SAGO PALM 14: 10－19 (2006)

Response of Sago Palm (*Metroxylon sagu* Rottb.) to NaCl stress

Ritsuko YONETA, Masanori OKAZAKI and Yoshiharu YANO

Graduate School of Bio-Applications and Systems Engineering

Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

Abstract: Sago palm (*Metroxylon sagu*) plants grown in a soil culture of 5 L plastic pots were dipped into 6 L plastic pots with a culture solution containing sodium chloride (NaCl) at a concentration of 0, 10, 50, 100, 200 or 400 mM to provide salt stress. The growth parameters, the water potential in sago leaflets, proline (Pro), glycinebetaine (GB), soluble chloride ion (Cl^-), total sodium ion (Na^+) and potassium ion (K^+) in sago leaflets and roots were measured to evaluate the response of sago palm to NaCl stress. The exchangeable Na^+ and K^+ concentrations in soil were also determined to estimate the uptake by sago palm. The greatest growth was obtained in the 10 mM NaCl culture solution, based on the growth parameters and water potential in sago leaflets, which implied that sago palm requires a small amount of NaCl for vital growth. Pro was under the detection limit in leaflets and roots. However, a small amount of GB production was found in leaflets. Soluble Cl^- concentrations in leaflets increased exponentially with an increase in NaCl concentrations. Na^+ was exponentially accumulated up to 100 mM in the roots and was not transported to the leaflets, indicating that sago palm can uptake and store Na^+ in roots using a regulation mechanism to maintain the appropriate Na^+concentration in leaflets. In addition, sago palm was able to uptake K^+ from the soil culture, and K^+ was accumulated in leaflets through the root systems in response to NaCl stress.

Key words: Compatible solute, *Metroxylon sagu*, Osmoregulation, Salt stress, Selective potassium uptake

塩化ナトリウムストレスに対するサゴヤシ (*Metroxylon sagu* Rottb.) の応答

米田理津子・岡崎正規・矢野義治

東京農工大学大学院BASE　〒184-8588　東京都小金井市

要旨　サゴヤシの耐塩性を研究する目的で，土耕中のサゴヤシポットを0, 10, 50, 100, 200, 400 mMの塩化ナトリウム(NaCl)水溶液の入ったプラスチックポット中に浸漬し，温室内で長期間栽培して塩ストレスを与えた．1ヶ月ごとにサゴヤシの生長を測定し，また1年後に葉の水ポテンシャルと土壌のpH, EC, CEC, 水溶性Cl^-, 交換性Na^+, K^+およびサゴヤシ小葉・根中のプロリン(Pro), グリシンベタイン(GB), 水溶性Cl^-, 全Na^+, K^+を測定した．サゴヤシの生長因子および水ポテンシャルの測定結果から，10 mMのNaCl水溶液中で栽培したサゴヤシの生育が最も良好であった．NaClストレスに対してProの蓄積は全くみられず，GBは小葉で少量検出された．一方，サゴヤシは培地のNaCl濃度が増加するにつれて小葉にCl^-，根にNa^+を蓄積したが，Na^+の小葉への移行は認められなかった．このとき，サゴヤシはK^+を培地から多量に吸収し，小葉に移行・蓄積させた．これらの結果から，サゴヤシは高NaClストレス下において，Na^+を根から小葉へ移行させない機構を持ち，K^+を小葉に蓄積し，K^+を適合溶質の一つとして利用している可能性があると結論した．

キーワード　塩ストレス, サゴヤシ, 浸透圧調節, 選択的カリウム吸収, 適合溶質

Introduction

Under salt stress, water absorption and photosynthesis of most plants are inhibited in plants because of a decrease in the water potential in soils and the turgor pressure in cells. Furthermore, salt stress disturbs metabolic processes such as photosynthesis and inhibits the absorption of essential elements (Kaku 1996). Though almost all crops die in a culture solution containing 100 mM NaCl, so-called halophytic plants can live in a solution of over 100 mM NaCl (Matoh 1999) . Halophytic plants show a decrease in leaf area and the number of stomates, and the cuticular layers of leaves thicken under salt stress. There are two different physiological mechanisms to regulate salts in halophytic plants. Some halophytic plants do not uptake salts from their root systems, while others actively take salts from their root systems to increase the osmotic pressure in leaves to uptake water. In the latter case, absorbed NaCl in leaf cells is transported to the vacuoles to prevent metabolic disturbances. That is, to cancel the gradient of osmotic pressure between the vacuoles and the cytoplasm caused by high salt concentrations, osmoregulating compounds, which do not inhibit metabolic activity, accumulate in the cytoplasm (Matoh 1999). In osmoregulating compounds, low-molecular-weight compounds which do not inhibit various metabolic activities are called compatible solutes when accumulated in high concentrations in cells (Nakamura and Takabe 1999). Proline (Pro), glycinebetaine (GB), sugar alcohols and inorganic ions are well-known compatible solutes (Wada 1999). K^+ absorption is inhibited in common crops under salt stress. However, salt-tolerant crops have the ability to selectively absorb K^+ well (Tadano 1986).

