# SALT TOLERANCE IN SOYBEAN (GLYCINE MAX L.): EFFECT ON GROWTH AND ION'RELATIONS

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### Abstract

A water culture experiment was conducted to study the growth and ions relations in four cultivars of soybean (Glycine max L., cv. Loppa, Egyptian, AGS-160 and ICAL-132) over a range of salinity (10-40 mM NaCl) at different stages of development. Increase in salinity caused a significant decrease in fresh and dry weight of shoot. Relative growth rate (RGR) decreased with increasing salt levels and at different stages of development. Cv. AGS-160 generally exhibited the lowest RGR and ICAL-132 the highest, while Loppa and Egyptian were intermediary. Sodium concentrations increased with increase in salinity while potassium concentration decreased.

Salinity tolerance was generally associated with efficient sodium regulation in shoot. Potassium concentration and K fluxes were the highest in control and decreased under salinity. The magnitude of this reduction was dependent on the tolerance of the cultivars. A high K/Na ratio was generally associated with better salt tolerance.

#### Introduction

Salinity and osmotic stress are known to suppress growth by reduction in osmotic potential and excessive accumulation of undesirable salts in the cytoplasm which may have direct or indirect effects on metabolism through disruption in mineral uptake and transport. Plant cells try to adjust to these harmful effects by utilizing both organic solutes and inorganic ions for osmotic adjustment (Maathius et al., 1992; Xiaomu et al., 1993). In addition, concentrations of sodium and chloride, the major constituent ions in our saline soils, are regulated by a number of membrane transport processes. There is evidence that both the rate and selectivity of ion absorption may be influenced by transpiration (Clipson & Flowers, 1987; Hanayama & Nakano, 1994), although conflicting data also exist (Lopez et al., 1999) and no consistent correlation emerges between salt resistance and exclusion of salts from the leaves. Very limited information is available about these regulatory mechanisms at different growth stages and its significance in adaptation of plants to saline conditions. The present studies were therefore, conducted to understand the effect of sodium chloride salinity on growth behaviour, ions uptake and its regulatory mechanism at various stages of growth in different cultivars of soybean.

#### Materials and Methods

Seeds of four soybean cultivars (Loppa, AGS-160, Egyptian and ICAL-132) were germinated in river sand and seven days old soybean seedlings were transferred to plastic boxes filled with three litres of ½ strength Hoagland solution. The lids of these boxes were perforated to hold 28 plants each. Four days after transplantation, (0, 10, 20 and 40 mM NaCl were added to Hoagland solution) which corresponded to EC<sub>w</sub> 1.04, 2.06, 3.06

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and 4.93 mS/cm, respectively. The solutions were not aerated, but renewed weekly. The experiment was performed in a growth room maintained at  $25^{\circ}$ C  $\pm$  3 with a photoperiod of 12 hours and light intensity of 4500 lux at the level of plant tops. These boxes with plants were weighed daily and the loss was made up with ½ strength Hoagland solution and brought back to the initial weight.

Six sequential harvests of four plants each were made at one week intervals. After every harvest, the remaining weight of each box was recorded and taken as the initial weight till the next harvest. The harvested plants were separated into root and shoot, rinsed twice with distilled water and then blotted dry. Length, fresh and dry weight of shoot were recorded and the shoot was analyzed for K and Na contents through flame photometer after extracting weighed dried material in 100 mM acetic acid for two hours at 90° C (Ansari & Flowers, 1986). Relative growth rate and ion fluxes were calculated at each harvest (Evans, 1972; Pitman, 1975).

## Results and Discussion

Depending upon the concentration of salt and duration of the growth period, salinity under the present experimental conditions had an adverse effect on most of the growth parameters recorded. These effects were not so pronounced at the initial two harvests, but at later harvests, showed an adverse effect specially at the higher level of salt (40 mM NaCl), where significant intercultivar differences were observed. The addition of salts resulted in reduction in plant height, shoot fresh and dry weight, relative growth rate (RGR). There was a general enhancement in Na accumulation, Na fluxes and reduction in K accumulation, K fluxes and K/Na ratios with increase in salinity.

