Aluminum Stress Effects of Nine Tropical Tree Species In The Hydroponic Assay

C Pidjath¹, 4*, S W Budi¹, D Sopandie², M Turjaman³

¹ Department of Silviculture, Faculty of Forestry, IPB University (Bogor Agricultural University), Bogor, Indonesia
² Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University (Bogor Agricultural University), Bogor, Indonesia
³ Forestry and Environment Research Development and Innovation Agency (FOERDIA), Ministry of Environment and Forestry Indonesia Bogor, Indonesia.
⁴ Department of Forestry, Agriculture of Faculty, Palangka Raya University (UPR), Palangka Raya, Central Kalimantan, Indonesia.

*Corresponding author e-mail: chartina8pz@gmail.com

Abstract. Aluminum (Al) toxicity usually occurs in acidic soils with a pH of 5.5 or lower. Plants present different degrees of adaptation to Al concentrations in the soil. Recent evidence indicates that different species have evolved different mechanisms to cope with this stress, and the understanding of this characteristic can lead to a viable option for the utilization of acid soils. The objective of this experiment was to investigate the effect of nine level Al concentration on nine tropical trees seedling (Enterolobium cyclocarpum Griseb, Adenanthera pavonina L. Samanea saman (Jacq.), Spathodea campanulata P. Beav Merr, Ochroma grandiflora Rowlee, Gmelina arborea Roxb, Calophyllum inophyllum, Cecropia peltata, and Calliandra calothyrsus) in the hydroponic culture. The treatments arranged in a Randomized Block Design with three replications. The relative root elongation and roots number in terms of increased under low (below 2 mM) and, decreased under high (above 4 mM) Al concentrations. Significant reductions in relative root elongation, lateral root number and biomass observed almost in all seedling treated with hight Al concentration. A. pavonina, E. cyclocarpum, C. inophyllum classify into tolerance and S. campanulata were the most susceptible to Al. Even though A. pavonina was tolerant to Al toxicity, there was a high concentration in its tissue. This species is likely has an Al tolerance mechanism by internal detoxication. On the contrary, S.saman as sensitive species absorbed Al less than other species; it indicated that S.saman was have an avoidance mechanism as Al exclusion.

1. Introduction
Aluminum is the third element of earths. It is one of the abundant metals in the soil layer which solubility is affected by the acidity of the soil. Soil acidity at pH <5.5 dissolves aluminum into the monomeric form Al³⁺ [1], and this form is the most toxic and dangerous for plant growth. The high solubility of heavy metals and aluminum (Al) is the main limiting factor for plant growth. Metal toxicity such as Al toxicity reduces plant productivity, especially in agricultural crops [2, 3, 4]. In the forest area, Al toxicity causes disturbance to the health of the forest, while in open lands due to mining a high level of Al toxicity can cause failure in the land re-vegetation process. Studies of the effect of Al on annual crops in post-mining land in several studies indicate Al influences growth. Based on the research results of [5]...
in post-coal mining land, the very high Al solubility (3.48-14.31 me.100g-1) at pH 2.7-3 caused the growth of Bridelia monoica tillers to be disrupted by the occurrence of curly root tip shape, decreasing apical root length and biomass. Likewise, in annual crops of Robusta coffee species growth stunted on Al-recorded land in post-coal mining land [6].

The selection of suitable plant species can overcome the problem of Al stress in post-mining acid land. Plants tolerant could suppress the absorption Al soluble in the soil and could neutralize the effect of Al toxicity in plant tissues [7]. The effect of Al on plants and adaptation mechanism to Al toxicity are well known for years. Woody species are relatively more tolerant of Al compared to other types of agricultural crops [7,8]. Different plant species or different genotypes within the same species have evolved special mechanism to alleviate stress Al and live under excessive Al environments [3]. Indonesia has many types and candidates of fast-growing tree species for plants rehabilitation. However, information about Al tolerant tropical rainforest species is not widely available, especially in fast-growing species.

