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Performance of different *Mesorhizobium* strains on nodulation, grain, straw yield and soil fertility status of chickpea

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Abstract
The field experiment was conducted in the Pulses Improvement Project MPKV, Rahuri, Dist. Ahmednagar during 2012-13 to 2014-15 in randomized block design with three replications The results indicated that the highest grain yield (2684 kgha^{-1}) and straw yield (2912 kgha^{-1}) was recorded in T_9, *Mesorhizobium* + PSB3 (Rahuri- Reference strain**)**, which was at par with T_5, *Mesorhizobium* + ORE 35 (Hisar) grain yield (2568 kgha^{-1}), straw yield (2766 kgha^{-1}).
The maximum number of nodules (54.10 /plant) were observed in T_9, *Mesorhizobium* + PSB3 (Rahuri- Reference strain**)**, which was followed by T_5, *Mesorhizobium* + ORE35 (Hisar) number of nodule (49.82), T_7, *Mesorhizobium* + PSB12 (Ludhiana) (49.30), T_8, *Mesorhizobium* + PSB (*Pseudomonas striata*) (Gulberga) (47.01).The higher dry weight of nodules was recorded in T_9, *Mesorhizobium* + PSB3 (Rahuri- Reference strain**)** (1044.8 gm/plant) which was at par with T_5, *Mesorhizobium* + *ORE35* (Hisar), (982.6 mg/plant) T_7, (982.4 mg/plant), T_8, (975.7 mg/plant).The maximum organic carbon content (0.60%), Available nitrogen was (210.6 kgha^{-1}), available phosphorous (16.67 kgha^{-1}), and phosphorous uptake (39.42 kgha^{-1}) was observed in T_9, *Mesorhizobium* + PSB3 (Rahuri- Reference strain**)**.

Keywords: Chickpea, yield, *Mesorhizobium*, rhizobium

Introduction

Microorganism living within plant tissues for all or part of their life cycle without causing any visible symptoms of their presence have been defined as endophytic bacteria are ubiquitous in most plant species, residing intercellular or intracellularly within host tissues and therefore are at advantage as composed to free living counter parts by being protected from environmental stresses and microbial competition. Many of reports found in literature suggested that plants endophytes have an excellent potential to be used as plant growth promoters in legumes, combined inoculation of PGPR with rhizobia in grain leghumes has received much attention in recent years because of their positive effects on nodulation, N fixation and yield of pulse crops (Dudeja *et al.* 2011). Endophytic microbes can promotes plant growth by altering plant physiology including osmotic pressure regulation, changes in stomatal responses adjusted in root size and morphology, improvement in nutrients acquisition via nitrogen fixation, phosphate solubilization, iron chelation and increased uptake of certain minerals, preventing pathogen infections via antifungal or anti bacterial agents, out compacting pathogens for nutrients by siderphone production, or by establishing the plants systemic resistance (Compant *et al.* 2005). However, for establishment of endophytes in different tissues, endophytic microbes must be compatible with the host plants and able to colonize the tissues of the host plants without being recognized as patho Legume-rhizobium symbiosis depends on the specificity of plant and bacterial species because of chemical signaling that resulted in formation of specialized structures i.e. nodules in which the bacteria are hosted and reduced atmospheric nitrogen into ammonium (Rao and Cooper, 1994; Bai *et al*., 2002) [17, 1]. It is established and studied fact that world supply of organic nitrogen is met via the symbiosis between root nodulating bacteria and leguminous host plants (Postgate, 1998) [15].
Plant growth promoting rhizobacteria (PGPR) are responsible to mediate the soil processes such as decomposition, nutrient mobilization, mineralization, solubilization, nitrogen fixation and growth hormone production (Dobbelaere *et al*., 2003; Khan *et al*., 2003) [3, 13].

