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Research article

COMPARATIVE STUDY OF BERSEEM CULTIVARS (*TRIFOLIUM ALEXANDRIUM* L.) IN SUPPORT OF NODULATION AND LEG HAEMOGLOBIN CONTENT UNDER SALINE CONDITIONS

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ABSTRACT: Aim of present study is to evaluate the effect of salinity on the growth of *Rhizobium* symbiosis with various cultivars of Berseem (WARDAN, BB3, JHB 052 and BB2). Two sets of plots were maintained in which one set contained non-inoculated while another set contained inoculated seeds with *Rhizobium* culture, which were irrigated with saline waters of different concentrations (0, 3, 6, 7.2, 10, 12 and 14 dSm^{-1}). The observations have been recorded only at 90 and 120 DAS but nodular leghaemoglobin had been estimated at 90^{th} day stage. Results indicated that nodulation and lb content had been increased generally upto 7.2 dSm^{-1} and declined thereafter. Inoculation with *Rhizobium* culture had invariably and significantly promoted nodulation and lb content at both durations particularly at lower EC levels and minimized the deleterious effect of salinity at 10 to 14 dSm^{-1}. Taken together, our findings indicate that cv. BB3 produced maximum nodulation and lb content almost at all saline irrigations but cv. WARDAN registered beneficial effect of *Rhizobium* culture inoculation as this cultivar registered greater lb content at 3 to 7.2 dSm^{-1} and lesser reductions at 10 to 14 dSm^{-1} when compared with uninoculated sets. We concluded that this experiment reveals the great impact of *Rhizobium trifolii* culture on berseem cultivars along with the use of saline soil and water for agriculture without using fertile land and normal water, hence there will be a substantial conservation of normal water and soil for further use.

Key words: Salt stress, *Trifolium alexandrium* L., *Rhizobium*

INTRODUCTION

Salinity is major problem of agriculture in arid semi-arid region of the world (Rao and Sharma, 1995). Salt affected land comprises of 19 % of the 2.8 billion hectares of arable land on earth and an increase in this menace is posing a serious threat to agriculture globally. Salinity affects plants in different ways such as osmotic effects, specific-ion toxicity and/or nutritional disorders (Läuchli, 1990). The chemical agriculture affected the soil environment is well known. Biofertilizer such as microbial inoculant which can promote plant growth and productivity have internationally been accepted as an alternative source of chemical fertilizer. Nitrogen fixing legumes improve soil fertility is well known, the development of biological nitrogen fixation by appropriate rhizobia strains for maximum yield is well desirable, especially on soils of low N status (Almadini, 2011). Salinity reduces the plant growth and productivity. Impact of salinity is easily visible in plant crop such as stunted growth (Almadini, 2011), chlorosis of green parts (Srivastava and Jana, 1984), leaf tip burning (Husain, et. al., 2003), necrosis of leaves (Wahid, et. al., 1999). This clears that salinity reduce plant growth and productivity. Berseem clover (*Trifolium alexandrinum* L.) belongs to family Fabaceae. *Trifolium alexandrinum* L., has been widely cultivated in Asia, particularly in Pakistan, India and United States (Chen, et. al., 2003). Legumes are important agricultural plants and entail one of the rich sources of proteins. *Rhizobium* is well known symbiotic nitrogen fixer. But report suggested that salinity affects the survival and distribution of *Rhizobia* in soil and the rhizospheres of plants (Knight, 1985; Jenkins, et. al., 1989) but not lethal to, the host plants and strains visibly possess variations in their sensitivity to salinity (Tate, 1995; Steinborn and Roughley, 1975; Rao, et. al., 2002; Singh, et. al., 2005; Gama, et. al., 2007). Therefore, aim of present study is evaluated the effect of salinity on growth of plant, *Rhizobium* and symbiotic relationship of Berseem clover and *Rhizobium*.

MATERIALS AND METHODS

Sampling of soil and preparation of field

The field experiment has been designed to study the efficacy of *Rhizobium trifolii* on nodulation and leghaemoglobin content under saline soil. The observations have been recorded only at 90 and 120 DAS. The experimental field was harrowed and ploughed thoroughly for sowing and cultivation of berseem crop and soil samples were subjected to physico-chemical analysis. This experiment was conducted from 5th November, 2006 to 31st March, 2007 at a farmhouse located on Kath road, Harthla, Moradabad, U. P., India. Minimum and maximum temperature and relative humidity were measured regularly during the whole course of experimentation from 5/11/06 to 31/03/07 (Table 1).

