RESPONSE OF MUSTARD (*BRASSICA CAMPESTRIS* L.), SUNFLOWER (*HELIANTHUS ANNUUS* L.) AND TEFF (ERAGROSTIS *TEFF* L.) TO INOCULATION WITH PLANT GROWTH PROMOTING RHIZOBACTERIA

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Carrier-based (Soil:FYM::1:1) plant growth promoting rhizobacteria (PGPR) isolates [*Bacillus subtilis, Klebsiella planticola*, *Proteus vulgaris, Azospirillum brasilense*(CDJA)*, Azotobacter chroococcum* (CBD 15)] were tested on mustard (*Brassica juncea* L. cv. Pusa Bold), sunflower (*Helianthus annuus* L. cv. Morden and teff (*Eragrostis teff cv. DZ-01-354*), for their ability to influence the growth and yield in the presence and absence of applied fertilizer nitrogen. The results revealed in general, with the increase in nitrogen level there was significant increase in the population of inoculant bacteria. The biomass and seed yield were not influenced markedly by the application of nitrogen fertilizer when tested under green house conditions.. Among inoculants, *Bacillus subtilis* resulted in significantly more biomass and grain yield than uninoculated control at 0 level of N. Further, the interaction of N and inoculants also revealed better performance.

Keywords: Bacterial population, Mustard, PGPR's, Sunflower, Teff, Yield

There is an enormous volume of literature available on the application of bacteria for improvement of plant performance (Bashan and Holguin 1997, Okon and Labendra Gonzalez 1994, Okon *et al*.1998, Saxena and Tilak 1994, Gupta *et al.* 2003, Tilak *et al*. 2006, Nautiyal and Tilak 2009). However, few bacteria like *Azotobacter* and *Azospirillum* have been developed as commercial products. The organisms under most scrutiny for potential use in agriculture are bacteria belonging to the genera *Pseudomonas* and *Bacillus* species (Kloepper *et al*. 1988, Powell and Rhodes 1994, Tilak *et al*. 2006, Tilak and Reddy 2006). Malik *et al*. (1999) reported significant increase in the growth and yield of rapeseed due to inoculation of *Kurthia* sp. However, there is no information on effect of *Proteus vulgaris, Klebsiella planticola*, *Bacillus subtilis, Azospirillum brasilense and Azotobacter chroococcum* on oilseeds and forage/fodder crops. The present study, therefore, embodies the response of mustard (*Brassica campestris* L.), sunflower (*Helianthus annus* L.) and teff (*Eragrostis teff* L.) to inoculation with aforesaid PGPR's under green house conditions.

MATERIALAND METHODS

Seeds of mustard cv. Pusa bold, sunflower cv. Morden and teff cv.DZ-01-354 were obtained from Division of Genetics, Indian Agricultural Research Institutre, New Delhi, and National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, respectively.

Bacterial cultures *Bacillus subtilis* (Bs), *Klebseilla planticola* (Kp), *Proteus vulgaris* (Pv), *Azospirillum brasilense* (CDJA) and *Azotobacter chroococcum* (CBD 15) were

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Table : 1 Inoculation effect of PGPR on rhizosphere population of mustard cv. Pusa Bold and teff cv. DZ-01-354 at 45 days of plant growth (average of 6 replications)

Nitrogen (kg ha^{-1})	Treatment	Population [cfu (x 10^5) g^{-1} soil] Mustard	Teff
0	Uninoculated control	1.8	2.2
	Bacillus subtilis	62.5	25.1
	Klebsiella planticola	17.2	16.9
	Proteus vulgaris	14.7	13.7
	Bacillus polymyxa	20.1	-
	Azospirillum brasilense (CDJA)	-	16.8
	Azotobacter chroococcum (CBD15)	21.0	20.3
*30	Uninoculated control	2.0	
	Bacillus subtilis	26.6	
	Klebsiella planticola	27.2	
	Proteus vulgaris	15.1	
	Bacillus polymyxa	12.6	
	Azotobacter chroococcum (CBD15)	37.0	
46	Uninoculated control		2.5
	Bacillus subtilis		20.0
	Klebsiella planticola		22.6
	Proteus vulgaris		14.4
	Azospirillum brasilense (CDJA)		23.7
	Azotobacter chroococcum (CBD15)		26.2
	$CD_{0.05}$		
	Nitrogen level (N)	5.55	1.50
	Inoculant (I)	4.82	7.95
	N x I	3.25	8.25

* 30 kg N/ha was not included with teff

obtained from Culture Collection,Division of Microbiology, IARI, New Delhi India.

