Ph.D. Thesis

Growth and Physiological Features of Sago Palm under Different Soil pH Conditions

Preuk CHUTIMANUKUL

Laboratory of Crop Production and Ecology
Major of Sustainable Resource Sciences
Graduate School of Bioresources, Mie University

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Chapter 1

General Introduction

Sago palm (*Metroxylon sagu* Rottb.) is a hapaxanthy (once-flowering), monocotyledonous plant belonging to the family Aracaceae, subfamily Calamoideae and genus *Metroxylon* (Uhl and Dransfield, 1987). *Metroxylon*, derived from the Greek language means "pith" for 'metra' and "xylem" for 'xylon' (Flach, 1977; Singhal et al., 2008). The genus *Metroxylon* spreads from Southeast Asia to Micronesia and Melanesia and it is divided into two sections, that is, *Metroxylon (Eumetroxylon)* and *Coelococcus* (Beccari, 1918; Rauwerdink, 1986). Tropical Asia has been home to most of the 2,500,000 ha of sago palm in the world (Oates and Hicks, 2002), of which *M. sagu* Rottb. is the only species in of *Metroxylon* (*Eumetroxylon*: although monophyly of this division remains uncertain) which is distributed in Southeast Asia (Thailand, Malaysia, Indonesia, Philippines), and north-western Melanesia (Papua New Guinea and the Solomon Islands). Five species are recognized within section *Coelococcus* which represents the eastern half of the distribution of the genus *Metroxylon*: one species is in Micronesia and the other four species are in Melanesia and Polynesia, from Vanuatu to Fiji and Samoa (McClatchey, 1999). Based on starch yield, the genus *Metroxylon* is the most productive among them and *M. sagu* Rottb. (true sago palm) of the division *Metroxylon* (*Eumetroxylon)* is considered to be the most promising (Ehara, 2015). Palms of the section *Coelococcus* also produce sago, i.e. starch extracted from the pith of the palm trunk. The word "sago" appears to derive from a Javanese word that means "starch obtained from palm pith", but it has become a common name for starch in general in many Southeast Asian languages (Ehara, 2015). However, Sago palm is also one of the oldest tropical plants exploited by humans for its stem starch. Starch can accumulate in the trunk of the sago palm until the flowering stage, with maximum starch content occurring just before the onset of the palm flowers. Around 25 ton ha^{-1} y^{-1} of starch productivity from sago plantation is under development in the Malaysian state of Sarawak and was investigated by Ishizaki, 1997. Moreover, Ehara (2006) reported that the sago

palm stores large quantities of starch in its trunk. The total starch storage in one trunk is approximately 300 kg dry weight. This means this plant is the highest in productivity, amongst the starch crops of the world. The two primary uses of the species *Metroxylon* are for the production of edible starch and durable leaf thatch (Singhal, 2008). On sago growth, at the mature stage, possesses a huge trunk and may reach a height of 6-10 m, with a circumference of 1.2 m (Flach, 1977). The plant reaches commercial maturity at 9-12 years of age, the fruit starts to develop and starch accumulation in the trunk reaches a maximum (Yatsugi, 1986). Sago reaches a maximum height of 25 m and a diameter of 40 cm, grows in clumps and has pinnate leaves about 6-9 m long. The stem of sago palm can measure fully grown approximately 15-20 m long (Singhal, 2008). *M. sagu* distinguishes has four stages during its life cycle (Rosette stage, Bole formation stage, Inflorescence stage and Fruit ripening stage) which one cycle taking 11-12 years (Flach, 1977). In addition, sago palm biomass can be processed into several utilizations both for human and animal consumption e.g. bio energy source, animal feed and improving material e.g. compost. Presently, we could say that the three leading world producers are Malaysia, Indonesia and Papua New Guinea, where sago is grown commercially for the production of sago starch and/or conversion to animal food or to ethanol. Indonesia has large forests (>700,000 ha) of wild sago palms, in which 5 million plants are for processing palm pulp into sago flour and by-products, set on Halmahera Island. The plant capacity is around 11,000 tons sago flour per year (Magda, 1993).

Sago palm grows and thrives well in peat swamp rainforests, in alluvial and peaty soil, where almost no other major crops can grow, due to poor drainage or the need for soil improvement (Sato et al., 1979; Jong, 1995), as it is tolerant to low pH, high Al, Fe and Mn in the soil, saline soil and heavily impermeable clay. Thus, its advantage is being able to thrive in under-utilized land in tropical countries. Sago palm has a natural adaptation to peat soils of low nutritional value and high acidity and therefore the soil needs no reclamation and is considered by farmers a minimal risk crop as it is least affected by flooding, drought, pest and disease infestations. Hence, sago palm is one of the most important bio-resources for not only

sustainable agriculture, but also rural development in cultivated areas of the tropics. Evidence showed that peat land is characterized by the accumulation of large amounts of partially decomposed plant material, low pH, high ground water level, and low nutrient content. Due to these characteristics peatland is unfavorable for use as cultivated fields. However, to supply foods in balance with increasing population peatland makes up 30 million ha in South East Asia (Radjagukguk, 1997; Miyamoto, 2009) a potential area for crop production.

However, many arable areas consist of acidic soil which can be cultivated but the yield will be not high because of limitations of crop yields due to soil acidity. In general, the third hydrogen is only lost at pH values above neutral and is not usually a factor. However, two hydrogen ions will readily be lost in acid soil pH range, which will be an issue. This acidity will gradually diffuse into the soil. According to Lindsay and Stephenson (1959), pH values as low as 1.5 can be found in a zone immediately around a fertilizer band. One of the most significant elements in organic acidification reaction is sulfur oxidation. In fact, sulfur is normally used when soil has a pH higher than desired and pH reduction is necessary. There are zones, such as mine spoil and mangrove reclamation areas where sulfur content is naturally high and therefore acidification is a serious problem. In the case where pyrite is present the reaction readily occurs, producing 2 hydrogen ions for every sulfur ion oxidized. Up to 300 cmol (H^+) kg^{-1} of free H_2SO_4 have been reported (Thomas and Hargrove, 1984). Thus the remaining unbound hydrogen ions in the soil solution reduce soil pH. It can be extremely harmful when pH merely changes by a little, because the chemical processes of the cell plants are sensitive to the concentration of hydrogen and hydrogen ions (Rohyadi et al., 2004). Besides, when the soil pH is low, during acidification, aluminum is released into soil. Al related processes in plants can be attributed to the complex chemistry of Al (Kinraide, 1991). Al hydrolyzes in solutions so that the trivalent Al species, Al^{3+}, dominates in acid conditions (pH < 5), whereas the $Al(OH)^{2+}$ and $Al(OH)_2^+$ species form as the pH increases. At near neutral pH the solid phase $Al(OH)_3$, or gibbsite, occurs whereas $Al(OH)_4^-$, or aluminates, dominates in alkaline conditions. Al toxicity (Al^{3+}) and symptoms in plants Al mainly affects plants by inhibiting radical growth. This can be

seen in the primary and lateral root apexes, which also become thick and turn brownish-gray (Roy et al., 1988; Rout et al., 2001). Radical inhibition coincides with a decline in cell division (Frantzios et al., 2001) and elongation of the root cells then induces significant rigidification of the cell walls by crossing with pectin (Jones et al., 2006). This alteration prevents the water absorption essential to transportation of nutrients through the apoplast, eventually causing a decrease in yield and grain quality (Zheng and Yang, 2005; Raman et al., 2002). However, many plant species can vary in their ability to grow in acid soils with severe Al phytotoxicity (Jones and Ryan, 2003). Therefore, Al tolerance mechanisms have been classified into two main types: a) those that exclude Al from the root cells and b) those that allow Al to be tolerated once it has entered the plant cells (Barceló and Poschenrieder, 2002). Plant species in tropical areas are very resistant to Al stress and some of these species can accumulate high concentrations of Al in the leaves (Jones and Ryan, 2003). By contrast, plants species seem to prefer to use the Al exclusion mechanism through organic acid exudation by its root system.

A current study consisting of three experiments are as follows: Chapter 2 shows the effect of different pH levels on growth and physiological characteristics of sago palm with high aluminum concentration in culture solutions. When sago palm seedlings were treated by the same concentration of aluminum and the different pH soil solution, show sago palm seedlings are adapted. Then, in Chapter 3 shows the effects of different aluminum concentrations in culture media on growth characteristics of sago palm seedlings. The most acidic condition of the soil pH solution was selected from the first experiment (Chapter 2) for use in this experiment. For this experiment (Chapter 3), the different aluminum concentrations were set. In order to know the characteristics of sago palm seedlings under acidic soil with aluminum concentrations, demonstrate how sago palm seedlings can be changed to survive. However, the 2 experiments above were investigated in pot conditions, but in Chapter 4, shows growth of sago palm seedlings under different soil pH conditions at the experimental farm in Kendari, Indonesia, which was investigated in the field. In the field experiment, we would like to investigate how

sago palms grow between the natural native acid soil and the neutral conditions as soil applied calcium. Hence, it was determined by comparison of the different soil pH levels between plots.

Formerly, Anugoolprasert (2012a, b) reported that sago palm can grow in different soil pH ranges from 4.3 to 7.0 under natural conditions. Some previous researches reported on the pH of tropical peat soils such as in Riau or Sarawak, where the pH ranged from pH 3.3 to pH 4.7, sago palm had the ability to grow (Kawahigashi et al., 2003; Miyamoto et al., 2009). However, there are few studies on the mechanisms of acid and Al resistance in sago palm, and the ability to live in widely different soil pH range under natural soil conditions is of interest to the investigation. The aims of all studies, (i) to compare growth characteristics of sago palm in wide ranges of pH conditions (Chapter2). (ii) the growth and physiological characteristics of sago palm grown in different soil pH conditions (Chapter2) and different Al concentrations under low pH conditions (Chapter3) in laboratory experimental study were investigated to clarify the Al-resistance mechanism of sago palm. (iii) to clarify the nutrient uptake and translocation in plant body of sago palm (Chapter2 and 3). (iv) to characterize the change in sago palm roots under high Al stress (Chapter 3). And (v) the growth ability in the field experiments between native soil conditions and soil applied with calcium, the condition of sago palm was investigated to clarify the effects of different pH levels in soil and make clear the resistant ability, mechanisms (Chapter 4) and clearly show how we can feedback information results from laboratory level and field experiments to sago palm cultivation.

Chapter 2

Effect of Different pH Levels on Growth and Physiological Characteristics of Sago Palm with High Aluminum Concentration in Culture Solution

Introduction

Generally, the pH of soil is a measure of acidity or alkalinity, where the pH scale ranges from 0 to 14, the value 7 can indicate values as neutral; below 7 can indicate an acid soil, and above 7 can indicate alkaline. Acidification of soil is a natural process with major ramifications on plant growth, which acid soils are soil with a pH of 5.5 or lower are one of the most important limitations to agricultural production worldwide (Kochian, 1995). Approximately 30% of the world's total land area consists of acid soils as much as 50% of the world's potentially arable lands are acidic (von Uexküll et al., 1995). As soils become more acid, particularly when the pH drops below 4.5, it becomes increasingly difficult to produce food crops. Thus, soil pH is an important factor for plant growth, as it affects nutrient availability, nutrient toxicity, and has a direct effect on the protoplasm of plant root cells (Alam et al. 1999). The detrimental effects of low pH to plants growing on acid soils can be direct or indirect, although some research reported that low pH can directly inhibit root growth (Koyama et al., 2001; Yang et al., 2005). However, most of forest soils are acidic and the pH of forest soils ranges from 4.0 to 6.0 as demonstrated (Kawada, 1989), the average is also approximately 5.1 (Takahashi et al., 2001). Some researchers have studied soils with natural pH differences (Anderson and Joergensen, 1997). Others have studied soils in which the pH was changed through anthropogenic intervention, e.g. liming, ash application, alkaline or acidifying pollution (Anderson, 1998; Chagnon et al., 2001; Thirukkumaran and Parkinson, 2000). Plants differ in their responses to pH conditions and the dependence of molecular conditions of the cell insures that cellular processes are sensitive to pH. Obviously, soil pH affects all chemical, physical and biological soil properties (Brady and Weil, 2002), which soil pH is probably at least as

important as soil C and N concentrations in influencing the size of the biomass. Soil pH also affects organic C solubility (Andersson et al., 2000) and increases the availability of biologically toxic Aluminum (Al) with decreasing pH. Al becomes solubilized resulting in increased activity of Al^{3+} ions (Hoekenga et al., 2003). However, several different forms of Al are appearance in soil solutions when the soil pH has changed, such as $AlOH^{2+}$ and $[Al(OH)_2]^+$ at pH 4.5, Al^{3+} at pH 5-7, and whereas at slightly alkaline conditions the amphoteric species $Al(OH)_4^-$ predominate at pH 7-8 (Delhaize and Ryan, 1995). Other complex ions $AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}$ (Al_{13}) and Al^{3+} are almost certainly toxic, but no rhizotoxicity has been detected for $AlSO_4^+$ and $Al(SO_4)_2^-$ or Al-F (e.g. AlF^{2+}). The status of $AlOH^{2+}$ and $Al(OH)_2^+$ is uncertain, although experimental results show toxicity of Al-OH (Kinraide 1997). Indeed, there is a significant correlation between low pH and high concentrations of Al^{3+} in soil (Rout et al., 2001).

Several plants were examined which that were related with Al, in this study, sago palm (*Metroxylon sagu* Rottb.) seedlings were used to investigate the effects of low pH and Al. Sago palm is economically acceptable, environmentally friendly, and promotes a socially stable agro-forestry system (Flach, 1997). It is an extremely hardy plant, thriving in swampy, acidic peat soils, submerged and saline soils where few other crops survive, growing more slowly in peat soil than in mineral soil (Flach and Schuilling, 1989). Formerly, the study to investigate the effect of Al under low pH concentration on growth and Al distribution in roots of sago palm has been done (Anugoolprasert et al., 2008, 2009). There are few studies on the Al-induced changes on growth responses of sago palm, but much evidence have been related to plant species and Al, such as root morphology (Hirano and Hijii, 1998). Martin (1986) reported that the molecular mechanisms underlying Al toxicity are not known, but because Al forms strong bonds with oxygen-donor compounds, it can interact with multiple sites in the apoplasm and symplasm of root cells. The binding of Al with these substances is probably an important factor in its toxicity. Some plant species have evolved mechanisms to tolerate Al stress, which helps them to grow in acidic soils with similar results Anugoolprasert et al. (2009). However, previous research

reported that sago palm can grow in a small scope of the soil pH range test, to which little relevant research has been was conducted regarding the effects of low pH with sago palm. The study of the effects of low pH on plants has an important role for further evaluation of the combined effects of pH and aluminum toxicity on plants. Thus in this chapter, the effects of different pH levels on growth and physiological characteristics of sago palm with high aluminum concentration in culture solution was investigated to evaluate the growth ability.

Materials and Methods

1. Plant materials and pH treatment

The seeds of *Manno* type sago palm that were collected from Sentani, Jayapura, Province of Papua, Indonesia, on July 2010, were transferred to Kendari, Province of Southeast Sulawesi, as explained by Rembon et al. (2008). 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 Noen 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 they were transplanted in a 1/5000a Wagner pot that was filled with vermiculite. 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), and 140 ppm $AlCl_3 \cdot 6H_2O$ were contained together. The pH value of the culture solution was adjusted by pH meter (Fujiwara Co., Ltd., pH/NO_3/Eh Meter, PRN-41, Japan) to 3.5, 5.7 and 7.9 with 1.0N H_2SO_4 and 1.0N KOH as required with 3 replications. Culture solutions were added every day, according to the amount of solution consumed and renewed every 2 days. During the experiment, an air pump was inserted into the pots to provide air for the roots in the hydroponics system (Fig. 2.1). The pots were placed in a greenhouse under natural sunlight at Faculty of Bioresources, Mie University. The experiment was conducted from 18 July to 10 October 2012,

totaling 91 days (approximately 3 months). The mean day and night temperatures during the Al treatment were 29 °C and 21 °C, respectively.

Fig. 2.1 The Hydroponics system with the culture solution used for planting the sago palm seedling in the experiment.

2. Photosynthetic rate, transpiration rate and stomatal conductance

At 3 months (13 weeks) after the start of culture, the leaflets of the most active leaves, or the 4th leaf position from the top of the treated plants were selected to measure net photosynthetic rate and transpiration rate by a potable photosynthetic meter (LCA-4, Analytical Development Co., Ltd., England) at saturation irradiance with incident photosynthetically active radiation (P.A.R.) of 800-1000 μmol $m^{-2}s^{-1}$. Light was provided using a halogen lamp i.e. KODAK EKTAGRAPHIC model AF-2 slide projector (Kodak eastern company, USA). The appropriate P.A.R. was obtained by changing the distance between the projector and leaves.

Thestomatal conductance was also measured at the 4th leaf position, from the top of the treated plants by leaf porometer (Decagon Devices, Inc., Pullman, WA). Stomatal conductance is a function of the density, size, and degree of opening, of stomata, which are pores in plants that open to the outside air. The leaf porometer measures stomatal conductance by putting the

conductance of a leaf in a series with two known conductance elements and comparing the humidity measurements between them.

3. Measurement of the photochemical system

The efficiency of excitation captured by open photochemical system II (Fv/Fm), the photochemical quenching coefficient (qP) and the non-photochemical quenching coefficient (qN) were measured in the same leaf position of photosynthesis rate measurement (the 4th leaf position from the top) at room temperature with a portable Mini PAM chlorophyll fluorometer (PAM-2000, Heinz Walz, Germany). The data acquisition software (Wincontrol-2000, Walz, Germany) was used to connect the fluorometer to the computer. The minimal fluorescence level (Fo) was obtained by measuring the modulated light, which was sufficiently low (<0.1 mmol m^{-2} s^{-1}) in order not to induce any significant variable change in fluorescence. The maximal fluorescence level (Fm) was measured by a 0.8s saturating pulse at 8,000 mmol $m^{-2}s^{-1}$.

4. SPAD

The Soil and Plant Analyzer Development (SPAD) value, indicating chlorophyll content, was determined by using a Chlorophyll Meter (SPAD-502, Minolta Co., Ltd., Japan). SPAD was measured every week for 3 points such as base, middle and tip parts of a leaflet attached to middle position of the upper most fully developed leaf in each plant after transplanting.

5. Chlorophyll content

The chlorophyll content of the leaflets at each leaf position was measured employing the method of Mackinney (1941). An area of 0.25 cm^2 from each leaflet (middle part of leaflet with 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 of leaflet area. The extractions were used to measure the absorbance at 663 nm and 645 nm in a 1cm cell using a spectrophotometer (UVmini-1240, Shimadzu, Japan) at final sampling. The

chlorophyll content can be calculated according to the specific absorption coefficient formulas as describe below;

Total chlorophyll (a+b) (mg/l)	=	8.02 E_{663}	+	20.20 E_{645}
Chlorophyll a (mg/l)	=	12.7 E_{663}	+	2.69 E_{645}
Chlorophyll b (mg/l)	=	22.9 E_{663}	+	4.68 E_{645}

The chlorophyll content was expressed as the content per unit leaflet area.

6. Sampling

After 3 months (13 weeks), the treated plants were sampled and carefully washed in distilled water. The plants were separated into the four parts: leaflets, petioles (including rachis), bases and roots. The fresh weight of each part was recorded and the leaflet areas were measured by using a photoelectric digital scanner (AAM-9, Hayashi Denko Co., Ltd., Japan). All of the fresh samples were dried in an oven at 80 ºC for 72 hrs to measure the dry weight.

7. Nutrient and ion concentrations in different plant parts

The dried samples were ground into a powder by using a coffee mill and kept in a zip lock bag to use for analyzing the ion concentrations later.

7.1 Aluminum concentration determined by aluminon calorimetric method.

The ground dried samples (0.05g) were put in porcelain crucibles and placed in a muffle furnace (Yamato FO 300, Japan) at 500 ºC for 4 hrs to obtain the ash. After burning, 10 ml of 6N HCl were added to dissolve the ash, and then the porcelain crucibles were placed on a hot plate at 140ºC, until the entire sample solutions evaporated (approximately 2 hrs). The evaporated solutions were dissolved again with 25 ml of 1% HCl and filtered with filter paper (5A 150 mm, ADVANCE, Japan), then diluted in the volumetric flask to 50 ml total with distilled water.

As a step of the aluminum analysis, 10 ml of 20% ammonium acetate (CH_3COONH_4), 0.5 ml of 1% mercapto acetic acid (thioglycolic acid) ($HSCH_2COOH$) and 2 ml of 0.2% aluminon (aurintricarbxylic acid ammonium salt) ($C_{22}H_{23}N_3O_9$) were mixed with 1 ml of sample solutions, the volume was then diluted in a measuring cylinder to 50 ml with distilled water. The mixed sample solutions in the volumetric flask were placed into boiling water for 2 mins and placed for cooling at room temperature, which showed red coloring indicating the Al^{3+} concentration. The mixed sample solutions were examined with the absorbance at 525 nm by a spectrophotometer (UVmini-1240, Shimadzu, Japan). The standard calibration was used for calculating the correct Al^{3+} concentration of samples solutions.

7.2 Nitrogen concentration analysis by the semi - micro Kjeldahl method.

7.2.1 Digestion step

Ground dried samples (0.3 g) and 8 ml of concentrated sulfuric acid (H_2SO_4) were put in a digestion flask (kjeldahl flask 100 ml). The sample solution flasks were shaken gently and then placed into a digestion block at 440 ºC for 4 mins. After that 8 ml of 30% hydrogen peroxide (H_2O_2) was added to the charred sample via the funnel on the fractioning head. The color of the solution in the flask had become clear. After adding hydrogen peroxide, excess hydrogen peroxide was boiled by heating for one more minute. Then the digested solution was taken off the heat and placed for cooling at room temperature. The cool solution was diluted with distilled water until the total volume in the flask reached to 100 ml.

7.2.2 Distillation Step

5 ml of digested solution was poured into a beaker and 2-3 droplets of violet solution (100 mg methylene blue and 100 mg methyl red dissolved in 95% ethanol) was added, followed by the addition of 2 ml of saturated sodium hydroxide (NaOH), the color will change to the green and was then poured gently into the A path in Fig 2.2. After the C tube (in Fig. 2.2) forms an air bubble, the conical beaker that contained 10 ml of 0.02N H_2SO_4 and 2-3 droplets of the violet solution was connected to the distillation system in the B position. After the condensate solution

in the conical beaker reached approximately 30 ml, the conical beaker was removed to the titration step.

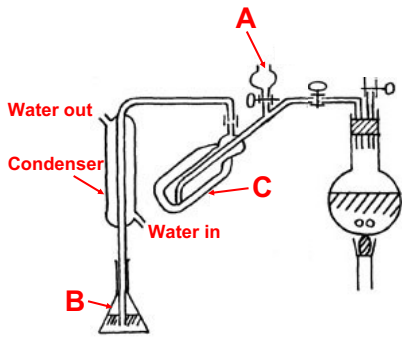


Fig. 2.2 The distillation system of the semi-micro Kjeldahl method for nitrogen (%) analysis.

7.2.3 Titration Step

The condensate solution was titrated with 0.02N NaOH until the solutions turned to green, indicating that the "endpoint" has been reached. Then the volume of the neutralizing base (Sodium hydroxide solution) was recorded, this was used to calculate the amount of nitrogen that came from the original sample.

7.2.4 Calculation

Percentage of nitrogen can be calculated according to the formula as describe below.

$$\% \text{ Nitrogen} = \frac{0.2802 \times F \times (\text{ml blank} - \text{ml titrate}) \times 100\ (\%)}{\text{Sample Weight (g)} \times 1000}$$

F = the factor for the correction factor of 1/50N NaOH (from approximately 0.9 to 1)

7.3 Phosphorus concentration determined by the ascorbic acid method.

The ground dried sample (0.25 g) was put in the conical beaker, to which 10 ml of the 60% Nitric acid (HNO_3) was then added. The sample and HNO_3 were mixed gently and placed on a hot plate at 90 ºC for 45 mins followed by a temperature adjustment to 140 ºC. The sample solution was placed on a hot plate until this solution turned to the clear (approximately 9-10 hrs). A sample solution was shaken frequently and HNO_3 was added occasionally when sample

solution had evaporated before the digestion was completed. The digested complete solution was placed at room temperature for cooling down. Then it was diluted in the volumetric flask to 25 ml with 1% HNO_3 and filtered with a filter paper (5A 150 mm, Advantec, Japan).

