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Morpho-physiological changes of four tropical tree seedlings under aluminum stress

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Abstract. *Pidjath C, Sopandie D, Turjaman M, Budi SW. 2021. Morpho-physiological changes of four tropical tree seedlings under aluminum stress. Biodiversitas 22: 1211-1220.* Phytotoxicity of aluminum due to acidic soil can cause a major threat to plant survival and health in the tropical region. Woody plant species are known to be well adapted to acidic soils. The research aimed to assess the growth and physiological response of tropical tree species *Calliandra calothyrsus* Meisn, *Samanea saman* Jacq. Merr, *Ochroma grandiflora* Rowlee, and *Calophyllum inophyllum* Linn exposed to high aluminum concentration in a nutrient solution. The completely randomized design was applied in this experiment with two treatments (0 and 4.0 mM Al exposure). Each treatment consists of three replicates for each species. The results revealed that the Al concentration of 4.0 mM inhibited plant growth, and nutrient uptake of all plants tested. Chlorophyll and carotenoid contents of *S. saman and C. calothyrsus* were higher when exposed to 4.0 mM Al, whereas *O. grandiflora and C. inophyllum* were lower. Hematoxylin staining showed high Al accumulation in the root epidermal and outer cortical cells of all plants except *C. inophyllum*. The Al concentration of 4 mM decreased the calcium and magnesium concentrations in shoots and roots in all plants tested. There was a high increase in Al concentration in shoots of *O. grandiflora*. Based on various parameters studied, we concluded that *C. inophyllum* could be proposed as a tolerant species, whereas *O. grandiflora* is more vulnerable to aluminum stress.

Keywords: Aluminum toxicity, chlorophyll, hematoxylin, lipid peroxidation, resistance

Abbreviations: MDA: Malondialdehyde; LA: Leaf area; DW: Dry weight; FW: Fresh weight

INTRODUCTION

Aluminum is the third most common element of the Earth crust composition. Aluminum forms aluminosilicate and aluminum oxide in the soil, which are harmless to plants. Nonetheless, it will alter into a trivalent cation (Al^{3+}) when pH drops below 5.5 and becomes available in the acidic soil. Furthermore, the existence of aluminum in certain level turns into an element that is harmful to plant development (Bojórquez-Quintal et al. 2017). Aluminum toxicity impacts morpho-physiology and biochemistry processes, thus restrain plant development (Kochian et al. 2015; Cárcamo et al. 2019; Silva et al. 2020). Aluminum stress primarily targets plant roots, where Al^{3+} injury and inhibits root elongation (Kopittke et al. 2015). Therefore, the Aluminum stress successively leads to disruption of water and nutrient absorption in plants (Gupta et al. 2013; Yang et al. 2013). Additionally, Aluminum stress has an indirect effect on plant growth. It inhibits photosynthesis, leaf expansion, biomass growth, reduces the amount of total chlorophyll, and carotenoids (Cárcamo et al. 2019), induces oxidative stress (Giannakoula et al. 2010). Peroxide lipid is an auto-oxidation reaction, where membrane lipids experience excess free radical oxygen (superoxide). In agriculture, crop productivity is restricted by Al toxicity in acid soils (Singh et al. 2017). In forest, Al toxicity may for example disturb early-stage forest growth (Amara et al. 2020), have a negative impact on forest health in general (Cronan and Grigal 1995), and hamper the reforestation in post-mining landscapes (Aurum et al. 2020). Nevertheless, many plants have adaptation mechanisms to alleviate Al stress. Two mechanisms have been identified: (i) avoidance of Al entering into the roots (exclusion), and (ii) transformation of the toxic Al forms into non-toxic forms within the plant cells (internal tolerance) (Ma 2007; Liu et al. 2014). Plants prevent Al from entering root cells by releasing organic acid (AO), phosphate (Pi), phenolic anions, increasing the pH of the rhizosphere, modifying the cell wall, accumulating inside the cell wall, redistribution of Al and Al waste (Kochian et al. 2004; Singh et al. 2017; Amara et al. 2020). Various tropical tree species grow well in acidic soils with high levels of Al (Brunner and Sperisen 2013). This provides an opportunity to reforestation degraded lands with trees. Based on several studies, crop and woody-plant species that grow in acidic soils show a specialized strategy in restraining aluminum translocation from roots to the leaves. Such strategies have been found for instance in Solanaceae

plants (He et al. 2019), Simplocos sp. (Schmitt et al. 2016), *Melaleuca cajuput* (Maejima et al. 2016), and *Eucalyptus camaldulensis* (Teng et al. 2018).

One effort to deal with high Al stress in acidic soils is using adaptive and tolerant local plants. *S. saman*, *C. calothyrsus*, *O. grandiflora*, and *C. inophyllum* are known to grow well in Indonesia. These species are fast-growing species and have potential as re-vegetation plants on degraded land. Woody-plant species such as *Samanea Saman* (Jacq.) Merr., (Wulandari et al. 2016); *Calliandra calothyrsus* Meisn (Soendjoto et al. 2014); *Ochroma grandiflora* Rowlee (Istiqomah et al. 2017) and *Calophyllum inophyllum* Linn (Chaturvedi et al. 2012) have been used for a re-vegetation plant on the degraded acid soils of post-mining areas in Indonesia and India. However, there are insufficient data about the impact of Al toxicity on morpho-physiological characteristics response and plant tolerances of those species.

