Kasetsart J. (Nat. Sci.) 27: 500-507 (2536)

Evaluation of Salt Tolerance in Four Species of Annual Sesbania

Patcharaporn Phupaibul [1], Toru Matoh and Eiichi Takahashi[2]

ABSTRACT

Hydroponic culture experiment was performed to evaluate salt tolerance potential of four species of annual Sesbania; *S. rostrata*, *S. cannabina*, *S. speciosa* and *S. aculeata*. Plant growth responded to salinity differently from species to species. About 24 to 58% reduction in growth was found at moderate salinity (30 mM). Among four species, *S. rostrata* showed the most favourable growth even under high salinity, followed by *S. cannabina*. The lower Na accumulation in the leaves than in other parts was apparently an adaptive feature in these legumes under salt stress condition. The changes in other mineral content differed from parts to parts. K and N content were adversely affected by the increasing salinity. A drastic decrease in Ca, Mg and P content could not be found. Leaf sap determination indicated that osmotic pressure in all species increased with the rising external osmotic pressure. The osmotic adjustment were mainly achieved by Na salt accumulation. The different response was found in S. aculeata which accumulated lower Na and higher K in its leaf sap. A notable role of organic constituents in osmotic adjustment was not observed.

Key words : annual sesbania, salt tolerance.

INTRODUCTION

A greater loss of organic matter and low fertility in the tropical soil need to be rectified (Arunin *et al.*, 1988). Instead of chemical nitrogen fertilizer, green-manuring with leguminous plants is the best option and effective means for providing available nitrogen to soil. Sesbania species, fast-growing legume, are potential low-cost nitrogen source for cropping systems and also source of biomass for feed, fuel or fibre (Evan and Rotar 1987). They are recognized as tolerating soil salinity and alkalinity, flooding and soil waterlogging. They are quite promising plants for improving fertility of saline soil.

This study was conducted to evaluate salt tolerance in four species of annual sesbania.

MATERIALS AND METHODS

Seeds of *S. rostrata*, *S. cannabina*, *S. speciosa* and *S. aculeata* were obtained from Department of Land Development. Sesbania seeds were soaked in tap water and sown in vermiculite on 16 July 1989. One-week-old seedlings were transfered to hydroponic culture in 140 litter containers each consisted of 20 seedlings (5 seedlings/species). The composition of the nutrient solution was 0.48 mM KH_2PO_4, 0.48 mM KCl, 0.75 mM $CaCl_2$, 0.82 mM $MgSO_4$ and 1.4 mM NH_4NO_3 with micronutrient (Hoagland and Arnon cited 1983). After one week, NaCl was added at 30 mM and the concentration was raised every three days to produce the final concentration of 180 mM. The treatments were 0, 30, 100 and 180 mM NaCl addition. The nutrient solution was renewed every two weeks. Plants were grown under salinization in a greenhouse for one month, then harvested. After harvest, two of five seedlings were taken and the leaf sap was prepared as followed. Leaf samples were confined into air-tight plastic bags and boiled for three minutes, then depressed with garlic squeezer. Sap osmolarlity was measured by the freezing point depression method using Knauer Model M Semi-Micro Osmometer (Knauer, West Germany). Inorganic anion and cation were determined by ion chromatography (Shimadzu IC 6A) and atomic absorption spectrophotometry (Shimadzu AA 640). Sugar and amino acid content were determined by antrone (Dreywood 1946) and the ninhydrin method (Moore and Stein 1954), respectively.

The other seedlings were divided into leaves,

1 Central Laboratory and Greenhouse Complex, Kasetsart University,Kamphaeng Saen Campus, Nakhon Pathom.
2 Dept. of Agricultural Chemistry, Fac ulty. of Agriculture, Kyoto University, Kyoto.

stems and roots. All parts were dried in an oven (70°C) and powdered. Dried plant materials were digested by Novozamsky method (Novozamsky *et al.*, 1983). The Na, K, Ca and Mg concentrations from the digest were determined by atomic absorption spectrophotometry (Shimadzu AA-640). Total N and P contents were determined colorimetrically using the indophenol blue (Weatherburn, 1967) and molybdenum yellow method (Official methods of analysis of fertilizer, 1977), respectively. Seedlings grown under 180 mM NaCl were damaged by insect so only the osmolarity of the leaf sap was determined.