PGPR having the P-solubilizing capacity are called as phosphate solubilizing microorganisms (PSM) or phosphate solubilizing bacteria (PSB) have been reported to increase P-concentration by converting insoluble forms to soluble ones through the production of organic acids (Maliha *et al*., 2004) [14] and hence increased the crop yields (Zaidi 1999; Gull *et al*., 2004) [18, 12]. Inoculation of soil with P-solubilizing bacteria is a promising approach that may alleviate the deficiency of phosphorus (Cakmakci, 2005) [2].
Therefore, the present study was undertaken to study the performance of different Mesorhizobium strains on nodulation, grain, straw yield and fertility status of chickpea at harvest.

Materials and Methods

A field experiment was conducted during *rabi* season of 2012-13 to 2014 -15 at Pulses Improvement project MPKV, Rahuri, Dist. Ahmednagar (M.S.). The experimental soil was medium black having pH (8.15), EC (0.32 dSm^{-1}), organic carbon (0.56%) and available N (188.5 kg ha^{-1}), P (10.65 kg ha^{-1}) and K (432.5 kg ha^{-1}) respectively. The nine endophytic bacteria of chickpea were obtained from microbial culture collection under All India Coordinated Research Project on Chickpea. The experiment was laid out in randomized block design with three replications. The recommended dose of fertilizer was applied uniformly to all plots as a basal through urea, singles super phosphate at the time of sowing. Chickpea were sown at spacing 30 x10 cm. In five plants form each plot were randomly uprooted along with soil core at 45 -50 days after sowing, roots were washed off to remove the adhering soil, nodule were removed from roots and counted. The dry weights of nodule and plants were determined after drying to constant weight. Soil samples (0-15 cm depth) were randomly taken with the help of soil auger to make a composite sample for evaluating the initial fertility status and after harvest The different methods was used as suggested by Alkaline permanganate, Subbiah and Asija (1956) [9] for analyzing available soil nitrogen, Olsen (1954) for available phosphorous, Walky and Black by Jackson (1967) [5] for organic carbon.

Results and Discussion

Root nodulation

The number of root nodules and dry weight of root nodules increased with crop age (Table 1). The pooled results revealed that there was significant influence of different *mesorhizobium* strains on grain yield, straw yield, nodulation and soil fertility status at harvest of chickpea. The inoculation of different *mesorhizobium* strains significantly favored the number and dry weight of root nodule over the uninoculated control. The significant highest nodule number were noted at 60 DAS with application of T_9, treatment, *Mesorhizobium* + PSB3 (Rahuri- Reference strain) (54.10 $plant^{-1}$) and in dry weight of nodule (1044.8 gm $plant^{-1}$).The result are in agreement with the finding of Dudeja and Giri (2014) [4]. This could be attributed to increased supply of nutrients though biological N_2 fixation and P solubilization as well as production of growth hormone by the endophytic bacteria (Rashied *et al*. 2012). Increased in nodulation and yield components of legume crops following inoculation with N2-fixing and P-solubilizing microbes have also reported by other researcher (Garcia *et al*., 2004; Gupta, 2004) [10, 11]. Results of this study contradicted with the findings of Paul and Verma (1999) [16] who observed increased nodule number and mass due to free-living diazotrophic inoculation.

