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Effect of different rhizobium inoculation on yield and nutrient uptake by soybean (*Glycine max* (L.) Merrill)

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Abstract
A field experiment was conducted at Instructional Farm, Rajasthan College of Agriculture, Udaipur (Rajasthan) in *kharif* 2016 to assess the effect of different *rhizobial* strains on nutrient content & uptake of soybean. The experiment was laid out in randomized block design with four replications. The experiment comprised with nine treatments of different *rhizobial* strains to the soybean and one treatment should be uninoculated. The results revealed that inoculation with different *rhizobial* isolates have significant influence on nutrient content and uptake in grain & straw of soybean.

Keywords: *Rhizobial* strains, Soybean, Nutrient, Content, Uptake

Introduction

Soybean (*Glycine max* L.) is one of the major grain legume crops, whose production is getting popularized and adopted in the region of Southern Rajasthan. The governmental and non-governmental bodies also undertaken for the purpose of making to aware of the importance of soybean to small land holding farmers not only as a crop for improving their economic status but also as an important high protein food. India ranks fifth in soybean production in the world. Soybean production is mainly confined to Madhya Pradesh (also known as bowl of soybean in India), Maharashtra, Rajasthan, Andhra Pradesh, Karnataka, Uttar Pradesh and Chhattisgarh (Pawar *et al*, 2011) [20]. In Rajasthan, soybean is mainly cultivated in the south eastern part of the state covering Kota, Bundi, Baran and Jhalawar districts which are known as Haroti region while it is grown in patches in some other districts like Sawai Madhopur, Bhilwara, Chittorgarh, Rajsamand, Dungarpur, Banswara and Udaipur (SOPA, 2001) [26]. Total area of soybean in Rajasthan in the year 2015-16 was 11.04 lakh ha with production of 7.86 lakh MT (Anonymous, 2015-16) [2].

Nitrogen is a limiting nutrient for growth and yield of soybean, *rhizobia* have a direct role to play in its supply to the growing plants (Kanimozhi and Panneerselvam 2010) [11]. *Bradyrhizobium japonicum* capable of forming root nodules on soybean. The ability to form nodules has been found to be highly host specific for different species of *rhizobia* (Jorden 1982).

Nitrogen fixing bacteria are able to form symbiotic associations with legumes and fix nitrogen through Biological Nitrogen Fixation (BNF). The bacteria involved in this process is able to utilize molecular nitrogen with the help of the nitrogen fixing enzyme nitrogenase or to convert atmospheric nitrogen into ammonia, a form that can easily be used by plants (FAO, 2006). The symbiotic association between the bacteria and the host legume is such that the host legume provides nutrition for the bacteria and the bacteria fix nitrogen (Keyser and Li, 1992; Unkovich and Baldock, 2005) [12, 29]. Although this association requires high amount of energy, its energy source is inexpensive and renewable and as such sustainable. BNF can improve soil fertility through the addition of nitrogen (Okogun *et al*., 2005) [19]. Herridge *et al*. (2008) [8] reported that grain legumes contribute more than 20 million tons of fixed N each year indicating that the contributions of BNF cannot be undermined. Tahir *et al*. (2009) [27] reported that the BNF capacity of legumes is a vital process for sustaining crop land management and is an effective and efficient source of N supply to plants under favorable atmospheric and environmental conditions.

Materials and Methods

The experiment was conducted at Instructional Farm, Rajasthan College of Agriculture, Udaipur (Rajasthan) in *kharif 2016* on sandy clay loam soil which is slightly alkaline in nature consisted of 9 treatments of different rhizobial strains, *viz*., SB-22 (T2), SB-272 (T3), SB-31 (T4), SB-431 (T5), SB- 401 (T6), SB-441 (T7), SB-442 (T8), SB-481 (T9) and SB-402 (T10), respectively and control (T1). These treatments were evaluated under randomized block design (RBD) with four replications. Soybean cultivar (JS - 9560) was taken as test crop.

