Ph.D. Thesis

Resistant Mechanism of Sago Palm (*Metroxylon sagu* Rottb.) against Aluminum Stress under Acidic Condition

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Chapter 1

General Introduction

Competition between biofuel production and food production has occurred in recent years in the context of the current social background regarding the exhaustion of fossil energy and the increase in the world population at the rate of 200,000 individuals per day. Various plants are receiving attention as sustainable energy resources for the production of bioethanol and biodiesel. However, worldwide arable lands are limited. Barren land with poor productivity should be used to produce plants to meet the demands for food and energy. Moreover, the development and/or improvement of new plant resources and their utilization is needed as a strategy to secure a sufficient amount of biomass for producing foods and biofuel sources that will not compete with food production (Ehara, 2009).

Sago palm (*Metroxylon sagu* Rottb.) is the only species in the *Metroxylon* (*Eumetroxylon*) section of genus *Metroxylon* of the family *Arecaceae*. This species is distributed in Southeast Asia (Indonesia, Malaysia, Brunei, Thailand and Philippine), Pacific Islands (Papua New Guinea and Solomon) and other Asian countries. Sago palm is once flowering (hypoxanthic) and tillering (soporiferous) perennially. It is mainly propagated vegetative from the suckers (tillers), although seeds are sometimes used. The growth of sago palm can roughly be divided into two broad stages: the rosette stage without trunk growth and the later stage with trunk growth (Jong and Flach, 1995). This palm produces a great plant length (more than 10 m) and fresh weight (more than 1,000 kg for the whole plant). Moreover, sago palm stores large quantities of starch in its trunk. The total starch storage in one trunk is approximately 300 kg dry weight (Ehara, 2005), the productivity of which is calculated to be four times the yield of rice on a yearly base per unit area (Yamada, 1990). In addition, sago palm has long been cultivated for food, fulfilling a need similar to banana and taro (Barrau, 1959; Takamura, 1990). This palm species is one of the oldest crops having been used as a carbohydrate resource by humans since ancient times (Takamura, 1990). The importance of sago palm as a staple food

has not changed in areas such as Siberut Island in West Sumatra, the Eastern Archipelago of Indonesia, Maluku and Papua, and Western Melanesia, Papua New Guinea. As a staple food, sago palm continues to be important in parts of Southeast Asia and in areas inhabited by the Melanesian people (Ehara et al., 2000). The carbohydrate or starch can be further processed into various basic raw materials for human and animal consumption as well as for use as an industrial energy source, such as ethanol. Moreover, there are many traditional ways for consumption. For example, villagers will use leaves to thatch roofs and house walls. These leaf materials are reputed to be used for several years. In some folk wisdom, the roots and young fruits are used in traditional medicine to relieve headaches and hypertension. Presently, sago palm is also used as an ornamental plant due to the long life span of the leaves and the beautiful red emerged leaves.

Sago palm grows in swampy, alluvial and peaty soils where almost no other major crops can grow without drainage or soil improvement (Sato et al., 1979; Jong and Flach, 1995). This palm is one of the most important bioresources for sustainable agriculture and rural development in swampy areas of the tropics. However, sago palm is recognized as an unexploited or underexploited plant because it has been harvested from natural forests and/or has been semi-cultivated under very simple maintenance. Further increase in its production is expected to be economically valuable in land development in swampy areas. Nevertheless, most soils in these areas have developed on marine clay sediments that were deposited during periods of high sea level, and contain high concentration of pyrite (FeS_2). When the pyrite is oxidized upon exposure to air, sulfuric acid is formed, which accounts for the soil acidity. The pH of this soil usually falls to below pH 4 and sometimes even to below pH 3 (Dent and Pons, 1995). In chemical terms, the pH scale refers to the concentration of hydrogen ions in a substance and each whole unit represents a factor of 10 times. For example, pH 5 is 10 times more acidic than pH 6. It can be extremely harmful when pH changes even only a little, because the chemical processes of plant cells are sensitive to the concentration of hydrogen and hydrogen ions (Rohyadi et al., 2004). Moreover, one of the major limitations of plant growth

on acid peaty soils is the prevalence of soluble aluminum ions (Al^{3+}), especially the inhibition on root growth (Malkanthi et al., 1995). The most commonly observable target of Al toxicity seems to be the plasma membrane of the root cell, particularly of the root apex (Pietraszewska, 2001). Recently, numerous reports describe the Al induced changes in root cell formation (Doncheva et al., 2005), irregular cell division, alterations in cell shape and cell wall thickening (Jones et al., 2006). According to Ma et al. (2001), Al^{3+} can inhibit root growth at micromolar concentrations within minutes or hours of exposure to Al in many plant species. Stunted roots are a consequence of Al-induced inhibition of root elongation, in which the main root tips and lateral roots become thicker and brownish (Pietraszewska et al., 1997). Although Al does not seem to interfere with seed germination, it does impair the growth of new roots and seedling establishment. For this reason, young seedlings are usually more susceptible than older plants (Nosko et al., 1988). However, there are many studies of the mechanism of Al toxicity and Al resistance that have been carried out in many plant species (Osaki et al., 1997). Osaki et al. (2003) reported that some native plants are endowed with a specific mechanism for Al resistance that can adapt to acid soil in tropical regions. According to Andersson (1988), plants may develop various degrees of tolerance for Al. Five types of tolerance mechanisms may be discerned:

(a) Al tolerance involves an exclusion mechanism. The roots of such plants contain less Al than average.

(b) A low uptake of Al in plant tops, usually due to immobilization of excess Al in the roots.

(c) An increasing pH of their rhizosphere, thereby lowering the solubility and availability of Al.

(d) A mechanism making the uptake of mineral nutrients possible ever in the presence of Al or involving low requirements for nutrients.

(e) A mechanism involves Al accumulation in the plant tops and a high internal tolerance to Al. Al probably binds to specific sites in the cell walls of the epidermis and mesophyll or inside the cytoplasm. This prevents Al from reaching sensitive metabolic sites within cell.

In addition, Matsumoto et al. (1998) reported that sago palm cannot be found in peaty soil in its natural habitat, but it has the mechanism to adapt well to peaty and swampy areas. For example, the mineral (especially N, P, Ca and Mg) requirement is quite low when compared with other plant species. Sago palm grows in peat soil, which extremely low pH (below pH 5) and still achieves high productivity (Flach and Schuiling, 1989; Nakamura et al., 2004). It is, therefore, assumed that sago palm is resistant to acidic pH and Al. However, few studies have compared the growth characteristics of sago palm at different pH levels as well as the resistance mechanism of sago palm against Al stress under acidic condition. In the current study, the nutrient accumulation in plant tissues of sago palm grown at different levels of soil pH in South Thailand was investigated to clarify the nutrient uptake and translocation in the plant body of sago palm in its natural habitat under the widely different soil pH. Moreover, the growth and physiological characteristics of sago palm grown at different levels of pH and Al concentrations under low pH condition in the laboratory experimental study were investigated to elucidate the acid- and Al-resistance of sago palm. Finally, the growth and nutrient accumulation in plant tissues of sago palm, rattan and yatay palm against Al stress under low pH condition were investigated to compare the Al resistance ability between sago palm and related species. The internal morphology of the leaflets and roots of sago palm and related species were observed to identify a localization of Al in plant tissues.

Chapter 2

Nutrient accumulation in plant tissues of sago palm in the rosette stage at different levels of soil pH in South Thailand

Introduction

Peatlands are the most widespread wetlands in the world and constitute 3% of the earth's land and freshwater surfaces (Paavilainen and Päivänen, 1995). In Southeast Asia, peat swamps cover an area of about 30 million hectares: two-thirds of the total area of the world's tropical peat swamps (Radjagukguk, 1997). In Thailand, the total area of peat swamp currently comprises about 0.13% of the country or about 0.48% of the whole forest area. The biggest peat swamp is located in Narathiwat Province, followed by Nakhon Si Thammarat Province (Nuyim, 2000).

Recently, land and population crises have become serious issues around the world (Yanbuaban et al., 2007). Human activities have increased in magnitude and have begun to extend toward the coastal lowland areas where peat swamps are widespread (Okubo et al., 2003). However, peat swamp soil is classified as having a very low potential for agriculture because of its physical and chemical properties, such as a high groundwater level and low nutrient content. In addition, most of these soils are highly acidic and generally contain highly exchangeable Al, in which almost no other crops grow without soil improvement (Osaki et al., 1998). Thus, actions to find new plant resources for future land uses are needed (Okubo et al., 2003).

Sago palm that stores large quantities of starch in its trunk is distributed in Southeast Asia, including South Thailand (Ehara et al., 2008a). This palm is one of the dominant species in tropical swampy and peaty soils and can grow in widely adverse conditions, such as acidic or saline-effected conditions. Flach and Schuiling (1989) reported that sago palm can be exploited without harmful effects on the existing ecological pattern and is really adapted to the humid tropical peat swamp. Considering the specific characteristics of sago palm, the efficient use of

carbohydrates from sago palm is currently expected, followed by an anticipated increase in utilization from the viewpoint of land development in swampy areas. Since sago palm is distributed even in brackish water areas near the coast and peaty areas where the strong acidic soil reaction is usually observed, it is considered that sago palm can grow in the widely different soil pH. In general, the soil nutrients are strongly affected by soil pH, which due to the interaction between soil particles and nutrients. The availability of various nutrients has been determined depending on a function of soil pH. Beside, the soil pH generally affects the plant growth through the increase or decrease in the nutrient uptake and the effects of soil pH on the plant growth are complex because the change in content varies with the individual nutrient. Yamamoto et al. (2003) reported that the growth and starch yield of sago palm grown in deep peaty soil were comparatively smaller than those in mineral soil, which soil pH of deep peaty soil usually lower than those of mineral soil. Therefore, it can be speculated that the growth and nutrient uptake of sago palm may be affected by the soil pH. In addition, Yamamoto (1996) and Yamaguchi et al. (1997) suggested that the duration from establishment of a young sago palm to the beginning of trunk formation was closely related to the soil properties. Thus, in this study, plant sampling of young sago palms in the rosette stage was conducted and the nutrient concentrations in plant tissues were analyzed to make clear the nutrient uptake and translocation in the plant body of young sago palms grown at three different sites in South Thailand where the soil pH was differed.

Materials and Methods

1. Experimental site

Young sago palms from the small clumps with no trunk formation in the rosette stage were sampled at the three sites of the natural sago palm growing-area in South Thailand from 30 January to 5 February, 2010 (Fig. 2.1). The site 1 was in Ban Kaokok, Tambon Tongnien, Khonom District, Nakhon Si Thammarat Province (9º16'22.14"N, 99º47'36.74"E). The sites 2 and 3 were in Rattaphum District, Songkhla Province (site 2: Ban Thachamuang, Tambon

Thachamuang, 7º07'25.22"N, 100º14'06.36"E; site 3: Phetkasem Road, Tambon Kamphangphet, 7º08'01.89"N, 100º15'20.17"E). The mean annual temperature in 2010 was approximately 27.6ºC in Nakhon Si Thammarat Province and 28.2ºC in Songkhla Province recorded at the nearest meteorological station of Thai meteorological department. The sites 1 and 3 located beside the canal, these sites were poorly drained and swampy (Fig. 2.2, Fig. 2.4). Contrarily, the site 2 where located near the residence of the villagers was well drained and comparatively dried (Fig. 2.3).

2. Soil physicochemical analysis

Soil samples were collected from a depth of 0-20 cm at each site (Fig. 2.5). Then, the soil samples were air-dried for 4 days at room temperature and prepared to analyze the soil physical and chemical property by sieving through a 2 mm mesh. Then, the soil samples were sent to analyze the soil chemical properties including pH, soil texture, organic matter and nutrient content at Soil Fertilizer Environment Scientific Development Project and Agricultural Production Science Research and Development Office in Kasetsart University, Bangkok, Thailand. At those places, the soil pH was measured at a soil : water ratio of 1 : 1 (w v^{-1}) by a pH meter. Total soil organic matter was measured by wet oxidation (Walkley and Black, 1934). The soil texture was determined by the percentage of sand, silt and clay, which the percentage of each particle type was determined using the hydrometer method. The total N concentration was measured by the semi-micro Kjeldahl digestion procedure. The amount of available P was determined by the Bray II method. The exchangeable K, Ca, Mg and Na were extracted with 1N ammonium acetate (NH_4OAc) solution (pH 7.0) and extractable Al was extracted with 1N KCl. All exchangeable cations and extractable Al were determined by atomic absorption spectrophotometer (170-30 AA, Hitachi, Japan). The extractable SO_4 was extracted with 0.08M $Ca(H_2PO_4)_2 \cdot H_2O$ and analyzed using the turbidimetric method.

3. Sampling and analysis of nutrient concentrations in plant tissues

Three young sago palms were selected from each site and were felled. After felling, the growth parameters including the plant length, number of live leaves and number of leaflets of

the third leaf position from the top were measured. The SPAD value (SPAD-502, Minolta, Japan) which has a positive correlation data with chlorophyll content per unit leaf area (Uddling et al., 2007) was measured at each leaf of the samples. From each plant, the third leaf from the top, unexpanded leaf (a needle-like leaf) and adventitious roots were sampled (Fig. 2.6). The third leaf was separated into four parts: leaflets, rachis, petiole and leaf sheath, which is recognized as the basal part of petiole that enclose the inside part of plant (Ehara, 2012). The adventitious root was divided into stele and cortex (epidermis, exodermis, suberized sclerenchyma cell and cortex), which were classified according to the method of Nitta et al. (2002). The separated samples were dried in an oven at 80ºC for 72 hours to measure the dry weight and then ground into powder in order to analyze the nutrient concentrations

3.1 Aluminum (Al) concentration

The ground dry sample (0.05 - 0.1 g) was put in the porcelain crucible and placed in a muffle furnace (FO 300, Yamato, Japan) at 500 ºC for 4 hours to obtain the ash. After burned, 10 ml of 6N HCl was added to dissolve the ash, then the porcelain crucibles were placed in hot plate at 100ºC until the entire sample solutions evaporated. The evaporated solution was dissolved again with 25 ml of 1N HCl and filtered with the filter paper (5A 150 mm, Advantec, Japan), and then diluted with distilled water to a total volume of 50 ml in the volumetric flask.

For the Al analysis, 10 ml of 20% ammonium acetate (CH_3COONH_4), 0.5 ml of 1% mercapto acetic acid (thioglycolic acid) and 2 ml of 0.2% aluminon (aurintricarbxylic acid ammonium salt) were mixed with 1 ml of sample solution; the volume was then adjusted to reach 50 ml with distilled water. The mixed sample solution in the volumetric flask was placed into boiling water for 2 minutes and placed for cooling at room temperature, which showed the red color indicating the Al^{3+} concentration. The mixed sample solution was determined with the absorbance at 530 nm by the spectrophotometer (UVmini-1240, Shimadzu, Japan). The standard calibration was used for calculating the correct Al^{3+} concentration of the sample solution.

3.2 Cation and anion concentrations

The approximately 0.05 g ground sample was put in the porcelain crucibles and placed in a muffle furnace (FO 300, Yamato, Japan) at 350ºC for 2 hours and 450ºC for 8 hours to obtain the ash. After burned, 100 µl of 1N HNO_3 and 1% HNO_3 were added to dissolve the ash for cation and anion analysis, respectively. Sample solution was filtered with the filter paper (5A 150 mm, Advantec, Japan) and diluted with distilled water to a total volume of 25 ml in the volumetric flasks. Then, these sample solution was filtered with 0.2 µm filter paper (Millipore omnipore^{TM} membrane filter paper, Ireland) before determining cation and anion concentrations by using HPLC with a conductivity detector (CDD-6A, Shimadzu, Japan)

3.2.1 Cation concentration analysis

3.3mM 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 liquid chromatograph pump (LC-9A, Shimadzu, Japan) at speed 1 ml per minute. This mobile phase was flown to the auto injector (SIL-6B, Shimadzu, Japan) and mixed with 10 µl of sample solutions to be homogenized, which was controlled by the system controller (SCL-6B, Shimadzu, Japan). The cation concentration was detected through the analytical column (IC-C3, Shimadzu, Japan), in the column oven (CTO-10A vp, Shimadzu, Japan) at 40ºC. The result was printed by a chromatopac (C-R 6A, Shimadzu, Japan). The standard solution of the cation concentration (for 100% was equated with 5 ppm K^+, 5 ppm Ca^{2+}, 5 ppm Mg^{2+} and 2 ppm Na^+) was measured for writing a standard calibration to calculate the correct ion concentrations of the sample solution.

3.2.2 Anion concentration analysis

1mM Hydroxybenzoic acid and 1.1mM N,N-diethylethanolamine were 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 liquid chromatograph pump (LC-9A, Shimadzu, Japan) at speed 1.5 ml per minute.

This mobile phase was flown to the auto injector (SIL-6B, Shimadzu, Japan) and mixed with 50 µl of sample solutions to be homogenized, which was controlled by the system controller (SCL-6B, Shimadzu, Japan). The anion concentration was detected through the analytical column (Shimadzu, IC-A1, Japan), in the column oven (CTO-10A vp, Shimadzu, Japan) at 40ºC. The result was printed by a chromatopac (C-R 6A, Shimadzu, Japan). The standard solution of the anion concentration (for 100% was equated with 40 ppm SO_4^{2-}) was measured for writing a standard calibration to calculate the correct ion concentrations of the sample solution.

3.3 Nitrogen (N) concentration

3.3.1 Digestion step

The ground dry sample (0.25 - 0.5 g) and 4 ml of concentrated sulfuric acid (H_2SO_4) were put in a digestion flask (kjeldahl flask 100 ml). The sample solution flask was shaken gently and then placed into a digestion block at 440ºC for 4 minutes. After that 10 ml of 30% hydrogen peroxide (H_2O_2) was added to the charred sample via the funnel on the fractioning head. The color of solution in the flask was changed to be clear and no color. After addition of hydrogen peroxide was complete, boil of excess hydrogen peroxide by heating for one more minute. Then the digested solution was taken off the heater 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.

3.3.2 Distillation Step

5 ml of digested solution was poured in the beaker and 2-3 droplets of the violet solution (100 mg methylene blue and 100 mg methyl red dissolve in 95% ethanol) was added, next 2 ml of the saturated sodium hydroxide (NaOH) were added, which the color will change to the green color and then poured gently into the A path in Fig 2.7. After the C tube (in Fig. 2.7) has the 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 to the volume approximately 30 ml, the conical beaker was removed to the titration step.

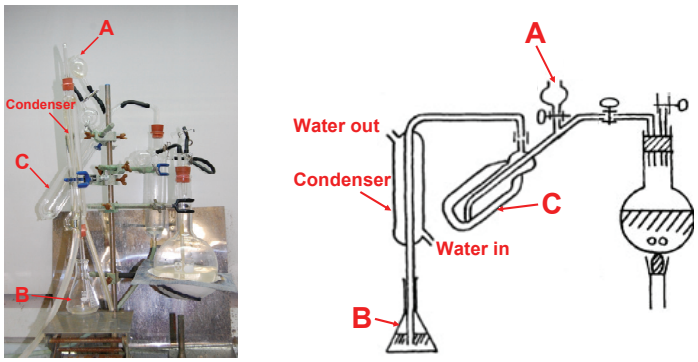


Fig. 2.7 The distillation system of the semi - micro Kjeldahl method for nitrogen (%) analysis.

3.3.3 Titration step

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

3.3.4 Calculation

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

$$\% \text{ Nitrogen} = \frac{0.2802 \times F \times (\text{ml blank} - \text{ml titrate}) \times (100/5) \times 100\ (\%)}{\text{Sample weight (g)} \times 1000}$$

F = the correction factor of 0.02N NaOH (from approximately 0.9 to 1)

3.4 Phosphorus (P) concentration

The ground dry sample (0.3 - 0.6 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 in the hot plate at 90ºC for 45 minutes and then the temperature was changed to 140ºC. The sample solution was placed in the hot plate until this solution turned to the clear solution. Sample solution was shaken frequently and HNO_3 was added occasionally when sample

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

For the P analysis, 200 µl of the sample solution was mixed with 4 ml of the P analysis solution [0.1M ascorbic acid ($C_6H_8O_6$), 0.004M bis diantimonate (III) dipotassium trihydrate ($C_8H_4K_2O_{12}Sb_2 \cdot 3H_2O$), 0.03M hexaammonium heptamolybdate tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) and 2.5M hydrogen sulfate (H_2SO_4)] and then adjusted the volume with distilled water until there was 25 ml in the volumetric flask. The mixed sample solution was placed at the room temperature for 15 - 20 minutes. This solution would change to the blue color that indicated the P concentration. The mixed sample solution was determined by the spectrophotometer (UVmini-1240, Shimadzu, Japan) with the absorbance at 880 nm. The standard calibration was used for calculating the correct phosphorus concentration of the sample solution.

4. Statistical analysis

The statistical difference of the data was determined using NCSS 2001 (Number Cruncher Statistical Systems). The effect of different levels of soil pH was determined by one-way ANOVA (analysis of variance), and the differences among the mean values of the three sampling sites were determined using the Tukey-Kramer test.

Results and Discussion

1. Soil physicochemical properties

The physical and chemical properties of soil samples at the three sampling sites are shown in Table 2.1. The main particles of the soil at sites 1 and 3 were clay particles (clay texture), while those at site 2 were silt particles (loam texture). The organic matter content of the soil at sites 1 and 3 was higher than that at site 2. These results might be due to year-round or several months of waterlogged conditions at sites 1 and 3, which related to the lower decomposition in

the soil at those sites rather than in the drained soil at site 2. The soil at site 1 had a neutral pH of 7.0, while the soil at sites 2 and 3 had an extremely acid pH of 4.3 and 4.4, respectively. There have been several reports on the pH of tropical peat soil in a sago palm plantation, such as in Riau or Sarawak, where the pH ranged from 3.3 to 4.7 (Purwanto et al., 2002; Kawahigashi et al., 2003; Miyamoto et al., 2009). Moreover, there have been several reports on the pH of the peat soil in either a sago palm plantation or the natural sago palm growing-area in South Thailand, such as Songkhla and Narathiwat Provinces, where the pH ranged from 3.5 to 5.4 (Osaki et al., 2003; Yanbuaban et al., 2007). From the results of the current study, it was confirmed that sago palm grows in the widely different soil pH range from 4.3 to 7.0 under natural conditions in Thailand.

Almost all concentrations of soil nutrients, such as those responsible for salinization (Na, Ca, and Mg) and extractable SO_4, were higher in the soil at site 1 (pH 7.0), followed by the soil at site 3 (pH 4.3) and site 2 (pH 4.4) (Table 2.1). On the other hand, the Al concentration was higher in the soil at sites 2 and 3 than in that at site 1. A tendency toward a higher Al concentration in the low pH soil than in the neutral pH soil was in agreement with that reported by Adams and Moore (1983), which suggests that the decrease in soil pH increased the amount of Al concentration in the soil solutions. According to Brady and Weil (2002), Al is a major constituent of most soil minerals, including clay. Although a low pH is defined as a high concentration of H^+ ions, Al also plays a major role in soil acidity. When H^+ ions are adsorbed on a clay surface, they do not usually remain as exchangeable cations for a long time but, instead, attack the structure of the minerals, releasing Al^{3+} ions in the process. In addition, the Al concentration in soil at the three sampling sites, 5-145 mg kg^{-1}, tended to be higher than the values reported for the peat soil of the sago palm cultivation in Sarawak, Malaysia, 5-14 mg kg^{-1} (Jong and Flach, 1995).

The N, P and K concentrations in the soil samples were in the range of 0.9 - 2.0 g kg^{-1}, 4 - 8 mg kg^{-1} and 50 - 90 mg kg^{-1}, respectively, that were almost the same low level at the three sampling sites (Table 2.1). A tendency toward poor macronutrient at the current sampling sites

was in agreement with the report of Kawahigashi et al. (2003), suggesting that a deficiency in the nutritional condition is common among peat soils, especially strongly acid peat soil, and few plants survive in such adverse conditions.

2. Nutrient concentrations in different plant parts

Table 2.2 shows the size of young sago palms in the rosette stage grown under natural conditions at the three sampling sites. In the current study, all young sago palms from three locations in South Thailand belonged to a non-spiny type. The average length of three sago palms from sites 1, 2, and 3 were 3.0, 5.0 and 3.9 m, respectively. The number of live leaves did not differ distinctively among the three sampling sites, being approximately 5-7. In the current study, the SPAD value of the leaflet from sites 2 and 3 tended to be higher than that from site 1. These results suggested that sago palm would show preferable growth under acid (sites 2 and 3) than neutral pH (site 1) conditions. However, the ages of the plant samples under natural conditions in the current study were unknown. Even so, this finding is noteworthy. The results of this study were similar to those observed in a high level acid tolerance of lowland rice, which had lower yields with a pH 6.0 treatment than with low pH treatments ranging from pH 3.5 to pH 5.0 (Thawornwong and Diest, 1974). An experimental study on the comparison of the growth of sago palm under acid and neutral soil conditions will be conducted in the future.

The nutrient concentrations in different plant parts and whole plant of sago palm from the three sampling sites are shown in Table 2.3. The N and P concentrations in all plant parts of sago palm from sites 2 and 3 tended to be higher than those from site 1. Beside, the N and P concentrations in the whole plant of sago palm from sites 2 and 3 were significantly higher than that from site 1 (Table 2.3), although the available P and total N in the soil did not differ among the three sampling sites (Table 2.1). Probably, the comparatively higher P and N uptake of sago palm under low pH conditions (sites 2 and 3) may be an important factor to explain the acid resistance of sago palm. Furthermore, the N concentration in the leaflets was significantly higher than that in other parts, while the P concentration in different plant parts of sago palms at the three sampling sites tended to be higher in the unexpanded leaf than in other parts (Table

2.3). This tendency toward a higher concentration of N in the leaflets and P in the unexpanded leaf than in other parts was in agreement with the experimental study reported by Prathumyot et al. (2011). The K^+ concentration was significantly higher in the lower (petioles, leaf sheaths and roots) than the higher (leaflets) parts (Table 2.3), which was in agreement with the previous reports (Matsumoto et al., 1998; Ehara et al., 2006; Prathumyot et al., 2011). In addition, the K^+ concentration in almost all plant parts and whole plant tended to be higher in sago palm from site 1 followed by that from sites 3 and 2 (Table 2.3), which had a tendency similar to that observed in the soil results at the three sampling sites (Table 2.1). However, the effect of the difference in soil pH between the neutral pH soil (site 1) and the low pH soil (sites 2 and 3) on the K^+ concentration was significant only in the case of the leaf sheaths and cortex of adventitious roots (Table 2.3). From the current results of macronutrients (N, P and K) in plant tissues, it is likely that sago palm grown at sites 2 and 3 could maintain or increase the uptake ability of macronutrients, which may be one of the major reasons that sago palm can adapt to growth in extremely acidic conditions.

The Ca^{2+} concentration in different plant parts of sago palm at the three sampling sites was significantly higher in the leaf sheaths than in other parts, such as the leaflets or petioles. In addition, the difference in the Ca^{2+} concentration in whole plant between the neutral pH soil (site 1) and the low pH soil (sites 2 and 3) was not significant (Table 2.3), though the concentration of Ca in the soil at the three sampling sites was differed distinctively (Table 2.1). It was considered that the widely different soil pH in the range from 4.3 to 7.0 might not have a remarkable effect on the Ca^{2+} accumulation in whole plant. These results support the previous report of Matsumoto et al. (1998) suggesting that sago palm has some mechanism to remobilize Ca from old to new leaves, which may account for the constant accumulation of Ca in plant tissues, especially in the leaves, during growth under natural conditions.

The Mg^{2+} concentration in almost all plant parts of sago palm from sites 2 and 3 tended to be lower than that from site 1 (Table 2.3), which tended to be similar to that observed in the soil samples (Table 2.1). Beside, the Mg^{2+} concentration in whole plant of sago palm from site 1

was significantly higher than that from sites 2 and 3. However, the difference in Mg^{2+} concentration in plant tissues was smaller than that in the soil among the three sampling sites. From site 1, the Mg^{2+} concentration tended to be the same level in all plant parts, while the Mg^{2+} concentration in different parts of sago palm from sites 2 and 3 tended to be higher in the lower parts, especially the cortex of adventitious roots, than that in the higher parts, such as the leaflets (Table 2.3). This result indicated that the translocation of Mg^{2+} from the roots to the top parts might be restricted by low pH conditions. According to Wu and Rebeiz (1985), absorbed Mg and N are important structural components located in the center of chlorophyll, as could be estimated from the result of the SPAD value of the leaflets in the current study. The higher chlorophyll production (higher SPAD value) of sago palm under low pH conditions (sites 2 and 3) could be attributed to the comparatively higher N accumulation in the leaflets, although the Mg^{2+} concentration in the leaflets of sago palm under low pH conditions was lower than those under neutral pH conditions (site 1).

The Al^{3+} concentration in almost all plant parts and whole plant, except the cortex of adventitious roots, did not display any significant differences among the three sampling sites (Table 2.3), although the Al concentration in the soil at site 1 was apparently lower than that at sites 2 and 3 (Table 2.1). In addition, the concentration of Al^{3+} in the higher plant parts was not very high in any sampling sites. Sago palm at the three sampling sites tended to store a higher Al^{3+} concentration in the cortex of adventitious roots than in other parts. It was considered that sago palm might prevent the excess Al^{3+} influx at the cells between the cortex and stele, namely, the endodermis. However, Rasmussen (1968) reported that the concentration of Al was the highest at the surface of the root cap and steadily decreased toward the center of the corn root, and, furthermore, suggested that the epidermal layer of the root could obstruct the Al movement into the cortex and conductive tissues. Therefore, the observation of the localization and distribution of Al in plant tissues of sago palm will be undertaken in the future research (Chapter 5).

The SO_4^{2-} concentration in almost all plant parts and whole plant of sago palm from site 1 was significantly higher than that from sites 2 and 3 (Table 2.3), which a significant difference was clearly exposed in the cortex of adventitious root (Table 2.1). The SO_4^{2-} concentration in different plant parts of sago palm from site 1 was significantly higher in the cortex of adventitious roots than that in other parts. There are several reports concerned with sulfate accumulation, suggesting that sulfate is generally regarded as an immobile element and its accumulation in the root zone is a frequent phenomenon in various plant species (Sunarpi and Anderson, 1996; Kowalska, 2004; Van der Welle et al., 2007). Nevertheless, this tendency toward a higher SO_4^{2-} concentration in the cortex of adventitious roots than in other parts was not observed in sago palm from sites 2 and 3, which may be attributed to the relatively small amount of SO_4^{2-} concentration in the soil at both sampling sites compared with that in the soil at site 1. In addition, the SO_4^{2-} concentration in the soil at the three sampling sites was in the range of 6 to 116 mg S kg^{-1}. According to Kyuma (2003), the SO_4^{2-} concentration in some representative acid sulfate soil and non-acid sulfate soil in the Bangkok plain for rice cultivation in Thailand was in the range of 181 to 5,235 mg S kg^{-1}. It is likely that the SO_4^{2-} concentration in the soil from the current sampling sites, especially the soil from sites 2 and 3 (6 and 22 mg S kg^{-1}, respectively), was comparatively lower than that from a former report.

