Aluminum sensitivity and optimum Ca and pH requirement of teak (*Tectona grandis* Linn. f.) clones used for forestry plantations in Australia

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Abstract
Teak is adapted to grow in tropical and subtropical regions and is considered to require fertile soils. Despite this, teak is often planted in soils that are highly leached, acidic and potentially Al-toxic. In Australia, several clones are commonly used for teak plantations and the aim of this study was to determine the optimum pH, Ca requirement and Al sensitivity of these clones. Biomass increase of nutrient solution-grown teak plants was used to determine the optimum pH and Ca. There were no significant differences in optimum pH and Ca between clones and, overall, the greatest growth was obtained with nutrient solutions containing 1 mM Ca and adjusted to pH 6. Teak plants still grew at pH 4, but developed foliar Ca deficiency symptoms at 0.1 mM Ca. The Al sensitivity was determined by staining excised roots with chromeazurol S, eriochrome R and hematoxylin. Roots of plants exposed to 50 and 300 μM Al for 1-7 days gave a strong reaction with hematoxylin, and chromeazurol S, but a weak response to eriochrome cyanine R. The greatest resistance to low pH and Al was observed in the two clones E and H.

Keywords
Al toxicity, Ca deficiency, clonal differences, root morphology, roots, staining.

Introduction
Teak is a valuable timber species due to its decay-resistance and colour of its heartwood timber. Teak is a tropical to subtropical tree originating in South and Southeast Asia, and it is adapted to summer rainfall areas with strong seasonality in rainfall (Tanaka *et al*. 1998). Teak occurs naturally on fertile soils derived from limestone, basalt and alluvium (Tanaka *et al*. 1998) and reflects the high nutrient requirement of teak, especially with regard to Ca saturation of the soil (Craven *et al*. 2007; Drechsel and Zech 1991; 1994). Due to its value, teak plantations have been established in a number of countries, including Australia. Soils used for teak plantations in Australia range from Ferrosols to Vertosols to Hydrosols. As a result of the high rainfall (2000-3000 mm per year) in the plantation regions, the soils tend to be acidic (pH measured in $CaCl_2$ <4), potentially Al-toxic, and low in fertility. However, teak has a high requirement for N, P and Ca with pH_{CaCl2} > 4.7 (Craven *et al*. 2007; Drechsel and Zech 1991; 1994), therefore, the sites need amelioration with lime and fertiliser ensure adequate establishment and growth of the planted trees. Most teak currently planted in Australia is of clonal origin, comprising circa twenty clones, and it is unknown if some of these clones are more suitable to the acidic, Ca deficient and Al-toxic soils.

The aim of this study was to determine: 1) the optimum Ca and pH requirement of teak clones and 2) the sensitivity of the teak clones to toxic Al concentrations. The studies were conducted in solutions culture and aimed to inform decisions as to which clones are most suitable for a range of soils used for teak plantations in Australia.

Materials and Methods
Tissue-culture propagated bare-rooted plantlets of twenty clones were obtained from Thai Orchid Labs (Bangkok, Thailand). These twenty clones are widely used in Australia, but are also supplied to teak plantations elsewhere in the world. The plantlets were gradually hardened-off in a controlled temperature glasshouse (28°C day, 23°C night) under 70% shadecloth until plants showed signs of resuming growth after which the plantlets were transferred to higher temperature (33°C day, 28°C night) under ambient light. Eventually, 14 clones (subsequently labelled A-O) were available in sufficient numbers for experimental work.

