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Effect of Different Aluminum Concentrations in Culture Media on Growth Characteristics of Sago Palm Seedlings

Preuk Chutimanukul[1], Takayuki Inagaki[1], Hitoshi Naito[2], Masakazu Goto[1] and Hiroshi Ehara[3,*]

[1] Graduate School of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie 514-8507, Japan

[2] College of Life Sciences, Kurashiki University of Science and The Arts, 2640 Nishinoura, Tsurajima-cho, Kurashiki, Okayama 712-8505, Japan

[3] International Cooperation Center for Agricultural Education, Nagoya University, Nagoya 464-8601, Japan

Corresponding author: H. Ehara (ehara@agr.nagoya-u.ac.jp)

Abstract: Three levels of aluminum concentration ($AlCl_3 \cdot 6H_2O$) were added to a Kimura B culture solution: 0, 150, and 300 ppm with 3 replications. The culture medium pH of all treatments was adjusted to 3.5. There were no differences in plant height, plant length, or base diameter among the three treatments. No significant difference was seen in the leaf number per plant, leaflet number per plant, leaflet number per leaf, or number of emerged leaves per plant among the three treatments. Root diameters and dry matter weights of roots and whole plants were significantly higher in non-Al-treated (0 ppm Al) sago palms. The number of dead leaves, SPAD value, and chlorophyll content were significantly higher in Al-treated (150 and 300 ppm Al) sago palms. The dry matter weight of leaflets, petioles, and bases were not significantly different among the three treatments. There was marked significant difference in the total leaflet area per plant between 0 and 300 ppm Al. The difference in a single leaflet area was negligible among the three treatments. The relative growth rate (RGR) and net assimilation rate (NAR) tended to be slightly deceased with Al treatments; however, the leaf area ratio (LAR), specific leaf area (SLA), and leaf weight ratio (LWR) showed same levels in all three treatments. Stomatal conductance was significantly lower in Al-treated than in the non-Al-treated sago palms. The photosynthetic rates and transpiration rates were not significantly different with the three treatments. Moreover, the uptake of P, Ca, and Mg was interrupted in the Al treatments; however, N and K uptake were not affected by Al treatment. The root color darkened with Al treatments, and was more distinct with higher Al concentrations. There was a significant difference in the number of root cells per mm^2 in the transverse section. From these data, it was clear that sago palm seedlings did not show obvious differences in morphogenesis of the top parts; however, its root diameter and cell differentiation in the cortex of the root were inhibited by high Al concentrations in the media.
Keywords: acidity, aluminum, growth analysis, *Metroxylon sagu*, nutrient concentration in plants

Introduction

Generally, acidic soils cover as much as 30–40% of the world's arable land area and up to 70% of the world's potentially arable land (Haug, 1983). However, it is usually extremes of acidity that cause environmental problems leading to difficulty growing plants. An important problem is various soil chemical factors, such as Al, Mn, and other cations in acid soils (Foy, 1992), that limit plant growth and reduce crop production and yield. One of these is aluminum (Al), a light metal that makes up 7% of the earth's crust and is the third most abundant element after oxygen and silicon (Ma et al., 2001). In general, aluminum toxicity has been recognized as an important growth-limiting factor of plant productivity in acid soils. The main cause of aluminum toxicity is the dramatic inhibition of root growth. Several studies have reported the effects of aluminum on plants. For example, Clarkson (1965) reported that decreased root growth is a result of the inhibition of cell division.

Ryan et al. (1993) recognized the root apex as a primary site of aluminum-induced injury in plants. However, the aluminum solubility involved in soil pH is such that it increases the availability of biologically toxic Al with decreasing pH (Flis et al., 1993). However, some plants are better able than others to grow in soils with low pH. Plant tolerance of low soil pH has become extremely important in the agricultural development of the humid tropics because many of those soils have low pH (Kamprath and Foy, 1985; Maranville et al., 1994).

Several tropical crops are economically important as food supplies and sources of energy. The sago palm (*Metroxylon sagu* Rottb.) is an outstanding tropical crop that dominates mainly in permanent freshwater swampy areas or peatlands of Southeast Asia (Sato et al., 1979; Jong, 1995). All parts of sago palms can be used for multiple purposes. Leaflets are used for house thatching, rachises are used for house building, cortices of trunks are used in factories as firewood, pith is used for starch extraction or animal feed as a residue, and starch is used for food production (Flach, 1997; Ehara et al., 2000). Therefore, the sago palm is economically acceptable, environmentally friendly, and promotes a socially stable agroforestry system (Flach, 1997). It is an extremely hardy plant, thriving in swampy, acidic peat soils or submerged in saline soils where few other crops survive, although it grows more slowly in peat soil than in mineral soil (Flach and Schuilling, 1989). The sago palm is expected to enhance agricultural production in lower productivity areas with problem soils.

Anugoolprasert et al. (2012a, b) reported that sago palms can adapt to different soil pH levels ranging from 3.6 to 5.7 in pot cultivation and from 4.3 to 7.0 under natural conditions in South Thailand. However, their paper compared the growth of sago palms under different pH conditions using data collected from different sites, i.e., results from sago palms grown in soils with different parent materials in different environments. On the other hand, Chutimanukul et al. (2014) reported that the growth of sago palms did not decelerate in acid conditions (natural soil) as compared with their growth in neutral soil conditions (calcium carbonate was applied) with the same parent material from experimental procedures at the experimental farm of Haluoleo University in Kendari, Southeast Sulawesi, Indonesia. The previous study, conducted in two plots, showed different levels of Al^{3+} in the soil.

There are a few studies regarding the effect of aluminum on the growth of sago palms (Anugoolprasert et al., 2014); however, information about the morphogenesis and physiological response of sago palms with different aluminum concentrations is still very limited. For the promotion of sago palm cultivation in problem soils, further studies from an ecophysiological perspective should be carried out to understand in greater detail the agronomic features of this rare plant species in severe environments. Thus, we analyzed the growth of sago palm seedlings grown in different aluminum concentrations in a culture media. Our aim is to clarify the effect of different aluminum concentrations in culture media that have the same parent material on the growth rate of the sago palm.

