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Effect of Phosphate Solubilizing Bacterial Strains on Plant Growth of Green Gram (*Vigna radiata*) and Mustard (*Brassica compestris*)

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Thirty-nine phosphate solubilizing bacteria (PSB) were isolated from the rhizosphere of green gram and mustard by using Pikovskaya medium containing tricalcium phosphate. All the PSB showed a large variation in P- solubilization on soild as well as in liquid media. Most of the isolates fell in <100% class of P- solubilization, while in liquid media 50-110 μg/ml. No correlation was established between P solubilization on solid and liquid media. The selected isolates 4GRP, 25MRP, 27MRP, 28MRP, 33MRP and 34MRP showed P- solubilization 168.3 μg/ml, 260.8 μg/ml, 255.4 μg/ml, 261.6 μg/ml, 220.5 μg/ml and 175.6 μg/ml respectively. The pH of the medium was decreased by PSB during the P-solubilization, maximum decrease in pH was found with 25 MRP. Under pot house conditions at 60 DAS in green gram and mustard, maximum plant dry biomass was recorded with 25MRP with URP followed by 33MRP with URP. In mustard maximum P uptake was observed in 25MRP with URP (284%) followed by 4GRP with URP (143%) at 60 DAS. In green gram maximum P uptake was observed in 25MRP with URP (224%) followed by 33MRP with URP (182%) at 60 DAS.

Key words: Phosphate solubilizing bacteria (PSB) - Green gram, mustard, Plant growth.

In soil, both macro and micronutrients undergo a complex dynamic equilibrium of solubilization and insolubilization that is greatly influenced by the soil pH and microflora. Phosphorous is component of some important cellular constituents and is involved in the energy transformation, photosynthesis in plants and other organisms. The concentration of total P in the soil ranges from 0.1 to 0.2 % (Kapoor, 1995). However, most of the iron is not available to plants as it is fixed with Ca, Al, Fe and Mn depending on soil pH, organic matter level and type of microorganisms (Barker, 1984). Even the applied phosphorus as phosphatic fertilizers is converted in to non availability form as soon as the phosphate ions react with Fe, Al or Ca depending on the soil pH. Phosphorus is commonly deficient in most natural soils, since it is fixed as insoluble iron and aluminium

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phosphates in acidic soils (especially those with pH lower than 5.0) or calcium phosphates in alkaline soils (pH above 7.0). However, insoluble calcium phosphates can be dissolved and made available to plants by soil and rhizosphere microorganisms via a mechanism that is thought to involve the release of organic acids (Cunnigham, *et al*., 1992; Goldstein, *et al*., 1995). The soil is indeed a habitat for diverse group of organisms which employ variety of solubilization reaction to release soluble phosphorous from insoluble phosphate (Illmer and Schinner, 1995; Singh and Kapoor, 1994). Numerous microorganisms, especially those associated with roots, have the ability to increase the plant growth and productivity (Chang, *et al*., 1986; Kloepper, *et al*., 1988). Therefore, it is important to develop technology for P solubilization for plant growth. Phosphate solubilizing microorganisms (PSM) have been isolated from various sources (Pandey, *et al.*, 2006; Nautiyal, *et al*., 2000; Rameshkumar, *et al*., 2005) and potential of these PSM have been utilized as bioinoculants for crops grown in soil low in available and amended with rock phosphate or tricalcium phosphate (de Freitas, *et al.,* 1997; Bagyaraj, *et al*., 2000). In the present study a large number of phosphate-solubilizing bacteria have been isolated and assessed for their impact on plant growth promotion of green gram and mustard crop under pot house conditions.

MATERIALS AND METHODS

Chemicals. The following chemicals, media and reagents were used for the present studies. The chemicals used were from Hi Media Laboratories, SRL, Glaxo and E. Merck etc. The chemicals were of AR grade. Tricalcium phosphate (TCP) was obtained from Hi media laboratories, Udiapur rock phosphate which is of metamorphic cum sedimentary origin and a flouapatite (69% apatite) containing 30% P_2O_5 was obtained from Rajasthan state mines and mineral corporation.