The experimental plots were divided into micro plots measuring 1x1m each (inner size) and these plots were lined with polythene sheets at a depth of 15 inches to avoid lateral leaching of salts. Two sets of plots were maintained in which one set contained non-inoculated while another set contained inoculated seeds with *Rhizobium* culture. Each set of salinity was based on randomized block design with triplicates. The experimental plots, which were irrigated with saline waters of different electrical conductivities (3, 6, 7.2, 10, 12 and 14 dSm^{-1}). The control sets were irrigated with tube well water. The saline solutions of different electrical conductivities were prepared by mixing the salts of NaCl, Na_2SO_4, $NaHCO_3$ and $CaCl_2$ in tubewell water. The quantity of salts dissolved was followed as mentioned by US Salinity Laboratory Staff Handbook (US Salinity Laboratory Staff, 1954).

Table 1 Average minimum, maximum temperature and relative humidity

S. No.	Month	Minimum temperature (^{0}C)	Maximum temperature (^{0}C)	Relative humidity (%)
1	November	14.76	26.80	61.40
2	December	10.19	22.39	58.00
3	January	8.22	20.10	48.32
4	February	9.32	22.42	43.41
5	March	13.74	27.22	52.00

Methods of Application of *Rhizobium* Inoculants

Seeds of the four cultivars viz. cv. WARDAN, BB 3, JHB 052 and BB2 were divided into two lots and one lot was treated with culture of *Rhizobium trifolii* and another lot was kept untreated (control).

Seed bacterization method has been found to be the suitable method of *Rhizobium* inoculation. First seeds were surface sterilized by 0.1$HgCl_2$ solution for 2 minutes. Dry the seed under sterile condition. A 10 % jaggery (gur) solution was used as sticker for *Rhizobium* cells to seed. First the solution was spread over sterilized dry seeds and mixed to build up a thin coat over the seeds. After ascertaining the proper coating of slurry over the seeds, the inoculant was sprinkled over the seeds and the content was again mixed thoroughly. Then content was dried in the shade by spreading thinly on a polythene sheet at least for overnight.

The seeds were sown 2 cm deep in furrow in linear rows, which were apart at a distance of 20 cm from each other and each experimental plot (1x1 m) had three rows. The sowing rate was 1 gm in each plot.

Sampling of nodules from saline soil

Nodulation (Number and Mass) are directly related to atmospheric nitrogen fixation, hence estimation of nodules at different growing stages can be helpful in accordance of fertilizers application particularly nitrogen. The observations were recorded on nodulation at 90 and 120 days after sowing. Number of the nodules was counted at 0.5 cm or more diameter of the nodule and they have not been differentiated in larger or smaller ones. The samples were collected randomly from inoculated and non-inoculated plots of each salinity treatment (3-14 dSm^{-1}) including controls. Before sampling, the field was well irrigated to take out the samples with intact roots.

The plant samples were brought to the laboratory and the roots were thoroughly washed under running tap water without damaging roots and nodules.

First of all nodules were separated from the roots by sharp edged blade carefully and their number and fresh weight was measured. After measuring the fresh weight the plant samples of nodules were kept in paper bags and these bags were kept in hot oven at 60^{0}C for 72 hrs. Thereafter their dried weights were determined. All observations were made in triplicates to avoid experimental error.

Quantitative estimation of leg haemoglobin (lb) content

The lb content was estimated in parallel at 90th day stage only. It was determined in fresh uniform sized root nodules measuring 0.5-1 cm. Nodules were carefully removed from the roots with sharp edged blade. These were washed with prechilled double distilled water. After washing, the nodules were blotted on filter paper, weighed and then finally crushed in prechilled sterilized pestle mortar containing 50.0 mM HCl, 5 mM $MgCl_2$, 20 mM KCl and 5 mM mercapto ethanol. The slurry was centrifuged at 40^0C at 8,000 rpm for 15 minutes. The pellets were discarded and supernatant (SN) was made to known volume i.e. 4 ml/ gm fresh weight of nodules. In this supernatant, lb content was estimated by using haemochromogen method (Hartree, 1955).

