al includes internal detoxification and exclusion of Al via root organic acid anions (OA) exudation. While most studies of OA exudation have been on crop plants, there were few studies on forest trees mainly on *Eucalyptus* and some tropical species (Naik et al. 2009). *Cryptomeria japonica* usually grown in acidic soils is one of the major silvicultural species in Japan. *Pinus thunbergii* is tolerant to salt stress and thus widely distributed in Japanese coastal parts. We have recently checked Al tolerance of these two species using callose contents in root tips which has been used as a physiological indicator of Al toxicity (Hirano et al. 2008). The result suggests that *C. japonica* is tolerant but *P. thunbergii* is sensitive to Al, thus, we hypothesized that the amount of exuded OA exudation might be higher in *C. japonica*. The objective of this study was to clarify the differences of OA exudation pattern between the two species after exposure to Al.

2. MATERIALS AND METHODS

Eight seedlings of 2-month-old *C. japonica* or 3 seedlings of 1-month-old *P. thunbergii* grown in perlite in a greenhouse at 20°C were transplanted into a glass vial containing 5 ml of 0.2 mM $CaCl_2$ solution (at pH 4.0) and incubated for 24 h in a growth chamber at 25°C. Seedlings were then transferred into new vials with 5 ml of treatment solutions and incubated another 24 h in the growth chamber. The treatment solutions contained 0, 0.1, 0.5, 1.0 mM $AlCl_3$ in 0.2 mM $CaCl_2$

(pH 4.0). Each treatment comprised three replicates. After treatment, the treatment solutions were collected and immediately sterile filtered through 0.2 μm syringe filters. The roots of the seedlings were sampled and their weights measured before and after drying. Organic acid anions in the treatment solutions were measured by ion chromatography.

3. RESULTS AND DISCUSSION

The roots of *C. japonica* and *P. thunbergii* exuded different patterns of OA (Table 1). Oxalates were significantly exuded by both tree species with treatments of Al but the concentrations were higher in *P. thunbergii* than in *C. japonica*. The treatment of Al significantly induced the exudation of citrate by both species but the amount of exudation was significantly higher in *C. japonica* than in *P. thunbergii*. Malate was exuded only by *C. japonica* in the presence of Al.

Table 1. Release of organic acid anions from roots of *C. japonica* and *P. thunbergii* seedlings after treatment with different concentrations of Al in 0.2 mM $CaCl_2$ solution for 24 h.

	Al concentration	Organic acid anions (μmol gfw^{-1} day^{-1})		
Tree species	(mM)	Oxalate	Citrate	Malate
C. japonica	0	n.d[1] ^{c}	n.d. ^{b}	n.d. ^{b}
	0.1	0.49 ± 0.05^{b}	0.39 ± 0.10^{a}	0.14 ± 0.02^{a}
	0.5	0.75 ± 0.10^{a}	0.41 ± 0.02^{a}	0.14 ± 0.02^{a}
	1.0	0.70 ± 0.04^{ab}	0.38 ± 0.12^{a}	0.13 ± 0.02^{a}
P. thunbergii	0	0.47 ± 0.25^{b}	n.d.^{b}	n.d.
	0.1	1.08 ± 0.65^{b}	0.10 ± 0.04^{a}	n.d.
	0.5	2.93 ± 0.59^{a}	0.10 ± 0.01^{a}	n.d.
	1.0	4.04 ± 0.48^{a}	0.11 ± 0.02^{a}	n.d.

[1] n.d.; OA concentrations were below detection limit in solutions (0.125 mg/l), values were set zero in the statistical analysis. Different letters indicate significant differences between treatments of Al ($P < 0.05$).

Our results suggest that both tree species have the potential of OA exudates to detoxify Al. The exudation patterns of OA differed between the two species. The amount of oxalate exuded by *P. thunbergii* was distinctly higher than that of oxalate, citrate, and malate exuded by *C. japonica.* However, *C. japonica* exuded higher amount of citrate compared to *P. thunbergii*, and citrate binds Al much stronger than oxalate. Thus, we cannot conclusively support our hypothesis with the exudation of OA that *P. thunbergii* is more sensitive to Al toxicity. It might be that the amount of OA exudation compared to the total organic C flux is not a critical mechanism of resistance to Al (Naik et al. 2009).

REFERENCES

Hirano, Y., Frey, B. & Brunner, I. 2008 Difference in aluminum-induced callose formation in roots among tree species. Woody roots and Ecosystem service, Abstract book. R026.

Naik, D., Smith, E. & Cumming, J.R. 2009 Rhizosphere carbon deposition, oxidative stress and nutritional changes in two poplar species exposed to aluminum. Tree Physiology 29: 423-436.

Vanguelova, E., Hirano, Y., Eldhuset, T.D., Sas-Paszt, L., Bakker, M.R., Püttsepp, U., Brunner, I., Lõhmus, K. & Godbold, D. 2007 Tree fine root Ca/Al ratio - Indicator of Al and acidity stress. Plant Biosystems 141: 460-480.