The Na^+ concentration in whole plant and almost all plant parts, except the leaflets, were significantly higher in sago palm from site 1 than that from sites 2 and 3 (Table 2.3), which tended to be similar to that observed in the soil results among the three sampling sites. Beside, the Na^+ concentration in the soil from site 1 was 835 mg kg^{-1} or about 0.08% NaCl that was 10 times higher than the value in the soil from sites 2 and 3 (Table 2.1). Prathumyot et al. (2011) reported that the absorption of macronutrients (N and P) by sago palm was not inhibited by salt stress even under 1.3% (224 mM) NaCl, although the chlorophyll production was depressed. Based on these findings, it was considered that the Na^+ concentration in the soil (0.08% NaCl) from site 1 in the current study might not affect the macronutrients (N and P) of sago palm from site 1 (neutral pH soil). In this study, the significant difference was found in the N and P

concentrations in whole plant tissues among the three sampling sites, the value were higher in low pH condition (sites 2 and 3) rather than in neutral pH condition (site 1). Therefore, at least it is able to say sago palm grown at comparatively low pH soil showed the higher accumulation of N and P than that at the neutral pH soil condition in this study. However, the concentration of Na^+ in the soil from site 1 in this study might cause to delay the chlorophyll production, which may account for declining the SPAD value of sago palm from site 1. Nevertheless, Prathumyot et al. (2011) suggested that the chlorophyll concentration could increase up to high level over a comparatively long time because of there was no lack of materials, such as N and Mg, for the chlorophyll production, which may account for the ability to grow under salt stress even with a reduction of the growth rate. In addition, the Na^+ concentration in the cortex of adventitious root was higher than that in the other parts of sago palm at the three sampling sites (Table 2.3). This current results support the former findings of Ehara et al. (2008a) showing that a dense distribution of Na was observed around the endodermis in the extension zone of the adventitious roots. Based on the finding of Prathumyot and Ehara (2010), the development of the Casparian strip located in the endodermal cell wall of the adventitious root of sago palm can be considered as an important mechanical factor relating to the avoidance mechanism for preventing the excess influx of Na^+ through an apoplastic partway into the stele and its translocation from root to shoot in sago palm.

The current study demonstrates some tendencies of the nutrient uptake and translocation in plant tissues of sago palm in its natural habitat. Based on the current results of macronutrients (N, P, K and Ca) in plant tissues, it is likely that sago palm grown at sites 2 and 3, pH 4.4 and pH 4.3, respectively, could maintain or increase the uptake of macronutrients, which may be a major reason that sago palm can adapt to growth in strongly acidic soil in a natural habitat. Furthermore, sago palm at the three sampling sites tended to store a higher Al^{3+} concentration in the cortex of adventitious roots than in other parts, such as the leaflets, and a similar tendency was observed for the accumulation of SO_4^{2-} and Na^+ in plant tissues. It was, therefore, assumed

that sago palm grown under any conditions of soil pH might exhibit an avoidance mechanism to prevent the excess influx of these ions in plant tissues.

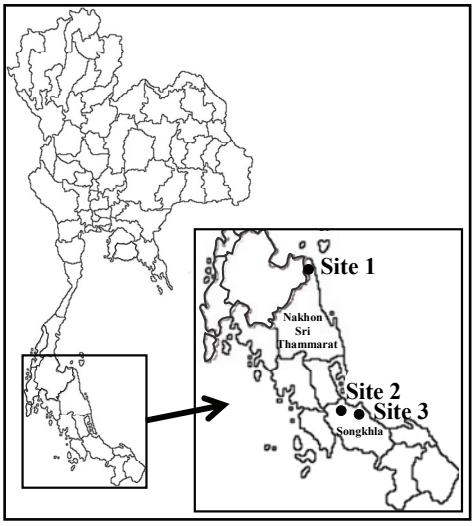


Fig. 2.1 Research area and sampling site distributed in South Thailand.

Fig. 2.2 Sago palm population at site 1 in Nakhon Si Thammarat Province, South Thailand.

Fig. 2.3 Sago palm population at site 2 in Songkhla Province, South Thailand.

Fig. 2.4 Sago palm population at site 3 in Songkhla Province, South Thailand.

Fig. 2.5 Soil sampling from each sampling site.

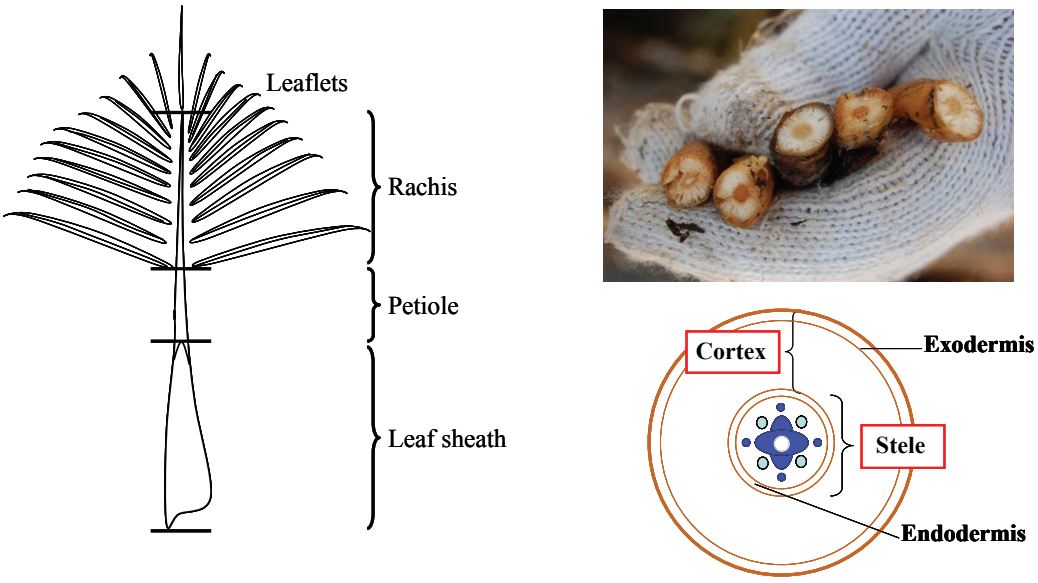


Fig. 2.6 Separation of leaf and adventitious root of sago palm from each sampling site.

Table 2.1 Characteristics of the physical and chemical properties of soil samples at the three sampling sites.

Sampling site	Acidity- Alkalinity		Liming	% particle size			Soil texture	Organic matter	
	pH (H_2O)	Level	($CaCO_3$ kg ha^{-1})	Sand	Silt	Clay		($g\ kg^{-1}$)	Level
Site 1	7.0	Neutral	-	33.0	16.0	51.0	Clay	118.7	High
Site 2	4.4	Extremely acidic	5,881.3	31.0	44.0	25.0	Loam	19.1	Medium
Site 3	4.3	Extremely acidic	6,718.8	23.0	34.0	43.0	Clay	43.5	High

Sampling site	Total N		Available P		Exchangeable K		Exchangeable Ca		Exchangeable Mg	
	($g\ kg^{-1}$)	Level	$mg\ kg^{-1}$	Level	$mg\ kg^{-1}$	Level	$mg\ kg^{-1}$	Level	$mg\ kg^{-1}$	Level
Site 1	1.5	Low	4.0	Low	90.0	Medium	3,196.0	High	680.0	High
Site 2	0.9	Low	5.0	Low	50.0	Low	196.0	Low	40.0	Medium
Site 3	2.0	Low	8.0	Low	80.0	Medium	716.0	High	90.0	Medium

Sampling site	Extractable Al		Extractable SO_4		Exchangeable Na	
	$mg\ kg^{-1}$	Level	$mg\ S\ kg^{-1}$	Level	$mg\ kg^{-1}$	Level
Site 1	4.5	Low	115.6	Low	835.0	Low
Site 2	135.0	High	6.2	Low	74.0	Low
Site 3	145.0	High	21.9	Low	86.0	Low

Table 2.2 Young sago palms in the rosette stage grown under natural conditions at the three sampling sites.

Sampling site	Plant length (m)	Number of live leaves	Number of leaflets of the third leaf from the top	SPAD
Site 1	3.0	6.7	25.3	54.0 b
Site 2	5.0	5.3	53.7	62.7 ab
Site 3	3.9	7.3	48.0	67.2 a

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

Table 2.3 Nutrient concentrations in different plant parts and whole plant of young sago palms grown under natural conditions at the three sampling sites.

Plant part	Sampling site	Nutrient concentration							
		N (mg g^{-1})	P (mg g^{-1})	K^+ (µmol g^{-1})	Ca^{2+} (µmol g^{-1})	Mg^{2+} (µmol g^{-1})	Al^{3+} (µmol g^{-1})	SO_4^{2-} (µmol g^{-1})	Na^+ (µmol g^{-1})
Leaflet	Site 1	8.0 cA	1.0 cAB	177.6 aD	45.3 aB	49.6 aA	9.5 aB	28.2 aC	13.1 aC
	Site 2	14.0 bA	2.4 bBC	172.6 aC	31.5 aBC	32.5 bBC	10.8 aB	27.5 aA	4.3 bD
	Site 3	15.8 aA	3.1 aA	175.3 aC	42.8 aB	27.8 bCD	12.2 aB	29.5 aA	9.5 aB
Unexpanded leaf *	Site 1	7.7	1.3	518.8	31.7	49.9	8.7	23.0	14.1
	Site 2	11.7	6.1	344.6	28.5	28.3	10.5	13.0	5.8
	Site 3	12.0	7.0	496.9	40.8	31.9	13.9	20.9	10.1
Rachis	Site 1	2.7 bB	1.1 bA	531.3 aC	38.5 aB	48.4 aA	13.2 aB	37.3 aBC	18.7 aC
	Site 2	4.2 abBC	2.8 aAB	343.3 bBC	33.0 aB	22.1 bC	12.8 aB	22.4 bA	6.9 cD
	Site 3	5.3 aBC	3.1 aA	468.2 abB	37.1 aBC	29.7 bCD	13.4 aB	16.9 bB	10.9 bB
Petiole	Site 1	2.3 bB	0.8 bABC	570.4 aC	40.6 aB	48.5 aA	10.2 aB	37.2 aBC	28.7 aC
	Site 2	3.4 abC	3.8 aA	436.2 bB	34.7 aB	31.3 bBC	11.2 aB	7.2 bB	10.7 bC
	Site 3	4.7 aBC	4.1 aA	695.7 aAB	43.3 aB	39.3 abBC	12.0 aB	8.7 bC	12.6 bB
Leaf sheath	Site 1	3.5 aB	0.6 bBC	1,084.6 aB	60.5 aA	49.9 aA	12.2 aB	23.7 aC	76.4 aB
	Site 2	3.4 aC	2.9 aAB	703.6 bA	43.3 bA	45.2 aAB	12.0 aB	6.9 bB	22.4 bB
	Site 3	4.1 aC	3.1 aA	732.6 bA	59.7 aA	48.9 aB	12.6 aB	8.5 bC	32.8 bA
Stele of adventitious root	Site 1	2.3 bB	0.4 bC	314.9 abCD	24.5 aC	48.3 aA	10.0 aB	59.8 aB	97.5 aB
	Site 2	5.9 aB	1.2 aC	201.8 bBC	8.9 bD	18.6 bC	9.8 aB	11.0 bB	30.1 bAB
	Site 3	5.5 aBC	0.9 aB	395.1 aB	9.5 bD	14.8 bD	9.8 aB	3.1 cD	32.7 bA
Cortex of adventitious root	Site 1	3.0 bB	0.9 bAB	1,164.3 aA	34.4 aBC	60.4 bA	22.1 bA	187.3 aA	446.4 aA
	Site 2	6.9 aB	1.7 aBC	431.2 bBC	25.5 aC	56.1 bA	28.1 aA	25.3 bA	36.5 bA
	Site 3	6.3 aB	1.0 bB	453.9 bB	32.2 aC	74.7 aA	38.0 aA	27.3 bA	38.3 bA
Whole	Site 1	3.5 b	0.9 b	546.0 a	37.3 ab	52.2 a	13.0 a	61.1 a	124.3 a
	Site 2	7.1 a	3.0 a	415.3 b	35.0 b	34.7 b	14.8 a	21.4 b	17.4 b
	Site 3	7.7 a	3.3 a	466.1 ab	47.6 a	38.1 b	15.8 a	16.5 b	21.0 b

Means followed by different letters are significantly different at the 0.05 level by the Tukey-Kramer test (n=3; *: data of the unexpanded leaf at the three sampling sites was from two plants). Lowercase letter indicates comparison among the sampling sites in each plant part. Capital letter indicates comparison among plant tissues within each sampling site.

Chapter 3

Effect of low pH on growth, physiological characteristics and nutrient absorption of sago palm

Introduction

Acidity is a major degradation factor of soil and covers extensive areas in the temperate and tropical zones. In Japan, most of the forest soils are acidic, which the pH ranges from 4.0 to 6.0 (Kawada, 1989) and the average pH is approximately 5.1 (Takahashi et al., 2001). In Thailand, acid soils (pH below 4) are scattered throughout the country with a total area of 22.8 million ha, which are mainly in the Northeast region, follow by Southern, Central Plain, Northern, Western and Eastern regions (10.4, 4.3, 4.0, 3.04 and 1.12 million ha, respectively) (Chareonchamratcheap et al., 1997). Although, the acid soils as a problem soil have long been experienced in the country, their magnitude and intensity are continuously increasing as a result of inappropriate soil, which use for agriculture in recent decades and still distributed widespread throughout the country (Rungsun et al., 2004). Acid soil toxicity is not a single factor but a complex factor that may affect the growth of many plant species though the different physiological and biochemical pathways (Foy and Fleming, 1978). The growth limiting factors for many plants have been associated with the acid soil infertility complex include toxicities of Al, Mn and other metal ions and deficiency or unavailability of essential elements, particularly Ca, Mg and P (Jackson, 1967). When the field crops are cultivated or introduced into this adverse soil, serious nutrition problems will be occurred. From this problem, the acid resistance crops become extremely important in the agricultural development (Maranville et al., 1994). Nevertheless, some native plants are growing well in these adverse soil conditions (Osaki et al., 2003).

Since sago palm can grow under acid conditions, it is assumed to be resistant to acid (Purwanto et al., 2002; Osaki et al., 2003). In the previous study, sago palm was considered to have a high adaptability to grow under widely different pH in the natural habitat, including low

pH soil. However, few studies have compared the growth characteristics of sago palm in growth media at various low pH conditions. Thus, the current experimental study was designed to compare the physiological features and growth characteristics of sago palm seedlings at different pH levels under a hydroponic system. The analysis of the effect of low pH on the growth characteristics with related physiological features may contribute to the development of a sustainable method of cultivation that is essential for the improvement of sago palm as an economic plant.

Materials and Methods

1. Plant materials and pH treatment

Fruits of sago palm were collected in the swampy areas of Rattapum, Songkhla, Thailand, on 1 August 2006. Fertilized and well-developed fruits were selected and treated physically to remove seed coat tissues. The cleaned seeds were placed in a plastic tray filled with tap water and then kept in darkness in a room kept at 30ºC in Thammasat University, Pratumtanee, Thailand, as reported by Ehara et al. (1998). The germinated seeds were brought to Mie, Japan, and transplanted to a 1/5000a Wagner pot filled with vermiculite and Kimura B culture solution containing (μM) 36.5 $(NH_4)_2SO_4$, 9.1 K_2SO_4, 54.7 $MgSO_4$, 18.3 KNO_3, 36.5 Ca $(NO_3)_2$, 18.2 KH_2PO_4 and 3.9 FeO_3 (Baba and Takahashi, 1958). The culture solution was adjusted to an initial pH of 5.5 using 1N HCl before irrigation into pots, as reported by Ehara et al. (2006). The pots were placed in a greenhouse under natural sunlight and maintained at over 15ºC, even at night, at Mie University. Culture solution was added daily in an amount equal to that consumed, and the culture solution was renewed twice weekly.

Three seedlings at the 7th leaf stage, with the 8th leaf emerging and the mean plant length of all plant materials at 39 cm, were cultured in Kimura B culture solution adjusted to pH 5.7, 4,5 and 3.6 with 1N HCl as required. The pots were placed in the same greenhouse under natural sunlight. An air pump was connected to the pots to provide air to the roots. Culture solution equal to that consumed was supplemented daily and each solution was renewed every

other day from 23 May to 9 October, 2007. The plant length and leaf number were measured once a week.

2. Photosynthetic rate, transpiration rate and stomatal conductance

On 5 October 2007, 18 weeks after the start of culture, the leaflets of the most active leaves or the 4th leaf position from the top were selected for measuring the net photosynthetic rate, transpiration rate, and stomatal conductance using a potable photosynthetic meter (LCA-4, Analytical development, England) at saturation irradiance with incident photosynthetically active radiation (PAR) of 800 - 1,000 μmol m^{-2} s^{-1}. As a light source, a halogen lamp was used. The appropriate PAR was obtained by changing the distance between the projector and leaves.

3. Chlorophyll content of the leaflets

The chlorophyll content of the leaflets at each leaf position was measured by the method of Mackinney (1941). An area of 0.25 cm^2 from each leaflet was punched out from each leaf and soaked in 10 ml of 80% (v/v) acetone to extract chlorophyll. 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). The chlorophyll content was expressed as the content per unit leaflet area, which can be calculated according to the specific absorption coefficient formulas as describe below;

Total chlorophyll (a+b) (mg/l)	=	8.02 A_{663}	+	20.20 A_{645}
Chlorophyll a (mg/l)	=	12.70 A_{663}	-	2.69 A_{645}
Chlorophyll b (mg/l)	=	22.90 A_{645}	-	4.68 A_{663}

4. Sampling and analysis of nutrient concentrations in plant tissues

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

exodermis, suberized sclerenchyma cell). Adventitious and lateral roots were classified according to the method of Nitta et al. (2002) as follows: adventitious roots were about 6 to 11 mm in diameter, and lateral roots, less than 6 mm in diameter. The separated samples were dried in an oven at 80ºC for 72 hours to measure the dry weight and then ground into powder in order to analyze the ion concentrations. The ground samples were reduced to ash in a furnace and extracted with 1N HNO_3, and the K^+, Ca^{2+} and Mg^{2+} concentrations were determined using a high-performance liquid chromatograph (HPLC) with a conductivity detector (IC-C3, CDD-6A, Shimadzu, Japan). The concentration of P was evaluated by atomic absorption spectrophotometry. The total N concentration was determined by the semi-micro Kjeldahl method, while the Al^{3+} concentration was determined calorimetrically by the aluminon method.

5. Statistical analysis

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

Results and Discussion

1. Plant growth

The numbers of emerged leaves, live leaves and dead leaves were counted throughout the treatments (Fig. 3.1 and Table 3.1). During the experiment, approximately 7 leaves emerged at each pH treatment. The number of dead leaves counted during the experiment was 4, 4 and 3 at pH 5.7, 4.5 and 3.6, respectively. There were no significant differences in the numbers of emerged, dead and live green leaves among the three pH treatments. These results indicate that the low pH conditions had no effect on leaf emergence and senescence.

The increment of plant length, total leaflet area and dry matter weight of the leaflets, petioles, roots and whole plant at the end of the pH treatments are shown in Table 3.2. The mean increment of plant length did not change at any pH throughout the experiment. The

leaflet area at the end of the treatment was the same at all three pH treatments. The total dry matter weight tended to be heavier at pH 4.5 than at pH 5.7. On the other hand, the total dry matter weight at pH 3.6 tended to be lighter than that at pH 5.7. There was no significant difference in the total dry matter weight between pH 5.7 and pH 3.6, the mean difference being 9%; the comparatively large variation in the value among the individuals in each treatment might be related to this variation. It is difficult to obtain uniform sago palm seedlings because of the low germination percentage and very large variation in the days of germination; therefore, there is a comparatively large variation in the growth parameters, which is a general characteristic of wild plants. In addition, the difference in the specific leaf area (SLA = leaflet area / leaflet dry weight) of the leaflets at different leaf positions from the top was negligible among the three pH treatments, which the SLA value at the higher leaf positions tended to be higher than at lower leaf positions in all the pH treatments (Fig. 3.2).

As described above, the dry matter weight of plants grown for 4.5 months at pH 4.5 was slightly heavier, and that at pH 3.6 was slightly lighter than that at pH 5.7. However, differences in plant size not only shoot elongation, leaf emergence and leaf area expansion but also dry matter increase, were statistically negligible. Beside, the morphological appearance of the root growth of sago palm was similarly in all the pH treatments (Fig. 3.3). Therefore, it was considered that the decrease of the pH in the growth media in the range from 3.6 to 5.7 might not have a marked effect on the growth rate of sago palm seedlings in the early stage of growth.

2. Physiological characteristics

The mean values of the chlorophyll content per unit leaflet area of all the leaf positions were 74.7, 73.5 and 76.0 μg cm^{-2} at pH 5.7, 4.5 and 3.6, respectively. The highest value of the chlorophyll content was observed in the 5th leaf from the top at each pH treatment. The difference, then, in the chlorophyll content among the three pH treatments were not significant at any leaf position (Fig. 3.4).

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. All the measured values decreased slightly under lower pH treatments, but the extent of the decrease was not distinct (Table 3.3). In sugar maple (Hogan, 1998), red oak (Reich et al., 1986) and hybrid poplar (Hogan and Taylor, 1995), the photosynthetic rate, transpiration rate and stomatal conductance did not decrease markedly in the pH range from 3.0 to 5.5. Generally, reduction in the photosynthetic rate of several plants has been recorded when the pH is extremely low, i.e., lower than the soil pH in field conditions (Taylor et al., 1986; Hogan and Taylor, 1995). 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 or growth inhibition (Kawahigashi et al., 2003).

As described above, although there was no significant difference in the total dry weight per plant among the three pH treatments, the total dry weight in pH 3.6 tended to be smaller than that at pH 5.7. Similarly, the photosynthetic rate at pH 3.6 tended to be lower than that at pH 5.7. The difference in photosynthetic rates among the three pH treatments could be attributed to differences in the stomatal conductance, which tended to be lower than that at pH 5.7 (Table 3.3). Yamamoto et al. (2003) reported that sago palm growth was slower and starch yield was smaller in acid peat soil than in mineral soil. Generally, the time from planting to harvesting (maturity), is longer in acid peat soil than in mineral soil, but even so sago palm grown in acid peat soil can be harvested even when the growth rate is depressed by environmental stress, such as low pH or poor fertility per soil volume. The current results on the dry matter weight and photosynthetic rate with related parameters suggest that the lower growth rate and lower yield of sago palm under acid conditions in the field is due to lower photosynthetic rate caused by the small stomatal conductance of the leaflet.

3. Nutrient concentrations in different plant parts

3.1 Leaflets, petioles, roots and whole plant

The concentrations of Al^{3+}, N, P, K^{+}, Ca^{2+} and Mg^{2+} in the leaflets, petioles, roots and whole plant at each pH treatment are shown in Table 3.4. In the current experiment, Al was not added into the culture solution, although Al^{3+} was detected in plant tissues probably due to its elution from vermiculite. The Al^{3+} concentrations in the whole plant under the three pH treatments, namely pH 5.7, pH 4.5 and pH 3.6 were 7.8, 8.6 and 9.4 μmol g^{-1}, respectively, which these results slightly increased under lower pH treatments. In addition, the Al^{3+} concentration in plant tissues tended to be higher in the roots than in the leaflets and petioles, and the difference between the roots and top parts, petioles and leaflets, was significant at both pH 4.5 and 3.6. A similar tendency was found in tea plants, in which the concentration of Al^{3+} in the root was higher than that in the shoot or leaves, especially in the large-leaf variety that grows in low pH soil (Fung and Wong, 2001). However, there was no significant difference in the Al^{3+} concentration in any of the plant parts among the three pH treatments. Considering the results from the current experiment, such as the difference in the Al^{3+} concentration in the roots and top parts under a lower pH condition and the lack of a significant difference in the Al^{3+} concentration in the top parts among the three pH treatments, the translocation of Al^{3+} from the roots to the top parts might be restricted.

The change in the P concentration with the decrease of the pH in culture solution was not significant; nevertheless, the P concentration in the leaflets and petioles was higher in the pH 5.7 treatment than that in the pH 4.5 and pH 3.6 treatments. In contrast, the P concentration in the roots was comparatively higher in the pH 4.5 and pH 3.6 treatments than in the pH 5.7 treatment. Furthermore, the P concentration in different plant parts under each pH condition was significantly higher in the petioles, followed by the leaflets and roots, in the pH 5.7 treatment. In contrast, there was no significant difference between the P concentration in the petioles and leaflets or the leaflets and roots, and there was no significant difference between the P concentration in the petioles, leaflets and roots in the pH 3.6 treatment (Table 3.4). This different trend, such as the vertical difference in the P concentration in the roots and top parts, especially between the pH 5.7 and pH 3.6 treatments, suggests that the translocation of P from

the roots to the top parts will be affected and depressed at lower pH, which is a similar tendency to that observed in wheat (Malkanthi et al., 1995).

The N concentration in the leaflets was apparently higher than that in the petioles and roots at all pH treatments (Table 3.4). A tendency toward a higher N concentration in the leaflets than in the other parts, such as the petioles or roots, is generally found in various plant species, such as winged and velvet beans (Anugroho et al., 2010). Moreover, the N concentration in the leaflets tended to be higher at higher leaf positions than at lower leaf positions at all pH treatments (Fig. 3.5). This tendency toward a higher concentration of N in the young than older leaves was in agreement with the report of Purwanto et al. (2002), which suggested that N was retranslocated from the mature leaves to the young leaves of sago palm growing on the peat soils.

In the leaflets and petioles, the N concentration tended to decrease under the lower pH treatments, while this tendency was not observed in the roots. However, the differences of N concentration among the three pH treatments were not significant in all plant parts. Kueh (1995) also reported that the foliar N level of sago palm was unaffected even by the application of N fertilizer. Purwanto et al. (2002) suggested that the native perennial crop may be responsible for the delay in the response of sago palm to fertilizer on the acid peat soil. In the current experiment, the unclear effect of the pH of the culture solution on the N concentration in plant tissues might be related to the absence of a difference with the pH in leaf appearance and shoot elongation in young sago palm seedlings.

The K^+ concentration in the roots and petioles was higher than that in the leaflets at all pH conditions. The effect of the pH on the K^+ concentration in plant tissues was significant only in the petioles. The Ca^{2+} concentration in the top parts, the petioles and leaflets, was higher than that in the roots under the three pH treatments. On the other hand, the Mg^{2+} concentration was higher in the roots and petioles than in the leaflets (Table 3.4). There are several reports concerned with the effect of low pH on nutrient uptake in rice (Thawornwong and Diest, 1974), wheat, barley, chili, cowpea (Malkanthi et al., 1995) and tea plants (Fung and Wong, 2001). In

these plant species, the Ca^{2+} and Mg^{2+} concentrations in the top parts decreased under strong acid conditions (pH lower than 4.0). These reports suggest that the suppressed uptake of Ca^{2+} and Mg^{2+} under a low pH condition is likely to be the result of a high H^{+} concentration. In the current study, however, there was no significant difference in the Ca^{2+} and Mg^{2+} concentrations in the whole plant under the three pH treatments.

3.2 Different parts of roots

Fig. 3.6 shows the Al^{3+}, N, P, K^{+}, Ca^{2+} and Mg^{2+} concentrations in different parts of roots (stele and cortex of adventitious roots and lateral roots) at all pH treatments. At pH 5.7 and 4.5, the Al^{3+} concentration in the lateral roots was higher than that in either the stele or cortex of the adventitious roots and a similar tendency was found at pH 3.6. A higher Al^{3+} concentration in the roots at a lower pH was apparent in the lateral roots. Beside, the Al^{3+} concentration in different root parts were tended to increase under lower pH treatments, which is a similar tendency to that observed in the leaflets and petioles. The decrease in the pH could lead to an increase in the Al^{3+} concentration in the culture solution, which is in agreement with the fact that the increased H^{+} ions attack the structure of the minerals, releasing Al^{3+} ions (Brady and Weil, 2002). However, there was no significant difference in the Al^{3+} concentration among the three pH treatments in plant tissues even in the lateral roots.

Regarding the P concentration in different root parts, the value of the lateral roots and both the cortex and stele of the adventitious roots tended to be higher at a lower pH, although the difference among the three pH treatments was not significant (Fig. 3.6). Generally, the available P in the culture solution is affected by the Al concentration at a lower pH, which may account for the low P absorption by the plant body (Fageria, 1985). The Al^{3+} concentration in plant tissues was significantly higher in the roots than in the petioles and leaflets at pH 4.5 and 3.6 in the current experiment. Even so, the absolute value of the Al^{3+} concentration in the roots was 10 to 12 μmol g^{-1} in the current experiment, which was a low level and might not significantly affect the P concentration.

The Ca^{2+} concentration slightly changed in all root parts but there were no apparent relationship with the effect of pH. The Mg^{2+} concentration almost same level in both the stele of the adventitious roots and the lateral roots among the three pH treatments, while the Mg^{2+} concentration in the cortex of the adventitious roots tended to decrease with the decrease of the pH in the culture solution. The N and K^{+} concentrations slightly changed in all root parts but there was no apparent relationship with the pH levels. However, the differences were not significant in all the nutrient concentrations of the root parts among the three pH treatments (Fig. 3.6).

In general, the structure component of chlorophyll is a central magnesium atom surrounded by a nitrogen-containing structure called a porphyrin ring, which has a long carbon-hydrogen side chain attached, known as a phytol chain (Wu and Rebeiz, 1985). In this experiment, there was no significant difference in either the Mg^{2+} or total N concentration in the leaflets with the pH (Table 3.4), which might be due to the small effect of the low pH or a lack of a significant difference in the chlorophyll production in the leaflets (Fig. 3.4). The chlorophyll production could be maintained for a comparatively long time, which may account for the ability to maintain growth under these adverse conditions.

In conclusion, sago palm seedlings can maintain leaf morphogenesis and nutrient uptake under a wide range of low pH conditions (pH 3.6 to pH 5.7) in culture solution for 4.5 months, which may account for the maintenance of dry matter production. However, a significant difference may occur in the growth rate of sago palm grown under unfavorable conditions, such as acid peat soil versus that under favorable conditions of mineral soil after culture for a longer time. In addition, the current study also demonstrates some tendencies of the nutrient uptake and translocation in plant tissues of sago palm in this experimental study in the laboratory, which were similar to the results of its natural habitat in Chapter 2 (Table 3.5). The finding in Chapter 2 (natural habitat) and Chapter 3 (the experimental study) was confirmed that sago palm has a high adaptability to grow under widely different pH range from 3.6 to 7.0.

