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Effectiveness of *Rhizobium* Under Salinity Stress

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Abstract: This research work was conducted to evaluate the efficacy of *Rhizobium trifolii* in enhancing the salt tolerance of *Trifolium alexandrinum* (Berseem or Egyptian clover), protein value of fodder and nitrogen fixation in the soil. Three salinity levels (8,12, 16 dS m^{-1}) were developed in a loamy soil in pots. A similar set of inoculated pots with *Rhizobium trifolii* was also arranged. Berseem crop was grown in both of the sets. The data indicated that total green and dry matter yield as well as root dry weight of berseem decreased consecutively with increase in salinity level while *Rhizobium* inoculation created a positive effect and these parameters remained significantly higher than the similar levels without inoculation. Nodulation and nodule dry weight was promoted markedly by inoculation and depressed significantly with consistent increase in salinity. Nitrogen percentage of berseem shoot increased with inoculation and decreased significantly at 8 dS m^{-1} but further increase in salinity had a little effect. Percent nitrogen of soil and nitrogen fixation in soil was enhanced significantly by inoculation and decreased significantly with increase in salinity. Inoculation was helpful to keep the protein content higher. Soil ECe and pH were lowered.

Key words: *Rhizobium* inoculation, berseem, salinity stress

Introduction

A legume provides a nutritious body building food for man and animals of the world. Air is 80% N_2, thus, there are around 6400 kg of nitrogen above every hectare of land and water. This free air nitrogen can be fixed through symbiotic or associated nitrogen fixation processes. Fixation of 100-150 kg N_2/ha/year are common by root nodulation bacteria of legumes (*Rhizobium* spp.) in temperate climate (Alexander, 1976). Since nitrogenous fertilizer is the most commonly expensive due to high-energy consumption in its conversion, the special ability of leguminous crops to work symbiotically with rhizobia to fix nitrogen and produce protein is becoming increasingly important in world agriculture. The agronomist interest in rhizobia is due to the N_2-fixing ability of these bacteria. Rhizobia are soil bacteria characterized by their unique ability to interact with root hairs of legumes and induce effective nitrogen fixing nodules. However, soils do not become productive immediately but gradually as the favorable microorganisms are given time to multiply. Changes in microbial population of soil are continuous and very important. The alkali salts, which lower the production of crops, similarly affect activities of desirable soil microorganisms. Saline and alkali soils constitute an unfavorable habitat for the growth and multiplication of rhizobia. Bernstein (1962) observed almost complete failure of nodule development on soybean when ECe of rhizosphere exceeded 7 dS m^{-1}. Nevertheless, it is not uncommon to expect the presence of many *Rhizobium* species in salt affected soils because legumes like sesbania and Molalities have been found to nodulate and play important role in reclamation. Forage legumes, such as Persian clover or shaftal (*Trifolium resupinatum* L.) and Egyptian clover or berseem (*Trifolium alexandrinum* L.) have been recommended for growing in sodic soils (Chillar and Bhumbla, 1972; Kumar *et al*., 1981; Sharma *et al*., 1983). Burton (1967) reported that Rhizobia are either totally absent or are scanty in salt affected soils. More recent work of Asthma *et al*. (2000) proved that number of primary roots, root proliferation and root uptake was enhanced even in salt stressed condition when *Rhizobium* inoculation and 2,4-D was applied to wheat.

Saline soils are generally deficient in nutrients and microbial activities and population is low. Adding *Rhizobium* bacteria in the soil can enhance it. Grain and forage legumes vary in their nutritional requirements and are better adopted to soil low in nitrogen provided proper rhizobial cultures are used for seed inoculation. Generally leguminous crops are salt sensitive and show the host-strain specifically also within the same cross inoculation group. Little literature is available related to soil salinity. Studies on the sensitivity of *Rhizobium* for its nodulation and symbiotic behavior in salt affected soils are not well documented. So this investigation was conducted to explore all such possibilities.