Sago palm belongs to a Palmaceae which grows in tropical Southeast Asia within 10 degrees north and south of the equator. Because sago palm provides 100 to 200 kg dry starch from its stem, it is considered an important agricultural plant for the 21st century. Since sago palm can grow in brackish water areas, it is believed to have salt tolerance (Yamamoto 1998). Under salt stress, most plants can regulate osmotic pressure to avoid high pressure and ion stress using compatible solutes, such as Pro and GB, to control osmotic pressure. However, information on osmosis in sago palm is limited. Flach et al. (1977) showed that young sago palm seedlings can withstand electrical conductivity (EC) of 10 mS m^{-1} without damage; the sago palm, therefore, can be considered fairly tolerant of saline conditions. Hirotsu et al. (2002) reported that two species of genus *Metroxylon*, *M. sagu* and *M. salomonense* (Warb.) Becc., produced Pro and GB. Yoneta et al. (2003) reported that *M. sagu* under salt stress (0～200 NaCl mM) produced GB (0.16～3.0 mg kg^{-1} DW) in the leaflets. Although these numerical values for GB are thought to be low compared to those of other halophytic plants, sago palm may use other compatible solutes, excluding Pro and GB, under salt stress if the plant is adapted to saline conditions. Ehara et al. (2003) reported that Na^+ was not transferred to leaflets from the roots of sago palm, although sago palm absorbed Na^+ in a culture containing various concentrations of Na^+ (0 to 872 mM of NaCl solution) when the plant underwent Na^+ treatment for 1 month. The objective of this study was to elucidate the response of sago palm to salt stress.

Materials and Methods

Greenhouse cultivation of sago palm.

Sago palm seedlings were collected from Papua New Guinea. They were transplanted to cultivation pots with soil and were cultivated in the greenhouse at the Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo. On Oct. 16, 2001, the sago palms were cultivated in 5 L plastic pots containing a soil culture. Two kg of Akadama Soil, taken from the subsurface horizon of Andosols, and 0.5 kg of pumice were used as soil cultures. Five g of MagAmp K (Hyponex Japan), a slow-release fertilizer, was applied. This fertilizer contains 13% nitrogen as NH_4-N, 42% phosphorus as P_2O_5, 13% potassium as K_2O and 32% magnesium as MgO. The

exchangeable ions and CEC in the original soil (Akadama Soil) are shown in Table 1. On May 28, 2002, the pots with the sago palms were moved and dipped in a NaCl solution of 0, 10, 50, 100, 200 or 400 mM in 6 L plastic pots with no replication. The water-saturated conditions with the NaCl solutions were maintained, and the NaCl solutions were renewed every month. The final samplings of soils, leaflets and roots were performed on December 9, 11 and 4, 2003, respectively. The soil cultures were continued, and dead leaves were removed on July 11, 2003. The temperature in the greenhouse was maintained at almost 25 ºC using an agricultural heating mat system (Daikin Industries FRJ600PK). The sago palms in the 400 and 200 mM NaCl solutions died 3 days and about 13 months after NaCl treatment, respectively.

Table 1. Exchangeable bases and CEC in original soil (Akadama Soil).

		($cmol_c\ kg^{-1}$ dry soil)
Exchangeable base	Na^+	0.235
	K^+	0.609
	Ca^{2+}	5.30
	Mg^{2+}	0.699
CEC		47.1

Determination of growth parameters.

The height, number of living and dead leaves and leaflets, stem diameter at ground level and length of leaves were determined monthly during the treatment period. The number of living leaves and leaflets, as well as the maximum number of living leaflets on each sago palm, was counted. Second leaflets of the living leaves were cut, and their water potential was measured by the pressure chamber method using a plant moisture tensiometer (Daiki DIK-7002).

Analysis of soil samples.

Soil samples were air-dried and sieved using a sieve with a 2-mm mesh and were analyzed 18 months after the initial cultivation. The soil pH (1:2.5, soil / water) was measured with a portable pH-meter (Horiba B-212). EC (1:5, soil / water) was measured with a conductivity meter (TOA Electronics CM-5S); EC (1:1, soil / water) was measured with a portable conductivity meter (Horiba B-173). CEC was measured by the Schorenberger method (Kamewada 1997). Exchangeable Na^+ and K^+ were extracted using 100 mL of 1 M ammonium acetate (pH 7.0) from 5 g of soil and were determined by an atomic adsorption spectrophotometer (AAS, Hitachi Z-5010). Soluble Cl^- was extracted using 25 mL of deionized water from 5 g of soil and was determined by capillary electrophoresis (CE, Waters Quanta 4000E).

Extraction and determination of Pro and GB from sago palm samples.