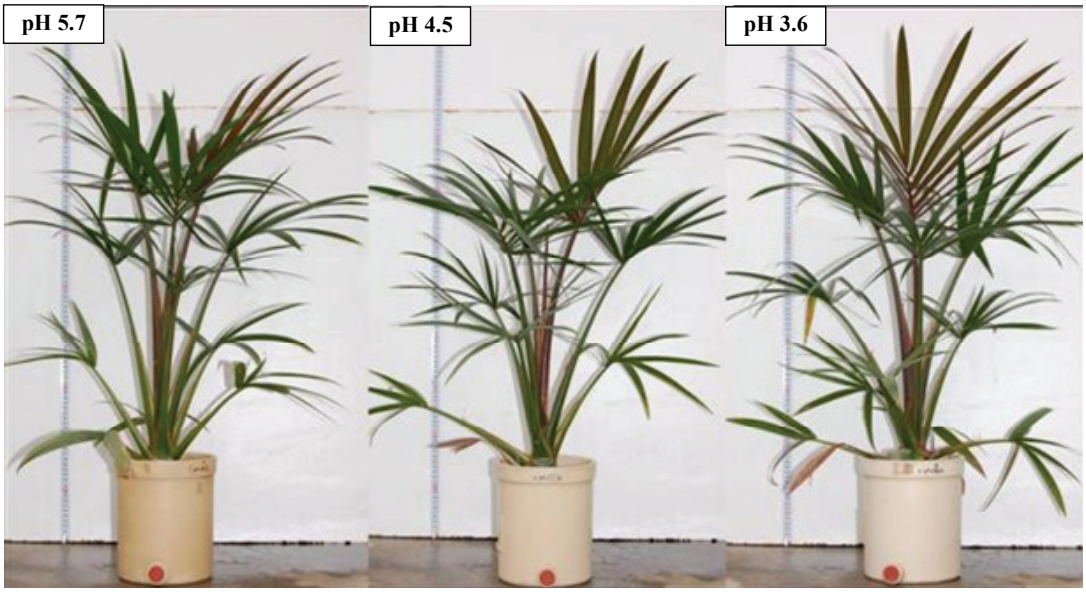


Fig. 3.1 Morphological appearance of sago palm seedlings at 4.5 months under different pH treatments.

Table 3.1 The number of emerged, live and dead leaves under different pH treatments.

pH treatment	Emerged leaves	Live leaves	Dead leaves
pH 5.7	7.3 a	10.0 a	4.0 a
pH 4.5	7.0 a	10.0 a	4.0 a
pH 3.6	7.0 a	10.7 a	3.0 a

Means with the same letters in a given column are not significantly different at the 0.05 level by the Tukey-Kramer test (n=3).

Table 3.2 Effect of low pH on increment of plant length, leaflet area per plant and dry matter weight.

pH treatment	Increment of plant length (cm)	Leaflet area per plant (cm^2)	Dry matter weight per plant (g)			
			Leaflet	Petiole	Root	Whole
pH 5.7	39.7 a	2,400.6 a	18.2 a	23.7 a	8.9 a	50.8 a
pH 4.5	41.1 a	2,457.2 a	19.5 a	22.0 a	10.8 a	52.3 a
pH 3.6	38.2 a	2,418.8 a	17.3 a	20.1 a	9.0 a	46.4 a

Means with the same letters in a given column are not significantly different at the 0.05 level by the Tukey-Kramer test (n=3).

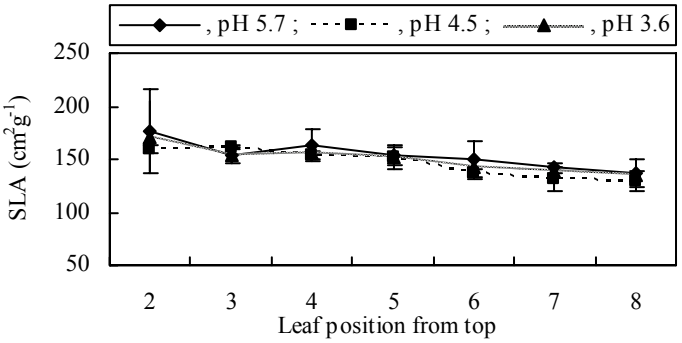


Fig. 3.2 Specific leaf area (SLA) at different leaf positions under different pH treatments. Data are mean values with the standard deviation (n = 3).

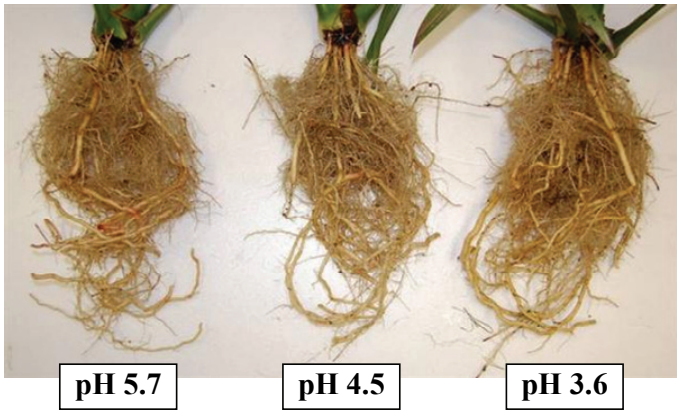


Fig. 3.3 The root morphological appearance of sago palm at 4.5 months under different pH treatments.

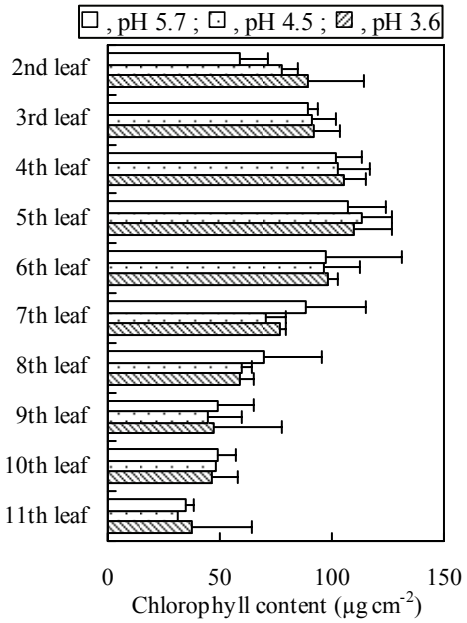


Fig. 3.4 Chlorophyll content per unit leaflet area at different leaf positions. Horizontal lines indicate the standard deviation (n = 3).

Table 3.3 Photosynthetic rate, transpiration rate and stomatal conductance under different pH treatments.

pH treatment	Photosynthetic rate (μmol m^{-2} s^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})
pH 5.7	11.15 a	2.26 a	0.12 a
pH 4.5	10.49 a	2.22 a	0.12 a
pH 3.6	10.22 a	2.21 a	0.11 a

Means with the same letters in a given column are not significantly different at the 0.05 level by the Tukey-Kramer test (n=3).

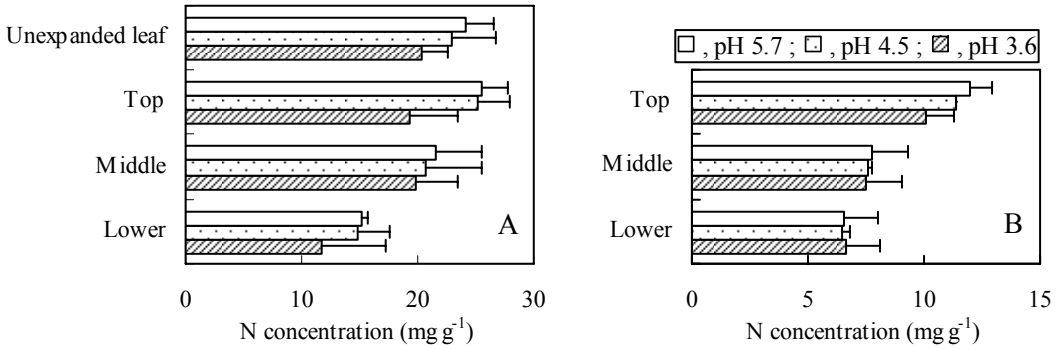


Fig. 3.5 N concentration in the leaflets (A) and petioles (B) at different leaf positions under different pH treatments. Horizontal lines indicate the standard deviation (n = 3).

Table 3.4 Effect of low pH on nutrient concentrations in different plant parts and whole plant.

Plant part	Nutrient concentration		
	pH 5.7	pH 4.5	pH 3.6
	---------- Al^{3+} (µmol g^{-1}) ----------		
Leaflet	7.4 aA	7.7 aB	8.7 aB
Petiole	7.5 aA	8.7 aB	8.9 aB
Root	9.6 aA	10.2 aA	12.1 aA
Whole	7.8 a	8.6 a	9.4 a
	---------- N (mg g^{-1}) ----------		
Leaflet	22.3 aA	22.3 aA	20.9 aA
Petiole	9.6 aB	9.6 aB	8.8 aB
Root	9.7 aB	9.7 aB	10.8 aB
Whole	13.9 a	13.9 a	13.6 a
	---------- P (mg g^{-1}) ----------		
Leaflet	2.1 aB	1.8 aAB	1.8 aA
Petiole	2.6 aA	2.1 aA	2.2 aA
Root	1.1 aC	1.6 aB	1.6 aA
Whole	2.1 a	1.9 a	1.9 a
	---------- K^{+} (µmol g^{-1}) ----------		
Leaflet	97.7 aC	95.9 aB	93.5 aB
Petiole	182.9 bB	225.3 aA	219.6 abA
Root	245.5 aA	230.1 aA	253.4 aA
Whole	156.5 a	177.4 a	178.2 a
	---------- Ca^{2+} (µmol g^{-1}) ----------		
Leaflet	50.4 aB	47.0 aB	42.4 aAB
Petiole	68.8 aA	68.1 aA	55.7 aA
Root	30.1 aC	31.1 aC	28.8 aB
Whole	55.2 a	52.5 a	45.7 a
	---------- Mg^{2+} (µmol g^{-1}) ----------		
Leaflet	43.8 aB	43.2 aB	41.1 aB
Petiole	60.7 aA	63.3 aA	56.7 aAB
Root	64.4 aA	63.0 aA	63.9 aA
Whole	55.2 a	55.6 a	52.1 a

Means with the same letters are not significantly different at the 0.05 level by the Tukey-Kramer test (n=3). Lowercase letter indicates comparison among the pHs in each plant part. Capital letter indicates comparison among plant tissues within each pH treatment.

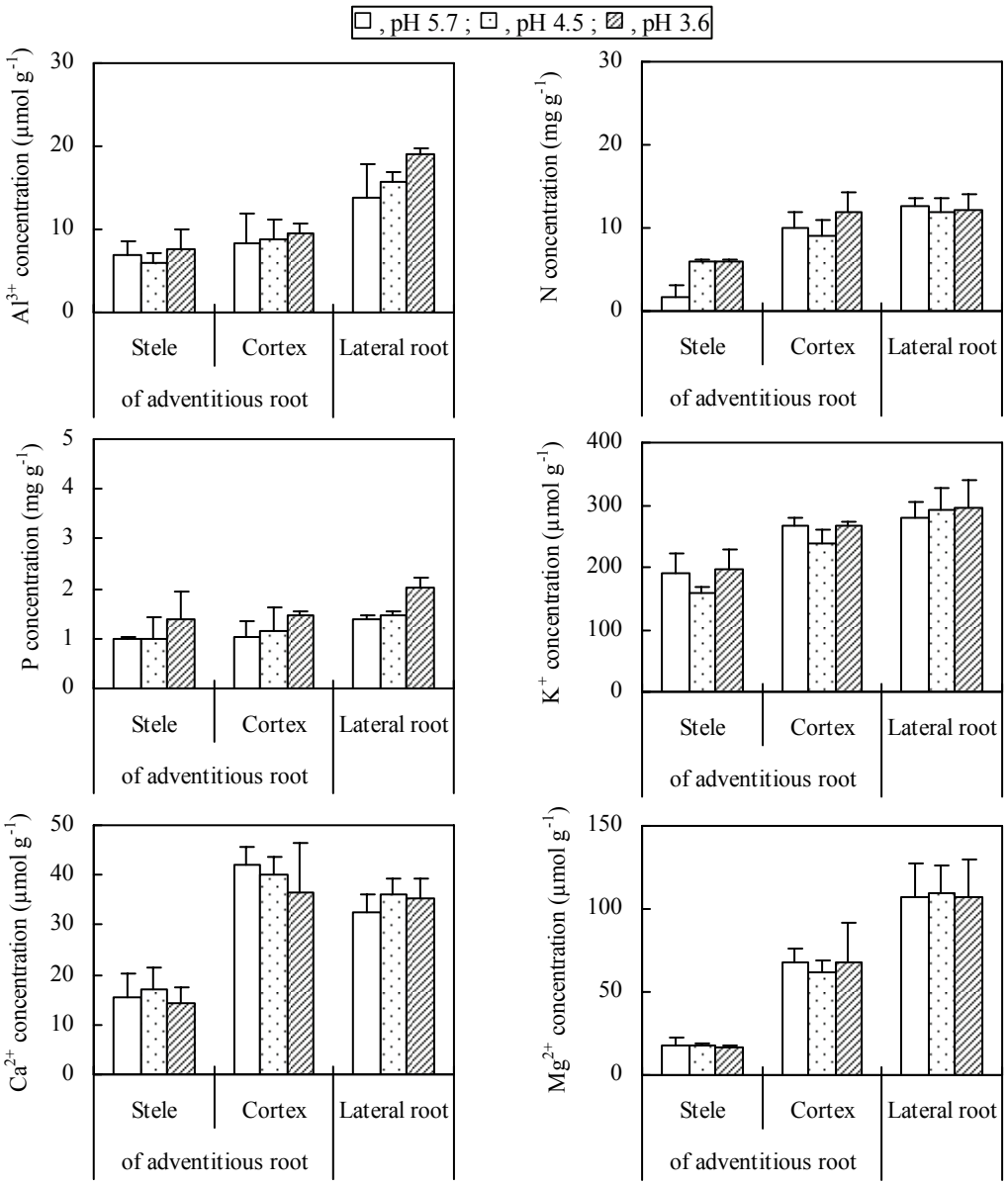


Fig. 3.6 Al^{3+}, N, P, K^{+}, Ca^{2+} and Mg^{2+} concentrations in different parts of roots (stele and cortex of adventitious roots and lateral roots) under different pH treatments. Vertical lines indicate the standard deviation (n = 3).

Table 3.5 The nutrient accumulation of sago palm grows at different levels of pH in comparison between the natural habitat and laboratory level study.

Nutrient	In natural habitat (Chapter 2)	Laboratory level study (Chapter 3)
Al^{3+}	leaflet ≤ petiole << root (Ac[1/])	leaflet ≤ petiole << root (Ac)
SO_4^{2-}	leaflet ≤ petiole <<< root (Ac)	leaflet < petiole <<< root (Ac)
Na^{+}	leaflet < petiole <<< root (Ac)	leaflet << petiole < root (Ac)
Ca^{2+}	root < leaflet < petiole	root < leaflet < petiole
Mg^{2+}	leaflet ≤ petiole < root	leaflet ≤ petiole < root
K^{+}	leaflet << root < petiole	leaflet << root ≤ petiole
P	root < leaflet < petiole	root < leaflet < petiole
N	petiole < root << leaflet	petiole < root << leaflet

[1/] Ac, Cortex of adventitious root

Chapter 4

Effect of aluminum concentration on growth and physiological characteristics of sago palm under low pH condition

Introduction

Aluminum (Al) toxicity is considered to be a serious factor limiting crop production in acid soil where the problem in such area is generally severe when the soil pH drops below 5 (Wright et al., 1989). According to Kochian et al. (2005), Al^{3+}, the toxic trivalent cation species, is the most abundant mononuclear Al species leading to rhizotoxicity in many plant species. The most common symptom of Al toxicity is stunted the root system that the lateral roots are shortening and the root tips often turn brown (Brady and Weil, 2002). The toxic symptom of Al to the root growth inhibition has been found in rice (Kikui et al., 2005), maize (Victor and Zobel, 1998) and wheat (Ohki, 1985). However, one of the most important effects of Al on the plant growth is the inhibition of nutrient uptake (Taylor, 1988). The inhibition of nutrient uptake caused by Al has been reported for several essential elements, including Ca, Mg, K (Baligar and Smedley, 1989) and Cu (Hiatt et al., 1963). The uptake of these elements was affected directly through antagonistic inhibition or precipitation and indirectly through phenomena such as disordering of the membrane functions (Osaki et al., 2003). In addition, the common responses of shoots to Al include: the reduction of stomatal aperture, the decrease in photosynthetic activity leading to chlorosis and necrosis of leaves and the decrease in shoot biomass and leaf number (Thornton et al., 1986). However, there are several reports that some plants species have adapted and grown well under acidic soil conditions, which no appearance symptom of the Al toxicity. It might be that sometime Al could stimulate plant growth or ion uptake (Konishi et al., 1985; Osaki et al., 1997).

Sago palm, as a starch producing plant, is one of the very few crops that can grow under the natural deep peat swamp with minimal drainage. By the cultivation of sago palm, it is possible to convert the vast areas of peat swamps into the productive agricultural lands without

sophisticated and expensive soil amendment, such as the drainage or compaction (Jong and Flach, 1995). However, the deep peat soils in swampy areas are usually characterized by low pH values, a deficiency in mineral elements and a high exchangeable Al (Sato et al., 1979). According to Foy and Fleming (1978), there was a good correlation between Al-resistant plants in nutrient solution and resistance to low pH conditions. In the previous chapter, sago palm has a high adaptability to grow under low pH conditions. It is, therefore, assumed that sago palm is resistant to Al. However, few studies have examined in the Al-induced changes on the growth responses of sago palm. The aim of the present study was to investigate the effect of Al concentration on the growth and nutrient absorption as well as some physiological characteristics of sago palm to elucidate the Al resistance under low pH condition.

Materials and Methods

1. Plant materials and Al treatment

Sago palm fruits were collected in the swampy areas of Rattapum, Songkhla, Thailand, on 1 August 2006. Fertilized and well-developed fruits were selected and treated physically to remove seed coat tissues. The cleaned seeds were placed in a plastic tray filled with tap water and then kept in darkness in a room kept at 30ºC in Thammasat University, Pratumtanee, Thailand, as reported by Ehara et al. (1998). The germinated seeds were brought to Mie, Japan, and transplanted to a 1/5000a Wagner pot filled with vermiculite and Kimura B culture solution containing (μM) 36.5 $(NH_4)_2SO_4$, 9.1 K_2SO_4, 54.7 $MgSO_4$, 18.3 KNO_3, 36.5 Ca $(NO_3)_2$, 18.2 KH_2PO_4 and 3.9 FeO_3 (Baba and Takahashi, 1958). The culture solution was adjusted to an initial pH of 5.5 using 1N HCl before irrigation into pots, as reported by Ehara et al. (2006). The pots were placed in a greenhouse under natural sunlight and maintained at over 15ºC, even at night, at Mie University. Culture solution was added daily in an amount equal to that consumed, and the culture solution was renewed twice weekly.

Seedlings at the 7th leaf stage, with the mean plant length of all plant material at 39 cm, were cultured in Kimura B culture solution without Al (referred to Al-0 hereafter) or containing

different levels of $AlCl_3 \cdot 6H_2O$ corresponding to 10, 20, 100 and 200 ppm Al (referred to Al-10, Al-20, Al-100 and Al-200, respectively, hereafter) at pH 3.6 for 4.5 months, from 23 May to 9 October 2007. Three seedlings were used for each different treatment. The pH of the culture solution was adjusted with 1N HCl as required. The pots were placed in the same greenhouse under natural sunlight. An air pump was connected to the pots to provide air to the roots. Culture solution equal to that consumed was supplemented daily and each solution was renewed every other day during the treatment to avoid accumulation of the excess Al over the assumed concentration. The plant length and leaf number were measured once a week.

2. Photosynthetic rate, transpiration rate and stomatal conductance

On 5 October 2007, 18 weeks after the start of culture, the leaflets of the most active leaves or the 4th leaf position from the top were selected for measuring the net photosynthetic rate, transpiration rate, and stomatal conductance using a potable photosynthetic meter (LCA-4, Analytical development, England) at saturation irradiance with incident photosynthetically active radiation (PAR) of 800 - 1,000 μmol m^{-2} s^{-1}. As a light source, a halogen lamp was used. The appropriate PAR was obtained by changing the distance between the projector and leaves.

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 mmolm^{-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. Chlorophyll content of the leaflets

The chlorophyll content of the leaflets at each leaf position was measured by the method of Mackinney (1941). An area of 0.25 cm^2 from each leaflet 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.

5. Observation of the leaflets under a light microscope

Before sampling, the stomata cells of both the left - and right - half leaflets of the 3rd leaf position from the top of the treated plants were investigated (Fig. 4.1). The middle of the treated leaflet was cut and then the number of stomata and stomata line per 1 mm^2 were observed by a light microscope (Axioplan, Zeiss, Germany). Images were recorded using the software program (Axio Vision Release 4.5, SP1, 2006).

For the observation of vascular bundle, the fresh leaves were cut in the immobilized within folded palafilm tape on the artificial piths, which were freehand cross sectioned (40 - 60 μm in thickness) by the plant microtome (MTH-1, NK system, Nippon medical and chemical instruments, Japan). The plant sections were observed with the light microscope and images were recorded similarly as describe above.

6. Sampling and analysis of nutrient concentrations in plant tissues

The treated plants were sampled and washed thoroughly in distilled water. The plants were separated into three parts: leaflets, petioles (including rachis and leaf sheath) and roots. The fresh weight of each part was recorded. The leaflet areas were measured using an automatic area meter (AAM-9, Hayashi-Denko, Japan). The roots were divided into lateral roots and adventitious roots, and the adventitious root was divided into stele and cortex (epidermis, exodermis, suberized sclerenchyma cell). Adventitious and lateral roots were classified according to the method of Nitta et al. (2002) as follows: adventitious roots were about 6 to 11 mm in diameter, and lateral roots, less than 6 mm in diameter. The separated samples were dried in an oven at 80ºC for 72 hours to measure the dry weight and then ground into powder in order to analyze the ion concentrations. The ground samples were reduced to ash in a furnace

and extracted with 1N HNO_3, and the K^+, Ca^{2+} and Mg^{2+} concentrations were determined using a high-performance liquid chromatograph (HPLC) with a conductivity detector (IC-C3, CDD-6A, Shimadzu, Japan). The concentration of P was evaluated by atomic absorption spectrophotometry. The total N concentration was determined by the semi-micro Kjeldahl method, while the Al^{3+} concentration was determined calorimetrically by the aluminon method.

7. Statistical analysis

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

Results and Discussion

1. Plant growth

Table 4.1 shows the number of emerged leaves, live leaves and dead leaves throughout the Al treatments. The new leaves emerged even at the higher levels of Al concentration in the growth media. However, the number of emerged leaves, 5 leaves at the 200 ppm Al treatment was significantly smaller than that at the other Al treatments (7-8 leaves). The number of live leaves during the experiment was 11, 10, 8, 8 and 6 under the no Al, 10 ppm Al, 20 ppm Al, 100 ppm Al and 200 ppm Al treatments, respectively, which was decreased by the increase of the Al concentration in the growth media. Beside, a significant difference in the number of live leaves was observed in the 200 ppm Al treatment compared with the no Al treatment. Contrarily, the number of dead leaves was significantly increased with the rise of the Al concentration in the media. It thus appears that the delay of new leaf emergence and acceleration of leaf senescence of sago palm were occurred at the higher Al treatments.

The effect of the Al concentration on the increment of length, total leaflet area and dry matter weight, and the morphological appearance of sago palm grown under the Al treatments for 4.5 months are shown in Table 4.2 and Fig. 4.2. The increment of length, total leaflet area

and dry matter weight of all plant parts were no significant differences even at the 100 ppm Al in the culture solution, compared with the no Al treatment. However, all the measurements at the 200 ppm Al treatment were significantly smaller than those at the no Al treatment. According to Zhang et al. (2007), the Al toxicity was estimated as a critical value of the Al concentration for the crop management. The critical values of the Al concentration for the plant grown in the hydroponic system were evaluated in many plant species, such as 1.5 mg l^{-1} Al for oat (*Avena sativa*), 0.8 mg l^{-1} Al for barley (*Hordeum vulgare*) and 8.9 mg l^{-1} Al for maize (*Zea mays*) (Foy and Brown, 1964). In the current experiment, the adventitious and lateral roots of sago palm seedlings at the 200 ppm Al treatment were stunted, brownish and thick (Fig. 4.3) and the root dry weight was 58% smaller than that at the no Al treatment, representing a significant difference. Consequently, the critical value to inhibit the growth of sago palm was considered to be approximately 200 ppm Al in the growth media. According to Jong and Flach (1995), the Al concentration in the peat soil of sago palm cultivation in Sarawak, Malaysia, was in the range from 5 to 14 ppm Al. Beside, from the field study of the sago palm grown at different level of soil pH in South Thailand, the Al concentration in these soils is in the range from 4.5 to 145 ppm Al (Table 2.1, Chapter 2). Considering from the results of the Al concentration in the natural peat soil, the critical Al concentration in the culture solution of the current experiment is much higher than the real concentration under the natural soil conditions.

At the 10 ppm Al treatment, the result showed the highest values in total leaflet area, as well as the total dry weight that was 37% higher than that at the other Al treatments (Table 4.2). Although, it is well known that aluminum toxicity had many effects on the growth of many plant species (Tomioka et al., 2005; Jiang et al., 2009; He et al., 2010), the results of the current experiment showed the stimulating effect of the lower Al concentration on the sago palm growth. Similar tendency toward the preferable growth under the lower Al concentration was also found in the tea plant (Morita et al., 2008), rice and some native plants (Osaki et al., 1997). According to Baker and Walker (1990), the metal resistant plants could demonstrate an increased need for the metals to which they are resistant and as a result to show a less beneficial

effect growth than maximal growth at normal availability levels. In the current experiment, however, there were no significant differences in these growth characteristics between the no Al and 10 ppm Al treatments. In addition, the number of live leaves of the 10 ppm Al-treated plants was smaller than that of the no Al-treated plants, although the total leaflet area was higher in 10 ppm Al treatment than that in the no Al treatment (Table 4.2). These results suggested that the leaf expansion of the 10 ppm Al-treated plants increased even compared with the no Al-treated plants. Moreover, the difference in the live leave numbers was not significant among the 20 ppm Al, 100 ppm Al and 200 ppm Al-treated plants, while the total leaflet area was significantly smaller in 200 ppm Al-treated plants than that in other Al-treated plants. It is likely that the leaf expansion might be affected by the higher Al treatment, which is a similar tendency to that observed in *Pinus sylvestris* L (Janhunen et al., 1995).

The specific leaf area (SLA = leaflet area / leaflet dry weight) of the leaflets at different leaf positions from the top is shown in Fig. 4.4. The difference in SLA was negligible among the Al treatments and the absolute value of SLA was larger at higher leaf positions. It was considered that the area expansion of leaflets depended on the elongation in leaflet length rather than that in leaflet width (from the observation, data not shown). According to Ehara (1993), the increase in SLA with increasing of nutrient concentrations in the culture solution did not depend on the leaf length increase but depended on an increase in the leaf width increase in the relationship between the leaf width and length for the leaf area expansion of rice seedling. From the investigation of SLA divided into dry weight and area of the leaflets, both the leaflet dry weight and leaflet area increased in the 10 ppm Al-treated plants, although the extent of the leaflet area increase was larger than that of the leaflet dry weight increase. However, the extent of the decrease in the leaflet dry weight was larger than that in the leaflet area in case of the 200 Al treatment. It is likely that the increase in SLA through the leaflet area expansion in the 10 ppm Al-treated plants might contribute to the large growth rate (Fig. 4.5).

2. Morphological observation of the leaflets

Table 4.3 shows the number of the vascular bundles in both left- and right-half cross section of leaflet of the 3rd leaf position from the top at difference concentrations of Al (no Al, 10 ppm Al and 200 ppm Al) in the growth media. The number of vascular bundles in both left- and right-half of leaflet of all the Al treatments were increased with the advancement of the leaf age (leaf age: leaf position from the bottom that is leaf emerging order after germination). This result might not be concerned with the effect of the Al treatments but concerned with the position of leaf, which is also suggested by Ehara (1993) that the vascular bundle number in the transverse section of rice leaf blade increased as the position advanced.

The number of the stomata cells, stomata lines and cell numbers between the stomata cells of the leaflet of the 3rd leaf position from the top before and after received effect of the Al treatments are shown in Table 4.4. The numbers of the stomata cells and stomata lines (per 1 mm^2) were larger in the leaflet of the 10 ppm Al-treated plants than the other treated plant in both left and right sides of leaves after the experimental period for 4 months. However, there was no distinctive difference in the cell number between the stomata cells on the same stomata lines and that between stomata lines among the three Al treatments.

3. Physiological characteristics

The chlorophyll content per unit leaflet area at almost all the leaf positions slightly decreased with the rise of Al concentration in the growth media, which the significant difference among the Al treatments was found in some leaves at the higher leaf positions (Fig. 4.6). However, the difference in the mean values of the chlorophyll content per unit leaflet area was not distinct, which tended to be similar to that observed in the result of some crop plants, such as tolerant type of soybean (Shamsi et al., 2007) and *Quercus glauca* Thumb. (Akaya and Takenaka, 2001).

The parameters of the photochemical system were determined by the efficiency of excitation captured by open PSII (Fv'/Fm'), the photochemical quenching coefficient (qP) and the non photochemical quenching coefficient (qN) that are shown in Fig. 4.7. All the measurements (Fv'/Fm', qP and qN) slightly decreased with the increase of Al concentration in

the growth media. However, there were no significant differences in all the measurements among the Al treatments. These results suggest that the photochemical processes of PSII are not inhibited by the Al stress, which was also found in both the Al susceptible and Al tolerant genotypes of wheat (Darko et al., 2002).

Table 4.5 shows the photosynthetic rate, transpiration rate and stomatal conductance of the 4th leaf position from the top, which was considered to be the most active physiologically according to the leaf development and chlorophyll content. The net photosynthetic rate and transpiration rate significantly decreased with the rise of Al concentration in the growth media, which the net photosynthetic rate in 200 ppm Al treatment was 33% smaller than that in the no Al treatment. The difference in the stomatal conductance between the no Al and 10 ppm Al treatments was not significant, but thereafter the stomatal conductance significantly decreased with the rise of Al concentration in the growth media. In the higher Al treatment, the decrease in the net photosynthetic rate was considered to be attributed to a decrease of the stomatal conductance (Table 4.5), the inhibition in photochemical capacity (Fig. 4.7), the reduction of the chlorophyll content (Fig. 4.6) or a combination of these factors. However, in the 10 ppm Al treatment, the limitation of the photochemical system or a decrease of stomatal conductance were not the main causes of the decrease of photosynthetic activity in the leaves but might be due to a small reduction of the chlorophyll content in the leaflets (Fig. 4.6). Nevertheless, the decrease of photosynthetic ability by Al stress is also related to the inactivation of many chloroplast enzymes, such as ribulose 1, 5-bisphosphate carboxylase per oxygenase (Rubisco) and fructose 1, 6-bisphosphate aldolase (FBPase), which may be induced by the oxidative stresses (Bengtsson et al., 1988). Loboda and Wolejko (2006) also found that Al could lower the photosynthetic rate of barley seedling through damage of thylakoid membrane and inhibition of electron transport. Therefore, the effect of Al toxicity to the photosynthetic tissues should be carried out as a further subject.

