

Preliminary Investigation of Growth and Physiological Characteristics of Sago Palm, Rattan and Yatay Palm against Aluminum Stress under Acidic Conditions

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Abstract

Although Al toxicity is a major factor limiting plant growth in acid soil, some plant species have developed several mechanisms to against Al stress. In the present study, we investigated the growth and physiological characteristics as well as the Al localization in plant tissues of sago palm, rattan and yatay palm to evaluate the Al resistant ability of the three species under acidic conditions. Seedlings were grown in media containing levels of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ corresponding in 0, 10 and 200 ppm Al, at pH 3.6. The results showed that sago palm had a comparatively high resistance to Al stress, maintaining rates of both plant length and net leaf product better than yatay palm and rattan. The macronutrients in whole plants of the three species decreased under the 200 ppm Al treatments, especially P, Ca^{2+} and Mg^{2+} . However, some macronutrients, such as N and K^+ , tended to decrease or increase differently depending on the palm species and Al concentration in the growth media. In addition to the Al^{3+} concentration in whole plants, it is likely that the Al resistance of yatay palm is due to its capacity as an Al-accumulator, whereas the Al resistant ability of sago palm and rattan can mainly be attributed to Al-exclusion, which sago palm has a higher Al resistance via exclusion than rattan. Moreover, Al localization of sago palm roots was observed only in the epidermal tissue layer, which would indicate that the radial transport of Al might be restricted by the development of suberin lamellae in the cell wall between the epidermis and hypodermis.

Keywords: aluminum localization; aluminum resistance; rattan; sago palm; yatay palm

1. Introduction

The aluminum cation, Al^{3+} , is toxic to many plant species and can inhibit plant growth at micromolar concentrations. The most commonly observable symptom of Al injury is the inhibition of root elongation, which can be recognized within hours of exposure to Al (Lazof *et al.*, 1994). Since Al is not a plant nutrient, it is not thought to be taken up by an active transport mechanism. Rather, it appears to enter the root cells passively by osmosis or with the flow of transpiration water, possibly through damage to the root membrane (Brady and Weil, 2002). Root growth inhibition by Al toxicity has been found in rice (Kikui *et al.*, 2005), maize (Victor and Zobel, 1998) and wheat (Ohki, 1985). However, one of the most important effects of Al on plant growth is the inhibition of nutrient uptake (Taylor, 1988). An inhibition of the nutrient uptake caused by Al has been reported for several essential elements, including Ca, Mg and K (Baligar and Smedley, 1989). The uptake of these elements was affected directly through antagonistic inhibition or precipitation and indirectly through phenomena such as disordering of the membrane functions (Osaki *et al.*, 2003). In addition, the common responses of shoots to Al include: reduction of stomatal aperture, decreased photosynthetic activity leading to chlorosis and necrosis of leaves, decrease in leaf numbers and a decrease in shoot biomass (Thornton *et al.*, 1986). Nevertheless some plant species, classified as Al resistant, can adapt to acidic conditions with a high Al

concentration, without showing any signs of toxicity.

Sago palm (*Metroxylon sagu* Rottb.), a starch producing palm, is one of the dominant species in natural peat swamps. Such swamps are poorly drained, highly acidic and generally contain a high level of exchangeable Al. Sago palm is therefore considered to be Al resistant. In our previous studies we found that sago palm maintains a low Al^{3+} concentration in all plant parts, even under 200 ppm Al in the growth media. It could be demonstrated that sago palm has high resistance to Al with mechanical restriction of the excess Al based on Al exclusion ability (Anugoolprasert *et al.*, 2009). However, many different mechanisms have been proposed for Al resistance in different plants both between and within species (Osaki *et al.*, 1997). It is therefore important to analyze the physiological features and morphogenesis which enable sago palm and related palm species to respond to Al stress, to elucidate their Al resistant ability. In the current study, the plant growth, some morphological and physiological characteristics, and nutrient concentrations in plant tissues were investigated. These were used to evaluate the Al resistance of sago palm under acidic conditions in comparison with rattan (*Calamus viminalis* Wild.), which belongs to the same tribe Calameae with sago palm, and yatay palm (*Butia yatay* Becc.), the starch producing palm, that is native to Latin America and belongs to the same family Arecaceae as sago palm.

2. Materials and Methods

2.1 Plant materials and Al treatment

Six seedlings each of sago palm, rattan and yatay palm were collected respectively in Songkhla Province (Southern Thailand), Rayong Province (Eastern Thailand) and Mie Prefecture (Central Japan). The seedlings were transplanted to a 1/5000a Wagner pot filled with vermiculite and Kimura B culture solution containing (μM) 36.5 $(\text{NH}_4)_2\text{SO}_4$, 9.1 K_2SO_4 , 54.7 MgSO_4 , 18.3 KNO_3 , 36.5 $\text{Ca}(\text{NO}_3)_2$, 18.2 KH_2PO_4 and 3.9 FeO_3 (Baba and Takahashi, 1958). The culture solution was adjusted to an initial pH of 5.5 using 1 N HCl before transfer into pots, following Ehara *et al.* (2006). The pots were placed in a greenhouse under natural sunlight and maintained at constant temperatures above 15 °C at Mie University. The culture solution was replenished daily in an amount equal to that consumed.

Seedlings of sago palm, rattan and yatay palm at the second to fourth leaf stages were cultured in Kimura B culture solution without Al (hereafter referred to as the control) and in two solutions of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ at 10 ppm and 200 ppm Al (hereafter referred to as Al-10 and Al-200, respectively). The treatments were conducted over a period of 3.5 months. Two seedlings were used in each treatment. The pH of the culture solution was adjusted to 3.6 using 1 N HCl as required. The culture solution was replenished daily in an amount equal to that consumed and the entire culture solution

was renewed twice weekly to avoid accumulation of excess Al. The pots were placed in the same greenhouse under natural sunlight. An air pump was connected to the pots to provide air for the roots. Plant length, number of leaves and transpiration rates were measured weekly.

