

Salt Resistance Mechanism of *Metroxylon sagu*, Starch-producing Palm

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Abstract

Sago palm (*Metroxylon sagu* Rottb.) distributed in Southeast Asia and Melanesia grows in swampy, peaty, and alluvial soils in areas with both fresh and brackish water where almost no other crops grow without drainage and/or soil improvement. This palm species stores a large amount of starch in the trunk. The starch of sago palm is processed into various basic raw materials and is receiving attention as a sustainable energy resource as well as for use in the production of bioethanol due to the current situation, in which competition between biofuel and food production is taking place. However, sago palm is harvested primarily from natural forests and is considered to be an unexploited plant. Thus, the Na⁺ and some other ion concentrations in different plant parts as well as the physiological and morphological features under NaCl treatments (86 to 342mM NaCl for 1 or 4 months in a hydroponic system) were investigated to study the salt resistance mechanism of sago palm to develop a sustainable method of cultivation that is essential for the improvement of sago palm as an economic plant. (1) Sago palm maintained a low Na⁺ concentration in the leaflets, which may be attributed to Na⁺ storage mainly in the roots. K⁺ absorption and distribution to the leaflets may not be affected by changes in the Na⁺ concentration in the roots and petioles in sago palm. (2) Based on X-ray micro-analysis, a dense distribution of Na was observed around the endodermis of the adventitious roots. Salt resistance of sago palm might be due to salt avoidance to mechanically restrict an excess of Na distribution in plant tissues as well as maintain the water status in the leaves by restricting the transpiration. (3) The development of Casparian strips in the endodermis can be considered as an important mechanical factor relating to the avoidance mechanism for preventing the excess influx of Na⁺ into the stele and its translocation from root to shoot in sago palm. (4) The factor limiting the photosynthetic rate under NaCl stress was the reduction in stomatal conductance that resulted from a trade-off with the decrease in the transpiration rate to maintain the water status in the leaves. (5) Although chlorophyll production was depressed, the absorption of macronutrients was not inhibited by salt stress and there was no lack of materials, such as N and Mg for chlorophyll production. The chlorophyll concentration could increase up to high levels over a comparatively long time. These factors may account for the resistance of sago palm to salt stress and its ability to grow even with a reduction of the growth rate.

Keywords: Casparian strip, Na⁺ absorption, sago palm, salt avoidance, transpiration rate

Introduction

Sago palm (*Metroxylon sagu* Rottb.) that is distributed in Southeast Asia and South Pacific areas grows in swampy, alluvial and peaty soils where almost no other crops can grow without drainage or soil improvement (Flach, 1977). It supplied carbohydrates and, like banana and taro, has long been cultivated (Barrau, 1959). This rare palm species stores a large amount of starch in the trunk, approximately 300kg (dry wt.) per tree (Ehara 2006). The importance of sago palm as a staple food is well recognized and the palm is still being in some areas of Southeast Asia and South Pacific (Ehara et al., 2000). Its carbohydrate can be further processed into various basic raw materials for food, animal feed and for industrial uses. Sago palm is one of the most important crops for sustainable agriculture and for rural development in swampy areas of the tropics.

Since, sago palm is distributed not only in fresh water areas but also in brackish water areas near the coast, it is considered to be salt-resistant. Flach (1977) reported that saline water treatment up to EC values of 6 to 7 mmho/cm did not affect leaf emergence in sago palm. However, there are few studies on the mechanisms of salt tolerance in sago palm. Thus, we investigated the Na⁺ and the other ions concentrations of different plant parts with some other physiological features under NaCl treatment to study the absorption and distribution of Na⁺ in sago palm.

Materials and Methods

Expt 1. Seedlings at the 8th leaf stage (mean plant length: 50cm) were treated with the culture solution lacking or containing NaCl concentrations of the rates of 86, 171 and 342mM NaCl (0.5, 1.0 and 2.0%) in a pot filled with vermiculite for 30 days (n=3) under a 25klx at 30°C and RH75%. Kimura B culture solutions (Baba and Takahashi, 1958) containing the different NaCl concentrations (pH5.5) were supplied every day according to the amount of solution consumed and then they were renewed once a week. The Na⁺ and K⁺ concentrations in the leaflets, petioles and roots determined using an ion chromatograph with a conductivity detector (Shimadzu CDD-6A, IC-C2, Japan).

Expt 2. The seedlings at the 8th or 9th leaf stage were used for the NaCl treatments. The culture solution containing 342mM NaCl was used from July 9 to August 9, 2004 in the phytotron under natural sunlight. Ion concentration in the plant tissue was measured. A portion in the region above the extension zone of the large roots of the treated plants was soaked in liquid nitrogen after sampling and kept at -70°C. The frozen root samples were freeze-dried and prepared as transverse sections. The sections were coated with gold ion and used for SEM observation and X-ray micro-analysis (Horiba EMAX-5770W, Japan).

