Academic Voices
A Multidisciplinary Journal
Volume 4, No. 1, 2014
ISSN 2091-1106

PLANT GROWTH PROMOTION ASSESSMENT IN RICE PLANT ENHANCED BY INOCULATION OF RHIZOBACTERIA

Umesh Prasad Shrivastava
Department of Botany, TU, Thakur Ram Multiple Campus, Birgunj, Nepal
Email: upshrivastava@gmail.com

Abstract

In the search of efficient biofertilizer, nine efficient strains of PGPR were evaluated by inoculation in two different rice varieties, Saryu-52 and Malviya Dhan-36 in the gnotobiotic conditions using two different media FCN- medium and soil extract medium. Agrobacterium sp. strain BN-2A showed better result in respect to other eight isolates in the total length, fresh weight, number of roots and chlorophyll-a content. By the inoculation Agrobacterium sp. strain BN-2A, total length increased in Saryu-52 (22.6%) and in Malviya Dhan-36 (52.1%), fresh weigh increased in Saryu-52 (42.4%) and in Malviya Dhan-36 (68.8%) and chlorophyll-a increased in Saryu-52 (76.6%) and in Malviya Dhan-36 (37.1%). Similarly in soil extract medium, inoculation of Agrobacterium sp. strain BN-2A alone showed better result in comparison to mixture of nine strains. To prove that colonization indeed occurs, gusA reporter gene was tagged with the most efficient isolate Agrobacterium sp. strain BN-2A and colonization in the rice root was confirmed by gusA staining and histochemical analysis of gusA staining. Therefore, BN-2A has best potential to be used as biofertilizer.

Key words

Plant Growth Promoting Rhizobacteria; PGPR; Bacterial inoculation; Gnotobiotic experiments; FCN- medium; Soil extract medium.

Introduction

Interactions between plants and beneficial bacteria can have a profound effect on crop health and yield and soil quality (Kloepper *et al*. 1989; Sturz and Nowak, 2000). Plant-associated bacteria may be beneficial, deleterious, and neutral groups on the basis of their effects on plant growth, they are called rhizobacteria. Beneficial rhizobacteria having ability of plant growth promotion are referred to as Plant Growth Promoting Rhizobacteria or PGPR (Glick, 1995). Plant growth-promoting rhizobacteria (PGPR) enhance plant growth either by direct or indirect mechanisms (Glick,

1995). Several PGPR that have been successful in promoting the growth of crops such as canola, soybean, lentil, pea, wheat, rice and radish have been isolated (Glick *et al.*, 1997; Timmusk *et al.*, 1999; Shrivastava and Kumar, 2011; Shrivastava, 2013a; Shrivastava, 2013b). The enhancement of plant growth by PGPR indicates their potential as biofertilizers in the field of agriculture. Bertrand *et al.* (2001) identified bacteria belonging to the genera *Pseudomonas, Varivorax, Agrobacterium* and *Phyllobacterium* as the most efficient PGPR associated with canola. Indole-3-acetic acid (IAA) production, inorganic phosphate solubilisation, siderophore production, ACC deaminase activities are major plant growth promoting characters of PGPR (Shrivastava and Kumar, 2011; Glick, 1995).

The ability to colonize roots has been considered the major factor that determines inoculum efficacy both for crop yield enhancement and for disease control (Schroth and Hancock, 1981; Weller, 1988). This has led to an emphasis on selection of plant-beneficial bacteria that are rhizosphere competent (i.e., beneficial bacteria that effectively colonize the root system) (Raaijmakers and Weller, 2001).

The rhizobacteria used in the present study was isolated from rhizosphere of rice plant and showed appreciable plant growth promoting abilities (Shrivastava, 2013a, b & c). The objective of the present work was to evaluate the root colonization of PGPR in rice plants and measurement of various plant growth promoting characters.

Materials and Methods

Bacterial strains

Nine efficient PGPR (ECI-10A, ECI-12A, AF-1D, AF-4B, AF-4C, AF-5A, PN-4D, BN-2A and BN-4A) isolated from rhizosphere of rice plants from Paras and Bara districts of Nepal and East Champaran and Varanasi districts of India were used as bacterial inoculation in rice plants (Shrivastava, 2013a, b & c).

