Title

The Protective Role of a Unique Tissue Detachment on Root Cap Cells in a Strong Aluminum-Resistant Tree *Acacia mangium*

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Introduction

The aluminum (Al) ion in acid soils is a major factor that inhibits root elongation at the root apex. Root cap cells play important roles in the protection of root apex against soil stresses such as soil compaction and pathogen attacks. Root cap cells, although they have strong binding capacity with Al, play minor roles in the amelioration of Al-caused root elongation inhibition (Ryan et al. 1993; Li et al. 2000).

Acacia mangium Willd. is a highly Al-tolerant leguminous tree, in spite of the fact that only a small amount of citrate is released against Al (Osawa and Kojima, 2006). We have recently noticed that the root apex of this species is surrounded by a large, cap-like structure of sloughed-off tissues. To understand its role in high Al tolerance mechanisms, we examined the effects of the cap-like structure in the Al-resistant root elongation in *A. mangium*.

Results and discussion

We show a representative *A. mangium* root under hydroponic condition in Figure 1a. A cap-like structure, which was apparently consisted of the plant tissues, covered the entire root apex up to the 5 mm region behind the tip. After the structure was readily sloughed off (Fig. 1b), the root was covered again with a new one of similar size in a week after the detachment (data not shown).

To evaluate roles of the structure on root growth, the whole structure and resulting tissues on root surface were carefully removed with forceps, and then the roots were exposed to 0.5 mM calcium solution containing 0, 100, or 500 μM Al (pH 4.5) for 48 h. Al induced a root bending in both roots immediately after the exposure, and the proportion in the occurrence of root bending was higher in Al-treated roots without the cap-like structure (Fig. 2 a, b). The addition of Al slightly reduced the elongation of roots both with and without a cap-like structure, and the degree in the reduction was larger in structure-less roots (Fig. 2c). In the absence of Al, the removal of a cap-like structure hardly decreased the root elongation, although it slightly increased the occurrence of root bending (Fig. 2c). In roots without cap-like structures, this facilitation of root bending may result in a sight decrease in the Al-resistant root elongation.

The present study revealed that the presence of a cap-like structure around root apices in *A. mangium* ameliorates an Al-facilitated bending of root growth direction. Root caps play major roles in root gravitropism through the localization of amyloplast granules and/or the basipetal flow of auxin (Swarup et al. 2005). Our further analysis of the cap-like structure identified that a major attached position of a structure to the root was confined to columella root cap region, and the rigidity between this connection was enhanced during prolonged Al exposure periods (data not shown). The undetached state of a cap-like structure may have a role in protecting inner root cap cell function from instant Al-caused damages. An apparent root bending induced by Al seems to require high concentrations, since no similar root bending in Al-sensitive plants has been reported. It is likely that a much lower concentration of Al may be sufficient for the complete arrest of root elongation until root cap cell dysfunction become recognizable as a root bending.

The present study did not find any detrimental role of a floating, cap-like structure around the root in Al-resistant root elongation, similar to previous findings on root caps (Ryan et al 1993; Li et al. 2000). In the present study, however, we did not evaluate potential roles of undetached tissues on Al tolerance mechanisms. A cycle of detachment and formation of cap-like structures imply that non-detached original tissues in the root surface area of root apex may play roles in the Al-resistant root elongation in *A. mangium*. Another study of *A. mangium*

revealed that more than half of fibrillary tissue initials separated from the root surface were present beyond the entire root elongation region (Endo et al., unpublished results).

In summary, we have found a novel cap-like structure of tissues for the protection of root cap cells at the root apex of *A. mangium*. Further characterization of the detachment pattern of the tissues from the root surface should provide better understanding of their roles in high Al tolerance mechanisms.

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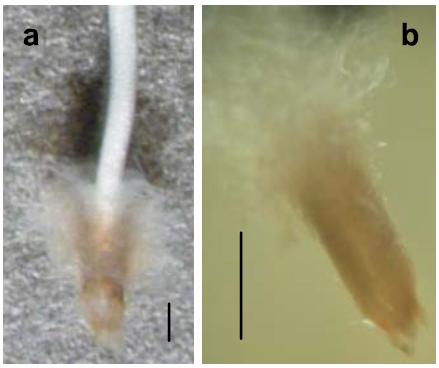

Figure 1. A cap-like structure attached to (a) and sloughed off (b) the root apex of *A. mangium*. Seedlings were grown under 1/5 strength Hoagland's solution (pH 4.5). A cap-like structure was removed off the root with forceps. Scale bar = 1 mm.

