Growth and Physiological Responses of Sago Palm against Aluminum Stress in Acidic Conditions

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Abstract

Sago palm (*Metroxylon sagu* Rottb.) grows in natural peat swamps, which are poorly drained and has high acidity and generally contain highly exchangeable Al. It is, therefore, considered to be acid- and Al-resistant. In this study, the growth, physiological characteristics and nutrient concentrations in the plant tissues of sago palm grown under a hydroponic system were investigated for 4.5 months. When sago palm seedlings were cultured at pH 5.7, pH 4.5 and pH 3.6, the leaf morphogenesis, nutrient uptake and dry matter production were maintained regardless of a small decrease in the photosynthetic rate through the decrease of stomatal conductance. In the case in which seedlings were grown at pH 3.6 with different levels of $AlCl_3 \cdot 6H_2O$ corresponding to 0, 10, 20, 100 and 200 ppm Al, the plant length and dry matter production increased with a mild Al concentration in the growth media, such as 10 ppm Al. This result was attributed to the increase in the P and N uptake. In contrast, all the growth parameters significantly decreased under the 200 ppm Al treatment. The critical value to inhibit the growth of sago palm was considered to be around 200 ppm Al in the growth media. In addition, sago palm maintained a low Al^{3+} concentration in all of the plant parts. Therefore, it could be concluded that sago palm has high resistance to Al with mechanical restriction of the excess Al based on the Al exclusion ability under the acid condition.

Keywords: acid resistance, aluminum resistance, dry matter growth, photosynthetic rate, stomatal conductance

Introduction

Competition between biofuel production and food production has occurred in recent years in the context of the current social background regarding the exhaustion of fossil energy and the growing world population. Various plants are receiving attention as sustainable energy resources for the production of bioethanol and biodiesel. However, worldwide arable lands are limited. Thus, the development and/or improvement of new plant resources and their utilization are needed as a strategy to secure a sufficient amount of biomass for producing foods and biofuel sources (Ehara, 2009).

Sago palm (*Metroxylon sagu* Rottb.) stores large quantities of starch in its trunk. The total starch storage in one trunk is approximately 300 kg dry weight (Ehara, 2005). As a staple food, sago palm continues to be important in parts of Southeast Asia and in areas inhabited by the Melanesian people (Ehara *et al*., 2000). The carbohydrate or starch can be further processed into various basic raw materials for human and animal consumption as well as an industrial energy source, such as ethanol. In addition, sago palm grows in swampy, alluvial and peaty soils where almost no other major crops can grow without drainage or soil improvement (Sato *et al*., 1979; Jong & Flach, 1995). Nevertheless, the deep peat soils in swampy areas are usually characterized by low pH values, a deficiency in mineral elements and a high rate of exchangeable Al (Sato *et al*., 1979). Some former field studies on the growth of sago palm reported that sago palm grew under acid

conditions (Purwanto *et al*., 2002; Osaki *et al*., 2003). According to Foy & Fleming (1978), there was a possitive correlation between Al-resistant plants in nutrient solution and resistance to low pH conditions. It is, therefore, assumed that sago palm is resistant to acidic pH and Al. However, few studies have compared the growth characteristics of sago palm at widely different pH levels as well as the Al-induced changes on sago palm growth. Thus, the objective of the present study was to compare the physiological features and growth characteristics of young seedlings of sago palm at different levels of pH and Al concentration under low pH condition in a hydroponic system to elucidate the acid- and Al-resistance.

Materials and Methods

Plant materials and treatments

Sago palm fruits were collected in the swampy areas of Rattapum, Songkhla, Thailand, on 1 August, 2006. Fertilized and well-developed fruits were selected and treated physically to remove seed coat tissues. The cleaned seeds were placed in a plastic tray filled with tap water and then put them in a dark temperature-controlled room at 30ºC in Thammasat University, Thailand. The germinated seeds were brought to Mie University, Japan and each of them was transplanted to a 1/5000a Wagner pot filled with vermiculite and Kimura B culture solution containing (μM) 36.5 $(NH_4)_2SO_4$, 9.1 K_2SO_4, 54.7 $MgSO_4$, 18.3 KNO_3, 36.5 Ca $(NO_3)_2$, 18.2 KH_2PO_4 and 3.9 FeO_3 (Baba & Takahashi, 1958). The initial pH of the culture solution was adjusted to 5.5 using 1.0N HCl before irrigation into pots. The pots were placed in a greenhouse under natural sunlight and maintained at over 15ºC, even at night, at Mie University. Daily additions were made to the culture solution, according to the amount of solution consumed, and the culture solution was renewed twice weekly.