As a step of the phosphorus analysis, 200 µl of the sample solution was mixed with 4 ml of the P analysis solution [100 mM ascorbic acid ($C_6H_8O_6$), 2 mM bis diantimonate (III) dipotassium trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$), 32mM hexaammonium heptamolybdate tetrahydrate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) and 2.5M sulfuric acid (H_2SO_4)] and then the volume was adjusted with distilled water until there was 25 ml in the volumetric flask. The mixed sample solution was placed at room temperature for 15-20 mins. This solution would change to color to blue which indicated the P concentration. The mixed sample solution was examined with the absorbance at 880 nm by using a spectrophotometer (UVmini-1240, Shimadzu, Japan). Standard calibration was used for calculating correct phosphorus concentration of the sample solution.

7.4 Cation concentration analysis by a high performance ion chromatograph (HPLC).

Dry powder samples (about 0.05 g) were put in porcelain crucibles and placed in a muffle furnace (Yamato FO 300, Japan) at 350 ºC for 2 hrs and 450 ºC for 8 hrs to obtain the ash. After burning, 100 µl of 1N HNO_3 was added to dissolve the ash. Sample solutions were filtered with the filter paper (5A 150 mm, ADVANCE, Japan) and diluted in measuring cylinder to 25 ml total with distilled water. These sample solutions were filtered with 0.2 µm filter paper (Millipore omnipore^{TM} membrane filter paper, Ireland) before determining the Na^+, K^+, Mg^{2+} and Ca^{2+} concentration by using cation concentration analysis. The 3.3 mM oxalic acid was diluted with filtrated water, which was filtered via 0.22 µm Milli Q Academic A10 (Millipore, USA) then used as the mobile phase. The mobile phase was degassed by degasser (DGU-12A, Shimadzu, Japan) and pumped with a liquid chromatograph pump (LC-20AD sp, Shimadzu, Japan) at a speed of 1 ml per minute. This mobile phase was flowed into the auto injector (SIL-10Ai, Shimadzu, Japan) and mixed with 10 µl of sample solutions to be homogenized, under the supervision of the system controller (SCL-10A vp, Shimadzu, Japan). The cation concentrations

were detected through the analytical column (IC-C3, Shimadzu, Japan) in the column oven (CTO-10A vp, Shimadzu, Japan) at 40 ºC. The results were printed by a chromatopac (C-R 6A, Shimadzu, Japan). The flow rate of the mobile phase was 1.0 ml/min. The Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations were detected with a conductivity detector (CDD-10Avp, Shimadzu, Tokyo, Japan). The standard solution of each cation concentrate (for 100% was equate with 2 ppm Na^+,5 ppm K^+, 5 ppm Mg^{2+} and 5 ppm Ca^{2+}) was measured for establishing a standard calibration to calculate the correct ion concentrations of sample solutions.

8. Statistical analysis

The statistical difference of the data was determined using SAS (Statistical Analysis System) for windows v9.0. The effect of different pH levels with high aluminum concentration were determined by one-way analysis of variance (ANOVA) and the differences among the mean values of the three treatments were determined using the Tukey's studentized range test (HSD). Terms were considered significant at the 5% level.

Results and Discussion

1. Plant growth

Three months after planting, the growth in plant height, plant length, leaf number, leaflet number and dead leaf number were measured. The leaflets, petioles, bases and roots of sago palm treated plants were sampled among the different three pH treatment levels (Fig. 2.3a, 2.3b, 2.3c). Morphological appearance of sago palm roots among differing three pH treatment levels under high aluminum concentration on 3 months after transplanting were observed (Fig. 2.4a, 2.4b, 2.4c). The root morphological appearance of low pH treatment (pH 3.5) was dark brown and when the pH level was high brightness was increased. Thus in, Fig. 2.4 it was clear that the appearance of roots at a higher pH level was brighter than lower pH level. (Hirano and Hijii, 1998) reported that the common effects of low pH (pH 3.5) on the morphology of white roots might enhance the branchiness of branching roots with a decrease in their length. Discoloration of roots may be an effect characteristic of low pH, because the white roots given by low pH treatment were browned more deeply than those given the other treatments.

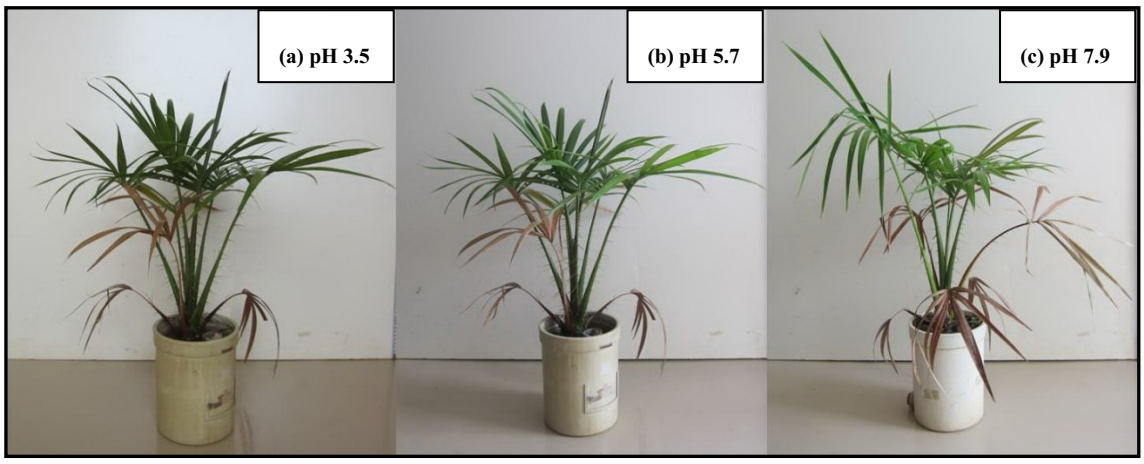


Fig. 2.3 Morphological appearance of sago palm seedlings among the three different pH treatment levels under high aluminum concentration at 3 months after transplanting.

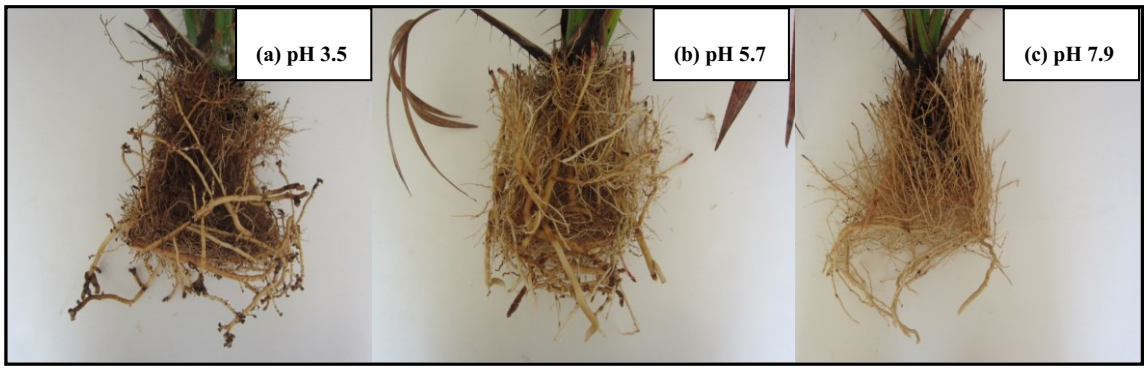


Fig. 2.4 Morphological appearance of sago palm roots among the three different pH treatment levels under high aluminum concentration at 3 months after transplanting.

From the results, Table 2.1 showed that growth by height, length, leaf number and leaflet number per plant differed in the three pH treatment levels. There were no significant differences in height, length, leaf number and leaflet number per plant. Although, pH 7.9 level tended to be higher than pH 5.7 and 3.5 the results could not indicate the best pH level in this experiment. Besides, the number of the emerged leaves, green leaves and dead leaves among the three different pH treatment levels undergoing high aluminum concentration were also counted throughout the treatments (Table 2.2). During the experiment, approximately 3 leaves emerged as a result of each treatment. Numbers of green leaves was counted among pH 3.5, 5.7 and 7.9 treatment levels during the experiment were, 5.5, 4.8 and 5.0, respectively. The numbers of dead leaves among pH 3.5, 5.7 and 7.9 treatment levels during the experiment were 4.0, 5.0 and 4.7, respectively. There were no significant differences in the numbers of emerged leaves, green leaves and dead leaves among different the three pH treatment levels. These results indicate that the different pH levels under environmental stress had no effect on leaf emergence and senescence. According to Anugoolprasert et al (2008) reported that the growth of sago palm seedling under low pH conditions had no effect on leaf emergence and senescence. This experiment was examined for three months, but in the case of the experiment of Anugoolprasert et al (2008) examinations lasted five months. Therefore, the time period may not have an effect to plant growth of sago palm.

The dry matter weights of plant parts among the three different pH treatment levels was harvested three months after transplanting. The results showed in Table 2.3 that the dry matter weights of leaflet, rachis, base and whole plant had no significant differences among the three different pH treatment levels. However, in case of dry matter weight of petioles was significantly different, finding pH 3.5 heavier than pH 5.7 and 7.9 (19.0, 11.5 and 10.2 g plant^{-1}, respectively). Dry matter weight of roots was significantly different at pH 7.9 and 5.7, but at pH 3.5 there was no significant difference (22.0, 17.1 and 12.1 g plant^{-1}, respectively). However, a decrease of root dry weights was affected by high aluminum (Al) concentration in the culture solution. Evidence bore out that soil acidification may reduce root growth and the uptake of essential elements from the soil (Nouchi, 1990; Persson and Madji, 1995). High concentrations of Al^{3+} may inhibit root growth directly, either by inhibition of cell division or cell elongation, or a combination of both (Marschner, 1991).

Table 2.1 Plant size and leaf characteristics of sago palm seedlings for 3 months at the three different pH concentrations in culture solution.

Treatment	Plant height (cm)	Plant length (cm)	Leaf number/plant	Leaflet number/plant
pH 3.5	52.3 ± 3.7 a	62.2 ± 10.3 a	9.5 ± 0.5 a	13.4 ± 1.2 a
pH 5.7	55.4 ± 9.1 a	65.4 ± 10.7 a	9.8 ± 0.3 a	13.0 ± 1.1 a
pH 7.9	61.1 ± 6.7 a	69.6 ± 5.8 a	9.7 ± 0.3 a	15.1 ± 0.7 a

Each value represents the mean ± SD (n=3). Different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

Table 2.2 The number of the emerged leaves, green leaves and dead leaves for 3 months at the three different pH concentrations in culture solution.

Treatment	Emerged leaves	Green leaves	Dead leaves
pH 3.5	3.0 a	5.5 a	4.0 a
pH 5.7	3.2 a	4.8 a	5.0 a
pH 7.9	3.2 a	5.0 a	4.7 a

Each value represents the mean ± SD (n=3). Different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

Table 2.3 Dry matter weight in each plant part of sago palm seedlings for 3 months at different pH concentration in culture solution.

Treatment	Dry matter weight (g plant $^{-1}$)					
	Leaflet	Rachis	Petiole	Base	Root	Whole
pH 3.5	13.1 ± 0.2 a	0.7 ± 0.3 a	19.0 ± 1.9 a	3.5 ± 0.2 a	12.1 ± 3.0 b	48.4 ± 4.8 a
pH 5.7	9.6 ± 2.0 a	0.6 ± 0.2 a	11.5 ± 3.0 b	2.9 ± 0.2 a	17.1 ± 4.8 ab	41.7 ± 8.1 a
pH 7.9	9.9 ± 2.5 a	0.5 ± 0.3 a	10.2 ± 1.7 b	3.5 ± 0.7 a	22.0 ± 5.5 a	46.1 ± 7.0 a

Each value represents the mean ± SD (n=3). Different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

The leaflet area per different leaf positions among the three pH treatment levels (Fig. 2.5) showing that the leaflet area per leaf was small at the lower leaf position, while larger at upper leaf position. Therefore, the leaflet area per leaf increased when the new leaf position emerged. There was same tendency in each leaf position among different three pH treatment levels, which at pH 3.5 had trended to be larger than at pH 5.7 and 3.5. However, most of the upper leaf positions (10th leaf position) included unexpanded and expanding leaves and was different than that from other leaf positions. The leaflet area, per leaflet at different leaf positions among the different three pH treatment levels was showed (Fig. 2.6), there was no practically difference between leaf positions. From the lowest leaflet position (4th leaflet position) to the top leaflet position (10th leaflet position), leaflet size at different leaf positions was similar. The total leaflet area per plant among different three pH treatment levels (Fig. 2.7) showed the results between the three pH treatments were not significantly different; the lower pH treatment level had tended to be higher than the higher pH treatment level. The total leaflet area per plant of pH 3.5, 5.7 and 7.9 was 1602.9, 1402.3 and 1277.1 cm^2 $plant^{-1}$, respectively.

As described above, plant height, plant length, leaf number, leaflet number per plant, emerged leaves, green leaves, dead leaves, dry matter weights of leaflet, rachis, petiole, base, root and whole plant, leaflet area per leaf, leaflet area per leaflet and total leaflet area per plant, there were no significant differences among the three different pH treatment levels. However, lower pH had values of each parameter rather than higher pH (pH 3.5, 5.7 and 7.9, respectively).

These results suggest that the pH concentration might have no effect to sago palm for three months after transplanting under high aluminum concentration.

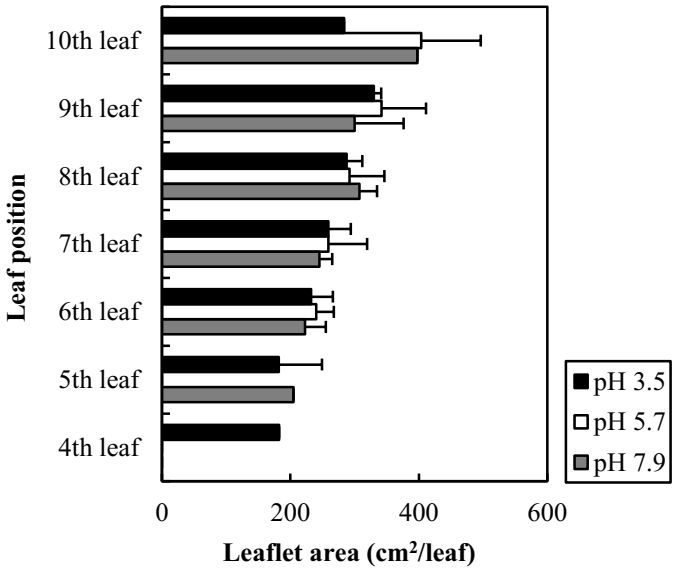


Fig. 2.5 Leaflet area per leaf at different leaf positions among the three different pH treatment levels under high aluminum concentration at 3 months after transplanting. Horizontal lines indicate the standard deviation (n=3).

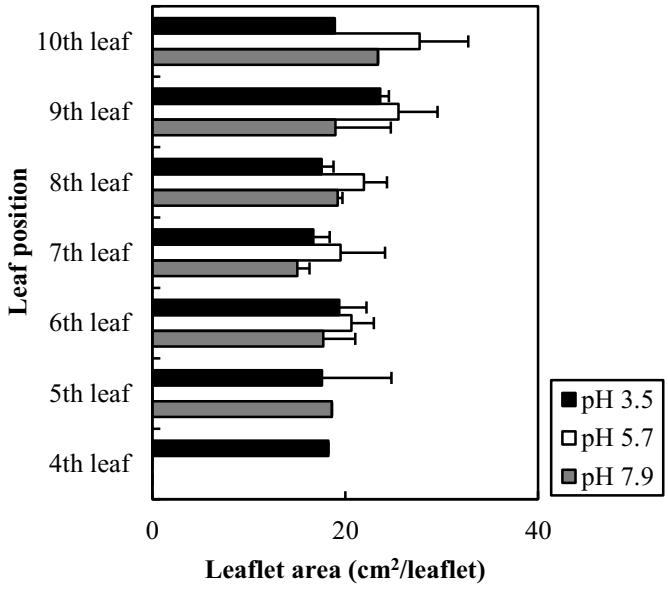


Fig. 2.6 Leaflet area per leaflet at different leaf positions among the three different pH treatment levels under high aluminum concentration at 3 months after transplanting. Horizontal lines indicate the standard deviation (n=3).

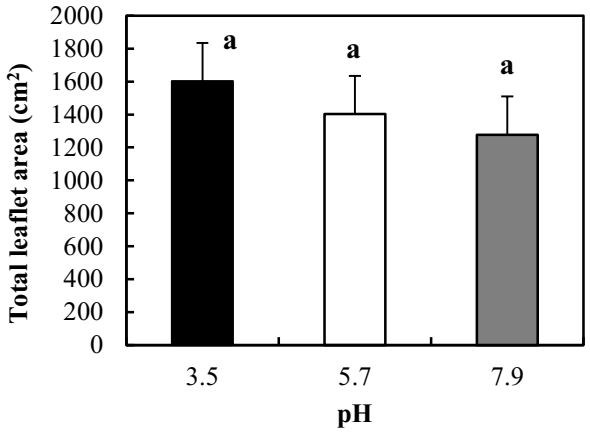


Fig. 2.7 Total leaflet area per plant among the three different pH treatment levels under high aluminum concentration at 3 months after transplanting. Vertical lines indicate the standard deviation (n=3). Means followed by different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

2. Physiological characteristics

The mean values of the chlorophyll content per unit leaflet area of all the leaf positions were 29.8, 26.6 and 17.1 μg cm^{-2} at pH 3.5, 5.7 and 7.9, respectively. There was a significant difference between pH 3.5 and 7.9, but pH 3.5 and 5.7, and pH 5.7 and 7.9 were none. However, pH 3.5 was highest, followed by pH 5.7 and 7.9 as in order (Fig. 2.8). The highest value of the chlorophyll content was observed in the 8th, 9th and 7th leaf position from the bottom at pH 3.5, 5.7 and 7.9 respectively. The difference of chlorophyll content among the three pH treatment levels tended to differ at any leaf position, pH 3.5 was highest followed by pH 5.7 and 7.9 (Fig. 2.9). According to Glynn et al. (2008) it was reported that the relationships between SPAD readings and total leaf chlorophyll concentrations in tree species. Because leaf chlorophyll concentrations change in response to external factors such as light and after various pruning regimes, building removal, or construction activities, quantifying chlorophyll concentrations may provide important information about tree growth and physiologic plasticity in response to changing environments (Larcher, 1995; Richardson et al., 2002).

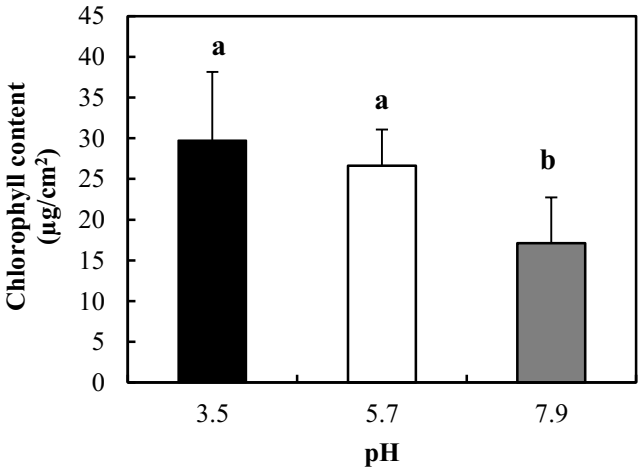


Fig. 2.8 The mean values of the chlorophyll content per unit leaflet area at different leaf positions among the three different pH treatment levels under high aluminum concentration at 3 months after transplanting. Vertical lines indicate the standard deviation (n=3). Means followed by different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

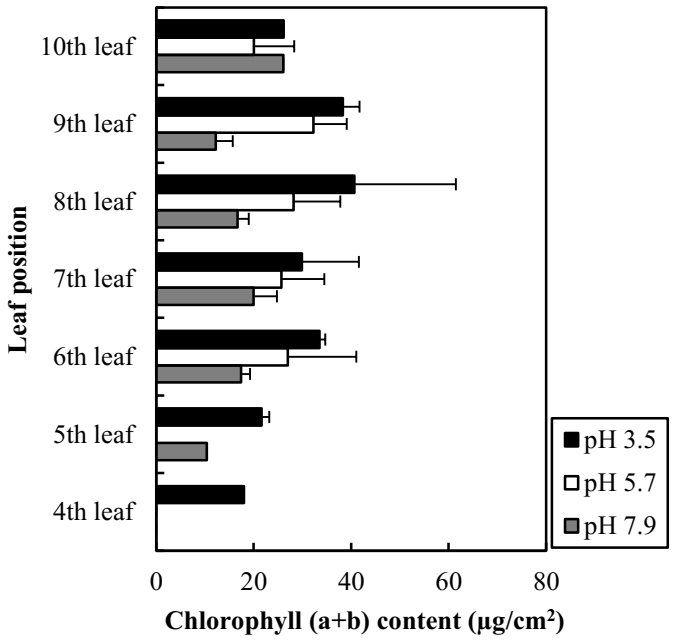


Fig. 2.9 Chlorophyll (a+b) content per unit leaflet area at different leaf positions among the three different pH treatment levels under high aluminum concentration at 3 months after transplanting. Horizontal lines indicate the standard deviation (n=3).

The amount of solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments. Low concentrations of chlorophyll can therefore directly limit photosynthetic potential and hence primary production (Filella et al., 1995).

The parameters of the photochemical system were determined by the efficiency of excitation captured by open PSII (Fv/Fm), photochemical quenching (qP) and non-photochemical quenching (qN) of the 4th leaf position from the top among the three pH treatment levels under high aluminum concentration at 3 months after transplanting were showed in Fig. 2.10. In Fv/Fm measurement, pH 5.7 (0.47) was highest, followed by pH 3.5 (0.44) and 7.9 (0.33), but there were no significant differences between pH 3.5 and 5.7, and pH 3.5 and 7.9. As measurement of qP, at pH 5.7 (0.46) was highest, followed by pH 3.5 (0.43) and 7.9 (0.32), but there were no significant differences between pH 3.5 and 5.7, and pH 3.5 and 7.9 as well. And measurement of qN, at pH 3.5 (0.13) and 5.7 (0.13) was significantly higher than pH 7.9 (0.10). A change in qP is due to closure of reaction centre, resulting from a saturation of photosynthesis by light. A change in Fv/Fm is due to a change in the efficiency of non-photochemical quenching. Dark adapted values of Fv/Fm reflect the potential quantum efficiency of PSII and are used as a sensitive indicator of plant photosynthetic performance, with optimal values of around 0.83 measured for most plant species (Björkman and Demmig, 1987; Johnson et al., 1993; Maxwell and Johnson, 2000). Values lower than this will be seen when the plant has been exposed to stress, indicating in particular the phenomenon of photo inhibition. Although a current experiment, the optimal values of Fv/Fm around 0.33-0.47 fell below rather than the optimal values measured for most plant species, because sago palm seedlings were treated by high Al concentration. Anugoolprasert et al. (2009) suggested that the photochemical processes of PSII are not inhibited by the Al stress, which was found in both the Al susceptible and Al tolerant genotypes of wheat and sago palm (Darko et al., 2002; Anugoolprasert et al., 2009). These results suggest that the photochemical processes of PSII are inhibited by the higher pH concentration.

