Agricultural
Communication

Biosci. Biotech. Res. Comm. 12(2): 377-383 (2019)

BBRC
Bioscience Biotechnology
Research Communications

Effect of inoculation of salt tolerant *Rhizobium* on nodulation and leghaemoglobin content of soybean

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ABSTRACT

A field experiment was conducted with a view to see the effect of inoculation of salt tolerant *Rhizobium* on nodulation and leghaemoglobin content of soybean at Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S) during the year 2015-2017. Legumes and the process of nodule initiation are both more sensitive to salinity than are rhizobia Both N_2 fixation activity and nodule respiration were inhibited sharply on exposure of plants to saline condition. The decrease in N2 fixation has been ascribed to direct effect on nitrogenase activity or an indirect effect to decrease in leghaemoglobin content, respiratory rate, malate concentration, nodules and altered their ultrastructure. Treatment T_7 (Liquid consortium + 100 % N) was singnificantly superior in number of effective nodules over rest of treatments however it was at par with treatment T_6 (Liquid consortium + 75 % N). It was also found that treatment T_7 (Liquid consortium + 100 % N) significantly superior in number of non- effective nodules over rest of the treatments however it was at par with treatment T_6 (Liquid consortium + 75 % N). The treatment absolute control recorded the least number of effective and highest number of non-effective nodules during flowering and harvesting stages respectively. The treatment T_7 (Liquid consortium + 100 % N) was significantly superior in Leghaemoglobin content of nodule over rest of all the treatments and it was at par with treatment T_6 (Liquid consortium + 75 % N). The treatment absolute control recorded the least in Leghaemoglobin content of nodule at 45 and 60 days. So there need to isolate strains and inoculation of salt tolerant *Rhizobium* which will enhance the nodule formation in legume crops.

KEY WORDS: SOYBEAN, LEGHAEMOGLOBIN CONTENT, SALT TOLERANT RHIZOBIUM, NODULATION

Article Information:*Corresponding Author:Krishna_pagare@rediffmail.com
Received 15/03/2019 Accepted after revision 25/06/2019
Published: 30th June 2019 Pp- 377-383

Published by Society for Science & Nature, Bhopal India.
Available at: https://bbrc.in/
Article DOI: http://dx.doi.org/10.21786/bbrc/12.2/21

INTRODUCTION

The decrease in N2 fixation activity has been ascribed to a direct effect on nitrogenase (Burns *et al.* 1985) or an indirect effect through decreases in leghaemoglobin content, respiration rate and malate concentration in nodules (Delgado *et al.* 1993, 1994). Legumes and the process of nodule initiation are both more sensitive to osmotic stress than are rhizobia (Russell, 1976; Tu, 1981; Velagaleti *et al.*, 1990). Salt tolerant rhizobia might have the potential to improve yield of legumes under salinity stress (EI-Mokadem *et al.*, 1991). Numerous studies have shown that soil salinity decreased rhizobial colonization and nodulation and dramatically reduced N2 fixation and nitrogenase activity of nodulated legumes (Elsheikh and Wood, 1995; Zahran, 1999). The osmotic environment within the rhizosphere may affect root colonization, infection thread development, nodule development, and the formation of effective N2-fixing nodules (Miller and Wood, 1996). Rhizobia induce the formation of nodules on the roots of legume plants, in which atmospheric nitrogen is fixed and supplied to the host plant, thereby enhancing growth under nitrogen-limiting conditions. The symbiotic interaction between rhizobia and their cognate leguminous plants is important for agricultural productivity. However, physiological stresses, such as salinity, negatively affect these symbiotic interactions by limiting nitrogen fixation (Zahran, 1999). An efficient Rhizobium-legume symbiosis under salt stress required also the selection of salt-tolerant rhizobia (Zahran, 1999). Nodulation and nodule dry weight was promoted markedly by inoculation with *Rhizobium triolii* in Berseem crop and depressed significantly with consistent increase in salinity (Hussain *et al.*, 2002). There is now increasing evidence that the use of beneficial microbes could enhance the resistance of plants to adverse environmental stresses, e.g., drought, salts, nutrient deficiency, and heavy metal contaminations (Glick *et al.*, 2007). An increase of tolerance to salinity of rhizobial bacteria might constitute another approach to improve plant productivity under symbiosis (Kenenil *et al.*, 2010).