Pro and GB were analyzed according to Nishimura et al. (2001) and Hirotsu et al. (2002). About 2 to 3 g of fresh sago leaflet and root samples were extracted with 7 to 8 mL deionized water in a 10 mL glass tube in a water bath at 80 ºC for 20 min. After cooling, the supernatant was filtered through a 0.45 μm membrane filter (Millipore Millex-HV Durapore Sterile EO). To 0.2 mL of the filtrate was added 0.05 mL of 500 mM sodium dihydrogen phosphate solution filtered through a 0.45 μm membrane filter (Advantec Toyo Mixed Cellulose Ester Type Membrane Filter), including 0.25 mM benzylamine as an internal standard.

Pro and GB in leaflets and roots were determined by CE (Waters Quanta 4000E). A fused-silica capillary 50 μm in diameter (total length 80.0 cm; effective length 75.0 cm) at 25 ºC was used to separate each peak well. The samples were injected in the hydrostatic mode (100 mm, 60 sec). The applied potential was 25 kV. The peaks of the samples were monitored at 185 nm with a positive power supply by a direct UV method. Migration time was 30 minutes. A solution of 100 mM sodium dihydrogen phosphate (filtered through a 0.45 μm membrane filter, Advantec Toyo Mixed Cellulose Ester Type Membrane Filter) at pH 2.25 was used as the electrolyte solution. Pro and GB were identified in the electropherogram with the standard addition technique and determined by linear regression curves of peak areas.

Determination of soluble Cl^-, total Na^+ and K^+ in sago palm samples.

To determine soluble Cl^- in leaflets according to Wada et al. (2003), sago leaflets growing in 0 to 100 mM NaCl solution were supplied. The sago palm growing in 200 mM NaCl solution died before sampling took place. About 0.5 g of fresh leaflets was extracted with 10 mL deionized water in a 10 mL glass tube in a water bath at 80 ºC for 30 min. After cooling, the supernatant was filtrated with a 0.45 μm membrane filter (Millipore Millex-HV Durapore Sterile EO) and soluble Cl^- was determined by CE (WaterS Quanta 4000E). A fused-silica capillary 75 μm in diameter and 60.0 cm in length (effective length 55.0 cm) at 25 ºC was used. The samples were injected in the hydrostatic mode (100 mm, 60 sec.). The applied potential was 20 kV. The peaks of the samples were monitored at 254 nm with a negative power supply by an indirect UV method. Migration time was 4.5 minutes. A mixed solution of sodium chromate and sulfuric acid (filtered through a 0.45 μm membrane filter, Advantec Toyo Mixed Cellulose Ester Type Membrane Filter) was used as an electrolyte solution.

To determine the amounts of total Na^+ and K^+ concentrations in leaflets and roots, fresh leaflets and roots were dried in a forced convection oven (Advantec Toyo FC-612) at 70 ºC for 3 days, although the sago palm grown in 200 mM NaCl solution died before the last sampling; dead plants were used for further analysis. About 0.1 g of oven-dried samples was digested for 1 week with 10 mL of 100 mM nitric acid and an additional 0.1 M nitric acid to make a constant volume of 100 mL. Na^+ and K^+ concentrations were determined by AAS (Hitachi Z-5010).

Evaluation of the absorption rates of Na^+ and K^+ by sago palm.

The concentrations of Na^+ and K^+ in the leaflets and roots before and after 450 days of NaCl stress were used to evaluate the absorption rates of Na^+ and K^+. The concentrations of Na^+ and K^+ in the first leaflet from the top were 0.736 mmol kg^{-1} DW and 268 mmol kg^{-1} DW, respectively. In the forth leaflet 6.43 mmol kg^{-1} DW for Na^+ and 84.5 mmol kg^{-1} DW for K^+ were obtained. From these results, the mean values, 3.59 mmol kg^{-1} DW for Na^+ and 176 mmol kg^{-1} DW for K^+, were used to estimate the adsorption rates. Meanwhile, the concentrations of Na^+ and K^+ in the roots were 13.7 mmol kg^{-1} DW and 107 mmol kg^{-1} DW, respectively.

Results

Growth response of sago palm to NaCl salinity.

The heights of the sago palms increased until Sep. 2002 (Fig. 1). The sago palm grown in 200 mM NaCl solution was obviously shorter than the others. The sago palm grown in 10 mM NaCl solution was the tallest after Sep. 2002. The growth parameters are shown in Table 2. The number of living leaves on the sago palms grown in 0 and 10 mM NaCl solutions was larger than those of others. However, a larger number of leaflets were recorded on sago palms grown in 50 and 100 mM NaCl solutions. Sago palms grown in 0 and 10 mM NaCl solutions had larger stem diameters at ground level. The longest leaf was found on the sago palm grown in 50 mM NaCl solution.

The water potential in leaflets of sago palm ranged from -2.00 to more than -3.80 MPa, and the highest water potential was observed in the sago palm grown in 10 mM NaCl solution (Table 3).