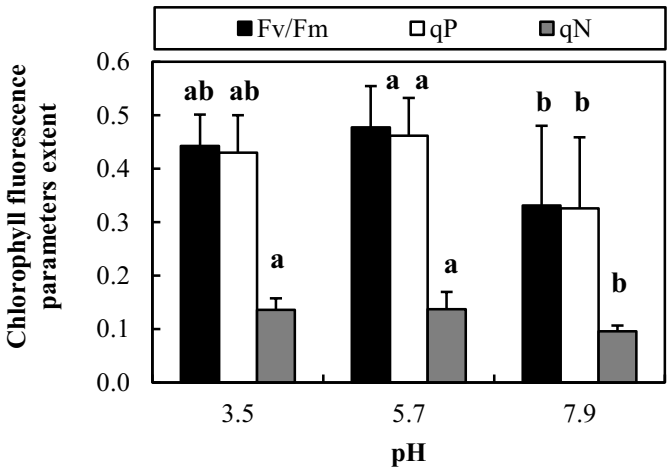


Fig. 2.10 Effect of pH concentration on the efficiency of excitation captured by open PSII (Fv/Fm), photochemical quenching (qP) and non-photochemical quenching (qN) of the 4th leaf position from the top among different three pH treatment levels under high aluminum concentration at 3 months after transplanting. Vertical lines indicate the standard deviation (n=3). Means followed by different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

Table 2.4 showed the photosynthetic rate, transpiration rate and stomatal conductance were measured at the 4th leaf position from the top, which was considered to be the most active physiologically according to the leaf development and chlorophyll content. Of all the measurements pH 3.5 was highest followed by pH 7.9 and 5.7. As to photosynthetic rate, the significant difference was detected between pH 3.5 and pH 7.9, 5.7 of which their values were 4.8, 3.7 and 3.3 µmol $m^{-2}s^{-1}$, respectively). There were no significant differences between pH 5.7 and 7.9. In the transpiration rate, the significant difference was detected between pH 3.5 and pH 7.9, 5.7 of which their values were 1.8, 0.8 and 0.8 mmol $m^{-2}s^{-1}$, respectively). There were no significant differences between pH 5.7 and 7.9. In the stomatal conductance, a significant difference was also detected between pH 3.5 and pH 7.9, 5.7 of which their values were 9.2, 7.6 and 7.1 mmol $m^{-2}s^{-1}$, respectively). There were no significant differences between pH 5.7 and 7.9 as well. Much evidences reported that reduction in the photosynthetic rate of several plants

has been recorded when the pH is extremely low, lower than the soil pH in field conditions. Anugoolpraset et al., 2008 showed the results that the photosynthetic rate, transpiration rate and stomatal conductance, there were no significant differences under low pH condition of which the growth media pH was investigated at 3.6 to 5.7. The soil pH of peat soil planted with sago palm in Salawak, Malaysia has been reported to be acidic, in the range of 3.9 to 4.5, and did not result in the appearance of symptoms of growth inhibition (Kawahigashi et al., 2003). However, in this experiment sago palm seedlings were treated by high Al in all treatments. Hence, this is a reason why the sago palm seedling at pH 3.5 exhibited significantly higher measurement of the photosynthetic rate, transpiration rate and stomatal conductance rather than other pH levels. Moreover, high concentrations of Al in leaves are usually found only in perennial species (Al accumulators) such as tea plants and certain Proteaceae (Chenery and Sporne, 1976). This Al accumulation does not necessarily reflect high Al tolerance of the leaf tissue but is most likely the result of root-induced chelation of Al^{3+} in the rhizosphere and translocation of chelated (non-toxic) Al into the leaf tissue where it is deposited into the epidermal layer (Matsumoto et al., 1976). In case of Al accumulator, acid tolerant species such as *Arnica montana* and *Deschampsia flexuosa* (L.) Trin. Al stimulates root and shoot growth at low concentrations (Pegtel, 1987), and in tea plants even at high Al concentrations (Konishi et al., 1985). By contrast, in view of the total dry weight per plant among the three different pH treatment levels in the current experiment, there was no significant difference to plant growth rate, which in pH 3.5 tended to be higher than that at pH 7.9 and 5.7, respectively (Table 2.3). These results suggest that amount of Al concentration approximately 140 ppm can stimulate the growth of sago palm seedling under low pH conditions.

The relationship between specific leaf area (SLA) and stomatal conductance in leaflets of sago palm among the three pH treatment levels under high aluminum concentration at 3 months after transplanting was showed in Fig. 2.11. The specific leaf area (SLA = leaflet area/leaflet dry weight) has a relationship with stomatal conductance. The SLA will have a negative relationship with photosynthetic rate, NAR or stomatal conductance in plant species which was

similar to the case of sago palm. Ehara et al. (1993) reported a positive relationship between SLA and CO_2refusion resistance through the stomata in rice and wheat, which means that the increase in SLA will affect stomatal conductance to be decreased in monocotyledon.

Table 2.4 The Photosynthetic rate, Transpiration rate and Stomatal conductance of sago palm seedlings for 3 months at the three different pH concentrations in culture solution.

Treatment	Photosynthetic rate (μmol $m^{-2}s^{-1}$)	Transpiration rate (mmol $m^{-2}s^{-1}$)	Stomatal conductance (mmol $m^{-2}s^{-1}$)
pH 3.5	4.8 a	1.8 a	9.2 a
pH 5.7	3.3 b	0.8 b	7.1 b
pH 7.9	3.7 b	0.8 b	7.6 b

Each value represents the mean ± SD (n=3). Different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

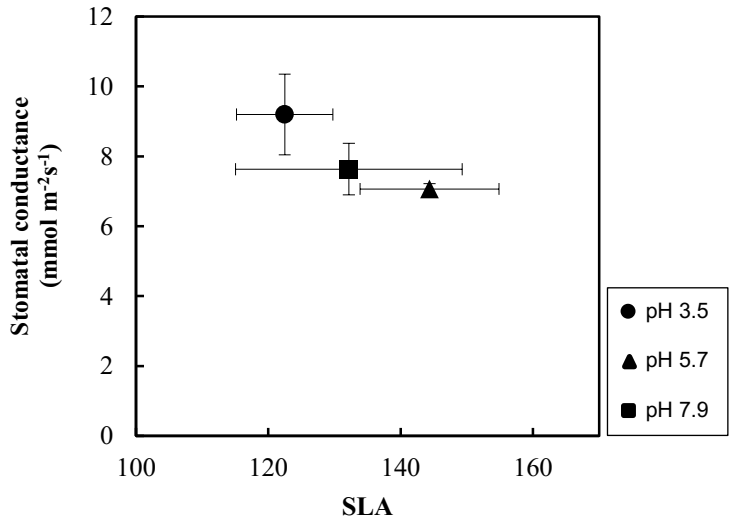


Fig. 2.11 Relationship between specific leaf area (SLA) and stomatal conductance in leaflets of sago palm among the three different pH treatment levels under high aluminum concentration at 3 months after transplanting. Vertical and horizontal lines indicate the standard deviation (n=3).

3. Nutrient and ion concentrations

3.1 The Al concentration in culture solution

The Al concentration in culture solution among the three pH treatment levels before transplanting is showed in Table 2.5. Culture solution put with Kimura B in each pH treatment was detected. Al concentration at pH 3.5 was highest followed by pH 5.7 and 7.9 (3.58, 2.61 and 2.01 ppm Al, respectively). The significant difference was not apparent between pH 3.5 and 5.7, and pH 5.7 and 7.9. However, at pH 3.5 there was a significant difference with pH 7.9. While culture solution was put with Kimura B and 140 ppm $AlCl_3 \cdot 6H_2O$ in each pH treatment was also detected. The significant difference was apparent among the three pH treatment levels. Al concentration at pH 3.5 was highest followed by pH 5.7 and 7.9 (36.77, 8.60 and 2.41 ppm Al, respectively). Too high Al^{3+} concentration are not different in higher pH (pH above 5) because Al^{3+} is not soluble in soil. Al^{3+} preferable under lower pH (pH 3.5) in same culture solution. At low pH that Al form is Al^{3+} (below 5) and higher pH (above 5), Al form will be formed $Al(OH)^{2+}$ and $Al(OH)^{+}$. Al^{3+} concentration will be decrease when pH is increased. Iqbal (2012) reported that the 2 Al compounds, aluminum chloride ($AlCl_3$) and aluminum hydroxide ($Al(OH)_3$), differed markedly in their ability to increase the bio availability of Al in soil. The bulk soil pH declined and the concentration of extractable Al increased with the addition of $AlCl_3$ to the soil.

Table 2.5 The Al concentration in culture solution among the three different pH treatment levels at before transplanting.

Treatment	Kimura B (ppm)	Kimura B + 140 ppm $AlCl_3 \cdot 6H_2O$ (ppm)
pH 3.5	3.58 ± 0.62 a	36.77 ± 1.27 a
pH 5.7	2.61 ± 0.18 ab	8.60 ± 0.62 b
pH 7.9	2.01 ± 0.78 b	2.41 ± 0.36 c

Each value represents the mean ± SD (n=3). Means followed by different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

In contrast, these measurements did not change with the addition of $Al(OH)_3$ to the soil. Al compound affected soil pH and Al^{3+} activity in soil solution. The $AlCl_3$ compound increased the concentration of extractable Al and decreased pH in soil. However, it has been indicated that these internal Al concentrations can be dangerous.

3.2 Nutrient and ion concentrations in different plant parts

3.2.1 Nutrient and ion concentrations in different parts of leaflets and petioles at different leaf positions

The Al^{3+} concentration in the leaflets and petiole at different leaf position among the three pH treatment levels at 3 months after transplanting is shown in Fig. 2.12. The Al^{3+} concentration of the plants in the three pH treatment levels (pH 3.5, pH 5.7 and pH 7.9, respectively) was in the range of 31.4-39.6, 31.3-38.3 and 6.2-8.9 μmol g^{-1}, that in petiole was in the ranged of 35.4-43.6, 14.1-23.2 and 8.4-19.9 μmol g^{-1}. These values of leaflets distinctively increased under pH 3.5 and 5.7 treatment levels at almost all the leaf positions, there was a significant difference among the three pH treatment levels. In contrast, the values of petiole decreased under pH 3.5 and 5.7 treatment levels at almost all leaf positions; there was no significant difference among the three pH treatment levels. At high pH treatment level, Al^{3+} accumulated in leaflets lowest and accumulated in petioles highest when compared with lower pH treatment level. Generally, the amount of Al^{3+} in soil solutions decreases in soil when pH increases in the shoots of cotton was reported by Adams and Moore (1983). Only a small fraction of plant species accumulates Al at a high level in above ground plant tissues (Jansen et al., 2002). Moreover, Jansen et al. (2002) has reported that Al-accumulating plants have been identified; these hyper-accumulators accumulate more than 1000 mg kg^{-1} of Al in the leaves but in this current experiment at pH 3.5, the amount of Al^{3+} can accumulated around 910-980 mg kg^{-1} of Al in the leaves. However, most of them are woody species which have been adapted to acidic soil. Much evidence showed that chelation of Al with small organic compounds plays an important role in the internal

detoxification of Al in the Al-accumulating plants although the mechanisms were also proposed (Taylor, 1991; Ma, 2000; Ma et al., 2001). Therefore, the chemical forms of Al were explored in buckwheat leaves with a very high Al concentration. Shen et al. (2004) reported that the form of Al in buckwheat leaves change with the concentration of Al. Besides which, high concentrations of Al in leaves are usually found only in perennial species (Al accumulators) such as tea plants and certain Proteaceae (Chenery and Sporne, 1976). However, Matsumoto et al. (1976) reported that this Al accumulation does not necessarily reflect high Al tolerance of the leaf tissue but is most likely the result of root-induced chelation of Al^{n+} in the rhizosphere and translocation of chelated (non-toxic) Al into the leaf tissue where it is deposited in the epidermal layer. In this current experiment it was showed that Al^{3+} concentration in all leaf positions was less than the root part (Fig. 2.20) among the three pH treatment levels. Sago palm can be adapted at lower pH conditions because we see the mechanisms of adaptation to acid mineral soils or soil solutions in this experiment. The mechanisms seem to be avoided because of Al^{3+} concentration in roots at pH 3.5 is very high, but they could not move into leaflet all. Thus, it can be indicated that exclusion of Al from uptake or at sensitive sites which is one of avoidance mechanisms.

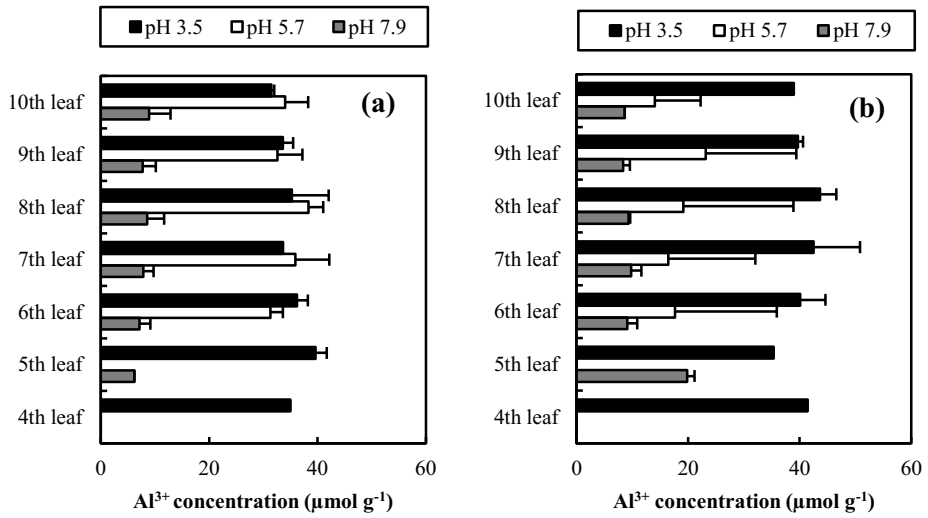


Fig. 2.12 Al^{3+} concentration in leaflets (a) and petiole (b) at different leaf positions. Horizontal lines indicate the standard deviation (n=3).

The P and N concentrations in the leaflets and petiole at different leaf positions of the plants among the three pH treatment levels at 3 months after transplanting is showed in Fig. 2.13 and Fig. 2.14, respectively. The P and N concentrations in the leaflets slightly increased when in lower pH conditions and tended to be higher at higher leaf positions than at lower leaf positions in all the pH treatments. The P concentrations in the petiole slightly increased when lower pH conditions and tended to be higher at higher leaf positions than at lower leaf positions in all the pH treatments. The N concentrations in the petiole slightly decreased when in lower pH conditions and tended to be higher at high leaf positions than at lower leaf positions in all the pH treatments. However, there were no significant differences in the P in leaflets and N in petiole concentrations of all the leaf positions among the three pH treatments.

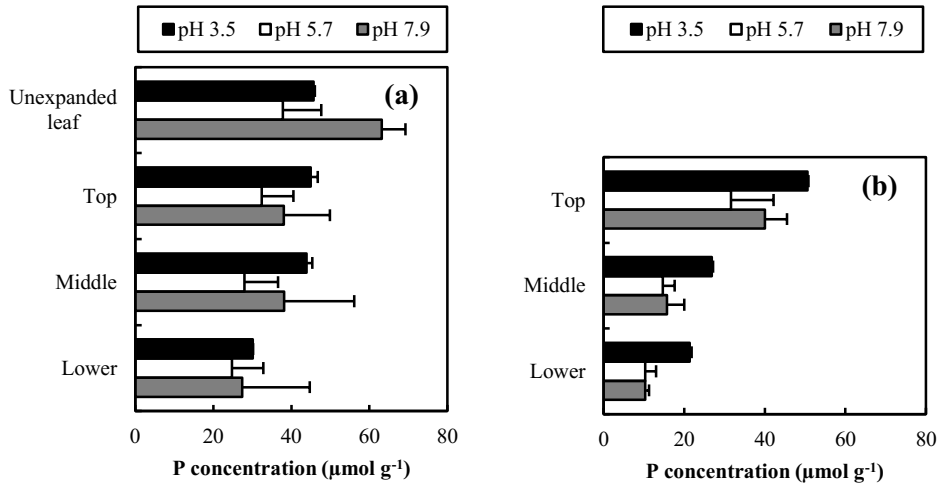


Fig. 2.13 P concentration in leaflets (a) and petiole (b) at different leaf positions. Horizontal lines indicate the standard deviation (n=3).

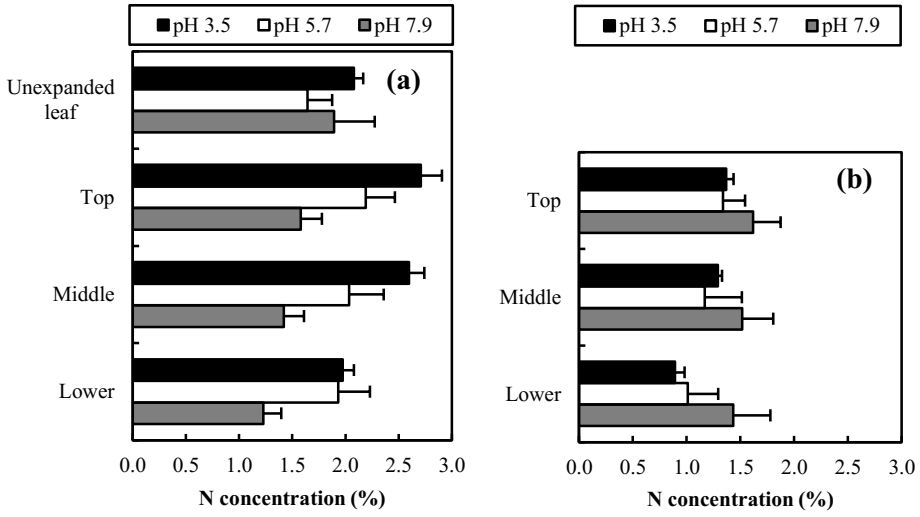


Fig. 2.14 N concentration (%) in leaflets (a) and petiole (b) at different leaf positions. Horizontal lines indicate the standard deviation (n=3).

Anugoolprasert et al. (2012) reported that N and P concentrations in the leaflets and petiole at different leaf positions were not affected when sago palm was treated under low pH culture media at pH range 3.6 to 5.7. However, this current experiment has found significant difference at all pH treatment levels, most likely caused by N concentration having a positive relationship with photosynthetic rate and chlorophyll content. In harmony with the results above which the photosynthetic rate and chlorophyll content was significantly highest (Fig. 2.8 and Table 2.4). Nevertheless, the effect of high Al had related to change among different pH treatment levels.

The K^+ concentration in the leaflets and petiole at different leaf positions was showed in Fig. 2.15. The K^+ concentration in the leaflets and petiole slightly decreased when in lower pH conditions and tended to be higher at high leaf positions than at low leaf positions in all the pH treatments. In addition, the K^+ concentration was larger in the petiole than in the leaflets at all leaf positions. Na^+ concentration in leaflets and petiole at different leaf positions was showed in Fig. 2.16. It slightly decreased when lower pH conditions and was no different between higher leaf positions and lower leaf positions in both the leaflets and petioles in all pH treatments. In addition, the Na^+ concentration was larger in the petiole than in the leaflets at all leaf positions.

In contrast, the Ca^{2+} and Mg^{2+} concentrations tended to be lower at high leaf positions than at lower leaf positions in both the leaflets and petioles in all pH treatments (Fig. 2.17 and Fig. 2.18, respectively). However, the difference of leaflets and petiole in K^{+} and Mg^{2+} concentrations among the three pH treatments was significant, but Na^{+} and Ca^{2+} concentrations among the three pH treatments were not significant.

The K^{+} and Na^{+} concentrations in the leaflets were less than that in the petioles. In general, many plant species accumulate Na^{+} in the death part or inactive leaf position. Ehara et al. (2008) reported that sago palm was able to maintain a low Na^{+} concentration in the leaflets of active leaves at high positions by storing Na^{+} mainly in roots and the lower leaf positions of petioles. Therefore, the K^{+} concentration was accumulated in petioles at high leaf positions caused by the relationship between the K^{+} and Na^{+} concentrations was exhibited.

For uptake and subsequent radial transport of Ca^{2+} and Mg^{2+} binding at cation exchange sites in the cell wall is of crucial importance. According to Marschner (1991), it was stated that this loading of the apoplast of roots maintains a high concentration of polyvalent cations in the vicinity of the plasma membrane or along the apoplasmic pathway which is necessary for high uptake rates of these cations. At low external pH, or in the presence of high concentrations of competing polyvalent cations, loading of particular cations is impaired. In addition, low external pH decreases the charge density of the cell walls so that the effects on cation uptake become more pronounced. Anyway, Kruger and Sucoff (1989) stated that the particular effect of Al^{3+} on the amounts of Ca^{2+} and Mg^{2+} in the apoplast of root cortical cells and the subsequent effects on Ca and Mg nutrition and growth. Moreover, there are several reports concerned with the effect of low pH on nutrient uptake in rice (Thawornwong and Diest, 1974). Their reports are suggesting that the Ca^{2+} and Mg^{2+} concentrations are likely suppressed by uptake of H^{+} or Al^{3+}. In the current study, there were significant difference in the Ca^{2+} concentration and tended to be decreased in Mg^{2+} concentration in the whole plant among the three treatment levels.

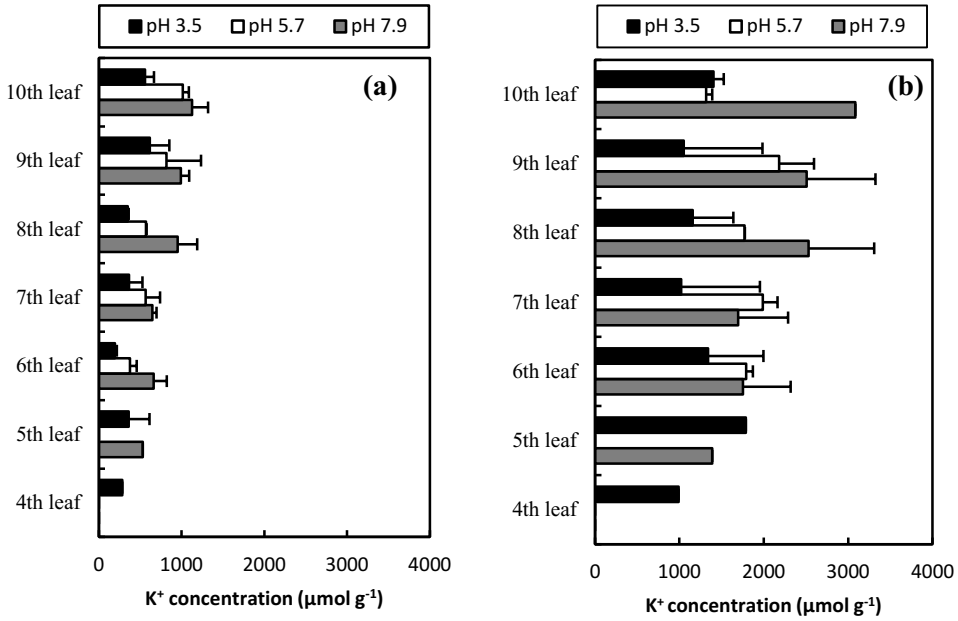


Fig. 2.15 K^+ concentration in leaflets (a) and petioles (b) at different leaf positions. Horizontal lines indicate the standard deviation (n=3).