This study aimed to determine the growth responses and morpho-physiological characters of four tropical trees exposed to aluminum stress in nutrient solution. The results of this study are expected to provide recommendations in determining suitable plants that are tolerant and adaptive to Al toxicity in acid soils of post-mining areas in Indonesia.

MATERIALS AND METHODS

Plant material and treatment

The research was conducted from July to September 2019 at IPB University, Bogor Indonesia. Observation and preparation of the experiment were arranged at the screen house in Faculty of Forestry, IPB University (-6.556550; 106.729155555556 E). Legume seeds of *Samanea saman* Jacq. Merr and *Calliandra calothyrsus* Meisn (Fabaceae) and non-legume ones of *Ochroma grandiflora* Rowlee (Malvaceae) and *Calophyllum inophyllum* Linn (Guttiferae) were obtained from Forest Tree Seed Technology Research and Development Center (BP2TPTH) Bogor, Indonesia. Seeds were treated according to methods used by (Pidjath et al. 2019). Seeds were grown in a sterile medium mixture of sand and rice husk (50:50 v/v). After seed germination, two-week seedlings of *S. saman* and *C. calothyrsus*, and four-week seedlings of *O. grandiflora* and *C. inophyllum* were transferred to adaptation solution for 14 days. The adaptation solution contained 0.02 ppm $CuSO_4.5H_2O$; 0.068 mM Fe-EDTA; 1.5 mM Ca $(N0_3)_2.4H_20$; 0.50 ppm H_3BO_3; 1.0 mM KCl; 0.05 ppm $ZnSO_4.7H_2O$; 0.50 ppm $MnSO_4.H_2O$; 1.0 mM KH_2PO_4; 0.4 mM $MgS0_4.7H_20$; 1.0 mM NH_4N0_3; 0.01 ppm $(NH_4)_6Mo_7O_{24}.4H_2O$ (Sopandie 1999). Four seedlings (in total, 24 seedlings for each species) in the same height were then transferred into standard nutrient solution in a pot (2.5 L) in the presence (4.0 mM), and absent Al (0 mM) of $AlCl_3.H_2O$ (Aluminum chloride hydrate). The nutrient was adjusted at pH 4.0 ± 0.02 with HCl and KOH 1 N every two days. The nutrient solution was replaced with new nutrition every ten days. The culture medium was aerated by aerator a 70-L minute^{-1}; pressure 0.37 MPa continuously for 30 days. Screen house temperature and humidity were ±25-35°C and 70%-90% respectively.

Data collection

The morphological plant characters such as root length, leaf area, stem height, diameter, and fresh weight were measured before treatment and 30 days after the end of treatment. Symptoms of Al toxicity on leaves and roots were visually observed for 30 days. Plant root length (cm) was obtained by measuring from the base of the stem to the tip of the longest root of the plant (Delhaize et al. 2012). Plant height (cm) and stem diameter (mm) were measured 1 cm from the stem base. Leaf area (cm^2) was measured using ImageJ software (Delhaize et al. 2012). Furthermore, the roots and shoots of the plants were dried in an oven at ±80ºC for 72 h, and then weighed as plant biomass (g). 0.5 g of dry samples of the roots and plant shoots were then dissolved with HNO_3 and $HClO_4$ (wet ash method) (Johnson and Ulrich 1959). The concentrations of calcium, magnesium, and aluminum were determined by atomic absorption spectrophotometer (Eviati and Sulaeman 2009), and the concentration values were presented in $mg \times g^{-1}$ of dry weight. Root anatomy was observed by making cross-sections of fresh plant roots. The plant roots (0.5–0.7 mm from the root tip) were cut into thin strips and stained with hematoxylin dye (Tistama et al. 2012). Root pieces were then observed using a compound microscope Olympus CX21 (Olympus, Japan), and Optilab Images (Miconos, Indonesia) with ×100 and ×400 magnifications. Chlorophyll, carotenoid, and MDA concentration were analyzed 14 days after treatments.

Chlorophyll and carotenoid contents were measured by spectrophotometer (Pharmaspec-1700, UV-Visible Spectrophotometer, Shimadzu Corporation, Japan) at wavelength 470, 645, and 662 nm. Chlorophyll and carotenoid concentration ($\mu g \times cm^{-2}$ leaf area) were measured following Lichtenthaler (1987) methods. Lipid peroxidation in roots and leaves was measured by malondialdehyde (MDA) concentration. The MDA concentration was determined using extraction methods by Wang et al. (2013). The adsorbent wavelength was measured at 450, 535, and 600 nm by spectrophotometer (Pharmaspec-1700, UV-Visible Spectrophotometer, Shimadzu Corporation, Japan). The MDA concentration values were presented in $\mu mol \times g^{-1}$ fresh weight. Plant analysis was carried out at The Laboratory of Plant Biology and Physiology at the IPB University, and at the Laboratory of Microbiology Forest and Nature Conservation Research and Development Center (FNCRDC).

Data analysis

The experiment arranged in a completely randomized design with three replication. Significant differences between treatment means were tested by a Duncan Multiple Range Test (DMRT), with values of $P < 0.05$ considered significant. Some data sets were transformed to logarithmic scale before analysis to equalize variances. Statistical analysis was carried out by using Excel 2013 and statistical software 1.5 STAR 2.0.1 for Windows.

