

Leaf area and ion contents of *Sesbania grandiflora* under NaCl and Na₂SO₄ salinity

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Abstract

A solution culture pot study was carried out to see the effect of two contrasting salt species i.e. NaCl and Na₂SO₄ on the growth and physiology of *Sesbania grandiflora*. Na⁺ contents in both the salt species were 0, 10, 20, 30, 40 and 50 mM. A significant difference was observed in case of osmotic pressure of sap which indicated that NaCl had a greater effect on osmotic pressure. There were non-significant differences between the two salt species for leaf area, K⁺ and Na⁺ contents and K⁺/Na⁺ ratio. A significant increase in osmotic pressure and Na⁺ content in sap was observed with increasing Na⁺ concentrations whereas in leaf area, K⁺ contents of sap and K⁺/Na⁺ ratio decreased with increasing Na⁺ concentrations. Leaf area of flag leaf was significantly higher in case of Na₂SO₄ as compared to NaCl treatments.

Keywords: *Sesbania grandiflora*; leaf area; salinity; ion contents; NaCl; Na₂SO₄.

Introduction

Sesbania grandiflora is commonly grown in India as a biofertilizer. Salinity is the major threat to crop production in these regions (Dhanapackiam & Muhammad Ilyas 2010a, 2010b). However, *S. grandiflora* is moderately tolerant to soil salinity but the Ece should not exceed 4 mm hos cm⁻¹ in the upper soil layer during germination. Yield decrease due to salinity is 0% of Ece 6.0, 10% at 7.4, 25% at 9.5, 50% at 13 and 100% at Ece 20 mm hos cm⁻¹. Ions which contribute to soil salinity include Cl⁻, SO₄²⁻, HCO₃⁻, Na⁺, Ca²⁺, Mg²⁺ and NO₃⁻ or K⁺. Most saline are high in Cl⁻ and Na⁺, though there are some soils in which the soluble salts consist mainly of Na⁺ and Mg²⁺ sulphates (Richards, 1954). According to study conducted by Shahid (1988), the salinity problem in India is mainly due to sulphates and chlorides and only partly due to carbonates and bicarbonates as these are relatively insignificant as compared to other anions. He found that SO₄²⁻ is dominating in all the salt crusts over the other anions. Among the cations, Na⁺ is dominant in all the salt crusts, accompanied by variable amounts of K⁺, Ca²⁺ and Mg²⁺. He concluded that sulphate minerals of sodium are dominant in soils of India, with lesser amounts of chlorides, where chloride is found as the second dominant anion. Most of the research work conducted on salt tolerance in crop plants uses NaCl in solution culture, which does not reflect true picture of salinity problems. Hence it was contemplated to investigate the effects of two contrasting salt species i.e. NaCl and Na₂SO₄ on the physiology of *S. grandiflora*.

Materials and methods

A solution culture pot study was carried out at Jamal Mohamed College, Trichy, Tamilnadu to see the effects of two contrasting salt species on the growth and physiology of *Sesbania grandiflora*. The test plant was *S. grandiflora* where as two salt species were NaCl at 0, 10, 20, 30, 40 and 50 mM and Na₂SO₄ at 0, 10, 20, 30, 40 and 50 mM

concentrations. In this experiment Na⁺ concentrations were comparable to 0, 10, 20, 30, 40 and 50mM in both salt species; hence the individual effects of Cl⁻ and SO₄²⁻ ions can be postulated.

The experiment was conducted under glass house conditions with no control of temperature and no supplementary lightings. Seeds of *S. grandiflora* were soaked for overnight in capillary matting under tap water during second week of July. Sprouted seeds were transferred to capillary matting on plastic supports, suspended over well aerated tap water containing 5 g of 'phostrogen'. Seven days old seedlings were transferred block by block during 3rd week of July to black painted plastics pots having 10 l of water capacity. The plants were supported by foam in 16 round holes at distance of 4.5 cm in black painted polystyrene sheets placed over the pots. In case of NaCl 4 M stock solution was made, while Na₂SO₄ could only by dissolved up to 2 M concentration. After two days of seedling establishment, salt stress was imposed by adding ½ doses in the first instance. The final dose was added after 3rd day of the first dose.

A modified long Ashton nutrient solution was used in combination with "Phostrogen" is a blended 10-27% NPK fertilizer, with 1.3%, Mg²⁺, 0.4% Fe³⁺ and 200 mg Kg⁻¹ Mn²⁺. Five grams of "Phostrogen" were added in 10 l of water directly into each pot at the time of transplanting while 10 g were added directly into each pot at the time of solution change after 21d of transplanting. Stock solution of FeEDTA (monosodium complex), MnSO₄.4H₂O, CuSO₄.5H₂O, ZnSO₄.7H₂O, H₃BO₃ and Na₂MoO₄.2H₂O, were made as 37.33, 22.3, 2.5, 2.9, 31.0, 58.5 and 1.21g l⁻¹ respectively. The composition of micronutrient in stock solution was Fe 2.8; Na 1.0; Mn 0.55; Cu 0.064; Zn 0.065; B 0.54; and Mo 0.048 µg cm⁻³ respectively.