All treatments reduced plant height to varying degrees (Table 1), and this reduction became more pronounced with the increase in salinity and duration of growth. Relative to controls, there was a decrease in fresh and dry weight of shoot (Table 1), and this reduction was more prominent than observed in case of plant height. Relative growth rate (RGR) of these cultivars, when compared on dry weight basis revealed that different cultivars exibited different growth rate and RGR decreased by increasing salt levels (Table 1). Comparatively, high RGR values have been observed at the initial harvests, which gradually decreased with the passage of time in the subsequent harvests. Such similar observations have been made by other workers (Sliman & Ghandorah, 1989; Rajab & Kenworty, 1990; Zaidi & Singh, 1993; Chang et al., 1994; Delgado et al., 1994; Seraj et al., 1998).

Ion concentrations calculated on unit dry weight basis showed that salinity caused an increase in the concentration of sodium at all harvests (Table 2). This increase was dependent on the age of plants and concentration of salt. Soybean cultivar ICAL-132 accumulated less Na in shoot at each harvest compared to other three cultivars which was followed by Loppa, Egyptian and AGS-160 in increasing order.

With the increase in sodium, the potassium concentration decreased (Table 2), presumably due to an exchange of vacuolar K with Na (Jeschke, 1980; Ragab & Kenworthy, 1990). In cultivar Loppa, at the first two harvests, potassium concentration was higher in comparison to other cultivars, which decreased with the passage of time. On the other hand, in cultivar ICAL-132 less potassium concentration was observed at the initial harvest, which increased in the subsequent harvests.

Table 1. Effect of salinity on growth of soybean plants at different harvests.

Cultivars/Treatments	inity on gr	THE OF BO	Harve		1020 441 1	<del></del>
(mM NaCl)	I	п	Ш	IV	v	VI
(III/I Traci)			Shoot heig			
AGS-160		=				
C	21.73	30.50	45.30	56.98	70.63	86.17
10	22.00	28.88	44.50	54.63	67.63	79.57
20	20.73	28.25	42.30	53.10	62.13	65.40
40	19.48	27.50	33.98	43.00	54.93	56.01
Loppa						
C	26.98	37.98	56.90	64.75	82.43	99.45
10	25.90	37.68	57.68	63.25	70.48	82.95
20	25.65	34.18	50.75	60.25	65.10	77.50
40	24.38	32.32	45.52	49.50	56.63	59.67
Egyptian						
C	22.00	35.20	53.88	65.45	97.25	99.22
10	22.25	32.25	52.63	56.30	75.33	80.20
20	21.75	31.00	45.88	54.93	64.48	72.30
40	21.25	30.87	38.00	41.15	62.80	66.57
ICAL-132						-
С	24.23	33.65	54.23	75.00	79.88	89.37
10	23.00	35.55	51.60	67.00	74.55	82.75
20	23.20	35.08	50.08	62.88	69.25	72.42
40	23.00	33.25	48.23	61.20	64.68	61.93
LSD values for cultivars and		0.77				
LSD values for harvests 0.94	4 at 0.05					
		<u>Fresh</u>	weight of	shoot (g/p	<u>lant)</u>	
AGS-160						
C	1.42	2.08	2.32	2.76	3.74	4.09
10	1.31	1.74	2.21	2.56	2.99	3.39
20	1.24	1.49	1.80	2.07	2.36	2.51
40	1.18	1.24	1.57	1.79	1.86	2.07
Loppa	1 40	1.00	2.10	2.02	2.00	<i>-</i> 11
C	1.40	1.88	2.19	3.02	3.80	5.11
10	1.29	1.60	1.88	2.45	3.36	4.40
20	1.26	1.47	1.59	2.11	2.28	2.93
40	1.19	1.04	1.28	1.51	1.89	2.13
Egyptian	1 77	2.06	2.27	2.46	2 90	5 41
C	1.77	1.70		2.46 2.38	3.80 3.61	5.41 4.68
10	1.47		2.07 1.54			3.50
20	1.46 1.46	1.46	1.34	1.95 1.92	2.93 2.39	3.19
40 ICAT 122	1.40	1.20	1.21	1.92	2.39	3.19
ICAL-132 C	1.11	2.05	2.32	2.53	2.57	4.28
10	1.11	1.70	2.32 1.94	2.53	2.37	4.28
20	1.23	1.70	1.75	2.32	2.26	2.94
40	1.08	1.43	1.73	1.95	2.26	2.94
LSD values for cultivars an			1.00	1.73	2.13	2.00
		0.03				
LSD values for harvests 0.0	4 at 0.05					