RESULTS AND DISCUSSION

Effects of aluminum toxicity on plant growth and root morphology

The study revealed that aluminum treatment (4 mM Al) significantly (P<0.05) affected almost all the growth parameters of *S. saman*, *C. calothyrsus*, and *O. grandiflora*, but not in *C. inophyllum* (Table 1). Generally, the growth parameters of these plants were inhibited by Al stress. The relative root length of *C. inophyllum* increased significantly (P<0.05) (26%) after exposure to Al (Table 1). The highest reduction in root elongation occurred in *S. saman*, which reached -61% of control, followed by O. *grandiflora* (-18%) and *C. calothyrsus* (-12%). The present study was in line with Pidjath et al. (2019) which showed reduced root elongation of sensitive plants (*S. campanulata, O. gradiflora, C. peltata*, and, *S. saman*) by -4 to -27% with the increase of Al concentration from 4 to 12 mM. This indicated that a high concentration of Aluminum in *S. saman, O. grandiflora*, and *C. calothyrsus* medium solution could inhibit plant root elongation (Table 1).

Aluminum interfered with the cellular division and cell elongation in the root tip and elongation zone, consequently disrupting root elongation in plants. According to Sharma et al.(2017), the major part of plant suffered from Al stress was in the roots, which causes undeveloped root systems, and inhibition of root elongation. Tistama et al. (2012) reported that the effect of Al concentration at the root tip was highly correlated with inhibition (-48.02%) of root elongation in *Jatropha curcas*. High Al accumulation within the root tip area caused deleterious effects to root epidermis and cortical root cells (Kopittke et al. 2015). Roots become short, stiff, stunted, cracked, brownest; branching and root hairs are reduced, and root tips are curved (Vardar and Ünal 2007). Root surface area was shown to decrease due to Al toxicity's detrimental effect, consequently reducing water and nutrient uptake (Azura et al. 2011). It indicated that root damage and Al concentration in the roots results in disruption of plant growth due to the limited absorption of nutrients.

Table 1. The average of morphological characters of *Samanea saman, Calliandra calothyrsus, Ochroma grandiflora,* and *Calophyllum inophyllum* at 0 and 4 mM $AlCl_3$ concentrations after 30 days

Morphological character	Treatment	Plant species			
		Samanea saman	***Calliandra calothyrsus***	***Ochroma grandiflora***	***Calophyllum inophyllum***
Root length (cm)	0 mM Al	91.97±1.31a	31.13±0.29a	45.10±0.64a	14.93±0.46b
	4 mM Al	35.67±0.69b	27.33±0.57b	36.83±0.83b	18.87±0.99a
Leaf area (cm^2)	0 mM Al	18.29±0.06a	12.69±0.03a	16.06±0.30a	5.22±0.07a
	4 mM Al	15.7±0.18b	9.83±0.06b	12.44±0.10b	5.65±0.21a
Stem height (cm)	0 mM Al	40.16±1.01a	31.78±1.47a	49.30±1.57a	20.63±0.03b
	4 mM Al	27.81±1.02b	27.79±0.84a	34.67±1.86b	22.23±0.02a
Stem diameter (mm)	0 mM Al	2.55±0.03a	1.96±0.03a	13.77±0.57a	3.65±0.03b
	4 mM Al	2.23±0.05b	1.94±0.02a	4.30±0.87b	3.89±0.02a
Root DW (g)	0 mM Al	0.59±0.08a	0.09±0.02b	1.41±0.16a	0.41±0.01b
	4 mM Al	0.26±0.03b	0.20±0.02a	0.57±0.14b	0.67±0.02a
Shoot DW (g)	0 mM Al	1.13±0.04a	0.94±0.02a	8.39±0.79a	1.23±0.01a
	4 mM Al	0.56±0.01b	0.58±0.01b	2.52±0.35b	1.17±0.03a
Total DW (g)	0 mM Al	1.72±0.11a	1.03±0.01a	9.79±0.79a	1.64±0.02b
	4 mM Al	0.82±0.02b	0.78±0.02b	3.10±0.44b	1.83±0.02a

Note: The numbers followed by the same letters in the same column show no significant different base on DMRT at the level P<0.05; (mean±SE; n=3); DW: dry weight.

Table 2. Shoot and root mineral uptake of *Samanea saman, Calliandra calothyrsus, Ochroma grandiflora,* and *Calophyllum inophyllum* at 0 mM and 4 mM $AlCl_3$ concentrations after 30 days

Plant species	Nutrient uptake ($mg \times g^{-1}$ DW)					
	Ca		Mg		Al	
	0 mM Al	4.0 mM Al	0 mM Al	4.0 mM Al	0 mM Al	4.0 mM Al
Shoot						
S. saman	18.4±1.33 a	9.03±1.02 b	2.13±0.15 a	1.30±0.15 b	0.43±0.03 b	1.22±0.09 a
C. calothyrsus	23.37±1.56 a	11.73±0.2 b	3.77±0.19 a	2.43±0.12 b	0.97±0.01 b	1.16±0.05 a
O. grandiflora	36.87±4.91 a	11.10±0.64 b	4.30±0.49 a	1.70±0.06 b	0.93±0.01 b	1.92±0.09 a
C. inophyllum	9.77±1.39 a	5.03±0.23 b	2.00±0.10 a	1.13±0.09 b	0.43±0.07 b	0.79±0.06 a
Root						
S. saman	26.57±5.27a	2.80±0.93b	3.63±0.71 a	0.50±0.15 b	0.82±0.07 b	2.43±0.12 a
C. calothyrsus	9.40±1.057 a	1.17±0.07 b	5.07±0.84 a	0.30±0.00 b	1.21±0.39 b	2.98±0.36 a
O. grandiflora	23.10±2.81 a	2.37±0.93 b	0.93±0.03 a	0.50±0.15 a	1.30±0.16 b	2.33±0.06 a
C. inophyllum	3.13±0.147 a	2.53±0.09 b	0.73±0.03 a	0.57±0.03 b	0.42±0.16 b	2.43±0.61 a