Expt 3. At ten months after germination, the longest adventitious root was taken as sample and preserved in 70% ethanol solution. The transverse sections of the root were prepared as 1st position: root tip, 2nd position: 10 mm from root tip, 3rd position: 20 mm from root tip, 4th position: 30 mm from root tip, 5th position: 40 mm from the root tip, 6th position: between the first and second lateral roots, and 7th position: above the second lateral root. The sections were stained according to Brundrett et al. (1988) and observed with a fluorescence microscope (Axio Imager A1; Carl Zeiss, Germany).

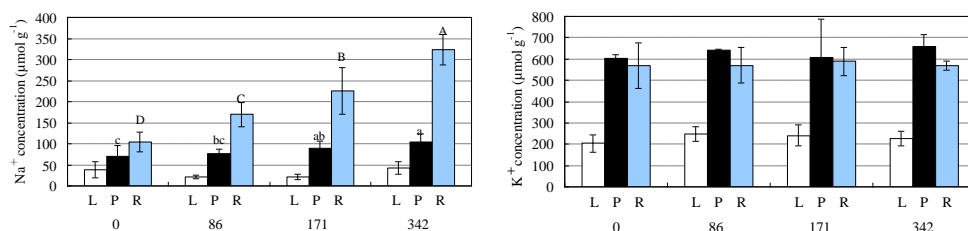
Expt 4. Four spiny seedlings were selected and transferred individually to a 7.3 L plastic pot filled with vermiculite. The mean value of plant length of all the plant materials was 79.3 cm. Two treated pots were connected individually to the first peristaltic pump that was used to supply Kimura B culture solution containing NaCl 224 mM (1.3% NaCl) (pH 5.5) to the treated pots for 6h and the 2nd pump that was used to supply the culture solution without NaCl for another 6h by turns twice a day. The 4th and 5th leaves from the top were used for the measurement of the photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Cs) by a portable

photosynthetic meter (Analytical Development Company limited LCA-4, England) under 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR.

Results and Discussion

Na⁺ and K⁺ concentrations in different plant parts

There were no significant differences in the Na⁺ concentrations in the leaflets between the three NaCl concentrations in the culture solution, the values of which ranged from 22 to 44 $\mu\text{mol/g}$ (Figure 1). In the petioles, Na⁺ concentrations under the 342mM NaCl treatment were significantly higher than those in the absence of NaCl treatment (one and half times), and the differences between the absence of NaCl treatment and the 86mM NaCl treatment, 86mM and 171mM NaCl treatments, and 171mM and 342mM NaCl treatments were not significant. The Na⁺ concentrations in the petioles tended to be higher at higher NaCl concentrations, but with a maximum increase of 1.5 times. The Na⁺ concentrations in the roots were significantly higher at higher NaCl concentrations, increasing 1.5, 2.2 and 3.1 times under the 86, 171 and 342mM NaCl treatments, respectively. Two types of salt tolerance include an avoidance mechanism and a resistance mechanism (Yeo and Flowers, 1983). Through the avoidance mechanism, a low Na⁺ concentrations maintained in the leaf blade. Sago palm can be considered to display an avoidance mechanism to maintain a low Na⁺ concentration in the leaflets by storing Na⁺ in the roots and petioles. The K⁺ concentrations in the leaflets, petioles and roots did not show any significant differences at three NaCl concentrations in the culture solution and in the absence of NaCl treatment, which seems very important to understand the mechanism of salt tolerance in sago palm (Figure 1). K⁺ accumulation may be associated with osmotic adjustment in sago palm.

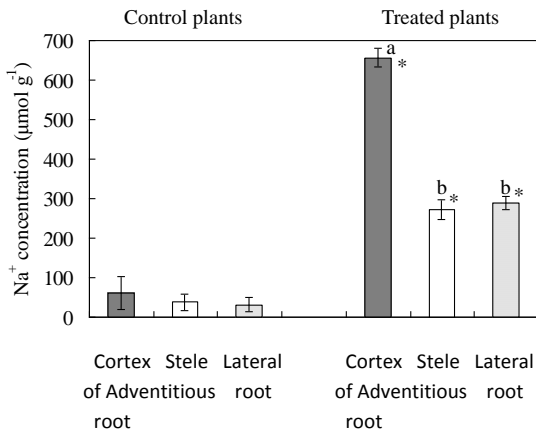


Different letters in the figure indicate significant differences (0.05 probability level, Tukey-Kramer test).