Materials and Methods

1. Plant materials and Al treatment

Fruits of *Manno* type sago palms were collected from Sentani, Jayapura, Province of Papua, Indonesia, on July 11, 2010. Seeds were cleaned by removing their seed coat tissues. The clean seeds were sown in a cell tray consisting of 36 cells (6 x 6 cells; each cell size: 43 mm W x 43 mm L x 40 mm D) filled with vermiculite (Tachikawa Heiwa Nouen Co., Ltd., Kanuma, Tochigi, Japan) and kept in a warm place, such as in an incubator, at 25–28°C for 5–6 months at Mie University, Japan. At the 6th leaf stage after germination, seedlings were transplanted in a 1/5000a Wagner pot filled with 400g of vermiculite. Three levels of aluminum concentration ($AlCl_3 \bullet 6H_2O$)—0, 150, and 300 ppm—with 3 replications were added to the Kimura B culture solution containing (μM) 365 $(NH_4)_2SO_4$, 547 $MgSO_4$, 183 KNO_3, 182 KH_2PO_4,

365 $Ca(NO_3)_2$, and 68 $FeC_6H_5O_7$ (Baba and Takahashi, 1958). Three plants were used for each plot (0, 150, 300 ppm Al). The pH values of the culture media in all concentrations were adjusted to 3.5 using a pH meter (HORIBA, Ltd., Twin pH meter B-212, Kyoto, Japan), with 1.0N H_2SO_4 and 1.0N KOH as suitable and beneficial. Culture solution was added every day, in accordance with the amount of solution consumed, and renewed every two days. During the experiment, a hydroponics system was set up; an air pump was connected to the pots to supply air for the roots in both control and treated plant groups. The pots were placed in the greenhouse under natural sunlight at the Faculty of Bioresources, Mie University. The experimental treatment was conducted from July 1 to August 29, 2014, for a total of 60 days (approximately 2 months). The mean day and night temperatures during Al treatment were 30°C and 22°C, respectively.

2. Plant growth and growth analysis

Plant growth parameters, such as plant height (from the soil surface to the top of the standing palm) and plant length (from the soil surface to the tip of the longest leaf: total length of the shoot), were measured, and the numbers of leaves, leaflets per leaf, dead leaves, and newly emerged leaves were counted every week during the experiment. The sago palm seedlings were sampled 2 times, at the beginning and end of the experiment. Plant samples were divided into four parts: leaflets, petioles with rachises, bases of shoots (the part of the leaf sheath holding the other leaves' sheaths inside), and roots. The diameters of the roots were measured using an LCD Digital Stainless Vernier Caliper Bio-Cal (NK Systems Ltd., Tokyo, Japan), and the leaflet area was measured using an AAM-9 automatic area meter (Hayashi Denko Co., Ltd., Tokyo, Japan). The dry matter weight was measured after drying at 70°C for 72 hr with a hot air oven. Plant growth was analyzed as to its relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), and leaf weight ratio (LWR).

3. Characteristics related to photosynthesis

Leaflets of the most active leaves (the uppermost to third expanded leaf from the top) were used to measure variables.

3.1 SPAD

The Soil and Plant Analysis Development (SPAD) value, indicating chlorophyll content, was determined using a SPAD-502 chlorophyll meter (Minolta Co., Ltd., Osaka, Japan. SPAD values were measured every week for three points: the base, middle, and tips of a leaflet attached to a middle position of the topmost fully developed leaf in each plant after transplanting.

3.2. Chlorophyll content

The chlorophyll contents of the leaflets with each treatment were measured according to the method of MacKinney (1941). An area of 0.25 cm^2 from each leaflet (middle part of a leaflet with the same SPAD measurement) was punched out from each leaf and soaked in 10 ml of 80% (v/v) acetone to extract chlorophyll. The chlorophyll content was expressed as the content per unit leaflet area. The extractions were used to measure the absorbance at 663 nm and 645 nm in a 1 cm cell using a spectrophotometer (Shimadzu UVmini-1240, Kyoto, Japan) at the final sampling.

3.3 Stomatal conductance

Stomatal conductance was measured using a leaf porometer (Decagon Devices, Inc., Pullman, WA, USA) once a week after transplanting.

3.4 Photosynthetic rate and transpiration rate

Photosynthetic and transpiration rates were measured using a portable photosynthetic meter (Analytical Development Company Ltd., LCA-4, Hoddesdon, England) at a saturation irradiance with incident photosynthetically active radiation (P.A.R.) of 800–1000 μmol m^{-2} s^{-1}. Light was provided using the halogen lamp of a KODAK EKTAGRAPHIC AF-

2 slide projector (Eastman Kodak Co., Rochester, NY, USA). The appropriate P.A.R. was obtained by changing the distance between the projector and the leaves at the final sampling.

4. Nitrogen, phosphorus, and ion concentrations in different plant parts

To analyze the total N, P, and ion concentrations, the dried samples were ground to a fine powder in a blender. The amount of total nitrogen (N) was determined by the semi-micro Kjeldahl method; 0.3 g of dried samples, 8 ml of concentrated sulfuric acid (H_2SO_4), and 8 ml of 30% hydrogen peroxide (H_2O_2) were used in the digestion step, followed by the distillation and titration steps, respectively. The phosphorus concentration (P) was determined with a spectrophotometer using the ascorbic acid method. For each dried sample, 0.25 g was extracted using 10 ml of 60% HNO3 at 140 ºC for 9–10 hr. After that, the sample solution was diluted in a measuring cylinder to 25 ml total with 1% HNO_3. The 200-μl sample solution and a 4-ml mixture of regents, including 2.5 M H_2SO_4, 2 mM $C_8H_4K_2O12Sb_2•3H_2O$, 32 mM $(NH_4)6Mo7O24•4H_2O$, and 100 mM $C_6H_8O_6$, were mixed and diluted in a measuring cylinder to 25 ml total with distilled water. The final solution was then examined at an absorbance of 880 nm in a spectrophotometer (Shimadzu UVmini-1240, Kyoto, Japan). The aluminum concentration (Al) was determined by the aluminon colorimetric method. In a muffle furnace (Yamato FO 300, Japan) at 500ºC for 4 hr, 0.05 g of each dried sample was reduced to ash. The sample solution was extracted by 10 ml of 6N HCl at 140ºC for 2 hr. The sample solution was added to 25 ml of 1% HCl and then diluted in a measuring cylinder to 50 ml total with distilled water. The 1 ml of sample solution was mixed together with 10 ml of 20% CH_3COONH_4, 2 ml of 0.2% $C_{22}H_{23}N_3O_9$, and 0.5 ml of 1% $HSCH_2COOH$ and diluted in a measuring cylinder to 50 ml total with distilled water. After that, the final solution was examined at an absorbance of 525 nm in a spectrophotometer (Shimadzu UVmini-1240, Kyoto, Japan). A high performance liquid chromatograph (HPLC) was used to analyze the cation concentration. Next, 0.05 g of the dried samples was reduced to ash in a muffle furnace (Yamato FO 300, Tokyo, Japan) at 350ºC for 2 hr and 450ºC for 8 hr. The sample solution was extracted by 100 μl of 1N HNO_3 and was diluted in a measuring cylinder to 25 ml total with distilled water. Sample solutions were passed through a column, Shim-pack IC-C4 (150 mm l. × 4.6 mm I.D., Shimadzu, Tokyo, Japan), and a guard column, Shim-pack IC-GC4 (10 mm l. × 4.6 mm I.D., Shimadzu, Tokyo, Japan). The eluent was 2.5 mmol/l oxalic acid, run at 40ºC, and 50 µl of injection volume. The flow rate of the mobile phase was 1.0 ml/min. Na^+, K^+, Mg^{2+}, and Ca^{2+} concentrations were detected with a conductivity detector (CDD-10Avp, Shimadzu, Tokyo, Japan).