Note: The number followed by the same letters in the same column shows no significant different base on DMRT at the level P<0.05; (mean±SE; n=3); DW: dry weight

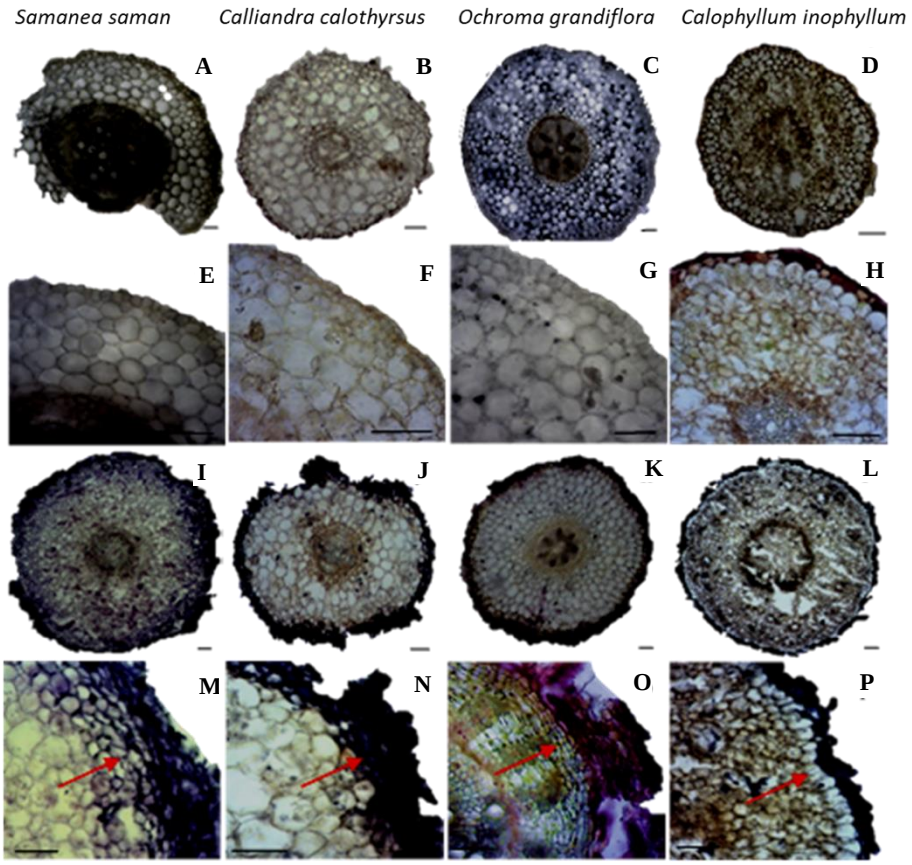

Figure 1. Root cross-section of *Samanea saman, Calliandra calothyrsus, Ochroma grandiflora,* and *Calophyllum inophyllum* 6 mm from the root tip at 0 mM Al (A-H) and 4 mM Al (I-P) with hematoxylin staining. Undamaged epidermis and cortex of the root are arranged in a magnification of 100× (A, B, C, D) and 400× (E, F, G, H). Treatment at 4 mM stress magnification of 100× (I, J, K, L) and 400× (M, N, O, P). The arrows indicate areas of Al accumulation with a darker color and the root part of the epidermis layer and the damaged outer cortex. Observation of Al distribution using a binocular microscope. 50µm scale bar.

There were negative responses in the aboveground features of *S. saman, C. calothyrsus*, and *O. grandiflora* due to aluminum stress. Leaf area, plant height, and stem diameter of *S. saman, C. calothyrsus*, and *O. grandiflora* species were reduced in comparison to the control (Table 1). The growth of *O. grandiflora* was more influenced by Al stress than the other three species. Almost all growth parameters of *O.grandiflora* declined with Al exposure. The leaf area, plant height, and stem diameter of *O. gandriflora* were decreased by -23, -30, and -69%, respectively as compared to control plants. Furthermore, the plant biomass also showed similar results (Table 1). The other growth parameters of *O. grandiflora* such as roots dry weight, shoot dry weight and total biomass dry weight were also decreased by -59, -70, and -68 % respectively as compared to control plants. This indicated that the growth of *O. grandiflora* is more susceptible to Al stress than *S. saman, C. calothyrsus*, and *C. inophyllum*. Inhibition of plant growth is an indirect response to Al stress. Al toxicity in the roots resulted in inhibition of nutrient translocation to shoots (Reyes-Díaz et al. 2015). As proposed by (Delhaize et al. 2012; Rehmus et al. 2014), reduction in plant biomass or root length could be used to determine plant resistance or sensitivity. Aluminum may inhibit plant growth by cellular modification in leaves, by disturbing stomata activity, by photosynthetic process obstruction, because of inhibition of nutrient uptake (N, Ca, Mg, P, K) (Ribeiro et al. 2013). Yang et al. (2015) reported concentration of Al at 4.4 mM inhibits photosynthesis and changes the morphology of *Eucalyptus* leaves. The differences in the plant adaptability to Al stress were considerable in *C. inophyllum* species in comparison to the three other species in this study. This study revealed changes in almost all growth character of *S. saman, C. calothyrsus*, and *O. grandiflora* induced by changing Al concentration, except in *C. inophyllum*. Even though the aluminum concentration in shoot and roots in *C. inophyllum* increased by 147 and 479% respectively, the plant growth parameters of *C. inophyllum* (leaf area, plant height, stem diameter, and biomass) increased in comparison to the other species in this research (Table 1 and Table 2). Total biomass of *C. inophyllum* increased 2 to 6 fold in *S. saman*, *C. calothyrsus*, and *O. grandiflora*. Similarly, root length and root biomass of *C. inophyllum* increased (26 and 62%, respectively). A similar result has been observed in *Quercus serrata,* where 15 days exposure to 2.5 mM increase in Al induced significant lateral root elongation (Moriyama et al. 2016). Each Al stressed plant