In Experiment 1, three seedlings at the 7^{th} leaf stage, with the mean plant length of all plant material at 39 cm, were selected and treated with Kimura B culture solution at three different levels of pH, 5.7, 4.5 and 3.6. In Experiment 2, three seedlings of the same size as those in Experiment 1 were treated with Kimura B culture solution without Al (referred to as control hereafter) or containing different levels of $AlCl_3 \cdot 6H_2O$ corresponding to 10, 20, 100 and 200 ppm Al at pH 3.6. The pH of the culture solution was adjusted with 1.0N HCl as required. The pots were placed in the same greenhouse under natural sunlight. An air pump was connected to the pots to provide air to the roots. The culture solutions in each pH and Al treatment were supplemented and renewed every other day from 23 May to 9 October, 2007.

Photosynthetic rate, transpiration rate, and stomatal conductance

The leaflets of the most active leaves or the 4^{th} leaf position from the top of the treated plants (18 weeks after the treatments) were selected for measuring the net photosynthetic rate, transpiration rate, and stomatal conductance using a potable photosynthetic meter (Analytical Development Co., Ltd., LCA-4, England) at saturation irradiance with incident photosynthetically active radiation (PAR) of 800-1,000 μmol $m^{-2}s^{-1}$.

Sampling and nutrient concentrations in plants

The treated plants were separated into three parts: leaflets, petioles including rachis, and roots. The leaflet areas were measured using an automatic area meter (Hayashi-Denko AAM-9, Japan). The separated samples were dried in an oven at 80ºC for 72 h to measure the dry weight and then ground into powder in order to analyze the ion concentrations. The ground samples were reduced to ash in a furnace and extracted with 1.0N HNO_3, and the K^+, Ca^{2+} and Mg^{2+} concentrations were determined using a high-performance liquid chromatography (HPLC) method with a conductivity detector (Shimadzu CDD-6A, IC-C3, 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 Al^{3+} concentration was determined calorimetrically by the aluminon method.

The statistical difference of the data was determined using NCSS 2001 (Number Cruncher Statistical Systems). The effects of treatments were determined by one-way ANOVA (analysis of variance), and the differences among the mean values of treatment were determined using the Tukey-Kramer test.

Results and Discussion

During the experiment, approximately 7 leaves emerged in each pH treatment. The number of dead leaves during the experiment was 4, 4 and 3 at pH 5.7, 4.5 and 3.6, respectively. There were no significant differences in the number of emerged, dead and living green leaves among the three pH treatments, which means that the low pH conditions had no effect on new leaf emergence and senescence. Plant growth in weekly increment of length and total leaflet area did not change with the pH treatments. Although there was no significant difference in the total dry weight per plant among the pH treatments, the total dry weight in the pH 3.6 treatment was 8.7% smaller than that in the pH 5.7 treatment (Table 1). Similarly, the photosynthetic rate in the pH 3.6 treatment was 8.3% smaller than that in the pH 5.7 treatment. The difference in photosynthetic rates among the three pH treatments could be attributed to differences in the stomatal conductance; the stomatal conductance in the pH 3.6 treatment was 7.5% smaller than that in the pH 5.7 treatment.

Table 1. Effect of low pH on weekly increment of plant length, leaflet area per plant and dry matter weight (Experiment 1)

pH Treatment	Weekly increment of plant length (cm)	Leaflet area per plant (cm^2)	Dry matter weight per plant (g)			
			Leaflet	Petiole	Root	Whole
pH 5.7	2.0 a	2400.6 a	18.2 a	23.7 a	8.9 a	50.8 a
pH 4.5	2.0 a	2457.2 a	19.5 a	22.0 a	10.8 a	52.3 a
pH 3.6	2.0 a	2418.8 a	17.3 a	20.1 a	9.0 a	46.4 a

Means with the same letters in a given column are not significantly different at the 0.05 level by the Tukey-

Under the Al treatments, the total dry weight and total leaflet area in the 10 ppm Al treatment were slightly larger than those in the other treatments, while those in the 200 ppm Al treatment were significantly smaller than those in the control treatment (Table 2). Moreover, the roots of sago palm seedlings under the 200 ppm Al treatment were stunted, brownish and thick, and the root dry weight was 58% smaller than that in the control treatment, representing a significant difference (Table 2). Consequently, the critical value to inhibit the growth of sago palm was considered to be approximately 200 ppm Al in the growth media, which is much higher than the real concentrations in the natural soil conditions.