2.2 Chlorophyll content of the leaflets

After harvesting, the chlorophyll content of the leaflets at each leaf position was measured using the method of Mackinney (1941). An area of 0.25 cm^2 was punched out and soaked in 10 ml of 80 % (v/v) acetone to extract chlorophyll. The extractions were used to measure the absorbance at the wavelengths 663 nm and 645 nm in a 1-cm cell using a spectrophotometer (UVmini-1240, Shimadzu, Japan). The total chlorophyll concentrations as μg per ml extract were calculated according to the specific absorption coefficient formulas as describe below;

$$\text{Total chlorophyll (a+b)} = 8.02 A_{663} + 20.20 A_{645}$$

In this equation, the letter A refers to the absorption at the wavelength specified in the subscript. The chlorophyll contents were then expressed as μg per cm^2 leaflet area.

2.3 Sampling and analysis of nutrient concentrations in plant tissues

The treated plants were sampled and washed thoroughly in distilled water. The plants were separated into three parts: leaflets, petioles (including rachis) and roots, and the fresh weight of each part was recorded. The leaflet areas were measured using an automatic area meter (AAM-9, Hayashi-Denko,

Japan). The longest root lengths were recorded across all samples. The initial and final root length data were used to calculate the relative root elongation rate, taking as a 100% reference the relative elongation rate of the control plant. In addition, the roots were divided into lateral roots and adventitious roots, classified according to the method of Nitta *et al.* (2002) as follows: adventitious roots are those 6 to 11 mm in diameter and lateral roots are those less than 6 mm in diameter. Adventitious roots were divided into stele and cortex (epidermis, exodermis, suberized sclerenchyma cells).

All samples were dried in an oven at 80 °C for 72 hours to determine the dry weight and then ground into powder in order to analyze ion concentrations. The ground samples were reduced to ash in a furnace and extracted with 1N HNO₃, and the K⁺, Ca²⁺ and Mg²⁺ concentrations were determined using a high-performance liquid chromatograph (HPLC) with a conductivity detector (IC-C3, CDD-6A, Shimadzu, Japan). The concentration of P was evaluated by atomic absorption spectrophotometry. The total N concentration was determined by the semi-micro Kjeldahl method, while the Al³⁺ concentration was determined calorimetrically by the aluminon method.

2.4 Al localization in plant tissues

To analyze the localization of Al in the plant tissues, a hematoxylin staining method adapted from the technique of Polle *et al.* (1978) was used. Root and leaflet samples were washed in distilled water for 15-30

minutes and then placed in a hematoxylin solution for approximately 20 minutes. The aqueous hematoxylin solution was prepared from 0.2 % (w/v) hematoxylin (C₁₆H₁₄O₆•H₂O) and 0.02 % (w/v) potassium iodate (KIO₃) dissolved in 100 ml of distilled water. After staining, the excess hematoxylin was removed with distilled water (15-20 minutes). Tissues samples were immobilized within artificial pith wrapped with palafilm tape, which was freehand cross sectioned (40-60 µm in thickness for leaflet and 60-80 µm in thickness for root) using a plant microtome (MTH-1, NK system, Nippon Medical and Chemical Instruments, Japan). The plant sections were observed under a light microscope and images were recorded using Axio Vision software (Release 4.5, SP1, 2006).

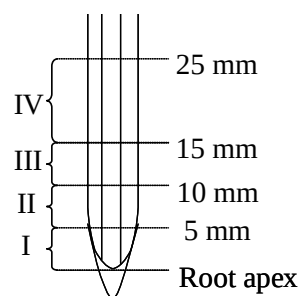


Figure 1 The position of root section for the observation under a fluorescence microscope.

2.5 Staining lignin and suberin in root tissues with berberine-aniline blue

Root samples were sectioned as described above, at various distances from the root tip (Fig. 1). The staining procedure was

performed according to the method of Brundrett *et al.* (1988). The sections were stained with 0.1 % (w/v) berberine hemi-sulphate, counter-stained with 0.5 % (w/v) aniline blue and then observed under both white and UV light and the images were recorded using the same Axio Vision software.

3. Results and Discussion

3.1 The effect of Al concentrations on the growth of sago palm, rattan and yatay palm

New leaf emergence was observed in all the seedlings of sago palm, rattan and yatay palm, even under the highest Al treatment (Table 1). In sago palm and yatay palm, the rate of new leaf production was higher under the 10 ppm Al treatment than the control. Under the 200 ppm Al treatment, rates of new leaf production were lower than both the 10 ppm Al treatment and the control, for both sago palm and yatay palm. In rattan, no change was observed across the three Al conditions. It appears that the emergence rate of new leaves of sago palm and yatay palm was affected by the Al concentrations in the growth media, while this tendency was not observed in rattan. The rate of leaf senescence in rattan apparently increased under the 200 ppm Al treatment, whereas the rate of leaf senescence in sago palm and yatay palm was almost constant across treatments. These results indicated that the acceleration of leaf senescence at the highest Al treatment was

apparent only in rattan. Considering the current results for leaf emergence and senescence, the growth response via the leaf formation of the three species to Al stress was different. In the 10 ppm Al treatment, the net leaf production of sago palm and yatay palm increased by 33 and 18 %, respectively, compared with the control, while the net leaf production of rattan was almost the same in the control and 10 ppm Al-treated groups. However, the net leaf product of sago palm, rattan and yatay palm decreased 33, 56 and 36 % respectively under the 200 ppm Al treatment. It thus appears that sago palm may be comparatively resistant to Al stress, which allows it to maintain net leaf product, in contrast to yatay palm and rattan.