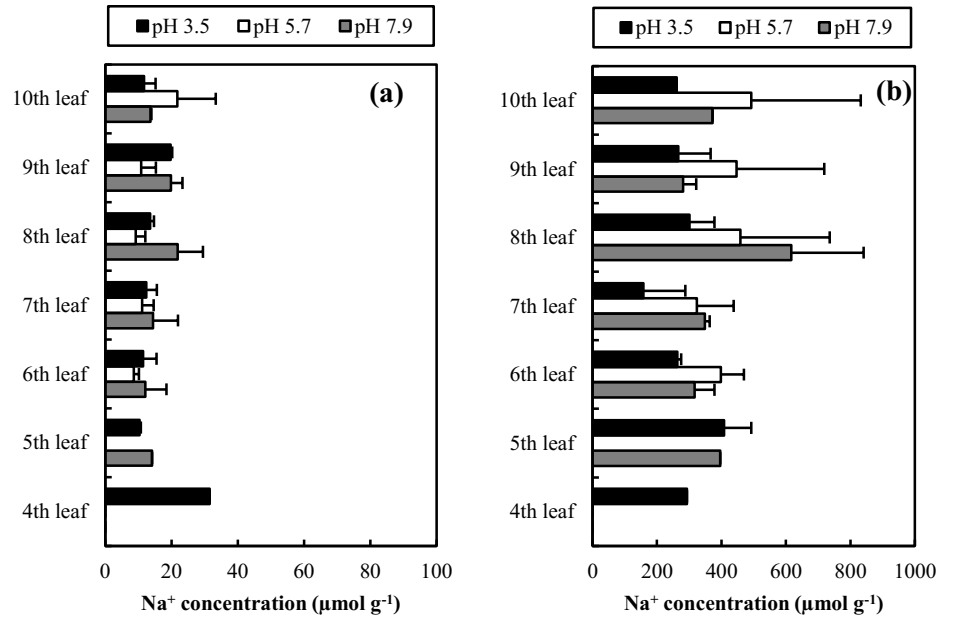


Fig. 2.16 Na^+ concentration in leaflets (a) and petioles (b) at different leaf positions. Horizontal lines indicate the standard deviation (n=3).

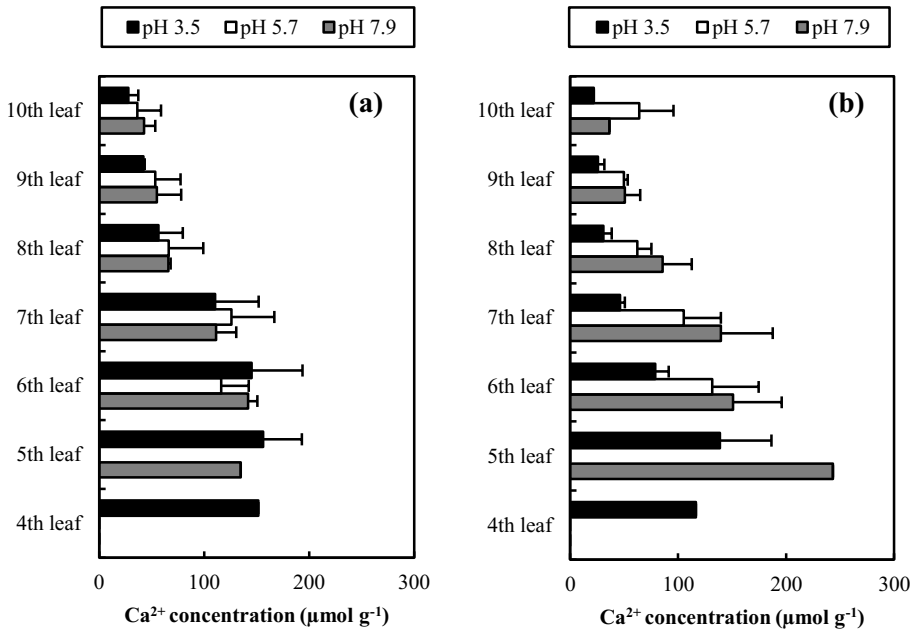


Fig. 2.17 Ca^{2+} concentration in leaflets (a) and petioles (b) at different leaf positions. Horizontal lines indicate the standard deviation (n=3).

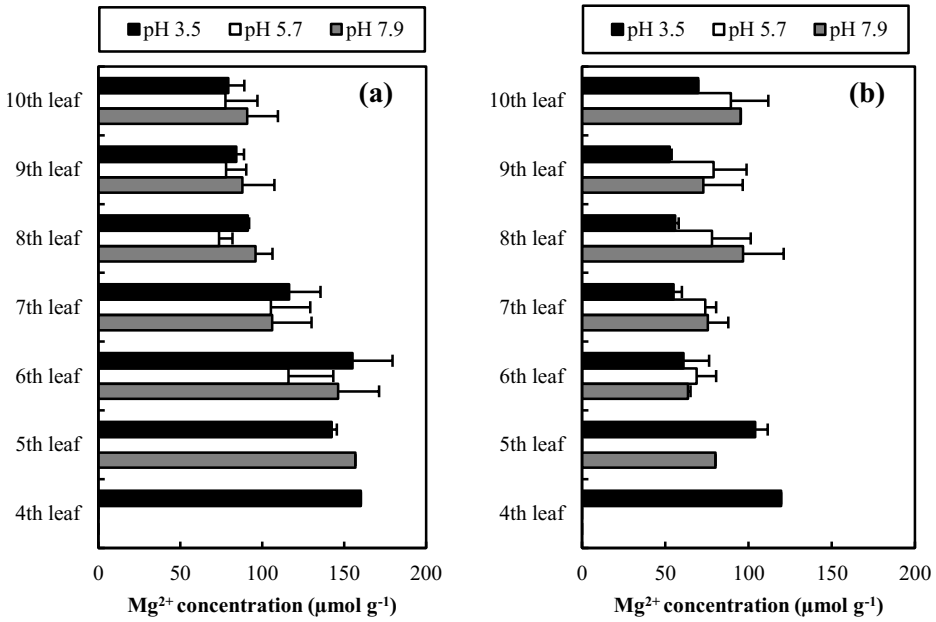
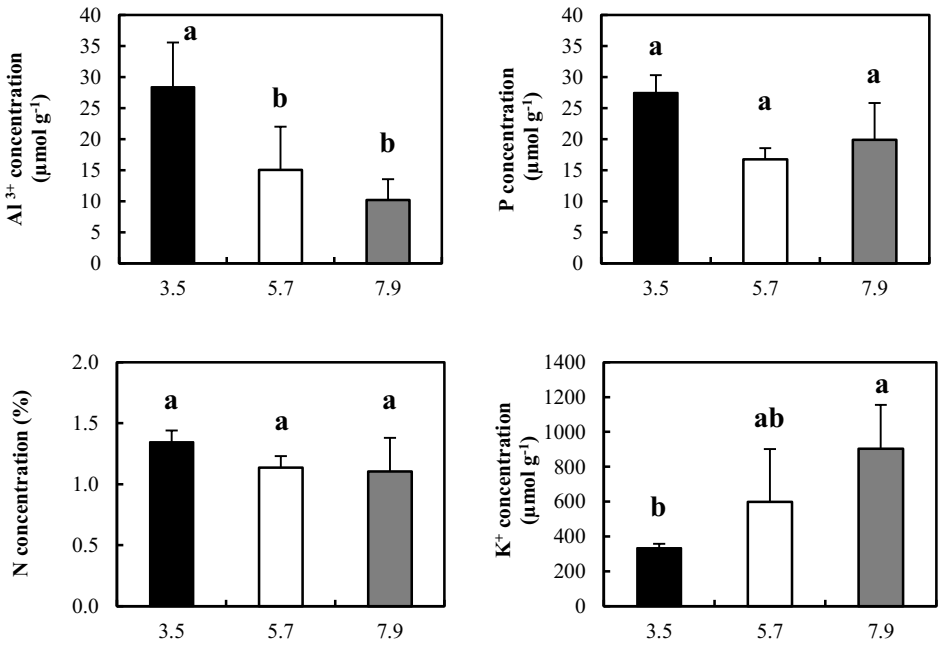


Fig. 2.18 Mg^{2+} concentration in leaflets (a) and petioles (b) at different leaf positions. Horizontal lines indicate the standard deviation (n=3).

3.2.2 Nutrient and ion concentrations in bases

The ion concentrations in bases were showed in Fig. 2.19. The Al^{3+} concentration tended to increase at lower pH among the three pH treatment levels. There was significant differences, pH 3.5 was highest followed by pH 5.7 and 7.9. In contrast, the K^+ and Ca^{2+} concentrations tended to decrease at lower pH among the three pH treatment levels. There were significant differences; pH 3.5 was lowest, followed by pH 5.7 and 7.9. However, at pH 5.7 there was no significant difference between pH 3.5 and 7.9 among the K^+ and Ca^{2+} concentrations. Moreover, the N (%), P, Na^+, and Mg^{2+} concentrations there were no significant differences among the three pH treatment levels.

The base of sago palm seedling has been a little or not examined, but this is an important part to nutrient accumulation. In this current experiment, the bases were distinguished to investigate as the base is a connector between root and petiole of sago palm, where nutrients can be accumulated. However, ion concentrations in the bases almost tended to be similar to the roots. The Al^{3+} concentration in the bases is less than the roots but higher than petioles.



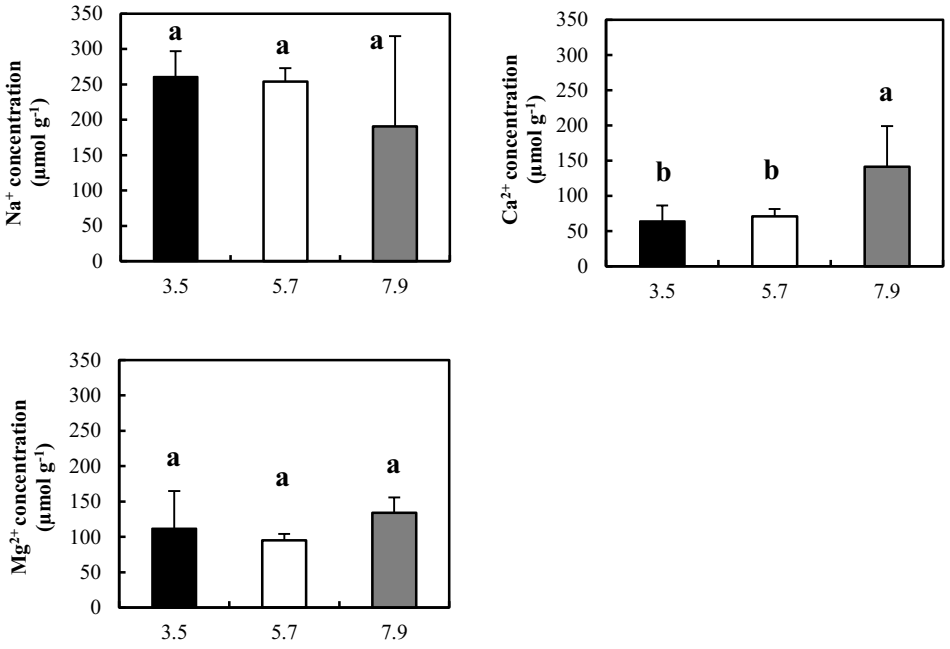


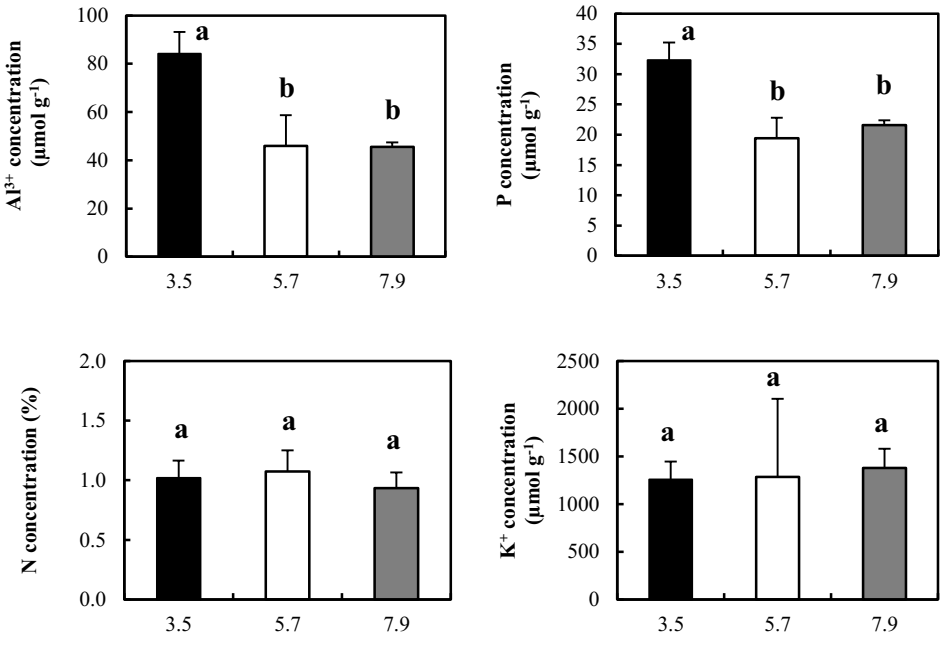
Fig. 2.19 Al^{3+}, P, N, K^{+}, Na^{+}, Ca^{2+} and Mg^{2+} concentrations in the bases. Vertical lines indicate the standard deviation (n=3). Means followed by different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

3.2.3 Nutrient and ion concentrations in roots

The ion concentrations in roots were showed in Fig. 2.20. The Al^{3+} concentration tended to increase distinctly at lower pH among the three pH treatment levels. There were significant differences at pH 3.5, which was highest, followed by pH 5.7 and 7.9. Similarly, the P concentration tended to increase distinctly at lower pH among the three pH treatment levels. There were significant differences; pH 3.5 was highest, followed by pH 5.7 and 7.9. In contrast, the Mg^{2+} concentration tended to decrease distinctly at lower pH among the three pH treatment levels. There were significant differences; pH 3.5 was lowest, followed by pH 7.9 and 5.7. Moreover, the N (%), K^{+}, Na^{+} and Ca^{2+} concentrations showed no significant differences among the three pH treatment levels.

According to Rout et al. (2001) it was reported that some Al passed through the epidermis and cortex, but considerable amounts were retained in cortical cells .Although a large fraction of the Al interacts with apoplastic targets, a small fraction enters the symplasm and interacts with

symplastic targets. Moreover, severity of Al toxicity depends on the concentrations of Ca^{2+} and other cations in the external solution, the ionic strength of solutions, pH, the presence of chelators, cell type and plant genotype (Kinraide and Parker, 1987). In addition, Tan and Keltjens (1990) reported that increasing P supply might play a role in ameliorating Al phytotoxicity, possibly through improved root growth and P uptake. Phosphorus efflux was speculated to be a potential mechanism of Al tolerance in wheat (Pellet et al., 1996). Zheng et al. (2005) found that the P content of the root apex of buckwheat was significantly correlated with the immobilization and detoxification of Al, indicating that there can be a significant P by Al interaction in roots. However, according to Haug and Vitorello (1996), long-term exposure to Al and inhibition of root growth generally lead to P, K, Ca and Mg deficiencies and the ultimate consequence is reduced plant biomass. But in this case, P and K efficiencies were contrasted. With the exception of Al-accumulating plants, little Al is transported into the shoot (Watanabe and Osaki, 2002), which it similar with sago palm case.



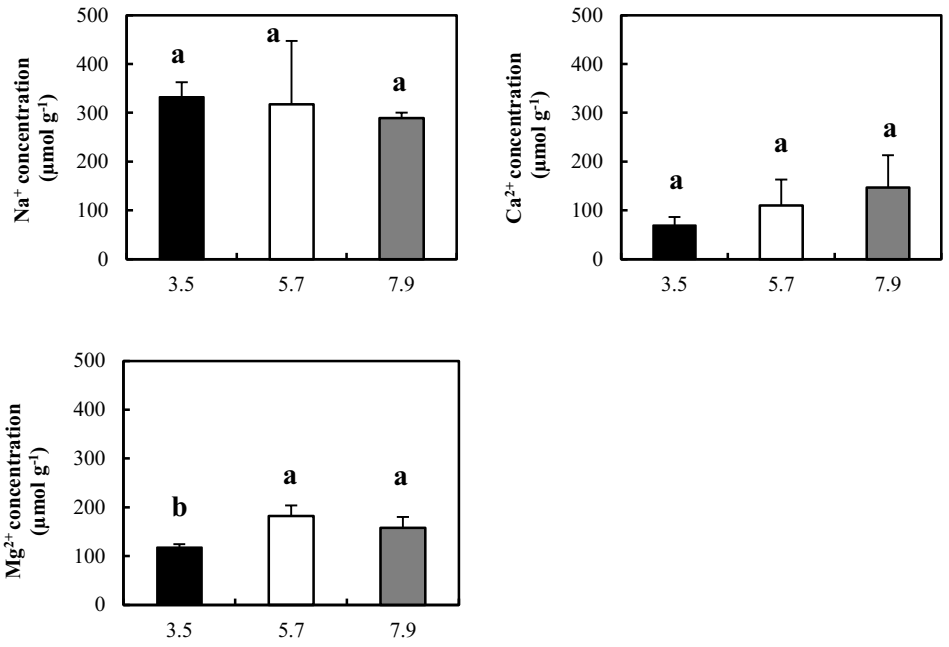


Fig. 2.20 Al^{3+}, P, N, K^{+}, Na^{+}, Ca^{2+} and Mg^{2+} concentrations in the roots. Vertical lines indicate the standard deviation (n=3). Means followed by different letters within a column are significantly different at the 0.05 level by the Tukey's HSD test.

Conclusion

The differences in growth of sago palm was not among the three pH treatments in the plant height, plant length, leaf numbers, leaflet numbers, emerged leaf numbers, green leaf numbers, dead leaf numbers and total leaflet area. The root weight was large at higher pH conditions; contrarily the petiole weight was largest at lower pH conditions. Then, there were no significant differences in the total weight. The photosynthetic rate, transpiration rate and stomatal conductance in leaflets showed a similar trend, the values of which were significantly higher at pH3.5 than at pH7.9 and 5.7, respectively. The Al^{3+} concentration in culture solution differenced depended on solution pH and was highest at pH 3.5, followed by pH 5.7 and pH 7.9. The Al^{3+}, N and P concentrations in almost all parts tended to increase at lower pH treatments. The Al^{3+} concentration accumulated into the roots was higher than that in other parts; the difference was significant at lower pH conditions. However, the N concentration in the leaflets was higher than the other parts and the difference was significant at lower pH conditions. The P concentration was also higher in the leaflets higher than other parts but the difference was not significant. From these results, it is concluded that in the range of pH level in the current experiment was quite wide. All of pH treatment levels had the same trend to several measured parameters. Moreover, it was found that at lower pH treatment levels had plant growth and nutrient uptake more than higher pH treatment levels and sago palm had an ability to grow under widely different pH ranges from 3.5 to 7.9.

Therefore, the next experiment should investigate the effect of aluminum on sago palm to clarify the mechanism of Al resistance and interaction among plant characteristics and Al toxicity in sago palm seedlings in more details.

Chapter 3

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

Introduction

Generally, soils that are acidic 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). Besides, it is usually the extremes of acidity that cause environmental problems, difficulties in growing plants. The important problem is various soil chemical factors that limit plant growth and also reduce crop production and yield such as Al, Mn and other cations in acid soils (Foy, 1992). One of them is aluminum (Al), it is 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 researches reported the effect of aluminum on plants; for example, Clarkson (1965) reported that the 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, aluminum solubility involved in soil pH is such that increases the availability of biologically toxic Al with decreasing pH (Flis et al., 1993). However, some plants are more 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 supplies for food and energy sources. The sago palm (*Metroxylon sagu* Rottb.) is one of the outstanding tropical crops and dominates in mainly permanent freshwater swampy areas or peatland in Southeast Asia (Sato et al., 1979; Jong, 1995). All parts of sago palms can be used for several purposes. Leaflets are used for

house thatching, rachis is used for house building, cortex of the trunk is used in factory activities such as firewood, pith is used for starch extraction or animal feed as 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, submerged in saline soils where few other crops survive growing more slowly in peat soil than in mineral soil (Flach and Schuilling, 1989). Sago palm is expected to enhance agricultural production at lower productivity areas where are covered by problem soils.

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

There are few studies about the effect of aluminum on the growth of sago palm (Anugoolprasert et al., 2014), however information about morphogenesis and physiological response of sago palm under different aluminum concentrations is still very limited. For promotion of sago palm cultivation in problem soils, further studies from eco-physiological point of view should be carried out to understand agronomic features of this rare plant species under severe environment more in detail. Thus, we analyzed the growth of sago palm seedlings grown in different aluminum concentrations in a culture media. Hence, the 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

The fruits of the *Manno* type sago palm were collected from Sentani, Jayapura, Province of Papua, Indonesia, on July 11, 2010 and treated to be clean seed by removing seed coat tissues. The clean seeds were sown in a cell tray consist 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 Noen Co., Ltd., Kanuma, Tochigi, Japan) and keep 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, the seedlings were transplanted in a 1/5000a Wagner pot filled with 400g of vermiculite. Three levels of aluminum concentration ($AlCl_3$•$6H_2O$): 0, 150 and 300 ppm with 3 replications were added into 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 value of the culture media in all concentrations was adjusted to 3.5 by a pH meter (HORIBA, Ltd., Twin pH meter B-212, Japan) with 1.0N H_2SO_4 and 1.0N KOH as suitable and beneficial. Culture solution was added every day, according to the amount of solution consumed and renewed every 2 days. During the experiment, the hydroponics system was set up; an air pump was connected into the pots to supply air for roots in both the groups of the control and the treated plants. The pots were placed in the greenhouse under natural sunlight at the Faculty of Bioresources, Mie University, Japan. The experimental treatment was conducted starting from 1 July to 29 August 2014 a total of 60 days (approximately 2 months). The mean day and night temperatures during the Al treatment were 30 °C and 22 °C, respectively. The experiment was set as show in Fig.3.1.

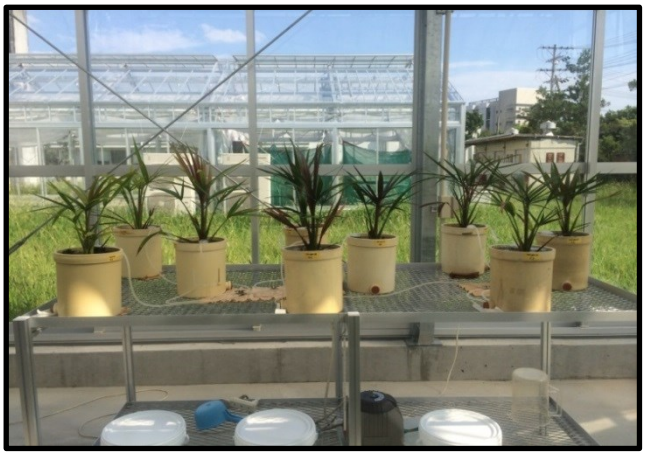


Fig. 3.1 The sago palms in each treatment (0 ppm; 150 ppm; 300 ppm) after transplanting from beginning in July 2014.

2. Plant growth and growth analysis

The plant growth parameters such as plant height (from soil surface to the top of the standing palm), plant length (from soil surface to the tip of the longest leaf: total length of shoot) were measured and leaf number, leaflet number per leaf, dead leaf number, new emerged leaf numbers were counted every week during the experiment. The sago palm seedlings were sampled 2 times at the beginning and end of the experiment. The plant samples were divided into four parts: leaflets, petioles with rachises, base of shoot (leaf sheath part holding the other leaves' sheath inside) and roots. Root diameter was measured by using a Digital LCD Stainless Vernier Caliper Bio-Cal (NK system, Japan) and the leaflet area was measured by using Automatic area meter (AAM-9, 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 consisting of 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

The leaflets of the most active leaves (the uppermost to third expanded leaf from the top) were used for the measurements of variables.

3.1 SPAD

The Soil and Plant Analyzer Development (SPAD) value, indicating chlorophyll content, used a Chlorophyll Meter (SPAD-502, Minolta Co., Ltd., Japan). SPAD was measured every week for 3 points such as base, middle and tip parts of a leaflet attached to middle position of the upper most fully developed leaf in each plant after transplanting.