The PGPR cultures were grown in their respective media (Tilak *et al.* 2010). Carrier based inoculant of PGPR was prepared by using sterilized charcoal: soil (3:1) as the carrier material following the method described by Jauhri *et al.* (1979). Forty milliliters of the broth culture (ca. 10^9 cells ml^{-1}) was mixed with 100 g sterilized carrier material under aseptic conditions. The inoculum was packed in pre-sterilized polypropylene bags and opened at the time of usage. The inoculum maintains a population of 10^8 cells ml^{-1} for a period of 3 months when stored at 4°C. Each inoculum carried 2.5 3.5 x 10^8 cells g^{-1} enumerated by the dilution plate method. Half an hour before sowing, the seeds were coated with different carrier-based inocula (as per treatments) by using a 10% sucrose solution as an additive. Coated seeds were air-dried for 15 min under shade and

Table 2 : Inoculation effect of PGPR isolates on plant height, biomass and grain yield of mustard cv. Pusa Bold (average of 6 replications)

Nitrogen kg/ha		Plant height (cm)	Mean yield (g / pot)	
			Dry shoot biomass	Grain weight
0	Uninoculated control	61.2	7.0	0.81
	Bacillus subtilis	68.5	9.1	1.15
	Klebsiella planticola	66.4	7.3	1.11
	Proteus vulgaris	65.0	4.7	1.14
	Bacillus polymyxa	60.6	7.7	0.75
	Azotobacter chroococcum	65.6	6.5	0.75
30	Uninoculated control	64.6	7.0	0.82
	Bacillus subtilis	60.5	6.6	0.75
	Klebsiella planticola	72.2	8.1	1.06
	Proteus vulgaris	71.4	8.5	1.05
	Bacillus polymyxa	68.5	7.8	0.72
	Azotobacter chroococcum	69.7	7.6	0.75
	$CD_{0.05}$			
	Nitrogen level (N)	5.55	1.25	0.17
	Inoculant (I)	4.82	1.15	0.28
	N x I	3.25	1.21	0.12

sown immediately. Seeds for the uninoculated control were also coated in a similar manner with the sterilized carrier. The seeds were air-dried for 10 min under shade before sowing (Tilak *et al*. 2010) .

The roots were thoroughly washed with tap water until no soil particles remained in water for six times. The washed root samples were crushed in 1 to 2 ml of sterile distilled water under aseptic conditions in a pestle and mortar and passed through a 1 mm mesh. The suspension contained both cultureable and non-cultureable microorganisms. Selective media were used for isolation of azotobacters (Jensen, 1954), azospirilla (Lakshmi-Kumari *et al*. 1980), *Klebsiella*, *Proteus and Bacillus* (Gupta *et al*.1998). For the isolation of *Bacillus,* the root suspension was subjected to heat shock at 80°C for 30 minutes in a water bath and then transferred to nutrient medium (Claus 1964). All the Petri-dishes were incubated at 28° (±1°C) for 28-30 h. These cultures were maintained on their respective isolation agar media and stored at 4°C for further use.