Isolation of PSB from rhizosphere of green gram and mustard. Rhizosphere soil of green gram cv. Asha and mustard crop cv. RH-30 growing at J. V. College Baraut campus were collected for isolation of PSB. Phosphate solubilizing bacteria (PSB) were isolated by using Pikovskaya medium (Pikovskaya, 1948) with the following composition (g/1) glucose, 10.0; $(NH_4)_2SO_4$, 0.2; NaCl, 0.2; $MgCl_2$, 0.1; KCl, 0.2; yeast extract, 0.5; $FeSO_4$, 0.02; $MgSO_4$, 0.02; tricalcium phosphate, 2.5; pH 7.0 and agar agar, 20.0 (whenever solid medium was used). The soil samples were serially diluted and plated on the Pikovskaya (PVK) medium plates. The plates were incubated at 30^0C for one week. After one week of incubation, colonies showing discrete halo zone around them indicating the dissolution of tricalcium phosphate were taken as P solubilizers. The colonies were picked up and streaked on PVK medium plates for purification. The isolated colonies obtained were grown on PVK medium slants for one week and subsequently stored in a refrigerator at 4°C. The pure cultures of phosphate solublizing bacteria were transferred to fresh slants at regular intervals.

Determination of P- solubilization by PSB strains under solid and liquid culture conditions. For assessing the P- solubilization on solid medium the bacterial growth of different cultures was suspended in 1 ml of sterilized distilled water and 40 µl of suspension containing approximately 10^7 cells/ml spotted on the PVK medium plates. After incubation of 4 days at 30^0C, the diameter of colony and of halo zone developed around colony was measured. P- solubilizing efficiency (PSE) was calculated as: PSE (%) = Z - C / C x 100, (Where, Z = zone diameter and C = colony diameter). Solubilization of TCP under liquid medium condition was examined by using PVK medium under stationary conditions. Fifty ml PVK medium was taken in to 150 ml Erlenmeyer flasks and inoculation was done with 1 ml suspension of different cultures containing approximately 10^7 cells/ml, uninoculated flasks were taken as control. The flasks were incubated at 30 ± 2°C for 4 days and the contents were centrifuged at 10,000 rpm for 10 min. The supernatant was analyzed for changes in pH and water soluble P- content. The pH of supernatant was recorded by using pH meter and phosphorous was estimated (John, 1970).

Evaluation of PSB strains for biomass and P uptake on green gram and mustard under pot culture condition. The selected PSB strains 4GRP, 25MRP, 27MRP, 28MRP, 33MRP and 34MRP were evaluated under pot house conditions for their effect on biomass production and P uptake with & without rock phosphate at different intervals (30,

45 & 60 days). Five Kg of loamy sand soil having pH, 8.21 and total P, 8.16 kg/ha was filled in earthen pots. The inorganic fertilizers 60 Kg/ha (326 mg urea/5 kg soil) and 30 Kg URP/ha (250 mg URP/5 kg soil) were mixed in upper 5 cm of soil. 30 kg P_2O_5 (469 mg SSP/5 kg soil) applied as SSP was used as check. Seed inoculation with selected PSB strains was done by dipping the mustard RH-30 and green gram seeds in suspension containing approximately 10^7 cells/ml. Five seeds per pot were sown, after 8 days thinning was done and only three plants were left in each pot. Three replications each of the following treatments were taken. (1) Control SSP (30kg/ha), (2) URP (30kg/ha), (3) 4GRP, (4) 4GRP + URP, (5) 25MRP, (6) 25MRP+ URP, (7) 27MRP, (8) 27MRP+ URP, (9) 28MRP, (10) 28MRP+ URP, (11) 33MRP, (12) 33MRP+ URP, (13) 34MRP and (14) 34MRP+ URP. Mustard and green gram plants were uprooted at 30, 45, 60 (days after sowing) and dried in oven at 60ºC to a constant biomass weight. Total P in both plant samples was determined by Vanadomolybdophosphoric yellow color method (Koenig and Johnson, 1942).

Establishment of PSB in the rhizosphere of mustard and green gram under pot house conditions. The freshly grown cultures of PSB were used as seed inoculants before sowing. The bacterial load per seed of mustard cv. RH-30 and green gram cv. Asha were determined after soaking the seeds for 30 minutes in culture broth which had 15-30 × 10^7 cfu/ml. The PSB number per seed varied between 8-15 x 10^5 cfu just before sowing. The crops were raised in earthen pots filled with 5 kg P- deficient soil. The PSB population in the mustard and green gram rhizosphere was checked on PVK medium at different days after sowing (DAS). The rhizospheric soil was serially diluted up to 10^{-5} and 1 ml from 10^{-4} and 10^{-5} dilutions were plated on PVK medium plates. The PSB were counted after incubating the plates at 30ºC ± 2ºC for 4 days.