3.2. Chlorophyll content

The chlorophyll content of the leaflets in each treatment was measured by the method of Mackinney (1941). An area of 0.25 cm^2 from each leaflet (middle part of leaflet that same with 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 (UVmini-1240, Shimadzu, Japan) at final sampling.

3.3 Stomatal conductance

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

3.4 Photosynthetic rate and transpiration rate

The photosynthetic rate and transpiration rate were measured by a portable photosynthetic meter (LCA-4, Analytical Development Co., Ltd., 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 a halogen lamp of a KODAK EKTAGRAPHIC model AF-2 slide projector (Kodak eastern company, USA). The appropriate P.A.R. was obtained by changing the distance between the projector and leaves at final sampling.

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

The dried samples were ground to a fine powder by a blender for analyzing the total N, P and ion concentrations. The amount of total nitrogen (N) was analyzed by the semi - micro Kjeldahl method; the 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 using spectrophotometer by the ascorbic acid method. The 0.25 g of dried samples was used for extraction by 10 ml of 60% HNO_3 at 140 ºC for 9-10 hr. After that, sample solution was diluted in measuring cylinder to 25 ml total with 1% HNO_3. The 200 µl sample solution and 4 ml mixture of regents including 2.5 M H_2SO_4, 2 mM $C_8H_4K_2O12Sb_2•3H_2O$, 32 mM $(NH_4)6Mo7O24•4H_2O$, 100 mM $C_6H_8O_6$ were mixed and diluted in measuring cylinder to 25 ml total with distilled water. Then the final solution was examined with the absorbance at 880 nm by a spectrophotometer (UVmini-1240, Shimadzu, Japan). The aluminum concentration (Al) was determined by the aluminon calorimetric method. The 0.05 g of dried samples were reduced to ash in a muffle furnace (Yamato FO 300, Japan) at 500 ºC for 4 hr. Sample solution was extracted by 10 ml of 6N HCl at 140 ºC for 2 hr. Sample solution was added 25 ml of 1% HCl and was diluted in 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% $C_{22}H_{23}N_3O_9$, 0.5 ml of 1% $HSCH_2COOH$ and diluted in measuring cylinder to 50 ml total with distilled water. After that, the final solution was examined with the absorbance at 525 nm by a spectrophotometer (UVmini-1240, Shimadzu, Japan). The cation concentration analysis was analyzed by a high performance liquid chromatograph (HPLC). The 0.05 g of dried samples were reduced to ash in a muffle furnace (Yamato FO 300, Japan) at 350 ºC for 2 hr and 450 ºC for 8 hr. Sample solution was extracted by 100 µl of 1N HNO_3 and was diluted in measuring cylinder to 25 ml total with distilled water. The 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 40 ºC, 50 µl of injection volume. The flow rate of the mobile phase was 1.0 ml/min. The 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

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

6. Statistical analysis

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

Results and Discussion

1. Plant growth and growth analysis

After treated Al for 8 weeks, the morphological growth of sago palm seedlings was presented in each treatment in the pots (Fig. 3.2), the morphogenesis of sago palm seedlings in each treatment was presented in Fig. 3.3, and the root morphogenesis of sago palm seedlings in each treatment was also presented in Fig. 3.4. In this study, the plant height, plant length, base diameter, leaf number per plant, leaflet number per plant, leaflet number per leaf and emerged leaves per plant for 8 weeks during the Al treatment were almost same level among three treatments (0, 150 and 300 ppm Al) and there was no significant difference in such parameters (Table 3.1 and 3.2). The root diameter (mean value of the data among the 5 different distances from the root tip) was significantly decreased in both Al treated plants (150 and 300 ppm Al) compared to non-treatment (0 ppm Al) and dead leaf was significantly increased in both Al treated plants compared to non-treatment, but in both Al treated plants there was no significant difference. The results in Table 3.1 suggest that Al concentrations in the media directly affected the root diameter and dead leaf. Piňeros and Kochian (2001) reported that the initial symptom of Al toxicity is the inhibition of root elongation, which has been suggested to be caused by a different mechanism, including Al interactions within the cell wall or plasma membrane. Dead leaf number has a relationship with leaf senescence, which during senescence, nutrients such as nitrogen, phosphorus and other metals in the leaf are reallocated to younger leaves and to growing seeds, or are stored for the next growing season (Buchanan-Wollaston, 1997). Leaf senescence is programmed as changes in many metabolic and morphological aspects of plant. However, leaf senescence can be induced by various environmental stresses particularly, by aluminum toxicity. Besides, it is suggested that aluminum enhanced the leaf senescence at faster rate in rice leaves (Muthukumaran and Vijaya Bhaskara Rao, 2013). In this study also, deal leaf number was increased significantly with Al treatments as shown in Table 3.2.

Table 3.3 shows that dry matter weight in 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 whole plant tended to decrease when Al concentration increased, significant differences were found only in root and whole plant between the non-treatment and Al treated palms. Several evidences reported about the effect of aluminum on plant and root growth such as the Al-induced phytotoxic symptoms, disruption of cell division and growth within the root has a significant effect on 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 Al concentration 200 ppm had an effect on dry weight such as leaflet, petiole and root in sago palm for 4.5 months. Considering the large difference in root between non-treatment and two Al treatments, it might be a factor affecting the difference in total dry weight of whole plant (Table 3.3). There was a markedly significant difference in 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 3.4). Considering the results of leaflet area in Table 3.4, the Al concentration higher than 150 ppm might affect the total leaflet area per plant due to Al toxicity which may be attributed to flexibility in leaflet membrane leading to a decrease in higher Al treatments. According to the results of Anugoolprasert et al. (2014) who found that in higher concentrations of Al treated sago palms, the total leaflet area per plant was decreased by effect of Al. There was no significant difference in the single leaflet area among the three treatments. However, higher Al concentration (300 ppm Al here) tended to decrease area of each leaflet slightly. This result was similar to Thornton et al. (1986) who reported that the rate of leaf expansion of seedlings of honeylocust grown in Al solution was reduced compared with the control. On the other hand, the single leaflet area in 150 ppm Al treatment has a including tendency rather than that in 0 ppm Al treatment.

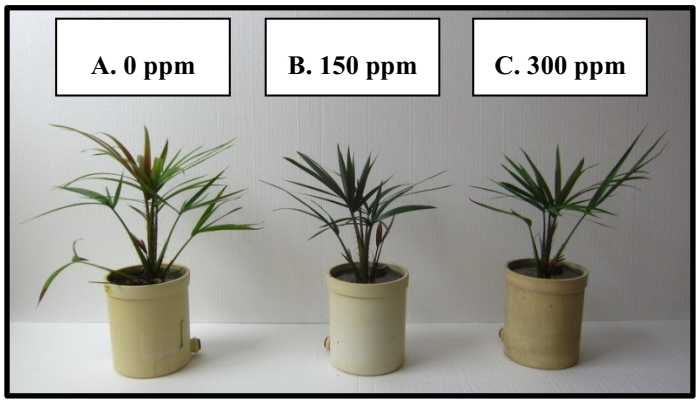


Fig. 3.2 The sago palm seedlings grown in each treatment in the pots (A. = 0 ppm Al, B. = 150 ppm Al, C. = 300 ppm Al) for 8 weeks at different aluminum concentrations in culture media.

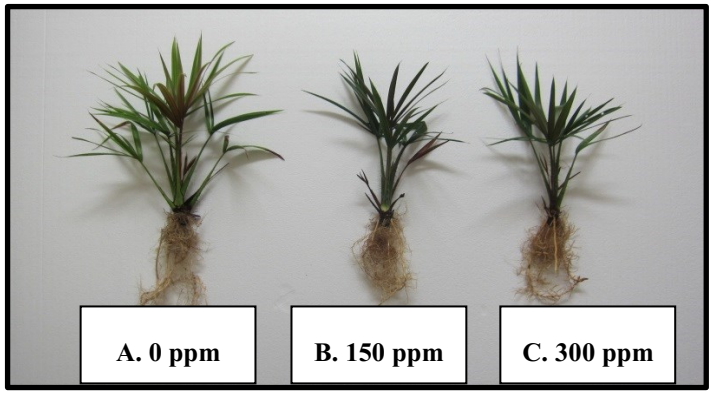


Fig. 3.3 Plant morphology of sago palm seedlings in each treatment (A. = 0 ppm Al, B. = 150 ppm Al, C. = 300 ppm Al) for 8 weeks at different aluminum concentrations in culture media.

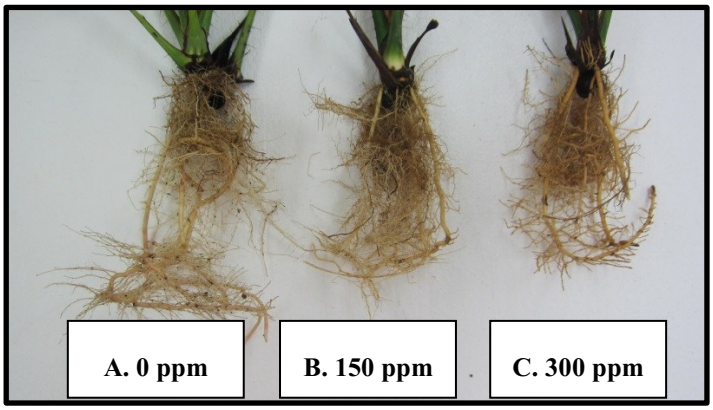


Fig. 3.4 Root morphology of sago palm seedlings in each treatment (A. = 0 ppm Al, B. = 150 ppm Al, C. = 300 ppm Al) for 8 weeks at different aluminum concentrations in culture media.

Table 3.1 Plant size 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)
0 ppm	34.6 ± 4.1 a	40.6 ± 4.7 a	2.9 ± 0.4 a	3.06 ± 0.2 a
150 ppm	33.9 ± 3.6 a	37.1 ± 2.6 a	2.3 ± 0.4 a	2.34 ± 0.2 b
300 ppm	34.3 ± 0.5 a	40.8 ± 3.2 a	2.5 ± 0.3 a	1.97 ± 0.2 b

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

Table 3.2 Leaf characteristics of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media.

Al treatment	Leaf number/plant	Leaflet number/plant	Leaflet number/leaf	Emerged leaves/plant	Dead leaf number/plant
0 ppm	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	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	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 difference at the 0.05 probability level, according to the Tukey's HSD test.

Table 3.3 Dry matter weight in 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 difference at the 0.05 probability level, according to the Tukey's HSD test.

Table 3.4 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 difference at the 0.05 probability level, according to the Tukey's HSD test.

Parameters of growth analysis were calculated using averaged value of three plants in dry matter weight or leaflet area of three plants at the 1st sampling (just before the Al treatments) and data from the individual of three plants at the 2nd sampling (at the end of the Al treatments), and then the mean value of growth parameters such as RGR and NAR were taken. In these kinds of parameters delivered from growth analysis, the procedures of significance test are not suitable. Therefore, the mean values from the different Al concentration plots were just listed with their standard deviation (n=3) (Table 3.5). The RGR tended to be slightly decreased with Al treatments. The value of RGR was smaller 12% in 150 ppm Al treatment and 16% in 300 ppm Al treatment rather than that in 0 ppm Al plot. The NAR of 150 ppm and 300 ppm Al treatments was slightly smaller that of 0 ppm Al treatment, the difference of which was about 12%. LAR showed slight difference in the three treatments. As shown in the results of SLA with slight difference within about 4% and single leaflet area with no significant difference among the three treatments, the change in leaflet morphogenesis such as single leaflet structure was small. 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) that 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 value of RLGR, LRGR, SRGR and RRGR tended to decrease with higher Al concentration, however the extent of decrease in RRGR was apparently larger than the other relative growth

parameters. The RRGR decreased by 30% and 32 % in 150 ppm and 300 ppm Al, respectively. Growth parameters were analyzed for 8 weeks (Table 3.5), RGR indicated the rate of increase of total dry weight of individual per day, which is the central parameter in plant growth analysis (Hunt, 2002) and consists of NAR and LAR. NAR that generally relates with photosynthetic performance showed slight difference in the three plots. LAR to investigate morphogenesis of individual plant; the LAR in the current experiment was same level among the three plots. SLA is an index of leaf thickness, which involves an assessment of the leaf's area in relation to its dry matter weight; this parameter was also same level among the treatments. LWR is the ratio of leaf weight to whole plant weight; the difference in this parameter was negligible, therefore LWR was considered to be very stable parameter under different Al concentrations. From these results, the difference in growth rate of individuals might be attributed to the difference in assimilation rate with accelerated leaf senescence, not too much in the morphological characteristics. In LAR, the dry matter might increase in maintenance a good balance with the increase in single leaflet area and total leaf area. Moreover, result of RRGR parameter showing decreased root growth, which might be attributed to transverse ruptures reported by Kopittke et al. (2008). They reported that 1) root elongation rate decreased by Al, accompanied by a decrease in the distance from the root tip to the proximal lateral root; and 2) kinks developed in some roots on exposure to Al, 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 inner cortical cell layers. The depression of root growth rate by the Al treatments was related to the differences in the root diameter (Fig. 3.6 and 3.8) and the root cell number per mm^2 (Table 3.9) in the current experiment.

Table 3.5 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 ($g\ g^{-1}$)
0 ppm	28.88 ± 2.98	0.533 ± 0.048	54.1 ± 1.7	166.1 ± 8.6	0.326 ± 0.015
150 ppm	25.46 ± 1.11	0.469 ± 0.052	54.7 ± 5.2	163.1 ± 14.5	0.335 ± 0.009
	(88.2%)	(80.0%)	(101.1%)	(98.2%)	(102.8%)
300 ppm	24.28 ± 1.92	0.469 ± 0.074	52.2 ± 4.7	159.1 ± 15.7	0.328 ± 0.006
	(84.1%)	(88.0%)	(96.5%)	(95.8%)	(100.6%)

Al treatment	Growth analysis parameter			
	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	30.31 ± 3.42	30.41 ± 3.28	26.37 ± 6.97	26.57 ± 1.30
150 ppm	27.12 ± 2.39	28.56 ± 1.37	24.82 ± 1.92	18.60 ± 0.75
	(89.5%)	(93.9%)	(94.1%)	(70.0%)
300 ppm	24.74 ± 1.37	27.58 ± 1.84	20.60 ± 3.20	18.07 ± 2.20
	(81.6%)	(90.1%)	(78.1%)	(68.0%)

Each value represents the mean ± SD (n=3). The values in the parentheses indicate relative value to 0 ppm treatment.

2. Characteristics relating to photosynthesis

2.1 SPAD and chlorophyll content

There were significant differences in SPAD and chlorophyll content per unit leaflet area between non-treatment and Al treatments (Table 3.6). As the results show, the SPAD value had a positive relationship with chlorophyll content and the both parameters were increased in 150 and 300 ppm Al treatments. 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) because the majority of leaf N is contained within the chlorophyll molecules. However, in this study, the chlorophyll content was not a similar trend with leaf nitrogen concentration (Table 3.7 and 3.8). The chlorophyll content was expressed per unit leaf area and different from the N concentration in leaflet dry weight basis. And the different tendency between chlorophyll content and N concentration was might reflected the difference in leaf morphogenesis such as smaller RLGR (leaflet area expansion rate) as well in Al-treated plants. Moreover, in Fig. 3.5, the observation that the leaf color of sago palm seedlings dramatically changed to dark green in both Al treatments which might be affected by Al. Fukrei et al. (2011) made the same observation and found that the foliar symptoms may be stunting, small, dark green leaves and late maturity. Rout et al. (2001) and Adams (1984) also reported about effects of aluminum toxicity on leaves that in plants, the foliar symptoms resemble those of phosphorus deficiency. Generally, inadequate P slows the process of carbohydrate utilization, while carbohydrate production through photosynthesis continues, and then these 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 on leaf photosynthesis, while resulting in increased SPAD value compared to phosphorous-sufficient plants in cotton. The changes in leaflet characteristics of sago palm in the current experiment might be related to P deficiency shown in Table 3.8. Now, it is still unclear about the mechanism of Al responses to Al toxicity in sago palm leaflets.

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 3.6). Stomatal density is an important component of stomatal conductance, so gas exchange through the stomata is determined by the width, length, 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 about stomata density but the tendency of stomata density is close 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 the decrease in thickness. However, there was no difference in SLA indicating leaf thickness among the three treatments, whereas the Ca concentration in the whole plant was much higher in the non-treatment than those in two treatments of Al (Table 3.8). Considering the former reports and our current results, the cause of slight decrease in growth rate by the Al treatments was the tendency of slightly small NAR, which might be through the difference in stomatal conductance. And Al concentration also had an effect on stomatal aperture considering a report by Anuggolprasert and Ehara (2013) that Al was detected preferentially in the upper epidermis and occasionally in the lower epidermis in the leaflet. Besides, regulations in potassium and chloride ion channels at the plasma membrane of guard cells, leading to stomatal closure by reducing transpiration (Leyman et al., 1999). Moreover, as Ohsumi et al. (2007) reported the importance of stomatal conductance, as well as leaf nitrogen content whereas in the current study, leaf nitrogen content was not significantly different among the 3 treatments (Table 3.8).

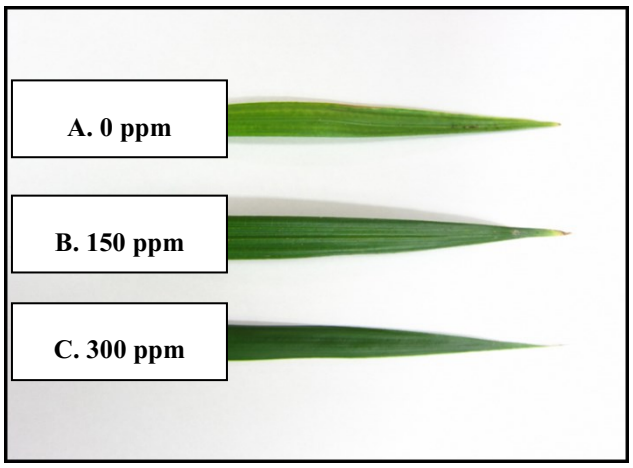


Fig. 3.5 Leaf morphology of sago palm seedlings grown in each treatment (A. = 0 ppm Al, B. = 150 ppm Al, C. = 300 ppm Al) for 8 weeks at different aluminum concentrations in culture media.

Table 3.6 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 ($\mu g/cm^2$)	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 difference at the 0.05 probability level, according to the Tukey's HSD test.

2.3 Photosynthetic rate and transpiration rate

The photosynthetic rate was not significantly different and actually same level among the three treatments (Table 3.7). Many studies have reported on photosynthesis in plants such as Hoddinott and Richter (1987) for example, reported that a decrease in photosynthesis and in the translocation of photosynthates in beans after direct injection of Al into the xylem. Moustakas et al. (1996) found that Al indirectly caused significant disturbances in the chloroplast architecture in plants, with a decrease in photosynthesis due to a reduction of electron transport in photosystem II. However, the impact of Al on photosynthesis is probably indirect in this experiment due to photosynthetic rate is environment-dependent traits and change with leaf ontogeny. It 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 of gas exchange activity on photosynthesis. Although the stomatal conductance decreased in 150 and 300 ppm Al treatments, there was no significant in photosynthetic rate among the three plots in this experiment. This might be related with the increase in SPAD and

chlorophyll content per unit leaflet area in 150 and 300 ppm treatments rather than in non-Al treatment (Table 3.6).

The transpiration rate was also not significantly different among three treatments, though the value of plants treated with 300ppm Al treatment was 20% smaller than that of non-treated plants (Table 3.7). The transpiration rate can indicate the loss of water vapor through the stoma of leaves. The result of transpiration rate in this experiment had tended to decrease in higher Al treated palms, which was in agreement with the report of Ohki (1986) who found that in wheat, Al toxicity decreased transpiration. The critical Al toxicity concentrations in wheat blade 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 stomatal opening (Schroeder, 1988).

Table 3.7 Photosynthetic rate and transpiration rate on different aluminum concentrations in culture media at 8 weeks after Al treatment.

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 difference at the 0.05 probability level, according to the Tukey's HSD test.

3. Mineral and ion concentrations in different plant parts

In this study, the nutrient concentrations such as N, P, K^+, Ca^{2+}, Mg^{2+} and Al^{3+} in the parts of leaflet, petiole (including rachis), base, root and whole plant of sago palm seedlings at different aluminum concentrations at 8 weeks after treatment were investigated and shown in Table 3.8. The N concentration among the parts of sago palm seedlings had a quite similar tendency in 0 and 150 ppm Al treatments, which in the leaflet and petiole it was significantly

higher than that in the base and root, while at 300 ppm Al treatment, the N concentration in the petiole was not significantly different with the base and root. In all parts of sago palm seedlings, there were no significant differences in N concentration among the three treatments.

The P was accumulated highly in base, followed by petiole, leaflet and root in all the treatments. Comparison among the three treatments, the P concentration in leaflet and base were slightly decreased even if Al concentration increased and there was a significant difference among the three treatments in petiole, root and whole, with the highest Al treated palms having the lowest P concentration.

The K^+ concentration among the parts of sago palm seedlings, showed a significant difference in root and petiole than that in base and leaflet in all treatments in which K^+ concentration was lowest accumulation in the leaflet, followed by base, petiole and root. Comparison among the three treatments, the K^+ concentration was not significantly different in leaflet, petiole, base, root and whole plant under Al stress.

The Ca^{2+} concentration in 0 ppm Al, there was no significant difference among the parts of sago palm seedlings, but in 150 and 300 ppm Al treatments, there was significant difference between leaflet and other parts: petiole, base, root and whole, which both leaflets of Al treated plants were markedly decreased. Comparison among the three treatments, the Ca^{2+} concentration in 300 ppm Al treatment, there was a significant difference in all parts compared with 0 ppm Al treatment. While in 150 ppm Al treatment, there was no significant difference in base and root compared with 0 ppm Al treatment.

The Mg^{2+} concentration among plant parts of sago palm seedlings was the same that tended to accumulate highly in root, followed by base, petiole and leaflet in all the treatments, which suggested that Mg^{2+} concentration might not re-translocate to the upper parts in sago palm seedlings. Comparison among three treatments, the Mg^{2+} concentration in leaflet, petiole, root and whole was slightly decreased even if Al concentration increased. There was markedly significant difference between 0 and 300 ppm Al treatments in leaflet and petiole, and root and

whole had significant difference between 0 ppm Al and Al treated treatments, whereas the base had no significant difference among the three treatments.

The Al^{3+} concentration among plant parts of sago palm seedlings tended to accumulate highly in the root, followed by base, petiole and leaflet in all the treatments. The Al^{3+} concentration of all treatments between petiole and leaflet were not significantly different. Comparison among the three treatments, the Al^{3+} concentration in leaflet, petiole, root and whole was increased when Al concentration increased. There was markedly significant difference in all plant parts among the three treatments.

The Al concentration has related to other minerals in soil and some ions in plant. According to Anugoolprasert et al. (2014), concentrations of Al had no significant difference in the N concentration in any root parts compared with the control. However, depending on 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 membranes permeability (Lidon and Barreiro, 2002). From these results, it can indicate that Al concentrations would not related with the N concentration in same plant part. For the P concentration, 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 which reduces the absorption of Al and its translocation to the shoots. In consequence, Al concentration in treated sago palm seedlings was indicated to be causing a lack of P concentration would relate to growth inhibition. In case of the K^+ concentration, it was demonstrated that Al may not block channels conducing the influx of K^+ in guard cells and 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 on higher Al concentration treatment in leaflet, root and whole compared with the non-treatment. The effects of Ca^{2+} on plant grown under conditions of Al stress have been recognized for a long time. According to Dogan et al. (2014) who 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 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 genotype, contrarily to the tolerant rye genotype (Silva et al., 2011). Therefore, in this study, Al toxicity may be involved in the inhibition of the cell division or root elongation by causing potential disruptions of Ca^{2+} for concentration of Al at more than 150 ppm in sago palm seedlings. In Mg^{2+} concentration, according to Mariano and Keltjens (2005) found that Al had negative effects on 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 decrease in K, Mg, Ca, and P contents and uptake (De Mendonca et al., 2003). As well as, in sago palm seedling, Al concentrations had an effect to decrease Mg^{2+} concentration compared with the non-Al treatment. Clearly, the Al treatments have a positive relation on Al concentration in different plant tissues, with much accumulation and increasing effect than that in the non-Al treatment particularly in root. In mostly acid soils, there are several limiting factors for 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 would interrupt the uptake of P, Ca and Mg in sago palm seedlings. It is not clear whether Al stress will make deficiency of major elements such as P, Ca and Mg or not, whereas N and K were in-deficiency in sago palm seedlings.