Plants were grown in aerated nutrient solution containing the following macronutrients (in mM): NH_4^+ (2.1), NO_3^- (3.9), PO_4^{3-} (0.13), SO_4^{2-} (1.17), K^+ (2.0), and Mg^{2+} (0.15). The micronutrients (in μM) consisted of Zn

(4.3), B (14.3), Co (0.01), Cu (0.03), Mn (14.3), Mo (0.15) and Fe (14.3). The nutrient solution also contained 1 mM MES buffer to minimise pH fluctuations between pH adjustments.
In experiment 1, twelve treatments were applied, consisting of three Ca concentrations (0.1 mM, 1.0 mM and 10 mM Ca, as chloride salt) and four pH values (pH 4.0, 5.0, 6.0 and 7.0). The pH of the complete nutrient solutions was adjusted with HCl or NaOH every second day and the nutrient solutions were replaced weekly. Between 5 and 10 plants per clone were grown in the solutions and the fresh weight of each individual plant recorded at the beginning and end of the experiment. The relative growth rate of the top 50% performing plants was calculated from the log-transformed weights (Hoffmann and Poorter 2002). This approach excludes plants with a poorly developed root system which may result in lower growth rates for individual plants despite all plants being clonal of origin.

In experiment 2, plants were grown in minimal salt solution consisting of 1 mM $CaCl_2$ and 5 µM H_3BO_3 . The aerated solution was adjusted to pH 4.0 ± 0.1 with HCl or NaOH. Thereafter, the three Al treatments (0, 45 µM, and 265 µM, as chloride salt) were added and plants transferred to the minimal salt solutions. The solutions (40 l per treatment) were replaced every second day, and the pH and Al concentration of the solutions measured on the fresh and spent solutions.

Roots of each clone and treatment were collected at day 1, day 3 and day 7, and soaked in deionised water for 24-48 hours at 4°C to desorb weakly bound Al. Roots were then rinsed again and divided into three batches. The batches were stained for 48 h at 4°C with eriochrome cyanine R, chromeazurol S and hematoxylin, respectively, and destained for 2 days with deionised water at 4°C. Roots were evaluated for the extent (apical 0-2 mm or 0-10 mm) and severity (none, weak or intense) of staining and possible root abnormalities (e.g. kinks or ruptures) under 40x magnification with a microscope in both light and dark-field illumination.

Results and Discussion
In experiment 1, the optimum Ca concentration was determined to be 1 mM which resulted in mean relative root growth rates of 0.02 g/g/d at pH 4 and pH 5, to 0.034 g/g/d at pH 6. The growth rates at 0.1 mM and 10 mM Ca were similar ranging from 0.013 g/g/d at pH 4, to 0.025 g/g/d at pH 6 (Figure 1a).