The increment of plant length of sago palm in the 10 ppm Al treatment was similar to that in the control, but decreased by about 47 % under the 200 ppm Al treatment. In the case of rattan, the incremental length decreased by about 24 and 54 % under the 10 and 200 ppm Al treatments respectively, compared with the control. A similar tendency toward a decrease in the incremental length was observed in yatay palm, which decreased by about 18 and 63 % under the 10 and 200 ppm Al treatments respectively, compared with the control. Based on this result, sago palm appears to be relatively resistant to Al stress, compared with the other palm species (Table 1).

In terms of root elongation, sago palm, rattan and yatay palm showed higher elongation rates in the 10 ppm Al treatment than that in the control (Table 1). From these

results, it appears that mild Al concentrations in the growth media tend to stimulate root growth in all three species, which is a similar tendency to that observed in tolerant genotypes of corn (Clark, 1977). However, the root elongation rate of all three species was depressed under the 200 ppm Al treatment. In addition, the root elongation rates of sago palm decreased by a greater amount than those of yatay palm and rattan, while yatay palm maintained the

greatest root elongation rate. In general, plant species have developed many mechanisms to tolerate increased Al concentrations. These include a high internal tolerance to Al, and an internal detoxification or exclusion of Al uptake by exudation of organic acids followed by the formation of less toxic Al complexes in the plant cells, allowing plants to modify the pH of the root interface (Jones, 1998).

Table 1 Growth parameters, chlorophyll content and transpiration rate of sago palm, rattan and yatay palm under different Al treatments.

Parameters	Treatment	Sago palm			Rattan			Yatay palm		
		Seedling 1	Seedling 2	Mean	Seedling 1	Seedling 2	Mean	Seedling 1	Seedling 2	Mean
Leaf number Expanded and new leave (A)	Control	6.0	8.0	7.0	9.0	9.0	9.0	6.0	6.0	6.0
	Al-10	9.0	10.0	9.5	9.0	9.0	9.0	6.0	7.0	6.5
	Al-200	5.0	6.0	5.5	9.0	9.0	9.0	4.0	4.0	4.0
Senescent leave (B)	Control	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	0.5
	Al-10	2.0	1.0	1.5	1.0	1.0	1.0	0.0	0.0	0.0
	Al-200	2.0	1.0	1.5	5.0	6.0	5.5	1.0	0.0	0.5
Net leaf product (A-B)	Control	5.0	7.0	6.0	8.0	8.0	8.0	6.0	5.0	5.5
	Al-10	7.0	9.0	8.0	8.0	8.0	8.0	6.0	7.0	6.5
	Al-200	3.0	5.0	4.0	4.0	3.0	3.5	3.0	4.0	3.5
Increment of plant length (cm)	Control	27.2	35.2	31.2	29.0	17.8	23.4	19.0	12.6	15.8
	Al-10	27.7	36.8	32.3	14.2	21.3	17.8	12.6	13.2	12.9
	Al-200	14.2	19.0	16.6	8.3	13.1	10.7	8.0	3.8	5.9
Root elongation rate (% of control)	Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Al-10	110.3	143.2	126.8	117.9	90.6	104.3	153.3	83.7	118.5
	Al-200	14.9	14.6	14.7	44.4	62.6	53.5	113.3	83.0	98.1
Chlorophyll content ($\mu\text{g cm}^{-2}$)	Control	41.8	51.3	46.5	32.5	51.2	41.8	83.7	69.2	76.4
	Al-10	47.8	60.1	53.9	53.9	33.6	43.8	76.4	67.4	71.9
	Al-200	29.2	33.9	31.5	25.9	34.9	30.4	65.9	48.7	57.3
Transpiration rate ($\mu\text{g cm}^{-2} \text{h}^{-1}$)	Control	4.6	6.6	5.6	3.5	3.6	3.6	7.5	8.6	8.1
	Al-10	4.6	4.6	4.6	4.2	3.5	3.9	8.2	7.0	7.6
	Al-200	4.9	4.4	4.6	2.4	3.2	2.8	9.9	7.8	8.8

3.2 The effect of Al concentrations on the physiological characteristics of sago palm, rattan and yatay palm

The chlorophyll content per unit leaflet area at almost all leaf positions slightly decreased with the rise of Al concentration in the growth media (data not shown). In addition, the mean values of chlorophyll content at different upper, middle and lower leaf positions of all three species tended to decline with the highest Al concentration in the growth media (Table 1). According to Marschner (1995), Al toxicity inhibits many metabolic processes including synthesis of nucleic acids and enzymatic activity. For example, high Al concentrations lead to a decrease in the chlorophyll content in the leaves and this in turn depresses the photosynthetic rate. From the current results, it is speculated that the reduction in chlorophyll content under the highest Al treatment may affect photosynthetic capacity, which at least partly accounts for the decreased growth rate of all three species.

Transpiration rates were measured at the end of the Al treatment period (Table 1). In sago palm, the transpiration rate decreased by 17% under both the 10 and 200 ppm Al treatments, compared to the control. The transpiration rate of rattan increased by 9 % under the 10 ppm Al treatment but decreased by 22 % under the 200 ppm Al treatment, compared to the control. In yatay palm, the transpiration rate of the 10 ppm Al-treated plants was lower by 6 % than control and that of the 200 ppm Al-treated plants was higher by

10 %. Changes in transpiration rates under Al stress have been reported in many plant species (Schlegel and Godbold, 1991; Zhang *et al.*, 2007; Ali *et al.*, 2011). Ohki (1985) observed that transpiration rates in *Triticum aestivum* decreased, while those in *Sorghum bicolor* increased after exposure to Al. These contradictory results demonstrate that the effect of Al on the transpiration rate is quite complex. From the current study, it appears that the change in the transpiration rate reflects the Al concentration in the growth media and varies among the three species.