RESULTS

Growth

The influence of salinity on fresh weight of Sesbania plants grown under water culture was shown in Figure 1. Sesbania plants responded to salinity differently from species to species. The growth of plants grown hydroponically decreased 24% to 58% by moderate salinity (30 mM), and progressively decreased with the increasing salinity (Figure 1). *S. speciosa* had the highest biomass under the absence of salt, however its biomass was severely reduced by the increasing in salinity. The growth of *S. rostrata* as well as that of *S. aculeata* were affected by moderate salinity (30 mM) but it was not further reduced by 100 mM NaCl. Generally, *S. rostrata* showed the highest biomass even under high salinity, followed by *S. cannabina*. Table 1 showed that *S. cannabina* had more leaf weight than the other species in either salt. However, there was no significant difference in leaf weight between *S. cannabina* and *S. rostrata* at high salinity. Shedding the older leaves may be one of the response to salt stress of these legumes, however, this was not reflected in the leaf weight. *S. rostrata* had higher stem weight. The average value of shoot/root ratio decreased with the increasing salt in *S. rostrata* and *S. aculeata* but not in the other two species.

Mineral content in plant parts

The mineral constituents in different parts of Sesbania species under salt stress were summarized in Figure 2 - 4. Salinity induced Na accumulation in all plant parts, particularly in stem (Figure 2). Na content was found to be relatively low in leaf tissue of *S. aculeata* and *S. rostrata*. *S. speciosa* accumulated considerably high Na in all plant parts. Salt stress caused reduction in K concentration in all plant parts, especially in roots and stems. K in leaves slightly reduced at 30 mM NaCl and drastically decreased with the increasing salinity in all species. Though K was suppressed by salinity, its content was maintained above value to be essential for normal plant growth (Epstein 1972). *S. aculeata* maintained almost con-

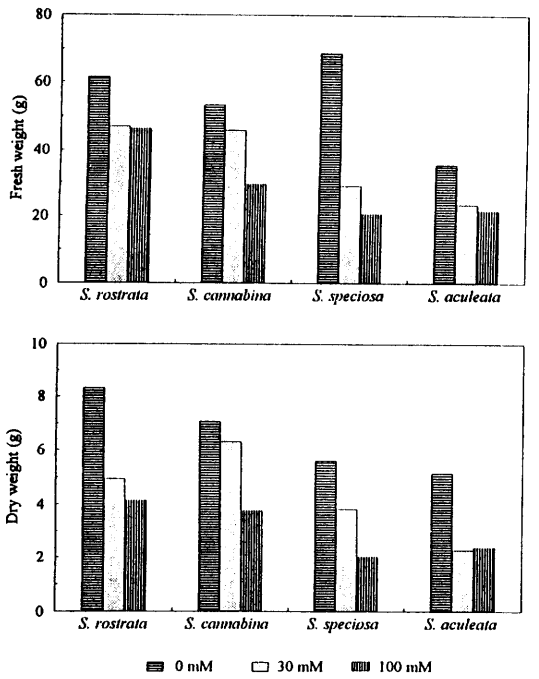

Figure 1 Effect of NaCl salinity on growth of Sesbania speices grown hydroponically.

stant K concentration in leaves up to 100 mm NaCl salinity. Ca concentration in all species was adversely affected by moderate salinity (30 mM) but notable increase in its concentration was found at high salinity (Figure 3). Reduction in Ca concentration induced by salinity was found in many plants (Maas and Grieve, 1987). From this experiment, the Ca concentration in all plant parts decreased at moderate salinity (30 mM) but increased again at 100 mM NaCl concentration without any explainable season. No obvious change in Mg concentration was detected in all plant parts. The judged salinity did not cause reduction in Mg concentration of all species. P tended to increase with the increasing salinity, especially in leaves and stems. The slight reduction in roots was observed (Figure 4). The total N was adversely affected by the increased salinity especially in stems (Figure 4). *S. cannabina* produced the highest N content in leaves while *S. rostrata* did in stems. In agreement with growth result, the total N content in all parts of *S. rostrata* and *S. aculeata* did not further decreased with the increase of salinity from 30 to 100 mM.

