



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2018; 6(5): 2048-2052

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Received: 01-07-2018

Accepted: 05-08-2018

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Effectiveness of native rhizobium on different genotypes of soybean

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Abstract

Rhizobium is the most well known species of a group of bacteria that acts as the most effective symbiotic fixer of nitrogen. Effectiveness of native *Rhizobium* on different genotypes of Soybean was studied with 10 advanced genotypes i.e. RSC 11-02, RSC 11-03, RSC 11-04, RSC 11-05, RSC 11-06, RSC 10-52, RSC 10-71, RSC 11-08, RSC 11-09, RSC 11-10. Data from field study revealed that highest plant height (120cm/plant) was associated with soybean variety RSC 11-06(T5). Highest fresh weight (209.33 g/plant) and dry weight (52.5 g/plant) of shoot were associated with soybean variety RSC 11-10 (T10). Furthermore, total the N uptake (712.38 mg/plant) observed was highest on soybean variety RSC 11-02 (T1). Highest no. of pods/plant (100.66) was observed in soybean variety RSC 11-10 (T10) and highest grain yield (33.02 q/ha.) was associated with soybean variety RSC 11-02 (T1). The poorest performance was observed in case of RSC 11-08 (T8) in overall findings while the better performance of RSC 11-02 (T1) on the basis of its effective symbiosis with native *Rhizobium* followed by RSC 11-10 (T10). Ten *Rhizobium* isolates from effective root nodule of each variety of soybean were isolated. These rhizobia isolates were also tested for their effectiveness in JS-9305 variety of soybean under glass house condition. Data from Glass house experiment revealed that, the highest plant height (145 cm/plant) was associated with isolate present in (T2) i.e. *Rhizobium* isolated from RSC 11-03 variety. Highest fresh weight of shoot (11.17 g/plant) and dry weight (2.92 g/plant) were associated with (T1) i.e. *Rhizobium* isolated from RSC 11-02. Further average no. of nodule/plant (14.33%) was observed highest in (T9) *rhizobium* isolated from RSC 11-09. Glasshouse experiment showed that none of the isolates obtained from different genotypes of soybean is not effective in JS-9305, as the nodulation in JS 9305 is very poor.

Keywords: soybean, isolation, *Rhizobium*, nitrogen, nodulation, experiment

Introduction

Soybean is recognized as golden bean because of its high nutritional values and economic importance. It is one of the most important oilseed crops in the world and also wonder crop of the twentieth century. It is a good sources of edible oil and protein. Chemical analysis revealed that soybean seed contains approximately 20% oil, 40% protein, 30% carbohydrates, 10% total sugar and 5% ash. In addition, soybean meal also has high content of minerals, particularly calcium, phosphorus and iron and its seed oil is rich in essential fatty acids.

Further symbiotic relationship between legumes like soybean and nitrogen fixing-bacteria, commonly known as *Rhizobia*. These are genetically diverse and physiologically heterogeneous group of bacteria and are able to elicit nodule formation on legumes. *Rhizobia* are soil-borne bacteria that selectively infect the roots of some legumes and have the following characteristics; gram negative, motile rod-shaped (approximately 0.5-0.9 μm in width and 1.2-3.0 μm in length) and heterotrophic (Pepper and Upchurch, 1991; Prescott *et al.*, 1996). This group comprise of the genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* (Denarie *et al.*, 1992) & *Sinorhizobium*. They are ubiquitous part of the soil micro-flora in a free-living state in the rhizosphere of legumes. An important characteristic of this symbiotic interaction is host specificity, where defined species of rhizobia forms nodules on specific legumes (Bottomley, 1992). Nitrogen is often the most limiting factor in plant growth and development (Albareda *et al.*, 2009). The use of nitrogen fertilizers is associated with severe pollution problems so the symbiosis between legumes and rhizobia for biological nitrogen fixation (BNF) is the best option because it uses photosynthetic energy and is environmentally cleaner. The biological nitrogen fixation (BNF) is the process by which soil bacteria collectively called rhizobia enter into a symbiotic association with legumes which leads to the formation of nitrogen fixing root nodules.