Table 1. (Cont'd.)	I	II	III	IV		VI
			veight of s			
AGS-160		-				
C ·	0.12	0.19	0.33	0.46	0.61	0.66
10	0.11	0.16	0.25	0.29	0.36	0.4
20	0.10	0.12	0.18	0.20	0.24	0.2
40	0.09	0.11	0.14	0.15	0.18	0.2
Loppa						
c ··	0.12	0.21	0.34	0.44	0.55	0.7
10	0.12	0.19	0.29	0.36	0.45	0.5
20	0.11	0.18	0.25	0.30	0.37	0.4
40	0.10	0.17	0.23	0.27	0.33	0.3
Egyptian						
c	0.14	0.21	0.34	0.53	0.70	0.8
10	0.14	0.16	0.26	0.39	0.49	0.6
20	0.12	0.15	0.24	0.35	0.42	0.4
40	0.11	0.13	0.21	0.30	0.34	0.3
ICAL-132						
<b>C</b> .	0.11	0.22	0.35	0.49	0.62	0.8
10	0.11	0.20	0.32	0.44	0.56	0.7
20	0.10	0.18	0.27	0.37	0.47	0.6
40	0.09	0.16	0.24	0.34	0.40	0.5
LSD values for cultiv	ars and treatments	0.013				
LSD values for harves	sts 0.016 at 0.05					
	I-II	II-III	III-IV	IV-V	V-VI	
		<u>Growth ra</u>	te (mg/g I	).W./day)		
AGS-160						
C	86.2	92.5	56.5	44.9	12.6	
10	66.7	72.4	27.6	36.1	12.8	
20	30.3	64.9	17.0	33.0	11.8	
40 Lama	20.5	49.2	11.0	29.5	12.3	
Loppa C	103.0	76.8	42.9	26.0	26.0	
10	88.5	69.6	42.9 34.0	36.9 35.6	36.9 28.5	
20	87.2	53.6	33.3	33.6 32.6	20.1	
40	07.2	33.0 40.0	33.3	32.0	20.1	

С	86.2	92.5	56.5	44.9	12.6
10	66.7	72.4	27.6	36.1	12.8
20	30.3	64.9	17.0	33.0	11.8
40	20.5	49.2	11.0	29.5	12.3
Loppa					
c '	103.0	76.8	42.9	36.9	36.9
10	88.5	69.6	34.0	35.6	28.5
20	87.2	53.6	33.3	32.6	20.1
40	85.0	48.8	29.9	29.5	11.2
Egyptian	00.0		->.>	->10	
C	59.4	81.6	74.9	47.2	34.3
10	30.2	78.7	65.4	38.1	33.8
20	30.2	78.5	63.5	28.8	13.3
40	30.7	78.3	58.5	22.0	9.6
ICAL-132	30.7	, 0.5	5,0.5	22.0	7.0
C	110.0	74.1	57.8	40.1	38.0
10	106.5	73.9	57.5	39.1	37.9
20	98.3	64.9	52.6	38.0	36.5
40	91.2	66.2	54.1	29.6	33.9
<del>- 10</del>	71.2	00.2		29.0	33.9

Table 2. Effect of salinity on ion concentration (%) in shoot at different harvests.