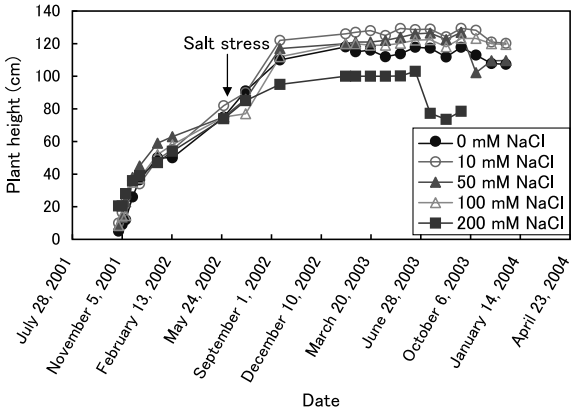

Fig. 1. Changes in the height of sago palms under different saline concentrations.

● 0 mM NaCl; ○ 10 mM NaCl; ▲ 50 mM NaCl; △ 100 mM NaCl; ■ 200 mM NaCl. Initial cultivation under NaCl stress was started on May 28, 2002.

Table 2. Sago growth parameters (except height)

Parameter	NaCl concentration (mM)	Feb. 17, 2003	Mar. 19, 2003	Apr. 18, 2003	May. 18, 2003	Jun. 17, 2003	Jul. 17, 2003	Aug. 18, 2003	Sep. 17, 2003	Oct. 17, 2003	Nov. 17, 2003	Dec. 16, 2003	Jan. 15, 2004
Weight (g)	0	5222	5335	5371	5378	5336	5203	5231	3616	5080	5133	5121	5121
	10	5417	5507	5549	5557	5546	5326	5370	4362	5210	5445	5388	5333
	50	5022	5119	5171	5145	5191	4802	4888	4921	5002	5047	4960	4897
	100	4536	4585	4604	4612	4591	4285	4328	4304	4326	4352	4298	4198
	200	4739	4766	4741	4606	4682	4562	−	−	−	−	−	−
Number of living leaves	0	6	5	5	4	5	5	4	5	4	4	4	3
	10	6	5	5	4	5	5	5	5	5	5	4	5
	50	5	5	3	3	3	3	3	4	4	4	3	3
	100	5	5	4	4	4	3	3	3	3	3	2	1
	200	3	3	3	3	1	1	−	−	−	−	−	−
Number of dead leaves	0	0	1	1	2	2	0	1	0	1	1	1	1
	10	0	1	1	1	2	0	0	1	1	1	2	0
	50	0	0	2	2	2	0	0	0	0	0	1	1
	100	1	2	2	3	3	0	0	0	0	0	1	2
	200	2	2	2	2	4	0	−	−	−	−	−	−
Number of leaflets	0	16	16	17	16	18	18	20	22	22	22	22	22
	10	16	16	16	14	12	14	18	18	18	18	18	18
	50	18	18	18	17	17	17	25	26	26	25	25	26
	100	16	16	18	19	19	21	23	24	24	24	24	24
	200	16	16	14	14	13	0	−	−	−	−	−	−
Stem diameter at ground level (cm)	0	4.5	5.0	5.0	5.9	6.5	5.4	4.9	6.4	6.5	6.1	5.6	5.6
	10	5.0	5.1	5.5	5.7	6.4	5.4	4.7	6.1	6.1	5.9	5.8	5.5
	50	3.5	4.0	4.5	5.1	5.3	5.5	4.0	6.1	5.9	5.9	5.2	5.1
	100	3.5	3.9	4.0	5.4	5.4	6.1	4.5	6.1	5.2	5.7	5.9	5.6
	200	3.4	3.4	3.5	3.8	3.8	4.6	−	−	−	−	−	−
Length of leaves (cm)	0	※	※	39.0	40.0	43.0	42.9	37.5	42.0	40.4	37.2	37.2	36.4
	10	※	※	35.0	36.0	37.0	40.8	35.9	40.8	41.3	38.2	38.2	41.3
	50	※	※	41.0	43.0	44.0	44.0	38.4	42.9	45.2	43.1	43.1	43.0
	100	※	※	40.0	40.4	42.2	41.0	36.6	40.8	41.5	38.5	38.5	30.2
	200	※	※	34.0	34.0	36.4	30.8	−	−	−	−	−	−

Soil was renewed and dead leaves were removed on July 11, 2003.
※ : no data
− : dead

Table 3. Water potential in leaflets of sago palm treated for 18 months.

NaCl concentation (mM)	Water potential (MPa)
0	−2.42
10	−2.00
50	<−3.80
100	<−3.80

Chemical properties of soils after cultivation.

The chemical properties of soil samples after 18 months of cultivation are shown in Table 4. The soil pH at different NaCl concentrations ranged from 8.4 to 8.9. The soil EC values (1:5 and 1:1) ranged from 0.32 to 5.1 and 0.85 to 5.2 dS m^{-1}, respectively, increasing as the NaCl concentrations increased. The CEC values of soil samples ranged from 49.1 to 61.8 $cmol_c$ kg^{-1}, with the exception of 71.5 $cmol_c$ kg^{-1} in the 50 mM pot. Soluble Cl^- and exchangeable Na^+ concentrations in soil ranged from 0.37 to 24.2 and 1.13 to 40.2 $cmol_c$ kg^{-1}, depending on the increase in the NaCl concentrations. Exchangeable K^+ concentrations in soil, ranging from 0.633 to 1.88 $cmol_c$ kg^{-1}, tended to decrease with increasing NaCl concentrations.