Methodology

The experiments were conducted under field conditions at Instructional cum Research Farm (*Bharri land*), College of Agriculture, Raipur (Chhattisgarh) during kharif season 2016-17, on MLT-2017 soybean. To evaluate the identification of effectiveness of native rhizobium on different genotypes of soybean. Effectiveness of native *Rhizobium* on different genotypes of Soybean was studied with 10 advanced genotypes i.e. RSC 11- 02, RSC 11-03, RSC 11-04, RSC 11-05, RSC 11-06, RSC 10-52, RSC 10-71, RSC 11- 08, RSC 11-09, RSC 11-10. The experiment was laid out in Randomized Block Design (RBD) with three replication & ten treatments. The layout plans and other details of the experiments are depicted in Table 1-

Table 1

Treatment	R ₁	R ₂	R ₃
T ₁ (Variety RSC 11-02)			
T ₂ (Variety RSC 11-03)			
T ₃ (Variety RSC 11-04)			
T ₄ (Variety RSC 11-05)			
T ₅ (Variety RSC 11-06)			
T ₆ (Variety RSC 10-52)			
T ₇ (Variety RSC 10-71)			
T ₈ (Variety RSC 11-08)			
T ₉ (Variety RSC 11-09)			
T ₁₀ (Variety RSC 11-10)			

This research were following observations: Number of nodule plant⁻¹ after 35 and 80 days, Fresh and dry weight of nodule & plant, N content of nodule & plant after 80 days, Plant height 30, 50 and 80 DAS day after sowing, Number of pods plant⁻¹ after 80 days, Yield after harvesting. Recommended dose of N, P₂O₅ and K₂O (RDF) applied @ 20, 60 and 40 Kg ha⁻¹ as basal dose. As a preventive measure Qunalphos 25 EC @ 2 ml lit⁻¹ was sprayed for the control of insects. The nitrogen content in the nodule and plant samples was estimated by Micro-Kjeldahl method as described by Jackson (1973) using auto digestion and distillation system. All the pre and post harvest observations were recorded and tabulated in a systemic manner. The final observations were statistically analyzed by randomized blocks design (Panse and Shukhatme, 1978) by using MS excel 2007.

Result and Discussion

The assessment of the treatment effects of the native rhizobium observations has been justified in a reasonable manner results obtained from the present investigation are depicted and discussed as bellow.

Plant height

Data presented of the plant height was summarized in Table 2 In general average plant height was significantly increases upto the harvest (plate 1). Data reveal that plant height of field grown soybean at 30 DAS, at 50 DAS and at 80 DAS increased significantly over all treatment. At 30 DAS, plant height increased significantly from 30.66 to 42.33 cm. The

highest plant height (42.33 cm) was recorded in RSC 11-02 (T₁) which was found comparable with (T₄), (T₇), and (T₁₀) while lowest plant height (30.66 cm) was recorded in RSC 11-05 (T₂). At 50 DAS, plant height increased from 57.33 to 89.0 cm. The maximum plant height (89.0 cm) was obtained under RSC 11-10 (T₁₀) which was found comparable with RSC 11-5 (T₄) and RSC 10-71 (T₇). The minimum plant height (57.33 cm) was found under RSC 11-03 (T₂). Further at 80 DAS, plant height increased from 67.0 to 120 cm. The maximum plant height (120.0 cm) was obtained under RSC 11-06 (T₅) which was found comparable with RSC 11-05 (T₄), RSC 10-52 (T₆) and RSC 11-10 (T₁₀). The minimum plant height (67.0 cm) was associated with RSC 11-03 (T₂).

Table 2: Influence of field grown soybean varieties with native *Rhizobium* on plant height (cm/plant)

Treatment	Variety	30 DAS	50 DAS	80 DAS
T ₁	RSC 11-02	42.333	78.667	82.33
T ₂	RSC 11-03	30.667	57.333	67.0
T ₃	RSC 11-04	34.667	72.667	80.0
T ₄	RSC 11-05	40.000	80.833	115.66
T ₅	RSC 11-06	38.667	77.333	120.0
T ₆	RSC 10-52	39.667	68.333	98.0
T ₇	RSC 10-71	39.667	81.667	95.0
T ₈	RSC 11-08	36.000	65.000	79.0
T ₉	RSC 11-09	36.333	68.000	70.83
T ₁₀	RSC 11-10	40.667	89.000	100.66
CD (0.05%)		2.286	5.476	6.78
SE(m)+/-		0.763	1.82	2.26