3.3 The effect of Al concentrations on the nutrient concentrations in different plant parts of sago palm, rattan and yatay palm

Table 2 shows the effect of Al concentration on nutrient concentrations in the leaflets, petioles, roots, dead leaves and whole plants. The Al^{3+} concentration in all plant parts of the three species increased with the rise of the Al concentrations in the growth media. Roots of the three species were obviously exposed most directly to Al stress in the growth media. In addition, the Al^{3+} concentration in all root parts of the three species tended to increase with increasing of the Al treatments (Fig. 2). In addition to the comparison of the Al^{3+} concentrations in the stele and cortex of adventitious roots between sago palm and rattan (belong to the same tribe) under the 200 ppm Al treatment, the Al^{3+} concentration in the root stele of sago palm was apparently lower than the value in the cortex samples. In contrast, Al^{3+} concentrations in rattan were

approximately equal in stele and cortex of adventitious roots. It is likely that sago palm

might have a mechanism to restrict the influx of Al^{3+} from the cortex into the stele.

Table 2 Nutrient concentrations in different plant parts and whole plants of sago palm, rattan and yatay palm under different Al treatments.

Plant part	Sago palm			Rattan			Yatay palm		
	Control	Al-10	Al-200	Control	Al-10	Al-200	Control	Al-10	Al-200
	----- Al^{3+} ($\mu\text{mol g}^{-1}$) -----								
Leaflet	13.7	14.7	15.2	10.7	11.0	16.0	11.2	13.9	22.4
Petiole	14.0	15.2	20.9	11.4	11.6	25.0	11.3	14.0	42.7
Root	14.4	15.6	22.8	12.4	12.4	23.4	14.8	15.3	35.2
Dead leaf	18.0	31.2	40.2	16.0	27.8	65.4	14.0	-	38.8
Whole	13.9	15.1	19.0	11.3	11.5	21.8	11.9	14.2	33.5
	----- N (mg g^{-1}) -----								
Leaflet	21.1	20.5	14.8	14.7	25.5	34.8	11.6	10.2	8.5
Petiole	9.2	9.2	10.2	10.5	18.7	22.2	10.7	7.0	5.1
Root	8.1	10.3	6.3	8.4	16.4	19.1	6.6	5.0	1.2
Whole	14.7	14.2	12.4	12.1	21.3	26.8	10.3	8.0	5.8
	----- P (mg g^{-1}) -----								
Leaflet	2.6	1.8	1.8	4.0	2.3	1.2	3.6	3.4	-
Petiole	3.7	2.4	1.3	5.7	2.4	0.8	3.0	3.0	-
Root	1.8	1.6	-	2.1	1.0	0.6	-	-	-
Whole	2.8	2.0	1.5	4.2	2.1	0.8	3.3	3.2	-
	----- K^+ ($\mu\text{mol g}^{-1}$) -----								
Leaflet	218.0	200.6	364.5	230.9	262.7	115.4	305.1	194.4	102.5
Petiole	599.1	470.3	719.4	431.7	331.9	367.3	415.7	224.7	52.6
Root	596.5	578.6	518.8	447.3	319.4	226.1	376.6	215.6	25.7
Whole	425.8	377.3	531.2	339.8	300.0	227.8	357.6	210.9	63.2
	----- Ca^{2+} ($\mu\text{mol g}^{-1}$) -----								
Leaflet	64.6	59.9	27.6	89.9	75.1	29.2	79.2	74.2	79.0
Petiole	133.0	93.2	50.0	107.2	86.6	62.8	66.9	58.7	25.6
Root	47.5	47.5	27.6	35.4	31.1	18.0	30.7	27.1	12.5
Whole	87.0	69.0	38.2	87.8	70.9	35.1	65.4	60.2	41.1
	----- Mg^{2+} ($\mu\text{mol g}^{-1}$) -----								
Leaflet	90.8	61.0	52.1	123.5	88.7	21.5	77.5	79.9	42.2
Petiole	163.4	130.6	52.5	186.9	139.7	43.5	110.3	97.5	37.7
Root	116.9	158.3	88.7	194.3	119.7	43.5	86.8	83.6	28.6
Whole	122.8	106.5	60.2	158.9	113.5	35.9	90.8	88.1	37.7

Values represent the mean of two plants. - = No measurement obtained.