Gnotobiotic experiments

Rice seeds (Saryu-52 and Malviya Dhan-36 varieties) were soaked overnight in sterile DDW and then dehusked gently. Surface sterilization was done by 70 % alcohol for 3 min in shaking conditions. It was washed 5 times with sterile DDW for 10-15 min each time. Again, it was treated with $HgCl_2$ (0.1 % w/v) for 3 min and washed 5 times with sterile DDW for 10-15 min. The seeds were treated again with 3.0 % (w/v) calcium hypochlorite in shaking conditions for 3 min. It was washed 5 times with sterile DDW (for 10-15 min at each step) and 20-25 seeds were aseptically kept on sterile cotton bed in petri plates. The cotton was properly moistened with sterile DDW. LB medium was also added on the cotton bed for cross checking the bacterial growth. The last wash of seeds was also inoculated on NA plate to check the complete sterilization of seeds. No bacterial growth in last wash was detected thereby ensuring proper surface sterilization of seeds. Petri plates containing seeds were kept in dark at 28°C for germination. Germinating seedlings were checked periodically for growth and contamination, if any. After three days the healthy seedlings of equal length (2-3 cm.) were transferred in sterile test tubes containing semi-solid FCN^- medium (Vincent, 1970). Nine efficient isolates (ECI-10A, ECI-12A, AF-1D, AF-4B, AF-4C, AF-5A, PN-4D, BN-2A and BN-4A) were grown in liquid $JNFb^-$ medium and 1 mL culture of exponential phase was taken and $O.D._{600}$ of all the isolates was adjusted at 0.20 by the same medium. The cultures were harvested and washed with normal saline (0.85 % NaCl) and re-suspended in 1.0 mL of PBS. 500 µL of bacterial suspension was inoculated in test tube planted with rice seedling aseptically and 100 µL of the bacterial suspension was spread over NA plate for viable bacterial cell counts. All the test tubes were kept in growth chamber under light-dark cycle (14 h light and 10 h dark) at 27°C and 25°C in day and night respectively for growth. Total fresh weight, total length and chlorophyll-a content were

calculated every week for consequent four weeks.

FCN- medium

It was prepared adopting the protocol of Vincent, (1970); which contains $CaCl_2$ (0.10 g/L), $MgSO_4.2H_2O$ (0.12 g/L), KH_2PO_4 (0.10 g/L), $Na_2HPO_4.2H_2O$ (0.15 g/L), Ferric citrate (0.005 g/L) and Trace elements (1 mL). Na2MoO4.2H2O (0.05 g), MnSO4.H2O (0.25 g), ZnSO4.7H2O (0.07 g), CuSO4.5H2O (0.0125 g), CoCl2.6H2O (0.0119 g), H3BO4 (0.003 g), and Water to 100 mL. pH of the medium was adjusted after autoclave.

Soil extract medium for Gnotobiotic experiment

1 kg of soil collected from rice field (BHU, Agri. Farm) was suspended in 1 L of sterile DDW in a sterile conical flask and mixed properly with glass rod and kept in shaking condition for 36 h. After 36 h of shaking, it was kept undisturbed for sedimentation for 1 h. Upper layer of water was filtered twice with filter paper. This soil extract medium was autoclaved after adding of 0.3% (w/v) of agar-agar. pH was adjusted to 6.8. This solution is referred as soil extract medium.

Estimation of chlorophyll-a

Leaves of rice plants cut into small pieces were immersed in 80% acetone, shaken for 5 min and kept at 4°C. The resulting suspension was centrifuged and supernatant was used for estimation of chlorophyll *a*. The total amount of Chl-*a* in the acetone extract was calculated after reading absorption at 665 nm using the method of Mackinney (1941).

Statistical analysis

Statistical analysis was done by Excel program of Microsoft Window XP 2003. t-test and test of significance of data were done using online tool available at http://www.quantitativeskills.com/sisa/statistics/t-test.htm website also. The value of significance ($P < 0.05$) was calculated with 95% of confidence interval.

Results and Discussions

Test of plant growth promoting features revealed that nine isolates having higher rate of N_2 fixation, IAA production, P solubilization, siderophore production and ACCD activity but their efficiency could be proved only if they show positive response to plants. To achieve this objective their PGP potentials were evaluated in gnotobiotic condition. Accordingly, two rice varieties namely, Malviya Dhan-36 and Saryu-52 and two growth media (FCN^- and soil extract) were selected. Response of plants in terms of growth, fresh weight, chlorophyll-a content etc were observed at desired time intervals. To prove that colonization indeed occurs, *gusA* reporter gene was tagged with the most efficient isolate BN-2A (*Agrobacterium* sp.) and colonization in the rice root was confirmed by *gus* staining and histochemical analysis of *gusA* staining. Prompted with the results of gnotobiotic experiments where *Agrobacterium* sp. strain BN-2A showed best response on two varieties of rice plant, an attempt was made to test the efficiency of this particular isolate in pot experiment in field conditions. All the experiments were performed with *Agrobacterium* sp. strain BN-2A alone or with combination of eight isolates (ECI-10A, ECI-12A, AF-1D, AF-4B, AF-4C, AF-5A, PN-4D, and BN-4A). Results are briefly presented below under separate headings.

Gnotobiotic Experiments (Semi-solid FCN^- medium)

With a view to screen the most efficient PGPR in terms of plant growth promotion, responses of two rice varieties namely, Malviya Dhan-36 and Saryu-52 following inoculation with nine selected isolates was tested. The parameters for testing responses included, enhancement of total length, fresh weight, number of roots and chlorophyll-*a* content.

Impact on total length It is evident from the data of Tables 1 and 2 that maximum enhancement in total length was observed with the inoculation of BN-2A in both the rice varieties. The increase in total length was 22.6% in Saryu-52 and 52.1% in Malviya Dhan-36. The response was poorest with the isolates PN-4D (1.6%) and ECI-10A (2.9%) in Saryu-52 and Malviya Dhan-36 respectively. However statistical analysis showed significance ($p \leq 0.05$) of the data.