showed a different level of symptoms. Shoots of *C. calothyrsus* showed the phytotoxic symptoms included curling of young leaves, dieback, dark green leaves, wilting and fallen leaves. Similar symptoms such as chlorosis and necrosis, wilting and fallen leaves were also observed in the shoots of *S. saman* and *O. grandiflora*. Böhlenius et al. (2018) reported that the Al-sensitive Poplar's stem height was shorter than Al-tolerant Poplars because of the dieback on the plant tip. By contrast, in the presence of Al our results noticed that Al only induced dark green symptoms in the *C. inophyllum* leaves. Aluminum stress changes the color of mature leaves to dark green (Karimaei and Poozesh 2016). It was indicating that plants have different visible responses on morphological characteristics to a high Al concentration.

Figure 1 shows Al distribution in the root cross-section of *S. saman, C. calothyrsus, O. grandiflora*, and *C. inophyllum*. The distribution of aluminum over the root surface was recognized by hematoxylin staining as the biomarker method. Dark purple color in the root epidermis layer and outer layer of the cortex of *S. saman, C. calothyrsus, O. grandiflora* showed high Al accumulation after exposure to Al, except in *C. inophyllum*, dark purple color was only seen in the root epidermis. (Figure 1.i-o). Consistent with the result, Miftahudin (2007) has found aluminum distribution in root apical identified as a dark purple color spread in the epidermal and sub-epidermal layers of root rice cross-section. Consistent with Batista (2013) Al was found to accumulate within the epidermal cells, the outer cortex layer, and in the central cylinder root in the corn plant. High Al accumulation in the outer cells of the root results in structural damage to the root surface (Silva et al. 2020). Furthermore, Figure 1 (i-o) transversal section view of the root treated with Al also showed an injury in the root tissue of the epidermis of *S. saman, O. grandiflora*, and *C. calothyrsus*. Al exposure caused damage to the epidermal layer to the outer cortex layer of *S. saman, C. calothyrsus*, and *O. grandiflora* whereas in *C. inophyllum* the damage was found only in the root epidermal layer. Silva et al.(2020) reported structural damage in the epidermal cell layer and root cap of soybean after 72 h of treatment. In line with Kopittke et al.(2015) who reported that Al caused lesions at the main root surface, where Al bonds to the outer cell and accumulates in the apoplast. It indicated that epidermis and the outer cortex layer of *S. saman, C. calothyrsus*, and *O. grandiflora* were susceptible to Al exposition. Figure 1(l, p), shows that dark color occurred only in the epidermal root layer of *C. inophyllum*, whereas in the other species, the color distribution was more concentrated and spread into the outer root cortical tissue. Furthermore, Al accumulation in root tissue caused less injury to the root surface of *C. inophyllum*, suggesting that the epidermal root layer of *C. inophyllum* could bind Al without experiencing stress symptoms (Table 2, Figure 1). The damaged cells in the *C. inophyllum* roots only appeared in the epidermal layer. The ability of *C. inophyllum* roots to restrain Al from entering cortical cells indicates that *C. inophyllum* roots are resistant to Al by holding Al in the epidermal layer. The epidermis is an effective barrier to prevent Al from penetrating into the root cortex (Batista et al. 2013). This result indicates that *C. inophyllum* is more resistant to Al stress than *S. saman, C. calothyrsus*, and *O. grandilora*, and the mechanism of Al resistance might be related to accumulation and binding of Al in the cell walls of epidermis layer. In the previous research, the plant growth's aluminum advantages reported on *Eucalyptus* sp., *Camellia sinensis*, *Quercus serrata*, and several other plants (Hajiboland et al. 2015; Yang et al. 2015; Moriyama et al. 2016). The results are in line with the reports that *Camellia sinesis* as a tolerant woody-plant species, accumulated Al mostly in its root cell walls (Hajiboland et al. 2015; Li et al. 2017). According to Yamamoto (2019), Al induced the accumulation of significant cell wall polysaccharides of hemicellulose, resulting in the thickening of the cell wall. The cell wall was composed of negatively charged carboxylic groups forming a pectin matrix with influential affinity binding sites against aluminum ions.