5. Root morphology and Al accumulation

In each treatment, root samples were sectioned at five distances (1, 2, 3, 4, and 5 cm) from the root tip and fixed to artificial pith wrapped with Parafilm. They were then transverse sectioned into 50 μm thicknesses using plant microtome (MTH-1, NK System, Nippon Medical and Chemical Instruments, Japan). The root sections were observed with a light microscope (Axioplan, ZEISS, Germany), and the images were recorded using a Nikon DS Camera Control Unit DS-L2 (Ver. 3.1). All of the cells in the cortex were counted and averaged per mm^2.

6. Statistical analysis

Statistical differences in the data were determined using Statistical Analysis System (SAS) for Windows v9.0. The effects of different aluminum concentrations were determined by one-way analysis of variance (ANOVA), and the differences among the mean values of the three treatments were determined using Tukey's studentized range test (HSD). Terms were considered significantly different at the 0.05 probability level.

Results

1. Plant growth and growth analysis

In this study, plant heights, plant lengths, base diameters, numbers of leaves per plant, numbers of leaflets per plant, numbers of leaflets per leaf, and numbers of emerged leaves per plant over the 8 weeks of Al treatment were almost the same for all three treatments (0, 150, and 300 ppm Al), and there were no significant differences in these parameters (Table 1). The root diameter (mean value of the data among 5 different distances from the root tip) was significantly decreased in both Al-treated plants (150 and 300 ppm Al) as compared to non-treated plants (0 ppm Al), and dead leaves were significantly increased in both Al-treated plants compared to non-treated plants; however, there was no significant difference between the Al-treated plants. Table 2 shows the dry matter weight of each plant part of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media. Although the dry matter weight in each plant part and the whole plant tended to decrease when the Al concentration increased, significant differences were found only in the root and the whole plant between the non-treated and Al-treated palms. There was a markedly significant difference in the total leaflet area per plant between 0 and 300 ppm Al, whereas there were no significant differences between 0 and 150 ppm Al, and 150 and 300 ppm Al (Table 3).

The parameters of growth analysis were calculated using the averaged value of the dry matter weight of three plants or leaflet area of three plants at the first sampling (just before Al treatment) and data from the

Table 1. Plant size and leaf characteristics of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media

Al treatment	Plant height (cm)	Plant length (cm)	Base diameter (cm)	Root diameter (mm)	Leaf number/plant	Leaflet number/plant	Leaflet number/leaf	Emerged leaves/plant	Dead leaf number
0 ppm	34.6 ± 4.1 a	40.6 ± 4.7 a	2.9 ± 0.4 a	3.06 ± 0.2 a	9.0 ± 0.9 a	41.0 ± 9.6 a	4.3 ± 1.2 a	3.3 ± 0.8 a	0.3 ± 0.6 b
150 ppm	33.9 ± 3.6 a	37.1 ± 2.6 a	2.3 ± 0.4 a	2.34 ± 0.2 b	8.5 ± 0.5 a	29.7 ± 4.0 a	3.5 ± 0.6 a	3.2 ± 0.3 a	1.3 ± 0.6 a
300 ppm	34.3 ± 0.5 a	40.8 ± 3.2 a	2.5 ± 0.3 a	1.97 ± 0.2 b	8.7 ± 0.8 a	31.7 ± 5.7 a	3.7 ± 0.4 a	3.3 ± 0.3 a	2.0 ± 0.0 a

Each value represents the mean ± SD (n=3). Different letters within a column indicate significant differences at the 0.05 probability level, according to Tukey's HSD test.

Table 2. Dry matter weight of each plant part of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media

Al treatment	Dry matter weight (g plant^{-1})				
	Leaflet	Petiole	Base	Root	Whole
0 ppm	3.82 ± 0.50 a	3.14 ± 0.98 a	1.04 ± 0.44 a	3.23 ± 0.25 a	11.24 ± 2.10 a
150 ppm	3.24 ± 0.07 a	2.93 ± 0.10 a	0.90 ± 0.10 a	2.00 ± 0.09 b	9.07 ± 0.61 b
300 ppm	2.94 ± 0.39 a	2.87 ± 0.25 a	0.71 ± 0.14 a	1.95 ± 0.25 b	8.47 ± 0.94 b

Each value represents the mean ± SD (n=3). Different letters within a column indicate significant differences at the 0.05 probability level, according to Tukey's HSD test.

Table 3. Total leaflet area and single leaflet area of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media

Al treatment	Total leaflet area per plant (cm^2/plant)	Single leaflet area (cm^2/leaflet)
0 ppm	629.23 ± 45.53 a	16.66 ± 4.33 a
150 ppm	515.60 ± 31.15 ab	17.47 ± 2.33 a
300 ppm	444.86 ± 18.44 b	14.33 ± 2.83 a

Each value represents the mean ± SD (n=3). Different letters within a column indicate significant differences at the 0.05 probability level, according to Tukey's HSD test.

Table 4. Growth parameters of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media

Al treatment	Growth analysis parameter								
	RGR (mg g^{-1} d^{-1})	NAR (mg cm^{-2} d^{-1})	LAR (cm^2 g^{-1})	SLA (cm^2 g^{-1})	LWR (g g^{-1})	RLGR (cm^2 cm^{-2} d^{-1})	LRGR (mg g^{-1} d^{-1})	SRGR (mg g^{-1} d^{-1})	RRGR (mg g^{-1} d^{-1})
0 ppm	28.88 ± 2.98	0.533 ± 0.048	54.1 ± 1.7	166.1 ± 8.6	0.326 ± 0.015	30.31 ± 3.42	30.41 ± 3.28	26.37 ± 6.97	26.57 ± 1.30
150 ppm	25.46 ± 1.11	0.469 ± 0.052	54.7 ± 5.2	163.1 ± 14.5	0.335 ± 0.009	27.12 ± 2.39	28.56 ± 1.37	24.82 ± 1.92	18.60 ± 0.75
	(88.2%)	(80.0%)	(101.1%)	(98.2%)	(102.8%)	(89.5%)	(93.9%)	(94.1%)	(70.0%)
300 ppm	24.28 ± 1.92	0.469 ± 0.074	52.2 ± 4.7	159.1 ± 15.7	0.328 ± 0.006	24.74 ± 1.37	27.58 ± 1.84	20.60 ± 3.20	18.07 ± 2.20
	(84.1%)	(88.0%)	(96.5%)	(95.8%)	(100.6%)	(81.6%)	(90.1%)	(78.1%)	(68.0%)

Each value represents the mean ± SD (n=3). The values in parentheses indicate relative values to 0 ppm treatment. Abbreviations: Relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), leaf weight ratio (LWR), relative leaf growth rate (RLGR), leaf relative growth rate (LRGR), stem relative growth rate (SRGR), and root relative growth rate (RRGR).