Table 1: Methods Adopted for Plant Analysis:

(i)	Nitrogen content	Colorimetric Method using spectrophotometer after development of colour with Nesseler's reagent	Snell and Snell (1949) [25]
(ii)	Phosphorus content	Vando-molybdo phosphoric acid yellow colour method	Jackson (1967) [9]
(iii)	Potassium content	Analysis of suitable aliquot of acid digested plant material by Flame photometer	Metson (1956) [18]
(iv)	Protein content	By multiplying % N in seed with a factor 6.25	A.O.A.C. (1960) [1]
(v)	Zn, Fe, Cu & Mn	Estimation on AAS	Lindsay and Norvell (1978) [17]
(vi)	Leghemoglobin content	Determination of leghemoglobin in legume nodules	Wilson and Reisenauer (1962) [31]
(vii)	Oil content	Extraction of oil from soxhlet extractor	Franz von soxhlet (1879) [7]
(viii)	Rhizobium population in soil (cfu g-1)	Standard serial dilution and plate count method	Scmidt and Colwell (1967) [22]

Nutrient uptake

N, P, K, and Fe, Mn, Cu, Zn uptake were computed from the data of N, P, K, and Fe, Mn, Cu, Zn content and yield using the following formulae:

$$\text{Micronutrient uptake (kg ha-1)} = \frac{\text{Nutrient content in plant material (\%) x Yield (kg ha-1)}}{100}$$

$$\text{Micronutrient uptake (g ha-1)} = \frac{\text{Nutrient content (mg kg-1) x Yield (kg ha-1)}}{1000}$$

Results

Effect of Inoculation on macro nutrient content and uptake in seed & straw of soybean

Data presented in Table: 2&3 revealed that inoculation of seed with different *Rhizobium* strains significantly increased in nutrient content & uptake in seed of soybean, *viz*. nitrogen, phosphorus and potassium increased significantly when seed was inoculated with different Rhizobium strains of soybean. The Highest N content in seed (6.557%) was recorded with inoculation of SB- 401 (T6), Maximum phosphorus content in seed (0.588%) was recorded in the inoculated with SB- 401 (T6). However, SB- 401(T6) was found statistically at par with all the rest of isolates except control (T1), maximum potassium content (1.163%) in seed was recorded in the inoculated with SB-401. The significantly highest total nitrogen uptake by soybean (150.49 kg ha-1) was recorded with inoculation of SB-401 isolate over control, highest total P uptake by soybean (16.84 kg ha-1) was observed with inoculation of SB-401 over control and SB-22, significantly highest total K uptake by soybean (74.31 kg ha-1) was observed with inoculation of SB-401 over control and the extent of increase was of order of 51.12 per cent over control.

An examination of data (Table 3) revealed that inoculation with *rhizobium* significantly affect the nitrogen, phosphorus and potassium content & uptake in straw of soybean. Maximum nitrogen content (1.092%) was recorded with inoculation of SB-401, Maximum phosphorus content (0.205 %) was recorded in the inoculated with SB-401, Significantly maximum potassium content (1.724%) was recorded in the inoculated with SB-401 (T6) over control.

Table 2: Effect of different *rhizobial* strains on macro nutrient content in seed & straw of soybean

Treatments	Nutrient content in seed					
	N %	P %	K %	N %	P %	K %
T1- Control	5.554	0.5570	1.103	1.040	0.1968	1.523
T2- SB- 22	6.062	0.5790	1.151	1.055	0.2018	1.681
T3- SB- 272	6.099	0.5792	1.152	1.060	0.2019	1.683
T4- SB- 31	6.152	0.5796	1.152	1.080	0.2026	1.683
T5- SB- 431	6.170	0.5798	1.152	1.084	0.2026	1.687
T6- SB- 401	6.557	0.5877	1.163	1.092	0.2053	1.724
T7- SB- 441	6.259	0.5812	1.159	1.087	0.2036	1.683
T8- SB- 442	6.312	0.5817	1.154	1.088	0.2037	1.688
T9- SB- 481	6.424	0.5788	1.154	1.090	0.2044	1.691
T10- SB- 402	6.504	0.5835	1.154	1.090	0.2049	1.687
SEm±	0.11	0.0066	0.014	0.017	0.0018	0.023
CD (P= 0.05)	0.327	0.0192	0.041	0.048	0.0053	0.065

Table 3: Effect of different *rhizobial* strains on macro nutrient uptake by seed, straw and total uptake of soybean