Plant Biomass

Data presented in Table: 3 revealed that the fresh weight and dry weight of shoot at 80 DAS flowering stage increased significantly in between 10 different treatments, At 80 DAS, fresh weight of shoot was increased significantly from 41.66 to 209.33 g/plant and maximum fresh weight of shoot (209.33 g/plant) was obtain under RSC 11-10 (T₁₀) which was found comparable with RSC 11-05 (T₄), RSC 11-06 (T₅) and RSC 11-09 (T₉). The minimum fresh weight of shoot (41.66 g/plant) was of RSC 11-03 (T₂). At 80 DAS, dry weight of shoot it was increased significantly from 12.5 to 52.5 g/plant. The maximum dry weight of shoot (52.5 g/plant) was obtain under RSC 11-10 (T₁₀) which was found comparable with RSC 11-02 (T₁), RSC 11-06 (T₅), RSC 11-09 (T₉) and RSC 11-05 (T₄), The minimum dry weight of shoot (12.5g/plant) was found under RSC 11-03 (T₂).

The N-uptake values in shoot increased significantly from 251.51 to 709.71 mg/plant. It's probably due to variety effect of native rhizobia. The highest shoot N-uptake value 709.71 mg/plant was associated with RSC 11-02 (T₁). While the lowest shoot N-uptake value 251.51 mg/plant was associated with RSC 11-03 (T₂) (Table:3). N-content values in shoot increased significantly from 1.33 to 2.11%. This is probably due to variety effect of native rhizobia. The highest N-content values in shoot 2.11% was associated with RSC 11-05(T₄). While the lowest N-content values in shoot 1.33% was associated with RSC 10-52(T₆).

Table 3: Influence of field grown soybean varieties with native *Rhizobium* on biomass accumulation (g/plant) and Nitrogen accumulation in shoot

Treatment	Variety	Fresh weight of shoot (g./plant)	Dry weight of shoot (g./plant)	N-content in shoot (%)	N-uptake in shoot (mg./plant)
T ₁	RSC 11-02	167.5	48.833	1.453	709.71
T ₂	RSC 11-03	41.667	12.5	1.823	251.25
T ₃	RSC 11-04	86	23	2.06	488.37

T4	RSC 11-05	119.333	31.333	2.11	490.88
T5	RSC 11-06	116	34.833	1.567	428.45
T6	RSC 10-52	93.333	23.5	1.33	447.28
T7	RSC 10-71	65.333	22.5	1.453	378.00
T8	RSC 11-08	69.333	19	1.91	347.07
T9	RSC 11-09	174.833	42.067	1.68	567.90
T10	RSC 11-10)	209.333	52.5	1.78	588.00
CD (0.05%)		8.603	5.19	0.298	80.946
SE(m)+/-		2.87	1.73	0.1	27.035

Nodulation

Average number of root nodules/plant of soybean was observed at 80 DAS (data presented in Table: (4) No. of nodules was significantly increased from 34.33 to 133.66 nodule/plant. The variation in nodulation may be due to variety effect on native rhizobia. Numbers of nodules (133.66/plant) were recorded as highest in RSC 11-06 (T5) among all the nodulated plants under field study the lowest number of nodules in RSC 11-09 (T9) was (34.33/plant).

Data of nodule fresh weight (presented in Table 4) indicated that highest fresh weight of nodules was associated with plants raised in experimental plots belonging to treatment T4 followed by treatment (T8). Fresh weight of nodules was significantly increased from 0.14 to 0.913 g/plant. Fresh weight of nodules 0.913 g/plant was recorded as highest among all the treatments, under field study. There was lowest fresh weight of nodules is 0.14 g/ plant was associated with RSC11-10 (T10).

Similarly, nodule dry weight (presented in Table 4) indicated that highest dry weight of nodules was associated with plants raised in plots belonging to treatment T2 followed by treatment T1. Dry weight of nodules was significantly increased from 0.108 to 0.414 g/plant. Dry weight of nodules 0.414 g/plant (RSC 11-06) was recorded as highest among all the nodulated plants, under field study. There was lowest dry weight of nodules is 0.108 g/plant in RSC 11-10 (T10).

N-content in nodule at 80 DAS Table (4), increased significantly from 0.787 to 1.577%, the highest nodule N-content value 1.577% was recorded in RSC 11-06 (T5), while the lowest nodule N-content value 0.787% were associated with RSC 11-10 (T10).