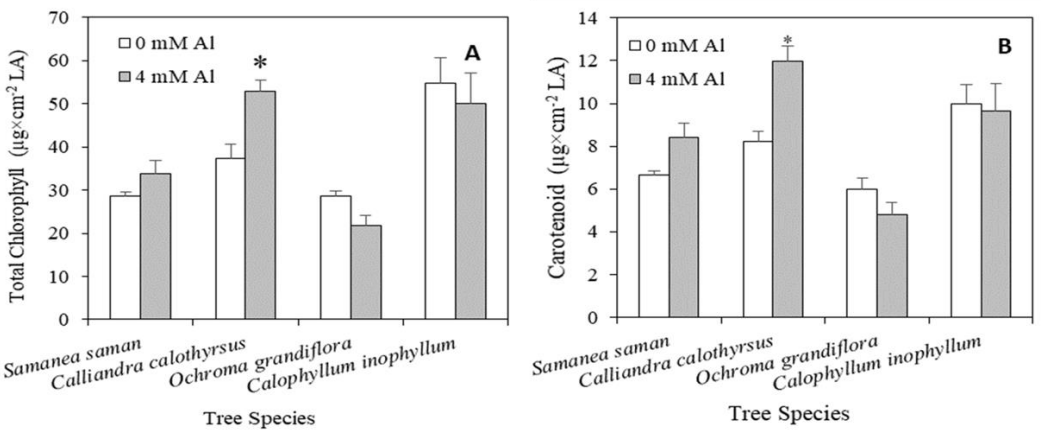

Figure 2. Total chlorophyll (A) and carotenoid (B) concentration (µg×cm^{-2} LA) of *Samanea saman, Calliandra calothyrsus, Ochroma grandiflora*, and *Calophyllum inophyllum* under 0 and 4.0 mM $AlCl_3$ concentrations at pH 4.0 (mean±SE; n=3) in hydroponic assay after 14 days. (*) Asterisks indicate a probability of a significant difference ($P<0.05$) between 0 and 4.0 mM $AlCl_3$ for a line; LA: leaf area

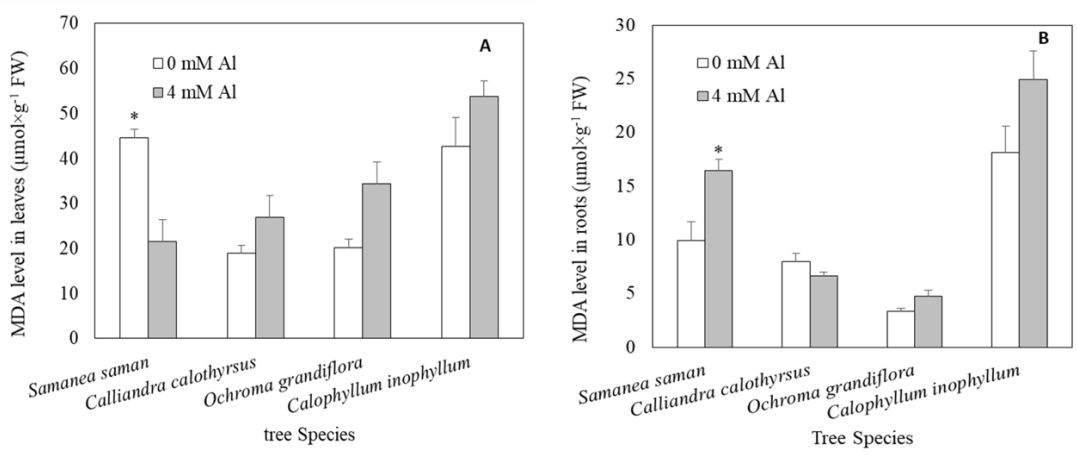

Figure 3. Concentration of Malondialdehyde (µmol×g^{-1} FW) in leaves (A) and root (B) of *Samanea saman, Calliandra calothyrsus, Ochroma grandiflora,* and *Calophyllum inophyllum* under 0 and 4 mM $AlCl_3$ concentrations at pH 4 (mean±SE; n=3) in hydroponic assay after 14 days. (*) Asterisks indicate a probability of a significant difference (P<0.05) between 0 and 4.0 mM $AlCl_3$ for a line: FW: fresh weight

Aluminum effects on calcium and magnesium concentration in plant

The present study showed that Al concentration significantly affected (P<0.05) plant nutrient concentration in the shoots and roots of *S. saman, C. calothyrsus, O. grandiflora*, and *C. inophyllum* (Table 2). Table 2 shows that the nutrient content of *S. saman, C. calothyrsus, O. grandiflora*, and *C. inophyllum* were varied under various treatments. The average Al concentration within the root of *S. saman, C. calothyrsus, O. grandiflora*, and *C. Inophyllum* (197, 147, 79 and 479% respectively) and in the shoot (186, 20, 106 and 85% respectively) significantly (P<0.05) increased as compared to control plants (Table 2).