Osmoregulating compounds (Pro and GB) in leaflets and roots of sago palm.

Pro concentrations in both leaflets and roots were negligible. Small amounts of GB, however, were detected in sago leaflets grown in 10, 100 and 200 mM NaCl solutions, at concentrations of 2.89, 1.12 and 2.96 mM kg^{-1} DW, respectively.

Ion concentrations in leaflets and roots of sago palm.

Soluble Cl^- concentrations in leaflets ranged from 85.6 to 4160 mmol kg^{-1} DW, exponentially increasing as NaCl concentrations increased (Fig. 2).

The Na^+ concentrations in roots ranged from 45.0 to 1020 mmol kg^{-1} DW (Fig. 3). Na^+ concentrations tended to increase with increasing NaCl concentrations up to 100 mM NaCl solution and decreased at 200 mM NaCl solution. The Na^+ concentration in the sago palm grown in 100 mM NaCl solution was about 200 times that of the sago palm grown in 0 mM NaCl solution. On the other hand, Na^+ concentrations in leaflets were extremely low except in the sago palm grown in 200 mM NaCl solution.

Table 4. pH, EC, exchangeable Na^+ and K^+ and soluble Cl^- in soil treated for 18 months.

NaCl concentration (mM)	pH	EC (dS m^{-1})		CEC ($cmol_c$ kg^{-1} dry soil)	Soluble Cl^- ($cmol_c$ kg^{-1} dry soil)	Exchangeable base ($cmol_c$ kg^{-1} dry soil)	
		(1:5)	(1:1)			Na^+	K^+
0	8.5	0.32	0.85	51.6	0.368	1.13	1.88
10	8.7	0.83	1.6	51.4	2.36	8.02	1.37
50	8.7	2.2	3.8	71.5	8.77	23.9	1.54
100	8.9	3.0	4.7	61.8	13.2	36.5	0.852
200	8.4	5.1	5.2	49.1	24.2	40.2	0.633

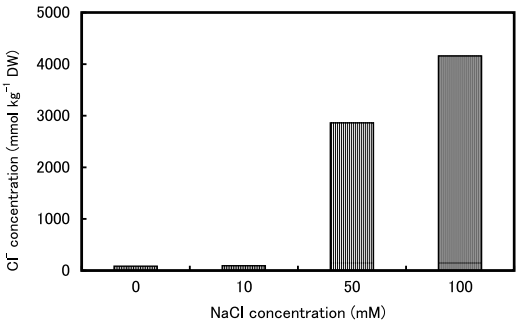

Fig. 2. Cl^- concentrations in sago palm leaflets treated for 18 months.

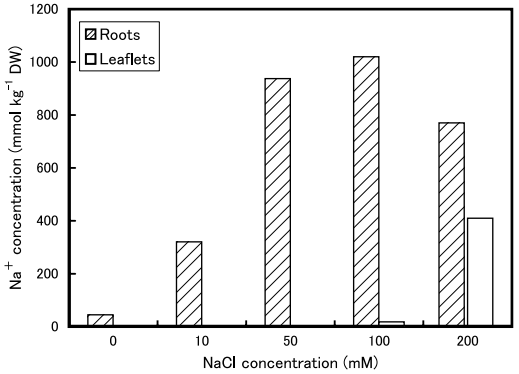

Fig. 3. Na^+ concentrations in sago palms treated for 14 to 16 months.

The K^+ concentrations in roots ranged from 12.9 to 190 mM kg^{-1} DW and depended on the increase in NaCl concentrations (Fig. 4). The K^+ concentrations in leaflets ranged from 183 to 735 mmol kg^{-1} DW and tended to increase as NaCl concentrations increased. Sago palms under NaCl stress accumulated Na^+ in roots and did not transport Na^+ to leaflets. Meanwhile, sago palms under NaCl stress (in the 50, 100 and 200 mM NaCl culture solutions) selectively absorbed K^+ and transported K^+ to the leaflets. However, K^+ uptake was not affected by the 10 mM NaCl stress from the K^+ concentration in the leaflets.

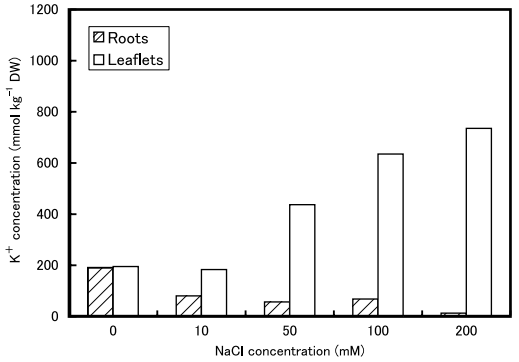

Fig. 4. K^+ concentrations in sago palms treated for 14 to 16 months.