Table 3.8 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 difference at the 0.05 probability level, according to the Tukey's HSD test. Lowercase letter indicates comparison among the treatments in each part of sago palm seedlings. Capital letter indicates comparison among the parts of sago palm seedlings on different aluminum concentrations in culture media at 8 weeks after treatment.

4. Root morphogenesis

Root size is indicated by root diameter as shown in Fig. 3.6. The root diameter at 5 distances from the root tip under different aluminum concentrations, in culture media at 8 weeks after Al treatment. In non-Al treatment, the 5 distances of root diameter were slightly increased, which in 1cm from root tip was smallest and in 5cm from root tip was largest. In both 150 and 300 ppm Al treatments there was a similar tendency of root size with non-Al treatment. Comparison among the three treatments, all distances of root diameter were markedly decreased

in 150 and 300 ppm Al treatments, respectively compared with non-Al treatment. From the results, it can indicate that an increase of Al concentration caused a decrease in root diameter at the same distance. Fig. 3.7 shows the effect of Al toxicity on root morphology of sago palm seedlings in culture media at 8 weeks after Al treatment. The observation of differential Al concentrations was exposed as morphological and structural responses of sago palm seedling roots. The root color changed to dark in Al treated treatments; the roots in higher Al concentration (300 ppm Al) became darker. Moreover, Al toxicity involved the number of cells in cortex per unit area in transverse section of roots is shown in Table 3.9 which describes the number of cells in roots among 5 distances of root length on different aluminum concentrations in culture media at 8 weeks after Al treatment. In non-Al treatment, there was a significantly large number among the distances from root tip of 1, followed by 2, and 3, 4 and 5 cm. In 150 and 300 ppm Al, 1 cm distance from root tip was significantly larger number than in the distances from root tip of 2, 3, 4 and 5 cm, which in 5cm distance from the root tip was significantly small. A smaller number of root cells per unit area at more distant part from the root tip indicated that cell root cell size was larger at more distant part, that is cell growth. Comparison among the three treatments (Table 3.9), there was a significant difference in the distance from the root tip of 1cm while the distances from root tip of 2 to 5 cm were significantly different between non-Al treatment and both Al treatments. Fig. 3.8 shows the mean value of root diameter for 5 distances from the root tip of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media. Variation of Al treatments affected root diameters, creating significant differences between the non-Al treatment and Al treatments. The mean value of root diameter for 5 distances from the root tip of sago palm seedlings grown for 8 weeks was significantly decreased in 300 ppm Al compared with other treatments, but there was no significant difference between 150 and 300 ppm Al. Fig. 3.9 shows the cross sections of sago palm root at different aluminum concentrations in culture media at 8 weeks after Al treatment. It can indicate that the distribution of root cells which was treated by Al or without Al.

From the results in Fig. 3.6, 3.7 and 3.8, it might suggest that root growth inhibition is a symptom of Al toxicity. Mostly, the inhibition of root growth is considered to be the result of inhibited cell elongation and expansion, prior to inhibiting cell division (Ciamporova, 2002). Silva et al. (2000) stated that prolonged exposures lead to Al interactions with the root cell division and the cytoskeleton. Thickened cell walls were frequently observed in Al-treated roots of oat (Marienfeld et al., 1995) and maize (Vázquez et al., 1999). Danilova et al. (1992) found that numerous vesicles indicated deposition of polysaccharide material into the cell wall via extreme exocytosis in epidermal and cortical cells of Al-treated soybean roots. Relationship between Fig. 3.9 and Table 3.9 is described by several studies such as Clune and Copeland (1999) found that at higher concentrations of Al, root growth was strongly inhibited, with cellular damage being observed primarily in peripheral root cap cells. After they were 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 Al induced inhibition of cell division in root tips of onions and the observation that Al binds to nucleic acids supported the view of Al induced inhibition of root cell proliferation as a primary target for Al toxicity. Thus, understanding of the cell size in the growing zone of root 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 1cm distance from root tip of sago palm seedlings. Vacuolation was observed in root cortex, dark cells were found in the vacuoles of Al 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 accumulation of both aluminum and callose occurred also in the developmental stage of cell ontogeny. Moreover, recently many evidences support 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 and thus pectin content may be related to 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 Al induced root growth inhibition 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, exclusion of Al from the root apex via exudation of root organic acids is the most important mechanism of Al resistance (Kochian et al., 2004). There are still no evidences of detection of organic acids from sago palm roots. Certainly, from the result in this study, it was cleared that Al induced alterations of root development and cell division as Al 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.

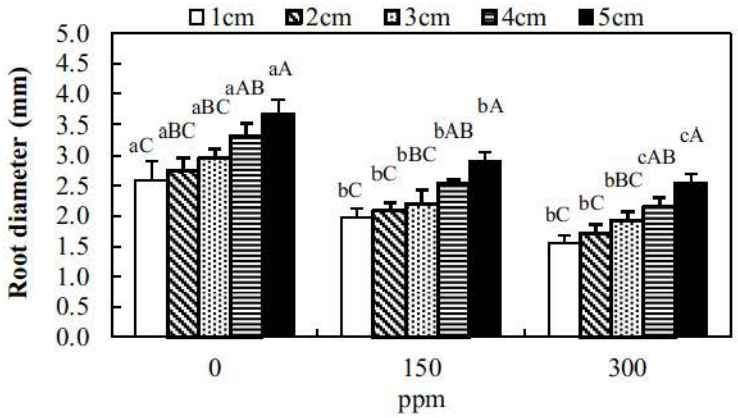


Fig. 3.6 Root diameter among 5 distances from the root tip of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media. Each value represents the mean ± SD (n=3). Different letters in the table indicate significant difference at the 0.05 probability level, according to the Tukey's HSD test. Lowercase letter indicates comparison among the treatments in each distance of root length. Capital letter indicates comparison among the distances of root length on same aluminum concentrations in culture media at 8 weeks after treatment.

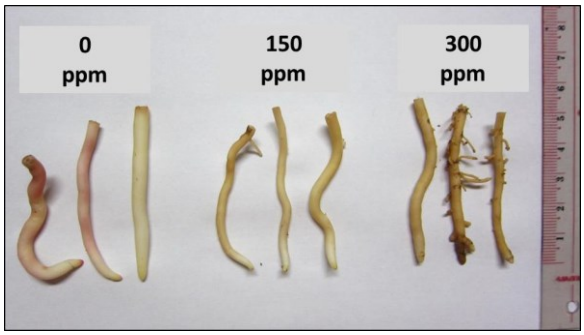


Fig. 3.7 Effect of Al toxicity on root morphology of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media. The Al treatments: A. = 0 ppm Al, B. = 150 ppm Al, C. = 300 ppm Al.

Table 3.9 The number of cells in cortex of roots at different 5 distances from the root tip of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media.

Al treatment	Root cell number / mm^2				
	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 aC	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 difference at the 0.05 probability level, according to the Tukey's HSD test. Lowercase letter indicates comparison among the treatments in each distance of root length. Capital letter indicates comparison among the distances of root length on same aluminum concentrations in culture media at 8 weeks after treatment.

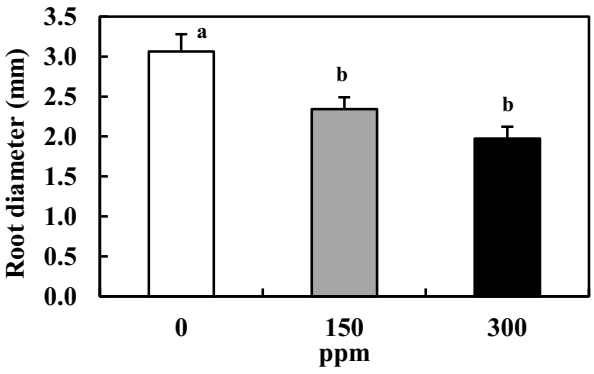


Fig. 3.8 Mean value of root diameter for 5 distances from the root tip of sago palm seedlings grown for 8 weeks at different aluminum concentrations in culture media. Each value represents the mean ± SD (n=3). Different letters indicate significant difference at the 0.05 probability level, according to the Tukey's HSD test.

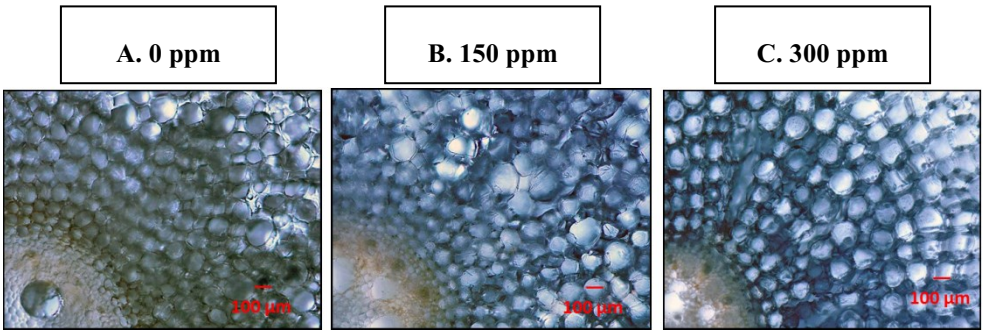


Fig. 3.9 Transverse section of sago palm root with different aluminum concentrations in culture media at 8 weeks after Al treatment. The Al treatments: A. = 0 ppm Al, B. = 150 ppm Al, C. = 300 ppm Al. 50 µm thickness by Plant Microtome MTH-1 (NK System), light microscope x100. Bar = 100 µm.

Conclusion

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

Therefore, future study should investigate about the mechanisms of external detoxification of Al such as the secretion of organic acids by roots of sago palm because these organic acids may chelate Al ions and transform them into a nontoxic form or prevent them from binding to the fixed negative sites of the cell wall and plasma membrane in sago palm root. However, this Al detoxification mechanism by organic acids has been found in many plant species, including both monocots and dicots, but for sago palm still no evidence with these mechanisms.

Chapter 4

Growth of Sago Palm Seedlings under Different Soil pH Conditions at the Experimental Farm in Kendari, Indonesia

Introduction

The sago palm (*Metroxylon sagu* Rottb.) grows swampy, alluvial and peaty soils where almost no other major crops can grow without drainage or soil improvement (Sato et al., 1979; Jong, 1995). Sago palm is one of the most important bioresources for not only sustainable agriculture but also rural development in swampy areas of the tropics (Ehara, 2009). Not only the importance of bioresources utility, but also the importance of a staple food security is continues and it is still grown in some areas of Southeast Asia and Melanesia. Its carbohydrate can be further processed into various basic raw materials for food, animal feed, and for industrial uses (Ehara et al. 2000). In Indonesia, sago palm has performed the important role in socio-economic and cultural aspect since hundred years ago. According to Oates (1999), by the early 14th century sago palm was the major agricultural product in the region between South Mindanaoa and Northern Borneo, South Sulawesi and the Maluku Islands. It was also reported that sago processing had been found in Sumatra in 1298 (Oates and Hicks, 2002). Sago palm can distribute in Sulawesi, according to Rasyad and Wasito (1986), sago palm stands in Sulawesi are distributed across various locations from northeastern Manado on the Minahasa Peninsula to Palopo, on the eastern shore of the Gulf of Boni. Semi-wild stands of sago palms were found around Kendari on the opposite side of the gulf in Southeast Sulawesi (The Society of Sago Palm Studies, 2015). At the mature stage, it produces a huge trunk that may reach 7-15 m in height and 1.2 m in average girth at the base of the palm (Flach and Schuiling, 1989). However, its size depends on several factors that include the planted area, planted density and physiological characteristics. Normally, sago palm dominates mainly in permanent or seasonal lowland freshwater swamps, preferably on mineral soils with a pH higher than 4.5. However,

according to Anugoolprasert (2012a), sago palm can grow in widely different soil pH range from 4.3 to 7.0 under natural condition in tropical area.

Plant growth is generally affected by relative concentrations of hydrogen ions (H^+). A plant's ability to endure various pH levels depends largely on its ability to take in and utilize nutrients at varying concentrations in the soil solution. Most crops grow well in soil that is neutral, mildly acidic, or mildly basic. Soils are naturally acidic or alkaline, so when correcting the pH of soil for healthy plant growth, it is important to better understand the growth response to soil pH and to be sensible about the long-term effects of different soil management on soil pH. Blagodatskaya and Anderson (1998) reported that the variations in soil pH are natural, due to the chemical characteristics of different types of soils. Generally, plant growth is limited by various factors relating to each soil type; for example, acidic soils significantly limit crop production worldwide, with approximately 50% of the world's potentially arable soils being acidic, illustrating an important constraint on agriculture (von Uexkull and Mutert, 1995). Some fertilizers can change the soil pH, and thus cause other nutrients to be more available, or can reduce certain nutrient availability to plants. Land utilization often faces problems such as unsuitability for plant growth or unwanted changes in soil pH. In the case where soil pH is exceedingly low, lime or dolomite can be used to improve the soil pH, bringing it to the desired level. Calcium carbonate ($CaCO_3$) is one of the liming materials used to improve or amend the soil, which is a common solution to acidity. However, the soils are characterized by high calcium carbonate ($CaCO_3$) content (more than 5%), which increases the amount of $CaCO_3$ in the soil, and as a result, the availability of most nutrients is considerably decreased (Chein and Somponges, 1987). Hence, the amount of lime or dolomite required to correct an acidic pH will vary from soil to soil. It is generally known that excessive use of lime or dolomite causes saline soils, and saline soils contain soluble salts in quantities that affect plant growth at various stages and create yield differences between crops, along with differences in the ion composition of crops at maturity (Sharma, 1997).

In this study, we analyzed the growth of sago palm seedlings grown in the native acid soil and in soil treated with calcium to increase the soil pH at the experimental farm in Southeast Sulawesi, Indonesia. Although the growth of the sago palm is generally considered to decelerate under a low pH condition, such growth depression is usually observed in peat soil compared with that in mineral soil, as reported by Sato et al. (1979). The purpose of the current study is to clarify the effect of different pH levels in soils that are amended with $CaCO_3$ and that have the same parent material on the growth of the sago palm.

Materials and Methods

1. Plant materials and Soil treatment

The seeds of Manno type sago palm that were collected from Sentani, Jayapura, Province of Papua, Indonesia, on July 11, 2010, were transferred to Kendari, Province of Southeast Sulawesi, as explained by Rembon et al. (2008). The germinated seeds grown in Kendari were used as planting materials. The two experimental plots were placed under natural sunlight in the experimental farm, Faculty of Agriculture, Halu Oleo University. In each plot (14.44 m^2) set in the 1 ha sago palm pilot farm, 16 sago seedlings at the third leaf stage (three leaves that have leaflets expanded) or 4 months after germination in a polyvinyl bag were transplanted. The compost with the trade name MOF-821 (Rembon et al., 2008) made from the sago pith residue after starch extraction (123 g C, 10 g N, 6 g P_2O_5, 20 g K_2O, 30 g CaO, 10 g MgO/kg) was applied in all the plots at the rate of 40 kg with physiological neutral fertilizers, 300 g urea, and 210 g superphosphate of lime (SP36 containing 36% P_2O_5). The two treatment plots were set up as follows: (1) control: native acid soil with fertilizers applied as described above; (2) calcium application plot: 3kg calcium carbonate ($CaCO_3$) was also applied to increase soil pH to above 6 prior to transplanting. The soil profile was characterized based on USDA classification methods (Pasolon et al., 2009). The experiment was conducted beginning on December 7, 2011, and 3 plants each among 16 plants in the experimental plot were taken as samples at the beginning of

the experiment as well as on June 24, 2012, and October 2, 2012. The plant growth parameters such as plant height (from soil surface to the top of the standing palm) and plant length (from soil surface to the tip of the longest leaf: total length of shoot) were measured, and the leaf number per plant, leaflet number per leaf, and dead leaf number were counted at the plant sampling in June and October 2012. The dry matter weight of leaflets, rachises, petioles, bases of shoots (leaf sheath part holding the other leaves' sheath inside) and roots, and the leaflet area was measured, and analyses of the growth from December 2011 to June 2012 and from June 2012 to October 2012 were performed.

2. Stomatal conductance and SPAD value

At 10 months after transplanting, the leaflets of the most active leaves or the 2nd leaf position from the top of the treated plants were selected to measure stomatal conductance by leaf porometer (Decagon Devices, Inc., Pullman, WA) at the sampling in October 2012. Stomatal conductance is a function of the density, size, and degree of opening, of stomata, which are pores in plants that open to the outside air. The leaf porometer measures stomatal conductance by putting the conductance of a leaf in series with two known conductance elements, and comparing the humidity measurements between them. The unit is used in mmol $m^{-2}s^{-1}$ for this experiment.

The leaflets of the most active leaves (the uppermost or second expanded leaf from the top) were used for measuring the Soil and Plant Analyzer Development (SPAD) value, indicating chlorophyll content, using the Chlorophyll Meter SPAD-502 (Minolta Co., Ltd., Japan) at the sampling in October 2012.

3. Sampling

At 10 months after transplanting, the control and the calcium application plots were sampled and were washed in distilled water carefully. The plants were separated into the four parts: leaflets, petioles (including rachis), bases and roots. The fresh weight of each part was

recorded. The leaflet areas were measured by using a photoelectric digital scanner (Hayashi-Denko Company, AAM-9, Japan). All of the fresh samples were dried in an oven at 80 ºC for 72 hours to measure the dry weight.

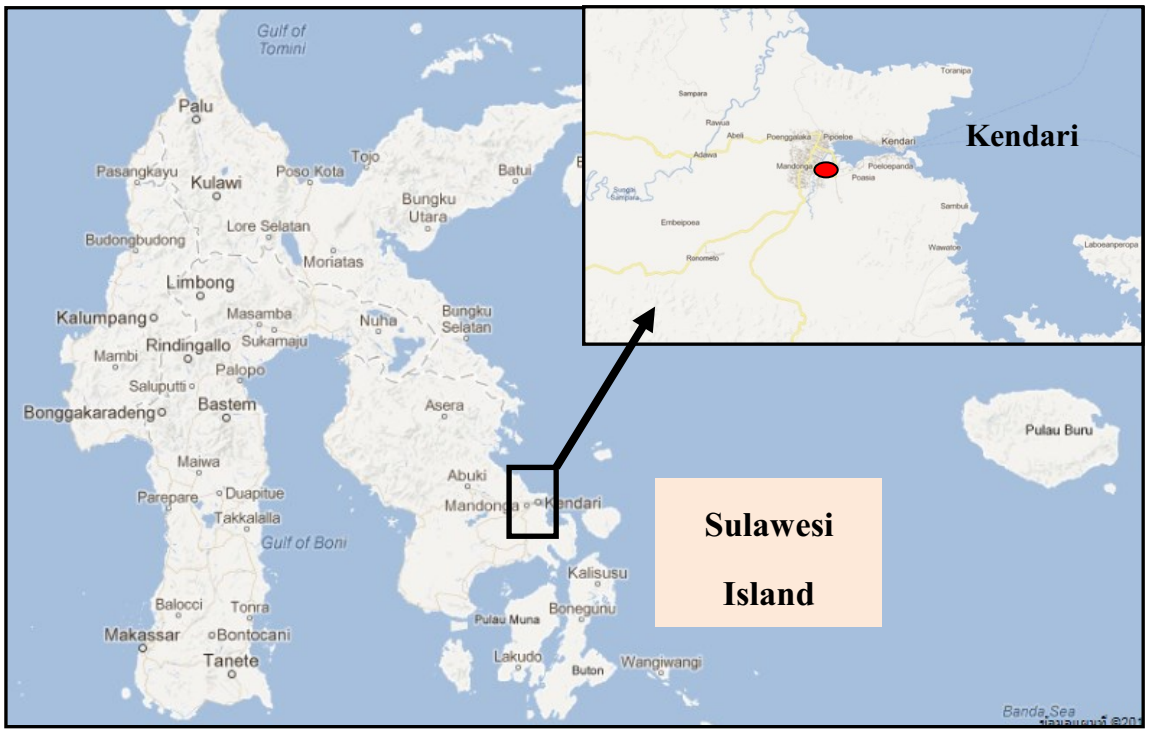


Fig. 4.1 The sago palm research area and sampling site in Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia.

4. Growth analysis

The term plant growth analysis refers to a useful set of quantitative methods that describe and interpret the performance of whole plant systems grown under natural, semi-natural, or controlled conditions. Plant growth analysis is an explanatory, holistic and integrative approach to interpreting plant form and function. It uses simple primary data in the form of dry weights, leaf areas, volumes and contents of plant components to investigate processes within and involving the whole plant. Plant growth analysis first illuminated plant physiology, then agronomy and now physiological and evolutionary plant ecology. A growth analysis will provide a first clue to answer these questions as follows (Hunt, 2003). The meaning of abridgements: W = weight, W_L = leaf weight, A = leaf area and t = time.

4.1 Relative growth rate; RGR

The basic idea of modeling growth and dry matter production as augmentation of capital led to the concept of the efficiency index in which the rate of growth is expressed as the rate of interest on the capital. This is identical with the present concept of relative growth rate. The mean RGR of different plant parts, such as shoots, roots, leaves and leaf area can also be estimated in this way. The sum of the RGR of the component parts of a plant equals the RGR of the whole. Partitioning of assimilate during growth can be assessed by calculating the RGR of the parts, and those parts of the plant likely to be most sensitive to environmental charges ascertained. The mean RGR, over the time interval from t_1 to t_2 as follows:

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1) \qquad (\text{weight} \bullet \text{weight}^{-1} \bullet \text{time}^{-1})$$

4.2 Net assimilation rate; NAR

Net assimilation rate is synonymous terms used for a growth characteristic whose original purpose was to remove, to a certain extent, the drawback of a large ontogenetic drift inherent in the concept of relative growth rate. This was accomplished by expressing the rate of dry weight increase at any instant on a leaf area basis, with leaf area representing an estimate of the size of the assimilatory apparatus. This expression can only be integrated if either the relationship between W and A is known, or the relationships between W and t, and A and t are known. It is usual either to assume an arbitrary relationship between W and A, or to determine the relationship graphically as follows:

$$NAR = (W_2 - W_1)/(A_2 - A_1) * (\ln A_2 - \ln A_1)/(t_2 - t_1) \qquad (\text{weight} \bullet \text{area}^{-1} \bullet \text{time}^{-1})$$

4.3 Leaf area ratio; LAR

A value obtained by dividing the total leaf area of a plant by its dry weight. The ratio is useful in relating total photosynthetic to total respiratory material within the plant, thereby giving information concerning the plant's available energy balance**.** Leaf area ratio is defined as the ratio between leaf area and total plant dry weight, and can be interpreted as a product of two

simpler ratios, namely of the specific leaf area, which is the leaf (A): leaf dry weight (W_L) ratio, and of the leaf weight ratio, which is the ratio of leaf dry weight (W_L) to total dry weight (W). The mutual relationships between leaf area ratio (LAR), net assimilation rate (NAR) and relative growth rate (RGR), at any instant are described by:

$$LAR = (A_2-A_1)/(W_2-W_1) \qquad (area \bullet weight^{-1})$$

or

$$LAR = RGR/NAR \qquad (area \bullet weight^{-1})$$

4.4 Specific leaf area; SLA

This is an index of the leafiness of leaf. A measure of density or of relative thinness, which involves an assessment of the leaf's area in relation to its dry weight. No particular symbol is in general use and it is described by:

$$SLA = (A_2-A_1)/(\ln A_2-\ln A_1)*(\ln W_{L2}-\ln W_{L1})/(W_{L2}-W_{L1}) \qquad (area \bullet weight^{-1})$$

4.5 Leaf weight ratio; LWR

This is an index of leafiness of the plant on a dry weight basis. A measure of the productive investment of the plant, dealing with the relative expenditure on potentially photosynthesizing organs. No particular symbol is in general use and it is described by:

$$LWR = (W_{L2}-W_{L1})/(\ln W_{L2}-\ln W_{L1})*(\ln W_2-\ln W_1)/(W_2-W_1) \qquad (weight \bullet weight^{-1})$$

5. Statistical analysis

The statistical difference of the data was determined using SAS (Statistical Analysis System) for windows v9.0. The effect of the growth analysis and plant physiological characteristics of sago palm seedlings under the native soil treatment and improved soil treatment was determined by one-way ANOVA (analysis of variance), and the comparison of differences among the mean values of the two sampling plots was determined using the T-test. Terms were considered significant at the 5% level.