Inoculation with RhM11 improved plant and nodule growth compared with those inoculated with RhM14 and CIAT 899 under saline condition in some common bean (Faghire *et al.* 2011). An alternate strategy to improve crop plants for salt tolerance is to introduce salt-tolerant plant growth promoting rhizobacteria (PGPR) that enhance crop growth in saline soil. It is suggested that root-colonizing bacteria that produce phytohormones may stimulate plant growth and help in nutrient recycling in the rhizosphere microcosm and thus the microbes can alleviate the effects of salinity in the environment. The evaluation of saline - tolerant bacterial strains to stimulate saline tolerance and promote growth of crop plants leading to better productivity in saline soil (Vivekanandan *et al.* 2015).

Therefore, inoculation of the salt tolerant *Rhizobium* under conditions of slat stress may help in nodule formation promote biological nitrogen fixation as well as leghaemoglobin content leguminous crops.

MATERIAL AND METHODS

Total 40 root nodules samples along with rhizosphere soil samples with intact root nodules of soybean plants were collected from saline tract of five districts of Western Maharashtra viz., Kolhapur, Sangali, Satara, Solapur and Ahmednagar to isolate salt tolerant *Rhizobium*. Total of 33 salt tolerant *Rhizobium* isolates were obtained from the root nodules of soybean grown in saline soils of Western Maharashtra. Isolation of Rhizobium from root nodule was done by the method of Samosegaran and Hoben (1985). The reference salt tolerant Rhizobium strain was used for comparison. To confirm the salt tolerance of *Rhizobium* isolates, they were tested against different concentrations of NaCl salt. For this, YEMA medium supplemented with 0.075, 0.15, 0.3, 0.6, 1.2, 1.8, 2.1, 2.4, 3.0, 3.6, 4.2, 4.8, 5.4, 6.0, 7.2, 8.4, 9.6 and 10.8 per cent NaCl. Out of 33 salt tolerant isolates, 20 isolates were categorized under extremely salt tolerance, (more than 5.4 % salt tolerance limit) only three efficient salt tolerant Rhizobium (STR-4. STR-14, STR-18) were selected to develop the liquid consortium. Classification of salt tolerant Rhizobium was done on the basis of salt tolerance limit (Cardoso *et al.*, 2014).

Using liquid consortium of salt tolerant *Rhizobium* in comparison with reference strain liquid formulation obtained from liquid biofertilizers production unit, Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri to study their performance on nodulation and leghaemoglobin content of soybean as detailed below. The land selection for experimental purpose was ploughed once and two harrowing were given. The farm yard manure (FYM) @ 10 ton ha^{-1} was uniformly spread all over the land before preparatory tillage operation. The soil was brought to fine tilth condition. The experiment was carried out in *kharif* -2016 in Randomized Block Design with three replications and eight treatments as given in (Table No. 1). The gross and net plot size were 1.80 x 3.0 m^2 and 1.20 x 2.60 m^2 respectively. The initial chemical properties of soil in terms of salinity, pH (1:2.5) and EC (dSm^{-1}) was 8.24 and 2.74 respectively.

The seeds of soybean (JS-335) were treated with consortium of salt tolerant Rhizobium @ 25 ml/kg of seeds at the time of sowing seed were dried in shade for 30

Table 1. Treatment details	
Treatment No.	**Treatment details**
T_1	Absolute control
T_2	Reference strain + 50 % N
T_3	Reference strain + 75 % N
T_4	Reference strain + 100 % N
T_5	Liquid consortium + 50 % N
T_6	Liquid consortium +75 % N
T_7	Liquid consortium+100 % N
T_8	Only 100 % N

T- Treatment
N- Nitrogen dose

minutes and were sown in lines with spacing 30 cm x10 cm and 1.5 cm deep) in each plot. Salt tolerant *Rhizobium* consortium was applied to the seeds and through drenching @ 3.0 lit/ha @ 10^8 cfu/ml after 45 days of sowing as per the application of N. 50: 75:00 N:P_2O_5:K_2O (kg ha^{-1}) were used to supply N, P_2O_5 and K_2O also initial chemical and biological properties of soil were studied.

The observations on nodulation were recorded at the flowering and harvesting stage. Before uprooting plants, light irrigation was given to plot so that it became easy for uprooting. The root system was dipped in water for removal of soil adhered to roots and then washed with water. The nodulation count for effective and non-effective nodules was taken for randomly selected five plants and then average figure was taken. The observations on lehaemoglobin content were recorded at 45 days and 60 days after sowing.