Table 1: Impact of bacterial inoculation on total length of rice variety Saryu-52 in gnotobiotic condition

Bacterial isolates	Total length (cm.)				
	Week				Average Increase (%)
	1st	2nd	3rd	4th	
Control	17.6 ± 1.52	19.7 ± 0.85	20.9 ± 1.15	21.1 ± 1.51	0
ECI-10A	21.9 ± 1.34	24.0 ± 1.23	25.1 ± 0.98	25.8 ± 1.33	22.6
ECI-12A	21.1 ± 1.27	22.3 ± 1.25	22.5 ± 0.87	22.6 ± 0.95	12.0
AF-1D	21.0 ± 1.48	23.5 ±1.34	24.5 ± 1.34	24.8 ± 1.51	18.3
AF-4B	21.9 ± 1.37	23.6 ± 1.35	24.2 ± 1.56	26.2 ± 1.36	21.0
AF-4C	17.7 ± 1.23	19.9 ± 2.05	21.4 ± 1.78	21.7 ± 1.71	1.7
AF-5A	17.8 ± 1.87	20.0 ± 2.14	22.9 ± 1.97	23.1 ± 1.85	5.4
PN-4D	17.9 ± 1.34	19.9 ± 1.76	21.1 ± 1.54	21.7 ± 1.43	1.6
BN-2A	20.8 ± 1.74	24.4 ± 1.29	25.2 ± 1.55	26.9 ± 1.73	22.6
BN-4A	18.1 ± 1.54	20.4 ±1.36	23.5 ±1.42	23.7 ± 1.82	7.8

Results are mean 3 experiments conducted separately with at least ten plants under identical conditions. Statistical analysis showed significance ($p \leq 0.05$) of data.

Table 2: Impact of bacterial inoculation on total length of Malviya Dhan-36

Bacterial isolates	Total length (cm.)				
	Week				Average Increase (%)
	1st	2nd	3rd	4th	
Control	12.8 ± 1.22	17.2 ± 1.35	17.9 ± 1.78	18.2 ± 1.27	0
ECI-10A	13.2 ± 0.97	17.8 ± 1.33	18.4 ± 1.45	18.6 ± 1.22	2.9
ECI-12A	15.1 ± 0.85	17.9 ± 1.51	18.1 ± 1.63	18.3 ± 1.15	5.9
AF-1D	14.4 ± 1.34	18.2 ± 1.82	19.1 ± 1.36	19.4 ± 0.86	7.9
AF-4B	13.0 ± 1.32	18.2 ± 1.21	18.4 ± 1.73	19.4 ± 1.44	4.2
AF-4C	16.8 ± 1.35	20.4 ± 1.53	21.4 ± 1.74	22.6 ± 1.19	23.4
AF-5A	15.1 ± 1.27	18.0 ± 1.25	18.8 ± 1.67	19.5 ± 1.28	8.7
PN-4D	14.6 ± 1.39	17.3 ± 1.36	18.7 ± 1.39	18.9 ± 1.24	5.8
BN-2A	16.8 ± 1.28	26.4 ± 1.36	28.6 ± 1.34	29.8 ± 1.25	52.1
BN-4A	17.0 ± 0.89	18.4 ± 1.35	20.0 ± 1.82	20.3 ± 1.37	15.8

Other conditions same as in Table 1.

Effect on fresh weight

It is evident from the data of Tables 3 and 4 that maximum increase in fresh weight of rice variety Saryu-52 and Malviya Dhan-36 occurred with the inoculation of the isolate BN-2A, the increase being 42.4% in Saryu-52 and 68.8% in Malviya Dhan-36. The least enhancement was noted by AF-4B (22.5%) in Saryu-52 and ECI-10A (9.2%) in Malviya Dhan-36.

Table 3: Effect of bacterial inoculation on fresh weight of rice variety Sartu-52.

Bacterial isolates	Fresh weight (mg)				
	Week				Average increase (%)
	1st	2nd	3rd	4th	
Control	120 ± 16	246 ± 16	360 ± 18	570 ± 19	0
ECI-10A	166 ± 20	319 ± 13	437 ± 13	667 ± 19	26.6
ECI-12A	162 ± 13	323 ± 17	432 ± 24	662 ± 23	25.6
AF-1D	188 ± 23	319 ± 16	448 ± 26	684 ± 19	32.7
AF-4B	173 ± 17	298 ± 25	410 ± 20	632 ± 28	22.5
AF-4C	188 ± 16	306 ± 21	414 ± 24	634 ± 25	26.8
AF-5A	161 ± 15	314 ± 19	463 ± 16	685 ± 27	27.7
PN-4D	164 ± 17	320 ± 16	457 ± 23	686 ± 21	28.5
BN-2A	199 ± 21	357 ± 22	472 ± 14	726 ± 18	42.4
BN-4A	169 ± 22	314 ± 17	449 ± 18	686 ± 23	28.3

Experimental details and data analysis were similar to Table 1.

Table 4: Effect of bacterial inoculation on fresh weight of rice variety Malviya Dhan-36.