Table 3 : Inoculation effect of PGPR isolates on plant height, head diameter, biomass and grain yield of sunflower cv. Morden (average of 6 replications)

Nitrogen level kg/ha	Treatment	Plant height (cm)	Head diameter (cm)	Mean yield (g / pot)	
				Dry shoot biomass	Grain weight
0	Uninoculated control	55.8	5.8	19.5	3.2
40	Uninoculated control	67.0	7.2	23.9	5.8
	Bacillus subtilis	75.3	9.5	32.8	6.3
	Klebsiella planticola	70.0	7.8	32.4	5.3
	Proteus vulgaris	63.3	7.3	26.5	4.9
	Azosprillum brasilense	69.8	6.6	26.1	3.8
	$CD_{0.05}$				
	Inoculants	6.75	1.57	3.55	0.42

Pot culture experiments were conducted using semi-arid sandy-loam type soil having a pH 7.6 with 172.2 kg / ha availabile N, 13.8 kg P_2O_5 and 182 kg K_2O / ha and organic carbon 0.405 %. Earthen pots 12" capacity were filled with 10 kg soil and phosphorus was applied as basal dressing at the rate of 60 kg P_2O_5 ha^{-1}. Mustard cv. Pusa Bold, sunflower cv. Morden and teff cultivar D-01-354 were used . Two levels of N (0 and 40 kg N/ha for sunflower, 0 and 30 kg N/ha for mustard and 0 and 46 kg N/ha for teff) were applied when seedlings were 10 days old. PGPR inoculations were done both in the absence and presence of nitrogen fertilizer. The treatments included uninoculated control, *B. subtilis, K. planticola, P. vulgaris, Azospirillum brasilense and Azotobacter chroococcum*. *Bacillus polymyxa* was included in place of *Azospirillum brasilense* in mustard. Each treatment was replicated 6 times. The plants were harvested at marutity (90-100 days of plant growth) and observations were made on plant height, head size, dry shoot biomass and grain yield. The plants were oven dried at 80°C for one day and dry weight of shoot was recorded. Rhizosphere population of specific bacteria was determined by serial dilution method and plating on specific media (Ranganayaki *et al*. 2006) at 45 days of plant growth.

The data were analysed statistically as described by Fisher (1958). The critical difference at the 5% significance level was calculated.

Table 4 : Inoculation effect of PGPR isolates on plant height, head diameter, biom ass and grain yield of teff cv. DZ 01-354 (average of 6 replications)

Nitrogen level kg/ha	Treatment	Plant height (cm)	Head diameter (cm)	Mean yield (g/pot)	
				Dry shoot biomass	Grain weight
0	Uninoculated control	83.6	25.3	56.9	1.19
	Bacillus subtilis	94.6	26.0	80.8	2.18
	Klebsiella planticola	91.5	27.9	68.8	1.56
	Proteus vulgaris	86.4	23.5	61.6	0.89
	Azospirillum brasilense	94.0	27.3	51.3	0.87
	Azotobacter chroococcum	89.4	28.5	64.7	1.22
46	Uninoculated control	89.4	27.7	47.4	0.65
	Bacillus subtilis	90.8	29.2	47.4	0.65
	Klebsiella planticola	101.5	29.1	50.0	0.65
	Proteus vulgaris	89.0	31.2	57.5	1.34
	Azospirillum brasilense	100.0	30.3	59.5	1.02
	Azotobacter chroococcum	89.6	23.5	50.1	1.11
	$CD_{0.05}$				
	Nitrogen level (N)	7.25	3.12	6.25	0.25
	Inoculant (I)	8.96	N.S.	3.50	0.32
	N x I	N.S*.	1.22	5.50	0.72
	*N.S.: Not Significant				

RESULTS AND DISCUSSION

Several investigations have been carried out to reduce the application of costly nitrogen fertilizer through the use of plant growth promoting rhizobacteria particularly belonging to the genera *Azospirillum, Azotobacter, Serratia* and *Psudomonas* (Okon and Labandra Gonzalez 1999, de Freitas and Germida 1990, Overbeek *et al*. 1995, Saubidet and Barneix 1998, Saxena and Tilak 1998). Information regarding inoculation of PGPR isolates on mustard, sunflower and teff is not available. Therefore, the present investigation was carried out to test the response of oilseed crops (mustard and sunflower) and fodder crop (teff) to different plant growth promoting rhizobacteria in order to develop a delivery system for these PGPR's .