Grain and straw yield

The pooled results revealed that there were significant influences of different treatments on grain yield of chickpea (Table 2). The inoculation of chickpea with *mesorhizobium* significantly higher grain yield (2684 kg ha^{-1}) of chickpea was recorded with the application of T_9 treatment, (*Mesorhizobium* + PSB3 (Rahuri reference strain), which was at par with T_5, (2568 kg ha^{-1}) and T_7 (2537 kg ha^{-1}). Similarly, inoculation produce higher straw yield (2912 kg ha^{-1}) (Table -2) by the application of T_9, treatment, (*Mesorhizobium* + PSB3 (Rahuri reference strain) which was at par with T_5 (2766 kg ha^{-1}) as compared to control.
The results of effect of different mesorhizobium strains on organic carbon, available nitrogen and available phosphorous, uptake of phosphorous at harvest of chickpea are presented in Table 3 and 4. The organic carbon, available nitrogen, phosphorous and phosphorous uptake status of soil after harvest of chickpea were significantly influenced due to application of different nutrients over initial status. Inoculation alone or in combination produced higher soil organic carbon (0.60%) available nitrogen (210.6 kg. ha^{-1}), phosphorus (16.67 kg. ha^{-1}) and uptake of phosphorous (39.42 kg. ha^{-1}) were observed due to application of T_9 treatment, (*Mesorhizobium* + PSB3 (Rahuri reference strain) as compared to control. Combined use of microorganisms having P-solubilizing capacity and producing PGR'S is gaining importance as an effective approach for enhancing yield of crops (Zaidi *et al*., 2003., Zaidi *et al*., 2004) [20]. In present study, significant increases in nodulation, yield and nutrient uptake were observed when both the *mesorhizobium* and phosphorous solubilizer inoculation were combined with fertilizer over un-inoculated control. However, *mesorhizobium* inoculation proved to be more effective in improving growth and yield of chickpea compared with absolute control. The positive effect of inoculation on plant growth and development observed in case of *mesorhizobium* in this study. These finding are supported by the work of previous researchers (Zaidi *et al*., 1999., Zaidi *et al*., 2003., Mirza *et al*., 2007) [18, 20].

Table 1: Effect of different Mesorhizobium strains on nodulation at flowering of chickpea

Tr. No	Treatment	No of nodules (gm/plant)			Pooled mean	Dry weight of nodule (gm/plant)			Pooled mean
		2012-13	2013-14	2014-15		2012-13	2013-14	2014-15	
1	Control	37.83	27.70	37.83	34.45	678.7	263.0	678.7	540.1
2	20 kg $P_2O_5ha^{-1}$	40.45	31.70	40.45	37.53	892.9	307.7	892.9	697.8
3	40 kg $P_2O_5ha^{-1}$	42.36	34.30	42.36	39.67	1029.2	330.3	1029.2	796.2
4	*Mesorhizobium*	46.41	37.70	46.41	43.51	1145.3	431.7	1145.3	907.4
5	*Mesorhizobium* + ORE35 (Hisar)	54.88	39.70	54.88	49.82	1253.4	441.0	1253.4	982.6
6	*Mesorhizobium + PS27* PSB2 (New Delhi)	47.75	44.00	47.75	46.50	1165.6	470.0	1165.6	933.7
7	*Mesorhizobium* + PSB12 (Ludhiana)	52.95	42.00	52.95	49.30	1217.9	511.3	1217.9	982.4

8	*Mesorhizobium* + PSB (Pseudomonas striat Gulberga)	48.67	43.70	48.67	47.01	1192.6	542.0	1192.6	975.7
9	*Mesorhizobium*+ PSB3 (Rahuri- Reference strain)	56.39	49.30	56.39	54.10	1289.7	555.0	1289.7	1044.8
	SE±	0.231	1.83	0.231	1.12	38.4	2.50	38.4	37.53
	CD at 5%	0.694	5.47	0.694	3.39	115.1	7.50	115.1	113.5

Table 2: Effect of different P solubilizing strains along with *Mesorhizobium* on grain, straw yield of chickpea

Tr. No	Treatment	Grain yield (kg ha^{-1})			Pooled mean	Straw yield (kg ha^{-1})			Pooled mean
		2012-13	2013-14	2014-15		2012-13	2013-14	2014-15	
1	Control	1873	1887	1873	1878	2147	2055	2147	2116
2	20 kg P_2O_5ha^{-1}	2058	2092	2058	2069	2357	2264	2357	2326
3	40 kg P_2O_5ha^{-1}	2177	2145	2177	2166	2360	2374	2360	2366
4	*Mesorhizobium*	2375	2183	2375	2311	2564	2392	2564	2507
5	*Mesorhizobium* + ORE35 (Hisar)	2723	2258	2723	2568	2926	2446	2926	2766
6	*Mesorhizobium* + *PS27* PSB2 (New Delhi)	2477	2215	2477	2390	2659	2384	2659	2567
7	*Mesorhizobium* + PSB12 (Ludhiana)	2674	2264	2674	2537	2861	2418	2861	2713
8	*Mesorhizobium* + PSB (Pseudomonas striat Gulberga)	2548	2196	2548	2431	2834	2363	2834	2677
9	*Mesorhizobium*+ PSB3 (Rahuri- Reference strain)	2829	2395	2829	2684	3046	2644	3046	2912
	SE±	188.3	90.2	188.3	66.14	172.1	100.0	172.1	62.93
	CD at 5%	564.6	270.4	564.6	199.99	516.1	300.0	516.1	190.29