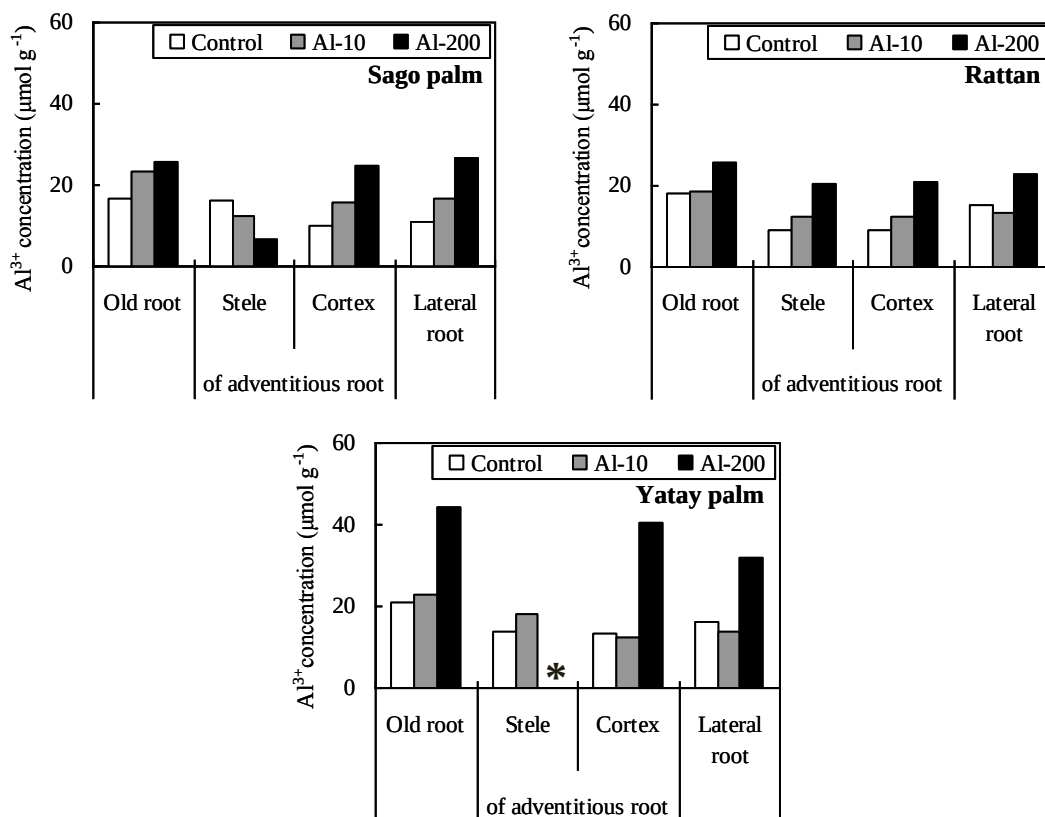


Figure 2 Al^{3+} concentration in different parts of old roots and new roots (stele and cortex of adventitious roots and lateral roots) of sago palm, rattan and yatay palm under different Al treatments. Data represent the mean of two plants. (* = No measurement obtained)

The Al^{3+} concentration in the leaflets of sago palm was lower than that in the petioles and tended to be highest in the roots across all Al treatments. Contrarily, yatay palm and rattan showed higher Al^{3+} concentration in the petioles than in the roots or leaflets. However, all three species tended to accumulate the Al^{3+} in dead leaves rather than in other plant parts. The highest Al^{3+} concentration in the whole plant (including leaflets, petioles and roots) under the 200 ppm Al treatment was $33.5 \mu\text{mol g}^{-1}$ dry weight

(903.9 mg kg^{-1}), recorded for yatay palm. Sago palm and rattan showed lower concentrations of 19.0 and $21.8 \mu\text{mol g}^{-1}$ dry weight (512.7 and 580.2 mg kg^{-1} dry weight), respectively (Table 2).

In previous experiments, the Al concentration in the culture solution used to examine the effect of Al on plant growth has ranged from 4 to 173 ppm for upland rice (Fageria and Carvalho, 1982), wheat (Ohki, 1985), snapbean (Miyasaka *et al.*, 1991) and tea plant that is one of the most Al tolerant

crop species (Konishi *et al.*, 1985). However, it should be noted that some of the Al added to the culture solution is lost by precipitation or polymerization, so free Al concentrations are much lower than applied concentrations. According to Zhang *et al.* (2007), Al toxicity was identified as a critical value of the Al concentration for crop management. The critical value of the Al concentration for plants grown in hydroponic systems were evaluated for many plant species, such as 1.5 mg l⁻¹ Al for oat (*Avena sativa*), 0.8 mg l⁻¹ Al for barley (*Hordeum vulgare*) and 8.9 mg l⁻¹ Al for maize (*Zea mays*) (Foy and Brown, 1964). Comparing the Al critical value and the Al concentrations used in those experiments to the growth response of sago palm, rattan and yatay palm recorded in the present study, all three species investigated here could be classified as Al resistant species. From the report of Chenery (1948), the thousands of plant species were classified by the Al concentration in plant tissues to sub-divide Al resistant species into Al-accumulators ($\geq 1,000$ mg Al kg⁻¹ dry weight) and Al excluders ($< 1,000$ mg Al kg⁻¹ dry weight). Most plants contain no more than 300 mg Al kg⁻¹ dry weight. The whole plant Al concentrations recorded in this experiment suggests that sago palm and rattan can be classified as Al-excluders, with sago palm exhibiting a higher Al resistance than rattan under acidic conditions. Contrarily, yatay palm is nearly an Al-accumulator.

In all three species, N concentration in the leaflets was higher than that in the

petioles and roots. The N concentrations in the whole plants of sago and yatay palm decreased with increasing Al concentrations in the growth media. This contrasts with rattan, where the N concentrations in the whole plants increased with increasing Al concentrations in the growth media (Table 2). It appears that the change in the N accumulation in plant tissues of sago palm and rattan was different, although rattan belongs to the same tribe Calameae as sago palm. Nichol *et al.* (1993) also observed the effect of Al on the different N sources: ammonium (NH₄⁺) and nitrate (NO₃⁻). According to these authors, Al inhibits the influx of NH₄⁺ but enhances the influx of NO₃⁻, which suggests that the results are consistent with a mechanism whereby Al binds to the plasma membrane phospholipids, forming a positively charged layer that influences ion movement to the binding sites of the transport proteins. A positively charged layer will retard the movement of cations and increase the movement of anions in proportion to the charge carried by these ions. These effects may inhibit the influx of cations but stimulate the influx of anions.

The P concentration in all plant parts and whole plants of sago palm and rattan tended to decrease under the higher Al treatments (Table 2). Although the P concentration of yatay palm under the 200 ppm Al treatment is not reported because of the small number of samples. The results from 10 ppm Al-treated plants suggest that the P concentration in the whole plant might be

depressed by the Al concentration in the growth media. According to Bollard (1983), the Al sensitivity of plants grown under acidic conditions is closely related to depressed P uptake, which is also observed in upland rice cultivars (Fageria and Carvalho, 1982) and barley (Loboda and Wolejko, 2006). Although sago palm and rattan belong to the same tribe Calameae, the P concentration in the 200 ppm Al-treated rattan and sago palm decreased by 45% and 80%, respectively, compared with the control plants, suggesting that rattan is more susceptible to P uptake than sago palm.