Study to select Al tolerant is usually done by maintaining plants in a greenhouse using certain high toxic metal or hydroponic media, then observing the ability of the root system by treating in Al and no Al concentration [9]. The purpose of the present study was to determine the tolerance of E. cyclocarpum, C. calothyrsus, S. saman, A. pavonina, G.arborea, O. grandiflora, C. inophyllum, C. peltata and S. campanulata fast-growing species at increasing level Al concentrations by observing plant growth responses and root morphological characters using a hydroponic assay.

2. Method

2.1. Materials

The materials are legumes as saga (Adenanthera pavonina L.), Trembesi (Samanea saman (Jacq.) Merr.), Kaliandra (Calliandra calothyrsus Meisn), Sengon Buto (Enterolobium cyclocarpum Griseb) and wide leaf species as Kecrutan (Spathodea campanulata P.B Beau), Balsa (Ochroma grandiflora Rowlee), Jati Putih (Gmelina arborea Roxb), Nyamplung (Calophyllum inophyllum L), sterile sand, haematoxylin and KI; dye solution, distilled water, nutrient solution [10], AlCl3, HCl and KOH. The equipment used includes pH meter, pipette, measuring cup, petri dish, test tube, balance sheet, binocular microscope, SP60 70 Liter Resun Aerator minute-1; a pressure of 0.37MPa, and a digital camera.

2.2. Procedure

The research carried out during July 2018 - February 2019 in the Ecological screen house Silviculture Department, Forest Faculty, as well as the Laboratory of Physiology and Plant Biology of Biology Department, Faculty of Mathematics and Natural Sciences, IPB. The experiment consisted of eight treatments of Al13+ concentration with control, namely 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 mM Al. The trial consisted of three repetitions and each repetition were lie of three plants so that the overall amounted to 81 experimental units for each species. Seeds of nine-forest species sterilized with 5% (v/v) NaOCl for 5 minutes. After a short while, each seed washed three times with distilled water. The seeds doused with hot water and soaked overnight. Afterward, the seeds then spread in sand and rice husk charcoal of 50:50 (v/v). Then, seeds germinated in a dark place until sprouting. Eighty-one sprouts with relatively uniform size were transferred to adaptation media solution [10] with modifications. The nutrition media concentration contained 1/5 of nutrition, Its consist of 1.5 mM Ca (N03)2.4H2O; 1.0 mM NH4NO3; 1.0 mM KCl; 0.4 mM MgSO4.7H2O; 1.0 mM KH2PO4; 0.50 ppm MnSO4.H2O; 0.02 ppm CuSO4.5H2O; 0.05 ppm ZnSO4.7H2O; 0.50 ppm H3BO3; 0.01 ppm (NH4)6 MoO24.4H2O; 0.068 mM Fe-EDTA; NaOH; HCl; AlCl3.6H2O and distilled water. The seedlings stems were wrapped by cotton to avoid injury and then, inserted to the hole of styrofoam boards. The styrofoam board floated in a pot which, has been filled with 11 L of nutrient culture media. This treatment intended to adjust the seedlings grew healthy and adapted in nutrient solution. During that, the nutrient media were maintained at pH 5 ± 0.5 by increasing or reducing the pH with 1 N HCl or 1 N KOH every 2 days. Then, nutrient solution added every three days with stock solutions. After 14 days in adaptation nutrient, the seedlings removed and
treated with AlCl₃·H₂O solution into eight liters full strength nutrient solution and adjust at pH 4 ± 0.02. Afterward, nutrient solutions replaced every 10 days with a new solution. The culture media were aerated by 24 h using a 70- liter minute⁻¹ aerator; pressure 0.37 MPa during 35 days.