Cultivars/Treatments	in snoo	in shoot at different harvests.  Harvests						
(mM NaCl)	I	JI .	III	IV	v	VI		
(	Sodium concentration (%)							
AGS-160		504		201001011	1.727			
C	0.10	0.06	0.06	0.07	0.09	0.13		
10	0.13	0.24	0.32	0.35	0.42	0.46		
20	0.44	0.46	0.76	0.83	0.89	1.08		
40	0.72	1.31	1.44	1.50	2.10	2.31		
Loppa								
C	0.08	0.06	0.06	0.05	0.09	0.13		
10	0.17	0.32	0.41	0.51	0.57	0.64		
20	0.30	0.51	0.67	0.91	0.95	1.27		
40	0.56	0.91	1.17	1.50	1.74	1.94		
Egyptian								
С	0.08	0.05	0.06	0.09	0.11	0.11		
10	0.20	0.27	0.29	0.37	0.38	0.40		
20	0.24	0.73	0.73	0.80	0.82	0.85		
40	- 0.56	1.09	1.21	1.26	1.53	1.85		
ICAL-132								
С	0.10	0.06	0.10	0.10	0.11	0.11		
10	0.22	0.22	0.23	0.25	0.28	0.30		
20	0.34	0.35	0.50	0.54	0.55	0.63		
40	0.46	0.69	0.74	0.79	0.81	0.99		
		3.89-02	7.46-02	7.11-02	0.13	0.12		
LSD values for Cultivars a	nd Treatmer	nts at 0.05						
		<u>Pota</u>	ssium con	centratio	<u>n (%)</u>			
AGS-160								
С	3.60	4.44	4.00	3.93	4.12	5.20		
10	3.48	3.68	3.79	3.98	4.14	4.62		
20	3.96	3.56	3.24	3.57	3.55	3.85		
40	3.96	3.34	3.16	3.51	3.31	3.25		
Loppa						-		
С	3.84	5.00	4.16	4.04	3.88	3.84		
10	3.84	4.89	4.14	4.04	3.91	3.84		
20	4.20	4.20	3.79	3.72	3.45	3.34		
40	3.96	3.80	3.17	2.92	2.91	2.92		

Table 2. (Cont'd.)

	<b>I</b>	II	Ш	IV	V	VI
Egyptian						
С	4.08	4.54	4.07	4.74	4.29	4.57
10	4.44	4.10	3.54	4.25	4.19	3.80
20	4.32	4.22	3.37	4.07	3.91	3.80
40	4.44	3.72	2.84	3.62	3.66	3.60
ICAL-132						
С	4.32	3.96	4.32	4.23	4.63	4.43
10	4.32	3.52	3.99	4.00	3.94	3.94
20	3.84	3.10	3.04	3.40	3.92	3.80
40	3.36	2.99	2.67	3.10	3.59	3.61
•		0.27	0.20	0.23	0.25	0.22
LSD values for Cultiv	vars and Treatment	s at 0.05				
		Pot	tassium: S	odium rat	ios	
AGS-160						
C	54.76	77.89	62.50	53.11	47.36	39.62
10	26.36	15.33	11.84	9.79	9.86	10.08
20	12.53	7.74	4.32	4.30	3.99	3.58
40	4.31	2.55	2.20	2.34	1.58	1.41
Loppa						
С	63.57	78.13	68.20	76.23	42.64	29.54
10	29.76	15.27	9.86	7.97	6.87	5.97
20	14.00	12.34	5.74	4.11	3.65	2.63
40	7.02	4.18	2.71	1.95	1.67	1.50
Egyptian						
С	81.60	85.66	67.83	52.64	39.00	41.55
10	21.76	16.00	12.21	11.48	10.94	9.50
20	18.00	5.79	4.62	5.09	4.78	4.49
40	7.87	3.41	2.35	2.87	2.40	1.95
ICAL-132						
С	86.40	56.57	45.00	42.30	42.09	40.23
10	20.00	16.32	17.33	15.81	14.07	13.13
20	11.43	8.37	6.16	6.30	7.12	5.98
40	7.37	4.33	3.58	3.90	4.47	3.65