Figure 1. Na⁺ and K⁺ concentrations in leaflets (L), petioles (P) and roots (R) in different NaCl treatments (0, 86, 171 and 342mM).

Na⁺ concentration in different parts of the roots

The Na⁺ concentration in the roots increased with the NaCl treatment (Figure 1). In the adventitious roots, the Na⁺ concentration was lower in the stele than in the cortex (Figure 2). Figure 3 shows the Na distribution from the cortex to the stele in the adventitious roots of the treated plants based on X-ray micro-analysis. The amount of Na was larger in the cortex than in the stele. A dense distribution of Na was observed in the inner region of the cortex near the endodermis. Based on our present findings, it appeared that the region with the endodermis was able to trap some of the excess influx of Na into the large roots. This mechanism must be very important to restrict the translocation of Na⁺ from root to shoot under salt stress.



Different letters in the figure indicate significant differences in different parts (0.05 probability level, Tukey-Kramer test). Asterisks indicate significant difference in each part (0.05 probability level, T-test).

Figure 2. Na⁺ concentration in different parts of the roots.

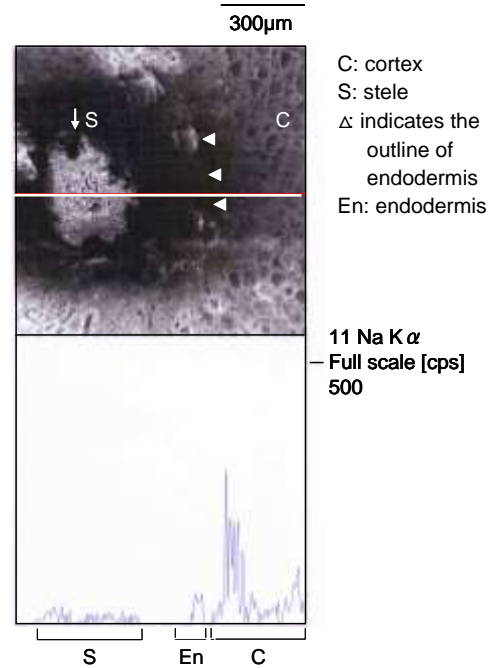


Figure 3. SEM of adventitious root and Na distribution based on X-ray micro-analysis.

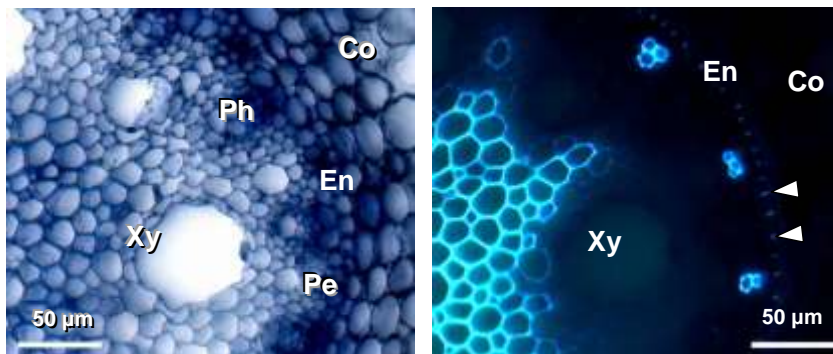


Figure 4. The structure and component of adventitious root at 10 mm from the root tip under visible light (left) and UV (right) microscope. Arrowheads indicate Casparian strips in the radial cell walls of endodermis. Co: cortex, En: endodermis, Pe: pericycle, Ph: phloem, Xy: xylem.

Casparian strip in the root

We found that the Casparian strip was located in the endodermal cell wall of both adventitious roots of sago palm (Figure 4). Taking into account the previous findings of Ehara et al. (2008) that a dense distribution of Na was observed around the endodermis in the extension zone of the adventitious root, therefore, the Casparian strip develops in the endodermis of which can be considered as one of the important mechanical factors relating to functional role of the avoidance mechanism for preventing the excess influx of Na⁺ through an apoplastic pathway into the stele and its translocation from root to shoot in sago palm.

Photosynthetic rate and related characteristics

After the 4th week of the treatment, the SPAD value of the control plants started to increase and became stable after the 6th week (Figure 5). Due to the slow rate of increase in the SPAD value of the treated plants, the difference in the SPAD value between the control and treated plants became more appreciable in the 5th to 7th week and gradually decreased after the 7th week. Finally, the SPAD value of the treated plants reached nearly the same level as that of the control plants. The SPAD value is a parameter positively related to the chlorophyll concentration in plant leaf or chlorophyll content per unit leaf area. Therefore, it can be suggested that the rate of increase in the chlorophyll concentration of the treated plants also may be low. Based on this result, it can be suggested that sago palm is able to produce chlorophyll even under NaCl stress, although the rate of chlorophyll production was low. The important structural components of chlorophyll are magnesium and nitrogen that are located in the centre of chlorophyll (Lack and Evans, 2001). We found that the total N and Mg²⁺ concentrations of the leaflets did not display any distinct differences between the control and treated plants, and it was therefore clear that the deficiency of N and Mg was not the cause of the low chlorophyll production.