2.3. Data collection and plant analysis

Three replications of each species harvested for each treatment in the screen house 35 days after exposing to Al. Plant height (cm) and root length (cm) measured before and after treatment. Plant height (cm) measured at the position 1 cm from root collar and lateral roots growth as lateral root number (the second root branch after the main root) counted after harvest. Root, shoot, and total dry weight (DW) (g) were oven-dried at 70°C for 72 hours, weighed, and ground. The variables analyses plant growth (relative plant height increment, root, shoot, and total biomass), root characteristics such as relative root elongation and relative lateral root number [11]. Analysis of total Al content in tissue with stress 0, 1 and 2 mM Al were followed AOAC methods (2012): 999.11 procedures at Integrated Laboratory IPB, Bogor.

2.4. Hematoxylin Staining

Al accumulation at root tips was detected by hematoxylin staining. Preparation of seedlings and solution were the same procedure as the first preparation. Tree seedlings treated with concentrations of Al at 0, 0.15, 0.5, 1, 6 mM for 14 days. Roots rinsed with distilled water for 30 minutes, and then immersed in a hematoxylin solution for 30 minutes. Afterward, the roots rinsed three times with distilled water for one hour by Polle methods [12]. The roots cut 1 cm long and observed under a microscope for the color of the root. The visual detection of Al was performed as describes by [13, 14]. Using the following criteria and scoring the light purple layer found at the tip of the root indicates the accumulation of Al. Score 0 = Root without any staining ≤25%; Score 1 = partial staining 25% < x ≤ 50%; Score 2 = Moderate staining 50% < x ≤75%; Score 3 = Deep staining of the whole root > 75%.

2.5. Statistical analysis

Statistical significance of treatment was analyses using Statistical Tool for Agriculture Research (STAR) 2.0.1 IRRI software analysis of variance (P<0.05) and (P<0.01) significance levels. Post hoc analysis was performed using DMRT test (P<0.05). The correlation existed among the variable with respect to Al tolerance relative to control, were submitted to Principal Component Analysis Past 2.17 Software Analysis.

3. Result and discussion

3.1. Growth responses to Al solution

The main target of damage due to Al exposure is at the root [15]. Growth of nine tropical tree species as expressed by root elongation, root number and shoot height increment reduced significantly with increasing level of Al concentration in hydroponic assays (Figure.1) Evaluation of Al sensitivity was carried out using the relative mean of root elongation, lateral root number and height increment on various level concentration Al to control an indicator. Growth and root morphology analysed by inhibition of the longest root growth and lateral root number. The roots became shorter and stunted by increasing Al stress. According to [16] aluminum formed Al³⁺ ions under acidic conditions pH <5.5 and it was toxic to plants. Al damage roots especially the root tip [17]. Al inhibited the root elongation and growth of S. saman, C. inophyllum, C. peltata and, S. campanulata roots. The first symptoms most easily recognized due to Al stress. Root toxicity symptoms are number of lateral root decrease obstruction of the length of the main root, the root became rough [18], thickened, and yellowing roots [19], damaged or rupture in the root tip apical region and root swelling[17, 20], deformed root hair [21], root became stubby, brittle [22] root tips shoes like and brown colour.
The root elongation decreased with increasing Al concentration in all species (Figure 1). Treat with higher concentration caused large inhibitions to the longest root relative to control (P<0.01). Given this statistic, after 35 days of treatment, there was no significant effect to relative root elongation of *E. cyclocarpum*, *C. calothyrsus*, *A. pavonina*, *G. arborea* and *O. grandiflora* from concentration 0.5 to 2 mM (DMRT test, P>0.05). Therefore, the relative root elongations of these species were 102-132%, 107-110%, 173-221%, 111-118% and 121-288% respectively. This result suggests that at the low concentrations Al were a positive effect or stimulated on root elongations for those five species. Moreover, concentrations Al from 4 to 12 mM were highly significant inhibited the relative root elongations of *S. saman*, *O. gradiflora*, *C. peltata* and, *S. campanulata* between 3 to 13%, 0 to 27%, 6 to 16% and 0 to 4% respectively (DMRT test, P>0.05). Relative root elongations of *Spathodea campanulata* show the lowest roots of other species, it suggests that *Spathodea campanulata* was the most susceptible of Al.