Materials and Methods

The study was carried out in pots at Salinity Research Institute, Pindi Bhattian. Pots were filled with 12 kg air dried, ground loam soil which was passed through 2 mm sieve (ECe = 0.97dS m^{-1}, pHs = 8.4, saturation percentage = 30 and soil nitrogen = 0.03%). Three salinity levels (8, 12, 16 dS m^{-1}) were developed with four salts; NaCl, Na_2SO_4, $CaCl_2.2H_2O$ and $MgSO_4.7H_2O$ in Ca^{2+} Mg^{2+}ratio of 2:1 and SO_4-^{2}: Cl^{-1} ratio of 1:1. Sodium adsorption ratio was fixed at 10 (m mol L^{-1}) ^{½}. Two treatments of inoculation (inoculated and un-inoculated) were kept at each salinity level. Seed of Egyptian clover or berseem (*Trifolium alexandrinum* L.) was inoculated with *Rhizobium trifolii*. Population of Rhizobial trifolii material was 5 x 10^9 cells of bacterial per ml of inoculum. A uniform dose of 20-75-0 Kg of NPK ha^{-1} was given to all pots. Five plants of Berseem were maintained in each pot and these pots were irrigated as and when required. The pots were kept open at the bottom in order to allow the leaching of salts, if any. Three cuttings were obtained. Soil and plant samples were analyzed for nitrogen as per method of Jackson (1960). Soil EC and pH were determined after the last cutting. The data were processed statistically applying split plot design described by Steel and Torrie (1986).

Results and Discussion

Yield and Yield Components: Data (Table 1 and 2) indicated that berseem inoculation with *Rhizobium* increased the total green fodder and dry matter yield significantly at all salinity levels, which was maximum in inoculated control pots. Mean total green fodder and dry matter yield depressed significantly with increasing salinity levels. Minimum Berseem yield was observed at 16dS m^{-1}. Mean total green and dry matter yield of Berseem enhanced significantly by *Rhizobium* inoculation. The results are in conformity with Batra and Ghai (1987) who reported that Egyptian clover, Persian clover and Sengi showed a significant reduction in yield at ECe 16dS m^{-1} compared with the normal soil while inoculation of seed with *Rhizobium* culture increased the green matter yield of these legumes.

In case of root dry weight (Table 3) treated pots with *Rhizobium* inoculum brought out significantly higher root dry weight of berseem at control and 8 dS^{-1}, but at 12 and 16dS m^{-1} salinity levels the differences between inoculated and un-inoculated treatments remained similar statistically. Hence, the higher salinity levels over masked the beneficial effects of inoculation. When main effects of salinity and inoculation were considered, it was noted that salinity had depressing effect and rhizobial inoculation had boosting impact on growth and dry weight of root. Maximum green fodder during first cutting was obtained in inoculated pots with control salinity whereas minimum was recorded the fodder yield was higher in all the inoculated pots at the equivalent salinity levels. When we compared means for different cuttings, it was

Table 1: Efficacy of *Rhizobium trifolii* for total green fodder (g pot^{-1}) of production of berseem under saline conditions.

	Inoculation Mean	Control	8dS m^{-1}	12dS m^{-1}	16dS m^{-1}
Un-inoculated	216.7b	177.7d	126.2f	95.08h	153.9B
Inoculated	260.1a	190.2c	164.9e	116.7g	183.0A
Mean	238.4A	184.0B	145.6C	105.9D	

Significance is at 5% probability level

Table 2: Efficacy of *Rhizobium trifolii* in total dry matter production (g pot^{-1}) of berseem under saline conditions.

Inoculation	Control	8dS m^{-1}	12dS m^{-1}	16dS m^{-1}	Mean
Un-inoculated	72.20b	65.10c	55.03e	36.43g	57.19B
Inoculated	84.50a	72.53b	61.70d	48.30f	66.76A
Mean	78.35A	68.82B	58.37C	42.37D	

Significance is at 5% probability level

Table 3: Efficacy of *Rhizobium trifolii* for root dry weight (g pot^{-1}) of berseem under saline conditions.

Inoculation	Control	8dS m^{-1}	12dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	16.63b	11.43c	7.93d	4.47e	10.12B
Inoculated	23.63a	25.37b	9.03d	5.77e	13.45A
Mean	20.13A	13.40B	8.48C	5.12D	

Significance is at 5% probability level

Table 4: Efficacy of *Rhizobium trifolii* for total green fodder (g pot^{-1}) at different cuttings of berseem under saline conditions.

Treatments	1st cutting	2nd cutting	3rd cutting
Control	78.30c	71.73de	66.67fg
Control + Rhizobial inoculation	102.94a	82.87b	74.27cd
8 dS m^{-1}	59.64h	76.80c	42.28j
8 dS m^{-1} Rhizobial inoculation	67.96ef	77.93c	44.29j
12 dS m^{-1}	44.03j	53.83l	28.29 ki
12 dS m^{-1} Rhizobial inoculation	62.98gh	61.50h	42.47j
16 dS m^{-1}	27.14i	41.60j	26.33l
16 dS m^{-1} Rhizobial inoculation	41.21j	43.87j	31.60k
Means for cuttings	60.53B	63.77A	44.53C

*Significance is at 5% probability level

Table 5: Efficacy of *Rhizobium trifolii* in dry fodder yield (g pot^{-1}) at different cuttings of berseem under saline conditions.