three individual plants at the second sampling (at the end of Al treatment); the mean value of the growth parameters, such as RGR and NAR, was then taken. For these kinds of parameters delivered from growth analysis, the procedures of significance tests are not suitable. Therefore, the mean values from the plots with different Al concentrations were just listed with their standard deviation (n=3) (Table 4). The RGR tended to be slightly decreased with Al treatment. The value of RGR was smaller, 12% with 150-ppm Al treatment and 16% with 300-ppm Al treatment, than that with 0-ppm A1 treatment. The NARs with 150-ppm and 300-ppm Al treatments were slightly smaller than that with 0-ppm Al treatment, the difference of which was about 12%. The LARs were slightly different among the three treatments. As shown in the results of the SLA, with slight differences within about 4% and a single leaflet area with no significant difference among the three treatments, changes in leaflet morphogenesis, such as single leaflet structure, were small. The relative leaf growth rate (RLGR) that is relative leaflet area growth; leaf relative growth rate (LRGR), stem relative growth rate (SRGR) and root relative growth rate (RRGR). These are relative growth rate of dry matter in leaflets, stem (petioles, rachises and base) and roots, respectively, showed similar tendency with RGR change with Al concentration increase. The RLGR, LRGR, SRGR, and RRGR values tended to decrease with higher Al concentrations; however, the RRGR decrease was apparently larger than that of other relative growth parameters. The RRGR decreased by 30% and 32 % with 150 ppm and 300 ppm Al, respectively.

2. Characteristics related to photosynthesis

2.1 SPAD and chlorophyll content

There were significant differences in the SPAD and chlorophyll content per unit leaflet area between non-treated and Al-treated samples (Table 5). As the results show, the SPAD value had a positive relationship with the chlorophyll content, and both parameters were increased with 150- and 300-ppm Al treatments.

Table 5. SPAD value, stomatal conductance, and chlorophyll content of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media

Al treatment	SPAD	Chlorophyll content (μg/cm^2)	Stomatal conductance (mmol m^{-2} s^{-1})
0 ppm	32.2 ± 9.0 b	2.8 ± 0.9 b	42.4 ± 17.5 a
150 ppm	61.5 ± 1.8 a	8.8 ± 0.8 a	25.5 ± 8.7 b
300 ppm	60.4 ± 2.2 a	8.0 ± 0.6 a	20.0 ± 1.9 b

Each value represents the mean ± SD (n=3). Different letters within a column indicate significant differences at the 0.05 probability level, according to Tukey's HSD test.

2.2 Stomatal conductance

The stomatal conductance was significantly lower in the Al treatments than in the non-treatment. Then, there was no significant difference between 150-ppm Al and 300-ppm Al treatments (Table 5).

2.3 Photosynthetic rate and transpiration rate

The photosynthetic rate was not significantly different, and was actually at the same level, with all three treatments (Table 6). Neither was the transpiration rate significantly different among the three treatments, although the values of plants treated with 300-ppm Al treatment were 20% smaller than those of non-treated plants (Table 6).

concentrations at the three treatment levels.

P was accumulated most highly in the base, followed by petiole, leaflet, and root in all treatments. Among the three treatments, the P concentrations in leaflets and bases were slightly decreased even if the Al concentration increased; and there was a significant difference among the three treatments in the petiole, root, and whole plant, with the highest Al-treated palms having the lowest P concentrations.

The K^+ concentrations among parts of sago palm seedlings were significantly different in the roots and petioles from those in bases and leaflets in all treatments in which the K^+ concentration had the lowest accumulation in the leaflet, followed by the base,

Table 6. Photosynthetic rate and transpiration rate of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media

Al treatment	Photosynthetic rate ($\mu mol\ m^{-2}s^{-1}$)	Transpiration rate ($mmol\ m^{-2}\ s^{-1}$)
0 ppm	7.44 ± 0.15 a	1.28 ± 0.11 a
150 ppm	7.51 ± 0.14 a	1.15 ± 0.26 a
300 ppm	7.60 ± 0.07 a	1.03 ± 0.07 a

Each value represents the mean ± SD (n=3). Different letters within a column indicate significant differences at the 0.05 probability level, according to Tukey's HSD test.

3. Mineral and ion concentrations in different plant parts

In this study, nutrient concentrations such as N, P, K^+, Ca^{2+}, Mg^{2+}, and Al^{3+} in the leaflets, petioles (including rachises), bases, roots, and whole plants of sago palm seedlings at different aluminum concentrations 8 weeks after treatment were investigated and are shown in Table 7. The concentrations of N among the parts of sago palm seedlings had quite similar tendencies in 0- and 150-ppm Al-treated plants; in leaflets and petiole it was significantly higher than in the base and root. However, at 300-ppm Al treatment, N concentrations in petioles were not significantly different from those in the bases and roots. In no parts of sago palm seedlings were there significant differences in N

petiole, and root. Among the three treatments, the K^+ concentration was not significantly different in leaflets, petioles, bases, roots, and whole plants under Al stress.

The Ca^{2+} concentrations in parts of sago palm seedlings treated with 0 ppm Al were not significantly different; however, in 150- and 300-ppm Al-treated plants, there were significant differences between leaflets and other parts: petioles, bases, roots, and whole plants, in which both leaflets of Al-treated plants were markedly decreased. Among the three treatments, the Ca^{2+} concentration with 300-ppm Al treatment was significantly different in all parts as compared with 0-ppm Al treatment. There were no significant differences in bases and roots of the 150-ppm Al treatment as compared to those of the 0-ppm Al treatment.

Mg^{2+} concentrations in plant parts of sago palm

Table 7. Nutrient concentrations in each part of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media