Generally, lateral root growth was strongly influenced by Al stress to all plants. Al concentration in solution inhibited lateral root number of all species (Figure 2). The increasing level of Al concentration significant reduced relative lateral root number (% of control) of *A. pavonina*, *S. saman*, *S. campanulata*, *O. gradiflora*, *G. arborea*, *C. inophyllum*, *C. peltata* and *C. calothyrsus*. The reduction of root branching also found in legume plant also reported by [23]. In contrast, there was no difference in relative lateral root number of *E. cyclocarpum* (DMRT test, P>0.05). The reduction in lateral root number in *O. gradiflora*, *C. peltata*, and *S. campanulata* were 50% higher than other species exposure with 4 mM to 12mM. Meanwhile, relative lateral root number of *A. pavonina* and *C. inophyllum were increased* slightly between 82-107%, 67-111 %, respectively. It is suggested that these species are tolerance to increasing level of Al concentration.

**Figure 1.** Relative root elongation of nine species in response to 35 days exposure to increasing Al$^{3+}$ 0.5, 1, 2, 4, 6, 8, 10, 12 mM Al. *P<0.05; **P<0.01 and DMRT test 0.05%. Bar denote mean±SE (n=3).
Growth of nine tree seedlings species were further inhibited by increasing Al$^{3+}$ concentration in solution (P<0.01) and DMRT test at P<0.05. According to Figure 3. Aluminum at a low concentration from 0.5 to 2 mM did not affect stem height of *E. cyclocarpum*, *C. calothyrsus*, *A. pavonina*, *G. arborea*, *O. gradiflora*, *C. peltata* and *S. campanulata*, except stem height increment of *S. saman* and *C. inophyllum* were decreased significantly more than 50% of control with an increasing Al. However, except *A. pavonina* and *C. calothyrsus*, almost all relative stem growth increment of those species decreased significantly by Al at 4 mM. Shoot growth of *S. campanulata* and *O. gradiflora* were considered sensitive to Al toxicity, 5%, and 8% respectively. These findings were similar to those study in Eucalyptus [24,25] Al stress was found to have significant toxic effect on the root, stem, and biomass growth of Eucalyptus seedling.
Table 1. Relative roots, shoots, and total DW of nine species in response to increasing AlCl₃ after 35 days exposure. *P<0.05; **P<0.01 and means followed by the same letter do not differ by the DMRT test at 5% (n=3).