The concentrations of Al^{3+}, N, P, K^{+}, Ca^{2+} and Mg^{2+} in the leaflets, petioles, roots and whole plants under the Al treatments are shown in Table 3. The Al^{3+} concentration in all the plant parts increased with the rise of the Al concentrations. Moreover, the Al^{3+} concentration in the leaflet was lower than that in the petiole and tended to be significantly higher in the root than in the top parts (leaflets and petiole) in all the Al treatments. Our current results in sago palm strongly support the assumption that Al^{3+} has a high binding ability with cellular components of the root and usually shows slight translocation to the upper parts of the plant (Ma *et al*., 1997). The total N and P concentrations in the whole plants of the 10 ppm Al-treated plant were higher than those in the control and other Al-treated plants, which could lead to an increase in the growth of sago palm under a mild Al concentration in the growth media. These results indicated that Al was unlikely to have induced the P and N deficiency in plant tissues but the uptake of these nutrients was higher

under a lower Al condition, as such evidence was also found in rice (Fageria, 1985) and some native plants (Osaki *et al*., 1997). The K^+ concentration in all the plant parts was independent of the Al treatment. Whereas the Ca^{2+} and Mg^{2+} concentrations in whole plants decreased by the increase of the Al concentrations in the growth media, a significant difference was clearly observed in the 200 ppm Al-treated plants. One interesting feature of these results is that Al^{3+} inhibited Ca^{2+} and Mg^{2+} absorption more than K^+ absorption in all the plant parts under the higher Al treatment. The possible mechanism to explain the different effects on cations is that the Al^{3+} toxicity was ameliorated by cations in the following order, H^+ approximately = C^{3+} > C^{2+} > C^+, and the amelioration was due to their binding to or screening the negative charges on the plasma membrane (Kinraide *et al*.,1992).

Table 2. Effect of Al concentration on weekly increment of plant length, leaflet area per plant and dry matter weight (Experiment 2)

Al concentration (ppm)	Weekly increment of plant length (cm)	Leaflet area per plant (cm^2)	Dry matter weight per plant (g)			
			Leaflet	Petiole	Root	Whole
Control	2.0 a	2418.8 ab	17.3 ab	20.1 ab	9.0 ab	46.4 ab
10	2.0 a	3008.6 a	23.0 a	27.1 a	13.6 a	63.7 a
20	1.9 ab	2092.0 b	15.7 bc	16.3 b	6.7 b	38.7 bc
100	1.9 ab	2151.7 b	15.1 bc	18.0 b	6.6 b	39.7 bc
200	1.7 b	1153.4 c	8.0 c	11.2 c	3.8 c	22.9 c

Means followed by different letters within a column are significantly different at the 0.05 level by the Tukey-Kramer test (n=3).

According to Chenery (1948), thousands of the plant species are classified, according to their Al concentrations in plant tissues, as Al-accumulators (≥ 1,000 mg Al kg^{-1} dry weight) or Al excluders (< 1,000 mg Al kg^{-1} dry weight). Some plant species known as the Al accumulators may contain more than 10 times this Al level without any Al injury. However, most plants contain no more than 300 mg Al kg^{-1} dry weight. In the current study, the range of Al^{3+} concentrations in whole plants of sago palm was from 9.4 to 15.6 µmol g^{-1} (254 to 420 mg kg^{-1}) dry weight even under the 200 ppm Al treatment (Table 3). Considering the result of the Al^{3+} concentration in whole plant tissues, sago palm is considered to have high resistance to Al with mechanical restriction of the excess Al based on its Al exclusion ability under acid conditions.

Table 3. Effect of Al concentration on nutrient concentrations in the leaflets, petioles, roots and whole plants (Experiment 2)