Nutrient concentration	Al treatment	Plant part				
		Leaflet	Petiole	Base	Root	Whole
N (mg g^{-1})	0 ppm	18.2 ± 1.6 aA	14.2 ± 1.7 aA	4.7 ± 4.2 aB	7.4 ± 1.4 aB	12.6 ± 0.6 a
	150 ppm	16.5 ± 2.1 aA	15.0 ± 1.9 aA	4.7 ± 1.6 aB	9.0 ± 0.4 aB	11.3 ± 1.4 a
	300 ppm	15.7 ± 2.1 aA	11.5 ± 0.4 aAB	5.7 ± 1.4 aB	9.2 ± 0.4 aB	10.5 ± 1.3 a
P (μmol g^{-1})	0 ppm	84.9 ± 7.3 aBC	103.7 ± 5.4 aB	139.9 ± 18.1 aA	61.0 ± 4.7 aC	97.4 ± 4.9 a
	150 ppm	72.8 ± 9.8 aBC	78.1 ± 6.2 bB	107.0 ± 7.1 bA	41.8 ± 1.9 bC	74.9 ± 2.6 b
	300 ppm	52.2 ± 2.6 bB	52.2 ± 2.7 cB	90.4 ± 1.9 bA	31.5 ± 0.8 cC	56.6 ± 0.3 c
K^+ (μmol g^{-1})	0 ppm	11.2 ± 0.4 aB	45.6 ± 9.4 aA	22.6 ± 5.8 aB	49.6 ± 9.6 aA	32.2 ± 5.8 a
	150 ppm	11.6 ± 1.8 aB	39.4 ± 8.1 aA	21.8 ± 1.9 aB	49.2 ± 3.0 aA	30.5 ± 2.0 a
	300 ppm	12.4 ± 4.0 aB	46.7 ± 5.8 aA	20.9 ± 1.9 aB	48.3 ± 7.6 aA	32.1 ± 3.6 a
Ca^{2+} (μmol g^{-1})	0 ppm	259.9 ± 33.0 aA	256.1 ± 37.7 aA	209.2 ± 41.8 aA	230.9 ± 11.9 aA	239.0 ± 9.1 a
	150 ppm	152.7 ± 42.7 bB	225.7 ± 38.0 bA	204.5 ± 13.2 aAB	222.3 ± 11.9 aA	201.3 ± 20.5 b
	300 ppm	148.5 ± 44.5 bB	221.8 ± 15.2 bA	160.5 ± 27.0 bAB	195.3 ± 7.3 bAB	181.6 ± 22.4 b
Mg^{2+} (μmol g^{-1})	0 ppm	155.3 ± 3.2 aC	205.0 ± 29.0 aB	181.4 ± 9.5 aBC	369.6 ± 13.5 aA	227.8 ± 8.8 a
	150 ppm	134.3 ± 15.5 abC	183.0 ± 13.6 abB	185.8 ± 28.3 aB	324.8 ± 14.4 bA	207.0 ± 8.1 b
	300 ppm	125.5 ± 8.0 bC	152.6 ± 23.5 bBC	170.9 ± 20.8 aB	288.9 ± 17.8 cA	184.5 ± 14.4 b
Al^{3+} (μmol g^{-1})	0 ppm	6.7 ± 0.7 bC	7.9 ± 0.7 cC	11.3 ± 0.9 cB	14.3 ± 1.3 cA	10.2 ± 0.4 c
	150 ppm	9.9 ± 0.7 aC	10.1 ± 1.8 bC	14.2 ± 1.0 bB	18.0 ± 1.8 bA	13.1 ± 0.8 b
	300 ppm	10.1 ± 1.9 aC	13.2 ± 1.6 aC	16.9 ± 0.9 aB	24.2 ± 1.2 aA	16.1 ± 0.6 a

Each value represents the mean ± SD (n=3). Different letters in the table indicate significant differences at the 0.05 probability level, according to Tukey's HSD test. Lowercase letters indicate comparisons among the treatments in each part of sago palm seedlings. Capital letters indicate comparisons among the parts of sago palm seedlings with different aluminum concentrations in culture media 8 weeks after treatment.

seedlings were the same in all treatments; they tended to accumulate highly in roots, followed by bases, petioles, and leaflets, suggesting that Mg^{2+} concentration might not re-translocate to the upper parts in sago palm seedlings. Among the three treatments, Mg^{2+} concentrations in leaflets, petioles, roots, and whole plants were slightly decreased, even if the Al concentration increased. There were markedly significant differences between 0- and 300-ppm Al treatments in leaflets and petioles, and there were significant differences in roots and whole plants of 0-ppm Al- or Al-treated plants, whereas there were no significant differences among the three treatments regarding the base.

In all treatments, the Al^{3+} concentrations in plant parts of sago palm seedlings tended to accumulate most highly in roots, followed by bases, petioles, and leaflets. In all treatments, the Al^{3+} concentrations were not significantly different between petioles and leaflets. When comparing the three treatments, the Al^{3+} concentrations in leaflets, petioles, roots, and whole plants were increased when Al concentrations increased. There were markedly significant differences in all plant parts among the three treatments.

4. Root morphogenesis

Root size is indicated by root diameter, as shown in Fig. 1: The root diameter at 5 distances from the root tip in different aluminum concentrations in culture media 8 weeks after Al treatment. In non-Al-treated

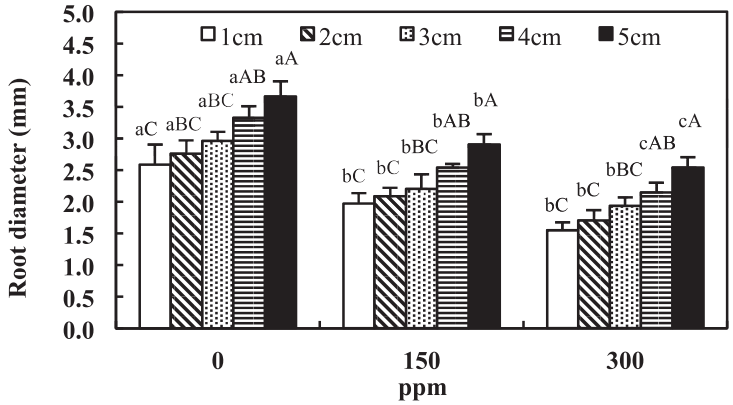

Fig. 1. Root diameters at five distances from the root tip of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media. Each value represents the mean ± SD (n=3). Different letters in the table indicate significant differences at the 0.05 probability level, according to Tukey's HSD test. Lowercase letters indicate comparisons among the treatments at each root length distance. Capital letters indicate comparisons among the root length distances of the same aluminum concentrations in culture media 8 weeks after treatment.

specimens, the 5 distances of the root diameter were slightly increased: 1 cm from the root tip it was smallest, and 5 cm from root tip it was largest. Both 150- and 300-ppm Al treatments showed a tendency similar to the root-size tendency with non-Al treatment. When comparing all three treatments, the distances of root diameters were markedly decreased in 150- and 300-ppm Al treatments, as compared with non-Al treatment. These results can indicate that increased Al concentrations cause a decrease in root diameters at the same distances. Figure 2 shows the effect of Al toxicity on the root morphology of sago palm seedlings in culture media 8 weeks after Al treatment. The observation of differential Al concentrations was exposed as morphological and structural responses of sago palm seedling roots. The

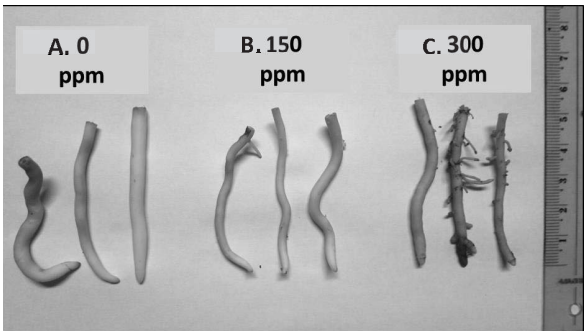

Fig. 2. Effect of Al toxicity on the root morphology of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media. The Al treatments: A. = 0 ppm Al, B. = 150 ppm Al, C. = 300 ppm Al.