| Species            | 0.5 mM | 1mM | 2mM | 4mM | 6mM | 8mM | 10mM | 12mM |
|--------------------|--------|-----|-----|-----|-----|-----|------|------|
| Root Dry Weight (% of control) |
| *E. cyclocarpum*    | 76     | 87  | 105 | 112 | 111 | 133 | 75   | 84   |
| *S. saman*         | 64b    | 72b | 63b | 103a| 79ab| 97a | 37c  | 56bc |
| *C. calothyrsus*   | 57de   | 50de| 56b-d| 86bc| 127b| 84e | 103ce| 85de |
| *A. pavonina*      | 115cd  | 122cd| 144bc| 183a| 145bc| 156ab| 137bc| 131bc|
| *G. arborea*       | 31b    | 39b | 40b | 22c | 17cd| 21c | 11de | 6e   |
| *C. inophyllum*    | 101    | 115 | 110 | 117 | 123 | 105 | 100  | 115  |
| *O. gradiflora*    | 114a   | 120a| 148a| 114a| 37b | 47b | 11b  | 9b   |
| *C. peltata*       | 109a   | 108a| 85ab| 90ab| 79ab| 58bc| 36c  | 30c  |
| *S. campanulata*   | 96ab   | 98a | 101a| 72bc| 75a-c| 59cd| 44d  | 44d  |
| Shoot Dry Weight (% of control) |
| *E. cyclocarpum*   | 120ab  | 132a| 108bc| 85de| 94c-d| 94cd| 89de | 83e  |
| *S. saman*        | 71b    | 50c | 62bc | 55bc| 65bc | 65bc| 31d  | 31d  |
| *C. calothyrsus*  | 43d    | 48cd| 48bc| 60b | 56ab | 40cd| 50bc | 33cd |
| *A. pavonina*     | 92de   | 87e | 116ab| 122a| 103cd| 105c| 109bc| 98c-e|
| *G. arborea*      | 76b    | 78b | 68b | 22c | 16c  | 25c | 16c  | 13c  |
| *C. inophyllum*   | 86     | 89  | 101 | 109 | 118 | 87  | 101  | 107  |
| *O. gradiflora*   | 143b   | 201a| 146b| 54d | 27de | 42de| 12e  | 12e  |
| *C. peltata*      | 83bc   | 72cd| 93ab| 59d | 34e  | 27ef| 18ef | 15f  |
| *S. campanulata*  | 101a   | 108a| 95a | 39b | 39b  | 37b | 26b  | 28b  |
| Total Dry Weight (% of control) |
| *E. cyclocarpum*   | 113ab  | 126ab| 108bc| 89d | 97cd| 100b-d| 87d  | 83d  |
| *S. saman*        | 70b    | 54b | 62bc | 63b | 67b  | 70b | 32c  | 35c  |
| *C. calothyrsus*  | 45c    | 48cd| 49c | 65b | 68a  | 47b | 59ab | 41b  |
| *A. pavonina*     | 98ef   | 95f | 123b| 136a| 113cd| 117bc| 116bc| 105de|
| *G. arborea*      | 65b    | 68b | 62b | 22c | 16c  | 24c | 15c  | 11c  |
| *C. inophyllum*   | 89     | 95  | 103 | 110 | 119  | 91  | 100  | 108  |
| *O. gradiflora*   | 138b   | 186a| 146b| 65d | 28ef | 43de| 12f  | 11f  |
| *C. peltata*      | 85ab   | 76bc| 92a | 62c | 38d  | 30de| 20e  | 17e  |
| *S. campanulata*  | 100a   | 106a| 96ab| 45b | 46b  | 41b | 29b  | 31b  |

Root, shoots, and total biomass of nine tropical seedling species affected by Al toxicity. Show in Table 1. Almost all species decreased significantly in the root, shoot and total DW by Al treatment, except root DW of *E. cyclocarpum* and *C. inophyllum* DMRT test at P<0.05, P<0.01). *S. saman*, *C. calothyrsus*, *G. arborea*, *O. gradiflora*, *C. peltata* and, *S. campanulata* significantly decreased in root dry weight with increasing Al in solution. It was likely *G. arborea*, *O. gradiflora*, *C. peltata* and, *S. campanulata* sensitive to Al. Roots, shoots, and total DW of these species reduced significantly more than 50% in the exposure of 4 mM to 12 mM AlCl₃. On the contrary, *E. cyclocarpum*, *A. pavonina*, and *C. inophyllum* have determined resistance to Al from low to high concentration. Relative total DW of these species was not inhibit by increasing Al from 0.5 to 12 mM. Furthermore, Al induces biomass of
both A. pavonina (root, shoot and total DW between 115 and 131%, 83 and 109%, 95 and 136%, respectively); and C. inophylum (root, shoot and total DW between 100 and 123%, 86 and 118%, 89 and 119, respectively). Although root length, root number, and height of tolerant species (E. cyclocarpum, A. pavonina, and C.inophylum) decreased, however, those tolerance species did not reduce on root DW, shoots DW and total DW. A similar observation was made by [26] aluminum had no detrimental effect on biomass of shoots of Red Oak and American beech.