The 0.5 and 1 ml aliquot of clear extract was taken in the test tube. To each tube, 1.5 ml of 1 N NaOH was added and kept for half an hour. After 30 minutes, 3 ml of pyridine solution and 1.5 ml of 10% (W/V) sodium bisulphide were added to each tube. Then distilled water was added to make the volume to 15 ml. The tubes were incubated for 30 minutes and the optical density was recorded at 535 and 556 nm. Calibration curve was prepared by using a standard solution of haemin 100 (µg/ml) by dissolving in 1N NaOH. Leghaemoglobin content is expressed as mg haemin / gm fresh weight of nodules. All observations were recorded in triplicates and data were subjected to statistical analysis of variance using three factorial randomized design methods (Bruning and Kintz, 1977).

RESULTS AND DISCUSSION

Nodulation was reduced in all genotypes (cv. WARDAN, BB 3, JHB 052 and BB2) at salinities above 3 dSm^{-1} but to lesser extent in the higher nodulation selection, which preferred inherently superior under both non saline and stress conditions (Rao, et. al., 2002). Saline environment provides unfavourable habitat for nodulation while inoculation of seeds with *Rhizobium trifolii* culture significantly enhanced nodulation in all varieties ($p<0.001$). Field experiments confirmed favourable effects of salinities upto 7.2 dSm^{-1} but higher salinities inhibited nodulation in all varieties, which may be due to shrinkage of the root hairs as earlier reported (Tu, 1981), however, inoculation with *Rhizobium* invariably enhanced nodulation (Table 2, 3, 4 and 5). The varieties had behaved differently as number of nodules was higher at 90th day stage and declined at 120 DAS thus indicating nodule senescence.

Saline irrigations had significantly increased ($P<0.001$) dry weight of nodules per plant upto 10 dSm^{-1} at 90th day stage in both sets, but upto 7.2 dSm^{-1} at 120 DAS except for cv. WARDAN. In this cultivar dry weight of nodule enhanced upto 10 dSm^{-1} in both sets at 90th and 120th day stage (Table 4 and 5). Dry weight of nodule significantly declined in inoculated and uninoculated sets at 12 and 14 dSm^{-1} at both stages. The inhibitory effects of salinity on nodulation (number and mass) have also been reported (Al-Mutawa, 2003; Anthraper and Dubois, 2003; Tejera, et. al., 2006; Lopez, et. al., 2008; Borucki and Sujkowska, 2008; Nabizadeh, et. al., 2011, Almadini, 2011).

Table 2 Effect of saline water irrigation on number of nodules in two cultivars of berseem (*Trifolium alexandrinum* L.)

Salinity of irrigation water (dSm-1)	Number of nodules/plant							
	Days after sowing							
	90		120		90		120	
	Cv. WARDAN				Cv. BB 3			
	NR	R	NR	R	NR	R	NR	R
Control	62.67±1.16	78.67±5.77	48.35±4.62	60.70±0.40	67.20±4.04	109.00±5.20	50.67±0.62	66.00±0.95
3	78.67±4.62	106.00±3.46	68.00±1.77	85.00±3.94	90.67±2.89	148.33±2.31	60.67±0.39	86.00±3.58
6	76.33±3.46	85.00±2.89	66.00±3.16	78.33±5.46	86.67±4.06	139.00±8.08	61.67±1.61	75.67±2.89
7.2	68.33±1.73	82.00±1.16	55.00±2.49	63.00±1.04	70.00±5.77	135.33±2.89	52.33±1.51	79.67±5.62
10	36.00±3.46	60.33±0.20	33.00±1.04	53.00±1.38	52.00±1.45	89.33±5.29	42.67±1.61	52.30±1.33
12	22.67±1.16	56.00±4.04	12.67±1.61	50.33±0.54	38.33±4.62	81.33±0.58	24.33±2.50	35.67±3.08
14	20.00±5.77	34.00±2.31	10.00±1.51	22.50±1.58	25.00±2.89	52.67±2.31	11.33±0.58	27.33±4.24

Mean ± SE (n = 3), NR: Non-treated, R: *Rhizobium* culture treated

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Table 3 Effect of saline water irrigation on number of nodules in two cultivars of berseem (*Trifolium alexandrinum* L.)