root color changed to dark in Al-treated plants; roots exposed to higher Al concentrations (300 ppm Al) became darker. Moreover, Al toxicity involving the number of cells in the cortex per unit area in the transverse section of roots is shown in Table 8, which

Table 8. The number of cells in the cortices of roots at five different distances from the root tips of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media

Al	Root cell number / mm^2				
treatment	1 cm	2 cm	3 cm	4 cm	5 cm
0 ppm	100.0 ± 2.0 aA	86.3 ± 4.0 aB	74.3 ± 4.7 aC	72.3 ± 5.5 aCC	72.3 ± 2.5 aC
150 ppm	82.7 ± 4.7 bA	71.3 ± 3.2 bB	65.3 ± 2.1 bB	63.0 ± 2.7 bBC	55.7 ± 3.2 bC
300 ppm	72.0 ± 2.7 cA	64.0 ± 3.6 bB	58.0 ± 2.0 bB	57.0 ± 1.0 bBC	50.7 ± 3.1 bC

Each value represents the mean ± SD (n=3). Different letters in the table indicate significant differences at the 0.05 probability level, according to Tukey's HSD test. Lowercase letters indicate comparisons among the treatments at each root length distance. Capital letters indicate comparisons among the root length distances of the same aluminum concentrations in culture media 8 weeks after treatment.

describes the number of cells in roots at 5 distances of root length at different aluminum concentrations in culture media 8 weeks after Al treatment. In non-Al-treated samples, there were significantly large numbers among the distances from the root tip of 1, followed by 2, 3, 4, and 5 cm. In plants treated with 150 and 300 ppm Al, the number at a distance 1 cm from the root tip was significantly larger than those at distances from the root tip of 2, 3, 4, and 5 cm, which was significantly small 5 cm from the root tip. A smaller number of root cells per unit area at parts farther from the root tip indicated that root cell sizes were larger at more distant parts, i.e., cell growth. A comparison of the three treatments (Table 8) shows a significant difference at 1 cm from the root tip, while distances of 2 to 5 cm from root tip were significantly different in non-Al treatment and both Al treatments. Figure 3 shows the mean value of the root diameter at five distances from the root tip of sago palm seedlings grown for 8 weeks in culture media with different aluminum concentrations. Variations of Al treatment affected root diameters, creating significant differences between non-Al-treated and Al-treated plants. The mean value of the root diameters at five distances from the root tip of sago palm seedlings grown for 8 weeks was significantly decreased in 300 ppm Al as compared with other treatments; however, there was no significant difference between 150 and 300 ppm Al. Figure 4 shows cross sections of sago palm roots at different aluminum concentrations in culture media 8 weeks after Al treatment, indicating the distribution of root cells treated with or without Al.

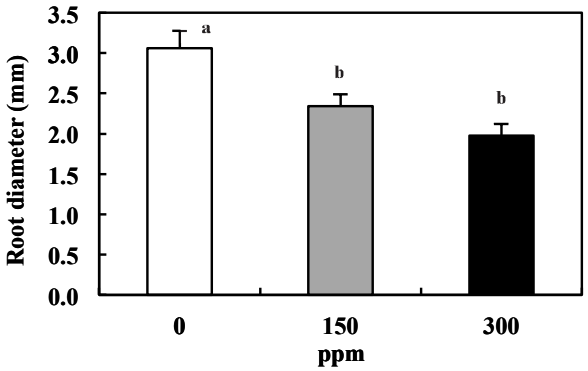

Fig. 3. Mean value of root diameter at five distances from the root tip of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media. Each value represents the mean ± SD (n=3). Different letters indicate significant differences at the 0.05 probability level, according to Tukey's HSD test.

Discussion

1. Plant growth and growth analysis

The results in Table 1 suggest that Al concentrations in the media directly affected the root diameter and numbers of dead leaves. Piňeros and Kochian (2001) reported that the initial symptom of Al toxicity is the inhibition of root elongation, which has been thought to be caused by a different mechanism, including Al interactions within the cell wall or plasma membrane. The number of dead leaves is related to leaf senescence. During senescence, nutrients such as nitrogen, phosphorus, and other metals in the leaf are reallocated to younger leaves and growing seeds or are stored for the next growing season (Buchanan-Wollaston, 1997). Leaf senescence

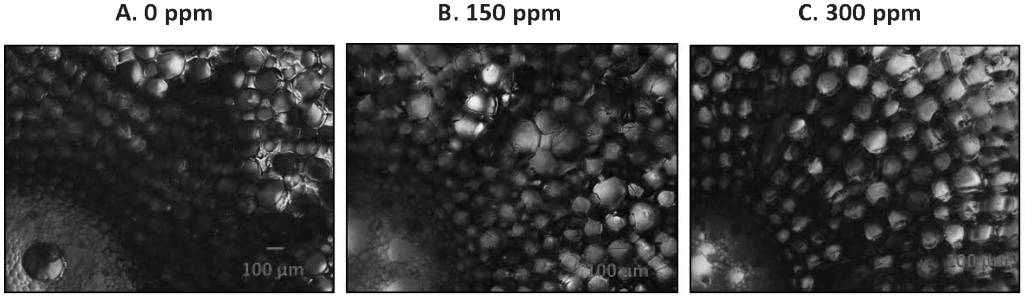

Fig. 4. Transverse section of sago palm roots with different aluminum concentrations in culture media 8 weeks after Al treatment. The Al treatments: A. = 0 ppm Al, B. = 150 ppm Al, C. = 300 ppm Al. 50-µm thickness by Plant Microtome MTH-1 (NK Systems Ltd.), light microscope x100. Bar = 100 µm.

is programmed changes in many metabolic and morphological aspects of plants. However, leaf senescence can be induced by various environmental stresses, particularly, by aluminum toxicity. Besides, it has been suggested that aluminum enhances leaf senescence at a faster rate in rice leaves (Muthukumaran and Vijaya Bhaskara Rao, 2013). Also in this study, the number of dead leaves increased significantly with Al treatment, as shown.

The large root differences between non-treated plants and those treated with both levels of Al may affect the difference in the total dry weight of whole plants (Table 2). Evidence reported about the effect of aluminum on plant and root growth, including Al-induced phytotoxic symptoms and the disruption of cell division and growth within the root, significantly affects plant growth (Liang et al., 2013; Liao et al., 2006). Khono et al. (1995) reported that excess Al caused a reduction in dry matter growth. Anugoolprasert et al. (2014) reported that an Al concentration of 200 ppm affected the dry weight of leaflets, petioles, and roots in sago palms for 4.5 months. Considering the results of the leaflet area recorded in Table 3, Al concentrations higher than 150 ppm might affect the total leaflet area per plant due to Al toxicity, which may be attributed to flexibility in the leaflet membrane that leads to a decrease in higher Al treatments. According to the results of Anugoolprasert et al. (2014), with higher concentrations of Al, the total leaflet area per plant was decreased in sago palms. There were no significant differences in the single leaflet areas with the three treatments. However, a higher Al concentration (300 ppm Al here) tended to slightly decrease the area of each leaflet. This result was similar to that of Thornton et al. (1986), who reported that the rate of leaf expansion of honey locust seedlings grown in Al solution was reduced as compared with the control. On the other hand, the single leaflet area in the 150-ppm Al treatment has an including tendency as opposed to that of the 0-ppm Al treatment.