RESULTS

Isolation of PSB from the rhizosphere of green gram and mustard. A total of 39 colonies showing discrete clear zone around them were

Table 1. P- solubilization efficiency and P- solubilization (µg/ml) by different PSB isolates in pikovskaya broth containing tricalcium phosphate (TCP)

Isolate No	Source of isolation	Solubilization efficiency	pH	Phosphate solubilized
1GRP, 4GRP, 5GRP, 6GRP, 7GRP, 8GRP, 18GRP,19GRP, 20GRP	Green gram	0.0	6.3, 4.3, 6.1, 5.9, 6.5, 6.3, 5.4, 5.8, 6.1	55.6,168.3, 52.1, 81.5, 55.6, 92.7, 63.8, 85.8, 77.8
2GRP, 3GRP, 9GRP, 11GRP	Green gram	99.9, 97.8, 125.2, 39.5	6.4, 5.3, 5.1, 5.2	57.3, 78.7, 93.9, 88.7
12GRP,13GRP, 14GRP,15GRP	Green gram	285.6, 81.6, 60.1, 51.2	6.2, 5.3, 5.6,5.3	98.4, 83.7, 80.5, 96.4
16GRP, 17GRP	Green gram	175.0, 21.5	6.1, 5.5	76.3, 80.8
21MRP, 22MRP, 23MRP, 24MRP, 38MRP, 39MRP, 40MRP	Mustard	0.0	5.7, 6.2, 5.8, 5.8, 5.3, 6.1, 5.5	64.3, 55.4, 66.8, 67.3, 91.2, 85.2, 69.9
25MRP, 26MRP	Mustard	105.3, 235.2	3.5, 5.6	260.8, 85.5
27MRP, 28MRP	Mustard	66.6, 69.9	3.7, 3.8	255.4, 261.6
30MRP, 31MRP	Mustard	94.3, 87.2	6.4, 4.9	59.7, 102.5
32MRP	Mustard	115.0	4.9	99.2
33MRP, 34MRP	Mustard	65.2, 70.2	4.0, 4.0	220.5, 175.6
35MRP, 36MRP	Mustard	65.1, 37.4	5.1, 5.2	84.0, 86.2
29MRP,37MRP	Mustard	98.7, 49.5	5.2, 5.3	98.3, 86.0

taken as potential P-solublizers. These PSB isolates were purified by streaking and maintaining on PVK medium for further studies. All the isolates were rechecked for P- solubilization on solid medium containing TCP. There was high variation in solubilization efficiency of different isolates. No correlation between colony diameter and halo/zone was recorded. Maximum P- solubilization efficiency was seen with 9GRP, 12GRP, 16GRP and 26MRP isolates on solid PVK medium plates. Some of the isolates did not show P- solubilization as shown in Table 1.

Table 2. Effect of PSB strains on mustard biomass under pot house conditions

Treatment	30 DAS		45 DAS		60 DAS	
	Biomass g/plant	% increase over control	Biomass g/plant	% increase over control	Biomass g/plant	% increase over control
Control	0.20	-	0.45	-	0.91	-
SSP(30 Kg/ha)	0.21	6	0.48	5. 4	1.23	35
URP(30 Kg/ha)	0.20	2	0.45	-	1.12	23
4GRP	0.28	43	0.68	49	1.61	77
4GRP +URP	0.39	103	0. 99	118	2.13	134
25MRP	0.23	16	0.57	27	1.66	82
25MRP+URP	0.49	147	1.69	270	3.16	247
27MRP	0.23	16	0.56	23	1.55	70
27MRP+URP	0.34	131	1.14	129	2.21	87
28MRP	0.24	23	0.57	3	1.61	76
28MRP+URP	0.36	139	1.27	158	2.35	158
33MRP	0.23	2	0.56	2.4	1.63	79
33MRP+URP	0.35	120	1.16	136	2.54	179
34MRP	0.25	27	0.59	29	1.68	85
34MRP+URP	0.37	141	1.18	138	2.62	187