Results and Discussion

1. Soil profile

The soil type of the experimental site was alluvial fans, which originally formed from the surrounding sedimented yellow podzolic soils. The texture of the virgin soil was loam, which contained 44% sand, 44% silt, and 12% clay, and its soil pH was 3.5 (KCl) to 4.2 (H_2O). The soil pH in the control and calcium application plots was 4.4 (KCl) to 5.4(H_2O) and 6.5 (KCl) to 6.9 (H_2O) respectively, and recognized as acid (control) and neutral (calcium application plot) (Table 4.1). Calcium application treatment increased Olsen P_2O_5, exchangeable Ca content, and CEC and decreased Al^{3+} content in the soil. Then K_2O (HCl 25%), Morgan K_2O, exchangeable Mg, and K contents in the soil tended to be lower in the calcium application plot than in the control.

Table 4.1 Property of the virgin soil and soils in experimental plots.

Plot	pH (HCl)	pH (KCl)	Total N (mg/kg)	P_2O_5 (HCl 5%) (mg/kg)	K_2O (HCl 5%) (mg/kg)	Olsen P_2O_5 (mg/kg)	Bray I P_2O_5 (mg/kg)	Morgan K_2O (mg/kg)
Virgin soil	4.2	3.5	400	20	60	-	5.0	27
Control	5.4	4.4	900	380	170	-	140.9	103
Calcium application	6.9	6.5	600	410	90	53	122.3	46

Plot	Exchangeable cation ($cmol_c/kg$)				CEC ($cmol_c/kg$)	Al^{3+} ($cmol_c/kg$)
	Ca	Mg	K	Na		
Virgin soil	0.73	0.20	0.05	0.13	2.34	0.84
Control	1.40	0.55	0.20	0.12	2.66	0.02
Calcium application	6.05	0.28	0.09	0.16	4.17	0.00

2. Plant growth and Growth analysis

Ten months after transplanting, the seedlings growth in plant height, plant length, leaf number, leaflet number and dead leaf number were measured. The leaflets, petioles, bases and roots were divided to sampling which three sago palm treated plants were sampled among the native acid soil plot (control) and the calcium application plot (Fig. 4.2A, 4.2B). The soil pH value was measured before treatment which the soil pH value in the native acid soil plot (control) and the calcium application plot approximately 4.6 and 6.0, respectively. The pH value was measured one a week until the experiment finished. However, some environmental factors may slightly effect on treated plants. From the results showed that the plant length of sago palms in the native acid soil plot and the calcium application plot from beginning until 5th month of two soil treatment plots were closely values and slightly increased, approximately 70-80 cm. Then, the plant length was obviously increased from 6th month until 10th month (October 2012) (Fig. 4.3).

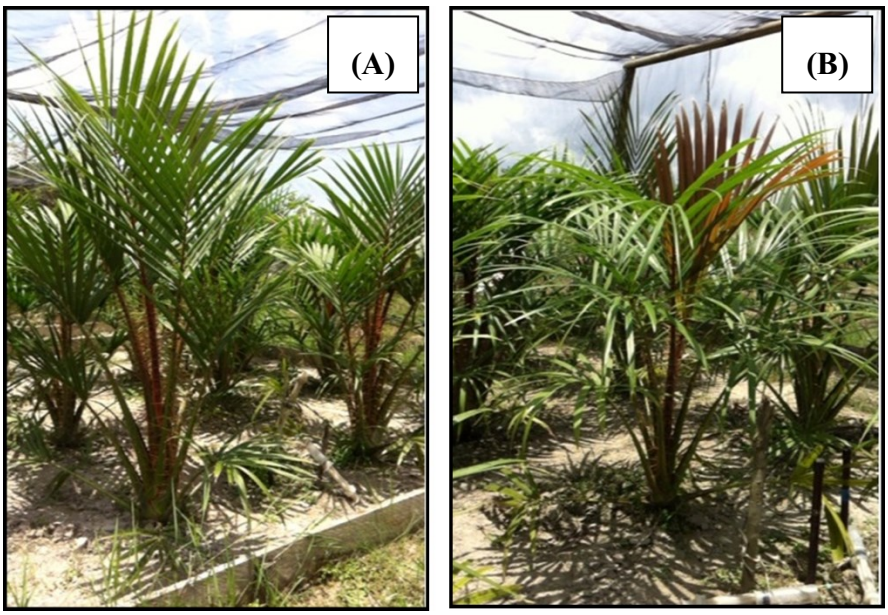


Fig. 4.2 The sago palms in the native acid soil plot (control) (A) and the calcium application plot (B) from December 2011 to October 2012.

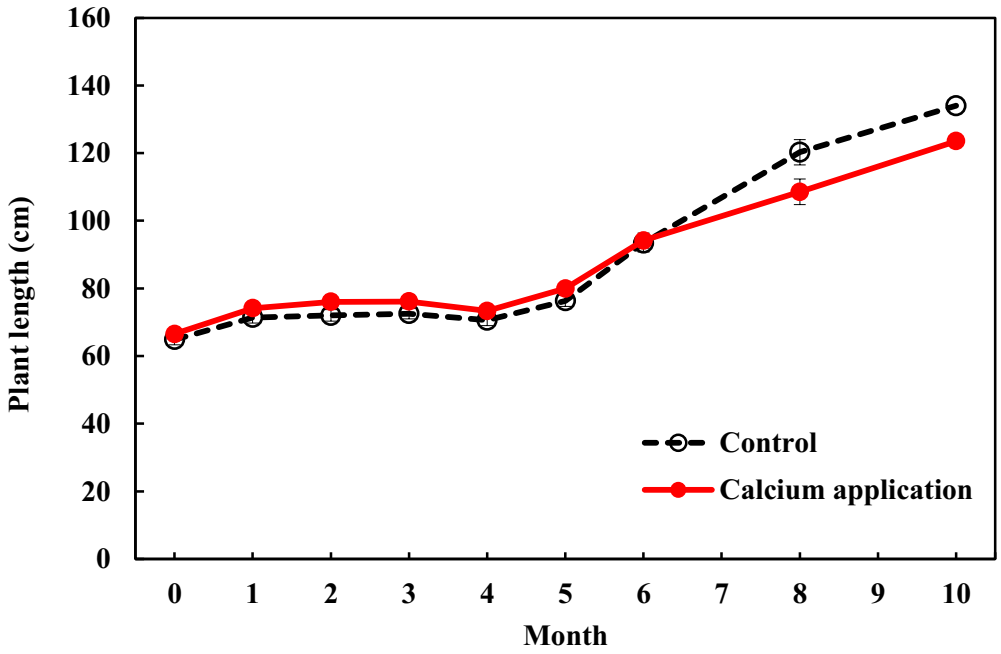


Fig. 4.3. The plant length of sago palm among the native acid soil treatment (control) and the calcium application plot from December 2011 to October 2012. Each value represents the mean (n=3).

The sago seedlings grew to around 100 cm in plant length in both the control and calcium application plots (Table 4.2). The growth in plant height, plant length, leaflet number per leaf, dead leaf number per plant, and dry matter weight of each part for 6 months after transplanting were almost the same as in the control and calcium application plots (Table 4.2 and 4.3). There was a significant difference in leaf number per plant; however, the difference was not so large. Additionally, in the results of growth analysis for 6 months, there were no remarkable differences between the control and calcium application plots (Table 4.4).

Table 4.2 Growth parameters of the control and calcium application plots at 6 months after transplanting (June 2012).

Plot	Plant height (cm)	Plant length (cm)	Leaf number/plant	Leaflet number/leaf	Dead leaf number
Control	87.3 ± 2.9 a	100.3 ± 2.8 a	11.9 ± 0.2 a	13.1 ± 0.3 a	0.5 ± 0.2 a
Calcium application	82.6 ± 3.1 a	96.4 ± 2.4 a	10.6 ± 0.3 b	13.1 ± 0.4 a	1.3 ± 0.3 a

Each value represents the mean ± SE (n=3). Different letters within a column indicate significant difference at the 0.05 probability level, according to the T-test.

Table 4.3 Dry matter growth parameters of the control and calcium application plots at 6 months after transplanting (June 2012).

Plot	Leaflet (g plant^{-1})	Rachis (g plant^{-1})	Petiole (g plant^{-1})	Base (g plant^{-1})	Root (g plant^{-1})	Whole (g plant^{-1})
Control	40.0 ± 2.3 a	3.6 ± 0.2 a	69.7 ± 5.1 a	9.4 ± 1.1 a	27.1 ± 2.5 a	149.7 ± 10.3 a
Calcium application	33.5 ± 0.3 a	3.0 ± 0.1 a	57.6 ± 2.2 a	5.0 ± 0.9 a	28.3 ± 1.6 a	127.3 ± 3.0 a

Each value represents the mean ± SE (n=3). Different letters within a column indicate significant difference at the 0.05 probability level, according to the T-test.

Table 4.4 Parameters of growth analysis from December 2011 to June 2012.

Plot	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})
Control	15.56 ± 0.34 a	0.543 ± 0.023 a	28.9 ± 0.6 a	113.2 ± 1.8 a	0.255 ± 0.002 a
Calcium application	14.44 ± 0.12 a	0.470 ± 0.024 a	31.5 ± 1.5 a	116.3 ± 4.8 a	0.271 ± 0.003 a

Each value represents the mean ± SE (n=3). Different letters within a column indicate significant difference at the 0.05 probability level, according to the T-test.

Growth parameters at 10 months after transplanting are shown in Table 4.5. There were no apparent differences in plant height and plant length between the control and calcium application plots. The leaf number per plant was 16.9 and 16.1 in the control and calcium application plots, respectively, and they were at almost the same level. Considering the result that the leaf number and dead leaf number were at the same level in the two plots, the different soil pH levels in the case of the same soil parent material had no apparent effect on leaf emergence and senescence. These results were in agreement with a former report by Anugoolprasert et al. (2012a), in which a different pH condition of culture solution, such as pH 3.6, 4.5, and 5.7, had no effect on the number of emerged leaves, live green leaves, or dead leaves for 4.5 months during summer in a greenhouse at Mie University, Japan.

Moreover, the leaf number per plant can be indicated that from beginning until 5th month of two soil plots were closely values and slightly increased. After that the plant length was obviously increased from 6th month until 10th month, the control was slightly different with the calcium application plot (Fig. 4.4). However, there was no significant difference among the control and the calcium application plot at ten months which the difference was also no apparent in leaf number per plant.

Table 4.5 Growth parameters of the control and calcium application plots at 10 months after transplanting (October 2012).

Plot	Plant height (cm)	Plant length (cm)	Leaf number/plant	Leaflet number/leaf	Dead leaf number
Control	124.4 ± 4.0 a	134.0 ± 1.0 a	16.9 ± 0.2 a	34.0 ± 0.7 a	3.1 ± 0.0 a
Calcium application	112.5 ± 4.6 a	123.5 ± 3.8 a	16.1 ± 0.4 a	30.5 ± 1.2 a	3.0 ± 0.2 a

Each value represents the mean ± SE (n=3). Different letters in the table indicate significant difference at the 0.05 probability level, according to the T-test.

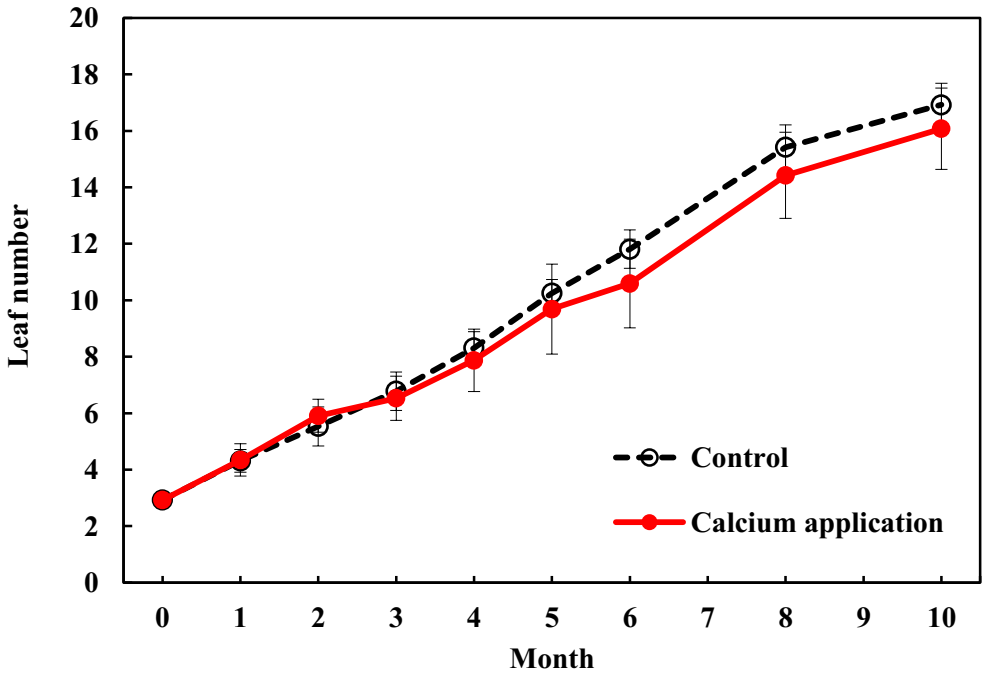


Fig. 4.4 The leaf number per plant of sago palms among the native acid soil plot (control) and the calcium application plot from December 2011 to October 2012. Each value represents the mean (n=3).

From the results, the leaflet area per leaf in each leaf position of sago palms between the control and the calcium application plot showed that each leaflet area in the lower leaf position was no difference because it is initial leaf stage, so it is small area. In upper leaf positions were slightly difference, which the control was larger than the calcium application plot. Although, the calcium application plot was larger than the control at the most upper leaf position due to it is consist of unexpanded leaf or expanding leaf, which area approximately 1666 cm^2(Fig. 4.5). Besides, the total leaflet area per plant of sago palms among the control and the calcium application plot were measured. The total leaflet area per plant of the control larger than the calcium application plot but there were no significant difference (10784 and 8316 cm^2, respectively) (Fig. 4.6). Therefore, the effects of environmental stress no effect on leaf area per leaf and leaflet area per plant among the control and the calcium application plot.

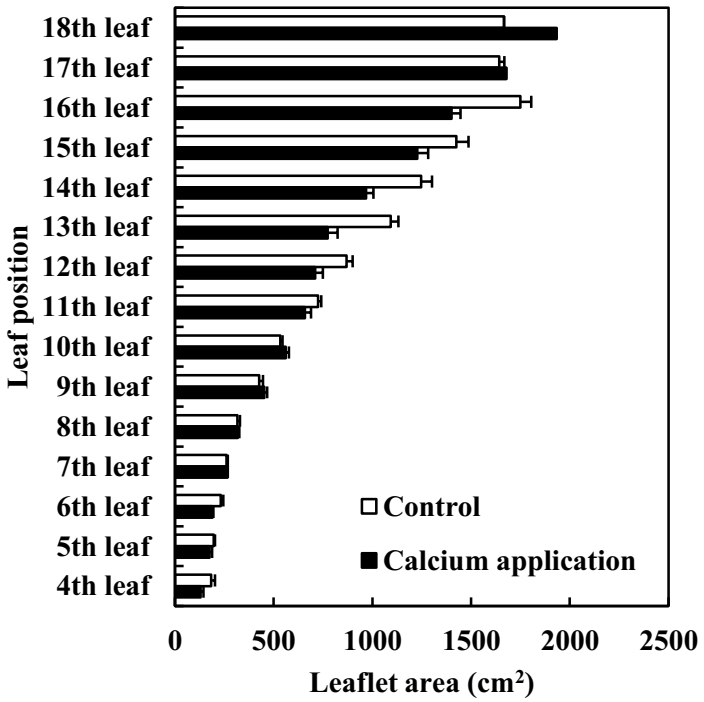


Fig. 4.5 The leaflet area per leaf of sago palms among the native acid soil plot (control) and the calcium application plot at 10 months after transplanting.

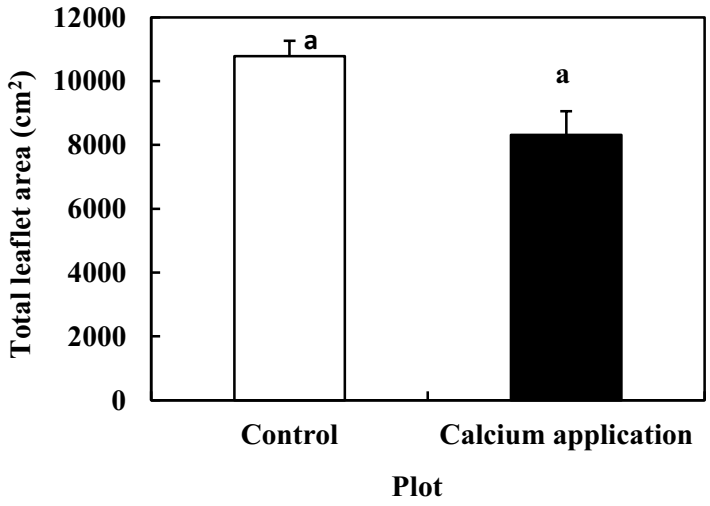


Fig. 4.6 The total leaflet area per plant of sago palms among the native acid soil plot (control) and the calcium application plot at 10 months after transplanting. Vertical lines indicate the standard deviation (n = 3). Means followed by different letters within a column are significantly different at the 0.05 level by the T - test.

The difference in dry matter weight tended to be different from plant part to part (Table 4.6). There were no significant differences in rachis and root dry weights between the two plots, though the leaflet, petiole, base, and total dry weights were larger in the control, with a lower soil pH condition, than in the calcium application plot with a higher soil pH condition. Considering that there were no significant differences in the leaf number per plant and leaflet number per leaf in the two experimental plots and that the leaf dry weight was larger in the control than in the calcium application plot, the size of a single leaflet might be larger in a lower soil pH condition than in a higher soil pH condition. It took longer than 6 months to observe the difference in the size of single leaflet, which might be related to the leaf emergence rate, one leaf per month in general (Jong, 1995). It was considered that a comparatively long period would be needed for the emergence of a leaf to reflect the effect of the experimental procedure.

Table 4.6 Dry matter growth parameters of the control and calcium application plots at 6 months after transplanting (October 2012).

Plot	Leaflet (g plant^{-1})	Rachis (g plant^{-1})	Petiole (g plant^{-1})	Base (g plant^{-1})	Root (g plant^{-1})	Whole (g plant^{-1})
Control	127.0 ± 6.6 a	14.0 ± 1.3 a	199.7 ± 19.6 a	38.1 ± 3.8 a	34.3 ± 1.0 a	413.5 ± 31.8 a
Calcium application	101.0 ± 10.4 b	10.6 ± 1.7 a	123.7 ± 12.8 b	26.2 ± 3.3 b	37.8 ± 1.9 a	299.2 ± 30.2 b

Each value represents the mean ± SE (n=3). Different letters within a column indicate significant difference at the 0.05 probability level, according to the T-test.

Table 4.7 shows the results of growth analysis for 4 months from 6 months after transplanting. There was a significant difference in relative growth rate (RGR), and the value was larger in the control than in the calcium application plot. RGR can be divided into net assimilation rate (NAR), which is a good index to evaluate photosynthetic activity, and leaf area ratio (LAR) to investigate the morphogenesis of an individual plant. The NAR of the control plants was large compared with that of the plants in the calcium application plot. The

components of the LAR, specific leaf area (SLA), indicating leaf thickness, and leaf weight ratio (LWR), which is the ratio of leaf weight to whole plant weight, were the same in the two experimental plots. Although there was a significant difference in NAR, there was no difference in the LAR. Based on these results, the difference in the growth rates of individuals might be attributed to the difference in assimilation rate, not to morphological characteristics.

Table 4.7 Parameters of growth analysis from June 2012 to October 2012.

Plot	RGR ($mg\ g^{-1}\ d^{-1}$)	NAR ($mg\ cm^{-2}\ d^{-1}$)	LAR ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)	LWR ($g\ g^{-1}$)
Control	10.18 ± 0.75 a	0.388 ± 0.036 a	26.7 ± 0.5 a	91.3 ± 1.1 a	0.293 ± 0.005 a
Calcium application	8.30 ± 0.97 b	0.297 ± 0.037 b	28.2 ± 0.2 a	92.8 ± 0.9 a	0.303 ± 0.001 a

Each value represents the mean ± SE (n=3). Different letters within a column indicate significant difference at the 0.05 probability level, according to the T-test.

3. SPAD value, stomatal conductance and stomatal density

The SPAD of sago palms among the native soil treatment and the improved soil treatment was investigated. So, there was no significant difference. The chlorophyll content (or SPAD) can be measure by the SPAD meter which is a commercially available portable piece of equipment that is used to measure greenness based on optical responses when a leaf is exposed to light that in turn is used to estimate foliar chlorophyll concentrations (Kariya et al., 1982). Considering that there was no difference in the LAR, dry matter might increase, keeping a good balance with the increase in the single leaflet area and total leaf area. Two parameters relating to the NAR, SPAD value and stomatal conductance, are shown in Table 4.8. There was no significant difference in the SPAD value indicating chlorophyll content per unit leaf area; however, the stomatal conductance was higher in the control than in the calcium application plot. Omori et al. (2000a) reported that stomatal density is related to thickness of leaf. Ehara

(2009) stated that higher stomatal density would be observed in sago palms grown in soil that contains higher exchangeable Ca. However, there was no significant difference in the SLA indicating leaf thickness between the two experimental plots, and the exchangeable Ca content in the soil was much higher in the calcium application plots. According to Awal et al. (2004) stated that the specific leaf area (SLA) differed according to the position of the leaf area on the plant. The distal or youngest leaf samples had the highest specific leaf area (SLA), which recommend that leaf area of a developing leaf stabilizes before its dry weight. Stomatal density on the abaxial surface of leaflets in different leaf position among two experimental plots is shown in Table 4.9. The study of the stomatal density in leaves by leaf position was conducted, however leaflet position and position on a leaflet were not investigated. Omori et al. (2000b) found about stomata density that even if comparison between the apical, middle and basal parts of the leaflet, stomatal density tended to be smaller at the apical part. In this study, stomatal density in control was higher than calcium application plot on both upper leaf and lower leaf positions. Although, there was no difference in stomatal density between upper and lower leaf positions in control, but calcium application plot was slightly depressed in lower leaf position than that in upper leaf position. However, there was little difference found in stomatal densities between different leaflet positions in a leaf and between different leaf positions in a plant, which was demonstrated by Omori et al. (2000b).

Table 4.8 Stomatal conductance and SPAD value at 10 months after transplanting in October 2012.

Plot	Stomatal conductance (mmol m^{-2} s^{-1})	SPAD
Control	62.4 ± 5.3 a	67.1 ± 2.0 a
Calcium application	43.5 ± 5.2 b	67.3 ± 2.4 a

Each value represents the mean ± SE (n=3). Different letters within a column indicate significant difference at the 0.05 probability level, according to the T-test.