Fig. 4.8 shows the net photosynthetic rate expressed on a chlorophyll content basis under the different Al treatments. Although, the net photosynthetic rate was significantly decreased

and the chlorophyll content was slightly decreased with the rise of Al concentration in the growth media, the decrease in the net photosynthetic rate expressed on chlorophyll content basis did not show significantly among the no Al and other Al treatments up to the 100 ppm Al treatment, but thereafter significantly decreased. These results indicated that sago palm can maintain the ability of CO_2 fixation in chlorophyll up to the 100 ppm Al treatment, while the chlorophyll production was affected and depressed at the lower Al treatment. In the 200 ppm Al treatment, however, the net photosynthetic rate expressed on a chlorophyll content basis was significant lower than that in the no Al treatment. These results may account for the decrease in the net photosynthetic rate was larger than the decrease in the chlorophyll content. It was considered that the ability of CO_2 fixation in chlorophyll or the chlorophyll efficiency was affected by the 200 ppm Al treatment more than the chlorophyll production.

4. Nutrient concentrations in different plant parts

4.1 Leaflets, petioles, roots and whole plant

The concentrations of Al^{3+}, N, P, K^{+}, Ca^{2+} and Mg^{2+} in the leaflets, petioles, roots and whole plant under the Al treatments are shown in Table 4.6. The Al^{3+} concentration in all plant parts increased with the rise of Al concentration, which the value was obviously different in the root. Moreover, the Al^{3+} concentration in the leaflets was lower than that in the petioles and tended to be significantly higher in the roots than the top parts (leaflets and petiole) in all the Al treatments. The current results in sago palm strongly support the assumption that Al^{3+} has a high binding ability with cellular components of the root and usually shows slight translocation to the upper parts of the plant (Ma et al., 1997). In addition, the Al^{3+} concentration in the petioles tended to be higher at lower leaf positions (old leaves) than at higher leaf positions (new leaves) in all the Al treatments, which means that the Al translocation from the lower leaf positions to the higher leaf positions was restricted (Fig. 4.9).

The total N and P concentrations in the leaflets and petioles under the 10 ppm Al treatment were higher than those in the other Al treatments. In the whole plant, the N concentration

tended to increase under the Al treatments up to the 100 ppm Al treatment, but thereafter decreased under the higher 200 ppm Al treatment, while the P concentration tended to increase under the Al treatments up to the 10 ppm Al treatment, but thereafter decreased with the rise of Al concentration in the growth media (Table 4.6). These results indicated that Al was unlikely to have induced the P and N deficiency in plant tissues but the uptake of these nutrients was higher under a lower Al condition, as such evidence was also found in sorghum (Tan and Keltjens, 1990), rice (Fageria, 1985) and some native plants (Osaki et al., 1997).

The accumulation of N concentration in the leaflets was significantly higher than that in the petioles and roots, which a tendency toward a higher N concentration in the leaflets than in the other parts is generally found in various plant species, such as winged and velvet beans (Anugroho et al., 2010). However, there were no distinctive differences in the N concentration in whole plant among the Al treatments, which these values of all the Al- treated plants were in the range from 12.6 to 14.8 mg g^{-1}. It appears that the Al treatments did not significantly depress the absorption and translocation of N to the leaves in sago palm, even under the 200 ppm Al treatment.

The P concentration was higher in the leaflets and petioles than that in the roots, which the difference in the P accumulation among the plant parts was clearly exposed in the higher Al treatments. Beside, the effect of the higher Al treatment (200 ppm Al) on the P concentration in plant tissues was not observed in the leaflets, in contrast to the tendency in the case of the petioles and roots, which was significantly decreased by the higher Al treatment. This results may attribute to the lower P concentration in the whole plant under the 200 ppm Al treatment (Table 4.6). It seems that sago palm could maintain the accumulation and translocation of P to the leaflets, even under the higher 200 ppm Al treatment, although the P absorption in the petioles and roots was rather restricted.

The K^+ concentration in all plant parts were decreased under the 10 ppm Al treatment, but thereafter tended to increase with the rise of Al concentration in the growth media, which the significant difference between the no Al and 200 ppm Al treatments was found in the petioles

and whole plant (Table 4.6). In addition, the K^+ concentration in the roots and petioles tended to be higher than that in the leaflets in all the Al treatments, which this tendency was also observed in the winged and velvet beans reported by Anugroho et al. (2010).

The Ca^{2+} concentration in different plant parts under each Al treatment tended to be stored in the petioles rather than in the leaflets and roots. In addition, the Ca^{2+} concentration in the leaflets, petioles and whole plant significantly decreased under the higher Al treatment, while a significant difference was unable to observe between the no Al and other Al treatments in that of the roots. The accumulation of the Mg^{2+} concentration was higher in the roots than that in the other parts, such as the leaflets and petioles, and the effect of the Al treatments on the Mg^{2+} concentration in plant tissues was a similar tendency to that observed in the Ca^{2+} concentration in all plant parts (Table 4.6). Considering the current results, the Ca^{2+} and Mg^{2+} concentrations in all plant parts seem to be a subject of caused to decrease by the increase of the Al concentration in the growth media, a significant difference was clearly observed in the 200 ppm Al-treated plants. Nevertheless, the difference of the Ca^{2+} and Mg^{2+} accumulations was not significant among the plant parts when the plants grown in the 200 ppm Al treatment, which was attributed to the decrease of both nutrients in all plant parts under the 200 ppm Al treatment.

One interesting feature of these results is that Al^{3+} inhibited Ca^{2+} and Mg^{2+} absorption more than K^+ absorption in all plant parts under the higher Al treatment. The similar result was also found in barley, which Al^{3+} inhibited divalent cation influxes more than those of monovalent cations (Nichol et al., 1993). According to Huang et al. (1992), the fact that $A1^{3+}$ induced the inhibition of an ion fluxes, particularly Ca^{2+}, may play an important role in the mechanisms of the $A1^{3+}$ toxicity in the higher plants. The possible mechanism to explain the differential effects on cations is that the Al^{3+} toxicity was ameliorated by cations in the following order, H^+ approximately = $C^{3+} > C^{2+} > C^+$, and the amelioration was due to their binding to or screening the negative charges on the plasma membrane (Kinraide *et al*.,1992).

4.2 Different parts of roots

The Al^{3+}, N, P, K^{+}, Ca^{2+} and Mg^{2+} concentrations in the roots that were divided as the stele and cortex of adventitious roots, and lateral roots are shown in Fig. 4.10. The Al^{3+} concentration in all root parts increased with the rise of Al concentration, which the difference in the Al^{3+} accumulation among the Al treatments was significantly exposed in the lateral roots. In addition, the Al^{3+} accumulation was significantly higher in the lateral roots than that in the stele and cortex of the adventitious roots in all the Al treatments, which may attribute to the root expansion of the lateral roots rather than the adventitious roots. In the adventitious roots, the Al^{3+} concentration tended to be lower in the stele than that in the cortex in all the Al treatments. The difference in the Al^{3+} concentration between the stele and cortex of the adventitious roots is clearly exposed in the higher Al treatment. It is likely that the cortex layer that means epidermis until endodermis of the sago palm root in the current experiment has some anatomical function for preventing the excess influx of Al^{3+} ion from the cortex to the stele. This fact was also observed in the roots of sago palm and its related specie for preventing the Na^{+} excess influx under salt stress (Ehara et al., 2008a, 2008b).

The P concentration in each part of the roots tended to increase up to 10 ppm Al treatment and decreased with the increase of Al concentration in the growth media, which is a similar tendency to that observed in the top parts (Fig. 4.10). However, there was no significant difference in the P concentration in any root parts compared with the no Al treatment. According to Foy and Fleming (1978), Al and P in the solution easily precipitate on the root surface or inside the root and a high Al concentration may induce the P deficiency as found precipitates of $AlPO_4$ in the root surface. They also suggested that both the uptake and transport of P from the root to the shoot can be negatively affected by Al, which was also found in rice (Fageria, 1985) and barley (Malkanthi et al., 1995). Moreover, Al plays an important role in the absorption and utilization of P (Konishi et al., 1985). In the current experiment, the increase in P concentration in the roots of the 10 ppm Al treated plants may result in the precipitation with Al. Sago palm that showed growth enhancement by lower Al concentration may contain some physiological mechanisms related with a greater ability to use the precipitated P. The

mechanism to detoxify Al both externally and internally of sago palm roots under the Al stress should be examined more precisely in the further studies.

The N concentration in all root parts decreased at once with the 10 ppm Al treatment and tended to increase with the rise of Al concentration, although there was no significant difference in the N concentration in any root parts compared with the no Al treatment (Fig. 4.10). According to Nichol et al. (1993), Al inhibited the influx of NH_4^+ but enhanced the influx of NO_3^-, which suggested that the results are consistent with a mechanism whereby Al binds to the plasma membrane phospholipids and forms a positively charged layer that influences the ion movement to the binding sites of the transport proteins. A positive charge layer will retard the movement of cations and increase the movement of anions in the proportion to the charge carried by these ions. These effects may inhibit the influx of cations but stimulate the influx of anions. Considering that reasons, sago palm may uptake NO_3^- than NH_4^+ from the culture solution under the Al stress for maintain the N concentration in the whole plant.

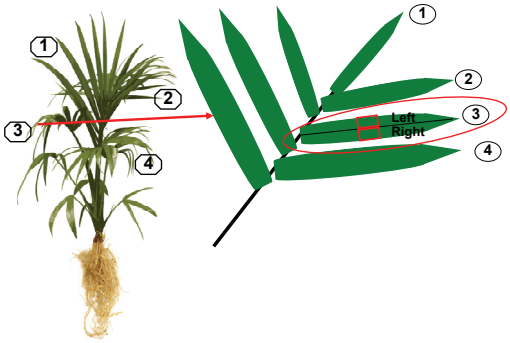
The K^+ concentration in different root parts decreased under the 10 ppm Al treatment, but thereafter tended to increase with the rise of Al concentration in the growth media, which the difference in the K^+ accumulation among the Al treatments was clearly exposed in the stele of the adventitious roots. However, there was no significant difference in the K^+ concentration in any root parts compared with the no Al treatment, which suggests that the K uptake is independent of the increase of the Al concentration in the growth media. In case of the Ca^{2+} and Mg^{2+} concentrations, both ion concentrations in the stele of the adventitious roots did not change apparently, while the values of these ions in the cortex of the adventitious roots and the lateral roots were significantly decreased by the 200 ppm Al treatment (Fig. 4.10). The decrease of the Ca^{2+} and Mg^{2+} concentrations in the root was also found in upland rice (Fageria and Carvalho, 1982) and *Betula pendula* Roth. (Kidd and Proctor, 2000). According to Marschner (1995), Al may inhibit the Ca^{2+} uptake by blocking the Ca^{2+} channels in the plasma membrane and inhibit the uptake of Mg^{2+} by blocking the binding sites of the transport proteins. Akeson and Munns (1989) reported that Al has more than 500-fold greater affinity for the

choline head of phosphatidylcholine, a-lipid constituent of the plasma membrane, than other cations such as Ca^{2+}. For this reason, Al can displace other cations that may form the bridges between the phospholipid head groups of the membrane bilayer and it has long been accepted that Al also directly blocks the ion transport proteins on the plasma membrane of the root cells (Huang et al., 1992). From the observable roots under the Al treatments, the root system under the 200 ppm Al treatment was obviously damaged and apparently differentiated from the other treatments, which may account for the decrease of the Ca^{2+} and Mg^{2+} concentrations in the lateral roots and the cortex of the adventitious roots.

In general, there are two main distinct classes of the Al-resistant mechanism. One class of mechanisms allows the plant to tolerate the Al accumulation in the root and shoot symplasm. The other class operates on the ability to exclude Al from the root apex, which is often related to the Al-triggered exudation of the organic acids (Kikui et al., 2005). According to Chenery (1948), thousands of the plant species are classified, according to their Al concentrations in plant tissues, as Al-accumulators ($\geq$ 1,000 mg Al kg^{-1} dry weight) or Al excluders (< 1,000 mg Al kg^{-1} dry weight). Some plant species known as the Al accumulators may contain more than 10 times of this Al level without any Al injury. However, most plants contain no more than 300 mg Al kg^{-1} dry weight. For example, the tea plants are typical Al accumulators, the Al content in these plants can reach as high as 30,000 mg kg^{-1} dry weight in old leaves (Matsumoto et al., 1976). In the current study, the range of Al^{3+} concentration in the whole plant of sago palm was from 9.4 to 15.6 μmol g^{-1} (254 to 420 mg kg^{-1}) dry weight, even under the 200 ppm Al treatment (Table 4.6). Considering the result of the Al^{3+} concentration in the current experiment, sago palm is considered to have the Al exclusion ability under low pH condition.

In conclusion, the present study revealed that the growth of sago palm was significantly decreased under the 200 ppm Al treatment, which might be associated with the significant decrease of the Ca^{2+} and Mg^{2+} uptake, and the decrease of chlorophyll production through a decrease in the Mg^{2+} accumulation in the leaflets. Consequently, the critical value to inhibit the growth of sago palm was considered to be approximately 200 ppm Al in the growth media.

Nevertheless, the increase of the growth with a mild Al concentration (10 ppm Al) in the growth media under low pH condition might be attributed to the positive effect on the major element (P, N and Ca^{2+}) uptake. Furthermore, sago palm maintains a low Al^{3+} concentration in all plant parts, even under the 200 ppm Al treatment. Therefore, it could be concluded that sago palm has high resistance to Al with mechanically restriction of the excess Al based on the Al exclusion ability under low pH condition.



Al treatment	Start treatment	Received treatment for 4 months
Al - 0	17th leaf	21st leaf
Al - 10	17th leaf	23rd leaf
Al - 200	16th leaf	20th leaf

Fig. 4.1 The 3rd leaf position from the top before and after received treatment for the leaflet observation.

Fig. 4.2 Morphological appearance of sago palm seedlings at 4.5 months under different Al treatments.

Table 4.1 The number of emerged, live and dead leaves under different Al treatments.

Al treatment	Emerged leaves	Live leaves	Dead leaves
Al - 0	7.0 a	10.7 a	3.0 b
Al - 10	7.7 a	10.0 a	3.7 b
Al - 20	7.0 a	8.3 ab	4.7 ab
Al - 100	6.7 a	7.7 ab	5.7 a
Al - 200	5.0 b	6.0 b	6.0 a

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

Table 4.2 Effect of Al concentration on increment of plant length, leaflet area per plant and dry matter weight.

Al treatment	Increment of plant length (cm)	Leaflet area per plant (cm^2)	Dry matter weight per plant (g)			
			Leaflet	Petiole	Root	Whole
Al - 0	38.2 ab	2,418.8 ab	17.3 ab	20.1 ab	9.0 ab	46.4 ab
Al - 10	42.1 a	3,008.6 a	23.0 a	27.1 a	13.6 a	63.7 a
Al - 20	37.5 ab	2,092.0 b	15.7 bc	16.3 b	6.7 b	38.7 bc
Al - 100	37.2 ab	2,151.7 b	15.1 bc	18.0 b	6.6 b	39.7 bc
Al - 200	26.3 b	1,153.4 c	8.0 c	11.2 c	3.8 c	22.9 c

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

Fig. 4.3 The root morphological appearance of sago palm seedlings at 4.5 months under different Al treatments.

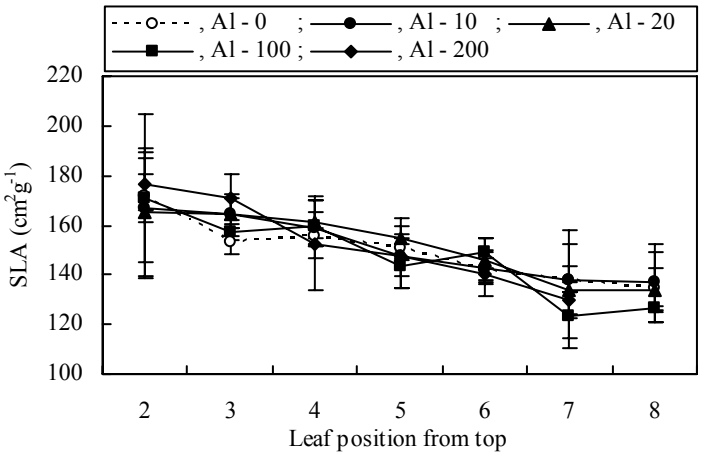


Fig. 4.4 Specific leaf area (SLA) at different leaf positions under different Al treatments. Data are mean values with the standard deviation (n = 3).

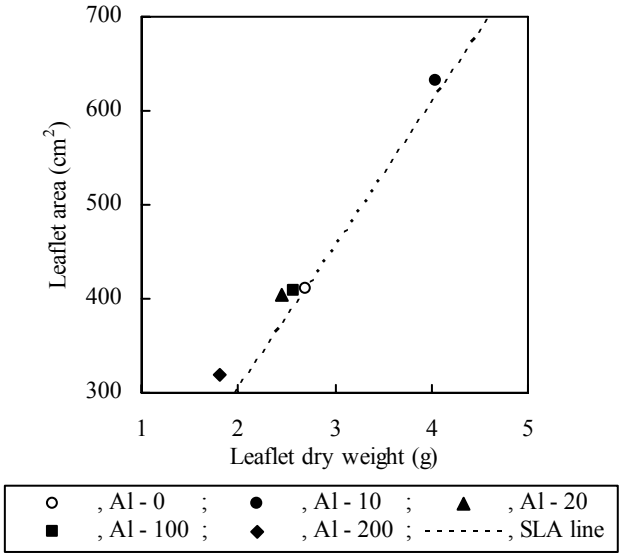


Fig. 4.5 Relationship between dry weight and leaflet area of the 14th leaf position under different Al treatments. The broken line indicates the SLA steady line (same ratio of LA to LDW).

Table 4.3 Number of vascular bundles in leaflet cross sections of the 3rd leaf position from top before and after received effect of the Al treatments.

Al treatment	Number of vascular bundle per 1 mm^2	
	Non effect	Effected
Al - 0	17 L [1)]	21 L [2)]
Left side	3.4 ± 1.1	3.6 ± 0.8
Right side	3.0 ± 0.6	3.5 ± 0.1
Al - 10	17 L [1)]	23 L [2)]
Left side	3.4 ± 0.1	3.8 ± 0.7
Right side	3.4 ± 0.1	3.7 ± 0.5
Al - 200	16 L [1)]	20 L [2)]
Left side	3.4 ± 1.1	4.2 ± 0.6
Right side	3.0 ± 0.7	4.2 ± 1.2

Each value represents the mean ± SD of three sampling sections.

[1),2)] leaf position from the bottom : leaf emerging order after germination

[1)], the 3rd leaf position from the top at the start of experiment

[2)], the 3rd leaf position from the top at the end of experiment

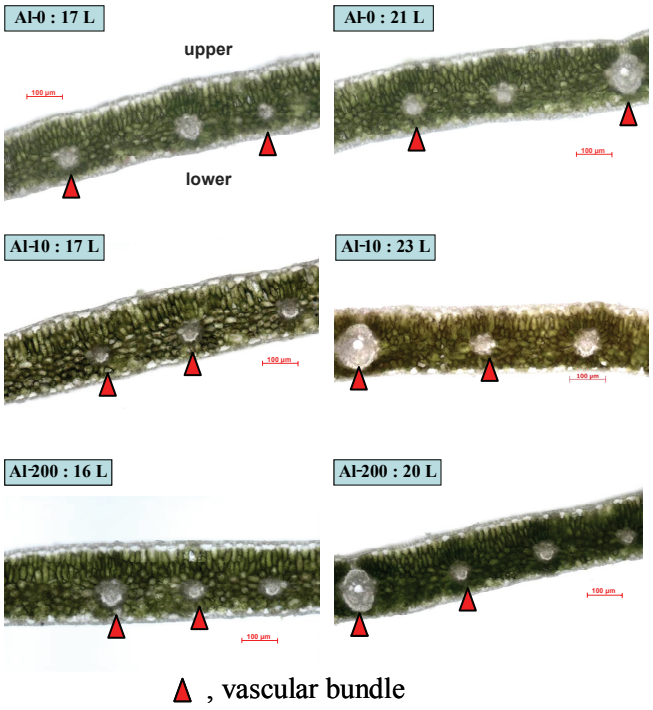
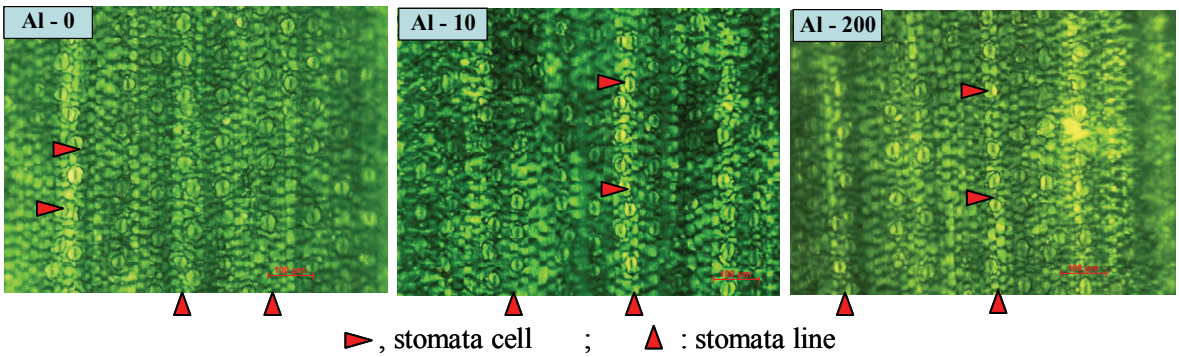


Table 4.4 Number of stomata cells, stomata lines and cell numbers between the stomata cell of the 3rd leaf position from top before and after received effect of the Al treatments.

Al treatment	Number of stomata per 1 mm^2		Number of stomata line per 1 mm^2		Number of cell between stomata in 1 mm^2			
					Horizontal (—)		Vertical (|)	
	Non effect	Effected	Non effect	Effected	Non effect	Effected	Non effect	Effected
Al - 0	17 L [1)]	21 L [2)]	17 L [1)]	21 L [2)]	17 L [1)]	21 L [2)]	17 L [1)]	21 L [2)]
Left side	132.4 ± 5.8	136.1 ± 7.4	28.7 ± 1.6	26.9 ± 1.6	1.5 ± 0.7	1.9 ± 0.7	1.5 ± 0.7	1.5 ± 0.7
Right side	136.7 ± 5.8	138.0 ± 1.6	25.6 ± 1.9	25.0 ± 0.0	2.3 ± 0.9	2.5 ± 0.9	2.3 ± 0.9	2.3 ± 0.9
Al - 10	17 L [1)]	23 L [2)]	17 L [1)]	23 L [2)]	17 L [1)]	23 L [2)]	17 L [1)]	23 L [2)]
Left side	132.2 ± 8.4	191.7 ± 2.8	28.9 ± 3.9	29.6 ± 4.2	2.3 ± 1.2	1.7 ± 0.6	3.0 ± 0.9	2.1 ± 0.9
Right side	140.7 ± 15.3	200.9 ± 11.3	26.9 ± 5.8	31.5 ± 1.6	2.1 ± 0.9	1.6 ± 0.8	3.5 ± 1.0	1.9 ± 0.9
Al - 200	16 L [1)]	20 L [2)]	16 L [1)]	20 L [2)]	16 L [1)]	20 L [2)]	16 L [1)]	20 L [2)]
Left side	132.2 ± 8.4	133.3 ± 2.8	23.7 ± 2.6	27.8 ± 2.8	3.1 ± 1.5	2.7 ± 1.4	3.3 ± 1.0	2.2 ± 0.9
Right side	140.7 ± 15.3	138.9 ± 2.8	26.4 ± 6.4	27.8 ± 2.8	2.6 ± 1.2	2.3 ± 1.9	3.2 ± 0.9	3.0 ± 0.9

Each value represents the mean ± SD three sampling sections. [1),2)] same as Table 4.3.



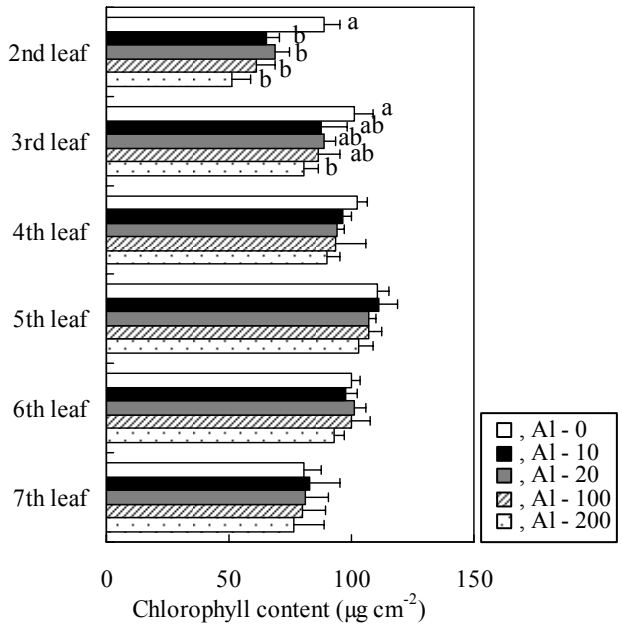


Fig. 4.6 Chlorophyll content per unit leaflet area at different leaf positions under different Al treatments. Horizontal bars represent the standard deviation (n=3). Different letters indicate a significant difference among the Al treatments at the 0.05 level by the Tukey-Kramer test.

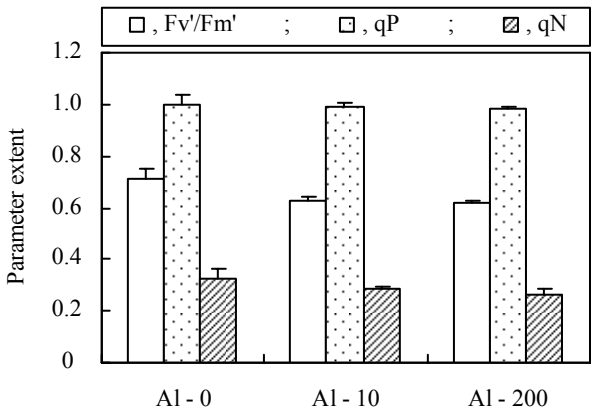


Fig. 4.7 Effect of Al concentration on the efficiency of excitation captured by open PSII (Fv'/Fm'), photochemical quenching (qP) and non-photochemical quenching (qN) of the 4th leaf position from the top. Vertical lines indicate the standard deviation (n = 3).

Table 4.5 Photosynthetic rate, transpiration rate and stomatal conductance under different Al treatments.

Al treatment	Photosynthetic rate (μmol m^{-2} s^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})
Al - 0	10.22 a	2.21 a	0.11 a
Al - 10	9.11 b	1.78 b	0.09 ab
Al - 20	8.93 b	1.68 b	0.07 b
Al - 100	7.93 c	1.55 c	0.06 c
Al - 200	6.86 d	1.11 d	0.03 d

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

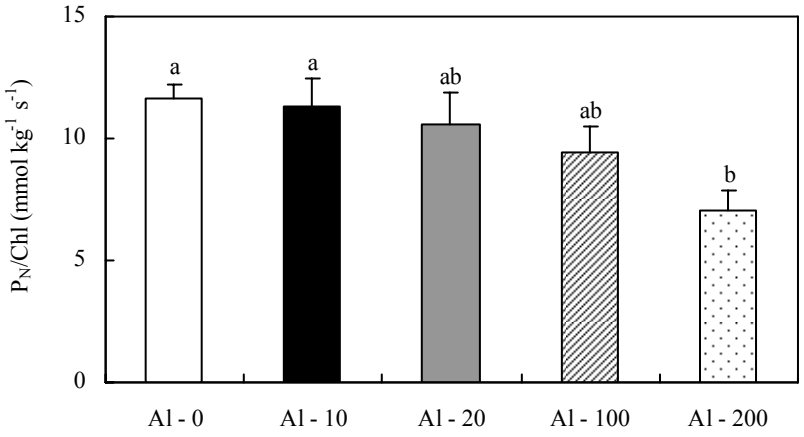


Fig. 4.8 Photosynthetic rate per chlorophyll content (P_N/Chl) of the 4th leaf position from the top under different Al treatments. Vertical bars represent the standard deviation (n=3). Different letters indicate a significant difference among the Al treatments at the 0.05 level by the Tukey-Kramer test.

Table 4.6 Effect of Al concentration on nutrient concentrations in different plant parts and whole plant.