Nodule N-uptake at 80 DAS Table (4), the N-uptake values increased significantly from 0.853 to 6.49 mg/plant. The highest nodule N-uptake value 6.49 mg/plant was recorded in RSC 11-06 (T5), while the lowest nodule N-uptake value 0.853 mg/plant was associated with RSC 11-10 (T10).

Table 4: Influence of field grown soybean varieties with native *Rhizobium* on N-accumulation in nodule (80 DAS)

Treatment	Variety	Avg. no. of nodule/ plant	Fresh weight of nodule (g./plant)	Dry weight of nodule (g./plant)	N-content in nodule (%)	N-uptake in nodule (mg./plant)
T1	RSC 11-02	44.667	0.451	0.238	1.123	2.673
T2	RSC 11-03	50.333	0.81	0.349	1.217	4.247
T3	RSC 11-04	42	0.249	0.143	0.930	1.323
T4	RSC 11-05	52	0.274	0.165	1.197	1.963
T5	RSC 11-06	133.667	0.913	0.414	1.577	6.497
T6	RSC 10-52	47.333	0.16	0.129	0.857	1.080
T7	RSC 10-71	44	0.464	0.31	1.233	3.797
T8	RSC 11-08	40	0.213	0.18	1.017	1.797
T9	RSC 11-09	34.333	0.233	0.158	1.123	1.717
T10	RSC11-10)	37.667	0.144	0.108	0.787	0.853
CD (0.05%)		6.445	0.054	0.008	0.058	0.209
SE(m)+/-		2.15	0.018	0.004	0.019	0.070

Rhizobium isolation

Rhizobium isolates were isolated from effective nodules of different soybean varieties (mentioned as treatment 1-10) of

with respect to soybean variety MLT 2017. Surprisingly there is a difference in growth and morphological characteristic of most of the isolates (Table (5)).

Table 5: Location from where *Rhizobium* isolates obtained for present investigation

S. No	Location/District with name of crop	Source	<i>Rhizobium</i> isolate No	Selected variety	Colour
1	IGKV research farm Raipur	Root nodule	1	(Variety RSC 11-02)	Whitish colour
2	IGKV research farm Raipur	Root nodule	2	(Variety RSC 11-03)	Whitish colour
3	IGKV research farm Raipur	Root nodule	3	(Variety RSC 11-04)	Whitish colour
4	IGKV research farm Raipur	Root nodule	4	(Variety RSC 11-05)	Red colour
5	IGKV research farm Raipur	Root nodule	5	(Variety RSC 11-06)	Whitish colour
6	IGKV research farm Raipur	Root nodule	6	(Variety RSC 10-52)	Light orange colour
7	IGKV research farm Raipur	Root nodule	7	(Variety RSC 10-71)	Red colour
8	IGKV research farm Raipur	Root nodule	8	(Variety RSC 11-08)	Light whitish colour
9	IGKV research farm Raipur	Root nodule	9	(Variety RSC 11-09)	Pink colour
10	IGKV research farm Raipur	Root nodule	10	(Variety RSC 11-10)	Pink colour

Total N in nodule and shoot

Total N-accumulation by field grown soybean is presented in Table (6) At 80 DAS, the total N-uptake increased significantly from 255.49 to 712.38 mg/ha. The variations is

probably due to the varietal effect of native rhizobia, the highest total N uptake 712.38mg/ha was observed in RSC 11-02 (T1), while the lowest total N-uptake value 255.49 mg/ha was associated with RSC 11-03 (T2).

Table 6: Influence of field grown soybean varieties with native *Rhizobium* on total N-accumulation

Treatment	Variety	N-content in shoot (%)	N-uptake in shoot (mg./plant)	N content in nodule (%)	N-uptake in nodule (mg./plant)	Total N uptake (mg./plant)
T1	RSC 11-02	1.45	709.71	1.123	2.673	712.38
T2	RSC 11-03	2.01	251.25	1.217	4.247	255.49
T3	RSC 11-04	2.12	488.37	0.93	1.323	489.69
T4	RSC 11-05	1.57	490.88	1.197	1.963	492.84
T5	RSC 11-06	1.23	428.45	1.577	6.497	434.94
T6	RSC 10-52	1.90	447.28	0.857	1.080	448.36
T7	RSC 10-71	1.68	378.00	1.233	3.797	381.79
T8	RSC 11-08	1.83	347.07	1.017	1.797	348.86
T9	RSC 11-09	1.35	567.90	1.123	1.717	569.61
T10	RSC1110)	1.12	588.00	0.787	0.853	588.85
CD (0.05%)		0.298	80.946	0.058	0.209	
SE(m)+/-		0.1	27.035	0.019	0.070	

Number of pods

Data of pod formation study are summarized in Table (7) The data revealed that no. of pods increased significantly from 20 to 100.66/plant. The highest no. of pods (100.66 / plant) was

observed in RSC 11-10 (T10), the variation in Number of pods may be effect of native rhizobia, Lowest no. of pods (20 per plant) was recorded in RSC 11-03 (T2).

