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EFFECT OF SALINITY ON *RHIZOBIUM* SPECIES, NODULATION AND GROWTH OF SOYBEAN (*GLYCINE MAX* L.)

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Abstract

A pot experiment was conducted to observe the growth, nodulation and ion accumulation of salt tolerant and salt sensitive cultivars of soybean (*Glycine max* L.) when grown in symbiotic association with rhizobium in the absence of mineral nitrogen at different salinity levels (0.05, 0.1 and 0.2% NaCl). The rhizobium strains pre-tested for salt tolerance were found more tolerant than the host plants. Plants were inoculated with a mixture of rhizobial strains at the time of sowing in sterilized sand. Salts showed a greater inhibitory effect on nodulation at 0.1% NaCl with decreased nodule number and weight than the plant growth. Soybean cultivar ICAL-132 showed better growth and produced more nodules by keeping low sodium in the shoot as compared to sensitive cultivar Loppa which failed to survive at 0.2% NaCl. Salinity decreased potassium concentration in all cultivars tested, but this reduction was also less in ICAL-132. K:Na ratios showed similar trends. A highly positive correlation was observed between nodules and shoot dry weight.

Introduction

Salinity adversely affects the growth of most agricultural crops with serious injury in legumes. This is due to a combination of disturbance in host metabolism *per se* and development of a root system devoid of root hairs, mucilagenous layer and infection thread formation as these plants rely heavily on the nitrogen fixed through nodulation in the presence of rhizobia (Tu, 1981; Sprent & Sprent, 199O).

Soybean (*Glycine max* L.) is an important oil seed grain legume which faces soil salinity hazards in its area of cultivation. It is generally regarded as more salt sensitive than other grain legumes (Velagaleti *et al.*, 199O). The response of different soybean varieties/cultivars to NaCl varied widely in their tolerance (Velagaleti *et al.*, 1990; Shereen, 1991). Similarly different rhizobial species also varied in their tolerance to NaCl from 0.5% for Rhizobia of *G. javanica* (Wilson & Norris, 1970) to 3% for *R.meliloti* (Subba Rao *et al.*, 1972). The rhizobial strains of *Acacia redolens* tolerates 1.8% salts (Craig *et al.*, 1991) whereas rhizobia of *Prosopis* species remain unaffected at 2.9% NaCl (Zhang *et al.*, 1991).

Limitation to the growth of soybean on saline soil may be due to a number of environmental and ecological factors that affect nodulation and nodule efficiency which have not been extensively studied. It has not yet been resolved whether NaCl exerts greater effect on nodulation and if so on rhizobia survival or infection process or nodule function. Studies were therefore, carried out to study the effect of salinity on

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plant growth, nodulation and on the rhizobium separately to assess which part of the symbiotic system is particularly susceptible to salt.

Materials and Methods

Effect of salinity on the growth of Rhizobium *in vitro*: Three different strains of *Rhizobium japonicum* (USDA - 110, TAL-102 and TAL-355) obtained from USDA and NIFTAL were used in this study. Yeast mannitol agar medium (YMA) was used in which salinity of the medium was adjusted with NaCl at 0.15%, 0.30% and 0.45% with a control without salt. The medium was poured into Petri dishes, 15ml/Petri dish. Culture suspensions prepared from single colonies were incubated at 30°C for one week and 1 ml of these culture were serially diluted from 10^{-1} to 10^{-7} followed by inoculation with 0.1 ml of each bacterial suspension into liquified medium and incubated at 30°C for one week in darkness. Observations were subsequently recorded on the number of colonies in Petri dishes and the number of viable cells/ml of broth were calculated by using the following formula:

Colony forming unit (cfu) of rhizobia = No. of colonies x dilution factor.

Effect of salinity on plant growth and nodulation: Three cultivars of *Glycine max* L. (cv. Loppa, Egyptian and ICAL-132) selected on the basis of their earlier performance (Shereen, 1991) were used. Eight seeds of each cultivar were sown in plastic pots which were later thinned to 4 per pot containing 7kg of sterile sand which was made saline and was irrigated with 1750 ml N-Free nutrient culture solution (Gibson, 1980) containing 0.05, 0.1, 0.2% NaCl (on sand weight basis) prior to planting. A non saline treatment was kept as control. A starter dose of 15 Kg N/ha as $(NH_4)_2SO_4$ was also applied to all the pots. Each treatment had 3 replications which were completely randomized. At the time of sowing, seeds were inoculated with a mixture of three different Rhizobial strains viz., USDA-110, TAL-355 and TAL-102 10^6 cfu/ml. Pots were daily irrigated with distilled water to meet the evapotranspiration losses.

