

Original Research Article

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Efficacy of Plant Growth Promoting Rhizobacteria on Mungbean Root and Shoot under Salinity Stress Conditions

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ABSTRACT

Keywords

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Total 41 Plant Growth Promoting Rhizobacterial (PGPR) isolates were obtained from salinity affected rhizospheric soil samples of mungbean and were characterized for different plant growth promoting characters in *in vitro* conditions. Two most potent isolates were tested alone and in combination with *Rhizobium* under salinity affected field of mungbean crop. Application of alone PGPR significantly improved different parameters of root and shoot i.e. length, fresh and dry weight under field conditions. However, combined application of two or three isolates gave highest root and shoot length, fresh and dry weight among all the treatments tested, suggesting synergistic effect of PGPR in salinity mitigation under saline stress conditions in mungbean crop. Combined application of all three bioinoculants produced higher root length (61.57%), shoot length (31.70%), root fresh weight (122.03%), shoot fresh weight (71.76%), root dry weight (153.33%) and shoot dry weight (100%) over control in pooled of two locations.

Introduction

Soil salinity is one of the major abiotic stresses affects crop production largely. Saline soil occupied nearly 6% of world's total earth's land surface that comprised of 900 million ha. of total globe area (Flower, 2004). In India, total 6.75 million ha. of land is affected by salinity (Mandal *et al.*, 2010). Salinity shows negative effect on plant morpho-physiological properties.

Extensive research is underway worldwide to develop strategies to ameliorate salt stress by developing tolerant varieties, shifting the crop calendars, production of transgenics, etc.

(Venkateswarulu and Shanker, 2009). Several PGPR have been isolated and exploited as an alternate to alleviate salt stress in different plants (Bacilio *et al.*, 2004). Nevertheless, these microorganisms "Induce Systemic Tolerance (IST)", alter the physiology of the plants and result in enhanced tolerance to abiotic stress (Yang *et al.*, 2008).

Co-inoculation of PGPR with *Rhizobium* may be more beneficial for plants growth due to different mechanisms like creating more infection sites by PGPR for rhizobial infection, antibiosis, siderophore production,

etc. (Plazinski and Rolfe, 1985). Experimental evidences suggested positive effect of PGPR and Rhizobial co-inoculation on root colonization, nodulation and nitrogen fixation in leguminous plants (Lucas García *et al.*, 2004). Keeping in view above, present field study was planned to evaluate the effect of PGPR co-inoculation on root and shoot growth of mungbean under saline stress conditions.

Materials and Methods

Test organisms

Total 41 bacterial isolates were obtained from salinity affected rhizospheric soil samples collected from mungbean and were screened for different plant growth promoting characters in *in vitro* conditions. *Bacillus subtilis* and *Serratia liquefaciens* were found most potent and used for further studies in field conditions. Native *Rhizobium* isolate was obtained from salinity affected mungbean nodule and was tested alone and in combination of above two isolates.

Experimental details

With the aim of determining the effect of native PGPR on mitigation of salt stress in mungbean (variety: Co-4) root and shoot growth under saline conditions, the multi-locational experiment was conducted at Navsari and Danti region of South Gujarat during *Rabi* season of 2015-16. Experimental site was selected on the basis of presence of salinity and alkalinity. Mungbean crop was planted and fertilizer dose of 25:50:00 NPK kg/ha was given as a basal dose. Spacing (30 X 10 cm) and plot size (2.1 X 3.0 m Gross; 1.8 X 2.6 m net) were applied and Randomized Block Design (RBD) treatment was selected for data analysis. Different nine treatments were given in field with three replications.