Plant part	Nutrient concentration				
	Al - 0	Al - 10	Al - 20	Al - 100	Al - 200
	---------- Al^{3+} (µmol g^{-1}) ----------				
Leaflet	8.7 cB	9.5 bcB	10.3 bB	10.2 bcB	14.3 aB
Petiole	8.9 bB	10.7 abB	11.6 abB	13.1 abB	15.1 aB
Root	12.1 cA	15.1 bcA	16.3 abA	17.7 abA	19.8 aA
Whole	9.4 b	11.2 b	11.9 ab	12.7 ab	15.6 a
	---------- N (mg g^{-1}) ----------				
Leaflet	20.9 aA	24.6 aA	23.0 aA	22.6 aA	21.0 aA
Petiole	8.8 aB	9.1 aB	8.7 aB	7.7 aB	6.3 aB
Root	10.8 aB	9.9 aB	9.8 aB	10.2 aB	11.3 aAB
Whole	13.6 a	14.8 a	14.6 a	14.1 a	12.6 a
	---------- P (mg g^{-1}) ----------				
Leaflet	1.8 aA	1.9 aA	1.8 aAB	1.7 aAB	1.6 aAB
Petiole	2.2 aA	2.3 aA	2.2 aA	1.9 aA	1.4 bA
Root	1.6 abA	1.8 aA	1.4 abB	1.1 bcB	0.9 cB
Whole	1.9 ab	2.0 a	1.9 ab	1.7 ab	1.4 b
	---------- K^{+} (µmol g^{-1}) ----------				
Leaflet	93.5 aB	92.6 aB	97.8 aB	93.4 aB	98.5 aB
Petiole	219.6 bA	199.5 bA	215.4 bA	220.0 bA	250.7 aA
Root	253.4 aA	209.3 bA	226.7 abA	250.7 aA	267.0 aA
Whole	178.2 b	162.8 b	170.7 b	173.9 b	200.0 a
	---------- Ca^{2+} (µmol g^{-1}) ----------				
Leaflet	42.4 aB	45.3 aB	42.6 aB	32.8 abB	25.5 bA
Petiole	55.7 abA	64.4 aA	65.2 aA	47.7 bA	28.9 cA
Root	28.8 abB	36.1 aB	36.8 aB	30.5 abB	21.7 bA
Whole	45.7 ab	51.4 a	51.3 a	39.2 b	26.7 c
	---------- Mg^{2+} (µmol g^{-1}) ----------				
Leaflet	41.1 aB	40.3 aB	40.9 aB	34.8 abC	29.0 bA
Petiole	56.7 abAB	60.4 aA	63.0 aA	46.6 bB	29.5 cA
Root	63.9 aA	65.1 aA	67.4 aA	66.8 aA	36.2 bA
Whole	52.1 ab	54.1 a	55.1 a	45.5 b	30.5 c

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

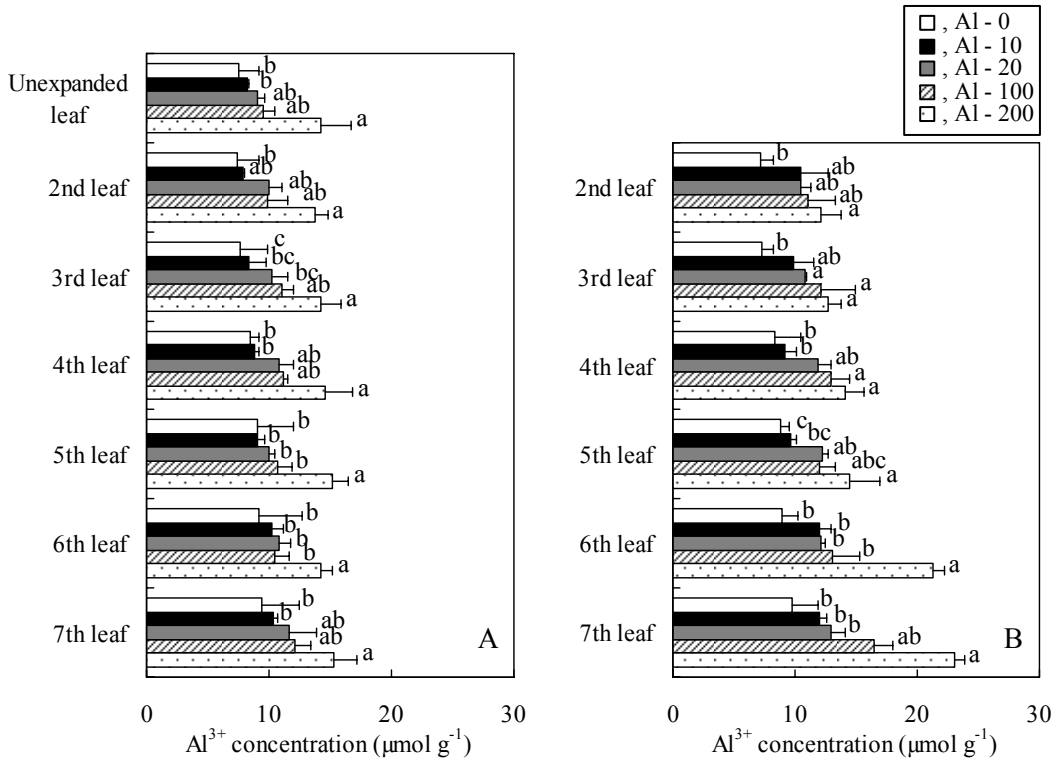


Fig. 4.9 Al^{3+} concentration in the leaflets (A) and petioles (B) at different leaf positions under different Al treatments. Horizontal bars represent the standard deviation (n=3). Different letters indicate a significant difference among the Al treatments at the 0.05 level by the Tukey-Kramer test.

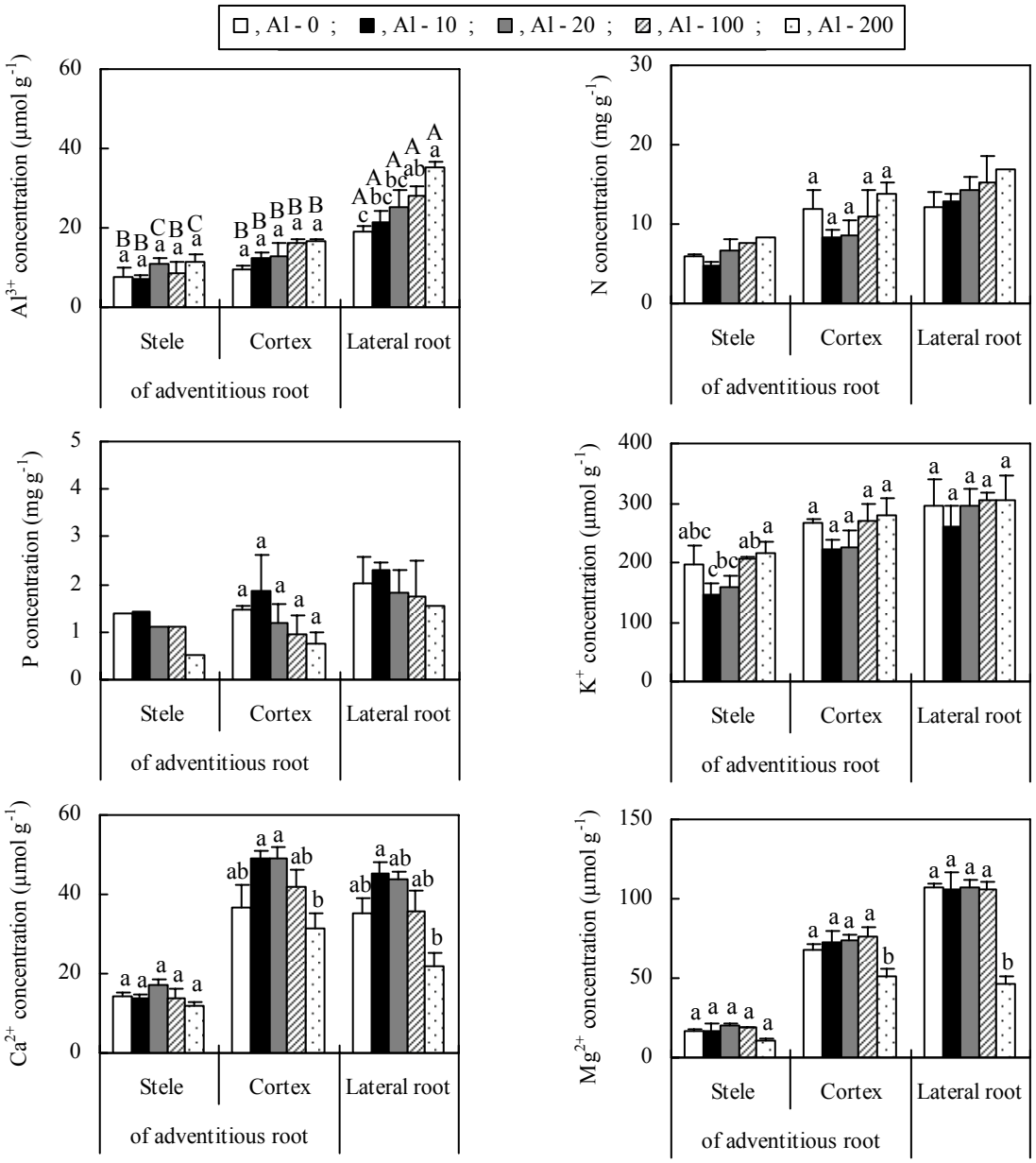


Fig. 4.10 Nutrient concentrations in different parts of roots (stele and cortex of adventitious roots and lateral roots) under different Al treatments. Vertical bars represent the standard deviation (n=3). Different letters indicate a significant difference among the Al treatments at the 0.05 level by the Tukey-Kramer test. Lowercase letter indicates comparison among the Al treatments in each plant part. Capital letter indicates comparison among plant tissues within each Al treatment.

Chapter 5

Comparison of growth and physiological characteristics of sago palm, rattan and yatay palm against aluminum stress under low pH condition

Introduction

The aluminum cation, Al^{3+}, is toxic to many plant species, which can inhibit the plant growth at the micromolar concentrations. The most commonly observable symptom of Al injury is the inhibition of root elongation, which can be recognized within several hours of exposure to Al (Lazof et al., 1994). Since Al is not a plant nutrient, it is not thought to be taken up by an active transport mechanism. Contrarily, it appears to enter the root cells passively by osmosis or with the flow of transpiration water, possibly through damages the root membrane (Brady and Weil, 2002). In general, there are several methods that can be used to assess the localization of Al in root tissues. The screening using hematoxylin staining of seedling roots (hematoxylin staining method) that requires less time and simpler pH management than other methods is very useful for selection or screening a relatively large population in breeding program (Anas and Yoshida, 2000). The hematoxylin staining is a very common technique for the evaluation of Al - resistance in wheat (Polle et al., 1978) and barley (Minella and Sorrells, 1992). Nevertheless, some plant species, known as Al resistant plant, can adapt to grow under acidic conditions with a high Al concentration without showing any signs of toxicity.

Sago palm is one of the dominant species in natural peat swamps, which are poorly drained and high acidity and generally contain a high exchangeable Al. It is, therefore, considered to be Al resistant. In the previous study (Chapter 4), sago palm maintains a low Al^{3+} concentration in all plant parts, even under the 200 ppm Al treatment, which it could be concluded that sago palm has high resistance to Al with mechanical restriction of the excess Al based on the Al exclusion ability under acid condition. However, there have been many different mechanisms proposed for Al resistance in various plants between or within species (Osaki et al, 1997). Therefore, it is important to analyze the physiological features and morphogenesis of sago palm

and related palm species for responding to the Al stress, which may enable to elucidate the Al resistant ability among the palm species.

In the current experiment, the growth, morphological and physiological characteristics, and nutrient concentrations in plant tissues were investigated to evaluate the Al resistant ability of sago palm under low pH condition in comparison with rattan (*Calamus viminalis* Wild.) that belongs to the same tribe Calameae with sago palm, and yatay palm (*Butia yatay* Becc.), the starch producing palm, that distributed in Latin America and belongs to the same family Arecaceae with sago palm.

Materials and Methods

1. Plant materials and Al treatment

Six seedlings of sago palm, rattan and yatay palm were collected in Songkhla Province (South Thailand), Rayong Province (East Thailand) and Mie Prefecture (Central Japan), respectively. The seedlings were transplanted to a 1/5000a Wagner pot filled with vermiculite and Kimura B culture solution containing (μM) 36.5 $(NH_4)_2SO_4$, 9.1 K_2SO_4, 54.7 $MgSO_4$, 18.3 KNO_3, 36.5 Ca $(NO_3)_2$, 18.2 KH_2PO_4 and 3.9 FeO_3 (Baba and Takahashi, 1958). The culture solution was adjusted to an initial pH of 5.5 using 1N HCl before irrigation into pots, as reported by Ehara et al. (2006). The pots were placed in a greenhouse under natural sunlight and maintained at over 15ºC, even at night, at Mie University. Culture solution was added daily in an amount equal to that consumed, and the culture solution was renewed twice weekly.

Seedlings of sago palm (S1 - S6), rattan (R1 - R6) and yatay palm (Y1 - Y6) at the 2nd to 4th leaf stage were cultured in Kimura B culture solution without Al (referred to Al-0 hereafter) or containing different levels of $AlCl_3 \cdot 6H_2O$ corresponding to 10 and 200 ppm Al (referred to Al-10 and Al-200, respectively, hereafter) at pH 3.6 for 3.5 months, from 12 June to 28 September, 2009. Two seedlings were used for each different treatment. The pH of the culture solution was adjusted with 1N HCl as required. The pots were placed in the same greenhouse under natural sunlight. An air pump was connected to the pots to provide air to the roots.

Culture solution equal to that consumed was supplemented daily and each solution was renewed every other day during the treatment to avoid accumulation of the excess Al over the assumed concentration. The plant length, leaf number and measurement of the transpiration rate were conducted once a week.

2. Chlorophyll content of the leaflets

The chlorophyll content of the leaflets at each leaf position was measured by the method of Mackinney (1941). An area of 0.25 cm^2 from each leaflet 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.

3. Sampling and analysis of nutrient concentrations in plant tissues

The treated plants were sampled and washed thoroughly in distilled water. The plants were separated into three parts: leaflets, petioles (including rachis and leaf sheath) and roots. The fresh weight of each part was recorded. The leaflet areas were measured using an automatic area meter (AAM-9, Hayashi-Denko, Japan). The roots were divided into lateral roots and adventitious roots, and the adventitious root was divided into stele and cortex (epidermis, exodermis, suberized sclerenchyma cell). Adventitious and lateral roots were classified according to the method of Nitta et al. (2002) as follows: adventitious roots were about 6 to 11 mm in diameter, and lateral roots, less than 6 mm in diameter. The separated samples were dried in an oven at 80ºC for 72 hours to measure the dry weight and then ground into powder in order to analyze the ion concentrations. The ground samples were reduced to ash in a furnace and extracted with 1N HNO_3, and the K^+, Ca^{2+} and Mg^{2+} concentrations were determined using a high-performance liquid chromatograph (HPLC) with a conductivity detector (IC-C3, CDD-6A, Shimadzu, Japan). The concentration of P was evaluated by atomic absorption spectrophotometry. The total N concentration was determined by the semi-micro Kjeldahl method, while the Al^{3+} concentration was determined calorimetrically by the aluminon method.

4. Observation of plant tissues

4.1 Morphological characteristics of the roots

After the treatments for 3.5 months, the length and diameter of adventitious root, number of lateral roots and average length and diameter of lateral roots from five adventitious roots were measured to elucidate the root morphological characteristics of the three palm species.

4.2 Al localization in plant tissues

For the localization of Al in the plant tissues, the hematoxylin staining method based on the technique of Polle et al. (1978) with some modifications was used. The aqueous hematoxylin solution consisted of 0.2% hematoxylin ($C_{16}H_{14}O_6 \cdot H_2O$) and 0.02% potassium iodate (KIO_3), (w/v) were dissolved in 100 ml of distilled water. Prior to evaluate of staining, the treated samples were washed in distilled water for 15-30 minutes and then placed into the hematoxylin solution for approximately 20 minutes. After staining, the excess hematoxylin was removed with distilled water for 15-20 minutes. The stained samples were photographed under the stereoscopic microscope and cut in the immobilized within folded palafilm tape on the artificial piths, which were freehand cross and transversal sectioned (40 - 60 μm in thickness for leaflet and 60 - 80 μm in thickness for root) by the plant microtome (MTH-1, NK system, Nippon medical and chemical instruments, Japan). The plant sections were observed with the light microscope and images were recorded by using the software program (Axio Vision Release 4.5, SP1, 2006), which used for connecting between the microscope and computer.

4.3 Staining Casparian strip with the berberine - aniline blue fluorescent staining

The fresh treated roots were immobilized within folded palafilm tape on the artificial pith, which was freehand cross sectioned (60 - 80 μm in thickness) by the plant microtome in the various positions from the root tip (Fig. 5.16). The staining procedure was performed according to the method of Brundrett et al. (1988) as followed:

I. Transfer freehand sections into the glass plate and stain sections in 0.1% (w/v) berberine hemi - sulphate in distilled water for 1 hour.

II. Rinse by distilled water for several changes.

III. Transfer the glass plate and stain sections in 0.5% (w/v) aniline blue WS ($C_{32}H_{25}N_3Na_2O_9S_3$) in distilled water for 30 minutes, then rinse as above.

IV. Transfer stained sections into 0.1% (w/v) $FeC1_3$ in 50% (v/v) glycerine (prepared by adding glycerine to filtered aqueous $FeCl_3$). After several minutes in this solution, transfer sections to microscope slides and mount in the same solution.

The sections were observed under the white light and UV light and the images were recorded same as describe in the observation of the Al localization in plant tissues.

Results and Discussion

1. Plant growth

Fig. 5.1 shows the morphological appearance of sago palm, rattan and yatay palm at the end of the Al treatments. New leaf emergence was observed in all the seedlings of sago palm, rattan and yatay palm, even under the higher Al treatment (Fig. 5.2). In sago palm and yatay palm, the newly emerged leaves was tended to be increased by the 10 ppm Al treatment and decreased by the 200 ppm Al treatment. In rattan, 4 or 5 new leaves emerged during the 3.5-month period at all the Al treatments. It is likely that the emergence rate of new leaves of sago palm and yatay palm was affected by the higher Al treatment, while this tendency was not observed in rattan. The total dead leaves of rattan apparently increased by the 200 ppm Al treatment, whereas the number of dead leaves, approximately 1 to 2 leaves, of sago palm and yatay palm was almost the same in all the Al-treated plants. These results suggested that the acceleration of leaf senescence at the higher Al treatment was apparent only in rattan. Considering the current results on leaf emergence and senescence, the growth response via the leave formation of all the palm species to Al stress was different. In the 10 ppm Al treatment, the live leaves or the net leaf product of sago palm and yatay palm increased by 33% and 18%, respectively, compared with the no Al treatment, while the live leaves of rattan were almost the same number in both the no Al and 10 ppm Al-treated plants. Nevertheless, the live leaves of sago palm, rattan and yatay palm decreased 33%, 56% and 36%, respectively, under the 200 ppm Al treatment. It is

likely that sago palm may be comparatively resistant against Al stress to maintain the net leaf product rather than yatay palm and rattan.

The specific leaf area (SLA = total leaflet area / total leaflet dry weight) of sago palm, rattan and yatay palm was investigated for clarifying in the response of leaf morphogenesis to Al concentration in the growth media (Table 5.1). The SLA of the sago palm leaflets was slightly decreased with the rise of the Al treatments, while the SLA of rattan and yatay palm was tended to be lower in the 10 ppm Al-treated plants and higher in the 200 ppm Al-treated plants compared with the control treated plants. Generally, SLA is a measure of leaf thickness, which the lower SLA seems to indicate the thick leave and the higher SLA seems to indicate the thin leave. From these results, it is likely that the leaflets of sago palm were tended to be increased in the thickness of the leaf blade with the rise of Al concentration in the growth media, especially in the 200 ppm Al treatment, which meant that the decrease of the leaflet area was higher than the decrease of the leaflet dry weight under the higher Al treatment. Contrarily, the thickness of leaf blade of rattan and yatay palm was tended to decrease by the higher Al treatment, which this tendency was in agreement with the report of Konarska (2010) suggesting that a reduction in the cell size composing in the leaf tissues are the symptoms observed under Al stress.

The increment of plant length of sago palm, rattan and yatay palm under the Al treatments was shown in Fig. 5.3. The increment of plant length of sago palm in the 10 ppm Al treatment tended to be similarly with that in the no Al treatment, whereas this parameter was decreased about 47% by the 200 ppm Al treatment. In the case of rattan, the incremental length decreased about 24% and 54% under the 10 ppm Al and 200 ppm Al treatments, respectively, compared with the no Al treatment. A similar tendency toward the decrease in the incremental length with the increase of the Al treatments was observed in yatay palm, which was decreased about 18% and 63% by the 10 ppm Al and 200 ppm Al treatments, respectively, compared with the no Al treatment. Base on this result, sago palm was considered to be relatively resistant to the Al stress, compared with the other palm species.

Since the most commonly observable symptom of Al injury is the inhibition of the root growth, the growth and morphological of the roots of the three palm species were also observed (Fig. 5.4 - Fig. 5.7). The observable roots of the three palm species in the 10 ppm Al treatment were quite similar with that in the no Al treatment. Contrarily, the root system in the 200 ppm Al-treated roots of the three palm species was apparently different from the no Al and 10 ppm Al-treated roots, which the adventitious and lateral roots were stunted, brownish and thick (Fig. 5.4). In sago palm, the 10 ppm Al-treated plants showed the higher length of adventitious roots than that in the no Al-treated plants, although the length and number of lateral roots of 10 ppm Al-treated plants were similarly with that of the no Al-treated plants. In the 200 ppm Al treatment, the length of the adventitious roots and lateral roots, and the number of the lateral roots of sago palm were markedly decreased in comparison with the no Al treatment (Fig. 5.5). However, the diameter of both the adventitious and lateral roots was tended to be maintained or increased even under the higher Al treatment, which is similarly to that observed in Japanese red cedar sapling (Hirano et al., 2003). Hirano et al. (2003) suggested that the reduction in root length could be offset by the increase in root diameter for reducing the effects of Al on the root biomass. In case of rattan, the length and diameter of adventitious roots of the 10 ppm Al-treated plants was similar with that of the no Al-treated plants, whereas the number of lateral root was tended to be higher in the 10 ppm Al treatment than the no Al treatment. Nevertheless, when rattan was treated with 200 ppm Al concentration in the growth media, the length was apparently depressed rather than the diameter of all root types. Beside, the lateral root was seemed to be affected by the 200 ppm Al treatment rather than the adventitious root (Fig. 5.6). In yatay palm, the difference in the length and diameter of both the adventurous and lateral roots was small among the three Al treatments, although the number of lateral roots decreased with the increase of Al concentration in the growth media, especially in the 200 ppm Al treatment (Fig. 5.7). These results indicated that there were quite different in the response of root morphology to the Al stress among the three palm species. According to Ma et al. (1997), the root growth seems to be an early indicator for Al resistance than the top growth, which

demonstrated that Al injury appears in the plant roots before any top damage is evident. The decrease in the number and length of the roots with the increase of the Al treatments is in accordance with the observation of *Lotus corniculata* (Blamey et al., 1991). Kollmeier et al. (2000) suggested that the reduction of the cell extension has been found to be the first effect of Al in the root growth inhibition, which involved to the reduction of basipetal auxin diffusion in the apoplast of apical root zones.

The root elongation of sago palm, rattan and yatay palm during the experiment is shown in Fig. 5.8. Sago palm and yatay palm showed the higher root elongation rate in 10 ppm Al treatment rather than that in the no Al treatment, while this tendency was not observed in the result of rattan. From these results, it thus appears that a mind Al concentration in the growth media could lead to a stimulation of the root growth in some palm species, such as sago palm and yatay palm, which is a similar tendency to that observed in tolerant genotypes of corn (Clark, 1977). However, the root elongation of the three palm species was depressed by the 200 ppm Al treatment. Beside, the root elongation of sago palm was extremely decreased rather than those of yatay palm and rattan, while yatay palm was seem to maintain the root elongation rather than the other palm species. In general, many plant species have developed several mechanisms to tolerate the increase of Al levels, for example, high internal tolerance to Al, internal detoxification or exclusion of Al uptake by exudation of organic acids followed by formation of less toxic Al complexes to keep the concentration of toxic Al forms in the plant at a low level, which is ability of plants to modify the pH of the root interface (Jones, 1998). Therefore, further studies on the exudation organic acid released from the root of the three palm species should be carried out.

2. Physiological characteristics

Fig. 5.9 shows the chlorophyll content per unit leaflet area at different leaf positions of sago palm, rattan and yatay palm under different Al treatments. The chlorophyll content per unit leaflet area at almost all the leaf positions slightly decreased with the rise of Al concentration in the growth media. In addition, the mean values of chlorophyll content at different upper,

middle and lower leaf position of sago palm and rattan increased under 10 ppm Al treatment (S-10; 15% and R-10; 2%, respectively) but thereafter decreased under 200 ppm Al treatment (S-200; 32% and R-200; 27%, respectively) compared with the no Al treatment (S-0 and R-0). In yatay palm, the chlorophyll content was decreased 6% and 27% by the 10 ppm Al (Y-10) and 200 ppm Al (Y-200) treatments, respectively, compared with the no Al treatment (Y-0). It is likely that the three palm species could maintain relatively higher the chlorophyll production to cope with the lower Al treatment, which is a similar tendency to that observed in tolerant genotype of soybean (Zhang et al., 2007). However, in the current experiment, a higher Al treatment caused declining the chlorophyll content in the leaflets of the three palm species. According to Marschner (1995), Al toxicity leaded to inhibit many metabolic processes including synthesis of nucleic acids and enzymic activity, for example, the Al can lead to a decrease of chlorophyll content in the leaves that lead in turn to depress the photosynthetic rate. Considering the current results, it was speculated that the reduction in chlorophyll content under the higher Al treatment may affect the photosynthetic capacity, which at least partly resulted for decreasing the growth rate of the three palm species.

Fig. 5.10 shows the transpiration rate of sago palm, rattan and yatay palm at the end of the Al treatments. In sago palm, the transpiration rate was decreased 17 % and 18% by the 10 ppm Al and 200 ppm Al treatments, respectively, compared with the no Al treatment. These results strongly support the previous experiment in Chapter 4, which the transpiration rate of sago palm significant decreased with the rise of Al concentrations in the growth media for 4.5 months. In addition, the transpiration rate of rattan increased 9% by 10 ppm Al treatment but thereafter decreased 22% by 200 ppm Al treatment, compared to the no Al treatment, in contrast to the tendency in the case of yatay palm, which the transpiration rate of the 10 ppm Al-treated plants decreased by 5% and that of the 200 ppm Al-treated plants increased by 5%, compared to the value of the no Al-treated plants. There were some reports for the change of transpiration rate that increase or decrease under the Al stress in many plant species (Schlegel and Godbold, 1991; Zhang et al., 2007; Ali et al., 2011). Ohki (1986) also observed that the transpiration

rates in *Triticum aestivum* decreased, while that in *Sorghum bieolor* increased after exposure to Al. These contradictory results demonstrate that the effect of Al on the transpiration rate is quite complex. From the current study, it is like that the change in the transpiration rate was depended on the Al concentration in the growth media and varies among the three palm species.

3. Nutrient concentrations in different plant parts

Table 5.2 shows the effect of Al concentration on nutrient concentrations in the leaflets, petioles, roots, dead leaves and whole plant of sago palm, rattan and yatay palm. The Al^{3+} concentration in all plant parts of the three palm species tended to increase with the rise of the Al treatments, which the difference in the Al^{3+} accumulation among the Al treatments was clearly exposed in the roots. Beside, the Al^{3+} concentration in all root parts of the three palm species was also tended to increase with the rise of Al concentrations in the growth media (Fig.5.11). The difference in the Al^{3+} accumulation among the root parts was clearly exposed in the 200 ppm Al treatment. For comparison the Al^{3+} concentration in the stele and cortex of adventitious roots between sago palm and rattan (belong to the same tribe) under the higher Al treatment, the Al^{3+} concentration in the stele of sago palm was apparently lower than the value in the cortex, while the difference in the Al^{3+} concentration between the stele and cortex of adventitious roots of rattan was not distinct. It appears that sago palm might have some mechanism to restrict the influx of Al^{3+} from the cortex into the stele rather than rattan.

In addition, the Al^{3+} concentration in the leaflets of sago palm was lower than that in the petioles and tended to be higher in the roots than the top parts, the leaflets and petioles, in all the Al treatments, whereas this tendency was different from that in the other palm species. Yatay palm and rattan showed comparatively higher Al^{3+} concentration in the petioles than in the roots or leaflets. However, the three palm species tended to be accumulated the Al^{3+} concentration in the dead leaves than that in the other plant parts. The highest value of Al^{3+} concentration in whole plant (including leaflets, petioles and roots) under the 200 ppm Al treatment of yatay palm was 33.5 μmol g^{-1} dry weight (903.9 mg kg^{-1}) and was apparently higher than that of sago

palm and rattan, 19.0 and 21.8 μmol g^{-1} dry weight, respectively (512.7 and 580.2 mg kg^{-1} dry weight, respectively).

In many experiments, the highest Al concentration in the culture solution in order to examine the effect of Al on the plant growth has been varied between 4 and 173 ppm Al in the culture solution for upland rice, wheat, snapbean and tea plant that is one of the most Al tolerant crop species (Fageria and Carvalho, 1982; Konishi et al., 1985; Ohki, 1985; Miyasaka et al., 1991). However, a free Al concentration of the Al addition in the culture solution is presumably lost by precipitation or polymerization, which is certainly much lower than that applied. Considering from the Al concentration in the culture solution of those experiments and the growth response of sago palm, rattan and yatay palm in the current study, the three palm species that can grown even under the 200 ppm Al concentration in the growth media could be considered as the Al resistant species. From the report of Chenery (1948) that informed in Chapter 4, the thousands of the plant species are classified by the Al concentration in plant tissues for the Al resistant plants as the Al-accumulators (≥ 1,000 mg Al kg^{-1} dry weight) or the Al excluders (< 1,000 mg Al kg^{-1} dry weight). In addition to the Al concentration in the whole plant of sago palm, rattan and yatay palm, it is likely that the Al resistant ability of sago palm and rattan may be as an the Al-excluder plants that mainly attributes to the avoidance mechanism, which sago palm may have a high Al resistance via exclusion ability more than rattan under acidic condition. Contrarily, the resistant ability in yatay palm may be nearly important as an Al-accumulator plant.

The accumulation of N concentration in the leaflets was higher than that in the petioles and roots of the three palm species, which a tendency toward a higher N concentration in the leaflets than in the other parts is similarly observed in the result of sago palm in Chapter 4. The N concentration in the whole plant of sago palm and yatay palm was decreased with increasing of Al concentration in the growth media, in contrast to the tendency in the case of rattan, which displayed that the N concentration in the whole plant was increased with increasing of the Al treatments. It appears that the change in the N accumulation in plant tissues of sago palm and

rattan was different, although rattan belongs to the same tribe Calameae with sago palm. Nichol et al. (1993) also observed the effect of Al on the different N sources that were ammonium (NH_4^+) and nitrate (NO_3^-). According to them, Al inhibited the influx of NH_4^+ but enhanced the influx of NO_3^-, which suggested that the results are consistent with a mechanism whereby Al binds to the plasma membrane phospholipids, forming a positively charged layer that influences ion movement to the binding sites of the transport proteins. A positive charge layer will retard the movement of cations and increase the movement of anions in proportion to the charge carried by these ions. These effects may inhibit in a differential manner of the influx of cations but stimulate the influx of anions. Therefore, further studies on the measurement of the type of N sources of the three palm species should be carried out.

The P concentration in all plant parts and whole plant of sago palm and rattan tended to decrease under the Al treatments. Although the P concentration of yatay palm under the 200 ppm Al treatment was not observed because of the lack of sample for analysis, it was presumed form the result of 10 ppm Al treated plants that the P concentration in the whole plant might be depressed by the Al concentration in the growth media. According to Bollard (1983), Al toxicity of plant grown under acidic conditions is generally considered to be closely related to depress the P uptake, which also observed in upland rice cultivars (Fageria and Carvalho, 1982) and barley (Loboda and Wolejko, 2006). In comparison between sago palm and rattan that belong to the same tribe Calameae, the P concentration in the 200 ppm Al-treated plant of rattan and sago palm decreased 45% and 80%, respectively, compared with the no Al-treated plants, suggesting that rattan showed more susceptive for the P uptake than sago palm. In addition, the Al concentration in the growth media caused declining the P concentration in the whole plant of sago palm in the current study, although this result was not similarly with the result of sago palm in Chapter 4 that the P concentration tended to increase under the Al treatments up to the 10 ppm Al treatment, but thereafter decreased with the rise of Al concentration in the growth media. One interesting feature of these results is that sago palm in Chapter 4 was older than that in the current experiment. This result suggested that the P accumulation of the younger sago

palm was affected by the Al stress rather than the older one, which was in agreement with the report of Meriga et al. (2010), demonstrating that younger seedlings of rice cultivars were found to be relatively more sensitive than older seedlings under Al stress. According to Rengel and Robinson (1989), the amounts of Al in the roots were larger in the younger Al-treated plants than that in the older Al-treated plants. This finding corresponded well with the higher sensitivity to Al stress in the younger stages plants than in the older stages plant.