Evaluation of the absorption rate of Na^+ and K^+ by sago palm.

The estimated absorption rates of Na^+ and K^+ by sago palms before and after 450 days of NaCl stress are shown in Table 5. The K^+ absorption rates by the roots of sago palm cultivated in 10 to 200 mM NaCl solution were -58.3 to -240 μmol kg^{-1} DW day^{-1}; larger amounts of K^+ were released as NaCl concentrations increased in solution. On the other hand, the K^+ translocation rates from the roots to the leaflets were 15.5 to 1430 μmol kg^{-1} DW day^{-1} and the Na^+ absorption rates by the roots were 69.6 to 2240 μmol kg^{-1} DW day^{-1}. Comparing to these translocation and absorption rates, the translocation rates of Na^+ to sago palm leaflets in 0 to 50 mM NaCl solutions were especially low, suggesting that neither a release of Na^+ from the leaflets nor a transformation of Na^+ occurred.

Table 5. Absorption rates of Na^+ and K^+ by sago palm.

NaCl concentration (mM)	Na^+ absorption rate (μmol kg^{-1} DW day^{-1})		K^+ absorption rate (μmol kg^{-1}DW day^{-1})	
	leaflet	root	leaflet	root
0	−4.48	69.6	41.6	186
10	−5.83	682	15.5	−58.3
50	0.877	2050	579	−111
100	32.3	2240	1020	−85.2
200	1040	1930	1430	−240

※ Only the rates of sago palm grown in 200 mM NaCl solution was calculated as "concentration devided 390 days."

Discussion

Sago palm (*Metroxylon sagu*), a tropical palm growing in brackish water areas and accumulating more than 200 kg fresh starch, seems to be somewhat salt-tolerant because it was able to grow for over 20 months in 100 mM NaCl solution (Table 1). According to the classification of crop tolerance to soil salinity (Maas 1985), sago palm is classified as "moderately sensitive" to "moderately tolerant" when the EC of the saturated soil extract and relative crop yield can be replaced by an EC of 1:1 (soil / water) extract and relative increase in height of sago palm to that grown in 0 mM NaCl solution during cultivation period, respectively. Maas (1985) introduced the concept of the relationship between relative crop yield and soil salinity;

$Y_r = 100 - B (EC_e - A)$

where Y_r is relative crop yield, A is the salinity threshold, B is the slope and EC_e is the EC of saturated soil extracts. In this study, the salinity threshold of sago palm was about 3.8 dS m^{-1}, the EC_e of cultivated soil of the sago palm grown in 50 mM, which is relatively high compared to general vegetable crops and fruit crops. The slope was 29 % per dS m^{-1} ,which was the largest value among the agricultural crops and caused an abrupt decline in growth. High salinity at 200 mM NaCl may have caused water stress and reduced the photosynthetic ability and the total growth of sago palm in the final stage of the experiment. Based on the growth parameters and water potential in leaflets, the largest growth occurred in the sago palm grown in 10 mM NaCl solution. A low NaCl concentration stimulates the activities of halophytic plants. Matoh (2002) reported that the growth of beet and Rhoses grass was stimulated by Na^+ even though K^+ supply was appropriate. The results of this study imply that sago palm requires a small amount of NaCl for better growth.

Although the highest GB concentration was detected in NaCl-stressed leaflets of sago palm grown in 200 mM NaCl solution, GB concentrations were not associated with the applied NaCl concentration. In this study, the GB and Pro concentrations were lower than those obtained by Hirotsu et al. (2002) because we improved the method for determining Pro and GB. However, the GB and Pro concentrations in sago palm were much lower than those in other halophytic plants. Cavalieri (1983) reported that large amounts of Pro and GB were detected in leaves of halophyte *Spartina alterniflora* Loisel., depending on the increases in the NaCl and nitrogen concentrations of the culture solution. The response of sago palm to excess NaCl seems to enhance the Na^+ concentrations in roots and to accumulate K^+ in leaflets but does not produce Pro and GB. This finding suggests that the tolerance of sago palm to excess NaCl is due to the ability to restrict Na^+ transport to the leaflets and to stimulate K^+ transport to the leaflets. The absorption rates of Na^+ and K^+ also suggest that a larger amount of K^+ was released as the NaCl concentration increased and that there was neither a release of Na^+ from the leaflets nor a transformation of Na^+. Na^+ concentrations found in this study were slightly higher in leaflets and remarkably higher in roots than those found by Ehara et al. (2003) under high NaCl concentrations because the cultivation procedures and periods were quite different. Ehara et al. (2003) reported, however, that Na^+ was not transferred to leaflets from the roots of sago palm under high NaCl concentrations, which was consistent with the results of this study. The new finding was that sago palm accumulated K^+ in leaflets, which are likely to use K^+instead of Na^+ to regulate osmotic pressure. Halophytic plants use two different strategies to deal with NaCl. One is to uptake Na intensively from the cultivation solution through the root and to transport Na to leaflets. The other strategy is to suppress the Na uptake and to hold Na in the root. Sago palm seems to use the latter strategy. Nishimura et al. (1999) classified several wild plants and crops grown in saline soil areas of China into two groups: GB-producing plants, which accumulate considerable Na, and those unable to produce GB, called a Na excluder. According to this definition, taking particular note of leaflets, sago palm is considered to be a Na excluder. The Na^+ concentrations in leaflets of the sago palm grown in 200 mM NaCl solution were high because the palm had already died, causing Na to accumulate passively. Excess NaCl significantly enhanced K^+ uptake by sago palm from the soil culture, resulting in low concentrations of exchangeable K^+ in soil 18 months after cultivation. Salt-torelant crops have the ability to selectively absorb K (Tadano 1986), and sago palm seems to have this ability.