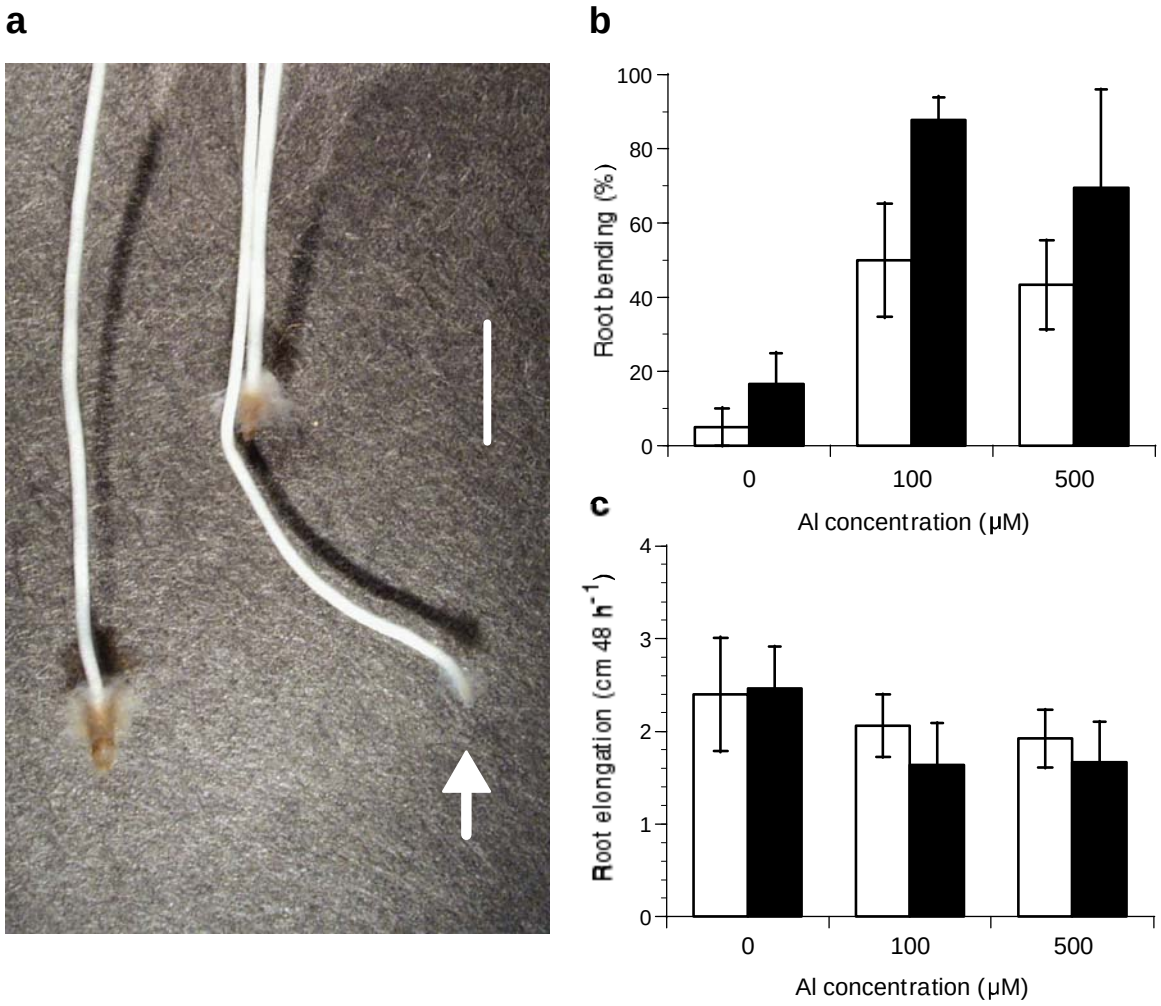

Figure 2. Roles of a cap-like structure on Al-tolerance root elongation in *A. mangium*.
a) A representative root bending after the exposure to 500 μM Al for 48 h. The root without a cap-like structure was indicated with the arrow. The scale bar represents 5 mm. b) The frequency of an inflective bending in the root with (white bar) or without (black bar) a root cap-like structure. Having carefully removed the halves of the root cap structure in the roots with forceps, the root length before and after the treatment was measured with exposure to 0.5 mM calcium chloride solution containing 0, 100 or 500 μM aluminum chloride (pH 4.5) for 48 h. The occurrence of an inflective bending was counted in the root that had an inflection point whose interior angle is less than 160°. Data were given as averages from three independent experiments with standard errors. Each experiment had at least five replicates. c) Effects of Al on the elongation of roots with (white bar) or without (black bar) a cap-like structure. Each value given as the mean and the vertical lines on the bars indicates standard deviation (n=16 to 23). Data were given from three independent experiments.