The K^+ concentration in all plant parts and whole plant of sago palm was decreased under the 10 ppm Al treatment, but thereafter tended to increase under the 200 ppm Al treatment. This result supports our previous finding in Chapter 4, which suggests that the K^+ uptake is independent of the increase of Al concentration in the growth media. Contrarily, the K^+ concentration in all plant parts and whole plant of rattan and yatay palm tended to decrease with the increase of Al concentration in the growth media. Beside, the K^+ concentration in the whole plant of rattan and yatay palm decreased by 32% and 82%, respectively, under the 200 ppm Al treatment compared with the no Al treatment. This tendency toward the decrease of K uptake by the Al stress was in agreement with the results in rice (Fageria, 1985) and oil palm (Cristancho et al., 2011). Wagatsuma et al. (1987) also reported that the K content in the roots decreased with the increase of Al content, which was attributed to the leakage of K from the roots as a result of the destruction of the plasmalemma by the Al toxicity.

The Ca^{2+} and Mg^{2+} concentrations in different plant parts of sago palm and rattan that belonged to the same tribe under the Al treatment was higher in the petioles, followed by the leaflet and root, while those of yatay palm was higher in the leaflets than in the other plant parts. In addition, the Ca^{2+} and Mg^{2+} concentrations in all plant parts and whole plant of the three palm species decreased with the increase of the Al concentration in the growth media, which was clearly exposed in the 200 ppm Al-treated plants. According to Wagatsuma et al. (1987), a reduction of the Ca and Mg uptake can be explained by the fact that Al occupied the absorption sites in the free space of root. Beside, the Ca^{2+} concentration in whole plant of the 200 ppm Al-treated plants of sago palm, rattan and yatay palm were lower by about 56%, 60% and 37% of

those of the no Al-treated plants, respectively. The Mg^{2+} concentration in whole plant of the 200 ppm Al-treated plants of sago palm, rattan and yatay palm decreased about 51%, 77% and 59% compared with the no Al-treated plants. Base on these results, it appears that the accumulation of Ca^{2+} and Mg^{2+} in whole plant of rattan was apparently depressed by the higher Al treatment rather than sago palm and yatay palm. Nevertheless, the Ca^{2+} and Mg^{2+} concentrations in all plant parts of the three palm species seem to be a subject of caused to decrease by the increase of the Al concentration in the growth media, which was in agreement with the results in Chapter 4.

In addition to the result of chlorophyll content that the important structural components are N and Mg as describe in Chapter 3, it was considered that the decrease in the chlorophyll content of sago palm and yatay palm might be related with the decrease of Mg^{2+} and N concentrations in the leaflets under the Al treatments. Beside, the decrease of the chlorophyll content of rattan might be due to the reduction of Mg^{2+} concentration in the leaflets, although the N concentration in the leaflet was increase with the rise of the Al treatments. It is likely that the N formation in the leaflets of rattan was not informed as the N component in the structure of chlorophyll. Therefore, the measurement of the N type in the leaflet of rattan should be carried out.

4. Al localization in plant tissues and Casparian strip in plant roots of sago palm, rattan and yatay palm

Fig. 5.12 shows the 40 -50 μm cross sections of the leaflets of sago palm, rattan and yatay palm under the Al treatments that were colored using hematoxylin to identify the leaf tissues where Al occurred or is deposited. The leaflets of the three palm species were selected from the second green leaf from the lowest position, which exposed to contain the highest Al^{3+} concentration. In the no Al and 10 ppm Al treatments, no Al was detected in the leaflets of the three palm species, in contrast to the 200 ppm treatment, the purple color formed by the compound of an aluminum haematein complex was found in the leaflets of the three palm species. In the case of sago palm, it seems that Al was accumulated preferentially in the upper

epidermis and occasionally in the lower epidermis. In general, the stomata are more numerous over the lower epidermis than the upper epidermis to prevent excess water loss by direct contact with the sun where the plant is most likely to lose water. Schnable and Zeiger (1975) found that a concentration of 1mM Al^{3+} in the culture solution inhibited the stomata opening in the illuminated epidermal strips of *Vicia faba*, by preventing the K^{+} accumulation and starch mobilization in the guard cells. Base on the current result, it is likely that sago palm may possess an effective mechanism to prevent the internal Al toxicity in the important tissues by transferring Al^{3+} to mainly accumulate in the upper epidermis, which was generally transparent to allow light to reach the mesophyll tissues for photosynthesis and lacked of the chloroplast. In the leaflets of rattan under the 200 ppm treatment, Al was detected in the bundle sheath cell that was the thick walled cell surrounding the vascular bundle. Beside, Al was also found to be located in the upper and lower epidermis of the rattan leaflets, which the higher Al accumulation in the upper epidermis was observed than in the lower epidermis. In the case of yatay palm, an aluminum haematein complex that located the Al^{3+} accumulation was observed in the inner of vascular bundle including the xylem and phloem, and was similarly observed in the upper and lower of epidermal layer of the leaflets. There are several reports in the Al accumulation in the leaves, which generally located in the epidermal cells, spongy parenchyma and vascular bundle in many plant species (Matsumoto et al., 1976; Haridasan et al., 1986). In most cases, there was the evidence that showed the Al localization in the upper and lower epidermis. Matsumoto et al. (1976) suggested that Al is absorbed from the soil via the roots and then passes into the vessels of the xylem and moves upward to the leaves. At the end of the vein, Al diffuses into the neighboring mesophyll cells, such as the palisade and spongy parenchyma, and passes from one cell to the other by diffusion and then reaches into the epidermal cells, which Al might be accumulated in these cell walls where Al is probably deposited and not very active metabolically.

The Al localization in the roots of sago palm, rattan and yatay palm under the Al treatments are shown in Fig. 5.13 - Fig. 5.15. In the three palm species, the result showed that the 10 ppm

Al-treated roots were stained weakly at the root cap, especially in the damaged zone. However, the 200 ppm Al-treated roots of the three palm species were markedly stained at the root cap, epidermis and outer hypodermis. Beside, Al was greatly accumulated in the root apical region, which a similar tendency was observed in the root of eddo (Kawasaki et al., 2008). Nevertheless, the degree and characteristic of the Al localization in the roots varied widely depending on the palm species. In the observation of 200 ppm Al-treated roots of sago palm, Al reached merely the epidermal tissue layer but not in the hypodermis or cortex, which would indicate that the radial transport of Al was restricted by the epidermal tissue layer (Fig. 5.13). In the 200 ppm Al-treated roots of rattan, Al was detected in the epidermal and 1-2 hypodermal tissue layers (Fig. 5.14). In addition to the similar tribe under the 200 ppm Al treatment, it is likely that the rattan root showed a higher Al accumulation than those of sago palm, which was in agreement with the result of the Al concentration in the roots of both palm species. In the 200 ppm Al-treated roots of yatay palm, a stronger purple stain was evident in the epidermal layer where quite damaged; following by the outer hypodermal tissue layer (Fig. 5.15).

The structure and component of adventitious roots of sago palm, rattan and yatay palm that observe around the external part of the root cortex under UV microscope in the various positions from the root tip are shown in Fig 5.17 - Fig. 5.19. When the root cross sections were stained by the berberine - aniline blue fluorescent staining, an intense bright blue fluorescence that indicated the suberin lamellae in the cell wall was observed between the epidermis and hypodermis of the roots of the three palm species. Beside, the fluorescence was observed in the thickened sclerenchyma cell wall but not in the crowed cortical cells, especially in the observation at the longest distance from the root tip. One interesting feature of these results is that the intense fluorescence was exhibited increasingly by the increase of the Al concentration in the growth media and the distance from the root tip. These observations suggested that the suberin lamellae in the cell wall between the epidermis and hypodermis might be the first barrier to restrict the radial movement of Al in the roots of the three palm species under acid

condition, which these results were supported by the finding in the localization of Al in the roots of the three palm species.

In addition, the Casparian strips of the three palm species were observed in the endodermis of the roots under the Al treatments (Fig. 5.20 - Fig. 5.22). In sago palm, the distances between the lowermost position of the Casparian strip and root tip under the no Al, 10 ppm Al and 200 ppm Al treatments were calculated as the percent of the root length that were 13%, 4% and 2.5%, respectively (Fig. 5.20). In rattan, the distances between the lowermost position of the Casparian strip and root tip were 6% (no Al treatment), 0.5% (10 ppm Al treatment) and 0.4% (200 ppm Al treatment) of the root length (Fig. 5.21). In yatay palm, the lowermost position of the Casparian strip from the root tip was detected at 9%, 1% and less than 1% of the root length under the no Al, 10 ppm Al and 200 ppm Al treatments, respectively (Fig. 5.22). Nevertheless, the Casparian strip in the 200 ppm Al-treated roots of the three palm species changed gradually to the suberin lamellae (the second state of endodermal development) and the thick U-shaped or O-shaped thickening in the cell walls of endodermis (the third state of endodermal development) at the upper part of roots (5 - 25 mm length from the root tip). It is likely that the distances between the lowermost position of the Casparian strip and root tip was shorten by the Al treatments, which might attribute to the inhibition of root elongation under the Al treatments (Fig.5.8). A similar tendency toward the decrease in the distance between the lowermost position of the Casparian strip and the root tip was in agreement with the observation in eddo roots (Kawasaki et al., 2008). In addition, one interesting feature of these results is that the difference of the Al^{3+} concentration between the stele and cortex of adventurous roots of sago palm was exhibited rather than that of rattan (Fig. 5.11). According to Prathumyot and Ehara (2010), the development of the Casparian strip located in the endodermal cell wall of the adventitious root of sago palm was considered as an important mechanical factor relating to the avoidance mechanism for preventing the excess influx of ions, such as Na^{+}, through an apoplastic partway into the stele and its translocation from root to shoot in sago palm. Although the Casparian strip was observed in the endodermal layers of the roots of both sago palm and

rattan that belonged to the same tribe Calameae with sago palm, the present results presumed that the efficiency of the Casparian strip to prevent the apoplastic passage of toxic ions, such as Al^{3+}, from the cortex to the stele of sago palm was higher than that of rattan.

In conclusion, the new leaf emergence of sago palm and yatay palm was affected by the higher level of Al concentration, while the acceleration of leaf senescence at the higher Al treatment was apparent only for rattan. In addition, the three palm species could maintain the chlorophyll production to cope with the lower Al treatment, while the chlorophyll content in the leaflets decreased under the higher Al treatment, which might account for declining the photosynthetic capacity that at least partly resulted in a correspondingly decreased growth rate of the three palm species. Beside, the change in the transpiration rate depended on the Al concentration in the growth media and also varied among the three palm species. The macronutrients in the whole plant of the three palm species tended to decrease under the higher Al treatments, especially P, Ca^{2+} and Mg^{2+}, which is generally found in various plant species. However, some macronutrients, such as N and K^{+} were decreased or increased differently depending on the palm species and Al concentration in the growth media. In addition to the Al concentration in the whole plant of sago palm, rattan and yatay palm, it is likely that the Al resistant ability of yatay palm may be nearly important as an Al-accumulator plant, whereas the Al resistant ability of sago palm and rattan (belongs to the same tribe Calameae with sago palm) were considered as an the Al-excluder plant that mainly attributes to the avoidance mechanism, which sago palm has a high Al resistance via exclusion ability more than rattan under acidic condition. Nevertheless, based on the growth response of the three palm species under the higher Al treatment, it is likely that sago palm was considered to be comparatively resistant against Al stress, which can maintain the net leaf product and increment of plant length rather than yatay palm and rattan.

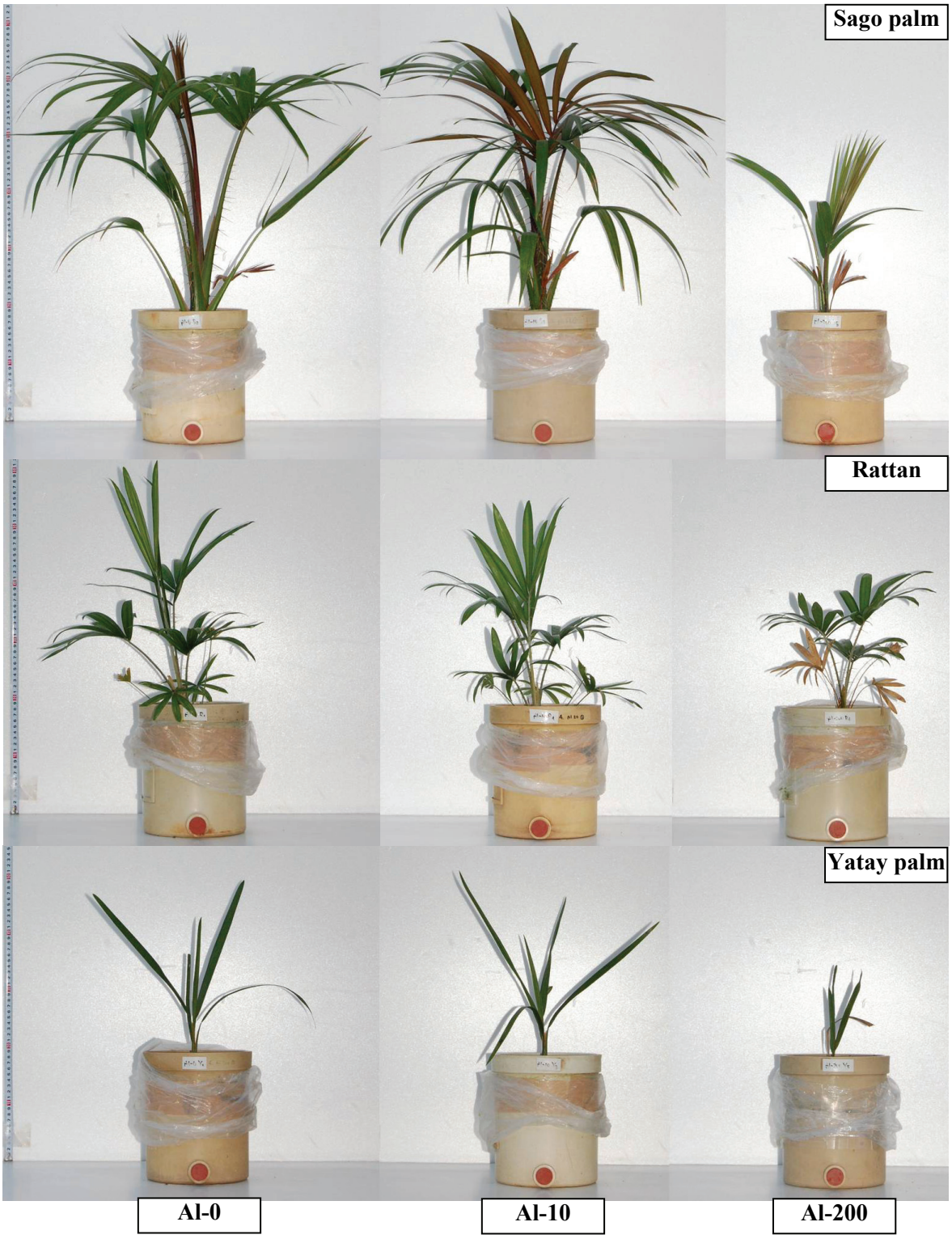


Fig. 5.1 The morphological appearance of the seedlings of sago palm, rattan and yatay palm at 3.5 months under different Al treatments.

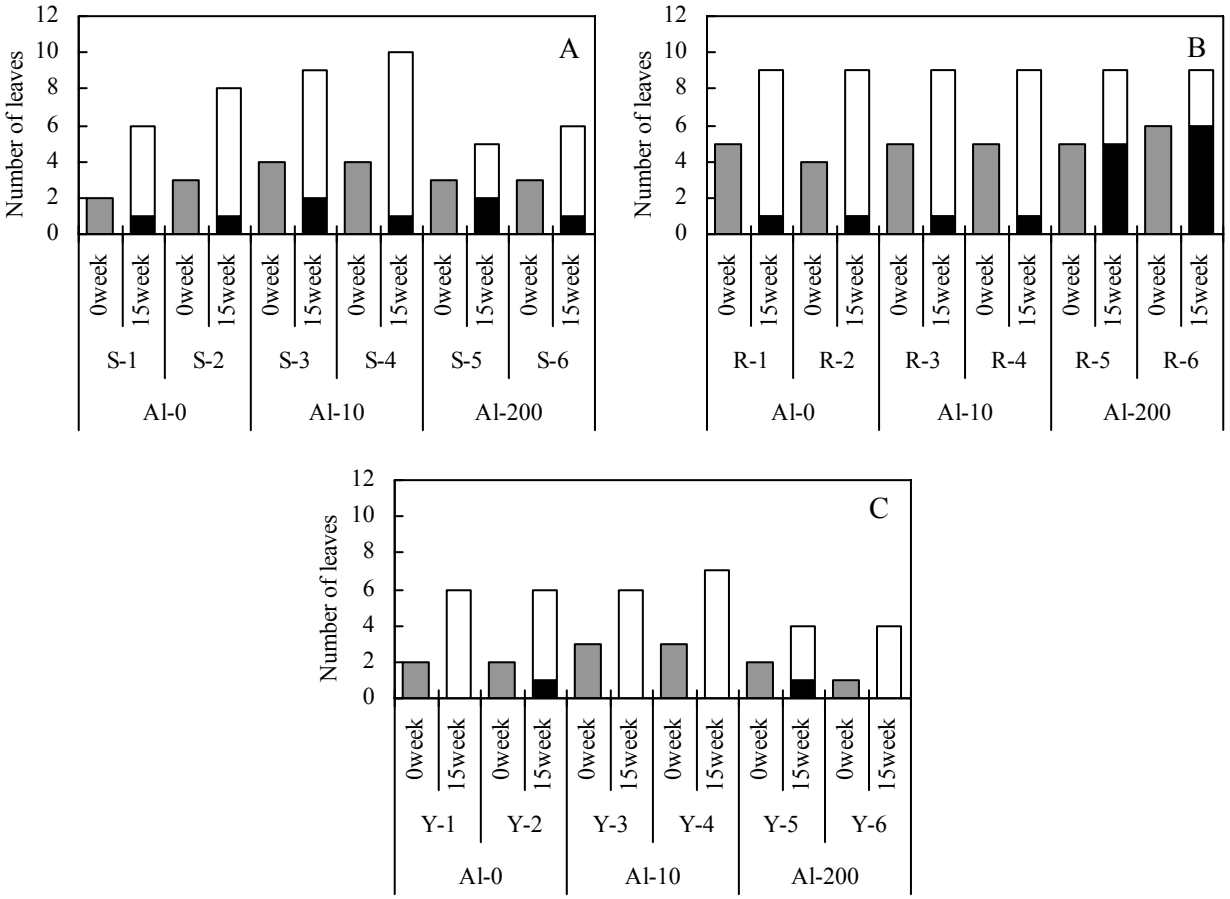


Fig. 5.2 Number of leaves during the experiment of sago palm (A), rattan (B) and yatay palm (C). ▩, Leaves existed at the start of the experiment; □, Live leaves during the experiment; ■, Leaves dead during the experiment.

Table 5.1 Specific leaf area (SLA) of leaflets of sago palm, rattan and yatay palm under different Al treatments

Palm type	Specific leaf area (SLA) of the leaflet ($cm^2\ g^{-1}$)		
	Al-0	Al-10	Al-200
Sago palm	168.9	160.4	176.2
Rattan	170.9	155.3	175.8
Yatay palm	114.0	83.7	124.8

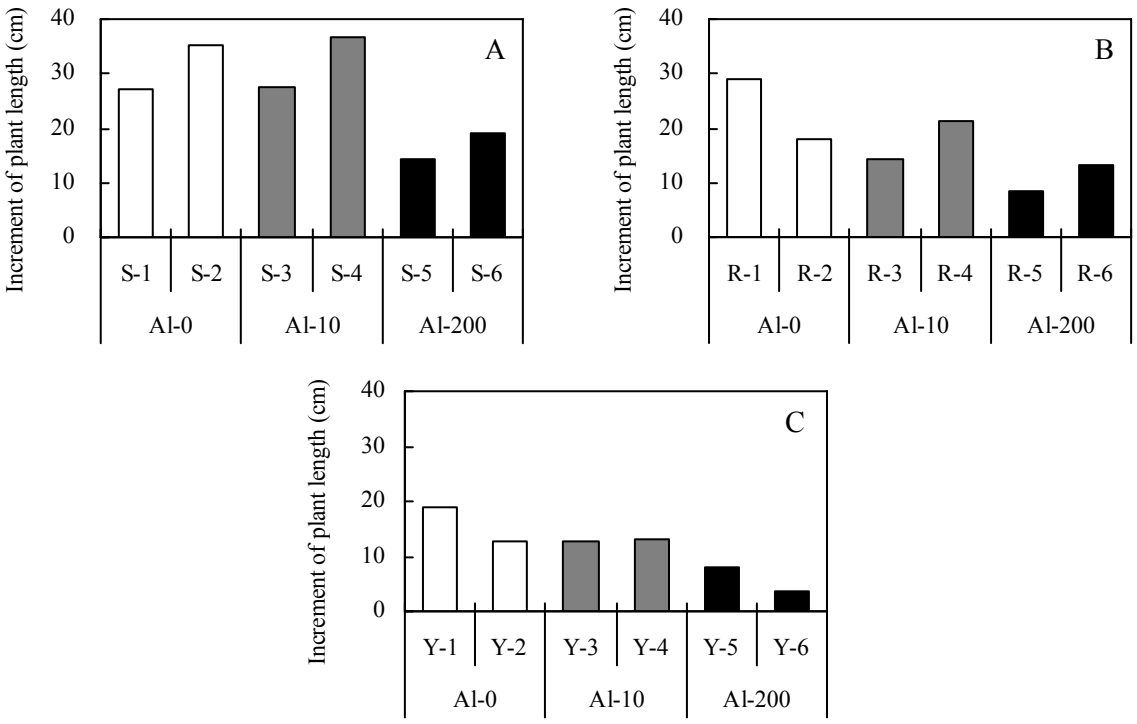


Fig. 5.3 Increment of plant length of sago palm (A), rattan (B) and yatay palm (C) under different Al treatments.

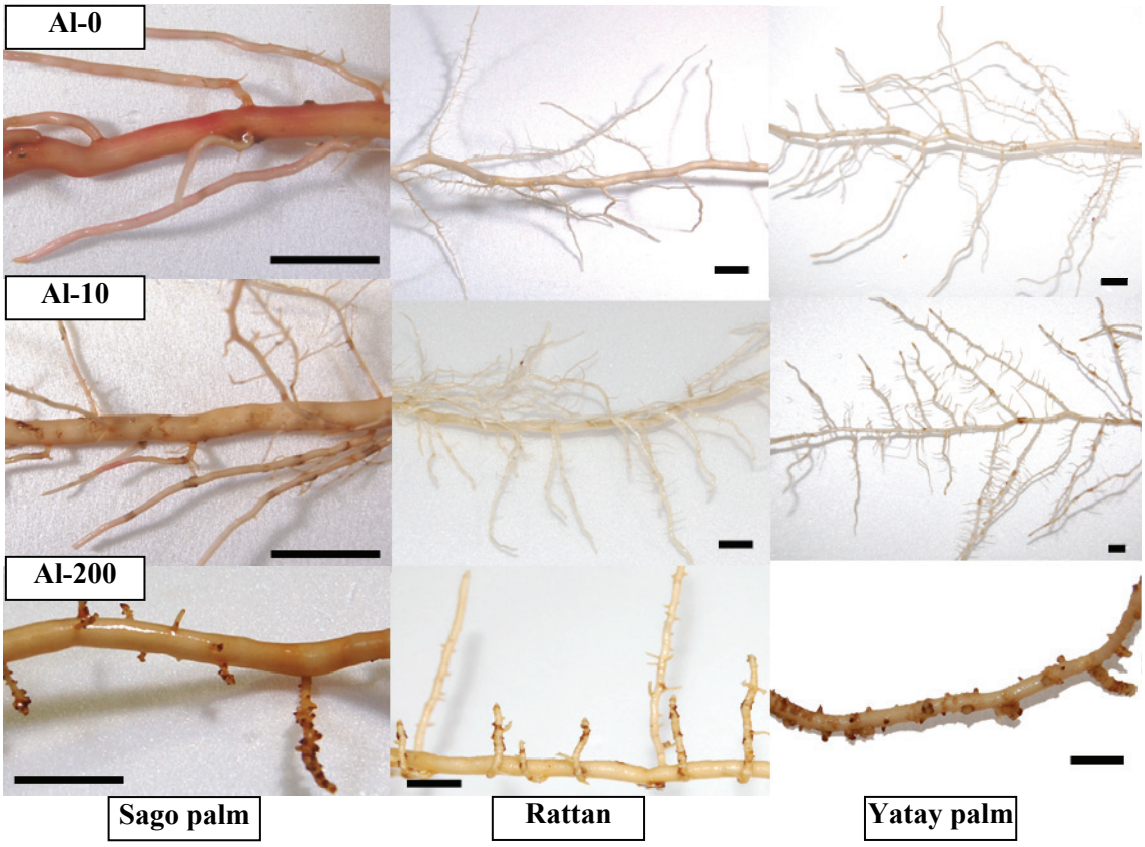


Fig. 5.4 The root morphological appearance of the seedlings of sago palm, rattan and yatay palm at 3.5 months under different Al treatments. Bars = 1 cm.

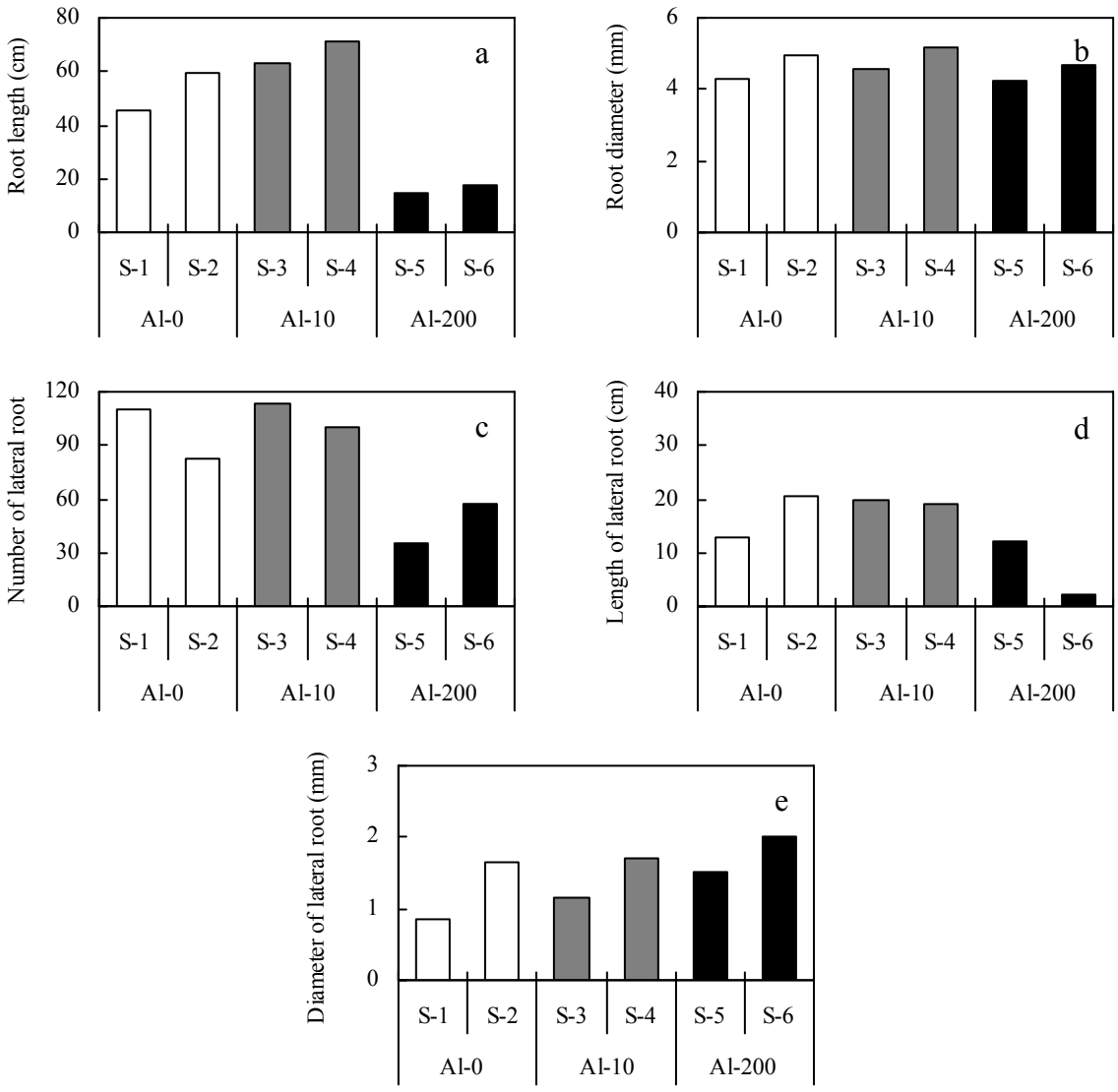


Fig. 5.5 Difference in root morphology of sago palm under different Al treatments: length of adventitious roots (a); diameter of adventitious roots (b); number of lateral roots (c); average length of lateral roots from five adventitious roots (d); average diameter of lateral roots from five adventitious roots (e).

