

UC Davis

**The Proceedings of the International Plant Nutrition Colloquium
XVI**

Title

Long-term and Highly Aluminum-resistant Root Elongation in a Camphor Tree *Cinnamomum camphora*

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Publication Date

2009-07-04

Peer reviewed

Introduction

A long-lasting tolerance to aluminum ion (Al) is an essential phenotype for perennial plants growing on strong acid soils for longer periods. There is increasing evidence that plants with superior Al tolerances are relatively easily found in woody plants, such as tropical plantation trees *Melaleuca cajuputi* and *Paraserianthes falcataria* (Tahara et al., 2005; Osawa and Kojima, 2006). Some woody plants, including tea, hydrangea, and *Melastoma malabathricum*, are also known as Al-accumulators that retain large amounts of Al in their aboveground organs (Matsumoto et al., 1976; Ma et al., 1997a; Osaki and Watanabe, 1997). However, the mechanisms responsible for high Al tolerance or high Al accumulation in woody plants remain to be elucidated.

Our preliminary screening has successfully identified that root elongation in seedlings of *Cinnamomum camphora*, an evergreen tree widely distributed or planted in China and its neighboring regions, is much less inhibited even at high Al concentrations in a simple ionic solution at least for several days. Our finding is consistent with a study that reported no growth reduction in *C. camphora* seedlings against Al in a nutrient solution for 5 weeks (Oda et al., 2002).

As a first step in understanding long-term Al tolerance mechanisms in seedlings of *C. camphora*, we employed a pulse Al exposure every two days for 60 days in measurements of root elongation and Al accumulation in each organ. To understand Al transport mechanisms in shoots, we also examined the Al accumulation patterns in branch cuttings of *C. camphora*.

Results and discussion

Al-resistant root elongation in C. camphora

To examine long-term high Al tolerance in root elongation of *C. camphora*, the seedlings were exposed every two days to 0.5-mM calcium chloride solution containing 0, 50, or 500 μM Al (pH 4.5) up to a total of 60 days. Furthermore, to see whether beneficial effects of Al are involved in the root elongation of *C. camphora*, we also applied 1/5 Hoagland's solution (reduced Pi: 100 μM) containing 500 μM Al to the seedlings every two days.

During pulse exposure of treatment solution, the root elongation at 500 μM Al in the calcium solution was slightly reduced to 70% of the control at the end of 60 days treatment (Fig. 1).

Figure 1

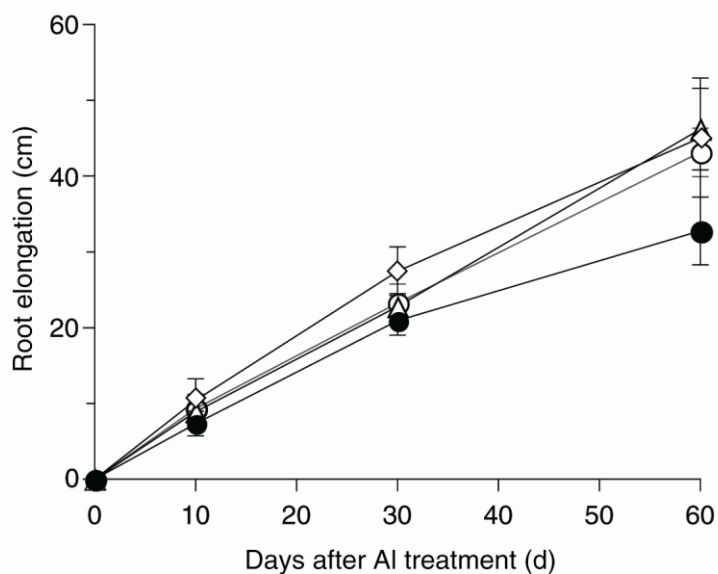


Figure 1: Long-term root elongation of *Cinnamomum camphora*.

Seedlings of four-months-old were intermittently exposed to 0.5-mM calcium chloride solution containing 0 (open circle), 50 (open triangle) or 500 (closed circle) μM Al (pH 4.5) every 2 days, followed by 1/5 Hoagland's solution for the next 2 days until the cycles is completed within 60 days. To evaluate Al in high-ionic condition, seedlings were exposed to 1/5 Hoagland's solution (100 μM Pi) containing 500 μM Al (open diamond) every two days. The length of the longest root at 0, 10, 30, and 60 days after Al treatment were measured with a ruler. Data indicate the mean and standard deviation ($n=3$).

We further checked the efficacy of the Al used in this study against root elongation of an Al-resistant wheat cultivar. After 48-h exposure to Al in the calcium solution, the root elongation of the Al-resistant wheat cv Atlas was 30% of the control at 50 μM Al, and 10% at 500 μM Al, respectively (data not shown). Compared to the control, neither the inhibition nor increase of root elongation in *C. camphora* was observed, both at 50 μM Al in the calcium solution and at 500 μM Al in 1/5 Hoagland's solution (Fig. 1). These results suggest that neither low Al levels nor the co-application of Al with nutrients facilitate the root elongation in *C. camphora*.

Al accumulation pattern in C. camphora

To understand whether the high Al tolerance in *C. camphora* is accompanied by high levels of Al accumulation or not, we examined changes in the Al contents in each organ of seedlings exposed to 500 μM Al in the calcium solution every 2 days for a total of 60-day period. Al contents in all organs, except for root apices at the day 60, increased with time after Al exposures. However, none of them, including root apices, exceeded over 1000 $\mu\text{g g}^{-1}$ DW, an indicative value for an Al accumulator plant (Fig. 2).

