Introduction

Afforestation provides a mean for Phytoremediation of acid sulfate soils. However, little is known about what tree species can grow on acid sulfate soils. In acid sulfate soils, low pH and excess aluminum are the primary factors that limit plant growth. Thus, understanding of the response of planting trees to these stresses hold a key to the success in afforestation on acid sulfate soils. *Melaleuca cajuputi* Powell (Myrtaceae) is one of the rare trees known to be able to grow on acid sulfate soils (Nakabayashi et al. 2001) with tolerance to excess aluminum (Tahara et al. 2005). *M. cajuputi* is a tree species that has an extremely high resistance to aluminum (Osaki et al. 1997, Nguyen et al. 2003, Tahara et al. 2008). Its root elongation and plant growth were not inhibited when it was grown hydroponically in a nutrient solution containing 2.5mM aluminum (Tahara et al. 2008). To date, the formation of complexes with ligands (e.g., malate, citrate and oxalate) has been considered to play a central role in aluminum resistance mechanisms (Ma et al. 2001). However, our previous study suggested that the high aluminum resistance of *M. cajuputi* cannot be explained by the roles of ligands alone (Tahara et al. 2008). In this study, to elucidate the mechanism of the high resistance to aluminum of *M. cajuputi*, we investigated aluminum accumulation in root with focusing on its localization and physiological responses to Al in the root apex of *M.cajuputi* and an aluminum-sensitive species *Melaleuca bracteata* F. Muell.

Materials and Methods

(1)Plant materials and Al treatment

M. cajuputi Powell and *M. bracteata* F. Muell were germinated on sand in a growth chamber (16h light/ 8 h dark; 30/26 ℃). Seedlings were watered daily with nutrient solution (Tahara et al. 2005). Seedlings one month old were transferred into 6 L plastic pots containing the aerated nutrient described above. They were then cultured hydroponically for one month in the growth chamber before the experiments. Seedlings were treated with culture solution (pH 4.5) containing 0, 0.1, 0.5, 1.0, and 2.0mM $AlCl_3$ for 21 days. After the treatment, the seedlings were washed with deionized water and were excised and used for analyses as described below. The each plant was transplanted to agar plates (pH5.5) that contained BCP, and the change in the pH of the rhizosphere was examined.

(2) Experiments of physiological properties

The influence of aluminum was examined by the following dyeings by using the root of 20 mm. Examination of physiologic response of root by hematoxylin and aniline blue dyeings. In addition the disability was examined by aluminum in the root cell.

Results and Discussion

As for *M.cajuputi,* the growth by the Al concentration was not inhibited. However, the root growth of *M.bracteata*, by the aluminum treatment was inhibited remarkable, and 83.2% inhibited the maximum more than control (Fig.1). The pH of rhizosphere of both plants has decreased by the BCP plate. However, the pH decrease has decreased as for *M. bracteata* with high concentration of aluminum, but *M.cajuputi* is not changed (Fig.2).

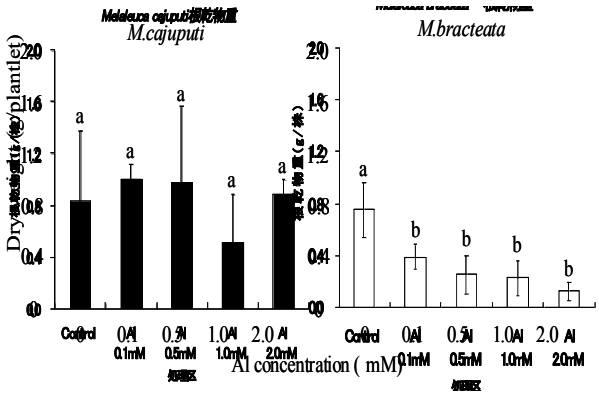

Fig.1 Effect of growth on the Al concentration.

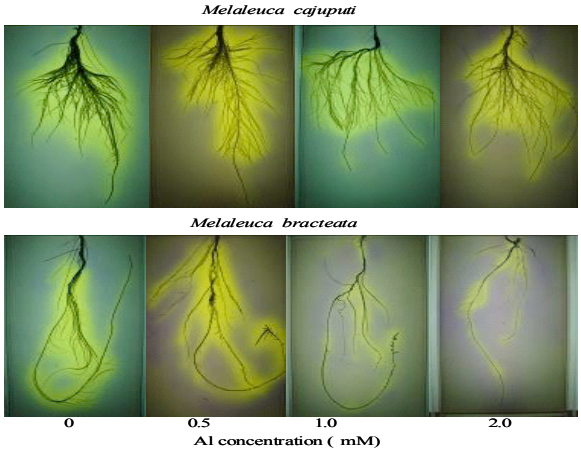

Fig2. Change of rhizosphere pH under Al treated

On the other hand, we investigated aluminum accumulation in root with focusing on its localization and physiological responses to aluminum in the root apex of *M.cajuputi* and an aluminum-sensitive species *M. bracteata*. Then, staining with hematoxylin revealed that aluminum was located in the root apex of aluminum-tolerant *M. cajuputi* and aluminum -sensitive *M. bracteata* (Fig.3,4). The extent of staining in *M. cajuputi* differed from that *M. bracteata* root. In *M. bracteata* roots, the staining intensity was high in the root cap and apical 5 to 20 mm after expose to various concentration of aluminum for 21 days. At the result, it was able to be confirmed of aluminum accumulated in the root *M.bracteata* more than *M.cajuputi*. As for *M.bracteata*, aluminum was absorbed in the inner-cell of the root tissue as shown in Fig.4. On the other hand, *M.cajuputi* had aluminum only in the surface of the root.