Solute composition of leaf sap

Osmotic pressure and concentration of inorganic and organic contituents of the sap were depressed from leaves of Sesbania at different salinity were shown in Table 2 and 3. Sap osmolarity was increased from 300 mOsmol kg^{-1}in the control plants to 600-800 mOsmol kg^{-1} in the 180 mM NaCl plants. The osmotic pressure was slightly increased at low salinity (30 mM) and drastically increased at high salinity (180 mM). With the increasing sap osmotic pressure, Na and Cl concentration were increased. The plants adjusted their osmotic pressure by storing Na salts, except for *S. aculeata*. K concentration increased only slightly or maintained constant up to 100 mM NaCl in outing medium, and it reduced at 180 mM in all species. The sum of Na and K salts always contributed approximately 80% to the sap osmotic pressure irrespective of salt treatment. The ratio of K to Na concentration (mM) of the sap was high in the control and the low salinity plants, particularly in *S. aculeata*. The ratio decreased with the increasing salinity. At high salinity (180 mM), the ratio drastically decreased to less than 1. The notably low concentration of Na was found in *S. aculeata*, while the reverse was obtained in *S. speciosa* at any salt concentration except 180 mM. Ca was adversely affected by salinity at low concentration but was raised when the salinity increased to 100

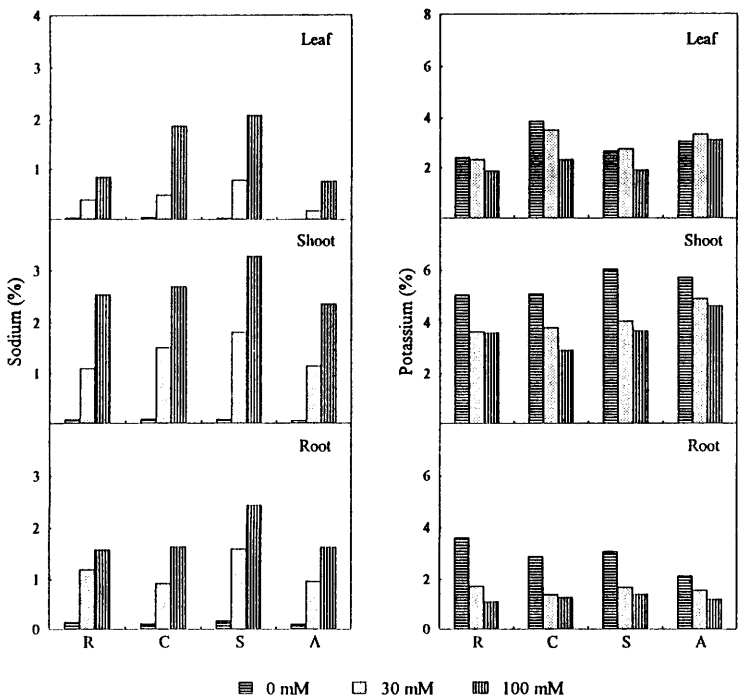

Figure 2 Changes in sodium and potassium concentrations in different parts of Sesbania species (R: S. rostrata, C: S. cannabina, S. S. speciosa, A: S. aculeata) by salinity.