Treatments	1st cutting	2nd cutting	3rd cutting
Control	22.63ef	24.20cd	25.31c
Control + Rhizobial inoculation	27.00b	28.4ab	29.1a
8 dS m^{-1}	20.67gh	22.00fg	22.43ef
8 dS m^{-1} Rhizobial inoculation	24.17cd	32.43de	24.93c
12 dS m^{-1}	15.43j	18.20 l	21.43fg
12 dS m^{-1} Rhizobial inoculation	18.40l	19.34 hi	24.17cd
16 dS m^{-1}	11.50k	9.40 l	15.53j
16 dS m^{-1} Rhizobial inoculation	14.14j	14.67 j	19.50hi
Means for cuttings	9.62B	9.97B	11.40A

*Significance is at 5% probability level

Table 6: Relative growth rate*(mg/g/day) of three cuttings of berseemas affected by inoculation of *Rhizobium trifolii* different under saline conditions

Treatments	1st cutting	2nd cutting	3rd cutting
Control	28.79	96.56	97.98
Control + Rhizobial inoculation	28.91	101.40	102.14
8 dS m^{-1}	26.57	93.67	94.25
8 dS m^{-1} Rhizobial inoculation	27.94	95.58	97.46
12 dS m^{-1}	24.00	87.92	92.87
12 dS m^{-1} Rhizobial inoculation	25.55	89.76	96.52
16 dS m^{-1}	21.42	67.90	83.11
16 dS m^{-1} Rhizobial inoculation	23.24	81.39	90.01
Means for cuttings	25.80	89.27	94.29

Significance is at 5% probability level

$$* RGR = \frac{\text{Loge } (\text{Log } W_2 - \text{Log } W_1)}{T_2 - T_1} \times 100 \text{ mg/g/day}$$

Table 7: Efficacy of *Rhizobium trifolii* in total nodulation (# nodules pot^{-1}) of berseem under saline conditions.

Inoculation	Control	8 dS m^{-1}	12 dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	338.3 b	238.7 c	119.3 e	3.3 g	174.9 B
Inoculated	416.7 a	273.3 c	172.7 d	54.0 f	229.2 B
Mean	377.5 a	265.0 b	146.0 c	28.6 d	

Significance is at 5% probability level

Table 8: Effect of *Rhizobium trifolii* on nodule dry weight (g pot^{-1}) of berseem under saline conditions.

Inoculation	Control	8 dS m^{-1}	12 dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	0.447 c	0.350 d	0.237 f	0.147 h	0.295 B
Inoculated	0.530 a	0.487 b	0.283 e	0.177 g	0.369 A
Mean	0.488 a	0.418 b	0.260 c	0.162 d	

Significance is at 5% probability level

Table 9: Nitrogen concentration (%) of berseem shoot under saline conditions inoculation

Salinity/*Rhizobium*	Control	8 dS m^{-1}	12 dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	0.460	0.420	0.415	0.400	0.424 b
Inoculated	0.554	0.484	0.466	0.448	0.488 a
Mean	0.507 a	0.452 b	0.440 b	0.424 b	

Significance is at 5% probability level

Table 10: Efficacy of *Rhizobium trifolii* in soil nitrogen (%) after berseem harvesting under saline conditions.

Inoculation	Control	8 dS m^{-1}	12 dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	0.070	0.050	0.050	0.050	0.056 B
Inoculated	0.091	0.080	0.082	0.060	0.079 A
Mean	0.079A	0.068B	0.067B	0.55C	

Significance is at 5% probability level

Table 11: Efficacy of *Rhizobium trifolii* in nitrogen fixation (g pot^{-1}) by berseem under saline conditions.

Inoculation	Control	8 dS m^{-1}	12 dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	4.740	2.921	2.714	2.153	3.132 B
Inoculated	7.555	6.276	6.294	3.784	5.977 a
Mean	6.147a	4.598b	4.504b	2.968c	

Significance is at 5% probability level

Table 12: Efficacy of *Rhizobium trifolii* for protein contents (%) of berseem under saline conditions.

Salinity/*Rhizobium*	Control	8 dS m^{-1}	12 dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	2.88	2.62	2.58	2.50	2.645 b
Inoculated	3.46	3.03	2.91	2.79	3.047 A
Mean	3.107A	2.823B	2.745B	2.645B	

Significance is at 5% probability level

Table 13: Effect of *Rhizobium trifolii* on ECe (dS m^{-1}) of soil after harvesting of Berseem under saline conditions.