Bacterial isolates	Fresh weight (mg)				
	Week				Average increase (%)
	1st	2nd	3rd	4th	
Control	60 ± 8	137 ± 7	220 ± 11	352 ± 18	0
ECI-10[a]	69 ± 4	150 ± 9	233 ± 12	374 ± 18	9.2
ECI-12[a]	90 ± 4	172± 8	260 ± 9	395 ± 24	20.2
AF-1D	96 ± 7	220 ± 8	303 ± 17	468 ± 13	47.8
AF-4B	88 ± 4	167 ± 9	273 ± 6	430± 13	28.7
AF-4C	86 ± 4	195 ± 7	306 ± 15	476 ± 14	40.0
AF-5A	76 ± 4	152 ± 8	238± 14	378 ± 24	13.3
PN-4D	85 ± 7	165 ± 6	283 ± 13	446 ± 17	29.4
BN-2A	100 ± 4	232 ± 6	380 ± 16	586 ± 15	68.8
BN-4A	85 ± 4	180 ± 9	326 ± 13	503 ± 17	41.1

 # Experimental details same as in Table 1.

Response on chlorophyll-a content

It is evident from the data of Tables 5 and 6 that out of all the isolates, inoculation with BN-2A caused highest increase in chlorophyll-*a* content in both the rice varieties namely, Saryu-52 (76.6%) and Malviya Dhan-36 (37.1%). There was minimum increase with the inoculation of BN-4A (21.6%) in Saryu-52 and with ECI-10A (10.8%) in Malviya Dhan-36.

Table 5: Impact of different bacterial inoculation on chlorophyll-a content of rice variety Sartu-52.

Bacterial isolates	Chlorophyll-a (µg/mg fresh weight)				
	Week				Average Increase (%)
	1st	2nd	3rd	4th	
Control	1.32 ± 0.56	2.7 ± 0.63	3.31 ± 0.56	2.65 ± 0.84	0
ECI-10A	2.28 ± 0.37	3.39 ± 0.54	4.11 ± 0.27	2.97 ± 0.45	33.6
ECI-12A	3.13 ± 0.26	4.82 ± 0.28	4.02 ± 0.87	4.45 ± 0.65	76.3
AF-1D	1.79 ± 0.38	3.17 ± 0.37	5.28 ± 0.98	4.71 ± 0.67	47.7
AF-4B	2.12 ± 0.35	3.42 ± 0.81	4.26 ± 0.51	3.42 ± 0.57	36.3
AF-4C	2.08 ± 0.46	3.11 ± 0.68	4.22 ± 0.67	3.65 ± 0.37	34.5
AF-5A	1.98 ± 0.47	3.56 ± 0.63	4.82 ± 0.82	4.25 ± 0.44	47.0
PN-4D	2.82 ± 0.27	3.28 ± 0.39	4.78 ± 0.56	4.7 ± 0.41	64.2
BN-2A	3.13 ± 0.52	4.52 ± 0.71	4.59 ± 0.52	4.32 ± 0.34	76.6
BN-4A	1.61 ± 0.28	3.94 ± 0.38	3.51 ± 0.91	3.24 ± 0.54	21.6

Experimental details same as in Table 1.

Table 6: Impact of bacterial inoculation on chlorophyll-a of rice varieties Malviya Dhan-36.

Bacterial isolates	Chlorophyll-a (µg/mg fresh weight)				
	Week				Average Increase (%)
	1st	2nd	3rd	4th	
Control	5.65 ± 0.34	5.71 ± 0.38	6.99 ± 0.61	5.69 ± 0.71	0
ECI-10A	5.69 ± 0.91	6.33 ± 0.67	7.41 ± 0.38	7.15 ± 0.42	10.8
ECI-12A	9.37 ± 0.37	6.54 ± 0.82	7.92 ± 0.51	5.82 ± 0.54	24.0
AF-1D	9.49 ± 0.34	5.86 ± 0.22	7.42 ± 0.41	5.94 ± 0.37	20.3
AF-4B	8.48 ± 0.33	8.73 ± 0.84	7.37 ± 0.83	6.02 ± 0.71	28.6
AF-4C	8.1 ± 0.64	8.06 ± 0.62	7.65 ± 0.29	6.6 ± 0.34	27.5
AF-5A	8.72 ± 0.82	6.61 ± 0.73	7.75 ± 0.71	7.35 ± 0.53	27.5
PN-4D	8.02 ± 0.92	6.58 ± 0.91	7.82 ± 0.53	8.74 ± 0.31	30.7
BN-2A	8.36 ± 0.25	8.28 ± 0.34	8.6 ± 0.84	7.52 ± 0.42	37.1
BN-4A	8.08 ± 0.37	8.57 ± 0.31	8.2 ± 0.41	6.3 ± 0.51	30.3

Experimental details same as in Table 1.

Response of Bacterial Inoculation on Rice Plant with Soil Extract Medium

Since BN-2A showed excellent plant growth promoting response in the FCN^- medium in gnotobiotic condition we became interested to test its response in medium made up of soil extract. Accordingly, response of plant was tested following inoculation of BN-2A and combination of eight isolates in soil extract medium. The response of plants in terms of morphological characters is evident from the photograph shown in Fig. 1 (A-C).