The highest increase in Al concentration in root and shoot was found in *C. calothyrsus* (2.98 mg×g^{-1}) and *O. grandiflora* (1.92 mg×g^{-1}) respectively exposed by 4 mM Al. According to Schmitt et al. (2016), the transport pathway of Al to leaves is through transpiration flow in *Symplocos* trees, while the high concentration of Al in the stem tissue of *S. paniculata* Al indicates Al absorption through the phloem. Table 2 shows that Al concentration in roots was higher than in shoots in all plant species. The root surface is where Al is first exposed. Al binds strongly to cell wall components especially to the carboxyl group of pectin (Maejima et al. 2016). Therefore, this study indicates that the inhibition of Al penetration to shoot through the roots may act as an avoidance mechanism to Al toxicity. It is consistent with (Silva et al. 2020) stated as the primary defense in plants to Al toxicity was by restraining Al in root cap, epidermis, cortex cell, and cell wall. Aluminum stress caused a significant decrease (P<0.05) in calcium and magnesium uptake in shoots and roots. In this research, the addition of Al in medium solution at 4.0 mM concentration decreased calcium concentration in roots and shoots of *S. saman, C. calothyrsus, O. grandiflora*, and *C. inophyllum* by -89, -88, -90, and -19%, respectively, and by -51, -50, -70, and -8%, respectively as compared to control (Table 2). Similarly, aluminum inhibited magnesium content in roots and shoots of *S. saman, C. calothyrsus, O. grandiflora*, and *C. inophyllum* by -86, -95, -46, and -23% respectively and -39, -35, -60, and -43%, respectively (Table 2). It has been widely reported that Al interferes in plant nutrient uptake mechanisms. Absorption of calcium and magnesium into the root was inhibited by Al because of the high-affinity ion Al^{3+} for Ca^{2+} and Mg^{2+} cations, through interactions in the cross membrane transporters or ion channels (Marschner 2012). In this present study, plants showed a different manifestation of Al stress. Table 2 shows that *O. grandiflora* species are more sensitive to Al stress than *S. saman, C. calothyrsus*, and *C. inophyllum* at 4 mM Al concentration. Aluminum inhibited calcium uptake in roots and shoots of *O. grandiflora* by -90 and -70% of controls. Aluminum causes a decrease in Ca^{2+} influx due to ion channels Ca^{2+} blocked and Ca^{2+} cell wall-bound displaced by Al (Liu et al. 2014). Similar results have also been shown in 4-week-old Norwegian spruce seedlings, in which the calcium concentration decreased at (77 to 92%) at 100 to 800 µM) Al stresses (Godbold and Jentschke 1998). Calcium is an essential element for growth, and it plays an important role in plant metabolic processes (Rengel 1992). As a result, the plasma cell membrane becomes rigid and disrupts the fluidity and permeability of plasma membrane (Maejima et al. 2016), and interferes with ion channel activity (Poschenrieder et al. 2008). Consequently, plants will suffer from a lack of nutrients. Therefore, they cannot grow normally. Wilting, leaf dropping, and dieback were observed on shoot tops of *C. calothyrsus* treated by Al. The plant exhibits similar symptoms of aluminum stress and a scarcity of calcium and magnesium.

Magnesium is a macronutrient, which is very important in plant metabolism. Magnesium is mainly involved in many enzyme activities and plant tissue structures (Guo et al. 2016). The highest reduction of magnesium concentration in the roots (-95%) occurred in *C. calothyrsus* as compared to control plant (Table 2).

Meanwhile, the lowest percentage of Mg concentration in shoot (-60%) was found in *O. grandiflora* as compared to control. Calcium and magnesium deficiency could be contributing to a decrease in chlorophyll, carotenoids, and total biomass of *O. grandiflora* (Table 1 and Figure 2). The results indicate that the translocation of magnesium from root into shoot was inhibited by Al. Magnesium deficiency can indirectly lead to inhibition of the photosynthesis process (Proklamasiningsih et al. 2012). Similarly, Rehmus et al. (2015) reported that the high Al treatment (1200 µM) decreased magnesium concentration in leaves and shoot biomass of *Heliocarpus americanus* tree seedlings. Aluminum inhibits magnesium uptake in the roots by blocking membrane transport and metal-binding site enzymes (Pandey et al. 2013). There were necrotic spots on the leaves mainly caused by disruption of physiological processes such as decreased carbon metabolism, decreased chlorophyll, and decreased carbon fixation (Guo et al. 2016; Hauer-Jákli and Tränkner 2019). Interestingly, *C. inophyllum* was more tolerant to Al stress, whereas high Al uptake in roots (579% of control) was still able to absorb Ca up to 8 times more than the other three species. Likewise, the magnesium concentration in the root system of *C. inophyllum* was higher (15 and 6 times) than in *C. calothyrsus* and *S.saman* respectively (Table 2). The results indicated that *C. inophyllum* was more tolerant to Al stress at a 4 mM concentration than other plants (Table 1). Chaturvedi et al. (2012) reported that *C. inophyllum* accumulates large amounts of heavy metals (Fe, Pb and Cu) in its tissues and has the potential as a phytomining.