T1 - Seed treatment (ST) of *Bacillus subtilis* (BS) and Soil Application (SA) of BS enriched FYM

T2 - ST of *Serratia liquefaciens* (SL) and SA of SL enriched FYM

T3 - ST of BS+SL and SA of BS+SL enriched FYM

T4 - ST of *Rhizobium* (R) and SA of R enriched FYM

T5 - ST of BS+R and SA of BS+R enriched FYM

T6 - ST of SL+R and SA of SL+R enriched FYM

T7 - ST of BS+SL+R and SA of BS+SL+R enriched FYM

T8 - Recommended Dose of Chemical Fertilizers (RDCF) + 8 t/h FYM

T9 - RDCF only

Seeds of mungbean was given Seed Treatment (ST) of respective culture (1×10^8 cfu/ml) @ 10ml/kg seed, dried in shed for 30 minutes before sowing.

Well decomposed farmyard manure was treated with respective cultures (1×10^8 cfu/ml) @ 0.2 lit/t and enriched for 10 days in shed with water spraying to maintain moisture and alternate day turning to avoid anaerobic condition, followed by Soil Application (SA) of 10 days PGPR enriched FYM @ 8t/h.

In the combination of culture treatments, the doses of individual bioinoculants were reduced in such a manner that the total volume of the culture remained constant i.e. 10 ml/kg of seed in seed treatment and 0.2 lit/t in FYM enrichment.

Observations recorded

Root and shoot length

Both the observations were recorded at 15 Days After Sowing (DAS) of mungbean crop at both the locations. Unit of observation was centimeters (cm) and it was recorded from five random plants from each treatments.

Root and shoot fresh weight

Fresh weight of root and shoot of mungbean were recorded from five random plants from each treatment at 15 DAS and were expressed in unit g/plant.

Root and shoot dry weight

Fresh root and shoot were dried in hot air oven and weight was measured and expressed in g/plant at 15 DAS.

Results and Discussion

From salinity affected mungbean field, total 14 rhizospheric representative soil samples were collected and were used for isolation of different PGPR isolates. Total 41 morphologically diverse isolates were obtained and were preserved in pure culture for further studies. All the isolates were screened for different plant growth promoting characters like ACC deaminase activity, nitrogen fixation, phosphorus solubilization, potash mobilization, zinc solubilization, antagonistic potential, IAA production, etc.

Two most potent isolates were identified on the basis of *in vitro* assay. Organisms were identified as *Bacillus subtilis* and *Serratia liquefaciens* by BIOLOG and 16's r-DNA methods. Both the isolates were tested in saline field conditions on mungbean crop, individual as well as in combination of native *Rhizobium*.

Effect of PGPR on root and shoot length

The effect of different PGPR on root and shoot length at 15 DAS is depicted in Table-1. Experimental data revealed that treatment that has received combined application of all the three bioinoculants (T₇) produced maximum root length (9.40, 8.60 and 9.00 cm at Navsari, Danti and in pooled respectively) than all other treatments under study. However, it was found at par with the treatment that received combined application of BS+SL (T₃) (8.63 and 8.23 cm at Navsari and Danti respectively). Significantly lowest root length was observed in RDCF (T₉) (5.70, 5.43 and 5.57 cm at Navsari, Danti and in pooled respectively).

The highest shoot length was found in treatment with SL+R (T₆) application (7.93, 7.03 and 7.48 cm at Navsari, Danti and in pooled respectively). The treatment was found at par with R (T₄) application (6.90 cm) and combination of all the three bioinoculants (T₇) (6.57 cm) at Danti location.

Significantly lowest shoot length was recorded in RDCF (T₉) (5.83, 4.77 and 5.30 cm at Navsari, Danti and in pooled respectively). Treatment RDCF was found at par with RDCF+FYM (T₈) application (6.07 cm) at Navsari.

Root and shoot are important for normal metabolic processes like absorption, translocation, etc. Thus, normal root and shoot development is essential for the plant to remain standing and survive in the soil. Abnormality in the root and shoot development prevents normal growth and metabolic activity of plant and shows direct impact on plant productivity. Salinity is the major abiotic factors that shows negative effect on root and shoot development by decreasing root absorption and affecting other metabolic processes.

