Title
Aluminum-detoxifying compounds in roots of Eucalyptus camaldulensis

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Introduction

In acid soils, aluminum (Al) toxicity is one of the major factors limiting plant growth. Al accumulated in root tips inhibits root elongation, which is the most notable symptom of Al toxicity in plants (Kochian et al. 2004). *Eucalyptus camaldulensis* is a Myrtaceae tree species that has a high resistance to Al (Tahara et al. 2005). Its root elongation and plant growth were not inhibited when it was grown hydroponically in a nutrient solution containing 1 mM Al. By contrast, root elongation of many herbaceous plants is suppressed at 1 to 50 μM Al. To date, Al exclusion from roots by secretion of Al-binding organic acids such as malate, citrate and oxalate has been considered to play a central role in Al resistance mechanisms. However, the high Al resistance of *E. camaldulensis* cannot be explained by the secretion of these organic acids (Tahara et al. 2008a). In this study, we tried to isolate new Al-binding compounds from the roots of *E. camaldulensis*.

Materials and methods

Seeds of *E. camaldulensis* were germinated on acid-washed and sterilized sand in a growth chamber (16 h light/8 h dark; 28/25°C; 200 μmol m^{-2} s^{-1} PPFD). Seedlings were watered daily with a nutrient solution (pH 4.0, Tahara et al. 2008b) and grown for 3 to 4 months. After seedlings were cultured hydroponically for 5 days, roots of seedlings were treated with 0.35 mM $CaCl_2$ solution (pH 4.0) containing 0 or 1 mM $AlCl_3$ for 24 h. The 5 mm apical portions of roots were excised, freeze-thawed, and centrifuged on an ultrafilter (10,000 nominal molecular weight limit) to obtain soluble compounds from root-tip cells.

Al-binding compounds which were soluble in the form of complex with Al were separated by gel-filtration chromatography. The cellular extract added with Al was subjected to a gel-filtration column (Sephadex G-25, GE Healthcare) to separate the complexes of Al-binding compounds with Al from free Al. The Al concentration of each fraction was determined with an inductively coupled plasma atomic emission spectrometer (Optima 4300DV, Perkin Elmer). In addition, Al-binding compounds which became insoluble in the form of complex with Al were separated by HPLC (LC-VP series, Shimadzu) equipped with a reversed-phase column. The cellular extract added with or without Al was subjected to HPLC analysis. Chromatographic peaks whose area was decreased by the addition of Al were judged to be those of Al-binding compound.

Results and discussion

When Al was added to the cellular extract from roots cultivated without Al and the extract was subjected to the gel-filtration column, one peak was observed by monitoring the Al concentration in each fraction. Two peaks were found when the roots were treated with 1 mM Al. These results

indicate that roots of *E. camaldulensis* contain Al-binding compounds which are soluble in the form of complex with Al, and the compounds increase with Al treatment. We also measured organic acids in each fraction, and found that the peak of citrate corresponded to that of the Al-complex. The increase of Al-binding compound can be explained partly by the increase of citrate.

We also found an Al-binding compound which was soluble without the presence of Al but became insoluble in the form of complex with Al. This compound is unlikely to be a well-known Al-binding compound such as malate, citrate and oxalate since these organic acids can be soluble in the form of Al-complex. We have developed a method for separating and purifying the compound by HPLC. Pyrocatechol violet assay (Menzies et al. 1992) confirmed that the compound had an ability to bind Al. The content of the compound in root tips of *E. camaldulensis* was compared with those of two Al-sensitive tree species, *Melaleuca bracteata* and *Populus nigra*. The content in *P. nigra* was much smaller than that in *E. camaldulensis* and the compound was not detected in *M. bracteata*.

Thus, *E. camaldulensis* contains at least two types of Al-binding compound; one remains soluble and the other becomes insoluble in the form of complex with Al. These Al-binding compounds might contribute to the high Al resistance of *E. camaldulensis* through internal Al detoxification.

References

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