Nutrient concentration	Plant part	Al concentration (ppm)				
		Control	10	20	100	200
Al^{3+} (μmol g^{-1})	Leaflet	8.7 cB	9.5 bcB	10.3 bB	10.2 bcB	14.3 aB
	Petiole	8.9 bB	10.7 abB	11.6 abB	13.1 abB	15.1 aB
	Root	12.1 cA	15.1 bcA	16.3 abA	17.7 abA	19.8 aA
	Whole	9.4 b	11.2 b	11.9 ab	12.7 ab	15.6 a
N (mg g^{-1})	Leaflet	20.9 aA	24.6 aA	23.0 aA	22.6 aA	21.0 aA
	Petiole	8.8 aB	9.1 aB	8.7 aB	7.7 aB	6.3 aB
	Root	10.8 aB	9.9 aB	9.8 aB	10.2 aB	11.3 aAB
	Whole	13.6 a	14.8 a	14.6 a	14.1 a	12.6 a
P (mg g^{-1})	Leaflet	1.8 aA	1.9 aA	1.8 aAB	1.7 aAB	1.6 aAB
	Petiole	2.2 aA	2.3 aA	2.2 aA	1.9 aA	1.4 bA
	Root	1.6 abA	1.8 aA	1.4 abB	1.1 bcB	0.9 cB
	Whole	1.9 ab	2.0 a	1.9 ab	1.7 ab	1.4 b
K^{+} (μmol g^{-1})	Leaflet	93.5 aB	92.6 aB	97.8 aB	93.4 aB	98.5 aB
	Petiole	219.6 bA	199.5 bA	215.4 bA	220.0 bA	250.7 aA
	Root	253.4 aA	209.3 bA	226.7 abA	250.7 aA	267.0 aA
	Whole	178.2 b	162.8 b	170.7 b	173.9 b	200.0 a
Ca^{2+} (μmol g^{-1})	Leaflet	42.4 aB	45.3 aB	42.6 aB	32.8 abB	25.5 bA
	Petiole	55.7 abA	64.4 aA	65.2 aA	47.7 bA	28.9 cA
	Root	28.8 abB	36.1 aB	36.8 aB	30.5 abB	21.7 bA
	Whole	45.7 ab	51.4 a	51.3 a	39.2 b	26.7 c
Mg^{2+} (μmol g^{-1})	Leaflet	41.1 aB	40.3 aB	40.9 aB	34.8 abC	29.0 bA
	Petiole	56.7 abAB	60.4 aA	63.0 aA	46.6 bB	29.5 cA
	Root	63.9 aA	65.1 aA	67.4 aA	66.8 aA	36.2 bA
	Whole	52.1 ab	54.1 a	55.1 a	45.5 b	30.5 c

Means followed by different letters within a row are significantly different at the 0.05 level by the Tukey-Kramer test (n=3). Lowercase letters indicate a comparison among the Al treatments in each part of a plant. Capital letters indicate a

References

Baba I and Y Takahashi. 1958. Solution culture. *In* Y. Togari ed., Sakumotsu Shiken Ho. Nogyo Gijutsu Kyokai, Tokyo. 327-343.

Chenery EM. 1948. Aluminium in the plant world: I. General survey in dicotyledons. *Kew Bull.* 2: 173-183.

Ehara H, S Susanto, C Mizota, S Hirose and T Matsuno. 2000. Sago palm (*Metroxylon sagu*, Arecaceae) production in the eastern archipelago of Indonesia: Variation in morphological characteristics and pith-dry matter yield. *Econ. Bot.* 54: 197-206.

Ehara H. 2005. Geographical distribution and specification of *Metroxylon* palms. *Jap. J. Trop. Agr.* 49: 111-116.

Ehara H. 2009. Potency of sago palm as carbohydrate resource for strengthening food security program. *J. Agron. Indonesia* 37: 209-219.

Fageria NK. 1985. Influence of aluminum in nutrient solutions on chemical composition in two rice cultivars at different growth stages. *Plant Soil* 85: 423-429.

Foy CD and AL Fleming. 1978. The physiology of plant tolerance to excess available aluminum and manganese in acid soils. *ASA spec.* 32: 301-328.

Jong FS and M Flach. 1995. The sustainability of sago palm (*Metroxylon sagu*) cultivation on deep peat in Sarawak. *Sago palm* 3: 13-20.

Kinraide TB, PR Ryan, and LV Kochian. 1992. Interactive effects of $A1^{3+}$, H^{+}, and other cations on root elongation considered in terms of cell-surface electrical potential. *Plant Physiol.* 99: 1461-1468.

Ma JF, SJ Zheng, H Matsumoto and S Hiradate. 1997. Detoxifying aluminum with buckwheat. *Nature* 390: 569-570.

Osaki M, T Watanabe and T Tadano. 1997. Beneficial effect of aluminum on growth of plants adapted to low pH soils. *Soil Sci. Plant Nutr.* 43: 551-563.

Osaki M, T Watanabe, T Ishizawa, C Nilnond, T Nuyim, T Shinano, M Urayama and SJ Tuah. 2003. Nutritional characteristics of the leaves of native plants growing in adverse soils of humid tropical lowlands. *Plant Foods Hum. Nutr.* 58: 93-115.

Purwanto BH, K Kakuda, H Ando, JF Shoon, Y Yamamoto, A Watanabe and T Yoshida. 2002. Nutrient availability and response of sago palm (*Metroxylon sagu* Rottb.) to controlled release N fertilizer on coastal lowland peat in the tropics. *Soil Sci. Plant Nutr.* 48: 529-537.

Sato T, T Yamaguchi and T Takamura. 1979. Cultivation, harvesting and processing of sago palm. *Jpn. J. Trop. Agr.* 23: 130-136.

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