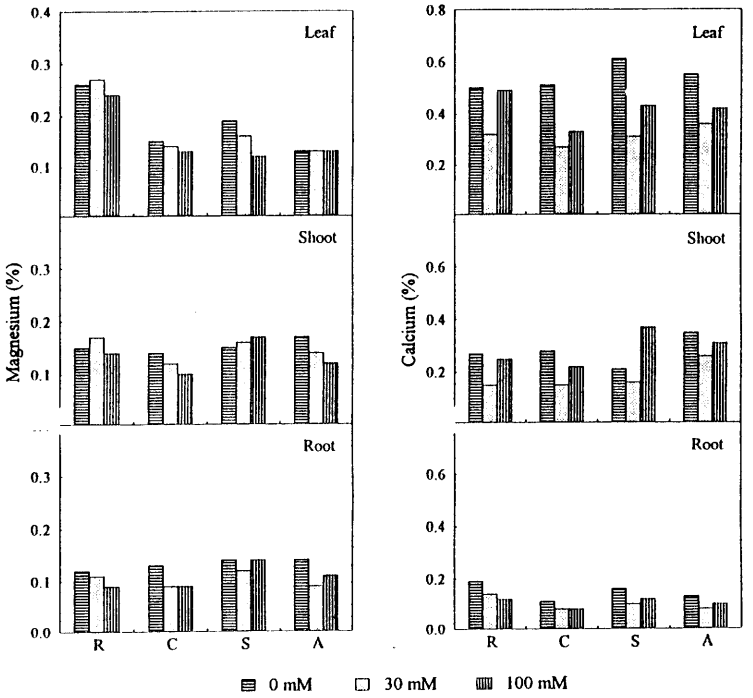

Figure 3 Changes in calcium and magnesium concentrations in different parts of Sesbania species (R: S. rostrata, C: S. cannabina, S. S. speciosa, A: S. aculeata) by salinity.

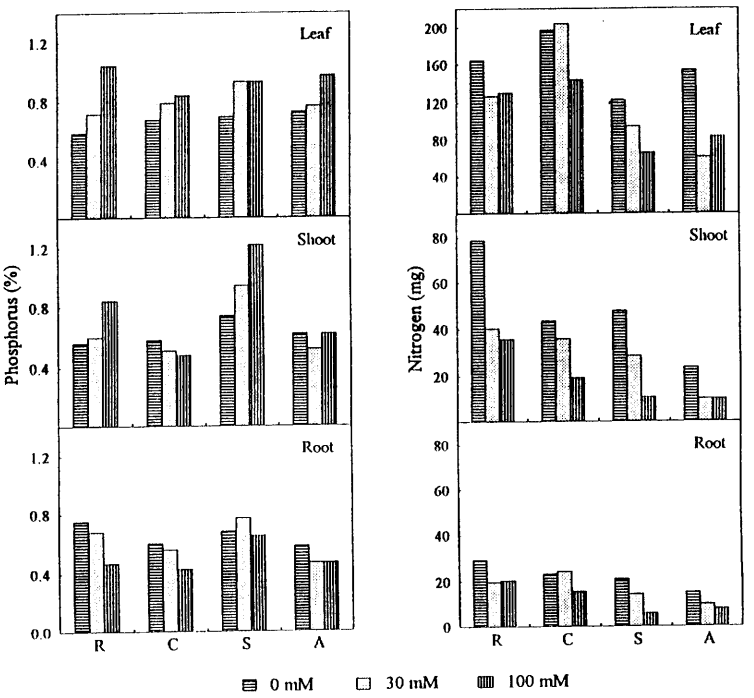

Figure 4 Changes in phosphorus and total nitrogen content in different parts of Sesbania species (R: S. rostrata, C: S. cannabina, S. S. speciosa, A: S. aculeata) by salinity.

Table 1 Growth of Sesbania species expressed in fresh weight (g) under different concentration of NaCl.

Sesbania species	NaCl	Leaf	Stem	Root	Shoot/Root
S. rostrata	0 mM	13.65+1.79	33.31+3.99	14.37+2.78	3.70+0.86
	30 mM	13.59+0.70	22.74+1.87	10.49+1.02	3.49+0.23
	100 mM	14.03+1.55	18.66+1.78	15.97+6.40	2.48+1.12
S. connabina	0 mM	19.41+0.37	20.72+2.36	12.97+3.47	3.80+1.48
	30 mM	19.63+2.97	15.96+1.41	10.25+3.09	4.39+1.44
	100 mM	13.37+3.27	8.32+1.53	7.34+2.27	3.76+1.11
S. speciosa	0 mM	17.59+1.93	32.16+3.30	19.00+1.44	2.84+0.45
	30 mM	10.10+2.74	10.72+3.28	8.25+1.87	2.94+0.72
	100 mM	9.37+0.83	5.25+1.10	4.75+1.31	3.83+0.90
S. aculeata	0 mM	15.06+1.98	13.57+2.12	6.36+1.79	5.69+1.66
	30 mM	10.77+2.11	7.07+1.25	5.50+1.89	4.03+2.01
	100 mM	10.37+0.24	6.08+0.54	5.31+1.32	3.63+0.92

mM. No obvious increase in total sugar concentration was detected. Slight increase in amino acids by the increased salinity treatment was found in *S. rostrata* and *S. aculeata*.