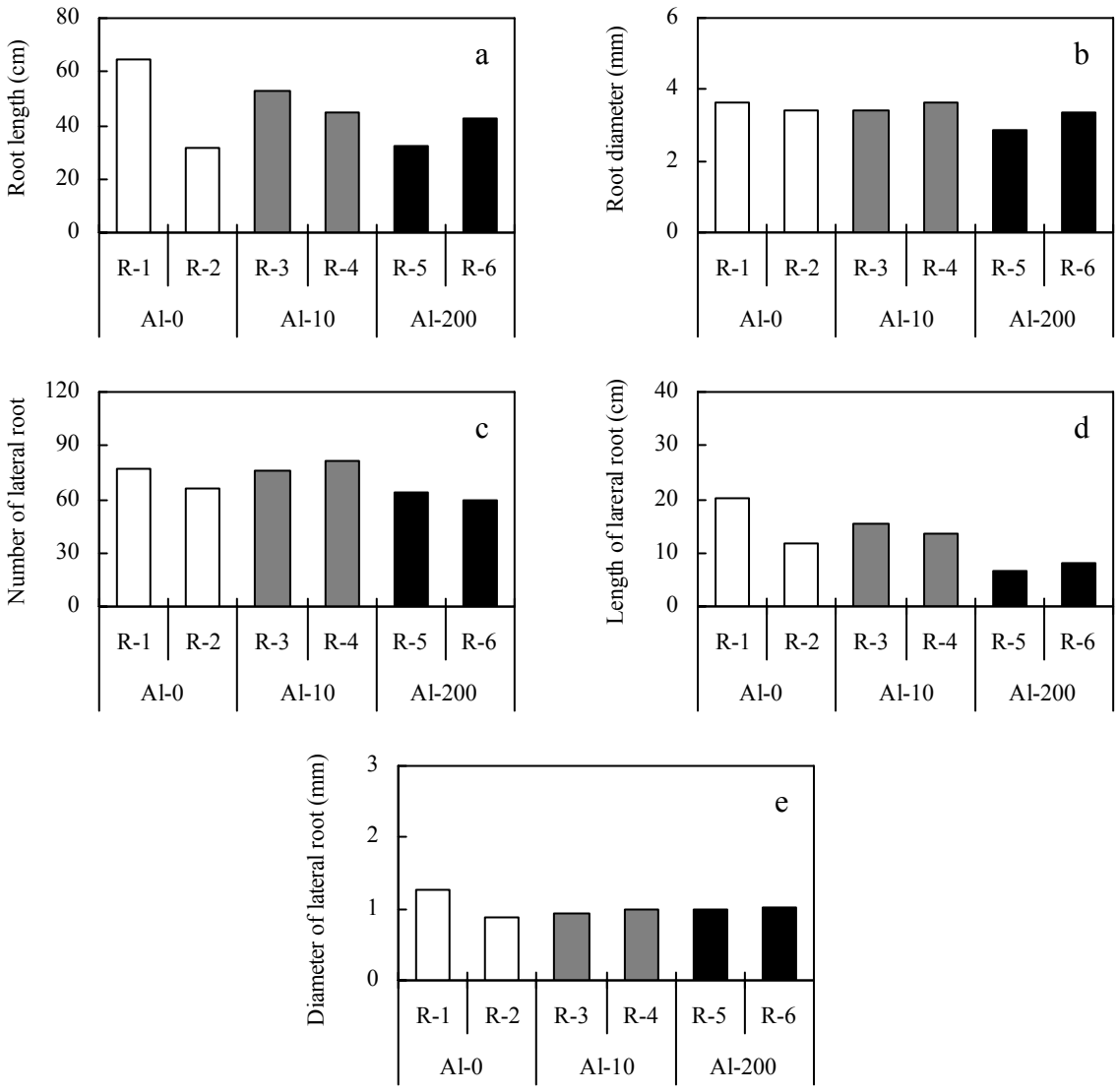


Fig. 5.6 Difference in root morphology of rattan under different Al treatments: length of adventitious roots (a); diameter of adventitious roots (b); number of lateral roots (c); average length of lateral roots from five adventitious roots (d); average diameter of lateral roots from five adventitious roots (e).

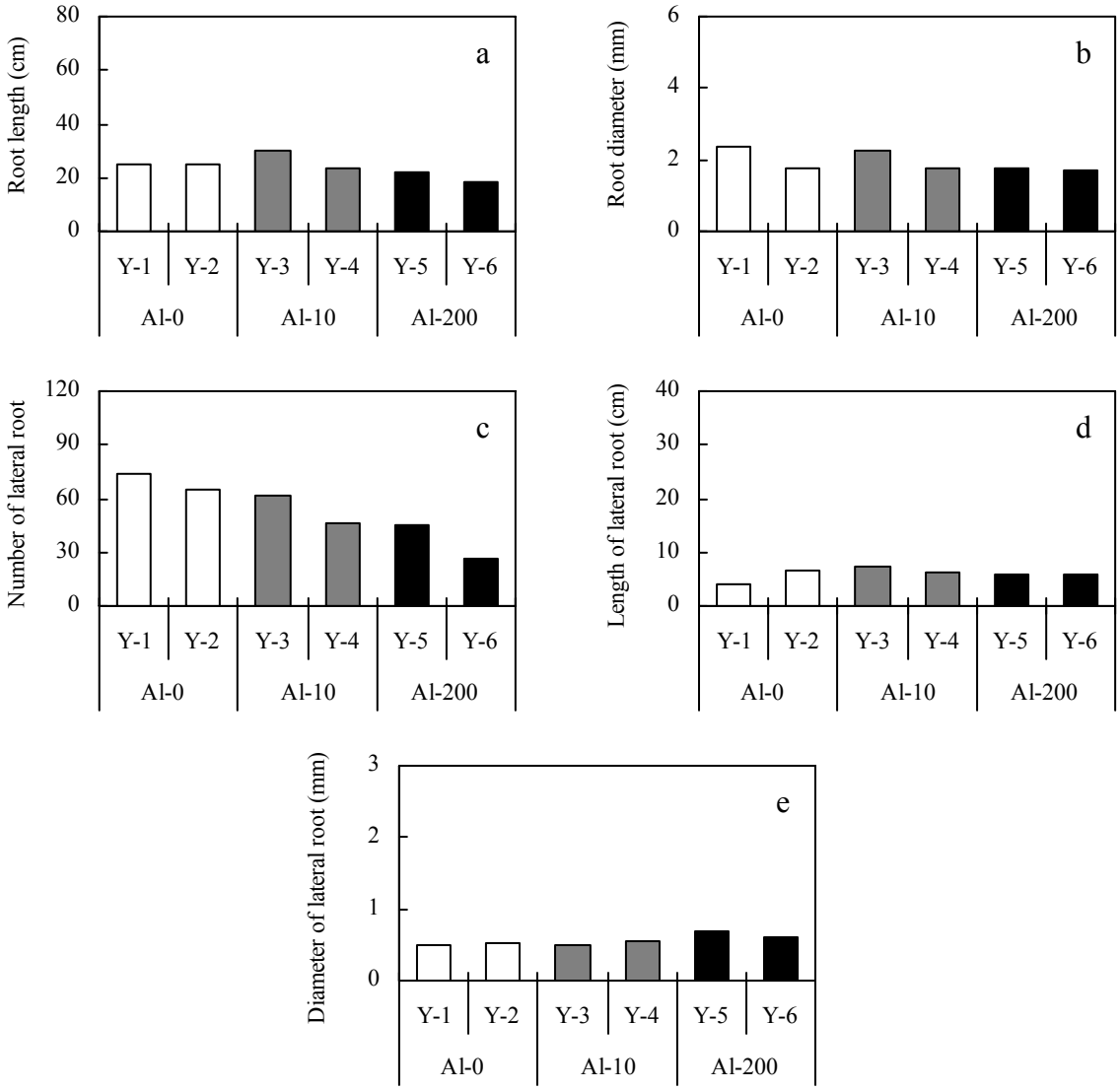


Fig. 5.7 Difference in root morphology of yatay palm under different Al treatments: length of adventitious roots (a); diameter of adventitious roots (b); number of lateral roots (c); average length of lateral roots from five adventitious roots (d); average diameter of lateral roots from five adventitious roots (e).

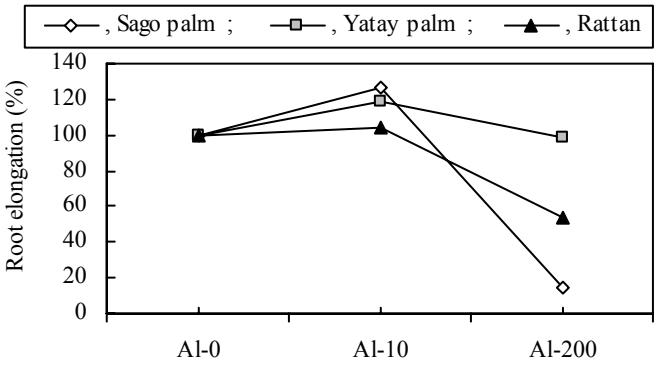


Fig. 5.8 Root elongation of sago palm, rattan and yatay palm. Initial and final root length data were used to calculate the relative root elongation rate, taking as a 100% reference in the relative elongation rate of no Al-treated plant.

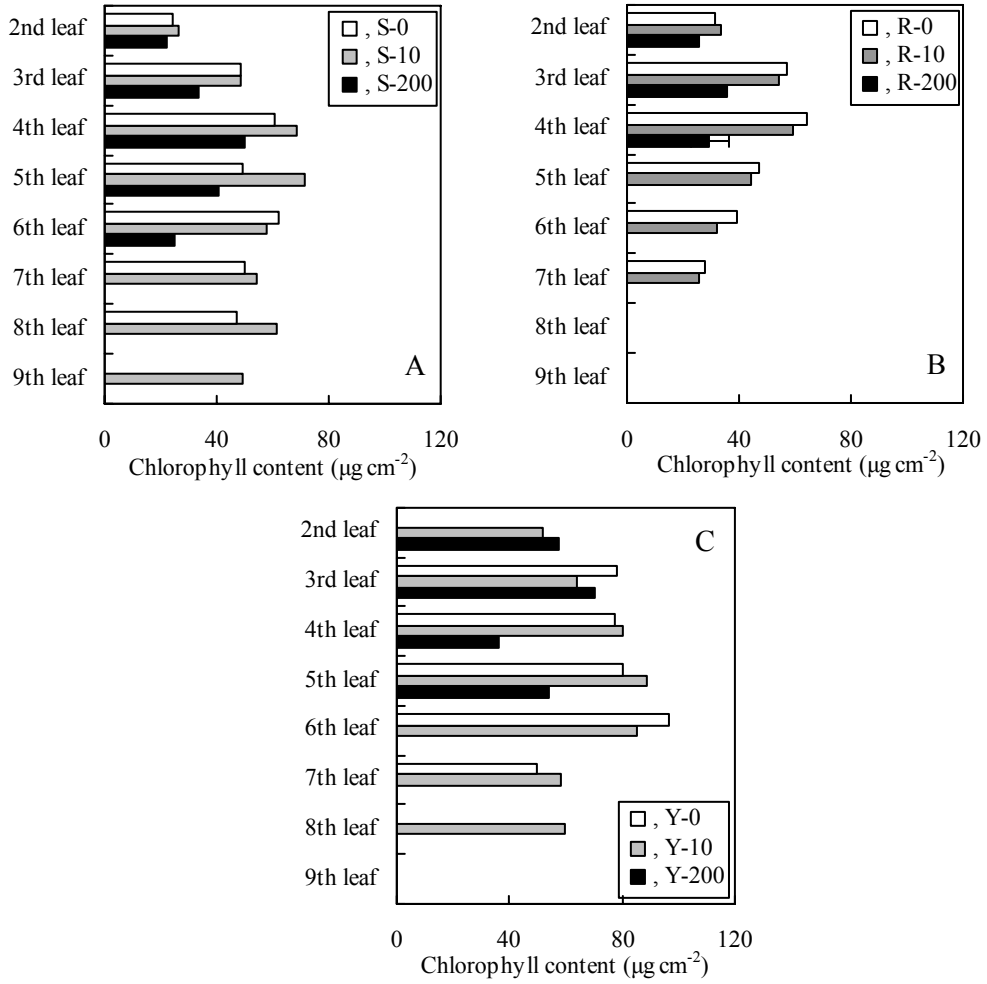


Fig. 5.9 Chlorophyll content per unit leaflet area at different leaf positions of sago palm (A), rattan (B) and yatay palm (C) under different Al treatments.

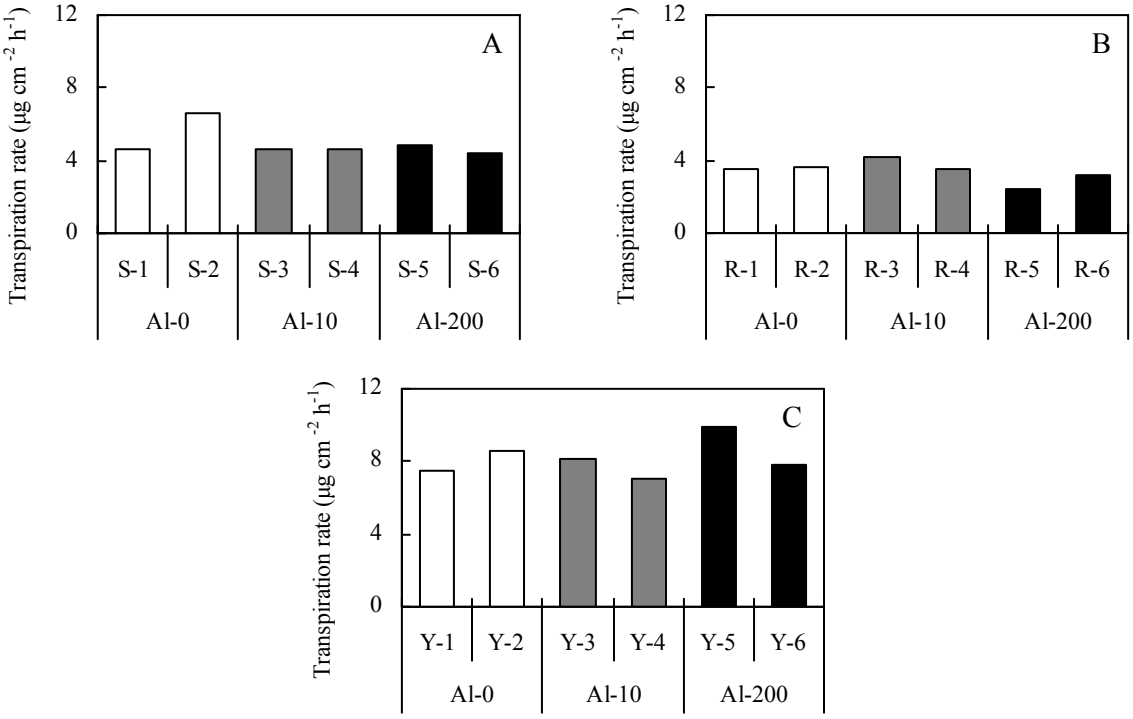


Fig. 5.10 Transpiration rate of sago palm (A), rattan (B) and yatay palm (C) under different Al treatments.

Table 5.2 Effect of Al concentration on nutrient concentrations in different plant parts and whole plant of the three palm species.

Plant part	Sago palm			Rattan palm			Yatay palm		
	Al - 0	Al - 10	Al - 200	Al - 0	Al - 10	Al - 200	Al - 0	Al - 10	Al - 200
	---------- Al^{3+} (µmol g^{-1}) ----------								
Leaflet	13.7	14.7	15.2	10.7	11.0	16.0	11.2	13.9	22.4
Petiole	14.0	15.2	20.9	11.4	11.6	25.0	11.3	14.0	42.7
Root	14.4	15.6	22.8	12.4	12.4	23.4	14.8	15.3	35.2
Dead leaf	18.0	31.2	40.2	16.0	27.8	65.4	14.0	-	38.8
Whole	13.9	15.1	19.0	11.3	11.5	21.8	11.9	14.2	33.5
	---------- N (mg g^{-1}) ----------								
Leaflet	21.1	20.5	14.8	14.7	25.5	34.8	11.6	10.2	8.5
Petiole	9.2	9.2	10.2	10.5	18.7	22.2	10.7	7.0	5.1
Root	8.1	10.3	6.3	8.4	16.4	19.1	6.6	5.0	1.2
Whole	14.7	14.2	12.4	12.1	21.3	26.8	10.3	8.0	5.8
	---------- P (mg g^{-1}) ----------								
Leaflet	2.6	1.8	1.8	4.0	2.3	1.2	3.6	3.4	-
Petiole	3.7	2.4	1.3	5.7	2.4	0.8	3.0	3.0	-
Root	1.8	1.6	-	2.1	1.0	0.6	-	-	-
Whole	2.8	2.0	1.5	4.2	2.1	0.8	3.3	3.2	-
	---------- K^{+} (µmol g^{-1}) ----------								
Leaflet	218.0	200.6	364.5	230.9	262.7	115.4	305.1	194.4	102.5
Petiole	599.1	470.3	719.4	431.7	331.9	367.3	415.7	224.7	52.6
Root	596.5	578.6	518.8	447.3	319.4	226.1	376.6	215.6	25.7
Whole	425.8	377.3	531.2	339.8	300.0	227.8	357.6	210.9	63.2

Table 5.2 Continued

Plant part	Sago palm			Rattan palm			Yatay palm		
	Al - 0	Al - 10	Al - 200	Al - 0	Al - 10	Al - 200	Al - 0	Al - 10	Al - 200
	---------- Ca^{2+} ($\mu mol\ g^{-1}$) ----------								
Leaflet	64.6	59.9	27.6	89.9	75.1	29.2	79.2	74.2	79.0
Petiole	133.0	93.2	50.0	107.2	86.6	62.8	66.9	58.7	25.6
Root	47.5	47.5	27.6	35.4	31.1	18.0	30.7	27.1	12.5
Whole	87.0	69.0	38.2	87.8	70.9	35.1	65.4	60.2	41.1
	---------- Mg^{2+} ($\mu mol\ g^{-1}$) ----------								
Leaflet	90.8	61.0	52.1	123.5	88.7	21.5	77.5	79.9	42.2
Petiole	163.4	130.6	52.5	186.9	139.7	43.5	110.3	97.5	37.7
Root	116.9	158.3	88.7	194.3	119.7	43.5	86.8	83.6	28.6
Whole	122.8	106.5	60.2	158.9	113.5	35.9	90.8	88.1	37.7

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

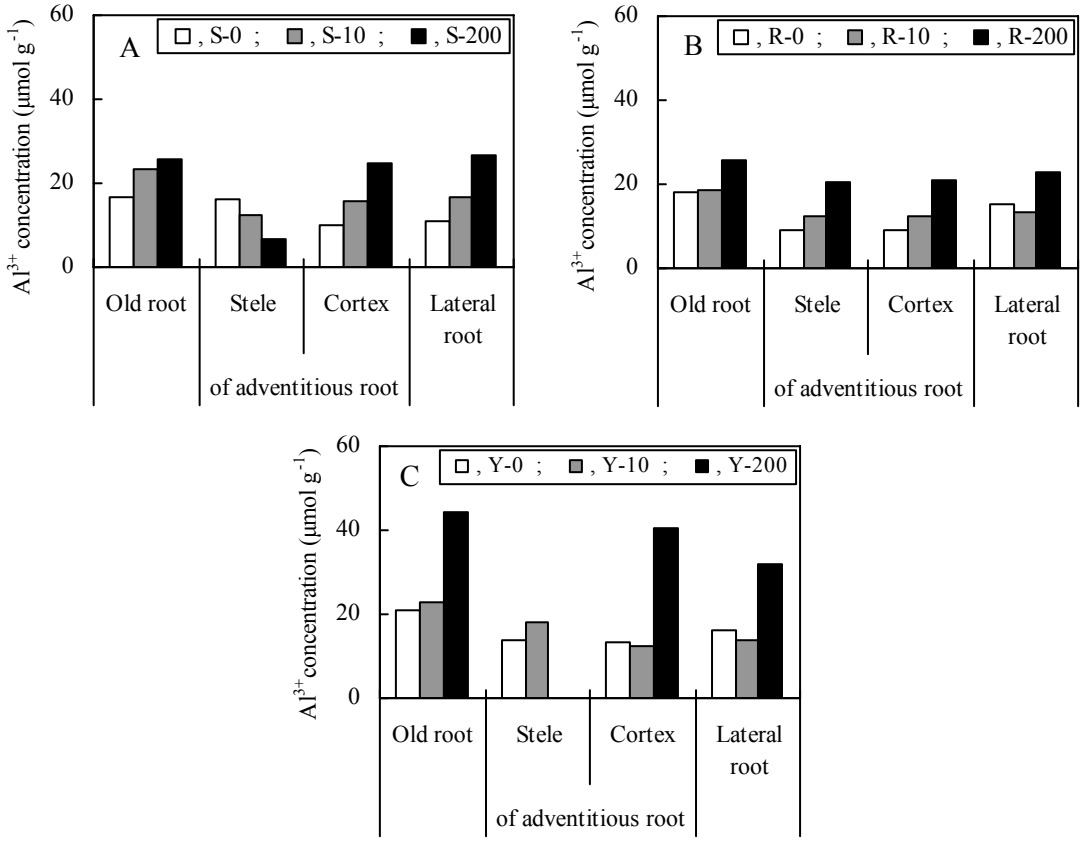


Fig. 5.11 Al^{3+} concentration in different parts of old roots and new roots (stele and cortex of adventitious roots and lateral roots) of sago palm (A), rattan (B) and yatay palm (C) under different Al treatments.

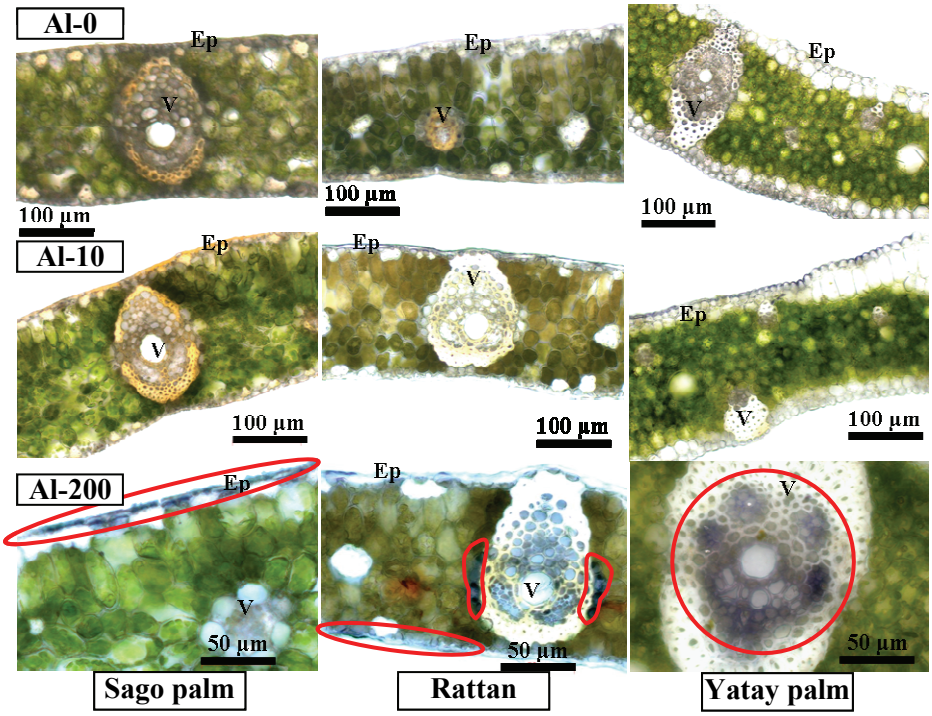


Fig. 5.12 Localization of aluminum by hematoxylin staining in the leaflet cross sections of sago palm, rattan and yatay palm under the Al treatments. Ep: epidermis, V: vascular bundle. Encirclements indicate the purple color of detectable Al in plant tissues.

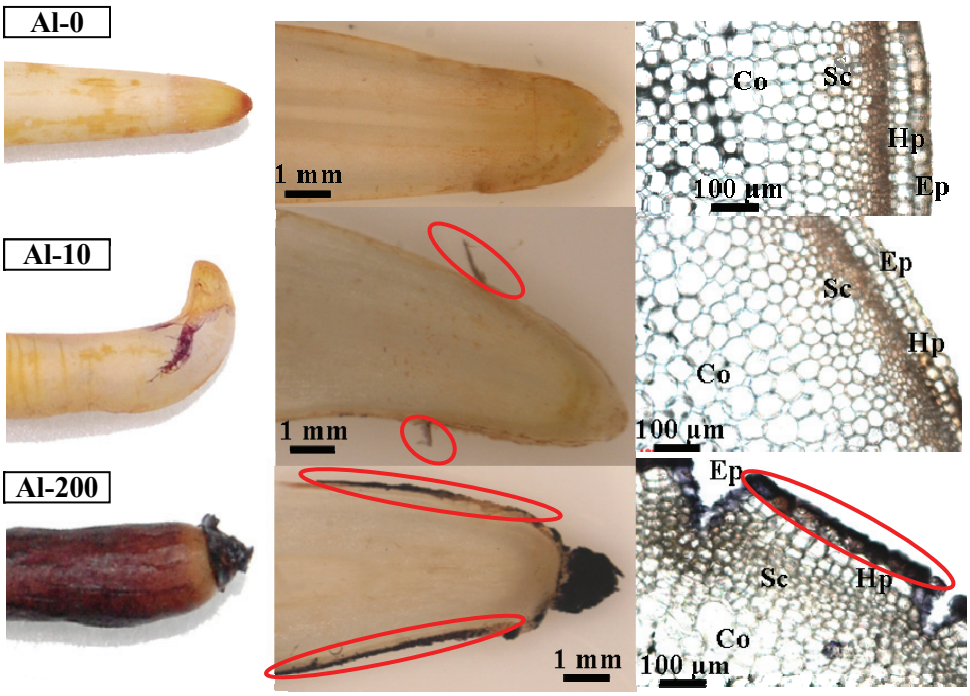


Fig. 5.13 Localization of aluminum by hematoxylin staining in the transversal and cross sections of adventitious roots of sago palm under different Al treatments. Ep: epidermis, Hp: hypodermis, Sc: sclerenchyma cell, Co: cortex. Encirclements indicate the purple color of detectable Al in plant tissues.

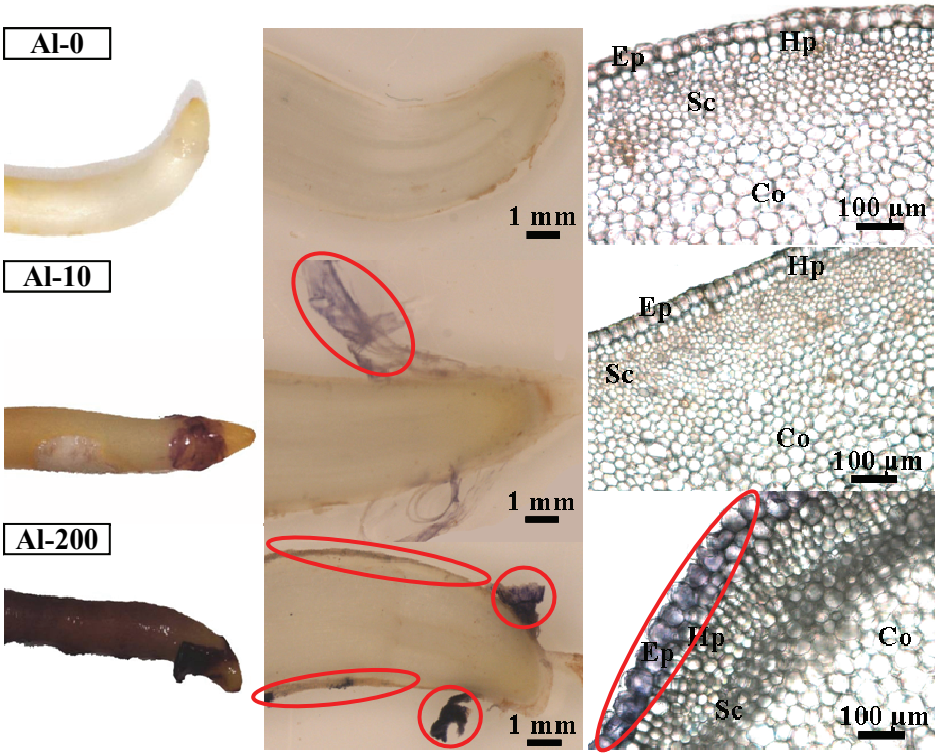


Fig. 5.14 Localization of aluminum by hematoxylin staining in the transversal and cross sections of adventitious roots of rattan under different Al treatments. Ep: epidermis, Hp: hypodermis, Sc: sclerenchyma cell, Co: cortex. Encirclements indicate the purple color of detectable Al in plant tissues.

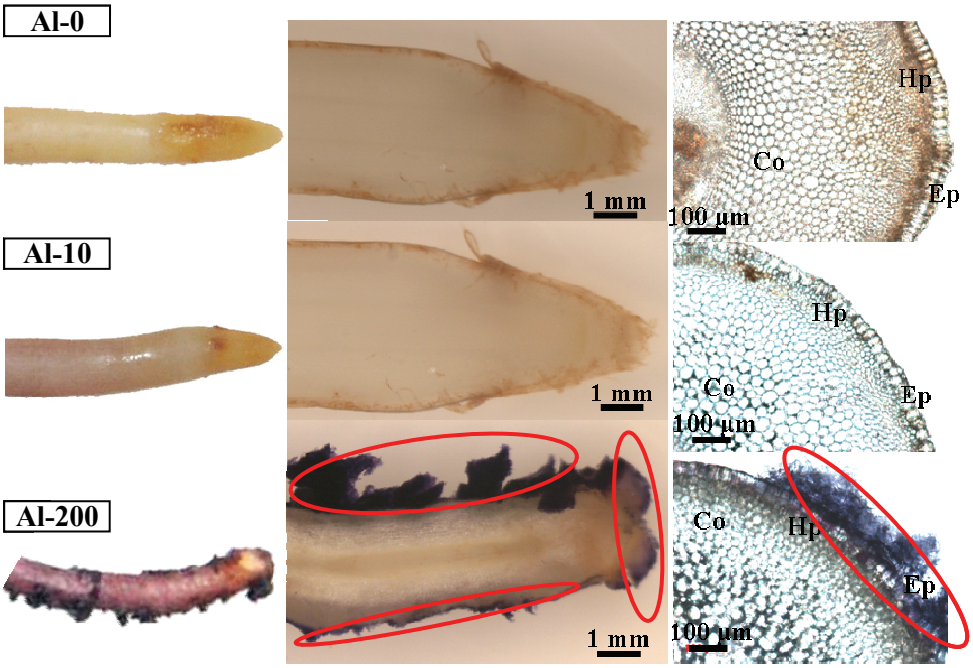


Fig. 5.15 Localization of aluminum by hematoxylin staining in the transversal and cross sections of adventitious roots of yatay palm under different Al treatments. Ep: epidermis, Hp: hypodermis, Sc: sclerenchyma cell, Co: cortex. Encirclements indicate the purple color of detectable Al in plant tissues.

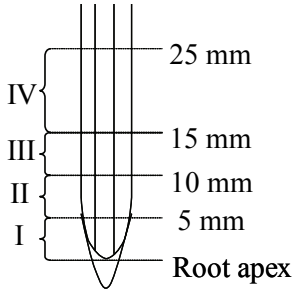


Fig. 5.16 The position of root section for the observation under a fluorescence microscope.