Figure 2

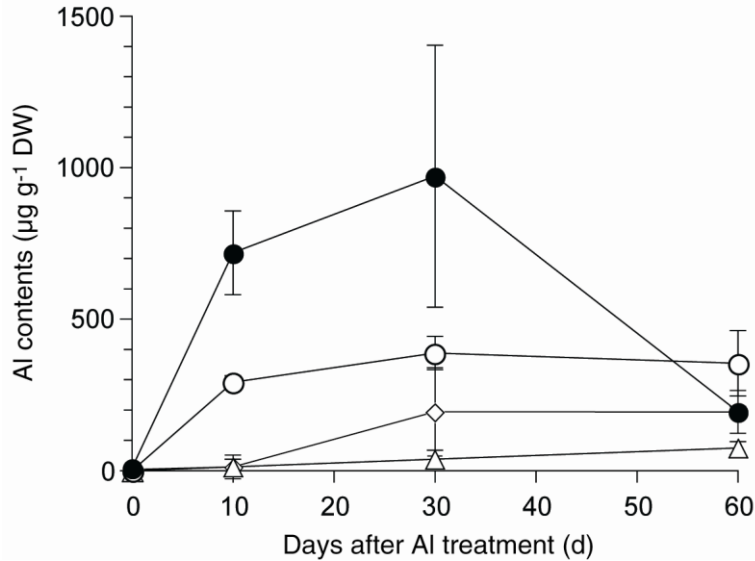


Figure 2: Al accumulation pattern in each organs of *C. camphora*

For Al quantification, lower leaves (open diamond), stems (open triangle), main roots (open circle), and root apex (5 mm length; closed circle) of each seedling exposed to the calcium solution containing 500 μM Al were sampled at 0, 10, 30, and 60 days after the start of pulse treatment every two days. Each 100 mg of dried samples was ashed with 60% (v/v) nitric acid and 30% (v/v) hydrogen peroxide. Al contents in the diluted solution were determined with graphite furnace atomic absorption spectrometry (Perkin Elmer, Germany). Data indicate the mean and standard deviation ($n=3$).

At day 60, the Al contents of root apices was significantly lower than those at day 10 and day 30, and equivalent to those in other organs (Fig. 2). An Al-off period of two-days after the last Al exposure (day 58) appears to be a major factor in the rapid decrease in Al accumulation in the root apex. One candidate for the rapid removal of Al in root apices could be the shift of Al-accumulating cells to the non-tip basal root region along with continuous root elongation. Furthermore, our organic anion analysis indicates that this Al exclusion mechanism from roots of *C. camphora* may be mediated only partly by the Al-inducible organic anions release, which is fewer than those in representative annual crops (Osawa et al., unpublished results).

Al translocation in branchlets

In Al accumulator plants such as tea, buckwheat, and *Melastoma malabathricum*, Al accumulation in shoots is accompanied by the formation of Al-organic anion complexes in roots (Ma et al., 1997b; Watanabe et al., 2005; Morita et al., 2008). As a first step to understanding the regulatory mechanisms of Al accumulation in woody plants, we examined the transport capacity of Al in branch cuttings of *C. camphora* under different concentrations of Al in calcium solution for 30 days. The amount of Al in the leaves of cuttings was increased according to Al concentrations in the media, although it was still below 1000 $\mu\text{g g}^{-1}$ DW at 500 μM Al. (Table 1).

Table 1: Al accumulation in the leaves of *C. camphora* branch cuttings.

Branch cuttings from six-months-old seedlings were exposed to 0.5-mM calcium chloride solution with 0, 50, 200, or 500 μM Al (pH 4.5) for 30 days. Each leaf attached between 6 to 8 cm from the cutting edge were separated and sampled for the Al quantification. To investigate the effects of chelatable anions on Al transport, branches were exposed to the calcium chloride solution containing 500 μM Al with an equivalent amount of citrate or phosphate for 30 days. Data indicate the mean with standard deviation ($n=3$).

Treatments		Al contents in leaves ($\mu\text{g g}^{-1}$ DW)
Al (μM Al)	chelator	
0	–	8 \pm 12
50	–	105 \pm 15
200	–	446 \pm 65
500	–	493 \pm 79
500	+ citrate	1280 \pm 450
500	+ phosphate	99 \pm 77

To understand the effects of Al-chelators on Al translocation in shoots, branch cuttings of *C. camphora* were exposed to 500 μM Al in the calcium solution containing either 500 μM citrate or 500 μM phosphate. The co-application of Al with citrate for 30 days increased Al accumulation in leaves as compared to the control (Table 1). In contrast, co-application of Al with phosphate resulted in less Al accumulation in leaves, presumably due to the formation of insoluble Al-phosphate in the treatment solution. These results suggest that apart from roots, Al forms in xylem transport of the shoots in a non-Al accumulative woody plant may be a factor for determining Al accumulation in leaves.

In summary, we have identified *C. camphora* as a tree with long-term, highly effective Al tolerance in its root elongation. Both the synergistic effects of less Al accumulation and the continued apical growth in the root apex could contribute to maintaining root elongation in the presence of high Al concentration. These relationships, although we could not ascertain which factor is more detrimental, will be a key to better understand high Al tolerance mechanisms – possibly other than organic anion release – in woody plants.

Acknowledgment

We thank Hiroyuki Ikeda, Takayuki Sasaki, and Yoko Yamamoto for providing plant seeds. This work was supported in Grant-in-Aid for Young Scientists (No. 18780111 to HO).

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