It was observed during solution renewing that Na₂SO₄ stock solution solidified quickly as soon as it come in contact with air, which needed a vigorous shaking. Keeping this difficulty in mind both NaCl and Na₂SO₄

Table 1. Main effects of salt species on fully expanded young leaf of *S. grandiflora*

Parameters	Salt species		S.E. of significance LSD		
	NaCl	Na ₂ SO ₄	Mean	Level	(P=0.05)
Leaf area (cm ²)	2	2	0.37	NS	-
Osmotic pressure ⁻¹	645	424	30.40	1%	-
Na ⁺ (mM sap)	112	97	6.45	NS	-
K ⁺ (mM sap)	108	108	5.19	NS	-
K ⁺ /Na ⁺ ratio	7	7	1.10	NS	-

S.E.= Standard error, NS = Non-significant,
LSD=Least significant differences by Turkey's test :
S.E. of Means x Q

salts were directly added to individual pot according to the layout of experiment.

Plant analysis

Plant analysis was carried out on the youngest fully expanded leaves and flag leaves harvested during last week of July, and 3rd week of August. After determining leaf area, the samples were prepared for storage. At each harvest, leaves were taken from plants. Leaves were kept separate during analysis and osmotic pressure; Na⁺, K⁺, concentration of cell sap was determined. After washing in tap water followed by rinsing with distilled water, fully expanded young leaves and flag leaves were blotted dry with tissue paper. For cell sap analysis fresh fully expanded young leaves or flag leaves were transferred in 1.5 cm² polypropylene micro centrifuge tubes and kept in deep freezer.

To extract cell sap, frozen plant leaves were thawed and crushed using a metal rod with tapered end. Small holes were made in the cap and the base of the tube and the sap centrifuged out into a second centrifuge tube at 700 x G. The cell sap was immediately frozen for further chemical analysis. For subsequent cell sap analysis, it was rethawed stirred and re-centrifuged with Bachman microfuge B for a period of 2 min. Osmotic pressure of cell sap was measured in a laboratory vapour pressure osmometer calibrated in mosmol kg⁻¹.

Cell sap extracts were diluted as required with distilled water, sodium and potassium concentration were measured using a spectrophotometer. Sodium was measured at 589 nm while potassium at 766 nm wavelength with 0.1 mm width of the slit against standard solutions. The statistical analysis of the data was performed by the analysis of variance method. When a

Table 2. Main effects of Na⁺ concentrations on fully expanded young leaf of *S. grandiflora*

Parameters	Na ⁺ conc. (mM)						S.E of significance SLD		
	0	10	20	30	40	50	Mean	Level	(P=0.05)
Leaf area (cm ²)	2	1.8	1.7	1.5	1.3	1.0	0.45	1%	2.01
Osmotic pressure (m Osmol kg ⁻¹)	284	296	315	406	519	573	31	1%	115.19
Na+(mM Sap)	4	15	45	93	128	221	18	1%	47.40
K+(mM Sap)	198	163	141	117	100	96	10	1%	39.10
K+/Na+ ratio	46	38	25	14	4	0	2	1%	8.11

S.E = Standard error, LSD=Least significant differences by Turkey's test: S.E of means x Q

significant "F" value was obtained for the treatment effects, Turkey's test at 5% probability level was applied to the treatment means (Snedecor & Cochran, 1980). In the figures, the vertical bars indicated the value of the least significant difference for comparison between treatment means at the last date of measurements except mortality of plants where it showed least significant difference.

Result and discussion

The results presented in this section are mainly concerned with behaviour of two salt species and their concentrations especially on leaf area, osmotic pressure and ionic contents of cell sap in the youngest fully expanded leaf on the main stem. The samples were taken from six plants just before the mortality of plants in high Na⁺ concentrations and similar observations were also recorded in case of flag leaves from six other plants. The data pertaining to the effect of salt species on leaf area, osmotic pressure and K⁺, Na⁺ contents in sap were shown in Table 1. There were non-significant differences between the two salt species for leaf area, K⁺, Na⁺ contents and K⁺/Na⁺ ratio. A significant difference was observed in the case of osmotic pressure of the cell sap, which indicates, that NaCl had a greater effect on osmotic pressure. Khan *et al.* (1995) also observed that on equimolar basis, Na⁺ uptake and K⁺ loss was less in Na₂SO₄ than in the NaCl stressed *Sorghum* plants.

