The Characteristics of Aluminum Uptake in Roots of *Melastoma malabathricum*, an Aluminum Accumulating Plant

Toshihiro Watanabe[1)*], Seiji Misawa[1)], Syuntaro Hiradate[2)], Steven Jansen[3)] & Mitsuru Osaki[1)]

[1)] *Graduate School of Agriculture, Hokkaido University, Kita 9, Nishi 9, Kitaku, Sapporo 060-8589, Japan,*
**nabe@chem.agr.hokudai.ac.jp*

[2)] *National Institute for Agro-Environmental Sciences, 3-1-3 Kan-nondai, Tsukuba, Ibaraki 305-8604, Japan*

[3)] *Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, U.K.*

Introduction

***Melastoma malabathricum* L.** is an aluminum (Al) accumulating woody plant that grows in tropical Southeast Asia in acid soils with high Al concentrations. This species accumulates more than 10,000 mg Al kg^{-1} in leaves in the form of **monomeric Al** and **Al-oxalate** complexes (Watanabe et al., 1998). It was also demonstrated that the Al-form for translocation from the roots to the shoots is an **Al-citrate** complex (Watanabe and Osaki, 2001). However, the exact mechanisms of Al uptake in *M. malabathricum* roots remain to be elucidated (**Fig. 1**). In the present study, La uptake in *M. malabathricum* was compared with Al uptake (Experiment 1), and the role of the mucilage exuded by *M. malabathricum* roots in Al uptake was examined (Experiment 2).

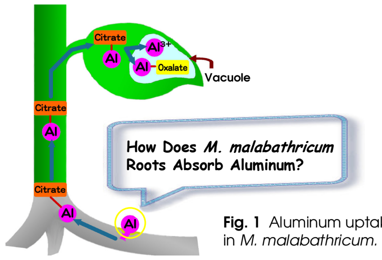


Fig. 1 Aluminum uptake, translocation and accumulation in *M. malabathricum*.

Materials and methods

Experiment 1: Responses of M. malabathricum roots to aluminum and lanthanum

M. malabathricum seedlings were transferred to containers filled with a standard nutrient solution (pH4.2) (Control), or with nutrient solution containing either 0.5 mM Al or 0.5 mM La. At 0.5, 3, 24 and 72 h after commencing the treatment, seedlings were harvested. Al in the roots was fractionated into symplastic, apoplastic, and residual fractions. The modified method of Tice et al. (1992) was used for the analytical fractionation, whereas 5 mM EDTA with 5 mM $CaCl_2$ was used as desorbing solution (pH4.2). Concentrations of the minerals and organic acids in each fraction were determined.

Fig. 2 *M. malabathricum* roots exude large quantities of mucilages (Exp. 2).

What is its function?

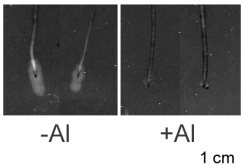


Fig. 3 Mucilage exudation is induced under "Al deficient" conditions (Exp. 2).

White zone indicates the mucilages.

Experiment 2: Role of the root mucilage in aluminum uptake in M. malabathricum

In general, the mucilage is considered to be effective in excluding Al (Horst et al., 1982). Although *M. malabathricum* is an Al accumulating plant, it exudes large quantities of mucilage from its roots (**Fig. 2**). Moreover, the mucilage exudation is induced in the absence of Al (**Fig. 3**).

Exp. 2-1. Selectivity for the absorption of cations in the mucilage

One mL of the mucilage exuded from roots of *M. malabathricum* grown in a nutrient solution was added to 4 mL of the cation mix solution (0.1 mM $AlCl_3$, 0.1 mM $BaCl_2$, 0.1 mM $LaCl_3$; pH4.2), shaken for 2 h and centrifuged at 12000 g. The supernatant was filtered on a membrane filter. Al, Ba and La concentrations in the filtrate were determined to calculate the concentration of these cations adsorbed to the mucilage.