Table 3. Effect of PSB strains on mustard P uptake under pot house conditions

Treatment	30 DAS		45 DAS		60 DAS	
	P up take mg/plant	% increase over control	P up take mg/plant	% increase over control	P up take mg/plant	% increase over control
Control	0.055	-	0.132	-	0.182	-
SSP(30 Kg/ha)	0.066	20	0.150	13	0.275	51
URP(30 Kg/ha)	0.063	14	0.142	7	0.271	76
4GRP	0.093	69	0.268	103	0.350	92
4GRP +URP	0.140	154	0.273	106	0.444	143
25MRP	0.098	78	0.195	47	0.321	76
25MRP+URP	0.215	290	0.753	470	0.699	284
27MRP	0.099	80	0.243	84	0.293	60
27MRP+URP	0.132	140	0.320	142	0.347	90
28MRP	0.095	72	0.184	39	0.288	58
28MRP+URP	0.135	145	0.333	152	0.430	136
33MRP	0.096	74	0.199	50	0.215	18
33MRP+URP	0.143	160	0.420	218	0.423	132
34MRP	0.092	67	0.210	59	0.258	41
34MRP+URP	0.152	176	0.375	184	0.397	118

P- solubilization by PSB in liquid medium containing TCP

All the isolates were checked in PVK liquid media under stationary conditions for P-solubilization and change in pH as shown in Table 1. The pH range was between 3.5- 6.3 in liquid, the isolate 25MRP decreased the pH of medium to 3.5. Not much change in pH was shown by 2GRP, 7GRP, 8GRP, 12GRP, 16GRP, 22MRP and 39MRP. The phosphate solubilization varied between (54.4-261.6 mg/ml) in PVK medium. The minimum P-solubilization (54.4 mg/ml) was shown

Table 4. Effect of PSB strains on green gram biomass under pot house conditions

Treatment	30 DAS		45 DAS		60 DAS	
	Biomass g/plant	% increase over control	Biomass g/plant	% increase over control	Biomass g/plant	% increase over control
Control	0.258	-	0.397	-	0.690	-
SSP(30 Kg/ha)	0.270	4	0.399	0.50	0.710	2
URP(30 Kg/ha)	0.275	6	0.407	2	0.705	2
4GRP	0.310	20	0.513	29	0.953	38
4GRP +URP	0.385	49	0.775	95	1.634	136
25MRP	0.305	18	0.515	29	0.975	41
25MRP+URP	0.410	58	0.814	105	1.79	159
27MRP	0.299	15	0.505	27	0.943	36
27MRP+URP	0.380	47	0.677	70	1.53	121
28MRP	0.321	20	0.520	30	0.920	33
28MRP+URP	0.395	53	0.690	48	1.58	122
33MRP	0.301	16	0.517	30	0.930	34
33MRP+URP	0.398	54	0.790	98	1.68	143
34MRP	0.320	24	0.518	30	0.922	33
34MRP+URP	0.340	31	0.705	77	1.61	133

Table 5. Effect of PSB strains on P uptake by green gram under pot house conditions

Treatment	30 DAS		45 DAS		60 DAS	
	P up take mg/plant	% increase over control	P up take mg/plant	% increase over control	P up take mg/plant	% increase over control
Control	0.067	-	0.195	-	0.258	-
SSP(30 Kg/ha)	0.075	11	0.199	2	0.263	2
URP(30 Kg/ha)	0.079	13	0.210	7	0.284	10
4GRP	0.112	67	0.284	46	0.398	54
4GRP +URP	0.173	158	0.653	234	0.721	178
25MRP	0.110	64	0.273	40.	0.395	53
25MRP+URP	0.315	370	0.786	303	0.836	224
27MRP	0.120	79	0.252	29	0.385	49
27MRP+URP	0.213	217	0.434	122	0.483	87
28MRP	0.111	65	0.235	20	0.374	45
28MRP+URP	0.237	253	0.534	173	0.598	131
33MRP	0.123	83	0.221	13	0.352	36
33MRP+URP	0.298	344	0.683	250	0.730	182
34MRP	0.125	86	0.213	9	0.373	44
34MRP+URP	0.267	298	0.568	200	0.640	148

by 22MRP and maximum by 28MRP (261.6 mg/ml). The highest P-solubilization was followed by 25MRP (260.8 mg/ml) and 27MRP (255.4 mg/ml). Low P-solubilization (<60 mg/ml) was noticed for 6 isolates, while 26 isolates exhibited P-solubilization between (60-100 mg/ml). Three isolates showed P-solubilization between (100-200) mg/ml, and four isolate showed between (200-260.8 mg/ml). Majority of the isolates fall in medium class P-solubilizers.