Growth parameters were analyzed for 8 weeks (Table 4). The RGR indicated each individual's rate of increase per day of the total dry weight, which is the central parameter in plant growth analysis (Hunt, 2002), and consists of the NAR and LAR. The NAR, which generally relates to the photosynthetic performance, showed slight differences among the three plots. To investigate the morphogenesis of individual plants, the LARs in the current experiment of the three plots were at the same level. The SLA is an index of leaf thickness that involves assessing the leaf's area in relation to its dry matter weight; this parameter was also the same among all plots. The LWR is the ratio of the leaf weight to whole plant's weight. The difference in this parameter was negligible; therefore, the LWR was considered to be a very stable parameter under different Al concentrations. From these results, the difference in the growth rates of individuals might be attributed to differences in the assimilation rate with accelerated leaf senescence rather than to morphological characteristics. In the LAR, the dry matter might increase to maintain a good balance with the increase in the single leaflet area and the total leaf area. Moreover, the result of the RRGR parameter shows decreased root growth, which might be attributed to the transverse ruptures reported by Kopittke et al. (2008). They reported that (1) the root elongation rate was decreased by Al, and was accompanied by a decrease in the distance from the root tip to the proximal lateral root; (2) kinks developed in some roots with exposure to Al, and then (3) soluble Al caused similar transverse ruptures to develop in sub-apical regions of the root through the breaking and separation of the rhizodermis and outer cortical layers from the inner cortical cell layers. The depression of the root growth rate by Al treatments was related to differences in the root diameter (Figs. 1 and 3) and the number of root cells per mm^2 (Table 8) in the current experiment.

2. Characteristics related to photosynthesis

2.1 SPAD and chlorophyll content

Leaf chlorophyll content may be used as an indicator of the light environment during plant growth (Boardman, 1977). The close relationship between leaf chlorophyll content and leaf nitrogen (N) content in agricultural crops such as rice, maize, and wheat was reported by Peterson et al. (1993), as the majority of leaf N is contained within the chlorophyll molecules. However, in this study, the chlorophyll content did not show a similar trend to that of leaf nitrogen (N) concentration (Tables 6 and 7). Chlorophyll content was expressed per unit of leaf area, and was different from the N concentration based on leaflet dry weight. The different tendencies of chlorophyll content and N concentration might reflect differences in leaf morphogenesis, such as smaller RLGR (leaflet area expansion rate) in Al-treated plants. Moreover, it was also observed that the leaf color of sago palm seedlings dramatically changed to dark green with both Al treatments, which might be affected by Al. Fukrei et al. (2011) made the same observation and found that foliar symptoms may include stunting, small, dark green leaves, and late maturity. Rout et al. (2001) and Adams (1984) also reported effects of aluminum toxicity on leaves of plants whose foliar symptoms resemble those of phosphorus deficiency. Generally, inadequate P slows the process of carbohydrate utilization, while carbohydrate production through photosynthesis continues; this results in a buildup of carbohydrates and the development of a dark green leaf color (Armstrong et al., 1999). According to Oosterhuis et al. (2008), phosphorus deficiency caused a reduction of leaf photosynthesis, resulting in increased SPAD values as compared to those of phosphorous-sufficient plants such as cotton. The changes in the leaflet characteristics of sago palms in the current experiment might be related to the P deficiency shown in Table 7. The mechanism of Al responses to Al toxicity in sago palm leaflets is still unclear.

2.2 Stomatal conductance

Stomatal density is an important component of stomatal conductance, so gas exchange through the stomata is determined by the width, length, and depth of single stomata and stomatal density (Parlange and Waggoner, 1970). Higher stomatal density would be observed in sago palms grown in soil that contains higher exchangeable Ca (Ehara, 2009). Although, in this study, there was no data regarding stomata density, its tendency is similar to that to stomata conductance. Omori et al. (2000) mentioned that stomatal density is related to the thickness of the leaflet of the sago palm if the stomatal conductance of a leaflet shows a tendency to decrease from base to tip with decreasing thickness. However, there was no difference in the SLA indicating leaf thickness among the three treatments, whereas the Ca concentration in the whole plant was much higher in the non-treated plant than plants treated at either level of Al (Table 7). Considering the former reports and our current results, the cause of the slight decrease in the growth rate with Al treatment was the tendency of a slightly small NAR, which might be caused by the difference in stomatal conductance. The Al concentration also had an effect on the stomatal aperture, considering a study by Anugoolprasert and Ehara (2013) that reported that Al was detected preferentially in the upper epidermis and, occasionally, in the lower epidermis in the leaflet. Additionally, regulations in potassium and chlori de ion channels at the plasma membrane of guard cells lead to stomatal closure by reducing transpiration (Leyman et al., 1999). Moreover, Ohsumi et al. (2007) reported the importance of stomatal conductance as well as leaf nitrogen (N) content, whereas in the current study, leaf nitrogen (N) content did not differ significantly among the three treatments (Table 7).

2.3 Photosynthetic rate and transpiration rate

Many studies have reported on photosynthesis in plants, such as Hoddinott and Richter (1987), who reported a decrease in photosynthesis and in the

translocation of photosynthates in beans after the direct injection of Al into the xylem. Moustakas et al. (1996) found that Al indirectly caused significant disturbances in the chloroplast architecture of plants, with a decrease in photosynthesis due to a reduction of electron transport in photosystem II. However, the impact of Al on photosynthesis in this experiment is probably indirect, as the photosynthetic rate is an environment-dependent trait that changes with leaf ontogeny. Photosynthesis is a physiological process affected by environmental factors, including aluminum toxicity stress or the age of the sago palm seedlings. Therefore, it may difficult to evaluate the potential effect of gas exchange activity on photosynthesis. Although stomatal conductance decreased in treatments treated with 150 and 300 ppm Al, there was no significant in the photosynthetic rate among the three plots in this experiment. This might be related to the increase in the SPAD and chlorophyll content per unit leaflet area in 150- and 300-ppm treatments but not in non-Al-treated treatment (Table 5).

The transpiration rate can indicate the loss of water vapor through the stoma of leaves. The transpiration rate in this experiment tended to decrease in palms treated with higher levels of Al, which was in agreement with the report of Ohki (1986), who found that Al toxicity decreased transpiration in wheat. Critical Al toxicity concentrations in blades of wheat were associated with decreases in transpiration. Al-treated plants induced stomatal closure (Sivaguru et al., 2003), and changes in Al-treated plants suggest the inhibition of K+ in guard cells, which is correlated to the stomatal opening (Schroeder, 1988).