Inoculation	Control	8 dS m^{-1}	12 dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	1.60 f	8.37 d	12.54 b	16.37 a	9.72 A
Inoculated	0.87 g	5.71 e	8.62 d	11.52 c	6.68 B
Mean	1.23D	7.04C	10.58B	13.94A	

Significance is at 5% probability level

Table 14: Effect of *Rhizobium trifolii* on pHs of soil after harvesting berseem under saline conditions.

Inoculation	Control	8 dS m^{-1}	12 dS m^{-1}	16 dS m^{-1}	Mean
Un-inoculated	8.4	8.4	8.3	8.2	8.3A
Inoculated	8.2	8.1	7.7	7.8	7.9B
Mean	8.3A	8.25A	8.0B	8.0B	

Significance is at 5% probability level

noticed that green fodder yield was the highest (63.77g pot^{-1}) at second cutting followed by first and third cuttings with 60.5 and 44.53g pot^{-1}, respectively. The differences within cuttings were significant statistically. Similar trend was obtained for dry matter yield of different cuttings (Table 5). However, maximum mean dry matter was gained at third cutting followed by first cutting, which in turn, was at par with second cutting. Relative growth rate (RGR) is the increase of plant material per unit of material assimilated in a unit time. It determines the growth of plant and efficacy of plant enhancement on account of different treatments. The data (Table 6) exhibited that Berseem had the highest value of RGR in control with inoculation at all cuttings, while the lowest value was observed at ECe 16 dS m^{-1} without inoculation in all cuttings. As a general trend the mean values of RGR showed an increase in the second and third cutting than first cutting. Relative growth rate increased on account of inoculation of *Rhizobium* at all the salinity levels. Hence, inoculation favored the relative growth rate of Berseem in saline condition. Saline media hindered the RGR and prevented the general growth of the legume at the higher salinity levels.

Nodulation: Data pertaining to nodule formation inferred that mean number of nodules decreases significantly with an increase in salinity level of soil and increased significantly by seed inoculation with *Rhizobium* (Table 7). Inoculation of seed increased the nodule formation significantly at control, 12 dS m^{-1} and 16 dS m^{-1} salinity levels, but at 8 dS m^{-1} increase in number of nodules per pot is non-significant statistically. Batra and Ghai (1985) also concluded that Egyptian and Persian clovers could not nodulate at 8 and 12dS m^{-1} respectively showing the host strain specificity within the same cross inoculation group. Nodules dry weight also reduced markedly due to higher levels of salinity (Table 8). Maximum mean nodule dry weight was obtained at control and minimum at 16 dS m^{-1} ECe level. Interaction between salinity and *Rhizobium* levels was found to be significant. At all salinity levels, the nodule dry weights were enhanced significantly by inoculation of *Rhizobium*. Similarly Batra and Ghai, (1987) proved that number and weight of nodules was reduced at ECe 15 dS m^{-1}. Mean nodule dry weight increased significantly by seed inoculation.

Nitrogen : Data of evinced that mean nitrogen percentage in berseem shoot decreased significantly due to soil salinity than control, but differences between 8,12 and 16 dS m^{-1} ECe levels were nominal and non-significant statistically (Table 9). Mean figures revealed that shoot nitrogen percentage rose significantly by inoculation. No interaction between salinity and inoculation was observed.

Nitrogen percentage of soil was badly lessened due to the presence. It decreased significantly with salinity increase, being minimum at 16 dS m^{-1} and maximum in control. The two middle levels of salinity (8 and 12 dS m^{-1}) had statistical equal nitrogen percentage. Inoculation had a significantly positive effect on soil nitrogen. Differences due to interaction between salinity and inoculation remained non-significant (Table 10). Mean nitrogen fixation in soil was suppressed with raising salinity and ascended significantly with inoculation of *Rhizobium trifolii* in control and different salinity levels (Table 11). No interaction between salinity and inoculation was observed.

Protein Contents: Protein percentage was influenced significantly by salinity and inoculation with *Rhizobium* (Table 12). All the there levels of ECe were statistically different as compared to control, but similar with one another when the main effect of salinity was considered. Plants with inoculation assimilated more protein than un-inoculated plants at all salinity levels.

Soil EC and pH: Data relating to electrical conductivity (ECe) indicated that salinity was reduced significantly by *Rhizobium* inoculation at all salinity levels. Mean figures showed that *Rhizobium* inoculation of seed deceased salinity level from 9.72 to 6.68 dS m^{-1} (Table 13) Mean pHs values of soil after harvest of crop indicated that this parameter was similar at initial two levels of salinity and significantly higher than the next two levels which were in turn similar to each other. Effect of inoculation was found to be significantly positive (Table 14)

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