A B C

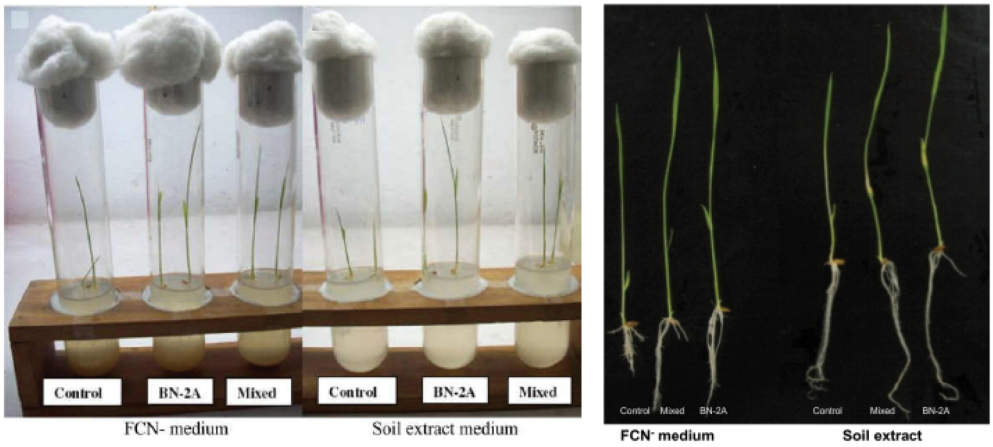
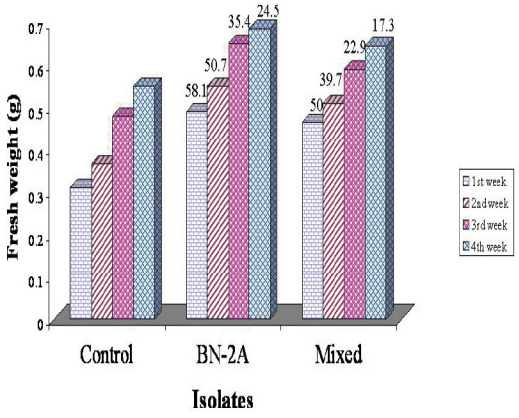


Fig. 1 (A-C): *Rice plant (Saryu-52) grown in gnotobiotic condition after 1st week of inoculation of bacterial inoculants.* **A**- *Growth in FCN- and* **B**- *soil extract medium,* **C**- *Rice plant grown after one week of inoculation of bacteria in two different media. Mixed isolates: ECI-10A, ECI-12A, AF-1D, AF-4B, AF-4C, AF-5A, PN-4D and BN-4A.*

Effect on total length and fresh weight

It is evident from the result of Fig. 2A that the total length of rice plant increased by 21.1% (range between 16.7 to 25.5%) in the presence of BN-2A whereas only 17.9% (range 12.1 to 22.9%) increase occurred by the inoculation of mixed cultures. Similarly there was 42.2% increase (range 24.5 to 58.1%) in the fresh weight in the presence of BN-2A but mixed cultures elicited 32.5% increase (range between 17.3 to 50%) (Fig. 2B).

A



B

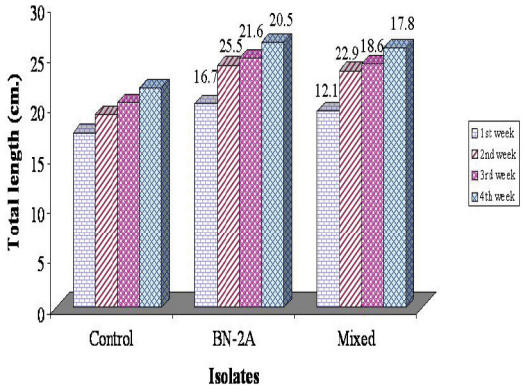


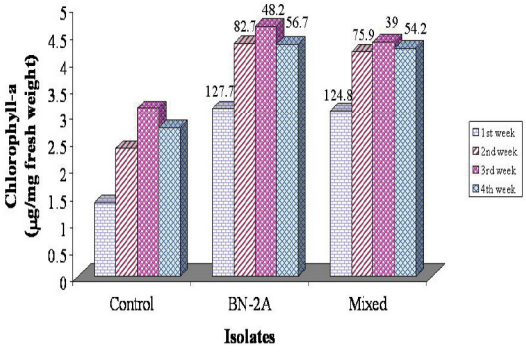
Fig. 2: *Total length* **(A)** *and Fresh weight* **(B)** *of rice plant (Saryu-52) after growth in soil extract medium and its response following bacterial inoculation. Control- no bacterial inoculation, BN-2A- pure culture of BN-2A alone, Mixed- consists of 8 isolates (ECI-10A, ECI-12A, AF-1D, AF-4B, AF-4C, AF-5A, PN-4D, and BN-4A).*

Number shown above the bar indicates % increase in respect to control.
Experiments were performed 3 times under identical conditions.
Results are means of 3 experiments conducted separately with at least ten plants under identical conditions. Value of significance $p < 0.05$.