Plant tops were harvested two months after emergence. Roots were separated from the sand and nodules counted. The samples were rinsed twice in distilled water and blotted dry. Shoot height, fresh and dry weight of shoot and number of nodules were

Table 1. Average number of colonies of *Rhizobium japonicum* strains (10^6 cell/ml) at different levels of salinity.

Strain Number	Control	% NaCl		
		0.15	0.30	0.45
USDA 110	30.00	40.00	30.00	0.00
TAL 355	16.27	10.33	5.80	0.00
TAL 102	92.33	106.33	100.33	0.00

LSD values for strains = 2.328795

LSD values for treatment= 2.68906 at 0.05

recorded and shoots were analysed for Na and K by flame photometer after digestion in sulphuric acid (Jackson, 1962).

Results and Discussion

Effect of salinity on Rhizobial growth *in vitro*: Salinity upto 0.3% NaCl had no effect on the number of colonies in all Rhizobial strains except TAL 355 which showed a 64% reduction over control at 0.3% NaCl. USDA-110 and TAL 102 were not affected upto 0.3% NaCl and even showed stimulation at 0.15% NaCl. At 0.45% NaCl none of the strain could survive (Table 1).

Effect of salinity on plant growth, nodulation and nutrient uptake: Shoot height decreased with the increase in salinity (Table 2). Maximum reduction was observed at 0.1% NaCl in cv. Loppa and Egyptian while ICAL-132 was able to grow upto 0.2% NaCl concentration. Fresh weight and dry weight decreased gradually at each level of salinity. As calculated from percent decrease over control, least reduction was observed in cultivar ICAL-132 and maximum in cultivar Loppa (60%) at 0.1% NaCl salinity level as compared to control. At 0.1% NaCl, cultivars Loppa and Egyptian respectively produced 66% and 40% dry weight of the control while ICAL-132 produced 70% at this level which survived at 0.2% NaCl as well producing 64% dry weight over the control where all plants of Loppa and Egyptian died. The reduction in

Table 2. Effect of salinity on shoot growth of soybean (*Glycine max* L.)

Cultivars	Treatment % NaCl	Shoot height (cm)	Fresh weight (g/plant)	Dry weight (g/plant)
Loppa	Control	28.43	3.64	0.91
	0.05	27.08	2.96	0.78
	0.10	21.34	1.46	0.60
	0.20	00.00	0.00	0.00
Egyptian	Control	33.03	3.75	1.10
	0.05	24.51	3.33	0.74
	0.10	18.27	2.26	0.43
	0.20	00.00	0.00	0.00
ICAL-132	Control	27.59	3.36	0.89
	0.05	22.97	3.07	0.81
	0.10	18.66	2.33	0.62
	0.20	16.70	2.19	0.57
LSD for varieties		1.99	0.27	0.10
LSD for treatments		2.30	0.31	0.12
LSD for v x t at 0.05		3.99	0.54	0.20

growth of many legumes due to salinity including soybean has been reported earlier (Tu, 1981; Lauter *et al.*, 1981; Haq & Larher, 1983; Malibari *et al.*, 1993) which lends support to the findings of the present study.

Results of nodulation (Table 3) showed more severe effects of salinity than on growth of plant. Nodule number, fresh and dry weight decreased significantly with the increase in the level of salinity. In control treatment, cultivar Egyptian showed the best nodulation followed by Loppa and ICAL-132 whereas in the presence of salinity this trend reversed and the reduction was generally more in Loppa followed by Egyptian while ICAL-132 seemed to suffer the least. All the cultivars were adversely affected by salt showing a sharp reduction in nodule number and weight even at 0.1% NaCl. Only cultivar ICAL-132 nodulated at 0.2% NaCl whereas the other two cultivars could not survive.

Salinity has generally been reported to inhibit the process of nodulation and nitrogen fixation, the effects varying with the types and levels of salinity, stage of growth and development and crop species (Kumar & Promila, 1983; Mirza & Tariq, 1993a, 1993b; Roomi *et al.*, 1994). In this study smaller number and weight of nodule could be a consequence of poor plant growth. A very strong positive correlation (0.9106, 0.8493 and 0.9429) has been found between shoot weight and nodule weight in all cultivars tested from which it may be inferred that nodule development is dependent on the biomass production. A number of earlier reports support this contention (Tu, 1981;

Table 3. Effect of salinity on nodulation of soybean (*Glycine max* L.)