3. Mineral and ion concentrations in different plant parts

The Al concentration is related to other minerals in the soil and some ions in plants. According to Anugoolprasert et al. (2014), different concentrations of Al did not cause significant differences from the control regarding N concentrations in any root parts. However, depending on the plant species, Al mediates the inhibition or stimulation of nitrate uptake, following a close link that implicates root acidification capacity and the chemical properties of membrane permeability (Lidon and Barreiro, 2002). These results can indicate that Al concentrations are not related to the N concentration in the same plant part. Meanwhile, P deficiency and Al toxicity usually coexist in acid soils (Kochian et al., 2004). Under low pH conditions, the interaction between Al and P results in the formation of complexes and especially low solubility compounds that reduce the absorption of Al and its translocation to the shoots. Consequently, the Al concentration in treated sago palm seedlings was believed to be causing a lack of P concentration related to growth inhibition. In the case of K^+ concentrations, it was demonstrated that Al may not block channels aiding the influx of K^+ in guard cells, and the transport of K^+ from cells may not respond to Al in sago palm seedlings. Anugoolprasert et al. (2014) stated, similarly, that the K^+ concentration was not significantly different in leaflets, roots, and whole plants with higher Al concentration treatments from those not treated. The effects of Ca^{2+} on plant grown under conditions of Al stress have been recognized for a long time. Dogan et al. (2014) reported that Ca^{2+} concentration was increased in roots but decreased in leaves at both levels of Al in *Urtica pilulifera* L. seedlings. An Al-induced increase in Ca^{2+} was found in root protoplasts of wheat (Lindberg and Strid, 1997). Huang et al. (1992) reported that a net calcium influx at the root apex was strongly inhibited by Al^{3+}, and Nichol and Oliveira (1858) reported that Al^{3+} reduced Ca^{2+} influx in barley (*Hordeum vulgare*). However, Al exposure led to an increase of Ca^{2+} accumulation in rye-sensitive genotypes, in contrast to the tolerant rye genotype (Silva et al., 2011). Therefore, in this study, Al toxicity may be involved in the inhibition of cell division or root elongation by causing potential disruptions of Ca^{2+} with concentrations of Al of more than 150 ppm in sago palm seedlings. In Mg^{2+}

concentrations, Mariano and Keltjens (2005) found that Al negatively affected the uptake of macro- and micronutrients such as Mg and Ca. In wheat, both sensitive and tolerant genotypes presented a decrease in Mg and K contents in roots, whereas Al contents increased (Silva et al., 2010). In rice plants, Al exposure led to decreased K, Mg, Ca, and P contents and uptake (De Mendonca et al., 2003). In sago palm seedlings, Al treatments decreased Mg^{2+} concentrations as compared with non-Al treatment. Clearly, Al treatment has a positive correlation to Al concentration in different plant tissues, causing much accumulation and increasing an effect as compared with non-Al treatment, particularly in the root. In mostly acid soils, several factors limit plant growth, including toxic levels of Al as well as deficiencies of some essential elements, such as N, P, K, Ca, Mg, and some micronutrients (Kochian et al., 2004). However, this study found that Al interrupts the uptake of P, Ca, and Mg in sago palm seedlings. It is not clear whether Al stress will cause deficiencies of major elements such as P, Ca, and Mg, whereas N and K were deficient in sago palm seedlings.

4. Root morphogenesis

The results shown in Figs. 1, 2, and 3 might suggest that the inhibition of root growth is a symptom of Al toxicity. Mostly, the inhibition of root growth is considered to be a result of inhibited cell elongation and expansion prior to inhibiting cell division (Ciamporova, 2002). Silva et al. (2000) stated that prolonged exposure leads to Al interactions with root cell divisions and the cytoskeleton. Thickened cell walls were frequently observed in the Al-treated roots of oats (Marienfeld et al., 1995) and maize (Vázquez et al., 1999). Danilova et al. (1992) found that numerous vesicles indicated the deposition of polysaccharide material into the cell wall via extreme exocytosis in the epidermal and cortical cells of Al-treated soybean roots. The relationship between Fig. 4 and Table 8 is described by several studies, such as Clune and Copeland (1999), that found that higher concentrations of Al strongly inhibited root growth, with cellular damage being observed primarily in peripheral root cap cells. After exposure to high Al concentrations, central cap and peripheral cap cells were diminished in size and number, and their contents appeared highly disorganized. The distinct boundary between cells in the root cap meristem and the zone of elongation was no longer apparent, and the outer layer of cells of the root cap appeared to be only loosely attached. Morimura et al. (1978) reported that Al induced the inhibition of cell division in the root tips of onions, and observations that Al binds to nucleic acids supported the view of the Al-induced inhibition of root cell proliferation as a primary target for Al toxicity. Thus, understanding of the cell size in the root growing zone has confirmed the inhibitory effect of Al on root elongation. In this work, cell length was reduced in the meristematic zone and elongation zone within 1 cm from the root tip of sago palm seedlings. Vacuolation was observed in the root cortex, and dark cells were found in the vacuoles of Al-treated sago palms. Similar changes were found by Eleftheriou et al. (1993). Dark deposits were observed inside the vacuoles of root cap cells in Al-treated seedlings of *Thinopyrum bessarabicum*. Kollmeier et al. (2000) reported that the higher accumulations of both aluminum and callose also occurred in the developmental stage of cell ontogeny. Moreover, considerable new evidence supports the view that root apoplast, especially cell wall pectin, plays an important role in Al resistance or toxicity in plants (Horst et al., 2010). The primary cell wall component, pectin, with its carboxylate group, is considered a major binding site of Al; thus, pectin content may be related to the accumulation of Al (Chang et al., 1999; Yang et al., 2011). Li et al. (2009) found that the disorganized distribution of pectin epitopes was related to the inhibition of Al-induced root growth in maize. Thus, cell wall pectin could play a major role in determining not only the extent of Al binding but also root growth inhibition, at least in monocotyledonous plants (Yang et al., 2011).

However, the exclusion of Al from the root apex via the exudation of root organic acids is the most important mechanism of Al resistance (Kochian et al., 2004). There is still no evidence of the detection of organic acids from sago palm roots. Certainly, from the results of this study, it is clear that Al induced alterations of root development and cell division as it induced root growth inhibition. The main symptom of Al toxicity is the inhibition of root elongation as a result of interaction of Al with root cells and their components in plants.

From these results, it was concluded that (1) higher Al concentrations at 150 and 300 ppm in growth media retarded the relative growth rate of individual sago palms by hindering the net assimilation rate; (2) the depression of dry matter growth with higher Al concentrations was apparent in the root part, which might be attributable to cell division prevented in the cortex of the root; (3) the effect of higher Al concentrations in growth media on the morphogenesis of top parts was not remarkable.

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