Impact on chlorophyll-a and number of roots

Unlike total length and fresh weight, chlorophyll-*a* content of the plant showed drastic increase. It is evident from the results of Fig. 3A that inoculation with isolate BN-2A caused 78.8% (range between 48.2 to 127.7%) whereas mixed isolates showed 73.5% (between 39-124.8% increase) increase in chlorophyll-a content. Furthermore the number of roots increased by 37.1% (range between 25 to 50%) in the presence of BN-2A and inoculation with mixed cultures showed 24.9% (between 12.5-40%) increase (Fig. 3B).

A



B

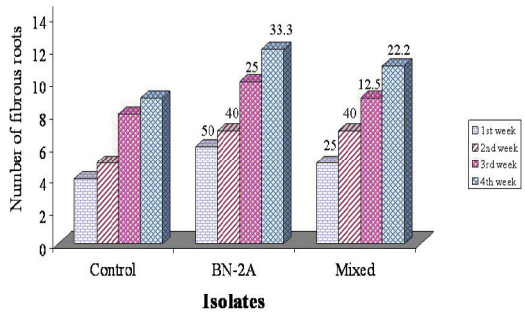


Fig. 3: Chlorophyll-a content (A) and number of roots (B) of rice plant following bacterial inoculation in soil extract medium.

Other conditions same as in Fig. 2.
Mixed: Same as in Fig. 2.

Discussion

Manipulation and exploitation of symbiotic and free-living PGPR in agriculture have become a significant component of modern agricultural practice in most of the countries (Bashan and Holguín, 1998). The most successful plant–bacteria relationships have been reported in leguminous *Rhizobium* for symbiosis and free-association in non-leguminous by *Azospirillum*, *Pseudomonas*, *Bacillus*, and *Azotobacter* (Bashan *et. al.*, 2004) for the plant growth promotion. Our results clearly show that inoculation of nine efficient PGPR strains (ECI-10A, ECI-12A, AF-1D, AF-4B, AF-4C, AF-5A, PN-4D, BN-2A and BN-4A) on two rice varieties (Saryu-52 and Malviya Dhan-36), shows differential response on both varieties. Out of nine isolates, BN-2A showed the best response for all the parameters tested in both the rice varieties. Our results resemble to the findings of Rothballer *et. al.* (2003) where they reported that the responses of inoculation in three varieties of wheat (Brazilian, Naxos and Atir) with *Azospirillum brasilense* Sp7 or Sp245 showed variable response in different wheat varieties. They reported that the Brazilian wheat cultivar showed the strongest response and thus may be able to gain greater benefit from its partnership with *Azospirillum*. They suggested that this might be due to adaptation process of the plant to a common bacterium. We also found that the association of PGPR and the plants depends upon their suitable adaptability which probably depends on the genetic makeup of both the crop and the PGPR. Furthermore the root exudates released by different types of plants vary that may be a cause of adaptability and compatibility factors for differential colonization of PGPR in different rice variety (Rothballer *et. al.*, 2003). Our results suggest that in addition to standard synthetic media soil extract may also support growth of the rice plants. This was evident from the fact that significant increase in growth and development of both the rice varieties occurred in soil extract.

Combined responses of PGPR have been reported in Granny Smith apple cultivar in which root inoculation with *Bacillus* M3 or OSU-142 and *Microbacterium* FS01 promoted significant tree growth and yield (Karlidag *et. al.*, 2007). The combination of *Bacillus* sp. strain M3, *Bacillus* sp. strain OSU- 142 and *Microbacterium* sp. strain FS01 in terms of growth and yield has been reported more effective in comparison to single culture. Almost similar findings have been reported by the application of *Bacillus* sp. strain OSU-142 and M3 in barley (Cakmakci *et. al.*, 2001), apricot (Esitken *et. al.*, 2003), raspberry (Orhan *et. al.*, 2006) and apple (Aslantas *et al.*, 2007). We also observed significant increase in total length, and fresh weight, number of roots and chlorophyll-*a* content in both the rice varieties (Saryu-52 and Malviya Dhan-36) by the inoculation of mixed isolates consisting of eight strains. This result is in agreement with the previous reports (Karlidag *et. al.*, 2007; Cakmakci *et. al.*, 2001; Esitken *et. al.*, 2003; Orhan *et. al.*, 2006; Aslantas *et. al.*, 2007). However inoculation of BN-2A alone to both the rice varieties (Saryu-52 and Malviya Dhan-36) produced better plant growth promotion than the mixed cultures. Although there was not vast differences but differences observed might be due to certain antagonistic effect or competition for nutrients by individual isolates. Our findings are similar to earlier reports which showed that certain mixtures of microbial strains did not show synergistic but at least comparable plant growth promotion, with respect to the separate application of microorganisms (Schmidt *et. al.*, 2004). There are other reports where it has been reported that *Azospirilla* are capable to colonize the root surface, and few strains can penetrate the root interior (Ramos *et. al.*, 2002).