Treatments	Nutrient uptake by seed (Kg/ha)								
	N	P	K	N	P	K	N	P	K
T1- Control	71.08	7.13	14.15	23.93	4.86	37.68	95.02	11.65	49.17
T2- SB- 22	96.08	9.18	18.25	30.50	5.83	48.61	126.58	15.01	66.86
T3- SB- 272	97.66	9.27	18.43	30.76	5.86	48.83	128.42	15.13	67.26
T4- SB- 31	101.20	9.54	18.95	31.98	6.00	49.83	133.18	15.54	68.79
T5- SB- 431	96.84	9.10	18.06	32.75	6.11	50.97	129.59	15.22	69.04
T6- SB- 401	116.47	10.44	20.66	34.02	6.40	53.66	150.49	16.84	74.31
T7- SB- 441	105.05	9.75	19.45	33.29	6.24	51.56	138.34	15.99	71.01
T8- SB- 442	101.97	9.36	18.57	33.34	6.23	51.75	135.32	15.59	70.32
T9- SB- 481	105.06	9.47	18.87	33.30	6.25	51.66	138.36	15.71	70.54
T10-SB-402	109.14	9.79	19.37	33.31	6.27	51.56	142.45	16.06	70.93
SEm±	5.13	0.43	0.84	1.56	0.27	2.35	6.32	0.63	2.99
CD(P=0.05)	14.883	1.251	2.443	4.530	0.780	6.820	18.331	1.826	8.677

Effect of Inoculation on micro nutrient uptake in seed & straw of soybean

As evident from the results (Table 4) inoculation of different *rhizobial* strains showed significant difference in micro nutrient uptake in seed & straw of soybean, *viz*. Zn, Mn, Fe and Cu uptake increased significantly when seed was inoculated with different Rhizobium strains of soybean. Significantly highest zinc uptake by seed (97.92 g ha-1) was observed with inoculation of SB-401, maximum uptake of Mn (77.71 g ha-1) by seed was recorded with inoculation of SB-401 over control and SB-22, maximum Fe uptake by straw (180.75 g ha-1) was observed with inoculation of SB-401, significantly maximum Cu uptake by straw was observed (15.77 g ha-1) with the inoculation of SB-401 over control.

A perusal of data (Table 5) showed that different *rhizobium* isolates significantly increased the total Zn, Mn, Fe and Cu uptake by soybean over control. The significantly highest total Zn uptake by soybean (222.29 g ha-1) was observed with inoculation of SB-401, highest total Mn uptake by soybean (274.28 g ha-1) was recorded with inoculation of SB-401, highest total Fe uptake by soybean (234.86 g ha-1) was observed with inoculation of SB-401 (T6) and significantly highest total Cu uptake by soybean (40.08 g ha-1) was observed under inoculation of SB-401 which was significantly superior over control.

Table 4: Effect of different *rhizobial* strains on micro nutrient (Zn, Mn) uptake by seed, straw and total uptake of soybean

Treatments	Nutrient uptake by seed (g/ha)		by Total upta			
	Zn	Mn	Zn	Mn	Zn	Mn
T1- Control	67.39	54.71	86.94	142.43	154.33	197.14
T2- SB- 22	87.17	68.13	113.27	180.91	200.44	249.04
T3- SB- 272	87.17	68.79	113.72	181.30	200.89	250.09
T4- SB- 31	90.28	71.03	115.85	185.47	206.13	256.50
T5- SB- 431	84.91	67.80	118.01	189.06	202.92	256.86
T6- SB- 401	97.92	77.71	124.38	196.58	222.29	274.28
T7- SB- 441	90.10	72.90	118.72	191.87	208.82	264.77
T8- SB- 442	86.25	69.82	115.91	192.96	202.17	262.79
T9- SB- 481	86.34	71.01	116.14	192.41	202.48	263.43
T10-SB-402	88.47	72.95	116.98	192.45	205.45	265.40
SEm±	4.01	3.28	5.23	8.32	8.21	10.99
CD(P=0.05)	11.635	9.530	15.185	24.147	23.825	31.901

Table 5: Effect of different *rhizobial* strains on micro nutrient (Fe, Cu) uptake by seed, straw and total uptake of soybean