Tropical tree species that grow in acidic soil tend to tolerant on low Al concentration. It has been reported [27,28] that Al has no effect on Melaleuca leucadendra, M. cajuputi, E. grandis, M. quinquenervia, and E. deglupta at low doses (ex.0.56 mM and 1 mM). In the same result, almost all types of experimental plants at low doses of Al (below 2 mM) showed resistance to Al stress, and almost whole species showed increasing growth compared to controls. A beneficial effect of low Al concentration on growth has been reported by [27,29,30] that enhancement of plant growth of Melaleuca cajuputi, Arnica montana, Deschampsia flexuosa, Hydrangeapaniculata induced by Al at low concentration.

While aluminum tolerance in trees is generally considered greater on low Al concentration (below 2mM), this research found that a higher aluminum concentration (above 4 mM) may reduce seedlings growth. Aluminum has affected almost all characteristic of nine trees species at a concentration from 4 to 12 mM. Moreover, it is apparent that the threshold of tree seedlings tolerant to Al toxicity is between 4 to 6 mM. G. arborea, O. gradiﬂora, C. peltata and, S. campanulata have a sensitive tendency of hight Al concentration. It showed that Al affects relative root elongation, root lateral number, stem height, and biomass. Furthermore, all characteristic of these species decreased drastically between 50 to 100% after exposed by Al after 35 days. Further investigation E. cyclocarpum, A. pavonina, and C.inophylum have an implication tolerant to Al toxicity at high concentration. In addition, Al concentration above 4 mM could not affect this biomass.

According to Principal Component Analysis (PCA), it applied to the correlation matrix of relative values of morphology characteristics relative to control. The analysis showed that the principal component of the 5 main components produced one main component that has an eigenvalue of > 1 and contributes 68% to the cumulative variation (Table 2).

| Characteristic | PC1 | PC2 |
|---------------|-----|-----|
| RE            | 0.26| 0.85|
| RLN           | 0.47| 0.21|
| PHI           | 0.48| -0.23|
| RDW           | 0.47| -0.43|
| TDW           | 0.52| -0.01|
| Eigenvalue    | 3.40| 0.97|
| CVA (%)       | 68  | 19  |

The characters contribute to PC1 are root dry weight, total height, and dry weight. Furthermore, the characters contribute to the second principal component (PC2) are relative lateral root number and relative root elongation. The resistance of nine plant species to Al toxicity to Al could be clustered into 2 categories by PCA analysis (Figure 4.) namely sensitive categories (ex. S.saman, G. arborea, O. grandiflora, C. peltata, S. campanulata) and tolerant categories (ex. E. cyclocarpum, C.calothyrsus, A. pavonina, and C.inophyllum).
As a result, *S. campanulata* was the most sensitive species to Al. Meanwhile, *A. pavonina* presents the greatest tolerance to Al. PCA conducted Al to visualize and classify character of morphology effect. It was reported by [31] that PCA gave information about which leaf physiological and biochemical features used during the experiment are the most determinant in response to Al toxicity. It is similar to [32] The research was found citrus tolerant could classified base on growth characteristic by PCA.

3.2. Aluminum distribution at root tips

Each type of plant has an avoidance mechanism for heavy metal or metal toxicity in the plant's root system. To observe Al absorbed into epidermal tissue and root bark tissue [14], rapid testing using root staining can indicate the sensitivity of plant roots to Al stress. Hematoxylin staining is a root staining technique to visualize and localize aluminum accumulates as an initial stage indicator in the root apices of the main root or lateral root tissue. The root turning blue when it forms a complex with Al [20] and Thirty seedlings per species were chosen for scoring in the hematoxylin staining assay [13]. Variation the hematoxylin index on nine species from complete staining to no staining showed in Table 2. Hematoxylin staining could be used to differentiate Al sensitive from Al tolerant [33]. All species showed low stained at a concentration of 0.15 mM Al, indicating that they were tolerant to Al in low concentration. It showed that the roots elude Al by rejecting Al with various avoidance mechanisms. Increasing Al concentration to 1 mM seedlings of *E. cyclocarpum, S. saman, C. calothyrsus, A. pavonina*, *G. arborea, C. inophyllum, O. grandiflora, C. peltata, S. campanulata* indicated moderate to tolerant characteristics.