Colorimetric estimation of Leghaemoglobin as pyridine haemochromogen

Leghaemoglobin content in the nodules of soybean was determined by using pyridine reagent as described by Hartree (1957). The standard working solution af haemin (1 mg haemin per ml solution) was prepared. The standard solution was pipette out corresponding to (0, 0.1, 0.2, 0.3....1.0 mg) concentrations. The colour was developed and the absorbance was measured at 500 nm as above. The calibration curve of optical density (O.D.) against mg of haemin was plotted.

The statistical analysis of data was carried out by employing Randomized Block Design (RBD). The critical differences were calculated at P = 0.05 level of significance for the *in-vivo* experiment. Wherever F test were significant and interpretation of the results was carried out in accordance by Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

Number of effective and non-effective nodule: It is revealed from the data that, the mean number effective nodules of soybean decreased with increase in the age of crop up to harvesting stage and the mean number non-effective nodules of soybean increased with increase in the age of crop up to harvesting stage. Effect of application of salt tolerant Rhizobium consortium on effective and non-effective nodules was significant at all intervals.

At flowering: From the data presented in (Table 2 and Fig. 2) it was found that treatment T_7 (Liquid consortium + 100 % N) was singnificantly superior in number of effective nodules (25.25) over rest of treatments how-

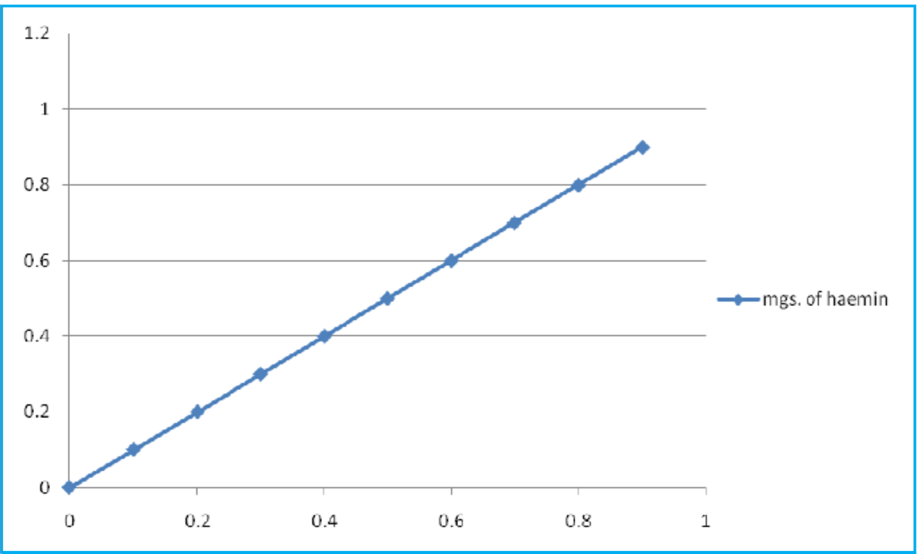

FIGURE 1. Calibration of a standard curve for leghaemoglobin (haemin) determination.

Table 2. Effect of liquid consortium of salt tolerant Rhizobium on number of effective and non effective nodules per plant of soybean at flowering and harvesting stages

Treatment		Number of effective nodules/plant		Number of non-effective nodules/plant	
		At flowering	At harvesting	At flowering	At Harvesting
T_1	Absolute control	15.88	5.79	16.60	21.80
T_2	Reference strain + 50 % N	17.74	8.20	15.12	19.17
T_3	Reference strain + 75 % N	21.64	11.33	11.55	15.47
T_4	Reference strain + 100 % N	23.32	12.24	10.76	14.21
T_5	Liquid consortium + 50 % N	18.49	9.12	13.87	18.33
T_6	Liquid consortium + 75 % N	24.70	14.93	8.75	12.20
T_7	Liquid consortium + 100% N	25.25	15.24	7.52	11.55
T_8	Only 100 % N	19.25	10.53	12.61	16.70
	S.E. +	0.18	0.10	0.41	0.21
	CD at 5 %	0.55	0.32	1.25	0.65

ever it was at par with treatment T_6 (Liquid consortium + 75 % N) (24.70). It was also found that treatment T_7 (Liquid consortium + 100 % N) significantly superior in number of non- effective nodules (7.52) over rest of the treatments however it was at par with treatment T_6 (Liquid consortium + 75 % N) (8.75). The treatment absolute control recorded the least number of effective and highest number of non-effective nodules (15.88) and (16.60) respectively.