Table 4.9 Stomatal density at 10 months after transplanting in October 2012.

Plot	Stomatal density (stomata mm^{-2})	
	Upper leaf position	Lower leaf position
Control	99.25 ± 2.19 aA	98.25 ± 2.23 aA
Calcium application	71.50 ± 1.92 bA	51.50 ± 1.14 bB

Each value represents the mean ± SE (n=3). Different letters in the table indicate significant difference at the 0.05 probability level, according to the T-test. Lowercase letter indicates comparison among the plots in each leaf position of sago palm seedlings. Capital letter indicates comparison among the leaf position of sago palm seedlings on same plot.

Considering these results, the difference in stomatal conductance in the current experiment might be attributed to stomatal aperture, which might account for the difference in growth rate through assimilation rate, that is, photosynthesis ability. Such differences in stomatal conductance and NAR would be reflected in difference in soil properties, such as the K_2O and/or exchangeable K content in soil, of the two experimental plots. Anugoolprasert et al. (2012b) reported that the sago palm adapts to a range of pH values from 4.3 to 7.0 even in soils that have different texture in southern Thailand. They supposed that the sago palm grown under any conditions of soil pH might exhibit an avoidance mechanism to restrict the distribution of any excess of undesirable nutrients in the plant, which would account for the mineral contents in the plant under a wide range of soil pH conditions. According to Anugoolprasert et al. (2012a), sago palm seedlings can maintain nutrient uptake under a wide range of low pH conditions (pH 5.7 to pH 3.6) in a culture solution.

Peaty soil is highly to weakly acidic and contains low levels of calcium, potassium, and phosphorus compared with mineral soil (Tie et al., 1991; Yamaguchi et al., 1994). Although the sago palm grows in mineral soil and in deep to shallow peat soil, the growth of the sago palm will be better in mineral soil or shallow peat soil (Sato et al., 1979). Kakuda et al. (2000) supposed that the reason the growth of the sago palm in peat soil will decelerate compared with that in mineral soil might be the small amount of nitrogen supply per unit of cubic capacity in

peat soil. Considering the former studies, nitrogen provision will be one of important factors limiting sago palm growth. On the other hand, Lina et al. (2009) reported that nitrogen did not significantly improve the growth parameters, such as cumulative increase in height, monthly growth rate, base diameter, number of leaves per palm, and number of leaflets per palm, in the early growth stages in the field experiment because nitrogen was provided by nitrogen fixation bacterium. Shrestha et al. (2007) reported that beneficial microbial interactions occur in the sago palm to enhance nitrogen-fixing activity. In our current experiment, the total nitrogen concentration in soil was higher in the control than in the calcium application plot, as shown in Table 4.1. However, there were no significant differences in plant size and growth rate between the two experimental plots, as shown in Table 4.2, 4.3, which might be related to microbial interaction. This experiment indicated that the growth and biomass production of sago palm seedlings at 10 months after planting tended to be better in acidic soil than in neutral soil conditions, as shown in Table 4.5, 4.6. This phenomenon may be due to nutrient competition between Ca and Mg with K. Table 4.1 indicates that lime application reduced the availability K_2O and the exchangeable K cation. In the current study, the growth of sago palm seedlings at soil pH 5.4 (H_2O) [4.4 (KCl)] was not small in comparison with that at soil pH 6.9 (H_2O) [6.5 (KCl)] on an experimental farm.

Conclusion

The growth in plant height, plant length, leaflet number per leaf, dead leaf number per plant, and dry matter weight of each part in the calcium application plots for 6 months after transplanting was almost same as those in the control and calcium application plots. The difference in dry matter weight tended to be different in each plant part. There was a significant difference in relative growth rate (RGR) and net assimilation rate (NAR) between the two plots from 6 months to 10 months after transplanting. It is still unclear whether sago palm will show a preferable growth at a lower soil pH condition. Based on the results of the current study, the experimental procedures at field level show that the growth of sago palm not decelerate under the original acidic condition compared with that under near neutral condition in soil having the same parent material.

Even if in the next, the experimental field should study on the effects of aluminum toxicity to characteristic changes in several sites of natural sago palm growing areas. Besides, also investigation about the mechanisms of Al resistance and detoxification of Al in sago palm should be examined.

Chapter 5

General Discussion

Sago palm (*Metroxylon sagu* Rottb.) is a species of the genus *Metroxylon* belonging to the Arecaceae family. It is a socio-economically important crop in Southeast Asia and its center of diversity is believed to be New Guinea or the Moluccas. In Southeast Asia and Melanesia, the starch produced from sago palms has long been used for food (Nakamura, 1990). Although, most crops grow well in soil that is neutral, mildly acidic or mildly basic, sago palm is one of the few tropical crops that can thrive in wide range of soil conditions, from harsh swampy, alluvial, and strong acid peat soils. From a report by Rasyad and Wasito (1986), sago palm stands in Sulawasi are distributed across various locations from northeastern Manado on the Minahasa Peninsula to Palopo, on the eastern shore of the Gulf of Boni. Thus, in Chapter 4, the experimental farm was established in Kendari, Indonesia, which is a suitable place to plant sago palms. A plant's ability to endure various pH levels depends largely on its ability to take in and utilize nutrients at varying concentrations in the soil solution. Soil pH or acidic soil is the limiting factor for plant growth, when the use of lime or dolomite would be beneficial to improve soil pH to the desired level. Calcium carbonate ($CaCO_3$) is one of the liming materials used to improve or amend soil and is a common solution to acidity. Thus, it is used as a chemical in order to improve soil pH level (above 6) for this experiment in native soil, which is peat soil. Even though, it is likely that sago palms are planted in peat soil areas primarily because other crops grow poorly in that soil, which Fukui (1984) mentioned that sago palm is mostly distributed across areas of peat soils rather than other soil types. From the results of growth analysis from 6 months to 10 months after transplanting showed that there was a significant difference in relative growth rate (RGR) and net assimilation rate (NAR). Similarly Kakuda et al. (2000) compared the nitrogen supply in peat soils and mineral soils and that it is a property of peat soils that mineralization and nitrogen supply occur more readily compared with mineral soils. However, the soil, with applied calcium carbonate ($CaCO_3$) did not show

preferable growth compared with native acid soil. The growth of the sago palm will not decelerate under acidic conditions compared with that under neutral conditions in soils that have the same parent material at the field level experimental procedures. Moreover, in Chapter 2, I focused on the different pH levels effects on growth and physiological characteristics of sago palm with high aluminum (Al) concentration because the actual condition of the experimental fields in Kendari, Indonesia or Southern of Thailand were investigated demonstrating that when the soil pH was different the amount of aluminum concentration was also different (Anugoolprasert, 2012a). Furthermore, soil acidity, associated infertility and mineral toxicities are major constraints to agricultural production in several parts of the world (Pariasca-Tanaka et al., 2009). If so, aluminum toxicity is a major problem limiting crop yield and forest productivity worldwide. The results showed that metabolic changes occurred in growth of aluminum-affected sago palm, which might be induced by aluminum excess. Similar results with Barcelo and Poschenrieder (2002) stated that metabolic changes in aluminum-affected plants are induced by both direct and indirect Al actions. The ion pumps (proton pump, proton ATPase) inside a plant cell membrane translocate hydrogen ions (protons) out of the cell. This pump's function is to maintain a weak alkalinity inside the cell and acidity outside the cell. At the same time, an inside is created and utilized in the transportation of other ions (Okazaki, 2015). I continuously undertook the experiment in Chapter 3, examining the different aluminum concentrations effects on growth characteristics of sago palm seedlings in culture media. As materials as used in Chapter 2 and Chapter 3, sago palms young seedlings at the 6th leaf stage after germination. Thus, the growth morphogenesis in upper side was not any different in leaf and petiole, but the difference was apparent in lower down levels, such as roots (adventitious roots are thick roots and lateral roots are thin roots). The results from Chapter 2 and Chapter 3 showed the root dry weight of aluminum treated sago palm was decreased, compared with non-aluminum treated sago palm. Aluminum absorbed by the plant tends to preferentially accumulate in the root apex, inhibiting root elongation and cell division (Kochian et al., 2005). Moreover, having a negative effect on physiology, mineral metabolism and plant growth, this

metal induces premature senescence manifested by the increase of ammonia content in plant tissues due to protein degradation (Balestrasse et al., 2006). Although it is considered that Al^{3+}, the toxic form of aluminum, is very scarce at plant cytosolic pH values, this ion may still be dangerous for the symplast due to its very high affinity towards metabolically important molecules (Vicherková and Minář, 1987). Some research evidences support our work that aluminum limited transportation of nutrients in sago palm. Aluminum also induces substantial disturbances in the trans-membrane transport of ions (NO^{3-}, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}) in plant roots (Nichol et al., 1993, Kochian 1995). For this mechanism, Al is prevented from moving through the plasma membrane to the cytoplasm in the root cells. This is achieved by the secretion of organic acids from the radical apex to the rhizosphere, which, in turn, modifies the pH and chelates the toxic Al ion (Kinraide et al., 2005). Further, these organic anions compete with phosphate groups for binding sites in the soil and thus block the sorption of P to other charged sites and form stronger complexes with Al^{3+}, Fe^{3+} and Ca^{3+} than phosphate does thus making the phosphorus available to plants. The second mechanism involves chelation of Al by specific proteins, short-chain organic acids, phenolic compounds and tannins that can bind and form complexes with aluminum ion (Al^{3+}) and subsequently compartmentalize it in the vacuole thus reducing Al-toxicity in the cell (Jones and Ryan, 2004).

When the roots were treated by Al, the root color changed to dark, Chapter 3 caused by the primary target of Al toxicity is the root apex. Generally, aluminum-tolerant plants may be grouped according to Al accumulates within their tissues (Foy, 1978), especially in their roots. Research investigated in wheat supports that much of the Al absorbed by roots penetrated the boundary between root apex and root cap accumulating in the nuclei and cytoplasm of cells adjacent to this zone. Some Al passed through the epidermis and cortex, but considerable amounts were retained in cortical cells (Henning, 1975). Al affects a host of different cellular functions, frustrating attempts to identify the principal effects of Al toxicity. Exposure to Al causes stunting of the primary root and inhibition of lateral root formation. Affected root tips are stubby due to inhibition of cell elongation and cell division (Samac and Tesfaye, 2003). The

resulting restricted root system is impaired in nutrient and water uptake, making the plant more susceptible to drought stress. Plants sensitive to Al toxicity have greatly reduced yield and crop quality (Jovanovic et al., 2006; 2007). As an initial symptom of its toxicity, a reduction and thickening of roots is observed in consequent impact on the nutritional status of the plants, because Al competes with other nutrients for absorption sites in the root system, triggering a nutritional imbalance. Reduction in the concentration of macronutrients implies decreased growth of both the shoot/ root systems and the appearance of nutritional disorders represented by nutritional deficiency in plants (Cruz et al., 2014). Moreover, Okazaki (2015) reported that in the mechanism of adaptation to aluminum stress plants with high aluminum tolerance have a root apical cell plasma membrane with an excellent aluminum exclusion mechanism. Subsequently, aluminum stress induces the synthesis of β-1, 3-glucan (callose) outside of the root apical cell plasma membrane, which inhibits the migration of several substances in the apoplast and the symplast between adjacent cells by depositing itself in the apoplast and around the plasmodesma (Wagatsuma, 2002). The cell wall and the cell membrane probably function as an important barrier to passive movement of aluminum into the symplasm (Wagatsuma, 1983). Therefore, a large amount of aluminum is bound to the plasma membrane, and the plasma membrane is eventually destroyed by aluminum (Wagatsuma, 1989). However, aluminum tolerance in plants is also regulated by citric acid, oxalic acid and malic acid released by roots. Releasing of these organic acids is in turn induced by aluminum stress (Ma et al., 2001). Different mechanisms seem to be involved in the secretion patterns of organic acids (Delhaize and Ryan, 1995). It has been suggested that organic acids are secreted through an anion channel located on the plasma membrane (Piñeros andKochian, 2001).

Consequently, in these studies sago palm can grow in wide range of soil pH from low pH conditions up to neutral conditions and high aluminum concentration. Even though, aluminum causes extensive root injury, leading to poor ion and water uptake. Aluminum is known to induce a decrease in mitotic activity in many plants, and the aluminum induced reduction in the number of proliferating cells is accompanied by them shortening of the region of cell division in

sago palm. Even so, Vanpraag and Weissen (1985) reported that plant species and ecotypes growing on acid soils had become extremely resistant to the inhibitory effects of aluminum on root absorption and growth in the course of time and phenological evolution.

Chapter 6

Conclusion

Comparing growth characteristics of sago palm in widely different soil pH condition are interesting points to investigate in this thesis. Besides, to make clear how we can feed back information and results from laboratory level and field experiments to sago palm cultivation. We also examined field level trials in the country of origin of sago palm such as Indonesia. Thus, we need to investigate growth conditions of these sago plants, but it takes a long time, about 30 years, if we want to determine the soil and conditions on the sago palm growth. It is entirely reasonable and effective to measure the characteristics of the growth responses, including photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, SPAD value, and growth rate such as the relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR). Also, it is very important to measure the chemical composition of mineral contents because it is quite useful to speculate what happens in sago palm, to know the plant growth activities as well as the absorption activities. Nowadays, several areas are producing the sago palm, it is in already started plantation that we need to know the more useful agriculture resume to improve the productivity of sago production. However, if we obtained the lifelong cycle of the sago palm, it would be time consuming and that is the reason why we examined the changes of morphological and physiological characteristics at the early stage of sago palm.

Firstly, we tried to focus on acidic conditions and aluminum concentrations on sago palm growth. Secondly, sometimes these negative effects are combined and make more negative effects of the sago palm growth, which is why we separately determined the effects of each acidic condition or aluminum concentration. Therefore, in Chapter 1, we focused on the acidic condition of soil and also the aluminum effect, because under the acidic conditions, the aluminum toxic forms can be solubilised and absorbed by plants. From the results, we have shown that it had negative effects depressing growth, thus, we firstly determined the effects of

acidic conditions on plant growth. In Chapter 2, we tried to determine the effects of aluminum concentration on growth characteristics. Thus, this examination aimed to find some useful agriculture resume to avoid the negative effects of acidic soil concentration and aluminum toxicity on the growth of sago palm. According to the previous results, when soil is acidic or has low pH, the toxicity in acid soil was apparent. Finally, in Chapter 3, we tried to find out the agricultural resume to protect this acidic condition on sago palm growth by considering the application of calcium carbonate ($CaCO_3$) into the experimental plot. Commonly, $CaCO_3$ is a chemical fertilizer, which can neutralize soil acidity. Moreover, we tried to find out some responses on two different soil pH conditions to sago palm growth in experimental plots such as the native acid soil plot, where the soil pH was 4.6 and the soil was applied with $CaCO_3$ to increase soil pH above 6.0. In addition, we expected low soil pH or acidic conditions might have negative effects, because of higher aluminum concentration, which we demonstrated sago palm growth responses; however the results did not show the difference between the two experimental plots. Besides, other nutrients in the soil were almost of the same content, particularly in the solubilised aluminum content. Nevertheless, the causes on organic matter from the compost were enough to reduce the negative effect of aluminum concentration, even under acidic soil condition where there was a small amount of nutrients from peat soil. In fact, this experiment did not achieve finding out if whether $CaCO_3$ treatment is affected or not because organic matter in soil was changed in aluminum solubilization.

The new finding of these results was that sago palm has ability to grow under widely different pH ranges and was considered to have a high resistance to aluminum, even under acidic soil conditions. The comparative wide range was also trying lead to an understanding of the acid resistant mechanism of sago palm on growth and physiological features under different soil pH conditions. However, this is the first step in expectation that the sago palm can grow under acidic soil at the early stage growth, even in the lower soil pH conditions. For future perspectives, we can utilize sago palm under problem soil anywhere in the world and develop the sustainable cultivation in sago palm.

Summary

Growth and Physiological Features of Sago Palm under Different Soil pH Conditions

Sago palm (*Metroxylon sagu* Rottb.) is economically acceptable, environmentally friendly, and promotes a socially stable agro-forestry system. It is an extremely hardy plant, thriving in swampy, acidic peat soils, submerged and saline soils where few other crops survive. Furthermore, sago palm is worthy of attention as a rare crop that can grow even in problem soil. Plant growth is generally affected by the relative concentrations of hydrogen ions (H^+) in the soil pH. Any mineral element may be present in deficient or toxic concentrations for a particular plant and subsequently, limit the growth of roots and tops. Thus, fertilizers and lime can be used to correct problems of mineral element deficiency or toxicity to sago palm growth. However, phytotoxic ions released from soil may affect the growth of sago palm. We have investigated the growth and physiological features of sago palm under different soil pH conditions and different aluminum concentrations at the laboratory level and the experimental farm level.

Expt. 1. Effect of Different pH Levels on Growth and Physiological Characteristics of Sago Palm with High Aluminum Concentration in Culture Solution

Nine seedlings of sago at the 6th leaf stage were used that were filled with vermiculite Kimura B culture solution with or without 140 ppm. $AlCl_3 \cdot 6H_2O$ was applied with different pH levels (pH 3.5, 5.7 and 7.9) and 3 replications each. The morphological and physiological parameters of sago palm seedlings were measured for 3 months.

There were no significant differences in plant height, length, green leaf numbers, dead leaf numbers, emerged leaf numbers, chlorophyll fluorescence, total leaflet area and total dry matter weight among the three pH treatment levels. The relationship between specific leaf area (SLA) and net assimilation rate (NAR), stomatal conductance, photosynthetic rate and transpiration rate in leaflets showed the same tendency, with pH 3.5 higher than pH 7.9 and 5.7, respectively.

The K^+, Ca^{2+}, and Mg^{2+} concentrations in almost all the plant parts slightly decreased at lower pH treatment. The N, P, Al^{3+} and Na^+ concentrations in all parts increased at lower pH treatment. However, only Na^+ concentration was not significantly different in all plant parts tissues among the three pH treatment levels. At lower pH (pH 3.5) that Al form is Al^{3+} and higher pH (pH 5.7 and 7.9), Al form will be formed $Al(OH)^{2+}$ and/or $Al(OH)_2^+$. Thus, the mononuclear Al^{3+} species and Al_{13} are considered as the most toxic forms, while Al^{3+} concentration will decrease when pH is increased. Generally, Al toxicity is considered the most important growth limiting factor for plants in acid soils. From these results, sago palm can be considered to Al-avoidance genotype; therefore some plant growth parameters increased the morphological appearance. It can be confirmed that sago palm has ability to grow under widely different pH range from 3.5 to 7.9. Moreover, sago palm can be considered to immune even when the range of pH level was widely extended.

Expt. 2. Effect of Different Aluminum Concentrations in Culture Media on Growth Characteristics of Sago Palm Seedlings

In this study, we investigated growth characteristics, morphogenesis and root development of sago palm seedlings grown under different aluminum concentrations in culture media. Sago palm seedlings at the 6th leaf stage were transplanted in 1/5000a Wagner pot filled with vermiculite. Three levels of aluminum concentration ($AlCl_3 \bullet 6H_2O$) were added into Kimura B culture solution: 0, 150 and 300 ppm with 3 replications. Culture media pH in all the treatments was adjusted to 3.5. The plant height, leaf numbers and dead leaf numbers were measured every week. Plant sampling was conducted at 8 weeks after transplanting in August 2014. Base diameter, root diameter and dry matter weight were measured. Transverse sections of adventitious root were used for root cell observation under light microscope.

There were no differences in plant height, length and base diameter among three treatments. Leaf growth development showed no significant difference in leaf and leaflet numbers per plant, leaflet numbers per leaf and the number of emerged leaves per plant among the three treatments.

Root diameter and dry matter weight of roots and whole plants were significantly higher in non-Al treated (0 ppm Al) sago palm. Dead leaf number, SPAD and chlorophyll content were significantly higher in Al-treated (150 and 300 ppm Al) sago palm. Dry matter weight of leaflets, petioles and base were not significantly different among the three treatments. There was a significant difference in total leaflet area per plant between 0 and 300 ppm Al. The difference in single leaflet area was negligible among the three treatments. Relative growth rate (RGR) and net assimilation rate (NAR) tended to be slightly decreased with Al treatments, however, leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) showed the same levels among the three treatments. The stomatal conductance was significantly lower in the Al treated than that in the non-Al treated sago palms. The photosynthetic rate and transpiration rate were not significantly different among the three treatments. The sago palm had growth responses to variation of Al stress at a low pH condition (pH 3.5). Al induced changes in numerous characteristics such as morphological and physiological responses. Moreover, the uptake of P, Ca and Mg was interrupted in the Al treatments; however that of N and K uptake was not affected by the Al treatments. Root color changed to dark in Al treated treatments, which was more distinct under higher Al concentration. There was a significant difference in the number of root cells per mm^2 in the transverse section. High Al concentration inhibited root growth, decreased root cell numbers and root diameter, and alleviated root structure of sago palm seedlings for 8 weeks after treated with Al. From this data it was clear that sago palm seedlings did not show any obvious differences of morphogenesis of top part, however its root diameter and cell differentiation in cortex of root were inhibited under high Al concentration in the media.

Expt. 3. Growth of Sago Palm Seedlings under Different Soil pH Conditions at the Experimental Farm in Kendari, Indonesia

The two plots were placed under natural sunlight at the experimental farm, Faculty of Agriculture, Halu Oleo University, Kendari, Indonesia. In each plot (14.44 m^2), Sixteen young

sago palm seedlings at the 3rd leaf stage were transplanted. The compost made from the sago pith residue after starch extraction was applied in all the plots. In the native acid soil plot (control: pH4.6) and the calcium application plot, 3kg calcium carbonate ($CaCO_3$) was applied to increase soil pH above 6 prior to the transplanting. The plant growth parameters were counted at the plant sampling in June and October 2012. The dry matter weights and the leaflet area were measured, and analyses of the growth from December 2011 to June 2012 and from June 2012 to October 2012, were performed.

In plant growth and physiological characteristics sago palm showed the growth in plant height, length, leaflet numbers per leaf, dead leaf numbers per plant, and dry matter weight of each part for 6 months after transplanting was almost the same as in the control and calcium application plots. The results of growth analysis for 6 months showed there were no remarkable differences between the control and calcium application plots. In growth parameters at 10 months after transplanting, there were no apparent differences in plant height, length, leaf numbers per plant, leaf numbers per leaf and dead leaf numbers between the control and calcium application plots. Moreover, the difference in dry matter weight tended to alter from plant part to part. There were no significant differences in rachis and root dry weights between the two plots. The results of growth analysis from 6 months to 10 months after transplanting showed a significant difference in relative growth rate (RGR) and net assimilation rate (NAR). However, leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) were not significantly different between the two plots. It is still unclear whether the sago palm will show a preferable growth rate at a lower soil pH condition. Based on the results of the current study, the experimental procedures at field level show that the growth of the sago palm will not decelerate under acidic conditions compared with that under neutral conditions in soils that have the same parent material.

As described before, the relationship among three experiments under acidic soil conditions and aluminum concentrations it was confirmed that sago palm has ability to grow under widely different pH ranges from 3.5 to 7.9. Sago palm was considered to Al-avoidance genotype as Al-

excluder, which has a high resistance to Al and grows well under a wide range of soil pH culture solutions. However, it can be considered that the critical value to inhibit the growth of sago palm is around 300 ppm Al in the growth media and had growth responses to variation of Al stress at a low pH condition (pH 3.5), which Al induced changes in numerous characteristics such as morphological and physiological responses, inhibited root growth, decreased root cell number and root diameter, and alleviated root structure. Besides, in the range of pH of same soil material in the field experiment, the sago palm can show preferable growth and dry matter production at the early stage of the growth even at the lower soil pH condition. Further in the experiment, we still expect to reconfirm and make clear how sago palm can grow even in the problem soils such as acidic soil.

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Preuk CHUTIMANUKUL

Mie University

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