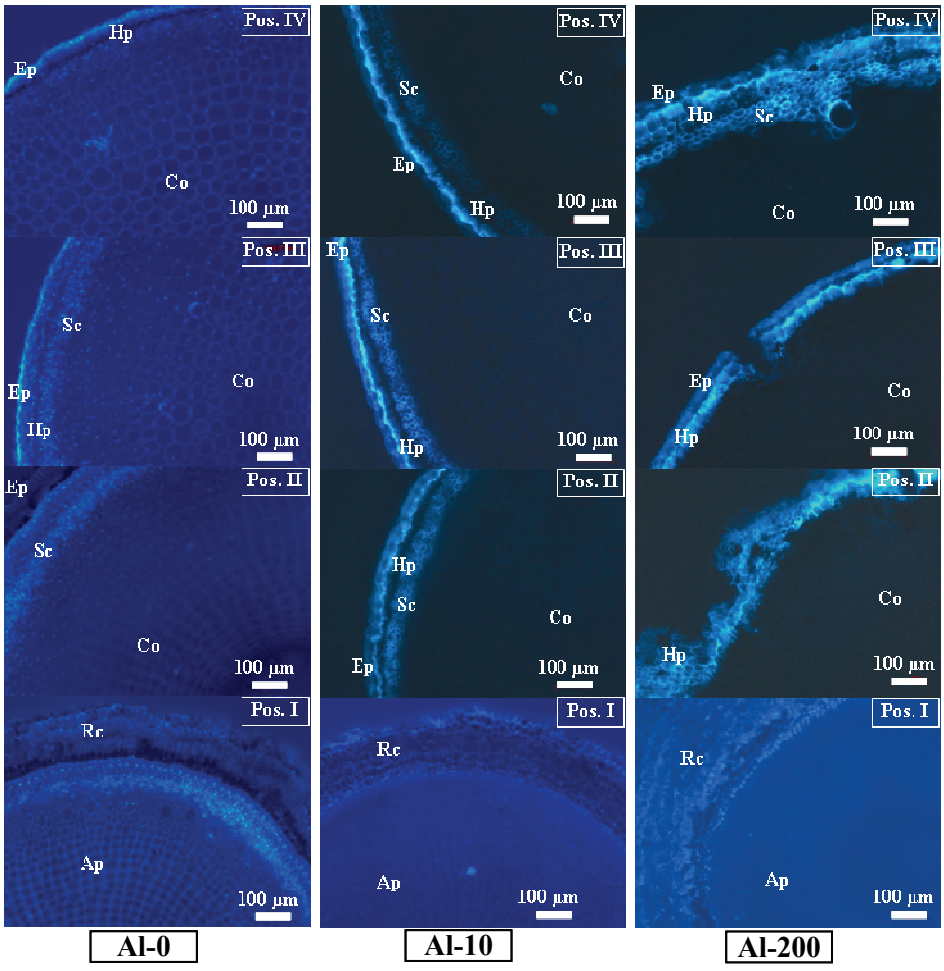


Fig. 5.17 The structure and component of adventitious roots of sago palm stained with berberin-aniline blue and observed around the external part of the root cortex under UV microscope in the various positions from the root tip under different Al treatments. Rc: root cap, Ap: apical meristem, Ep: epidermis, Hp: hypodermis, Sc: sclerenchyma cell, Co: cortex.

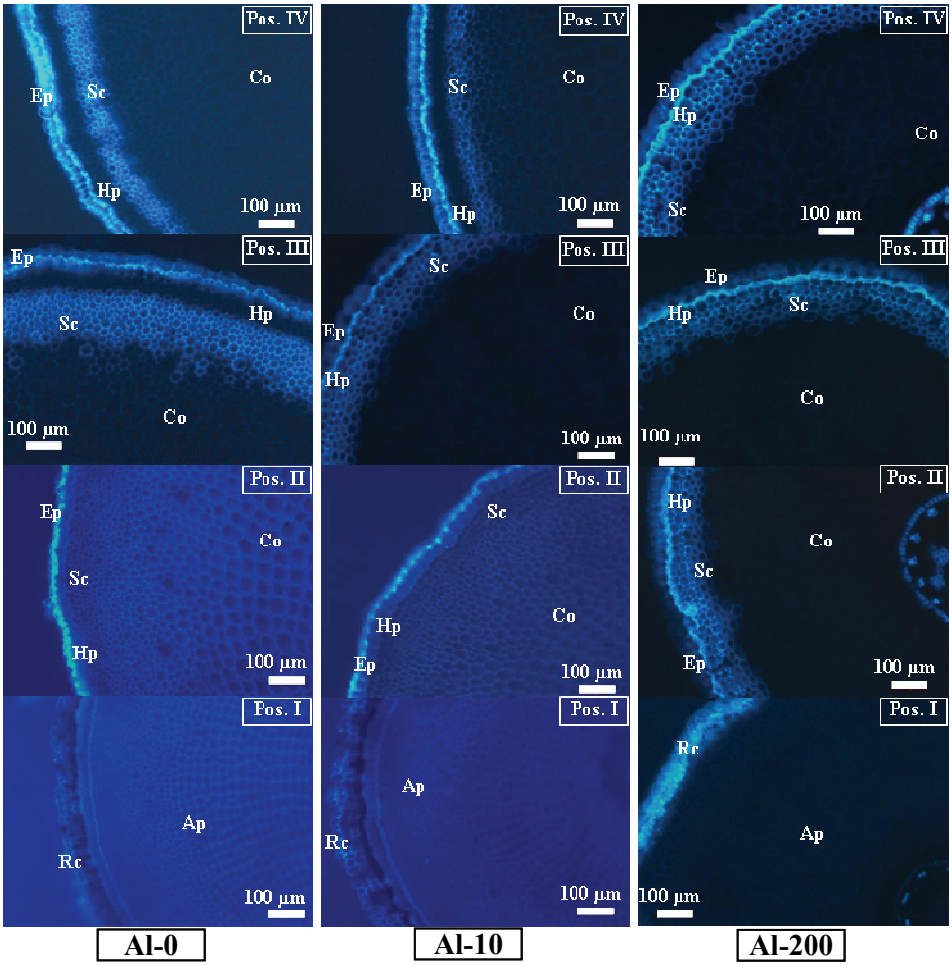


Fig. 5.18 The structure and component of adventitious roots of rattan stained with berberin-aniline blue and observed around the external part of the root cortex under UV microscope in the various positions from the root tip under different Al treatments. Rc: root cap, Ap: apical meristem, Ep: epidermis, Hp: hypodermis, Sc: sclerenchyma cell, Co: cortex.

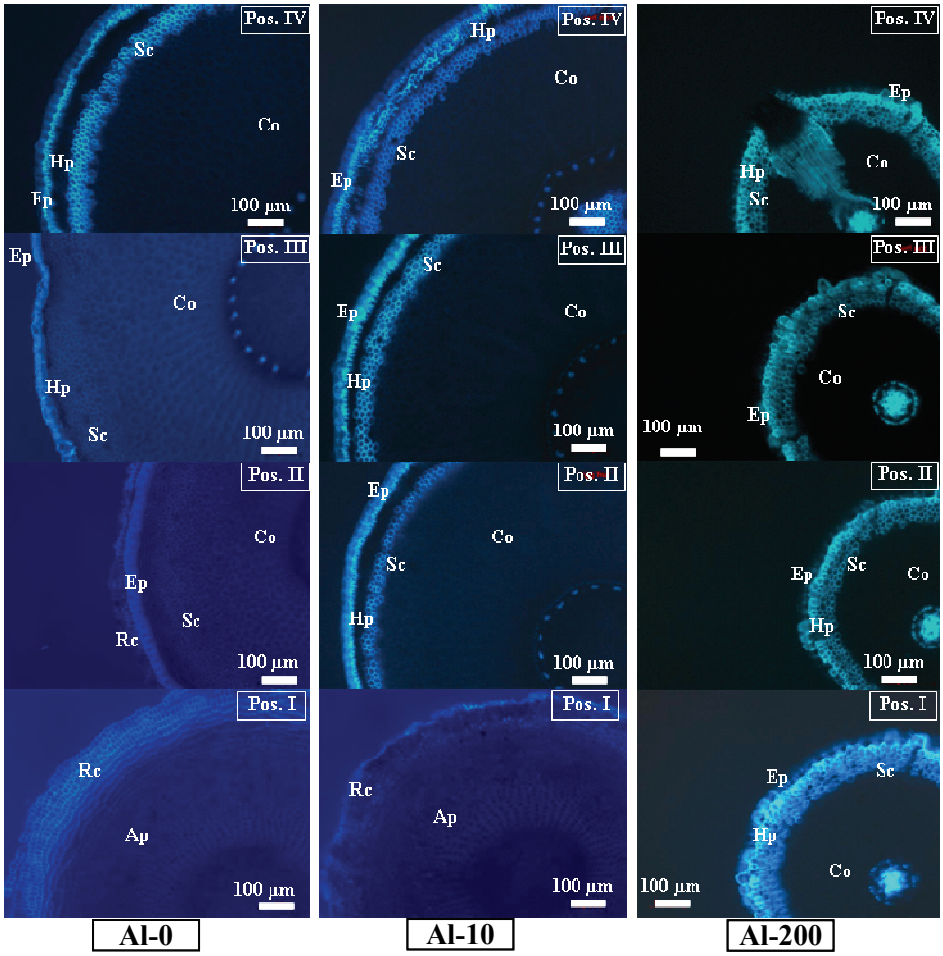


Fig. 5.19 The structure and component of adventitious roots of yatay palm stained with berberin-aniline blue and observed around the external part of the root cortex under UV microscope in the various positions from the root tip under different Al treatments. Rc: root cap, Ap: apical meristem, Ep: epidermis, Hp: hypodermis, Sc: sclerenchyma cell, Co: cortex.

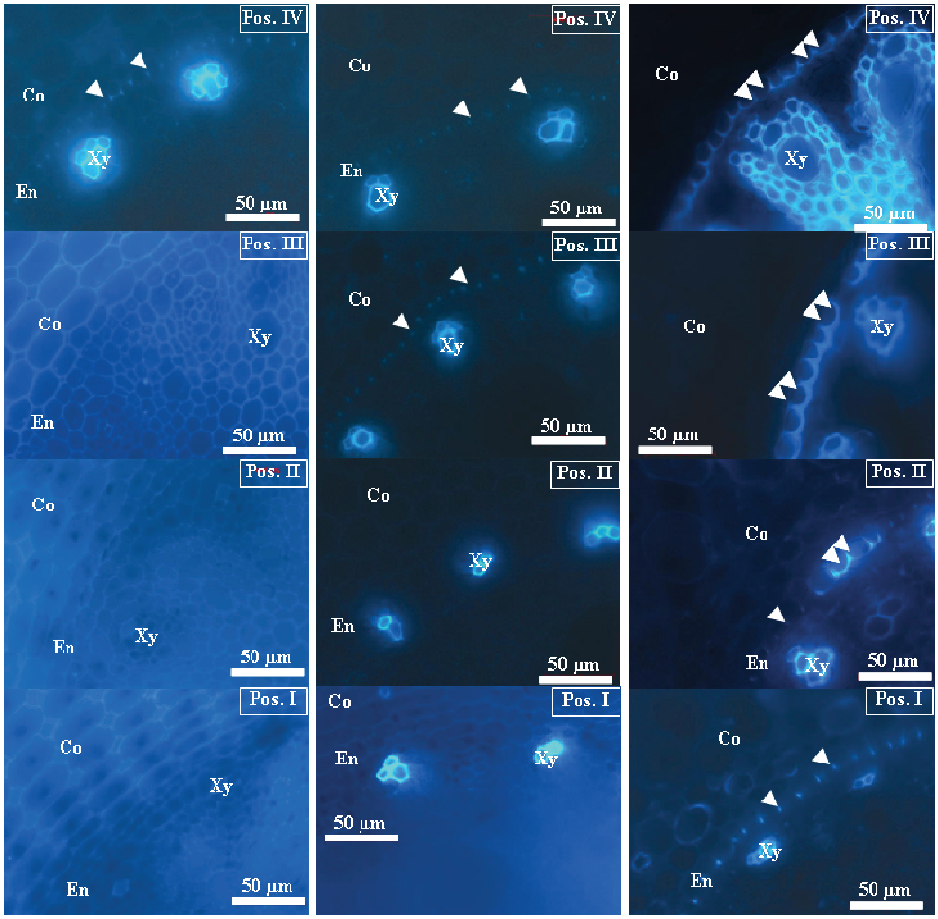


Fig. 5.20 The structure and component of adventitious roots of sago palm stained with berberin-aniline blue and observed around the internal part of the root cortex under UV microscope in the various positions from the root tip under different Al treatments. △ indicate Casparian strips in the radial cell walls of endodermis. △△ indicate U-shaped thickening in the cell walls of endodermis. Co: cortex, En: endodermis, Xy: xylem.

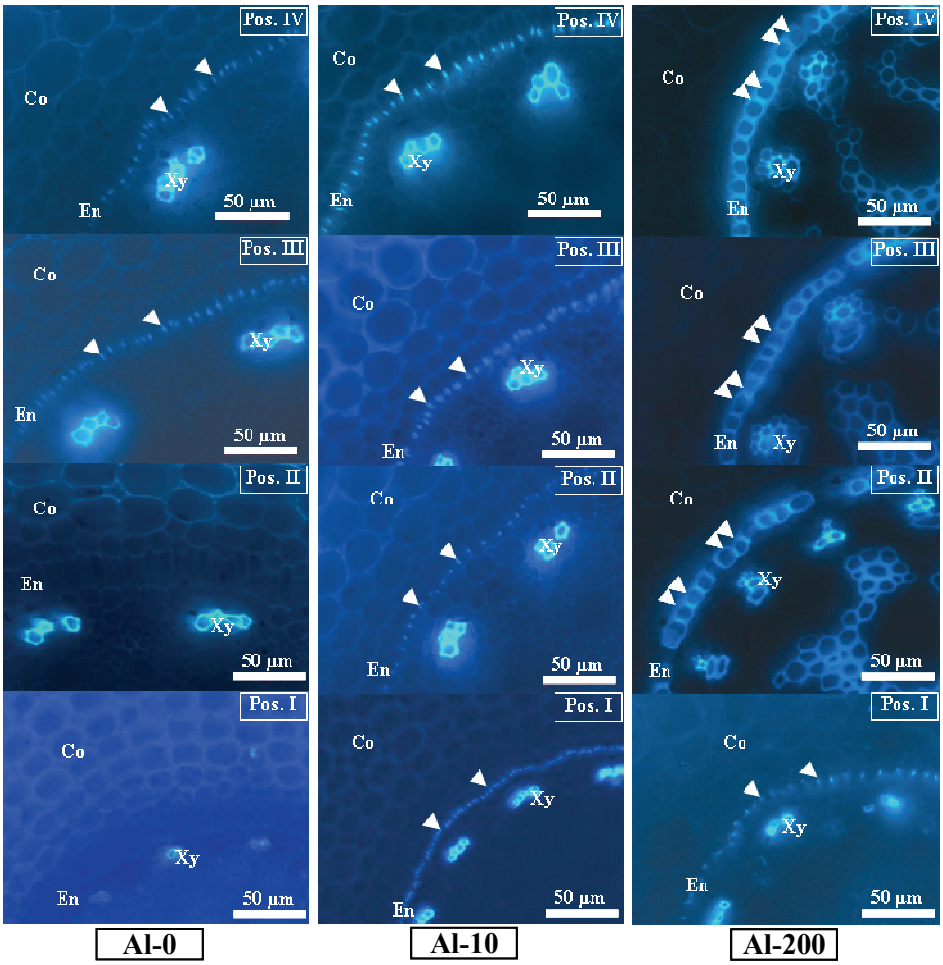


Fig. 5.21 The structure and component of adventitious roots of rattan stained with berberin-aniline blue and observed around the internal part of the root cortex under UV microscope in the various positions from the root tip under different Al treatments. △ indicate Casparian strips in the radial cell walls of endodermis. △△ indicate O-shaped thickening in the cell walls of endodermis. Co: cortex, En: endodermis, Xy: xylem.

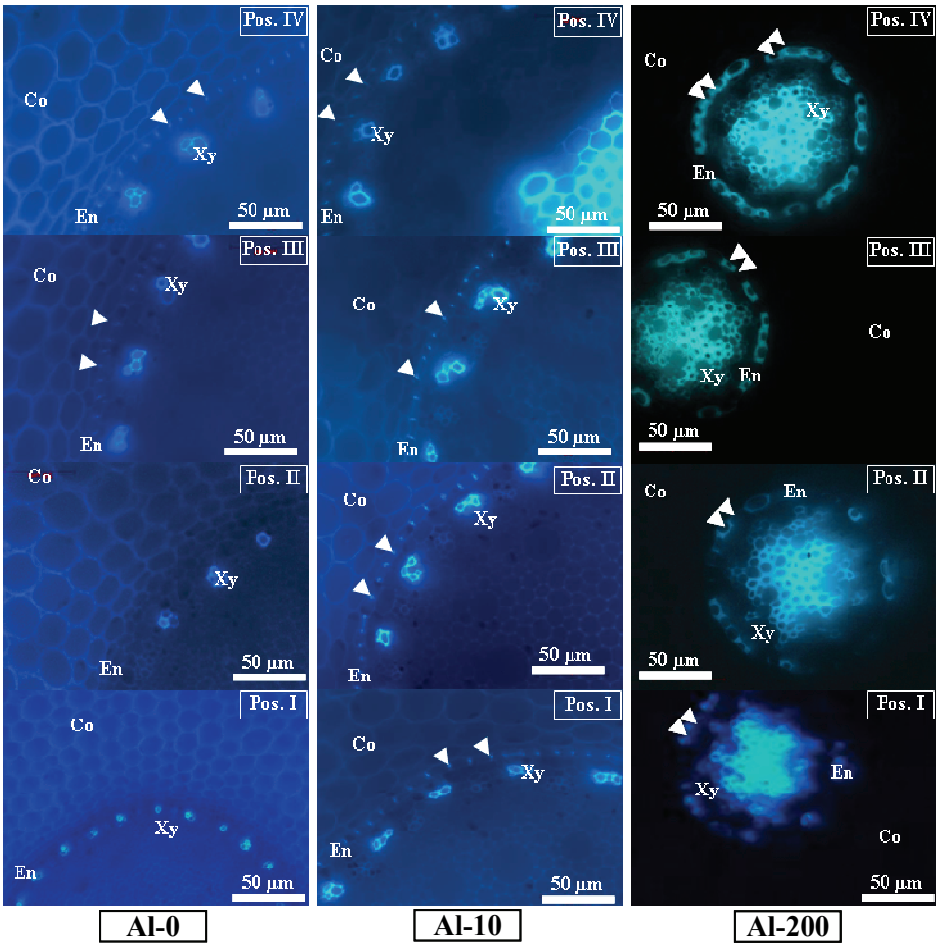


Fig. 5.22 The structure and component of adventitious roots of yatay palm stained with berberin-aniline blue and observed around the internal part of the root cortex under UV microscope in the various positions from the root tip under different Al treatments. △ indicate Casparian strips in the radial cell walls of endodermis. △△ indicate U-shaped thickening in the cell walls of endodermis. Co: cortex, En: endodermis, Xy: xylem.

Chapter 6

Summary of resistant mechanism of sago palm against aluminum stress

Competition between biofuel production and food production has occurred in recent years as a result of the current social background of the exhaustion of fossil energy and the increase in the world population. Various plants are receiving attention as sustainable energy resources for the production of bioethanol and biodiesel. However, arable lands are quite limited worldwide. Thus, the development and/or improvement of new plant resources and their utilization are needed as one of the strategies to secure a sufficient amount of biomass for producing foods and biofuel sources (Ehara, 2009). Sago palm (*Metroxylon sagu* Rottb.), as a starch producing plant, is one of the dominant species in tropical swampy, alluvial and peaty soil that are usually characterized by the low pH values, a deficiency in mineral elements and a high exchangeable Al. It is, therefore, assumed that sago palm is resistant to acidic and Al. However, few studies have compared the growth characteristics of sago palm at widely different pH levels as well as the Al-induced changes on sago palm growth. In the current study, the growth and physiological features of young sago palms grown at different pH levels under the field condition and experimental study in the laboratory were investigated to evaluate the ability of the acid resistance. Moreover, the growth, nutrient absorption and some physiological characteristics of sago palm, rattan and yatay palm were investigated to evaluate the Al resistant ability of sago palm under low pH condition in comparison with related palm species. The Al localization in the plant tissues of sago palm and related palm species was also observed to make more clearly in the Al resistant mechanism.

1. Nutrient accumulation in plant tissues of sago palm in the rosette stage at different levels of soil pH in South Thailand

In the first experiment, three young sago palms were collected at each sampling site from three locations in South Thailand to investigate the nutrient accumulation in plant tissues of sago palm in the rosette stage with no trunk formation grown at different levels of soil pH. The

tendency in the case of Mg^{2+} concentration was displayed a significantly higher Mg^{2+} concentration in the whole plant of sago palm grown at the neutral pH soil (site 1) than those at the low pH soil (sites 2 and 3), which was similarly to that observed from soil sampled. Contrarily, the N and P concentrations in whole plant of sago palm grown at the low pH soil (sites 2 and 3) were significantly higher than those at the neutral pH soil (site 1), although there were no differences in the available P and N in the soil at the three sampling sites. In addition, the effect of the difference in soil pH between the neutral pH soil (site 1) and the low pH soil (sites 2 and 3) on the K^+ and Ca^{2+}concentrations in the whole plant were indistinct. It is likely that sago palm grown at the low pH soil (sites 2 and 3) could maintain the uptake of macronutrients, which may be one of the major reasons that sago palm can adapt to growth in extremely acidic conditions. Furthermore, sago palms at the three sampling sites tended to store a higher Al^{3+} concentration in the cortex of adventitious roots than in other parts, such as the leaflets, and a similar tendency was observed for the accumulation of SO_4^{2-} and Na^+ in plant tissues. It was, therefore, assumed that 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 plant tissues, which may account for the ability of sago palm to grow in a range of soil pH from 4.3 to 7.0 under natural conditions.

2. Effect of low pH on the growth, physiological characteristics and nutrient absorption of sago palm

In the second experiment, the dry matter production, photosynthetic characteristics and nutrient concentrations in plant tissues of sago palm seedlings cultured for 4.5 months in a hydroponic system at pH 5.7, 4.5 and 3.6 were examined. Plant growth in weekly increment of length, leaf emergence, leaf senescence and total leaflet area was similar at all the pH treatments. There was no significant effect of pH on the dry matter weight, although it tended to be lighter at pH 3.6 than at pH 5.7. Similarly, the photosynthetic rate and its related parameter were not significantly affected by the pH. However, the photosynthetic rate at pH 3.6 tended to be lower than that at pH 5.7, which was attributed to a decrease in the stomatal conductance. The effect

of low pH on the nutrient concentrations in plant tissues was not distinct. It was, therefore, concluded that sago palm seedlings could maintain leaf morphogenesis and nutrient uptake in growth media at a pH ranging from 3.6 to 5.7 for 4.5 months, which led to a high growth rate and maintenance of dry matter production even at pH 3.6.

3. Effect of aluminum concentration on growth and physiological characteristics of sago palm under low pH condition

In the third experiment, sago palm seedlings were grown in culture solution at pH 3.6, containing the levels of $AlCl_3 \cdot 6H_2O$ corresponding in 0, 10, 20, 100 and 200 ppm Al for investigating the effect of Al concentration on the growth and physiological features under low pH condition. The total dry weight and total leaflet area in the 10 ppm Al treatment were slightly large, while those in the 200 ppm Al treatment were significantly smaller than that in the no Al treatment. The critical value to inhibit the growth of sago palm was considered to be around 200 ppm Al in the growth media. The total N and P concentrations in the whole plant under the 10 ppm Al treatment were higher than those in the other Al treatments, which could lead to an increase in the growth of sago palm under a mild Al concentration in the growth media. The K^+ concentration in all plant parts is independent from the Al treatment, while the Ca^{2+} and Mg^{2+} concentrations in the whole plant were significantly decreased under the higher Al treatments. The Al^{3+} concentration was significantly higher in the roots than in the top parts, which the absolute Al^{3+} accumulation in all plant parts was not so large even at 200 ppm Al treatment. It was, therefore, considered that a high resistance to Al of sago palm might exhibit an avoidance mechanism to maintain the low Al^{3+} concentration in the top parts by storing Al^{3+} in the roots and possess the mechanically restriction of the excess Al based on the Al exclusion ability under acidic condition.

4. Comparison of growth and physiological characteristics of sago palm, rattan and yatay palm against aluminum stress under low pH condition

In the fourth experiment, young seedlings of sago palm, rattan and yatay palm were grown in the culture solution at pH 3.6, containing the levels of $AlCl_3 \cdot 6H_2O$ corresponding in 0, 10 and

200 ppm Al to investigate the growth, morphological and physiological characteristics, and nutrient concentrations in plant tissues for evaluating the Al resistant ability of sago palm under low pH condition in comparison with rattan (*Calamus viminalis* Wild.) that belongs to the same tribe Calameae with sago palm, and yatay palm (*Butia yatay* Becc.), the starch producing palm, that belongs to the same family Arecaceae with sago palm. The results showed that the new leaf emergence of sago palm and yatay palm was affected by the higher level of Al concentration, while the acceleration of leaf senescence at the higher Al treatment was apparent only for rattan. In addition, the three palm species could maintain the chlorophyll production to cope with the lower Al treatment, while the chlorophyll content in the leaflets decreased under the higher Al treatment, which might account for declining the photosynthetic capacity that at least partly resulted in a correspondingly decreased growth rate of the three palm species. In addition, the change in the transpiration rate depended on the Al concentration in the growth media and also varied among the three palm species.

The macronutrients in whole plant of the three palm species were tended to decrease under the higher Al treatments, especially P, Ca^{2+} and Mg^{2+}, which were generally found in various plant species. However, some macronutrients, such as N and K^{+} were decreased or increased differently depending on the palm species and Al concentration in the growth media. In addition to the Al concentration in plant tissues of sago palm, rattan and yatay palm, it is likely that the Al resistant ability of yatay palm may be nearly important as an Al - accumulator, whereas the Al resistant ability of sago palm and rattan (belongs to the same tribe Calameae with sago palm) mainly attributes to the avoidance mechanism, which sago palm has a high Al resistance via exclusion ability more than rattan under acidic condition. Nevertheless, based on the growth response of the three palm species under the higher Al treatment, it is likely that sago palm was considered to be comparatively resistant against Al stress, which can maintain the net leaf product and increment of plant length rather than yatay palm and rattan.

From the observation of Al localization in the roots stained with hematoxylin, no Al was detected in the leaflets of the three palm species under the no Al and 10 ppm Al treatments, in

contrast to the 200 ppm Al treatment, the purple color formed by the compound of an aluminum haematein complex was found in the leaflets of the three palm species. In the case of sago palm, Al was accumulated preferentially in the upper epidermis and occasionally in the lower epidermis. It is likely that sago palm may possess an effective mechanism to prevent the internal Al toxicity in the important tissues by transferring Al^{3+} to mainly accumulate in the upper epidermis, which was generally transparent to allow light to reach the mesophyll tissues for photosynthesis and lacked of the chloroplast. In the leaflet of rattan under the 200 ppm Al treatment, Al was detected in the bundle sheath cell (the thick walled cell surrounding the vascular bundle) and upper and lower epidermis, which the higher Al accumulation in the upper epidermis was observed than in the lower epidermis. In the case of yatay palm under the 200 ppm Al treatment, the Al^{3+} accumulation was observed in the inner of vascular bundle including the xylem and phloem, and was similarly observed in the upper and lower of epidermal layer of the leaflets.

In addition to the Al localization in the roots of the three palm species, the result showed that the 10 ppm Al-treated roots were stained weakly at the root cap, in contrast to the roots of the 200 ppm Al-treated plants of the three palm species, which were markedly stained at the root cap, epidermis and outer hypodermis. In sago palm, the observation of root cross sections found that Al reached merely the epidermal tissue layer but not in the hypodermis or cortex of the 200 ppm Al-treated plants, which would indicate that the radial transport of Al was restricted by the epidermal tissue layer. In the roots of the 200 ppm Al-treated plants of rattan, Al was detected in the epidermal and 1-2 hypodermal tissue layers. In yatay palm, a stronger purple stain was evident in the epidermal layer where quite damaged by the higher Al treatments, following by the outer hypodermal tissue layer

From the fluorescent observation of the roots of the three palm species stained with berberine-aniline blue, the development of suberin lamellae in the cell wall between the epidermis and hypodermis might be the first barrier to restrict the radial movement of Al in the roots of the three palm species under low pH condition. Although the Casparian band was

observed in the endodermis of the three palm roots under the entire Al treatments, the distances between the lowermost position of the Casparian band and root tip were shorten by the Al treatments, which might attribute to the inhibition of root elongation under the Al treatments. Nevertheless, the Casparian strip in the 200 ppm Al-treated roots of the three palm species changed gradually to the suberin lamellae (the second state of endodermal development) and the thick U-shaped or O-shaped thickening in the cell wall of endodermis (the third state of endodermal development) at the upper part of roots (5 - 25 mm length from the root tip). According to Prathumyot and Ehara (2010), the development of the Casparian strip located in the endodermal cell wall of the adventitious roots of sago palm was considered as an important mechanical factor relating to the avoidance mechanism for preventing the excess influx of ions, such as Na^+, through an apoplastic partway into the stele and its translocation from root to shoot in sago palm. Although the Casparian strip was observed in the endodermal layers of the roots of both sago palm and rattan that belonged to the same tribe Calameae with sago palm, the present results presumed that the efficiency of the Casparian strip to prevent the apoplastic passage of toxic ions, such as Al^{3+}, from the cortex to the stele of sago palm was higher than that of rattan.

As described above, these studies demonstrate the resistance mechanism of sago palm against acidic and Al stress. Under widely different pH, young sago palms maintain the dry matter growth and the uptake of the essential nutrient in plant tissues, which some tendencies of the nutrient uptake and translocation in plant tissues of sago palm in its natural habitat were similar to the results of the experimental study in the laboratory. Based on the growth response of the three palm species under the higher Al treatments, it was considered that sago palm had a comparative resistance against Al stress, which can maintain the net leaf product and increment of plant length rather than yatay palm and rattan. Moreover, sago palm maintained a lower Al^{3+} concentration in all plant parts, even under the 200 ppm Al treatment. Beside, the Al located merely the epidermal tissue layer but not in the hypodermis or cortex of the 200 ppm Al-treated roots, which would indicate that the radial transport of Al was restricted by the development of

suberin lamellae in the cell wall between the epidermis and hypodermis. Furthermore, the growth such as the plant length and dry matter production of sago palm might be stimulated with a mild Al concentration, such as 10 ppm Al, in the growth media under acidic condition that was attributed to the increase in the P and N uptake. From these findings, it could be concluded that sago palm has a high resistance to Al with mechanical restriction of the excess Al based on the Al exclusion ability under acidic condition.

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* In Japanese.

** In Japanese with English abstract.