Entry of bacteria into plant tissue for colonization is a matter of debate. In general, it is felt that bacteria can enter into plant tissue via stomata, lentil cells, wounds, areas of emergence of lateral roots and germinating radicals (Hallmann *et al.*, 1997). However, there is a general agreement that the main entry for bacteria may be through wounds that naturally occur as a result of plant growth or through root hairs and at epidermal conjunctions. Cracks or plant wounding induced either by biotic or abiotic factors are ubiquitous in any agro-system and is probably a major factor for bacterial entrance. Wounds also create suitable conditions for the approaching bacteria by allowing leakage of plant exudates, which serve as a food source for the bacteria. However, wounds and lateral roots are not absolutely required for entrance of all the bacteria (Hallman *et al.*, 1997). It has been demonstrated that seedlings grown with minimal disturbance in liquid media or on water-agar were penetrated by bacteria long before lateral root emergence. Penetration of bacteria inside plant tissues even without wounds and emergence of lateral roots has been presumed to be due to the presence of a combination of cellulolytic and pectinolytic enzymes (Hallmann *et al.*, 1997; Reinhold-Hurek and Hurek, 1998). This presumption is supported by documenting the presence of cellulolytic and pectinolytic enzymes in numerous bacteria more specifically in endophytic such as *Azoarcus* sp., *Azospirillum irakense* and *Pseudomonas fluorescence* (Hallman *et al.*, 1997). All the above modes of entry have been well documented in several endophytic bacteria (Hallmann *et al.*, 1997).

Tagging of few important biomarker genes such as β-galactosidase (*lacZ*), β-glucuronidase (*gusA*) or green fluorescent protein (*gfp*) to the bacteria has been employed by many workers to track bacteria in the plants (Stoltzfus and de Bruijn, 2000; Gyaneshwar *et al.*, 2001). We have confirmed the colonization of inoculated bacteria by tagging β-glucuronidase (*gusA*) gene; but data not shown here due word limit prescribed in this journal.

In conclusion, the nine strains tested for plant growth promotion studies on the rice plants have the potentials of plant growth promotion, but, isolate BN-2A seems most efficient based on the response on rice plant. The isolate BN-2A is capable to colonize in the

interior root tissues as well in the rhizosphere of the plant and may be exploited efficiently for plant growth promotion in the agriculture.

Acknowledgement

Financial supports by ICCR, Government of India is highly acknowledged. The author is also grateful to A. Kumar, Professor, School of Biotechnology, Banaras Hindu University, Varanasi, India for guidance and all facilities.

References

Aslantas, R., Cakmakci, R., & Sahin, F. (2007). Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Scientia. Horti.,* 111(4), 371-377.

Barraquio, W.L., Revilla, L., & Ladha, J.K. (1997). Isolation of endophytic diazotrophic bacteria from wetland rice. *Plant Soil,* 194, 15-24.

Bashan, Y., Holguin, G., & de-Bashan, L.E. (2004). *Azospirillum*-plant relationships: Physiological, molecular, agricultural, and environmental advances (1997-2003). *Can. J. Microbiol.,* 50, 521-577.

Bertrand H, Nalin R, Bally R, Cleyct-Marcl JC (2001) Isolation and identification of the most efficient plant growth-promoting bacteria associated with canola (*Brassica napus*). *Biol Fertil Soils* **33**:152-156.

Cakmakci, R., Kantar, F., & Sahin, F. (2001). Effect of N_2-fixing bacterial inoculations on yield of sugar beet and barley. *J. Plant Nutri. Soil Sci.,* 164, 527-531.

Esitken, A., Karlidag, H., Ercisli, S., Turan, M., & Sahin, F. (2003). The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* l. cv. *Hacihaliloglu*). *Aust. J. Agric. Res.,* 54, 377-380.

Glick, B.R. (1995). The enhancement of plant-growth by free-living bacteria. *Can. J. Microbiol.* 41, 109-117.

Glick, B.R., Liu, C.P., Ghosh, S., & Dumbroff, E.B. (1997). Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Soil Biol. Biochem.* 29, 1233-1239.

Gyaneshwar, P., James, E.K., Mathan, N., Reddy, P.M., Reinhold-Hurek, B., & Ladha, J.K. (2001). Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. *J. Bacteriol.* 183, 2634-2645.

Hallmann, J., QuadtHallmann, A., Mahaffee, W.F., & Kloepper, J.W. (1997). Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* 43, 895-914.

Herrero, M., de Lorenzo, V., & Timmis, K.T. (1990). Transposon vectors containing non-antibiotic resistance markers for cloning and stable chromosome insertion of foreign genes in gram-negative bacteria. *J. Bacteriol.* 172, 6557-6567.

Karlidag, H., Esitken, A., Turan, M., & Sahin, F. (2007). Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. *Scientia Hort.,* 114, 16-20.

Kloepper, J.W., Lifshitz, R., & Zablotowicz, R.M. (1989). Free-living bacterial inocula for enhancing crop productivity. *Trends Biotechnol.,* **7**, 39-44.

Kloepper, J. W., Lifshitz R., & Zablotowicz R. M. (1989). Free-living bacteria for enhancing crop productivity. *Trends Biotechnol.*, 7, 39–43.

Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *J. Biol. Chem.*. 140, 315-322.

Orhan, E., Esitken, A., Ercisli, S., Turan, M., & Sahin, F. (2006). Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Scientia Horti.,* 111, 38-43.

Raaijmakers, J. M., & Weller, D. M. (2001). Exploiting genotypic diversity of

2,4-diacetylphloroglucinol-producing Pseudomonas spp.: characterization of superior root-colonizing P. fluorescens strain Q8r1-96. *Appl. Environ. Microbiol.,* 67, 2545–2554.

Ramos, H.J.O., Roncato-Maccari, L.D.B., Souza, E.M., Soares-Ramos, J.R.L., Hungria, M., & Pedrosa, F.O. (2002). Monitoring *Azospirillum*-wheat interactions using the *gfp* and *gusA* genes constitutively expressed from a new broad-host range vector. *J. Biotechnol.* 97, 243-252.

Reinhold-Hurek, B., & Hurek, T. (1998). Interactions of gramineous plants with *Azoarcus* spp. and other diazotrophs: Identification, localization, and perspectives to study their function. *Crit. Rev. Plant Sci.,* 17, 29-54.

Rothballer, M., Schmid, M., & Hartmann, A. (2003). *In situ* localization and PGPR-effect of *Azospirillum brasilense* strains colonizing roots of different wheat varieties. *Symbiosis,* 34, 261-279.

Schmidt, C.S., Agostini, F., Simon, A.M., Whyte, J, Townend J, Leifert C, Killham K, Mullins C (2004) Influence of soil type and pH on the colonisation of sugar beet seedlings by antagonistic *Pseudomonas* and *Bacillus* strains, and on their control of *Pythium* damping-off. *Euro. J. Plant Pathol.* 110,1025-1046.

Schroth, M. N., & Hancock, J. G. (1981). Selected topics in biological control. *Annu. Rev. Microbiol.,* 35, 453–476.

Shrivastava, U. P. (2013a). Characterization of Diazotrophic Rhizobacteria under Various Conditions. *Int. J. Appl. Sci. Biotechnol.,* 1(3), 110-117.

Shrivastava, U. P. (2013b). Isolation and initial characterization of diazotrophic plant growth promoting rhizobacteria (PGPR) from rice rhizosphere of Parsa and Bara district of Nepal. *Int. J. Pharma. Life Sci.,* 4, 2481-2488.

Shrivastava, U. P. (2013c). Isolation and Characterization of Plant Growth Promoting Rhizobacteria (PGPR) from Rice Rhizosphere of Varanasi and East Champaran District of India. *Academic Voices* 3 (1), 81-90.

Shrivastava, U. P. & Kumar, A. (2011). Biochemical Characterization of Siderophore Producing Plant Growth Promoting Rhizobacteria (PGPR) Isolated from Rice Rhizosphere. *Nep. J. Integrated Sci.* 1, 31-37.

Shrivastava, U. P. & Kumar, A. (2013). Characterization and optimization of 1-Aminocyclopropane-1-Carboxylate Deaminase (ACCD) activity in different rhizospheric PFPR along with *Microbacterium* sp. strain ECI-12A. *Int. J. Appl. Sci. Biotechnol.* 1(1), 11-15.

Stoltzfus, J.R., & de Bruijn, J. (2000). Evaluating diazotrophy, diversity, and endophytic colonization ability of bacteria isolated from surface-sterilized rice. In: *The quest for nitrogen fixation.* Ladha, J.K. & Reddy, P.M. (eds.), International Rice Research Institute, Manila, Philippines, pp 63-91.

Stotsky G, Devanas MA, Zeph LR (1990) Methods for studying bacterial gene transfer in soil by conjugation and transduction. *Adv Appl Microbiol* **45**:157-169.

Sturz, A. V., and J. Nowak. 2000. Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Appl. Soil Ecol. 15:183–190.

Timmusk, S., Nicander, B., Granhall, U., & Tillberg, E. (1999). Cytokinin production by *Paenibacillus polymyxa*. *Soil Biol. Biochem.,* 31, 1847-1852.

Vermeiren, H., Vanderleyden, J., Hai, W. (1998). Colonization and *nifH* expression on rice roots by *Alcaligenes faecalis* A15. In: Malik, K.A., Mirza, M.S., & Ladha, J.K. (eds) *Nitrogen fixation with non-legumes,* Kluwer Academic, Dordrecht, Netherlands, 167-177.

Vincent, J.M. (1970). A manual for the practical study of root nodule bacteria. Blackwell Scientific Publications Ltd., Oxford, U.K.

Weinstein, M., Roberts, R.C., & Helinski, D.R.

(1992). A region of the broad-host-range plasmid rk2 causes stable *in planta* inheritance of plasmids in *Rhizobium meliloti* cells isolated from alfalfa root nodules. *J. Bacteriol.,* 174, 7486-7489.

Weller, D. M. (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.* 26, 379–407.

Wilson, K.J. (1995). Molecular techniques for the study of rhizobial ecology in the field. *Soil Biol. Biochem.,* **27**, 501-514.