Table 3: Effect of different Mesorhizobium strains on organic carbon and available nitrogen of harvest of chickpea

Tr. No	Treatment	Organic carbon (%)			Pooled mean	Available nitrogen (kg ha^{-1})			Pooled mean
		2012-13	2013-14	2014-15		2012-13	2013-14	2014-15	
1	Control	0.48	0.51	0.47	0.49	189.1	191.5	188.5	189.7
2	20 kg P_2O_5ha^{-1}	0.51	0.53	0.50	0.51	192.5	193.5	191.7	192.6
3	40 kg P_2O_5ha^{-1}	0.53	0.52	0.54	0.53	194.1	195.6	193.5	194.4
4	*Mesorhizobium*	0.56	0.57	0.58	0.57	198.2	199.6	200.5	199.4
5	*Mesorhizobium* + ORE35 (Hisar)	0.55	0.58	0.57	0.57	201.4	201.8	203.4	202.2
6	*Mesorhizobium* + *PS27* PSB2 (New Delhi)	0.54	0.56	0.53	0.54	204.9	205.6	206.8	205.8
7	*Mesorhizobium* + PSB12 (Ludhiana)	0.56	0.57	0.55	0.56	207.4	208.6	207.9	207.9
8	*Mesorhizobium* + PSB (Pseudomonas striatGulberga)	0.54	0.56	0.53	0.54	205.8	207.5	206.8	206.7
9	*Mesorhizobium*+ PSB3 (Rahuri- Reference strain)	0.60	0.62	0.59	0.60	211.8	210.3	209.8	210.6
	SE±	0.02	0.01	0.01	0.1	0.79	0.45	0.53	0.57
	CD at 5%	0.6	0.03	0.04	0.02	2.34	1.31	1.57	1.72

Table 4: Effect of different Mesorhizobium strains on available Phosphorous and uptake of Phosphorous at harvest of chickpea.

Tr. No	Treatment	Available Phosphorous (kg ha^{-1})			Pooled mean	Phosphorous uptake (kg ha^{-1})			Pooled mean
		2012-13	2013-14	2014-15		2012-13	2013-14	2014-15	
1	Control	10.67	10.56	10.89	10.71	28.83	28.78	28.83	28.81
2	20 kg P_2O_5ha^{-1}	11.69	11.55	11.61	11.62	27.40	27.46	27.51	27.46
3	40 kg P_2O_5ha^{-1}	11.93	11.92	11.88	11.91	29.53	29.56	29.50	39.53
4	*Mesorhizobium*	12.57	12.52	12.60	12.56	31.23	31.26	31.28	31.26
5	*Mesorhizobium* + ORE35 (Hisar)	13.61	13.65	13.58	13.61	36.40	36.42	36.44	36.42
6	*Mesorhizobium* + *PS27* PSB2 (New Delhi)	12.26	12.28	12.38	12.31	32.33	32.28	32.38	32.33
7	*Mesorhizobium* + PSB12 (Ludhiana)	15.07	15.12	15.22	15.14	37.60	37.64	37.61	37.62
8	*Mesorhizobium* + PSB (Pseudomonas striatGulberga)	13.71	13.68	13.64	13.68	34.60	34.67	34.62	34.63
9	*Mesorhizobium*+ PSB3 (Rahuri- Reference strain)	16.70	16.68	16.64	16.67	39.40	39.45	39.42	39.42
	SE±	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02
	CD at 5%	0.07	0.06	0.07	0.13	0.04	0.04	0.05	0.06

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