Several PGPR can mitigate deleterious effect of soil salinity on root and shoot by increasing root absorption and by producing higher level of auxins and cytokinins. Other investigators have also obtained similar results to this study at different locations. Ahmad *et al.*, (2011) performed an experiment to study the effect of different PGPR in mitigation of salt stress in mungbean and found that the root and shoot length of mungbean seedlings were significantly reduced by salinity, with the minimum root length (13.43 cm) and shoot length (10.43 cm) at 6 dS/m EC level. However, co-inoculation of *Rhizobium* and PGPR significantly reduced the inhibitory effect of salinity and improved root length (16.13 cm) and shoot length (14.47 cm) than control.

Effect of PGPR on root and shoot fresh weight

Effect of different PGPR on root and shoot fresh weight/plant at 15 DAS is given in Table-2. Experimental data revealed that

application of PGPR, alone or in combination showed significant improvement in root fresh weight. Combined application of all the three cultures (T₇) produced significantly highest root fresh weight (1.48, 1.13 and 1.31 g at Navsari, Danti and in pooled respectively) over RDCF (T₉) (0.64, 0.53 and 0.59 g at Navsari, Danti and in pooled respectively). However, RDCF was found at par with RDCF+FYM (T₈) (0.72 g) in pooled data.

Table-2 also pointed the effect of different treatments on shoot fresh weight in mungbean at 15 DAS. The data revealed that combined application of two cultures i.e. SL+R (T₆) (1.55, 1.50 and 1.53 g at Navsari, Danti and in pooled respectively) produced highest shoot fresh weight and it was found at par with the treatment that received the application of all the bioinoculants (T₇) (1.46 g at Navsari and Danti). Lowest shoot fresh weight was found in RDCF (T₉) (0.88, 0.82 and 0.85 at Navsari, Danti and in pooled respectively) and it was found statistically at par with RDCF+FYM (T₈) application (0.99 g) at Navsari.

Table.1 Effect of PGPR on root and shoot length

No.	Treatment	Root length (cm)			Shoot length (cm)		
		Navsari	Danti	Pooled	Navsari	Danti	Pooled
T1	BS	6.90	7.13	7.01	6.30	5.77	6.03
T2	SL	7.30	7.47	7.38	6.47	6.03	6.25
T3	BS+SL	8.63	8.23	8.43	6.50	6.07	6.28
T4	R	7.63	7.37	7.50	7.40	6.90	7.15
T5	BS+R	7.37	6.87	7.12	6.70	5.90	6.30
T6	SL+R	7.70	7.70	7.70	7.93	7.03	7.48
T7	BS+SL+R	9.40	8.60	9.00	7.40	6.57	6.98
T8	RDCF + FYM	7.07	6.60	6.83	6.07	5.77	5.91
T9	RDCF (Control)	5.70	5.43	5.57	5.83	4.77	5.30
S.Em±		0.36	0.16	0.19	0.12	0.18	0.11
CD at 5%		1.07	0.48	0.55	0.36	0.54	0.32
L X T							
S.Em±		--	--	0.27	--	--	0.16
CD at 5%		--	--	NS	--	--	NS
CV%		8.24	3.83	6.50	3.49	4.52	4.16

Table.2 Effect of PGPR on root and shoot fresh weight

No.	Treatment	Root fresh weight (g)			Shoot fresh weight (g)		
		Navsari	Danti	Pooled	Navsari	Danti	Pooled
T1	BS	0.95	0.90	0.93	1.12	1.01	1.06
T2	SL	0.97	0.95	0.96	1.25	1.17	1.20
T3	BS+SL	1.06	1.01	1.03	1.42	1.31	1.37
T4	R	0.94	0.95	0.94	1.00	1.03	1.01
T5	BS+R	1.08	0.96	1.02	1.29	1.22	1.26
T6	SL+R	0.89	0.94	0.92	1.55	1.50	1.53
T7	BS+SL+R	1.48	1.13	1.31	1.46	1.46	1.46
T8	RDCF + FYM	0.82	0.62	0.72	0.99	0.92	0.95
T9	RDCF (Control)	0.64	0.53	0.59	0.88	0.82	0.85
S.Em±		0.04	0.03	0.06	0.04	0.02	0.02
CD at 5%		0.10	0.08	0.19	0.11	0.07	0.06
L X T							
S.Em±		--	--	0.03	--	--	0.03
CD at 5%		--	--	0.09	--	--	NS
CV%		6.98	4.79	5.89	5.23	3.54	4.51