The results related to Na⁺ concentrations fully expanded young leaves of *S. grandiflora* were presented in Table 2. A significant effect on leaf area, osmotic pressure, K⁺, Na⁺ contents in cell sap and K⁺/Na⁺ ratio were observed with increasing Na⁺ concentration. Osmotic pressure of cell sap was increased with higher Na⁺ concentrations. A similar trend was also observed in the case of Na⁺ contents cell sap. The data suggest that osmotic pressure increased due to increase in Na⁺ uptake by plants growing in higher concentrations. Potassium contents decreased as Na⁺ concentrations were increased. The data related to K⁺/Na⁺ ratio indicate that exchange of K⁺ with Na⁺ occurred with increase in salinity. The data related to effect of salt species on various physiological parameters of the flag leaf are presented in Table 3. Leaf area of the flag leaf was significantly higher in NaCl treated plants. A non-significant difference was observed in case of K⁺ and Na⁺ contents of cell sap and K⁺/Na⁺ ratio in both the salt species.

The data conserving to the effect of Na⁺ concentrations on flag leaf are shown in Table 4. A significant increase in osmotic pressure and Na⁺ contents in cell sap was observed with increasing Na⁺ concentrations whereas leaf area, K⁺ contents of cell sap

and K^+/Na^+ ratio decreased in higher Na^+ concentrations.

During this experiment, no interaction between salts and concentration was observed for Na^+ , K^+ , K^+/Na^+ ratio of fully expanded young leaf and flag leaf. During the course of study it was also found that plants under both salt species with low Na^+ concentrations had dark green leaves compared to control but yellowing necrosis and rapid mortality was observed with high Na^+ concentrations. The results indicated the specific effect of ions; Cl^- and SO_4^{2-} , but particularly of Na^+ . Dark green

Table 3. Main effects of salt species on flag leaf of *S. grandiflora*

Parameters	Salt species		S.E of significance LSD		
	NaCl	Na ₂ SO ₄	Mean	level	(p=0.05)
Leaf area (cm ²)	3	3.5	0.75	5%	2.0
Osmotic pressure (m Osmol kg ⁻¹)	484	348	17	1%	51.32
Na ⁺ (mM Sap)	104	71	13	NS	-
K ⁺ (mM Sap)	124	113	5	NS	-
K ⁺ / Na ⁺ Ratio	37	43	1	NS	-

S.E = Standard error, NS = Non significant, LSD = Least significant differences by Turkey's test: S.E of means x Q

colour with smaller leaves of plants under salt stressed conditions was also observed by Bernstein (1975) while rapid death of older leaves was confirmed by Greenway and Munns (1980) and Khan *et al.* (1995). It was also observed data related to chemical analysis of fully expanded young leaf and flag leaf that NaCl had higher cell sap osmotic pressure compared to Na₂SO₄. The toxic nature of former might be due to osmotic and ionic effect of Cl^- because Na^+ contents of leaves sap in both salt species were similar. The impact of NaCl growth and nodulation in *S. gradiflora* has been studied by us (Dhanapackiam & Muhammad Ilyas 2010a, 2010b).

Increasing the salt concentrations of both species not only resulted in increase of osmotic pressure but also increased uptake of Na^+ in leaves. K^+ contents in leaf cell sap and K^+/Na^+ ratio decreased with increasing salt concentration in both species. This indicates that toxic concentration of Na^+ can replace K^+ in salt sensitive species and this may disturb the osmoregulation mechanism in cells as result of which the plants die (Khan *et al.*, 1995). Hence, K^+/Na^+ ratio is important tool for determining the salt tolerance mechanism of crop plants. The findings are in agreement with the results of other

Table 4. Main effects of Na^+ concentrations on flag leaf of *S. grandiflora*

Parameters	Na ⁺ Conc.(mM)						S.E of significance LSD		
	0	10	20	30	40	50	Mean	Level	(P=0.05)
Leaf area (cm ²)	3.5	3.0	2.4	2.1	2.0	2.0	0.92	1%	3.30
Osmotic Pressure (m Osmol kg ⁻¹)	217	281	321	380	425	470	21.30	1%	76.25
Na ⁺ (mM Sap)	2	12	40	88	117	198	16.76	1%	60.00
K ⁺ (mM Sap)	192	151	124	103	81	53	6.22	1%	22.27
K ⁺ /Na ⁺ Ratio	110	91	63	43	10	2.34	2.34	1%	8.38

S.E = Standard error, LSD=Least Significant Differences by Turkey's Test: S.E of means X Q

workers (Marschner, 1971; Jeschke & Nassery, 1981; Rashid, 1986).

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