Munns (2001) showed that there is no specific Na^+ transporter and Na^+ entry is gained by competition with other cation transporters, especially K^+. Na^+ enters the cell through high-affinity K^+ carriers or through low-affinity channels that are selective for K^+. The cation

channels, which are not selective for Na^+, could also allow a large amount of Na^+ to enter from high-NaCl solutions (Munns 2001). Na^+ reaches the xylem through the apoplastic and symplastic pathways (Arima 2002) and is transported to the leaves. However, several plant species, including maize (Drew and Lauchli 1987), reed (Matsushita and Matoh 1991) and sago palm (Ehara 2003), trap Na^+ in the mesocotyl tissues between the root and shoot or in the stem during upward transport. Under NaCl stress, sago palm may have some mechanism which is responsible for restricting Na^+ transport and for selectively absorbing K^+ through the K^+-carrier, K^+-selective channels and unselective channels (Munns 2001) and finally accumulating it in the cytoplasm of leaflets (Jeschke 1984) to protect against high salinity. The selective uptake of K^+ over Na^+ was observed in sago palm, and it is important for the salinity tolerance of sago palm. Sago palm also has the ability to selectively uptake K^+ from the cultivation solution, even under high salinity, and it can transport K^+ to the leaflets. The K^+-Na^+ ratios of sago leaflets grown in 0 to 100 mM NaCl ranged from 2 to 190 (Table 6), markedly higher than those of wheat genotype leaves grown in 150 mM NaCl (Munns 2001) and those of *Phragmites communis* (Takahashi 1991). Thus, sago palm may have high K^+ selectivity in a solution with a high NaCl concentration. Further study with regard to plants characterized by low Na^+ and high K^+ uptake is required.

Table 6. $K^+ - Na^+$ ratios of sago leaflets.

NaCl concentration (mM)	K^+–Na^+ ratio
0	124
10	190
50	110
100	35
200	2

Acknowledgement

We appreciate Mr. Anthony Power, Sago Industries Limited, Port Moresby, Papua New Guinea, for giving us sago palms and Dr. Toshiaki TADANO, Tokyo University of Agriculture, for determining water potential.

References

Arima, Y. 2002 Absorption and Transfer. *In: Dictionary of Plant Nutrition and Fertilizer*. (Editorial Board of Dictionary of Plant Nutrition and Fertilizer ed.) Asakurasyoten (Tokyo) 127-136. (in Japanese)

Cavalieri, A. J. 1983 Proline and glycinebetaine accumulation by *Spartina alterniflora* Loisel. in response to NaCl and nitrogen in a controlled environment. Oecologia 57: 20-24.

Drew, M. C. and A. Lauchli 1987 The Role of the mesocotyl in sodium exclusion from the shoot of Zea-Mays-L (CV PIONEER 3906). Journal of Experimental Botany 38: 409-418.

Ehara, H., M. Matui and H. Naito 2003 Absorption and translocation of Na^+ in sago palm under NaCl treatments. Sago Palm 11: 35-36. (in Japanese)

Flach, M., F. J. G. Croops, and G. C. van Roekel-Jansen 1977 Tolerance to salinity and flooding of sago palm seedings; Preliminary investigations. *In: Sago-76: Papers of the 1st International Sago Symposium*. (Tan K. ed.) Kemajuan Kandi Sdn. Bhd., Kuala Lumper 190-195.

Hirotsu, S., M. Okazaki, K. Toyota, Y. Yano, K. Seki, H. Tsujimoto, Y. S. Ham and A. P. Power 2002 Determination of osmoregulating compounds in sago palm. *In: Abstracts of the 11th Conference of the Japanese Society of Sago Palm Studies*. Japanese Society Sago Palm Studies (Tokyo) 11-16.