Table.3 Effect of PGPR on root and shoot dry weigh

No.	Treatment	Root dry weight (g)			Shoot dry weight (g)		
		Navsari	Danti	Pooled	Navsari	Danti	Pooled
T1	BS	0.26	0.29	0.28	0.34	0.30	0.32
T2	SL	0.28	0.26	0.27	0.35	0.35	0.35
T3	BS+SL	0.29	0.29	0.29	0.41	0.37	0.39
T4	R	0.26	0.27	0.27	0.38	0.30	0.34
T5	BS+R	0.31	0.30	0.31	0.39	0.37	0.38
T6	SL+R	0.32	0.31	0.32	0.51	0.44	0.48
T7	BS+SL+R	0.37	0.39	0.38	0.47	0.44	0.46
T8	RDCF + FYM	0.21	0.22	0.22	0.25	0.24	0.25
T9	RDCF (Control)	0.15	0.15	0.15	0.21	0.24	0.23
S.Em±		0.01	0.02	0.02	0.03	0.02	0.02
CD at 5%		0.03	0.06	0.05	0.08	0.05	0.06
L X T							
S.Em±		--	--	0.02	--	--	0.02
CD at 5%		--	--	NS	--	--	NS
CV%		7.23	6.77	7.02	6.36	9.17	7.68

Salinity is the abiotic factor that affects normal metabolic process of root and shoot and thus shows negative impact on root and shoot weight. Application of PGPR improves root and shoot length and ultimately fresh

weight of root and shoot in mungbean. Several investigators have reported similar results at different locations. Ahmad *et al.*, (2011) recorded 173 and 145% increase in root and shoot fresh weight respectively by

the combined application of different PGPR under salinity stress in mungbean over control treatment.

Effect of PGPR on root and shoot dry weight at 15 DAS

Root and shoot dry weight as influenced by different PGPR application is given in Table-3. The experimental data revealed that application of PGPR, alone or in combination significantly improved root dry weight in mungbean under saline stress conditions over RDCF (T₉) (0.15 g at Navsari, Danti and in pooled respectively). However, combined application of all three bioinoculants (T₇) (0.37, 0.39 and 0.38 g at Navsari, Danti and in pooled respectively) showed highest root dry weight over all other treatments under study.

Table-3 also indicated that combined application of SL+R (T₆) improved shoot dry weight (0.51, 0.44 and 0.48 g at Navsari, Danti and in pooled respectively) and it was found at par with the treatment that received application of all the three bioinoculants (T₇) (0.47, 0.44 and 0.46 g at Navsari, Danti and in pooled respectively). Lowest shoot dry weight was recorded in RDCF (T₉) (0.21, 0.24 and 0.23 g at Navsari, Danti and in pooled respectively) and it was found at par with RDCF+FYM (T₈) application (0.25, 0.24 and 0.25 g Navsari, Danti and in pooled respectively).

Salinity can reduce root absorption, product assimilation and thus dry root and shoot weight. Many investigators have clearly stated that salinity had negative impact on dry weight of root and shoot in mungbean and the effect became worst with increasing level of soil salinity (Hossain, 2004; Rabi, 2004).

The application of PGPR *B. cerus* can mitigate salt stress in mungbean and could improve root and shoot-leaf dry weight

168.75 and 286.29% respectively as compared to treatment without PGPR application as reported by Chakraborty *et al.*, (2011).

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