Salinity of irrigation water (dSm-1)	Number of nodules/plant							
	Days after sowing							
	90		120		90		120	
	Cv. WARDAN				Cv. BB 3			
	NR	R	NR	R	NR	R	NR	R
Control	51.33±3.46	60.00±2.31	30.33±0.56	36.33±1.16	55.00±1.75	63.00±2.89	38.00±2.78	48.67±1.73
3	60.33±1.16	78.33±1.73	50.00±2.05	53.33±1.73	89.33±2.49	95.00±1.51	57.00±1.15	66.67±1.16
6	58.67±2.31	74.00±2.31	50.00±2.31	58.00±2.89	85.00±1.94	84.00±1.73	52.33±1.34	63.00±1.51
7.2	54.00±1.47	64.00±1.16	35.00±1.75	48.00±1.73	65.67±1.16	65.00±1.12	46.67±3.46	55.00±1.73
10	29.33±1.73	33.33±1.16	25.67±2.89	34.33±2.31	40.33±2.30	54.00±1.78	34.67±1.06	40.33±0.19
12	26.67±3.56	30.67±1.73	20.67±1.79	26.67±0.58	29.67±1.16	39.33±1.86	20.00±1.73	29.33±0.77
14	20.33±1.16	23.67±0.58	17.67±0.82	20.00±0.37	18.67±1.12	30.67±1.71	16.67†±4.04	18.00±0.75

Mean ± SE (n = 3); NR: Non-treated, R: *Rhizobium* culture treated

Table 4 Effect of saline water irrigation on dry weight of nodules in two cultivars of berseem (*Trifolium alexandrinum* L.)

Salinity of irrigation water (dSm-1)	Number of nodules/plant							
	Days after sowing							
	90		120		90		120	
	Cv. WARDAN				Cv. BB 3			
	NR	R	NR	R	NR	R	NR	R
Control	0.05±0.006	0.07±0.005	0.03±0.003	0.06±0.007	0.06±0.006	0.10±0.006	0.05±0.003	0.09±0.003
3	0.08±0.004	0.10±0.01	0.05±0.001	0.08±0.01	0.07±0.006	0.14±0.002	0.07±0.005	0.11±0.008
6	0.07±0.003	0.09±0.01	0.05±0.002	0.07±0.006	0.08±0.007	0.14±0.006	0.08±0.006	0.12±0.01
7.2	0.07±0.01	0.08±0.002	0.04±0.01	0.07±0.001	0.06*±0.02	0.12±0.01	0.06±0.003	0.10±0.008
10	0.04±0.003	0.05±0.002	0.03*±0.01	0.04±0.002	0.05±0.01	0.07±0.01	0.04†±0.003	0.06±0.005
12	0.03±0.01	0.04±0.001	0.02±0.006	0.03±0.001	0.03±0.006	0.05±0.006	0.03†±0.003	0.05±0.009
14	0.01±0.006	0.03±0.001	0.02±0.006	0.02±0.001	0.03±0.001	0.04±0.005	0.02†±0.01	0.03†±0.01

Mean ± SE (n = 3), NR: Non-treated, R: *Rhizobium* culture treated, † Non significant for days, * Non significant for treatment

Table 5 Effect of saline water irrigation on dry weight of nodules in two cultivars of berseem (*Trifolium alexandrinum* L.)

Salinity of irrigation water (dSm-1)	Number of nodules/plant							
	Days after sowing							
	90		120		90		120	
	Cv. WARDAN				Cv. BB 3			
	NR	R	NR	R	NR	R	NR	R
Control	0.04±0.003	0.05±0.002	0.03±0.005	0.04±0.007	0.05±0.04	0.07±0.006	0.04±0.002	0.05±0.001
3	0.06±0.005	0.07±0.002	0.04±0.006	0.06±0.002	0.06±0.005	0.09±0.003	0.05±0.003	0.08±0.006
6	0.06±0.006	0.07±0.009	0.04±0.005	0.06±0.006	0.07±0.002	0.08±0.001	0.06±0.005	0.08±0.005
7.2	0.04±0.009	0.06±0.004	0.04†±0.01	0.05±0.003	0.06±0.004	0.08±0.003	0.05±0.006	0.06±0.002
10	0.03±0.002	0.04±0.001	0.03†±0.01	0.04*†±0.01	0.04±0.003	0.06±0.006	0.03±0.002	0.04±0.003
12	0.02±0.007	0.04±0.003	0.02†±0.01	0.03†±0.01	0.03±0.003	0.04±0.005	0.03±0.003	0.03±0.003
14	0.02±0.002	0.03±0.006	0.02†±0.006	0.03†±0.01	0.03±0.002	0.03±0.002	0.02±0.002	0.02±0.003