At harvesting: The treatment (Table 2 and Fig. 2) T_7 (Liquid consortium +100 % N) was significantly superior in number of effective nodules (15.24) over rest of treatments however it was at par treatment T_6 (Liquid consortium + 75 % N) (14.93). Also it was found that treatment T_7 (Liquid consortium + 100 % N) significantly superior in number of non- effective nodules (7.52) over rest of treatments however it was at par treatment T_6 (Liquid consortium + 75 % N) (12.20). The treatment absolute control recorded the least number of effective and highest number non-effective nodule (5.79) and (21.80) respectively.

Effect of liquid consortium of salt tolerant Rhizobium on Lehaemoglobin content of nodule

The data (Table 3 and Fig. 3) showed that, the mean Leghaemoglobin content of soybean nodule increased with increase in the age of crop. Effect of application of salt tolerant Rhizobium consortium on Leghaemoglobin content was significant at all intervals.

The treatment T_7 (Liquid consortium + 100 % N) which was significantly superior in Leghaemoglobin content of nodule (0.290 mg g^{-1} fresh of nodule weight) over rest of all the treatments and it was at par with treatment T_6 (Liquid consortium + 75 % N) (0.276 mg g^{-1} fresh of nodule weight). The treatment absolute control recorded the least in Leghaemoglobin content of nodule (0.184 mg g^{-1} fresh of nodule weight).

The treatment T_7 (Liquid consortium + 100 % N) was significantly superior in Leghaemoglobin content of nodule (0.321 mg g^{-1} fresh of nodule weight) over rest of all the treatments and it was at par with treatment T_6 (Liquid consortium + 75 % N) (0.305 mg g^{-1} fresh of nodule weight). The treatment absolute control recorded the least in Leghaemoglobin content of nodule (0.202 mg g^{-1} fresh of nodule weight).

Similarly, Hussain *et al.* (2002) reported that 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*. 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. Similarly, Adewusi *et al.*, (2008) reported that rhizobial inoculation increased nodule biomass thus encouraged sustainable environmental friendly agriculture by responding perfectly in biological nitrogen fixation. Similarly Faghire *et al.* (2011) showed that in controls, inoculation with RhM11 improved plant and nodule growth compared with those inoculated with RhM14 and CIAT 899. NaCl treatment generally had a negative affect on plant and nodule growth. Under The nodular phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) exhibited higher activities and were less affected by salinity in plants inoculated with the reference strain CIAT899 than those inoculated with local strains and concluded that plants inoculated with CIAT899 and RhM11 showed more salinity stress tolerance than those inoculated with RhM14.

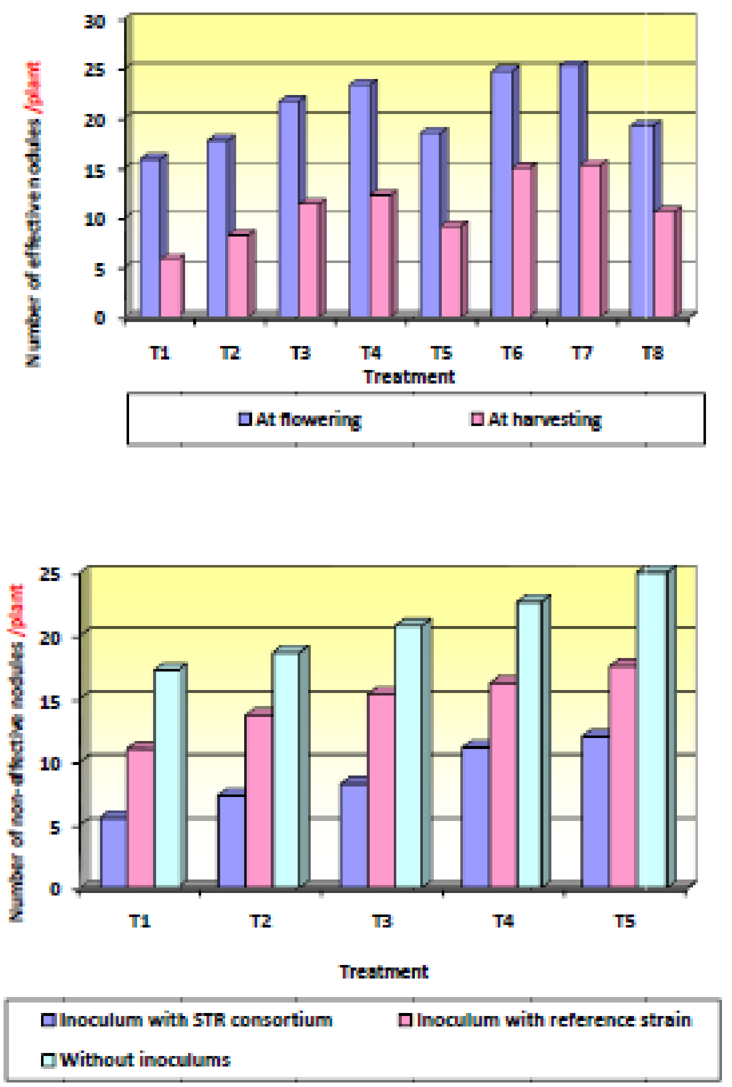

FIGURE 2. Effect of liquid consortium of salt tolerant Rhizobium on number of effective and non-effective nodulesper plant of Soybean at flowering and harvesting stages