The K^+ concentration in plant parts and whole plants of sago palm was lower under the 10 ppm Al treatment than the control and highest under the 200 ppm Al treatment, which suggests that the K^+ uptake is independent of the increase in Al concentration in the growth media. Contrarily, the K^+ concentration in plant parts and whole plants of rattan and yatay palm tended to decrease as the Al concentration in the growth media increased. The K^+ concentration in the whole plants of rattan and yatay palm decreased by 32% and 82% respectively under the 200 ppm Al treatment compared with the control (Table 2). This tendency toward a decrease of K uptake under Al stress is in agreement with results reported for rice (Fageria, 1985) and oil palm (Cristancho *et al.*, 2011). Wagatsuma *et al.* (1987) also report that the K content in the roots decreases with an increase in Al content, attributed to the leakage of K from the roots as

a result of the destruction of the plasmalemma by the Al.

In sago palm and rattan, Ca^{2+} and Mg^{2+} concentrations under Al treatment were highest in the petioles, followed by the leaflets and roots, while concentrations in yatay palm were higher in the leaflets than in the other parts. Ca^{2+} and Mg^{2+} concentrations in all plant parts decreased as Al concentrations in the growth media increased across all three species. This was most clearly observable in the 200 ppm Al treatment, where Ca^{2+} concentrations in whole plants were lower by 56% (sago palm), 60 % (rattan) and 37 % (yatay palm) compared to the control, while Mg^{2+} concentration decreased by 51 % (sago palm), 77% (rattan) and 59% (yatay palm) respectively. Based on these results, it appears that the accumulation of Ca^{2+} and Mg^{2+} in rattan was depressed at the highest Al treatment more than in sago palm and yatay palm. Nevertheless, the Ca^{2+} and Mg^{2+} concentrations in all plant parts of all three species decreased as Al concentration in the growth media increased (Table 2). According to Wagatsuma *et al.* (1987), the reduction of Ca and Mg uptake can be explained by the fact that Al occupies the absorption sites in the free space of the root.

In general, the structural component of chlorophyll is a central magnesium atom surrounded by a nitrogen-containing structure called a porphyrin ring, which has a long carbon-hydrogen side chain attached, known as a phytol chain (Wu and Rebeiz, 1985). The

lower chlorophyll content of sago palm and yatay palm grown under the 200 ppm Al treatment might be related to the decrease of Mg and N concentrations in the leaflets. The decrease of the chlorophyll content of rattan might be due to the reduction of Mg^{2+} concentration in the leaflets, although the N concentration in the leaflets increased in line with Al concentrations. It is likely that N in the leaflets of rattan was not available to act as the N component in the structure of chlorophyll.

3.4 Al localization in plant tissues of sago palm, rattan and yatay palm

Leaflet cross sections were colored using hematoxylin to identify the leaf tissues where Al was concentrated (Fig. 3). In the control and 10 ppm Al treatments, no Al was detected, in contrast to the 200 ppm Al treatment where the purple color formed by the compound of an aluminum haematein complex was found in the leaflets of all three species. In the case of sago palm, Al was detected preferentially in the upper epidermis and occasionally in the lower epidermis. Schnable and Zeiger (1975) found that a concentration of 1mM of Al^{3+} in the culture solution inhibited stomata opening in illuminated epidermal strips of *Vicia faba*, by preventing K^+ accumulation and starch mobilization in the guard cells. Based on the current results, it is likely that sago palm may possess a mechanism to protect important tissues from internal Al toxicity by causing Al^{3+} to preferentially accumulate in the upper epidermis. In the leaflets of rattan under the 200 ppm Al

treatment, Al was detected in the bundle sheath cells: the thick walled cells surrounding the vascular bundle. In addition, Al was also found to be located in the upper and lower epidermis of the rattan leaflets, but Al was observed in the upper epidermis than in the lower epidermis. In the case of yatay palm, an aluminum haematein complex that marked the Al^{3+} accumulation was observed in the inner vascular bundle including the xylem and phloem, and was similarly observed in the upper and lower epidermal layer of the leaflets. There are several reports of preferential Al accumulation in the epidermal cells, spongy parenchyma and vascular bundle within the leaves of many plants (Matsumoto *et al.*, 1976; Haridasan *et al.*, 1986). In most cases, the Al is localized in the upper and lower epidermis. Matsumoto *et al.* (1976) suggest that Al is absorbed from the soil via the roots and then passes into the vessels of the xylem and moves upward to the leaves. At the end of the vein, Al diffuses into the neighboring mesophyll cells, such as the palisade and spongy parenchyma, and passes from one cell to the other by diffusion. Then, Al passes into the epidermal cells where Al is probably deposited and is not metabolically active.

Al localization in roots under the different Al treatments is shown in Fig. 4. In all three species, the 10 ppm Al-treated roots showed weak staining at the root cap, especially in areas where the high Al concentration had caused lesions in the epidermis. In contrast, the 200 ppm Al-treated

roots of all three species were markedly stained at the root cap, epidermis and outer hypodermis. Moreover, Al strongly detected in the root apical region, which also observed in the root of eddo (Kawasaki *et al.*, 2008). However, the degree and chemical composition of the Al localization in the roots varied widely depending on the species. In the observation of 200 ppm Al-treated roots of sago palm, Al reached only the epidermal tissue layer but not the hypodermis or cortex, which would indicate that the radial transport of Al was restricted by

the epidermal tissue layer. In the 200 ppm Al-treated roots of rattan, Al was detected in the epidermal and 1-2 hypodermal tissue layers. This indicated that rattan exhibited the wider spread of Al in the roots than that of sago palm, although both palm species are belonged to the same tribe Calameae. In the 200 ppm Al-treated roots of yatay palm, the strongest staining was evident in areas of the epidermal layer that showed damage, followed by the outer hypodermal tissue layer.