Mean ± SE (n = 3); NR: Non-treated, R: *Rhizobium* culture treated, † Non significant for days, * Non significant for treatment

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Table 6 Effect of saline water irrigation on leghaemoglobin content in four cultivars of Berseem at 90 days after sowing (DAS)

Salinity of irrigation water (dSm-1)	Number of nodules/plant							
	Days after sowing							
	90		120		90		120	
	Cv. WARDAN				Cv. BB 3			
	NR	R	NR	R	NR	R	NR	R
0	45.00±2.02	48.00±1.30	50.00±0.88	58.00±1.55	42.00±1.24	45.00±1.46	40.00±0.85	43.00±1.03
3	48.00±0.30	53.00±0.78	54.00±0.48	60.00±1.47	46.00±1.64	49.00±0.68	45.00±1.44	49.00±1.57
6	50.00±1.16	54.00±0.95	56.00±1.35	62.00±1.76	47.00±1.68	52.00±1.14	46.00±1.21	50.00±1.14
7.2	45.00*±2.89	50.00±1.02	52.00±1.08	59.00±1.33	43.00±1.02	45.00*±3.46	42.00±1.11	46.00±1.34
10	40.00±0.78	44.00±2.12	47.00±1.80	49.00±0.57	38.00±1.42	42.00±2.21	36.00±1.78	38.00±1.42
12	38.00±1.05	42.00±0.92	43.00±1.33	45.00±1.34	36.00±1.87	40.00±1.81	35.00±1.78	36.00±1.68
14	35.00±1.78	38.00†±3.18	40.00±0.78	42.00±1.22	34.00±1.22	35.00±1.20	33.00±1.07	35.00±1.41

Mean ± SE (n = 3), NR: Non-treated, R: *Rhizobium* culture treated, † Non significant for days, * Non significant for treatment

Salinity does not affect colonization of roots by rhizobia (Singleton and Bohlool, 1984) but does retard initiation or growth of new nodules and reduces the efficiency of fully formed nodules, which had developed earlier under non-saline condition (Bernstein and Ogata, 1966). Salinity (48mM) also inhibits nitrogen fixation as well as nodule formation in *Neonotonia wightii* (Wilson, 1970) while Wilson and Norris (1970) also demonstrated reduced nodule formation due to salt stress in soybean. High salt concentrations in the soil reduce the absorption of nutrients of the plants, hence this affects the fertility of the soil and crop yield negatively (Al-Busaidi, et. al., 2009; Asik, et. al., 2009; Nagaz, et. al., 2007). NaCl salinity is described to cause an inhibition on the growth of wheat plants (Turan, et. al., 2007). Zadeh and Naeini (Zadeh and Naeini, 2007) also confirmed that high level of salinity (12 dSm^{-1}) violently affects yield in cultivars of canola. Shahzad, et. al. (2008) reported that the use of plant growth promoting bacteria with P-enriched compost in an integrated manner improves the growth, yield and nodulation in chickpea. Agarwal and Ahmad, (2010) and Saharan, et. al. (2011) also confirmed that rhizobacteria are commonly used as inoculants for improving the growth and yield of agricultural crops. Calcium treatment also has a great influence on reduction of negative effects of salt stress on crop yield (Dehaghi, et. al., 2007)**.**