Hirotsu S. 2003 Ion contents in *Spartina alteniflora* and salt, desiccation stresses. *In: Graduation thesis of Tokyo University of Agriculture and Technology*. 23-31. (in Japanese)

Jeschke, W. D. 1984 K^+-Na^+ exchange at cellular membranes, intracellular compartmentation of cations, and salt tolerance. *In: Salinity tolerance in plants, strategies for crop improvement*. (Staples R.C. and G.H. Toenniessen ed.) John Wiley & Sons (New York) 37-66.

Kamewada, K. 1997 Exchange capacity of soil (CEC, AEC). *In: Methods of Soil Environmental Analysis*. (Editorial Board of Methods of Soil Environmental Analysis ed.) Hakuyusya (Tokyo) 208-215. (in Japanese)

Kumar, S. G., A. M. Reddy and C. Sudhakar 2003 NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Science 165: 1245-1251.

Kaku, S. 1996 Salt stress. *In: Plant Physiology*. (Masuda Y. and M. Kikuyama ed.) The Society for the Promotion of the University of the Air (Tokyo) 104-105. (in Japanese)

Maas, E. V. 1985 Crop tolerance to saline sprinkling water. Plant and Soil 89: 273-284

Matoh, T. 2000 Salt tolerant mechanism in halophyte. Agriculture and Horticulture 75: 783-786. (in Japanese)

Matoh, T. 2002 Sodium. *In: Dictionary of Plant Nutrition and Fertilizer*. (Editorial Board of Dictionary of Plant Nutrition and Fertilizer ed.) Asakurasyoten (Tokyo) 119-120. (in Japanese)

Matsushita, N. and T. Matoh 1991 Characterization of Na^+ exclusion mechanisms of salt-tolerant reed plants in comparison with salt-sensitive rice plants. Physiologia Plantarum 83: 170-176.

Mulholland, M. M. and M . L. Otte 2001 Erratum to "The effects of nitrogen supply and salinity on DMPS, glycine betaine and proline concentrations in leaves of *Spartina anglica*". Aquatic Botany 1575: 1-8.

Munns, R. 2001 Avenues for increasing salt tolerance of crops. *In: Plant Nutrition-food security and sustainability of agro-ecosystems*. (Horst W. J. et al. ed.) Kluwer Academic Publishers (Netherlands) 370-371.

Nakamura, T. and T. Takabe 1999 Glycinebetaine in plants. *In: Environmental Response in Plants − Survival Strategy and Its Molecular Mechanism −*. Shujunsya (Tokyo) 86-90. (in Japanese).

Nishimura, N., J. H. Zhang, K. Takeda, N. Ichizen, K. Tian, T. Tadano and S. Yamazaki 1999 Contents of glycine betaine and other solutes in the wild plants and crops sampled in Huang-Huai-Hai Plain. *In: Proceedings of China-Japan Joint Symposium: Development of Technology for Sustainable Biological Production in Saline Soil Areas of Huang-Huai-Hai Plain in China*. The Chinese Academy of Sciences (Beijing) 158-170.

Nishimura, N., J. H. Zhang, M. Abo, A. Okubo and S. Yamasaki 2001 Application of capillary electrophoresis to the simultaneous determination of betaines in plants. Analytical Science 17: 103-106.

Tadano, T. 1983 Salt tolerance of crop plants and its mechanism. Chemistry and Biology 21: 439-445. (in Japanese)

Tadano, T. 1986 Genetic resources and biotechnology in improvement of stress tolerance of crop plants, 3-b, physiological mechanisms of salt tolerance in crop plants. Agricultural Technology 41: 337-342. (in Japanese)

Tadano, T. 1987 Salt tolerance in plants. Report of Agricultural Research Administration in Tropical Areas 59: 1-14. (in Japanese).

Takahashi, E. 1991 Excess of sodium − salt damage and salt tolerance − . *In: Modern Plant Physiology 4, Environmental Response*. (Shinmen T. ed.) Asakurasyoten (Tokyo) 132-141. (in Japanese)

Takahashi, E. 2002 Potassium. *In: Dictionary of Plant Nutrition and Fertilizer*. (Editorial Board of Dictionary of Plant Nutrition and Fertilizer ed.) Asakurasyoten (Tokyo) 81-85. (in Japanese)

Wada, K. 1999 Mechanism of salt tolerance. Heredity 53: 58-62. (in Japanese)

Wada, N., J. Zhang, N. Jinno, A. Okubo and S. Yamazaki 2003 Component analysis of a halophyte, *Suaeda japonica*, grown on the shore of Ariake Sea. Bunseki Kagaku 52: 843-846. (in Japanese)

Yamamoto, Y. 1998 Sago Palm, Tropical agriculture series, Tropical crops directory No. 25. Association for International Cooperation of Agriculture and

Forestry (Tokyo) pp.7-9. (in Japanese)

Yoneta, R., M. Okazaki, K. Toyota, Y. Yano and A. P. Power 2003 Glycine betaine concentrations in sago palm (*Metroxylon sagu*) under different salt stress conditions. *In: Abstracts of the 12th Conference of the Society of Sago Palm Studies*. Japanese Society Sago Palm Studies (Tokyo) 18-22.