Table 7: Influence of field grown soybean varieties with native *Rhizobium* on No. of pods after 80 days.

Treatment	Variety	No. of pod plant ⁻¹
T1	RSC 11-02	83.333
T2	RSC 11-03	20
T3	RSC 11-04	52
T4	RSC 11-05	86.667
T5	RSC 11-06	50.33
T6	RSC 10-52	60.333
T7	RSC 10-71	42.667
T8	RSC 11-08	44
T9	RSC 11-09	97
T10	RSC11-10)	100.66
CD (0.05%)		7.426
SE(m)+/-		2.48

Grain yield

Yield data presented in (Table 8) revealed that grain yield increased significantly over the treatments. The variation in yield may be due to variety effect on native rhizobia. The

grain yield increased significantly from 12.027 to 33.02 q/ha. The highest grain yield (33.02 q / ha) was associated with RSC 11-02 (T1), while RSC 11-08 (T8) gave lowest grain yield (12.02 q per ha).

Table 8: Influence of field grown soybean varieties with native *Rhizobium* on yield

Treatment	Variety	Grain yield (q./ha.)
T1	RSC 11-02	33.023
T2	RSC 11-03	17.527
T3	RSC 11-04	17.487
T4	RSC 11-05	23.7
T5	RSC 11-06	15.633
T6	RSC 10-52	22.3
T7	RSC 10-71	24.883
T8	RSC 11-08	12.027
T9	RSC 11-09	20.11
T10	RSC11-10)	28.103
CD (0.05%)		1.534
SE(m)+/-		0.512

Conclusion

Effectiveness of native *Rhizobium* on different genotypes of soybean was studied with 10 advanced genotypes i.e. RSC 11-02, RSC 11-03, RSC 11-04, RSC11-05, RSC 11-06, RSC 10-52, RSC 10-71, RSC 11-08, RSC 11-09 and RSC 11-10 (T1-T10). The experiment was conducted during Kharif season 2017-18 at Instructional Cum Research Farm Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). Observations comprises (1) plant height (2) plant biomass (3) nodulation (4) N-uptake by nodule and plant (5) No. of pods (5) grain yield after harvesting.

Ten *Rhizobium* isolates from effective root nodules of each variety of soybean were isolated. Those rhizobia hence also tested for their effectiveness in JS-9305 variety of soybean under glass house condition. Selected rhizobia used for basis of growth performance and select for future screening. Maximum plant height (120 cm) at 80 DAS was obtained in field condition RSC 11-06 (T5), the maximum fresh weight of shoot (209.33 g/plant) was obtained in RSC 11-10 (T10). Further the dry weight of shoot (52.5 g/plant) was highest in RSC 11-10 (T10). The maximum N content of shoot (2.11%)

was obtained under RSC 11-05 (T4), While RSC 11-02 (T1) gave highest N uptake (709.71 mg/plant).

The highest No. of nodules (133.66 nodule/plant) was obtained under RSC 11-06 (T5), whereas fresh weight of nodule (0.91 g/plant) was of RSC 11-06 (T5), RSC 11-06 (T5), RSC 11-06(T5) gave highest dry weight of nodule(0.41 g/plant), the maximum N content of nodule (1.577%) was obtained under RSC 11-06 (T5), and highest N uptake value of nodule 6.497 mg/plant was recorded in RSC 11-06(T5). The total N uptake (712.38 mg/plant) was obtained under RSC 11-02 (T1).

At pod formation stage the highest No of pod (100.66 /plant) was obtained under RSC 11-06 (T5). The highest grain yield (33.02 q per ha) was associated with RSC 11-02 (T1). It is due to the varietal effect of native *rhizobia*.

Acknowledgment

I am grateful to the Department of Agril. Microbiology, IGKV. Raipur (C.G.), for providing facilities for this research.

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