Cultivars	Treatment % NaCl	Nodule No.	Nodule Fresh weight (mg/plant)	Nodule Dry weight (mg/plant)
Loppa	Control	11.70	189.23	32.71
	0.05	4.50	110.83	19.93
	0.10	0.67	4.75	0.89
	0.20	0.00	0.00	00.00
Egyptian	Control	32.65	488.70	97.48
	0.05	7.40	171.30	31.42
	0.10	2.75	59.53	9.85
	0.20	0.00	0.00	0.00
ICAL-132	Control	13.89	312.17	63.79
	0.05	8.68	222.37	42.91
	0.10	1.32	53.33	9.26
	0.20	0.30	14.90	2.89
LSD for cultivars		4.94	59.90	12.28
LSD for treatments		5.70	89.17	14.16
LSD for v x t at 0.05		9.88	19.80	24.53

Lauter *et al.*, 1981; Singleton & Bohlool, 1984).

The ionic composition of shoot showed that Na increased and K decreased due to salinity in all cultivars (Table 4). The increase in Na due to salinity was more in sensitive than a tolerant cultivar. In cv. ICAL-132 even at 0.2% salinity Na was nearly half of the concentration in cultivar Loppa at a lower 0.1% salinity. The reduction of K in these cultivars shows their inability to maintain selectivity under saline condition. In cultivar ICAL-132 however, K accumulation remained almost unchanged upto 0.1% salts and increased slightly at 0.2%. This also reflected in the K:Na ratios. This behaviour is confirmed by the correlation between Na and K (ICAL-132, $r=0.5176$) (Egyptian and Loppa $r= -.6286$ and 0.6235, respectively). A negative correlation was observed between sodium accumulation in shoot and dry weight of nodule in all three cultivars (Loppa, $r = -0.3576$; Egyptian, $r = -0.8098$ and ICAL-132, $r = -0.8423$).

Regulation of sodium movement into the plant system particularly retention in the root and restriction to the shoot has an important role in salinity tolerance (Suba Rao *et al.*, 1990; Munns, 1993). The same mechanism may be applicable to the sensitivity of cultivar Loppa in this study where the sodium regulation seems affected at the concentration of 0.1% NaCl resulting in an unrestricted flow of sodium ions into the shoot system.

In our study, it appears that salinity tolerance was achieved with efficient regulation of sodium vs. potassium uptake under saline condition as was observed in most of the earlier studies with legumes (Lauchli & Wieneke, 1979; Lauchli, 1984; Gorham *et al.*, 1986). This efficient sodium regulation in a tolerant cultivar (like cultivar ICAL-

Table 4. Effect of salinity on ionic composition of shoot of soybean (*Glycine max* L.).

Cultivars	Treatment % NaCl	Na (%)	K (%)	K:Na ratio
Loppa	Control	0.27	2.10	7.93
	0.05	0.50	2.03	4.03
	0.10	0.71	1.82	2.58
Egyptian	Control	0.18	2.48	13.51
	0.05	0.34	2.48	7.38
	0.10	0.60	2.20	3.69
ICAL-132	Control	0.19	2.10	10.86
	0.05	0.24	2.10	8.63
	0.10	0.34	2.08	6.10
	0.20	0.41	2.23	5.49
LSD for varieties		0.05	0.09	
LSD for treatments		0.06	0.10	
LSD for v x t at 0.05		0.11	0.18	

132 of this study) may involve a series of physiological process including: (a) effective regulation of sodium influx through efficient K/Na selectivity at the plasmalemma (b) higher retention of Na in their root (c) Na reabsorption from the xylem sap by the xylem parenchyma transfer cells during upward transport (Lauchli & Wieneke, 1979; Suba Rao *et al.*, 1990; Yeo, 1994).

Velagaleti (1990) observed that the growth of leguminous plants as well as the process of nodule initiation are more sensitive to salinity of rhizosphere than the rhizobia responsible for nodulation. It is apparent from the present study that high salinity decreases plant growth. The poor nodulation here may be a consequence of decreased plant growth and not due to decrease in rhizobial growth because plant growth and nodulation was severely affected at 0.1% NaCl (Table 2,3) where rhizobial growth *in vitro* was unaffected (Table 1).

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