The population of inoculant bacteria were estimated in the rhizosphere of mustard at 45 days of plant growth. The results revealed that, in general, with the increase in the nitrogen level (N_{30} in case of mustard and N_{46}

in case of teff) there was an increase in the population of inoculant PGPR's. At N_0 nitrogen level *B. subtilis* could build up to a level of 34.8 and 11.4 times over uninoiculated control in the rhizosphere with mustard and teff respectively. (Table 1). However, at higher levels, *Azotobacter* could colonize better than other bacteria and resulted in maximum population, which was of 18.5 and 10.5 times over higher levels of N with mustard and teff, respectively.

Plant height showed variations both among the nitrogen levels and PGPR isolates in case of all the three crops. *Bacillus subtilis* and *K. planticola* significantly affected the plant height, over uninoculated control. In the absence of nitrogen amendment, maximum plant height was obtained with mustard in treatment inoculated with *B. subtilis* closely followed by *K. planticola* and the increase was of 10.28 %. However, at 30 kg N/ha *K. planticola* registered an increase of 11.7% over 30 kg N/ha application (Table 2). The increase in dry biomass and grain yield due to *B. subtilis* at no nitrogen level was 29.5 and 42 per cent over uninoculated control, respectively. Application of 30 kg N/ha brought an increase of 17.5 percent in grain yield over 30 kg N/ha application alone (Table 2).

Treatment of sunflower seed with *K.planticola* in the presence of 40 kg N/ha brought in an increase in head diameter (32 %), shoot biomass (35.6%) and grain yield (8.6%) over corresponding control i.e.40 kg N/ha alone. (Table 3)

Bacillus subtilis and *K. planticola* significantly affected teff's height, head diameter, straw and seed yield. when they were applied without N amendment. In the absence of nitrogen amendment, maximum plant height was obtained in treatment inoculated with *B. subtilis* closely followed by *Azospirillum* treatment. However, in the presence of nitrogen, *K. planticola* followed by *Azospirillum* influenced the plant height of teff plant(Table 4).

Head diameter was influenced due to application of 46 kg N/ha. The uninoculated but N fertilized control showed an increase of 9.5% over uninoculated and unfertilized control. In the absence of N fertilizer, inoculation of *Azotobacter* recorded significantly more head diameter and was 12.7% more than that obtained in uninoculated control. However, in the presence of 46 kg N/ha, maximum head diameter was obtained in treatment inoculated with *Proteus vulgaris* (Table 4).

The dry shoot biomass and seed yield of teff was influenced by application of nitrogen fertilizer. In the absence of nitrogen, inoculation of *B. subtilis* resulted in maximum dry matter and seed yield and was significantly higher than all other treatments. The increase was about 1.5 and 2.0 times more than uninoculated control, respectively (Table 4). In the presence of nitrogen fertilizer, inoculation of *P. vulgaris* resulted in maximum seed yield and was 2.6 times higher than the corresponding uninoculated control i.e. 46 Kg N ha^{-1} alone The application of higher doses of chemical fertilizers may help to increase teff production in the presence of *P. vulgaris* inoculation.

The investigations showed that PGPR isolates such as *Bacillus subtilis, Klebsiella planticola, Proteus vulgaris* and *Azotobacter chroococcum* increased plant development by affecting its different parts. *B. subtilis* positively affected height, head diameter, straw and seed yield of all crops at no nitrogen level. In general, the application of PGPR's performed better without N application indicating thereby that the organisms could establish in the rhizosphere and promote the growth of plants by producing growth promoting substances (Kloepper *et al*. 1988) . The beneficial effects of PGPR's on mustard

and teff may be attributed to the increased population of rhizosphere bacteria .

The application of higher doses of chemical fertilizers undoubtedly help to increase crop production. However, this may result in consequences where soil fertility will be depleted or become acidic and devoid of macro and micro nutrients for crops to grow and microorganisms to proliferate. Therefore, it is imperative to use ecofriendly inputs like beneficial PGPR's to improve the production of crops which will be of immense significance for small and marginal farming communities to reduce the use of fertilizers and other inputs under rainfed conditions in semi-arid tropical soils of India. However, further studies are warranted to test the efficacy of PGPR's with different cultivars (genotypes) of the crops in order to derive information on host specificity.

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