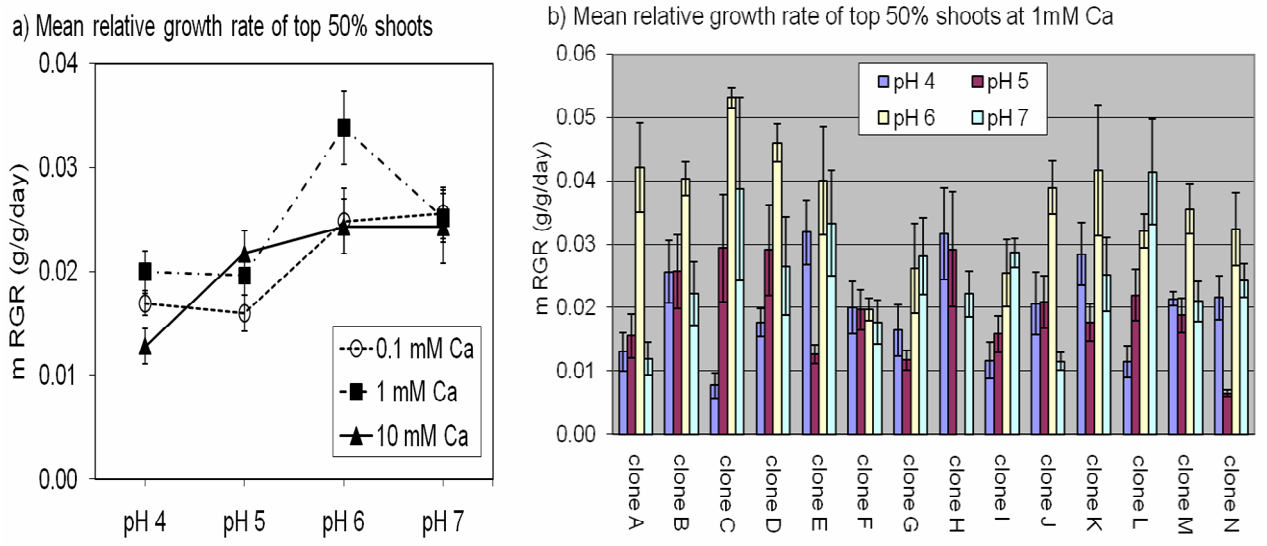

Figure 1. a) Mean relative shoot growth rate of teak clones at four pH values and three Ca concentrations in balanced nutrient solutions. B) Effect of pH on the mean relative shoot growth rate of teak clones at 1 mM Ca.

The optimum pH was pH 6, but no effect of either lower or higher pH on leaf and root health could be observed. Therefore, teak would be able to tolerate acidic soils, although it would grow better in near neutral soils. At 1 mM Ca, the clones with the greatest ability to tolerate low pH based on growth rates at pH 4 were clones B, E, H and K (Figure 1b). At 0.1 mM Ca, the leaves developed interveinal chlorosis which progressed to necrosis (Figure 2). Although there were clonal differences in response to pH and Ca, the differences were not statistically significant as exemplified for the 1 mM Ca treatment (Figure 1b).

The pH of the complete nutrient solutions dropped to below pH 4 within 2 days, despite the nutrient solution containing twice as much nitrate than ammonium nitrogen. Addition of 1 mM MES buffer did not maintain the pH constant for more than 1 day but limited the change in pH, compared to unbuffered solutions (data not shown).

Figure 2. Visual Ca deficiency symptoms on teak leaves grown in nutrient solution containing 0.1 mM Ca.

For experiment 2, a minimal solution adjusted to pH 4 was chosen to avoid hydrolysis of Al or interaction between added Al and nutrients such as sulphate or phosphate in the solution. Also, by avoiding pH adjustment and by replacing the solution every second day, development of highly rhizotoxic polymeric Al_{13} either due to pH adjustment or due to root-respiration mediated acidification of the apoplast (Kopittke *et al*. 2004) was avoided. In experiment 2, no growth rates were determined because the effect of Al toxicity on plant growth is only discernable in soil systems where water and nutrients become limited due to Al toxicity.

The solution Al concentration dropped within 1 day of adding plants, but upon replacement of the Al solutions with fresh solution, the decrease in solution Al was less pronounced (Figure 3a). The plant roots removed Al from the bulk solution either due to uptake or adsorption onto the root biomass. Since there was little difference in the amount of Al removed from the 45 and 265 µM Al solutions (Figure 3a), adsorption onto root biomass seems a more likely explanation than uptake. The adsorption of Al by roots resulted in a release of Ca and K ions from the roots for up to 7 d (data not shown), whereas release of phosphate was higher at day 1 but dropped back to control values thereafter (Figure 3b). This suggests that the release of Ca and K may be due to displacement of cations from the apoplast (esp. for Ca) but also due to membrane leakage (for K and P); both processes have been implicated in expression of Al toxicity in plant roots.