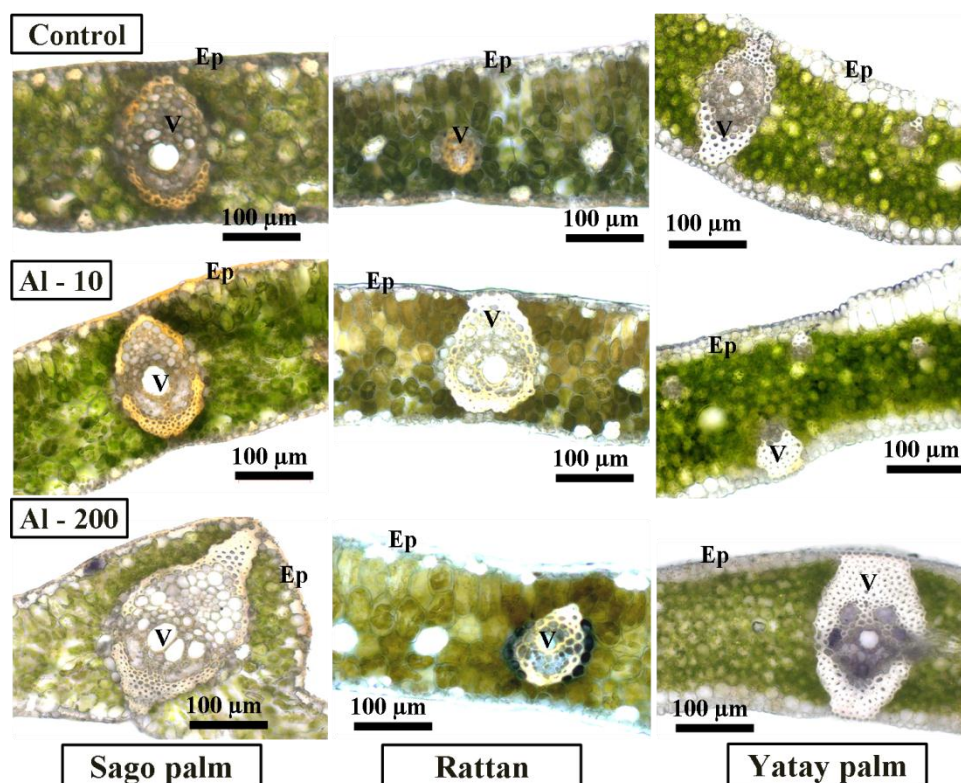


Figure 3 Localization of aluminum by hematoxylin staining in the leaflet cross sections of sago palm, rattan and yatay palm under the Al treatments. (Ep = epidermis, V = vascular bundle)

The structure of the external cortex of adventitious roots was observed under a UV

microscope at various distances from the root tip (Fig. 5). When the root cross sections were

stained with berberine-aniline blue fluorescent staining, an intense bright blue fluorescence indicating the suberin lamellae in the cell wall was observed between the epidermis and hypodermis of the roots of all three species. Fluorescence was also observed in the thickened sclerenchyma cell wall but not in the crowded cortical cells, especially in the observation most distal from the root tip. One interesting feature of these results is that fluorescence increased in line with increasing

Al concentrations in the growth media (Fig. 5). These observations suggest that the suberin lamellae in the cell wall between the epidermis and hypodermis might be the first barrier for the radial movement of Al in the roots of the three species under acid conditions. However, the findings on the localization of Al suggest that sago palm has a more efficient mechanism for restricting the movement of Al^{3+} through the root tissues than rattan or yatay palm.