Treatments	Nutrient uptake by seed (g/ha)					
	Fe	Cu	Fe	Cu	Fe	Cu
T1- Control	38.40	17.09	130.63	11.02	169.03	28.10
T2- SB- 22	48.20	21.63	167.73	14.46	215.93	36.08
T3- SB- 272	48.63	21.79	168.30	14.51	216.93	36.30
T4- SB- 31	50.01	22.35	171.46	14.72	221.47	37.07
T5- SB- 431	47.70	21.31	174.86	15.08	222.56	36.38
T6- SB- 401	54.11	24.31	180.75	15.77	234.86	40.08
T7- SB- 441	50.97	22.83	177.23	15.13	228.20	37.96
T8- SB- 442	48.76	21.79	177.21	15.15	225.96	36.94
T9- SB- 481	49.54	22.11	176.34	14.98	225.88	37.09
T10-SB-402	50.80	22.72	176.09	14.94	226.90	37.66
SEm±	2.30	1.02	8.09	0.74	9.85	1.64
CD(P=0.05)	6.668	2.964	23.488	2.140	28.592	4.765

Discussion

The combined inoculation of seed with *Rhizobium* was more beneficial or increase in nitrogen, phosphorus, potassium content and uptake by seed and straw due to increased solubility of phosphorus and higher nitrogen fixation in nodules, leading to increased availability of nitrogen and phosphorus. The increase availability of nitrogen and phosphorus also helped to utilize more potassium from the soil by the plant. Thus, the greater content and uptake of nitrogen, phosphorus, potassium by seed and straw might be due to *Rhizobium* inoculation. These results corroborate the findings of Vikram and Hamzehzarghani (2008) [30], Khan *et al.* (2014) [13], Rathore *et al.* (2010) [21] and Kumawat *et al.* (2002) [16].

Rhizobium improves the N and P status of soil. Inoculation with different *rhizobium* strain benefits the plant than either group of organisms alone and may have added advantage in the degraded agro ecosystem. Rhizobium inoculation might have contributed something towards enhanced plant growth and increased the soluble P. Increased nodulation under *Rhizobium* inoculation might be to due to close association with the microbial population and their activities resulting in improving soil fertility status. These finding are similar to the results obtained by Singh *et al.* (2012) [12], Khandelwal (2012) [14] and Kumari *et al.* (2012) [15].

The inoculation of different isolates of *rhizobium* were non significantly influence on the Zn, Mn, Fe and Cu after harvest of the crop. Above properties don't change in one cropping season. These results corroborate with the finding of Tiwari and Kumar (2009) [28], Choudhary and Yadav (2011) [4] and Das *et al.* (2013) [5].

Seed inoculation with *Rhizobium* isolate significantly increased the available N in soil after harvesting of crop. It might be due to release of the native P from the soil and concentration of phosphorus increased in soil solution which helped in early root growth and nodules formation due to which symbiotic N2-fixation increased in soybean roots. Due to increase in symbiotic N2- fixation, available N in soil also increased.

A significant increase in total and effective root nodules plant-1 at 60 DAS observed due to seed inoculation with different *Rhizobium* inoculation over control. Inoculation of seed with *Rhizobium* might have increased the concentration of an efficient and healthy strain of *Rhizobium* in rhizosphere, which in turn resulted in greater fixation of atmosphere nitrogen in soil for use by the plants and consequently resulting in to higher plant growth, which in term resulted into higher production of assimilates and their partitioning to different reproductive structures such as the result of increased plant growth in terms of total and effective root nodules at 60 DAS due to overall better nutritional environments in the rhizophere. Chattopadhyay and Dutta (2003) and Vikram and Hamzehzarghani (2008) [30] also reported similar results for black gram.

Conclusion

On the basis of one year experimental findings the inoculation with SB - 401 isolate significantly increased the macronutrient content and uptake in seed & straw of soybean. The inoculation of SB – 401 *rhizobium* strains were not significantly influenced on micronutrient content in seed & straw over control but micronutrient uptake found statistically significant as compared to other isolates and uninoculated treatments. Hence, it may be concluded that SB - 401 isolate was more effective in terms of nutrient content and uptake by soybean.

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