Evaluation of PSB for biomass production in mustard under pot house conditions

The effect of six selected PSB strains on plant growth of mustard was studied under pot house conditions in a P- deficient sandy soil as shown in Table 2. The recommended dose of 30 kg P_2O_5/ha was applied in the form of URP or SSP and observation were recorded at 30, 45 and 60 DAS. The plant biomass with SSP or URP at 30, 45 and 60 DAS did not increase as compared to control. The lowest response (2%) to plant biomass over control was noticed with URP and maximum increase (147%) was with 25MRP with URP in comparison to control at 30 DAS. Highest plant dry biomass was observed by 25MRP with URP (1.69 g/pl) followed by 28MRP with URP (1.146) at 45 DAS. Maximum plant biomass weight was found with 25MRP with URP (3.16 g/pl) followed by 34MRP with URP at 60 DAS.

Evaluation of PSB for phosphate uptake in mustard under pot house conditions

The effect of various treatments on the P uptake at different DAS is shown in Table 4. At 30 DAS, in comparison to control there was only 20% increase in case of SSP treatment, and 14% increase with URP. The 27MRP showed 80% increase followed by 25MRP (78%), 33MRP (74%), 28MRP (72%), 4GRP (69%) and 34MRP (67%) in comparison to control. The maximum P- uptake (0. 215 mg/plant) at 30 DAS was observed, when 25MRP was inoculated with URP, which was 290% increase over uninoculated control. It was observed that P uptake was more when PSB were inoculated with URP as compared to without URP at 30, 45 and 60 DAS. The increase in P uptake with URP (4%) and SSP (12%) was found in comparison to control. The maximum P uptake was observed in 25MRP with URP (470%) followed by 33MRP with URP (218%) at 45 DAS. At 60 DAS it was observed that SSP and URP treatment showed more P uptake, in comparison to 30 DAS and 45 DAS. The SSP and URP increased by 51% and 76% respectively in comparison to control. The P uptake ranged between (50-284%) and the maximum P uptake was observed in 25MRP with URP (284%) followed by 4GRP with URP (143%) at 60 DAS.

Table 6. Establishment of PSB (x 10^3 cfu/g soil) in mustard and green gram rhizosphere under pot house conditions at different DAS

	Mustard			Green gram		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
Control	1	ND	ND	1	ND	ND
SSP(30 Kg/ha)	5	4	1	6	4	2
URP(30 Kg/ha)	4	3	2	7	3	1
4GRP	15	7	4	13	9	4
4GRP +URP	18	12	7	17	11	6
25MRP	12	8	5	10	8	2
25MRP+URP	10	10	6	13	7	3
27MRP	17	12	4	17	13	5
27MRP+URP	22	13	5	18	12	3
28MRP	12	8	3	12	7	3
28MRP+URP	15	10	4	19	14	7
33MRP	21	15	7	12	8	2
33MRP+URP	18	8	2	22	15	2
34MRP	12	5	3	13	7	3
34MRP+URP	10	8	2	10	6	2

ND*- Not detected

Evaluation of PSB for biomass production in green gram under pot house conditions

The plant biomass of green gram with SSP or URP at 30, 45 and 60 DAS did not increase as compared to control as shown in Table 4. The lowest response (4%) to plant biomass over control was noticed with SSP and maximum increase (48%) by 25MRP with URP in comparison to control at 30 DAS. Highest plant dry biomass was observed by 25MRP with URP (0.814 g/pl) followed by 33MRP with URP (0.79 g/pl) at 45 DAS. Maximum plant biomass weight was found with 25MRP with URP (1.79 g/pl) followed by 33MRP with URP at 60 DAS.