The leaves of Sesbania plants, subjected to 100 mM NaCl for one month, were taken from three different positions. Young leaves were consisted of apex and unexpanded leaves while the older ones were in lower position. The analyses of the sap osmotic pressure and solute concentrations were shown in Table 4. Sap osmotic pressure was lowest in the youngest leaves. However, there was significant difference in sap osmolarity of the second and third leaves in all species but not for *S. cannabina*. Na concentration increased as K decreased with leaf age. The sugar content decreased was in the second leaves of all species except in *S. aculeata*, and tended to increase in the third leaves to the similar concentration level of the youngest leaves. The amino acid composition was generally decreased with increasing plant age in *S. rostrata* and in *S. cannabina*.

DISCUSSION

In term of growth, *S. rostrata* was the most resistant to salt stress, followed by *S. cannabina*. Sesbania is mainly cultivated for green manure, purpose so the most desirable character should be high biomass production even under saline conditions. Due to the rapid growth, *S. rostrata* had an advantage over the other species especially under the salt stress condition. As Sesbania plants contained considerably high N in their leaves, the more leafy species was desirable for soil fertility improvement. *S. cannabina* was found to be suitable for this purpose. Moreover, its almost constant shoot/root ratio regardless of the increasing salinity suggested that *S. cannabina* could produce abundant nitrogen source even under the presence of high salt. *S. rostrata* and *S. cannabina* showed high potential to produce relatively favorable growth even under drought condition as well (data was not shown).

In the presence of salinity, Na concentration was relatively high in the stems and roots but quite low in the leaves. However, leaf sap analysis suggested that Sesbania plants employed Na salts as osmotica of their leaves. Accordingly, the plants may be provided with an excellent regulation system for Na transport to the leaves. Na exclusion from leaves was suggested to be one of adaptive features of leguminous plants to withstand salt stress condition (Lauchli, 1984). It allows the leaves to maintain normal physiological functions. Yeo and Flowers (1984) found that the lower shoot Na content in rice variety "Pokkali" was due to the diluting salt effect by its rapid vegetative growth. It was possible that *S. rostrata* could minimize the shoot Na content because of its rapid growth.

The N content in leaves and roots was affected differently among Sesbania plants (Karadge and Chavan 1983). They noted that the total N content in *S. aculeata* was not adversely affected by salinity. On the contrary, the present results showed that the total N content in all plant parts of the four species, grown hydroponically, was reduced by salinity. However, the shoot N content of *S. cannabina* was slightly inhibited by 100 mM NaCl in both N-supplied and nodulated plants (data was not shown). It indicated that Sesbania plants could fix N even under saline condition.

Table 2 Comparison of solute concentrations in *S. rostrata* and *S. cannabina* exposed to different concentration of NaCl.

	S. rostrata				*S. cannabina*			
	0 mM	30 mM	100 mM	180 mM	0 mM	30 mM	100 mM	180 mM
Osmotic (mOsmol)	378.00±3.00	372.00±72.00	480.00±15.0	675.00	352.50±7.50	405.00±0.00	480.00±45.00	600.00
Na (mM)	43.38±0.26	33.74±5.14	82.65±8.70	192.90	18.67±0.88	23.24±3.59	103.18±11.67	329.72
K (mM)	148.35±0.25	121.54±15.42	121.17±4.13	89.85	95.80±8.86	160.01±2.18	128.09±6.44	51.74
Ca (mM)	10.02±0.13	3.71±0.49	5.47±0.39	1.83	6.74±0.85	1.83±0.51	4.93±0.32	1.30
Cl (mM)	40.56±5.51	76.95±2.69	103.11+3.03	197.62	29.38±5.79	51.61±5.81	80.14±0.21	268.58
NO_3 (mM)	3.04±0.95	0.35±0.11	2.82±1.55	0.34	12.96±8.55	7.03±0.97	7.21±0.53	0.00
SO_4 (mM)	2.51±0.29	0.04±0.01	2.76±0.30	2.93	1.67±1.10	1.25±0.15	4.07±0.09	3.98
Total sugar (mM)	52.31±1.67	30.77±2.25	42.19±0.19	44.26	27.77±0.00	36.76±4.87	38.45±3.56	34.69
Amino acid (mM)	22.53±2.67	20.13±4.23	33.61±1.71	55.59	14.80±1.33	30.07±0.38	30.03±3.31	26.57
O.P of Na+K	364.29	295.03	387.26	537.23	217.49	348.18	439.41	724.71
1.9 x ([Na]+[K])								
Na+K contribution (%)	96.40	79.30	80.70	79.60	61.70	86.00	91.50	120.80
K/Na	3.42	3.60	1.47	0.47	5.13	6.89	1.24	0.16
External O.P.	8.00	60.00	190.00	315.00				

Table 3 Comparison of solute concentrations in *S. speciosa* and *S. aculeata* exposed to different concentrations of NaCl.