Fig.3 Hematoxylin – stained roots of *M.cajuputi* (*Mc*) and *M.bracteata* (*Mb*).

To investigate the accumulation of callose, we stained root for callose with aniline blue (Fig.5). In roots of the Al-sensitive *M. bracteata* treated with 0.1 – 2.0mM aluminum, the portion about 20mm from the root apex was strongly

stained, whereas no staining was detected in aluminum–tolerant *M. cajuputi* roots.

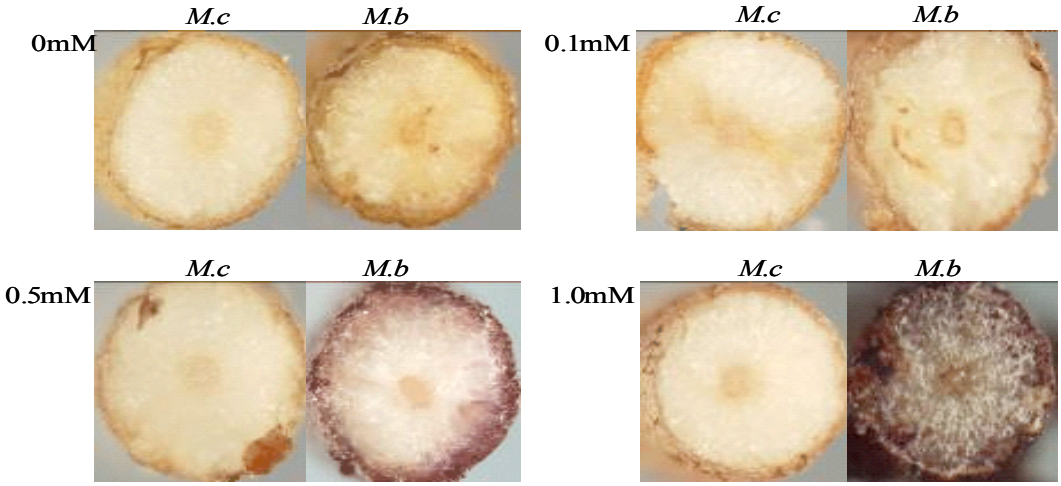

Fig.4 Hematoxylin – stained section of roots of *M.cajuputi* (*Mc*) and *M.bracteata* (*Mb*).

We concluded that *M.cajuputi* was an aluminum tolerance because the mechanism that aluminum doesn't invade the internal tissue of the root was possessed. And, it is thought that *M.bracteata* is aluminum receptivity because aluminum can invade the internal tissue of the root.

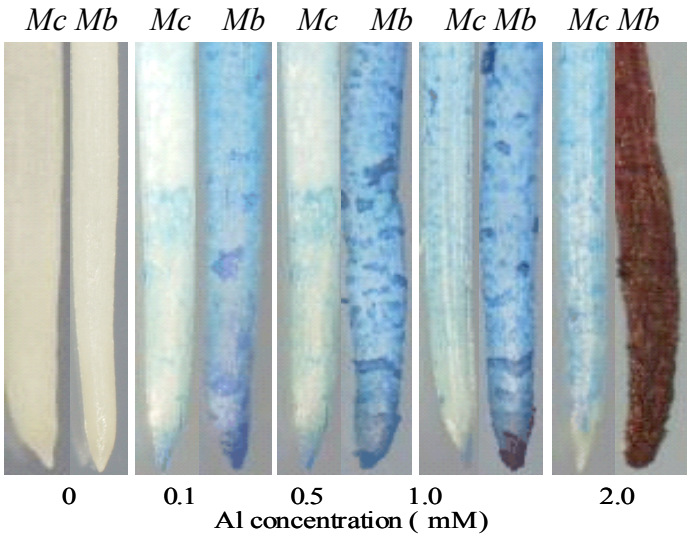

Fig.5 The roots of *M.cajuputi* (*Mc*) and *M.bracteata* (*Mb*) after staining with aniline blue to indicate callose.

References

Nakabayashi K et al. Effect of Embankment on Growth and Mineral Uptake of Melaleuca cajuputi Powell under Acid Sulphate Soil Conditions.Soil Sci. Plant Nutr. **47**, 711-725, 2001

Tahara K, Norisada M, Hogetsu T, Kojima K., Alminum tolerance and aliminum-induced deposition of callose and lignin in the root tips of *Melaleuca* and *Eucalyptus* species. J. For.

Res. **10**, 325-333, 2005

Osaki M, Watanabe T, Tadano T Beneficial effects of aluminum on growth of plants adapted to low pH soils. Soil Sci. Plant Nutr. **43**, 551-563, 1997

Nguyen NT, Nakabayashi K, Thompson J, Fujita K. Role of exudation of organic acids and phosphate in aluminum tolerance of four tropical woody species. Tree Physiol. **23**, 1041-1050 2003

Tahara K, Norisada M, Yamanoshita T, Kojima K., Role of aluminum-binding ligands in aluminum resistance of *Eucalyptus camaldulensis* and *Melaleuca cajuputi*. Plant Soil, **302**, 175-187, 2008