It is interesting to observe that inoculation with *Rhizobium* culture had invariably and significantly promoted nodulation (number and mass) ($p<0.001$) as compared to its corresponding sets under saline water irrigated sets of four varieties. This was similar to the findings of Ahmad and Sandhu (1988). It has also been reported that the *Rhizobium* population is also sensitive to salinity stress and thus higher levels of salinity had inhibited nodulation, might be due to root hair infection by the *Rhizobium*. Hussain, et. al. (2002) also proved that nodulation and nodule dry weight were promoted markedly by *Rhizobium* inoculation and depressed significantly with higher salinities. In contrast to this Eleshikh and Wood (1995) reported that a salt tolerant cultivar of soybean sustained nodulation irrespective of salt tolerant of *Rhizobium* strain. A salt tolerant soybean host cultivar 'Manchu' sustained nodulation irrespective of the salt tolerance of the strain of bacterium; no significant differences in shoot or root dry weight, nodule number or nodule weight were found whether a salt-sensitive *Bradyrhizobium* strain or a salt – tolerant *Rhizobium* strain was used for inoculation Eleshikh and Wood (1995). Hafeez, et. al. (1988) also reported inhibition in nodulation of *Vigna radiata* by about half at a salinity regime of 5.0 dSm^{-1} when compared to 1.4 dSm^{-1}. They also found that nodulation was completely depressed at 10 dSm^{-1} regardless of the plant growth. Inhibitory effect of salinity on nodulation of chick pea occurred at 4.0 dSm^{-1} and nodulation was completely inhibited at 7.0 dSm^{-1}; the plants died at 8 dSm^{-1} (Elsheikh and Wood, 1990). Cv. BB 3 produced maximum number of nodules almost at all saline irrigations in both sets at 90th and 120th day stages and it was followed by cv. WARDAN, BB 2 and JHB 052, whereas, maximum nodule dry weight was recorded in both sets of cv. WARDAN at 120 DAS. It is also noticeable that the inoculation had evidently enhanced nodulation in all the four varieties with saline water.

Leghaemoglobin is an important biochemical parameter, which is present in root nodules. It constitutes about 25-30% of the total soluble nodule protein and is actively involved in nitrogen fixation. It regulates the supply of oxygen for nitrogen fixation. It has been measured in uniform sized root nodules at 90 DAS in all four varieties. The characteristic red, pink or brown colour of the nodule is due to this pigment. Salinity stress caused a delay in the initiation of N-fixation (Almadini, 2011) and NR activity, nitrogen uptake and total nitrogen content in canola (Bybordi and Ebrahimian, 2011), which may be due to inhibitory effect of salinity on leghaemoglobin content. Fernandez-Pascual (Ferna´ndez-Pascual, et. al., 1996) reported that Nitrogenase activity and leghemoglobin content decreased in Lupinus albus at 100 mol m^{-3} NaCl. Salt induced inhibition in leghaemoglobin content has also been reported (Ram, et .al., 1989) in chick pea which could be due to the failure of root hairs by *Rhizobium* under salinity stress. Data are presented in table 6 for all the varieties and have been differentially affected by different saline waters used for irrigations and *Rhizobium* culture applications. Nodules have also expressed variation under saline irrigations, however, inoculation with *Rhizobium trifolii* culture had significantly enhanced leghaemoglobin content compared to non-inoculated sets which confirm the findings of Ram, et. al. (1989). Leghaemoglobin content significantly increased at 3 to 7.2 dSm^{-1} and declined thereafter in all the cultivars of berseem ($p<0.001$). Our findings are accordance with those findings of Nabizadeh, et. al. (2011) who reported that leghaemoglobin content decreased as the salinity levels increased. However, seed inoculation with *Rhizobium* culture had further increased leghaemoglobin content at all salinity levels as compared to corresponding non-inoculated sets of all cultivars (Table 6). Kouas, et. al. (2010) observed that Nitrogen fixing ability decrease 77% at 200 mM NaCl as compared to the control. The inhibition of N_2 fixation under moderate salinity may be related to a decrease in bacteroid respiration (L'taief, et. al., 2007) and nodule leghemoglobin contents (Lopez, et. al., 2008).

The varieties differed in the leghaemoglobin content and maximum content was observed in cv. BB 3 at 3 to 14 dSm^{-1}. Seed inoculation in this cultivar invariably had higher leghaemoglobin when compared with its corresponding non-inoculated sets. Second highest content of leghaemoglobin was found in cv. WARDAN at all saline regimes. Cv. WARDAN registered greater beneficial effect of *Rhizobium* culture seed inoculation at all salinity levels as compared to all other three varieties as this cultivar registered greater leghaemoglobin content at 3 to 7.2 dSm^{-1} and lesser reductions at 10 to 14 dSm^{-1} when compared with uninoculated sets. Overall experiment showed that *Rhizobium* inoculation can overcome the severe problem of increasing salinity for the growth of berseem cultivars at different growth stages. It is an environmentally sustainable approach to increase crop production.

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