	S. speciosa				*S. oculeata*			
	0 mM	30 mM	100 mM	180 mM	0 mM	30 mM	100 mM	180 mM
Osmotic (mOsmol)	327.00±18.00	432.00±18.00	522.00±3.00	645.00	309.00±15.00	400.50±4.50	480.00±15.00	840.00
Na (mM)	26.53±4.19	72.85±8.05	134.81±3.37	255.70	17.46±2.10	25.28±10.84	46.21±13.46	300.12
K (mM)	112.43±13.35	133.55±12.87	114.62±3.39	46.88	131.12±9.47	164.92±2.97	167.66±16.87	74.07
Ca (mM)	17.59±6.47	2.42±0.19	4.18±0.35	2.69	9.76±1.71	4.19±0.31	6.15±0.53	2.77
Cl (mM)	27.46±0.40	72.33±21.73	121.23±8.32	282.59	55.07±083	93.28±9.50	94.59±0.75	197.88
NO_3 (mM)	18.56±7.20	4.67±2.92	7.45±1.93	0.00	14.60±5.30	9.63±0.02	5.01±2.98	0.51
SO_4 (mM)	9.78±0.51	6.42±1.57	10.83±0.65	3.51	4.08±0.58	1.52±0.19	9.22±1.33	5.01
Total sugar (mM)	54.19±1.67	35.45±8.06	47.63±1.50	29.64	36.39±2.62	33.58±0.94	43.13±1.12	36.76
Amino acid (mM)	18.91±0.42	33.76±7.81	40.20±2.59	31.29	18.76±1.03	23.18±0.72	35.67±0.19	49.57
O.P of Na+K	264.02	392.16	473.92	547.90	282.30	261.38	406.35	710.96
1.9 x ([Na]+[K])								
Na+K contribution (%)	80.70	90.80	90.80	89.10	91.40	90.20	84.70	84.60
K/Na	4.24	1.83	0.85	0.18	7.51	6.52	3.63	0.25

Table 4 Solute concentration in leaves of different position Sesbania species grown under 100 mM NaCl.

	S. rostrata			*S. cannabina*			*S. speciosa*			*S. aculeata*		
	first L.	second L.	third L.	first L.	second L.	third L.	first L.	second L.	third L.	first L.	second L.	third L.
Osmotic (mOsmol)	432.0	540.0	477.0	445.0	549.0	547.5	435.0	529.5	650.0	423.0	538.5	595.5
		±60.0	±12.0		±15.0	±67.5		±19.5	±4.0		±3.5	±3.5
Na (mM)	31.1	84.9	125.5	20.4	84.9	172.2	50.6	158.4	228.1	38.4	94.3	161.9
		±5.8	±5.0		±24.8	±28.2		±26.9	±25.6		±14.3	±30.4
K (mM)	140.1	151.4	107.8	141.9	129.1	81.2	138.0	71.0	33.2	133.5	134.8	85.8
		±4.4	±8.5		±20.1	±11.7		±15.4	±8.4		±7.1	±9.5
Total sugar (mM)	60.1	56.1	60.7	52.4	48.8	60.1	50.0	46.0	58.3	48.2	62.8	76.1
		±12.1	±2.8		±5.6	±0.5		±1.4	±8.4		±1.9	±0.2
Amino acid (mM)	55.7	53.2	33.6	78.3	88.7	31.8	52.8	62.2	58.1	55.2	82.3	69.0
		±7.5	±8.3		±14.9	±6.5		±2.1	±3.0		±2.3	±9.2