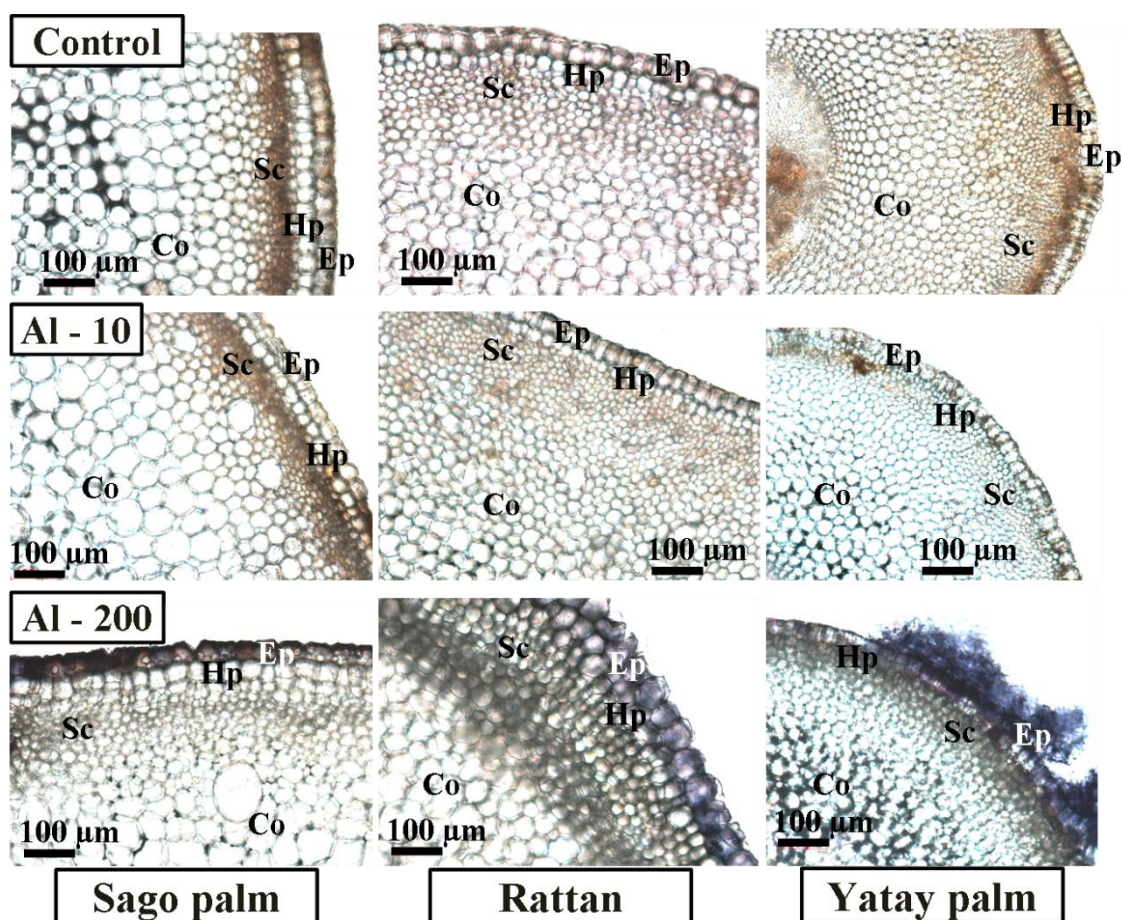


Figure 4 Localization of aluminum by hematoxylin staining in the root cross sections of sago palm, rattan and yatay palm under the Al treatments. (Ep = epidermis, Hp = hypodermis, Sc = sclerenchyma cell, Co = cortex)

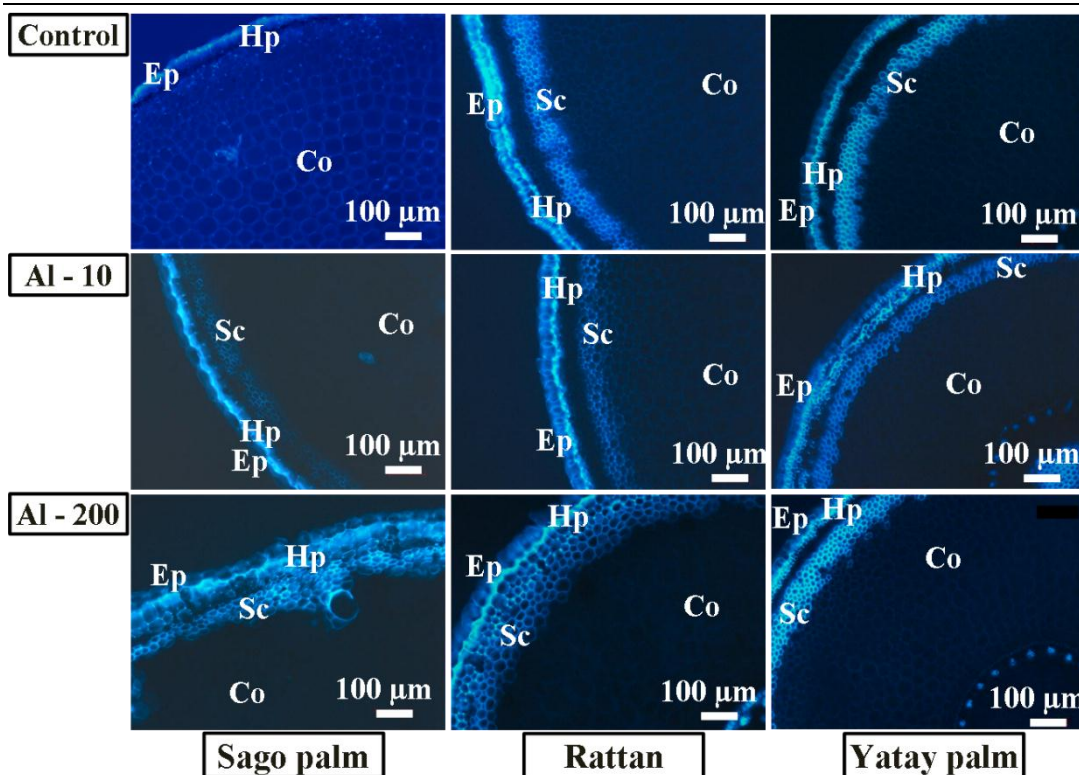


Figure 5 The structure and component of adventitious roots of sago palm, rattan and yatay palm under different Al treatments stained with berberin-aniline blue and observed around the external part of the root cortex at 15-25 mm from the root tip under UV microscope. (Ep = epidermis, Hp = hypodermis, Sc = sclerenchyma cell, Co = cortex)

4. Conclusions

This study has preliminary demonstrated the greater Al resistance of sago palm under acidic conditions than rattan or yatay palm. Based on the growth response of all three species under higher Al treatments, it has been shown that sago palm tend to maintain both net leaf production and increment of plant length better than yatay palm or rattan under conditions of Al stress. Sago palm maintained a lower Al^{3+} concentration in all plant parts, even under 200 ppm Al treatment. In addition, the Al reached only in the epidermal tissue layer

rather than the hypodermis or cortex of the 200 ppm Al-treated roots, which would indicate that the radial transport of Al might be restricted by the development of suberin lamellae in the cell wall between the epidermis and hypodermis. These findings suggest that sago palm may have a high resistance to Al with mechanical restriction of the excess Al based on Al exclusion under acidic conditions.

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