Morphological response due to Al stress is the occurrence of thickening at the tip of the root and root branches. Al can cause root membrane damage, root thickening, rolling, and short [34]. The next treatment of increasing Al concentration shows changes in root character to the absorption of hematoxylin. Al-induced plants have changed in the shape of the root tip and root wall injury exposed by 1 mM concentration (Figure 3). Under the microscope root color turns purple to dark purple and root surface damaged with changes on root shape and root tips.
**Table 3.** Classification of nine tree seedling species based on staining score NO: No staining ≤ 25% (tolerant); PS: partial staining 25% < x ≤ 50% (tolerant); MS: Moderate staining 50% < x ≤ 75% (moderately tolerant); DP: Deep staining > 75% (susceptible).

| Species                 | 0.15mM | 0.5mM | 1mM | 6mM |
|-------------------------|--------|-------|-----|-----|
| *E. cyclocarpum*         | PS     | PS    | DS  | DS  |
| *S. saman*              | PS     | MS    | DS  | DS  |
| *C. calothyrsus*        | PS     | MS    | DS  | DS  |
| *A. pavonina*           | PS     | MS    | MS  | DS  |
| *G. arborea*            | PS     | MS    | DS  | DS  |
| *C. inophyllum*         | PS     | MS    | MS  | DS  |
| *O. grandiflora*        | MS     | MS    | DS  | DS  |
| *C. peltata*            | PS     | MS    | MS  | DS  |
| *S. campanulata*        | PS     | PS    | PS  | MS  |

**Figure 5.** Morphology and Histochemical detection of Al by Hematoxylin assay on roots of nine tropical tree species after 14 days in control and 1 mM Al exposure. Ec: *E. cyclocarpum*; Ss: *S. saman*; Cc: *C. calothyrsus*; Ap: *A. pavonina*; Ga: *G. arborea*; Ci: *C. inophyllum*; Og: *O. grandiflora*; Cp: *C. peltata*; Sc: *S. campanulata*; a (control 0mM) and b (1 mM AlCl₃) concentration.

Root characteristics with the histochemical assay in 1 mM Al showed *A. pavonina*, *C. inophyllum* and *S. campanulata* roots may have the ability to prevent Al from sticking to the root surface (fig. 5). Root absorb aluminum, primary penetrate the epidermis layer of cell walls in the species of *Cryptomeria japonica* whereas in *Pine thunbergii* Al penetrates deeper into cortical and lumen cells [35]. The primary effect of aluminum toxicity was the restriction of root growth. Color density at the root after staining using Hematoxylin dye shows the amount of absorption at the root surface and root tip. Hematoxylin staining may determine the characteristics of the root type whether it is an exclusion or inclusion. The dark blue or purple in root indicates that the root absorbs much Al on the surface or at the root tip. While light-colored roots indicate that the roots may no absorbed aluminum. This indicates that dark plant roots tend to absorb Al into the tissue and this shows the inclusion mechanism. Meanwhile, bright-colored in roots are indicate that the roots have the ability to avoid Al toxicity and indicate that the plant has a rejection mechanism or exclusion mechanism. The mechanism involved may be an external tolerance exclusion mechanism, by preventing Al from entering into symplast, immobilization by sensitive metabolic parts through cell wall, selective permeability of the plasma membrane, pH barrier in the rhizosphere and root apoplast, and exudation of chelating ligands (exudation of organic acids), phosphorus exudation and Al efflux [14,36,37].
Root damage correlates with Al accumulation in root tip so that the detection of Al at the root tip is histologically step in knowing Al toxicity in plants. As shown in Figure 3, the root color is getting darker and the roots look damaged at the root tips and root walls. Root damage correlates with Al accumulation in the root tip. Ruptures along transverse root were proposed relate to the binding of Al to the cell wall thus increasing cell wall rigidity and decreasing elasticity [15,20,37].