Exp. 2-2. Effect of the removal of the mucilage on cation uptake

M. malabathricum seedlings from which mucilage was removed were transferred to containers containing the standard nutrient solution with 0.5 mM $AlCl_3$ at pH4.2 (-Muci). As a control, the seedlings with the mucilage were also treated (+Muci). At the end of the 10-day treatment period, the seedlings were sampled to determine their mineral concentrations in shoots.

Exp. 2-3. Determination of the Al-form in the mucilage

One mL of the mucilage exuded from roots of *M. malabathricum* grown in a nutrient solution was added to 4 mL of 0.1 mM $AlCl_3$ (pH4.4) and shaken for 2 h and centrifuged at 12,000 g to obtain the mucilage-Al. ^{27}Al NMR was used to determine the Al form in the mucilage.

Results and Discussion

Experiment 1: Responses of M. malabathricum roots to aluminum and lanthanum

Although the Al concentration in the symplastic fraction increased with time of treatment (**Fig. 4**), that in the apoplastic fraction only increased until 3 h, and stayed at this level until the end of the experiment. The Al level was higher in the symplastic fraction after 72 h than in the apoplastic fraction, exceeding 4 μmol g fw^{-1}. In contrast, even after 72 h La did not penetrate into the root cells, and very small quantities of La were found in the apoplastic and residual fractions (**Fig. 4**). These results suggest that high affinity of the root apoplast to Al contributes to specific Al uptake in *M. malabathricum* roots. When *M. malabathricum* was treated with Al, the concentration of citrate, a ligand for Al translocation, increased in the symplastic region in corresponding to Al penetration into this region (**Figs. 4 and 5**), which indicates that induction of citrate synthase occurs when Al enters the symplast.

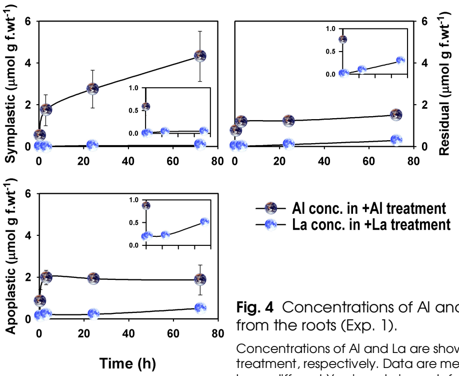


Fig. 4 Concentrations of Al and La in Each fraction extracted from the roots (Exp. 1).

Concentrations of Al and La are shown only in the +Al treatment and +La treatment, respectively. Data are means of four replicates ± SE. The inset has a different Y-axis scale in each fraction.

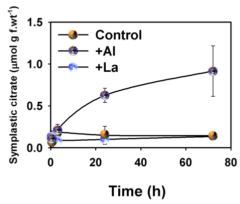


Fig. 5 Concentrations of citrate in the symplastic fraction extracted from the roots (Exp. 1).

Data are means of four replicates ± SE.

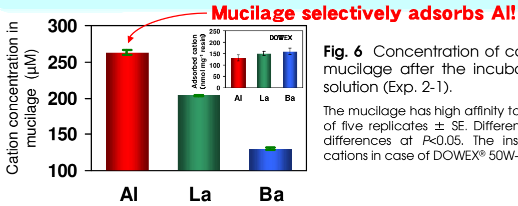


Fig. 6 Concentration of cations adsorbed to the mucilage after the incubation in the cation mix solution (Exp. 2-1).

The mucilage has high affinity to adsorb Al. Data are means of five replicates ± SE. Different letters indicate significant differences at $P<0.05$. The inset indicates adsorption of cations in case of DOWEX® 50W-X8 ($-SO_3^-$).