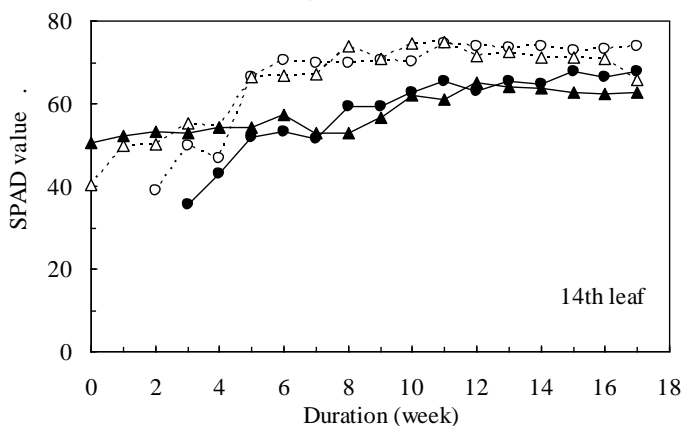


Figure 5. Changes in SPAD value of the leaflets at the 14th leaf of the control plants (C1, C2) and treated plants (T1, T2) during the NaCl treatment. Open and close symbols indicate the control and treated plants, respectively. Circle and triangle symbols indicate plant replication No. 1 and 2 of the control and treatment.

The photosynthetic rate, transpiration rate and stomatal conductance in both leaves of the treated plants decreased by 40%, compared to the value of the control plants (Figure 6). Based on the water content, which was not appreciably different between the control and NaCl treatment, it is suggested that a decrease in the stomatal conductance, which leads to the decrease in the transpiration rate and also to the decrease in the photosynthetic rate is the mechanism that sago palm used to avoid water loss and maintain the water status in the plant body under saline conditions. The decrease in the photosynthetic rate can be interpreted as a trade off with the decrease in the transpiration rate. The decrease of the photosynthetic rate might be caused by 2 main factors, i.e. reduction in the stomatal conductance and low chlorophyll production in the leaves.

In conclusion, sago palm growing under NaCl treatment can maintain a low Na⁺ concentration in the leaflets by storing Na⁺ mainly in the roots and petioles at lower leaf positions with a fair reduction of the growth rate. This accounts for the slow morphogenesis and the decrease in the stomatal conductance and results from the maintenance of the water status in the plant body.

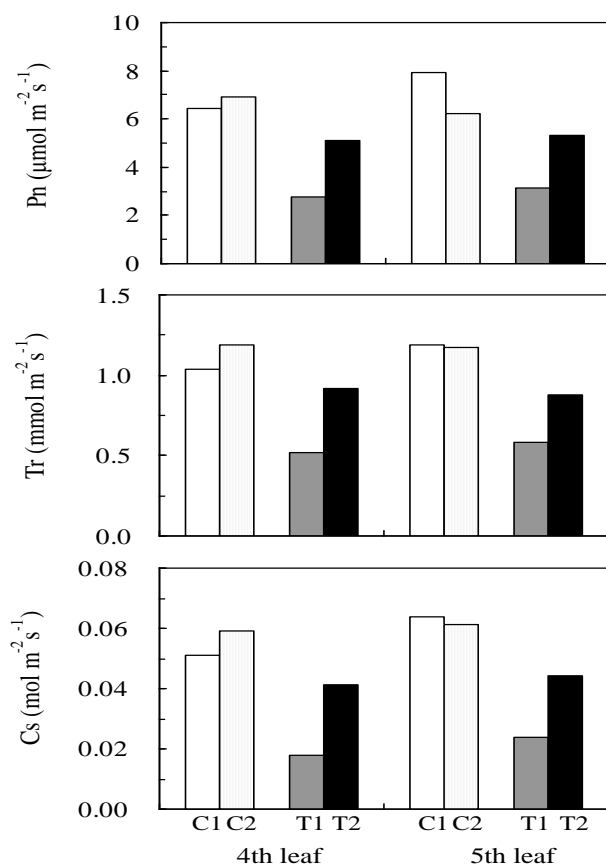


Figure 6. Photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Cs) of the 4th and 5th leaves from the top of the control plants (C1, C2) and treated plants (T1, T2) at 900 PAR in October 2007.

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