The results are in line with, Rejili *et al.* (2012) reported that on the selection and characterization of salt-tolerant Rhizobia strains, able to fix nitrogen symbiotically under salt conditions, might constitute a strategy for improving legume symbiosis in adverse conditions and might constitute a better economic and sustainable alternative to chemical fertilization. Similarly, Sharma *et al.* (2013) reported that on the salinity tolerance of naturally occurring rhizobia, isolated from the root nodules of three leguminous plants, viz., sesbania (*Sesbania sesban*), lablab (*Lablab purpureus*) and pigeonpea (*Cajanus cajan*), growing at research farm in Dubai (United Arab Emirates). The Rhizobial isolates were also found to be effective in nodulating 21-day old seedlings grown in potting soil and irrigated with saline water up to 12 dSm^{-1} after inoculation. The tolerance to high levels of salinity and the survival and persistence in severe and harsh desert conditions made these Rhizobia highly valuable inoculum to improve productivity of the leguminous plants cultivated under extreme environments.

Present finding correlates, Vishal *et al.* (2013) reported that the inoculation with *Rhizobium* culture had invariably and significantly promoted nodulation and leghaemoglobin content at both durations particularly at lower EC levels and minimized the deleterious effect of salinity at10 to 14 dSm-1.Salinity is considered a limiting factor

Table 3. Effect of liquid consortium of salt tolerant Rhizobium on Leghaemoglobin content of soybean at 45 and 60 days

Treatment		Leghaemoglobin content (mg g^{-1} fresh weight of nodule)	
		At 45 days	At 60 days
T_1	Absolute control	0.184	0.202
T_2	Reference strain + 50 % N	0.204	0.224
T_3	Reference strain + 75 % N	0.251	0.276
T_4	Reference strain + 100 % N	0.269	0.293
T_5	Liquid consortium + 50 % N	0.219	0.243
T_6	Liquid consortium + 75 % N	0.276	0.305
T_7	Liquid consortium + 100% N	0.290	0.321
T_8	Only 100 % N	0.235	0.257
	S.E. +	0.001	0.001
	CD at 5 %	0.004	0.003

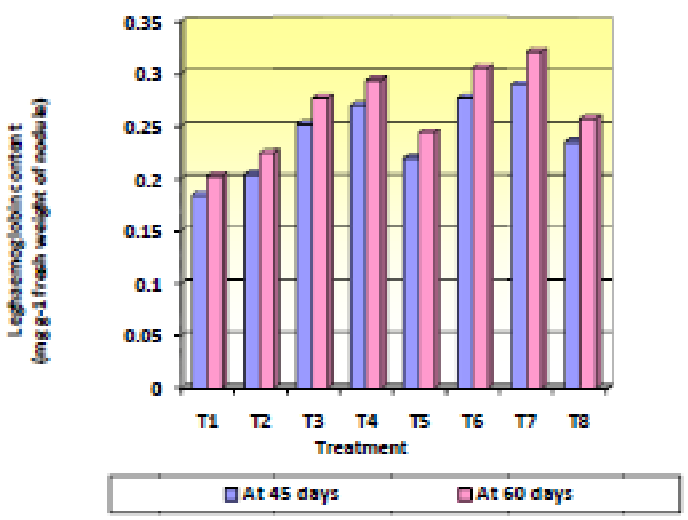

FIGURE 3. Effect of liquid consortium of salt tolerant Rhizobium on fresh weight of shoot and root of soybean at flowering and harvesting stages

in nodulation and leghaemoglobin content in legume-Rhizobium associations, which can adversely affect the yield of legume crops. Rhizobia can tolerate high concentrations unlike legume plants. Therefore, in saline soils, the multiplication of these Rhizobium strains will not be affected in the rhizosphere of the plant host. So, there is currently need isolation and development salt tolerant Rhizobium strains would enhance the plant growth through nodulation and leghaemoglobin content of plants under saline conditions which indirectly increase the nitrogen fixing ability of legume crop.

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