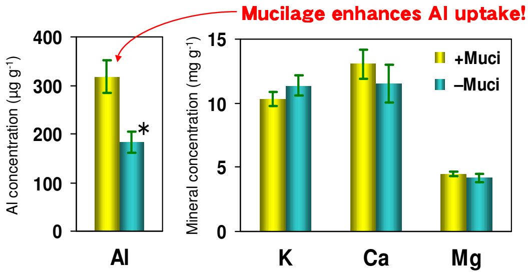


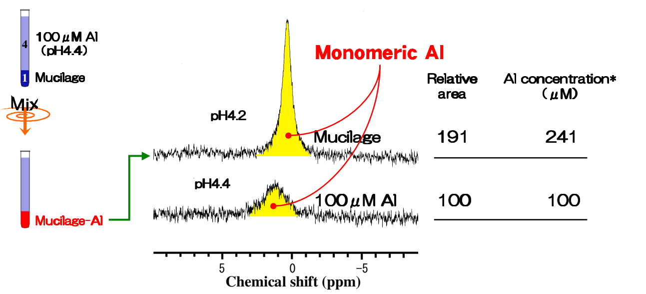
Fig. 7 Concentrations of Al, K, Ca and Mg in shoots of *M. malabathricum* grown in a nutrient solution with Al (Exp. 2-2).

In –Muci treatment, the mucilage was removed from the roots throughout the Al exposure (10 days). Data are means of three replicates ± SE. *, significantly different from +Muci treatment at $P<0.05$.

Experiment 2: Role of the root mucilage in aluminum uptake in M. malabathricum

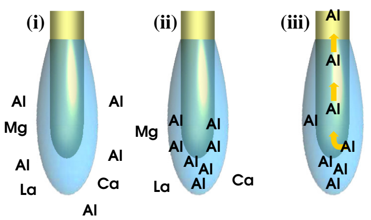
The mucilage exuded from *M. malabathricum* roots adsorbed more Al than Ba (**Fig. 6**). This can be explained by the general phenomenon that the amount of the cation absorbed by the cation exchangers increases with the number of charges on the cation. Interestingly, the mucilage adsorbed more Al than La (**Fig. 6**). As both Al and La are trivalent cations, it was suggested that the cation exchange sites in the mucilage have a specific configuration for absorption of Al. In order to elucidate the contribution of the mucilage on Al uptake in *M. malabathricum*, the effect of the removal of mucilage from roots on Al uptake was examined. Whereas K, Ca and Mg uptakes were not affected, Al uptake significantly decreased by the mucilage removal (**Fig. 7**).

The ^{27}Al NMR analysis showed that the most of concentrated Al in the mucilage is monomeric Al, whereas the ratio of $Al(OH)^{2+}$ (and/or $Al(OH)_2^+$) in the mucilage is lower than that in the external solution, depending on the difference in pH (**Fig. 8**). This result indicates that Al binds very weakly to the mucilage and can be absorbed easily by the roots. Taken together, Al is concentrated in the mucilage as the monomeric form, which contributes to higher Al uptake rate of *M. malabathricum* roots.



	Relative area	Al concentration* (μM)
Mucilage	191	241
100 μM Al	100	100

Fig. 8 ^{27}Al NMR spectra of Al in the mucilage (above) and 100 μM Al solution (below) (Exp.2-3).
$AlCl_3$ solution (0.1 mM, in 0.1 M HCl) was used as an external reference for calibration of chemical shift (0 ppm).
*The mucilage used in NMR analysis was digested, and Al concentration in the mucilage was determined.
Relative peak area in the spectra was calculated (100 μM Al = 100) and compared with actual Al concentration.



Conclusion

(i) The mucilage exudation from roots is induced under low Al conditions.

(ii) Trace Al in the medium is selectively concentrated in the mucilage as a monomer.

(iii) Concentrated Al in the mucilage is absorbed by roots.

The reason why *M. malabathricum* actively absorbs Al should be elucidated in future.

References

Horst, W.J., Wagner, A., and Marschner, H. 1982. Z. Pflanzenphysiol. **109**: 95-103.

Tice, K.R., Parker, D.R., and DeMason, D.A. 1992. Plant Physiol. **100**: 309-318.

Watanabe, T., Osaki, M., Yoshihara, T., and Tadano, T. 1998. Plant Soil **201**: 165-173.

Watanabe, T., and Osaki, M. 2001. Plant Soil **237**: 63-70.