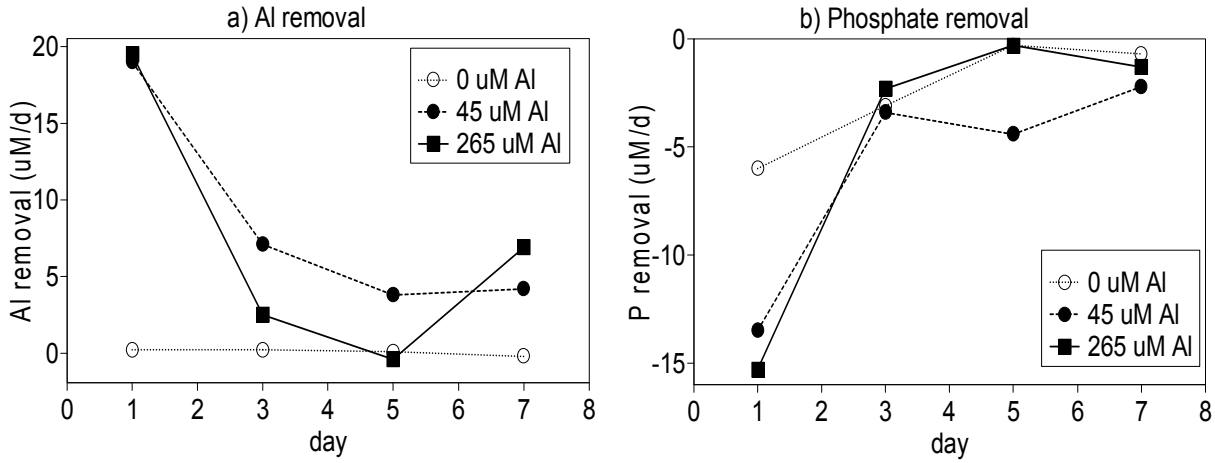

Figure 3. Removal of (a) Al and (b) P from solutions containing 1 mM $CaCl_2$, 5 µM H_3BO_3, and 0, 45 or 265 µM $AlCl_3$. Positive values denote removal of the element (either by uptake or adsorption) and negative values indicate increase in solution concentration due to release from roots. Values are from one replicate comprising 14 clones.

Depending on the Al concentration and duration of treatment, root tips of whole plants stained purple with CAS, magenta with ECR or dark violet with hematoxylin, whereas roots from the Al-free control did not stain (results not shown). Clones which stained positive with two out the three stains at a given concentration and duration were considered Al-sensitive. Clones which reacted with only one stain, or whose staining

reaction was only transient, were considered Al resistant. Clones B, L,M, N and O were resistant to 50 µM Al, but not 300 µM Al. Clones E and H were resistant to 50-300 µM Al. The most Al-sensitive clones were D and J. Clones F and G were sensitive to 50 µM Al within the first three days, but developed resistance at day 7. There were no clones which developed resistance over time to 300 µM Al.

Since the three stains detect different physico-chemical processes, this result was not surprising: hematoxylin is a dye reacting with Al and phosphate, thereby forming a purple precipitate in roots. The phosphate is released from roots due to Al-induced membrane damage (Ownby 1993). Therefore, hematoxylin indicates the extent of Al damage (or sensitivity towards Al), but does not indicate the amount of Al bound to roots. By contrast, eriochrome cyanine R staining is related to the amount of Al bound to the cell wall and chromeazurol S detects not only the amount of Al but also the extent of Al hydrolysis in roots (Wehr *et al*. 2009).

Conclusion

The most Al resistant clones at both 45 µM and 265 µM Al were clones E and H. The clones with best growth at pH 4 were clones B, E, H and K. Thus, it appears that clones E and H are tolerant to both low pH and Al. The results from this solution culture study reveal that teak is relatively tolerant of low pH and some clones have a greater resistance to rhizotoxic Al concentrations. Notwithstanding, teak grows best at pH 6 and with a Ca solution concentration of 1 mM. The Ca requirement of this plant in the field is high (Craven *et al*. 2007; Zech and Drechsel 1991) whereas the Ca concentration frequently encountered in leached acidic soils is low (Menzies *et al*. 1994). Therefore, addition of Ca prior to planting in the field may be advisable, either as limestone, gypsum or Ca-chloride. While there are observable differences in pH and Ca requirement and Al sensitivity of the clones, the results need to be confirmed in the field since field- grown plants often show greater Al tolerance in soil than in solution culture (Narasimhamoorthy *et al*. 2007).

Acknowledgements

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