In glycophytes salinity tolerance may depend at least in part, on the efficiency with which the root system can limit transport of Na and Cl to the shoot. If the rate of transport of NaCl to the shoot exceeds that at which those ions can be accumulated in the leaf cell vacuoles, then the plant will die either of ion toxicity or cellular dehydration (Greenway & Munns, 1980, 1983; Flowers & Yeo, 1986). More resistant glycophytic, species/varieties generally have lower shoot salt concentration than the sensitive ones (Yeo et al., 1977). The low sodium contents in shoot of ICAL-132 may be the reason for its better performance at moderate salt stress. Similar results have been observed in two cultivars of Glycine max for chlorine content (Lauchli & Wieneke, 1979; Yang & Blanchar, 1993).

Several studies of salt tolerance in crop plants have considered retranslocation and exclusion as important means by which plants can regulate the salt concentration in different parts of their shoot. Lacan & Durand (1995) reported the entry of ions into the roots and their transport through the cortex to the xylem vessels and on the other hand reabsorption from the xylem vessels to the neighbouring cells and the external medium. There was a low degree of selecive uptake of K<sup>+</sup> over Na<sup>+</sup>. However, Na<sup>+</sup> depletion of the xylem stream by reabsorption limited, although weakly, its translocation to the shoots. Na<sup>+</sup> reabsorbed was mainly re-excreted into the external medium.

Kramer et al., (1977) revealed the existence of transfer cells like xylem parenchyma cells in the proximal region of the root of *Phaseolus coccineus*, where the accumulation of sodium greatly exceeded those in the apical root and leaves. Lauchli (1976) concluded that in Phaseolus coccineus plants, Na accumulation in xylem parenchyma takes place due to Na reabsorption from the xylem sap in exchange for K, possibly by a Na/K exchange process operating at the plasmalemma of the transfer cells. Jeschke (1980) similarly reported that the tonoplast of the xylem parenchyma cells in bean roots may be important in Na accumulation in the vacuole. Whatever, the mechanism of Na reabsorption, this process may contribute effectively in Na exclusion from the leaves and as described for different crops, may operate in soybean (Glycine max L). This was further supported by the data regarding ionic ratios (Table 2), where K/Na decreased at each harvest in the same order as was observed in K concentration. When intercultivar comparison was made, it was observed that the more tolerant ICAL-132 generally had higher K/Na ratios than the other cultivars. Similar results have been reported by other workers for salt tolerance studies in soybean (Lacan & Durand.1996; El-Samad & Shaddad, 1997).

Ion fluxes, calculated from ion contents of shoot over sequential harvests, present a picture of net transport of ions during specific period. It was observed that in the control plants, there was a very nominal gain in the influx of sodium in all species (Table 3). Under saline condition the highest value of Na was observed in cultivar Loppa, which was unable to limit the rate of Na accumulation in the shoot and had a higher JNa than those of salt resistant cultivar ICAL-132, which had lowest value and proved particularly efficient in keeping JNa lower than other cultivars. These results are in agreement with Ansari (1982) and Hajibagheri et al., (1987), who found that the values of ion fluxes increase much rapidly in salt sensitive than salt tolerant grasses.

Like K concentration, the K fluxes (Table 3) were the highest in the control condition. The presence of NaCl decreased JK in all species, the magnitude of reduction depended on the tolerance/sensitivity of the cultivars. Highest values of JK were observed in cultivar ICAL-132 followed by Loppa, Egyptian and AGS-160. This becomes even

Table 3. Effect of salinity on ion fluxes (JNa) expressed as mgs/gm shoot dry weight/day at different harvests.