Evaluation of PSB for phosphate uptake in green gram under pot house condition

The effect of various treatments on the P uptake at different DAS is shown in Table 5. At 30 DAS, in comparison to control, there was only 11% increase in case of SSP treatment and 13% increase with URP. The maximum P- uptake (0.315 mg/pl) at 30 DAS was observed, when 34MRP was inoculated with URP, which was 370% increase over uninoculated control. It was observed that P uptake was more when PSB were inoculated with URP as compared to without URP. At 45 DAS there was increase in P uptake with URP (7%) and SSP (2%) in comparison to control. The maximum P uptake was observed in 25MRP with URP (303%) followed by 33MRP with URP (250%) and 4GRP with URP (234%). At 60 DAS it was observed that SSP and URP treatment did not exhibit more P uptake, in comparison to 30 DAS and 45 DAS. The SSP (2%) and URP (10%) increased in comparison to control. The P uptake ranged between (36-224%), and maximum uptake was observed in 25MRP with URP (224%) followed by 33MRP with URP (182%).

Population of PSB in the rhizosphere of green gram and mustard

The population of PSB in the rhizosphere of green gram and mustard was checked by using PVK medium at different DAS. The PSB count of different treatment varied from 4-22 $\times 10^3$ cfu/g soil in the rhizosphere of mustard and varied from 5-22 $\times 10^3$ cfu/g soil in the rhizosphere of mustard at 30 DAS. At 45 DAS and 60DAS in both green gram and mustard the population of PSB was less as compare to 30 DAS as shown in Table. 6.

DISCUSSION

Many soil bacteria are responsible for solubilization of insoluble phosphates in the soil and are present in the root zone of plants. These microorganisms can be isolated from rhizosphere by dilution plating on tricalcium phosphate (TCP) containing Pikovskaya medium (Pikovskaya, 1948) showing halo zone of P- solublization. The Phosphate solubilizing microorganisms (PSM) are present in almost all types of soil, although their number varies depending upon the soil types and climatic conditions. Various bacteria, fungi and actinomycetes are known to solubilize phosphorus and enumerated from different sources such as soil (Roy choudhary and Kaushik, 1989), root nodules (Surange and Kumar, 1993), compost (Thakkar, *et al*., 1993) rhizosphere of various plants (Kundu, *et al*., 2002; Alvaro *et al*., 2004). Seed inoculations of these microorganisms have shown positive effect on crop yield (Gupta, 2004; Panda, *et al*., 2004). The 39 phosphate solubilizing strains were isolated from the rhizosphere of green gram and mustard crop. P- solubilizing efficiency of all the isolates were measured from zone diameter and colony diameter on PVK medium plates. Large variation among different isolates for P solubilization was observed which may be due to variation in their metabolic activity. Such result has also been reported by (Kim, *et al*., 1997, Maheshkumar, *et al*., 1997). All the isolates were checked for P-solubilzation in PVK broth containing TCP. Phosphate solubilization in broth containing TCP was more due to complex mineral structure and low P content. Similar results have been reported by several workers (Kapoor, *et al*., 1989, Singh and Kapoor, 1994). There was no correlation between P- solubilization efficiency and P-solubilized under liquid condition (Ahmed and Jha, 1998). The isolation of microbes having higher P-solubilization efficiency both on solid as well as liquid medium were presumed to be potential P-solubilizers. The decrease in pH of the medium was due to the release of organic acid by PSB (Singh, *et al*., 1982; Mishra, 1985; Tripura, *et al*., 2007). However, no correlation between reduction in pH and P- solubilization was seen probably due to quality and quantity of acid produced by different organisms (Gaur, *et al*., 1973; Surang, 1985). The reliability of clear zone formation was

questioned as many isolates which did not produce any visible zone on agar plates could solubilize various types of insoluble inorganic phosphates in liquid medium (Gupta, *et al*., 1994). Under pot house conditions increase in plant dry biomass was recorded by application of PSB with URP, as compared to PSB alone both in green gram and mustard. At 60 DAS in both green gram and mustard maximum plant dry biomass was recorded in 25MRP with URP followed by 33MRP with URP. The PSB count in the rhizosphere of green gram and mustard decreased at 60 DAS as compared to 30 DAS and 40 DAS. Results from the present study indicate that strain 25MRP and 33MRP were excellent PSB under pot house conditions. Beside the supply of two major nutrients (N&P), PSB inoculation might also produce growth promoting substances (Liba, *et al*., 2006; Rajkumar, *et al*., 2006; Jat and Ahalwat, 2004). Thus a detailed research is required before recommending the use of 25MRP and 33MRP as biofertilizer.

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