Aluminum effect on the physiological characters

Aluminum treatment significantly influenced ($P<0.05$) the total chlorophyll and carotenoid content of *C. calothyrsus*, but no significant effect of Al to physiological responses of *S. saman, C. calothyrsus,* and *C. inophyllum* was found. The total chlorophyll and carotenoid in the leaves of *C. calothyrsus, S. saman, C. calothyrsus*, and *C. inophyllum* are presented in Figure 2. Chlorophyll and carotenoid concentration in *O. grandiflora* and *C. inophyllum* leaves were decreased by -9 and -24%, -3 and -20% respectively) when exposed to 4.0 mM of Al. A similar result has been reported that chlorophyll content of *Picea abies* decreased due to Al exposure (Slugeňová et al. 2011). The same results were also found in *Eucalyptus* sp. seedling when treated by Al at concentration of 4.4 mM (Yang et al. 2015). According to Reyes-Díaz et al. (2015), the reduced chlorophyll content by Al is an indirect effect of Al toxicity. In our study, the lowest total chlorophyll and carotenoid contents (-24 and -20% respectively) occurred in *O. grandiflora* leaves (Figure 2), related to this result, found that a decrease of magnesium content in the shoot by (-60%), and also the highest concentration of MDA (70% relatives to control) in *O. grandiflora* leaves (Table 2, Figure 2). The decrease of chlorophyll content in leaves might be caused by Al stress that was suppressed due to carotenoid and MDA role in *C. calothyrsus*. Chlorophyll reduction may be related to low magnesium content and oxidative stress in shoots due to Al exposure (Table 2, Figure 2). Aftab et al. (2010) found a decrease in total chlorophyll content and an increase in lipid peroxide in *Artemisia annua* L. exposed to Al. Yang et al. (2015) pointed out that the competition between aluminum and magnesium in plant roots corresponded to nutrient plant deficiency. Khan et al. (2020) revealed that heavy metals interfere by inhibiting enzyme activity and exchange of magnesium which is used as chlorophyll biosynthesis. Furthermore, aluminum toxicity can also damage chloroplasts and thylakoids, thereby disrupting chlorophyll function to carry out photosynthesis (Yang et al. 2015). On the contrary, this study has found that the total chlorophyll and carotenoid content in leaves of *C. calothyrsus* increased significantly ($P<0.05$) when compared to control. Hajiboland et al. (2013) the increased aluminum concentration induced the increment of chlorophyll and carotenoid in woody plant species leaves. According to Uarrota et al. (2018), carotenoids play roles in the photosynthesis process, protecting membrane lipids by removing free radicals ($^1O_2$).

The concentration of malondialdehyde (MDA) in the roots and leaves plants have been used to assess lipid peroxidation content (Siqueira et al. 2020). MDA concentration decreased significantly ($P<0.05$) in leaves and increased significantly ($P<0.05$) in *S. saman* roots after 14 days exposure by Al concentration 4.0 mM Al at pH 4.0±0.02. Meanwhile, aluminum did not affect MDA concentration in leaves and roots of *C. calothyrsus, O. grandiflora*, and *C. inophyllum*. However, Figure 3 shows that increasing Al concentration on growth medium occurs with the rise in MDA in the leaves by 26, 43, and 70%, in *C. inophyllum*, *C. calothyrsus*, *O. grandiflora* respectively, and in the roots of *S. saman, O. grandiflora* and *C. inophyllum* (66, 43, and 37% respectively).

According to the present study, the peroxide lipid concentration in the leaves of *O. grandiflora* was increased by 70% compared to control than in other three species. This result was in line with decreasing chlorophyll and carotenoid content in leaves (Figure 2). It indicated that increased oxidative stress in plant leaves led to the reduction in chlorophyll and carotenoid formation in leaves of *O. grandiflora*. Similar phenomenon of rising peroxide lipid levels has been earlier seen in *Eucalyptus platyphylla* when exposed to Al (Lima et al. 2016). According to Kochian et al. (2004), phospholipid metabolism could have interfered with lipid peroxide, in which phospholipids are the main component of lamella constituents in the leaf chloroplasts. In contrast to these earlier results, the increase in lipid peroxidation of *C. calothyrsus* was followed by enhancing of chlorophyll and carotenoid leaves concentration (Figures 2 and 3). This study reveals that high MDA content in the leaves of *C. calothyrsus* did not reflect lipid peroxide stress, but the increased MDA content in the leaves indicated increased antioxidants. According to (Schmid-Siegert et al. 2016), polyunsaturated fatty acids (PUFA) activity induced MDA in the chloroplast membrane. Additionally, malondialdehyde could protect and develop plants by activating gene regulation (Morales and Munné-Bosch 2019). Lipid peroxidation is distinguished by increased expression of MDA concentrations (Yamamoto 2019). Figure 3 shows that

MDA concentrations in the roots of *S. saman* were higher (66%) than in *C. calothyrsus*, *O. grandiflora*, and *C. inophyllum* (17, 43, and 30% respectively). The increasing MDA root concentration of S. *saman*, *C. calothyrsus*, and *O. grandiflora* may induce the inhibition of root elongation and root biomass (Table 1). These results were in line with Choudhury and Sharma (2014), who reported that Al inhibited the root elongation of *Cicer arietinum* due to lipid peroxide. Peroxide lipid induces programmed cell death (PCD), causes cell organelle (for example in mitochondria and vacuoles) dysfunction, which results in cell death, and leads to loss of cell membrane integrity in the plasma membrane (Yamamoto 2019).

In conclusion, these four types of forest tree seedling show a variety of responses to the Al stress. The response of *O. grandiflora* was more sensitive to Al treatment compared to *S. saman*, *C. calothyrsus*, and *C. inophyllum*. The highest amount of Al concentration was found in *O. grandiflora* shoots, while the highest Al concentration was found in *C. calothyrsus* at the roots. The accumulation of Al on the root surface changes the morphological structure of the outer layer of the root (epidermis and root cap), thereby inhibiting the absorption of magnesium and calcium into the roots. In contrast, the high Al resistance was found in *C. inophyllum*. Even though the Al accumulation in the roots was high, *C. inophyllum* had excellent growth performance even with Al stress. However, the Al resistance mechanism in the root system of *C. inophyllum* was still unclear. Further studies are required to understand the Al avoidance mechanisms of plants.

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