As can be seen at Figure 3 and Table 3, Al concentration in S.saman, C. calothyrsus, A. pavonina, G. arborea, O. grandiflora, C. peltata root were exposed by Al at 1mM and 6 mM, absorbed more hematox dye than E. cyclocarpum and S. campanulata.

3.3. Aluminum concentration in plant tissue

After 35 days to Al exposure, each species showed differences in Al absorptions of Al, except S. saman. Figure 4. Illustrates Aluminum uptake in all species increased with increasing Al concentration in the treatment. Concentration Al in S. saman and C.inophyllum tissue treated with 2 mM Al are 380.23 and 378.62g Kg\(^{-1}\) respectively. Furthermore, in the same treatment Al accumulated in A.pavinina and O.grandiflora tissue are 2389.65 and 2018.65 g Kg\(^{-1}\) respectively. It was five to six times compared to S. saman and C.inophyllum.

![Figure 6](image-url)

**Figure 6.** Total aluminum uptake in plant tissue of nine species after 35 days exposure to 0, 1, 2 mM Al\(^{3+}\). Bar denote SE (n=3). Different letters within the column of each plant species indicate significant difference (P<0.05) by DMRT test.

There was no significant difference in total Al concentration in S. saman at Al exposure 1 and 2 mM Al. Aluminum concentration in S. saman and C.inophyllum tissue lower than other plant tissue. It was apparent that S. saman and C.inophyllum could alleviate Al into root tissue. According to [38,39] plant may refuse Al toxicity by avoidance (Al exclusion) and/or tolerant mechanism (accumulate and detoxification of Al inside the cell). For exclusion mechanism, Al detoxication happens in plant rhizospheres through the exudation of organic acid anions. As a result, it may S.saman has an ability to reject aluminum from the root by aluminum resistance mechanism. Reported by [7,24] E. camaldulensis avoids Al uptake through the production oxalate as an organic ligand, which could form complexes with Al\(^{3+}\), conferring protection to the plant roots.

On the contrary, although Al concentration in A. pavonina tissue was high, this species could grow for 35 days without any stress symptoms. This result indicates that A. pavonina species is a tolerant species of Al stress with avoidance mechanism and able to absorb high doses Al and store into tissue or cell. [25] Avoidance mechanisms may occur in the cell wall to chelate Al and block its entry through the cell membrane. These mechanisms include the complexation of Al by lignin, the exudation of polysaccharides that bind Al in the cell wall (i.e., pectin), and sequential citrate rinses to remove
exchangeable Al in the apoplast. It has been reported that [40] there were three different schemes plants deal with Al toxicity: root tip Al exclusion, Al tolerance based on Al sequestration, and Al transport from the root to shoot for subsequent sequestration in leaf cell vacuoles.

4. Conclusion

Stress of Aluminum caused negative effects on root of E. cyclocarpum, S. saman, C. calothyrsus, A. pavonina, G. arborea, C.inophyllum, O. gradiflora, C. peltata and, S. campanulata at AlCl₃ concentration above 4 mM. Therefore, root properties were stronger affected by Al than other properties. Aluminum at low concentration (below 2 mM) could induce plant growth, mostly in all growth characteristic (root elongation, lateral root number, plant height increment, and biomass). A.pavonina and C.inophylum were no affected by stress Al in the biomass. Based on the PCA analysis, these nine species clustered into 2 groups, for instance: E. cyclocarpum, A. pavonina, C.inophylum as tolerant plants); S. saman, C. calothyrsus: G. arborea, O. gradiflora, C. peltata, S. campanulata as a sensitive plant. Plant species differ in their response to elevate Al into the root. A. pavonina seems has an avoidance mechanism to Al stress. Therefore, high Al concentration in root did not affect the plant growth. On the other hand, O.gradiflora and S. campanulata absorbed Al into root and showed sensitive to Al toxic. C.inophylum and S.saman have low concentration of Al in root tissue. It is indicated that they have tolerant exclusion mechanism to Al.

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