In all four species of Sesbania, the leaf sap osmolarity increased proportionally with the rising external osmotic pressure, suggesting that these plants could be regarded as an osmoconformer (Stewart and Ahmad, 1983; Gorham *et al.*, 1980). The osmotic adjustment was achieved by the increase in osmotica in order to maintain a constant difference in osmotic pressure between leaf sap and the external medium. Sesbania plants adapted themselves to salinity by using mainly Na salts for osmotic adjustment. Among four species, *S. aculeata* showed the different response, that was, it accumulated lower Na concentration and relatively higher K in the leaf sap. Gorham *et al.*, (1988), Karadage and Chavan (1983) reported that the increased Ca supply to *S. bispinosa* could lessen the toxic effect of Na and improve salt-damaged growth, because Ca functions as an osmoticum to increase the solute osmotic pressure. However, the contribution of Ca to the sap osmotic pressure was little in the current experiments. Organic compounds such as proline, glycinebetaine was synthesized in some halophyte to balance accumulated inorganic ion in the vacuole under salt stress condition (Storey and Jones 1979, Gorham *et al.*, 1980). *S. aculeata* was reported to accumulate pinitol under salt stress (Gorham *et al.*, 1984). However, evident increase in the total sugar and amino acid could not be observed from this experiment.

LITERATURE CITED

Arunin, S., C. Dissataporn, Y. Anuluxtipan and D. Nana. 1988. Potential of sesbania as a green manure in saline rice soils in Thailand. *In* Green Manure in Rice Farming, Proceeding of a symposium on sustainable agriculture, The International Rice Research Institute: 83-95.

Dreywood, R. 1946. Quantitative test for carbohydrate material. Ind. Eng. Chem. Anal. Ed. 18: p.499.

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons Inc., New York.

Evans, D.O. and P.P. Rotar. 1987. Productivity of Sesbania species. Trop. Agric. 64: 193-200.

Gorham, J., E. Mcdonnell and R.G. Wyn Jones. 1984 Pinitol and other solutes in salt-stressed *Sesbania aculeata*. Z. Pflanzenphysiol. 114: 173-178.

Gorham, J., L.L. Hughes and R.G. Wyn Jones. 1980. Chemical composition of salt-marsh plants from Ynys Mon (anglesey): the concept of physiotypes. Plant Cell Environ. 3: 309-318.

Gorham, J., O.S. Tomar and R.G. Wyn Jones. 1988. Salinity-induced change in the chemical composition of *Leucaena leucocephala* and *Sesbania bispinosa*. J. Plant Physiol. 132: 678-682.

Jones, J.B. 1983. A Guide for the Hydroponic and Soiless Culture Grower. Timber Press Portland, Oregon : 39-44 p.

Karadge, B.A. and Chavan P.D. 1983. Physiological studies in salinity tolerance of *Sesbania aculeata* poir., Physiol. Plant. 25: 412-418.

Lauchli, A. 1984. Salt exclusion: An adaptation of legumes for crops and pastures under saline conditions, *In* R.C. Staples and G.H. Toenniessen,(eds) Salinity Tolerance in Plants, Strategies for Crop Improvement by John Wiley and Sons Inc. New York: 171-186.

Maas, E.V. and C.M. Grieve. 1987. Sodium-induced calcium deficiency in salt-stressed corn. Plant Cell Environ. 10: 559-564.

Moore, S. and W.H.Stein 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 211: 907-913

Novozamsky I., V.J.G. Houba, R.V. Eck and W.V. Vark. 1983. A novel digestion Technique for multi-element plant analysis. Commun.Soil Sci. Plant Anal. 14: 239-248.

The National Institute of Agricultural Sciences official methods of Analysis of Fertilizers, 1977 Ministry of Agriculture and Forestry Tokyo: 21-29.

Stewart, G.R. and I. Ahmad. 1983. Adaptation to salinity in angiosperm halophytes. Essential and functional metals plants. *In* D.A. Robbs and W.S. Pierpoint.(eds) Academic Press, New York: 33 p.

Storey, R. and R.G. Wyn Jones. 1979. Responses of *Atriplex spongiosa* and *Suaeda monoica* to salinity. Plant Physiol. 63: 156-162.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. chem. 39: 971-974.

Yeo, A.R. and T.J. Flowers. 1984. Mechanisms of salinity resistance in rice as physiological criteria in plant breeding, *In* R.C.Staples and G.H. Toenniessen, Salinity Tolerance in Plants, Strategies for Crop Improvement, John Wiley & Sons Inc., New York, 1984. pp. 231-254