Cultivars/Treatments	Harvests					
(mM NaCl)	I-II	11-111	III-IV	IV-V	V-VI	
•	Sodium fluxes (JNa)					
AGS-160						
C	0.0043	0.0643	0.0561	0.0574	0.0891	
10	0.3066	0.3293	0.1544	0.2575	0.1251	
20	0.4620	0.9080	0.2501	0.3923	0.4431	
40	1.1868	0.8903	0.2778	1.5127	0.6801	
Loppa						
C	0.0409	0.0439	0.0115	0.0873	0.1140	
10	0.4658	0.4215	0.3147	0.2934	0.3202	
20	0.7123	0.5750	0.6655	0.3655	0.8297	
40	1.2145	0.9452	0.9604	0.8870	1.0402	
Egyptian						
C	0.0348	0.0535	0.1178	0.0756	0.0445	
10	0.1820	0.2543	0.3518	0.1626	0.1852	
20	0.9656	0.5678	0.6062	0.2687	0.1735	
40	1.1200	1.1211	0.7889	0.7558	0.6921	
ICAL-132						
С	0.0775	0.1187	0.0666	0.0617	0.0450	
10	0.2455	0.1782	0.1770	0.1411	0.1699	
20	0.4055	0.5323	0.3400	0.2368	0.3867	
40	0.9193	0.5695	0.4991	0.2544	0.6712	
		<u>Pota</u>	ssium fluxes	<u>(JK)</u>		
AGS-160						
С	3.6218	3.1543	2.1146	2.2018	2.3538	
10	2.2990	2.9008	1.4000	1.7265	1.4924	
20	1.7676	1.6792	1.1213	1.1216	1.0139	
40	1.0490	1.2500	0.9954	0.6384	0.3109	
Loppa						
C	4.7605	2.0833	1.5322	1.1895	1.6084	
10	4.1848	1.8756	1.2188	1.2077	1.1481	
20	3.6583	1.4646	1.0976	0.7534	0.6110	
40	3.0261	0.6450	0.4884	0.8418	0.2558	
Egyptian						
C	3.3326	2.7256	4.4618	1.3529	2.2539	
10	0.7185	2.0716	3.7565	1.4766	0.9499	
20	1.1145	1.5639	3.5473	0.8782	0.4100	
40	0.0227	1.1504	3.1646	0.8422	0.3272	
ICAL-132						
С	3.9096	3.6940	2.3076	2.4633	1.6609	
10	2.8062	3.5373	2.3449	1.3979	1.6162	
20	2.1236	2.0880	2.3058	2.2648	1.4337	
40	2.2262	1.3710	2.2491	1.8164	1.4732	
		_			_	

Table 3. (Cont'd.)

Table 3. (Cour d.)							
	I-II	II-III	III-IV	IV-V	V-VI		
	Comparison of fluxes irrespective of salinity level						
		So	dium fluxes	(JNa)			
AGS-160	0.49	0.55	0.19	0.56	0.33		
Loppa	0.61	0.50	0.49	0.41	0.58		
Egyptian	0.58	0.75	0.47	0.32	0.27		
ICAL-132	0.41	0.35	0.27	0.17	0.32		
		Pot	assium flux	es (JK)			
AGS-160	2.18	2.25	1.41	1.42	1.29		
Loppa	3.91	1.52	1.08	1.00	0.91		
Egyptian	1.30	1.88	3.73	1.14	0.99		
ICAL-132	2.77	2.67	2.30	1.99	1.55		

more clear, if in the absence of any interaction of these data on Na and K fluxes are presented as the average effect irrespective of salinity levels (Table 3). El-Samad & Shaddad (1997) reported similar finding, where the tolerance was associated with K accumulation. The data presented here generally supports the view that difference in salt tolerance can be related not only to the difference in total ions uptake, but also the rate of uptake, balance among ions taken up and nature of the ions (Ansari et al., 1987; Ansari, 1990).

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