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Synergistic Effects of the Inoculation with Plant Growth-Promoting Rhizobacteria and an Arbuscular Mycorrhizal Fungus on the Performance of Wheat

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Abstract: The synergistic effects of plant growth-promoting rhizobacteria and an arbuscular mycorrhizal (AM) fungus (*Glomus fasciculatum*) on plant growth, yield, and nutrient uptake of wheat plants were determined in field conditions. The triple inoculation of Azotobacter chroococcum with Bacillus and *Glomus fasciculatum* significantly increased the dry matter by 2.6-fold above the control. Grain yield of plants inoculated with *A. chroococcum* together with Bacillus sp. and *G. fasciculatum* was 2-fold higher than that of non-inoculated plants, at 135 days after sowing (DAS). The maximum increase in grain protein (255.2 mg g^{-1}) was observed in plants inoculated with *A. chroococcum* with Bacillus sp., *G. fasciculatum*, and Penicillium variabile, while the minimum grain protein (113.7 mg g^{-1}) was with a single inoculation of *G. fasciculatum*. The higher N content (33.6 mg $plant^{-1}$) and P content (67.8 mg $plant^{-1}$) in wheat plants were observed with the co-inoculation of *A. chroococcum* with Bacillus sp. and *G. fasciculatum*. The N and P contents of the soil at 135 DAS differed among treatments. Addition of *P. variabile* to single or double inoculation treatments negatively affected the measured parameters. Populations of *A. chroococcum*, phosphate solubilizing microorganisms, percentage root infection, and spore density of the AM fungus in some treatments increased at 80 DAS. The findings show that the multiple inoculations with plant growth promoting rhizobacteria consistently increased the growth and yield, N and P concentrations, and quality of wheat grains.

Key Words: Plant growth promoting rhizobacteria, AM fungus, *Azotobacter*, wheat

Introduction

Most tropical and some subtropical soils are acidic. In these regions, strong sorption coupled with low inherent phosphorus stocks leads to widespread phosphorus deficiency. Even where inorganic and organic P forms are abundant in soils, their concentration in the soil solution is usually in the micromolar range (Frossard et al., 2000). Therefore, phosphatic fertilizers as P sources were applied to soils to meet the crop P demands. After its application, a major part of the P is fixed and becomes less available for uptake by the plants. It has been reported that the plant growth promoting rhizobacteria (PGPR) including phosphate-solubilizing microorganisms (PSMs) are able to solubilize the unavailable forms of P in soil by acidification, chelation, and exchange reaction in the soil environment (Maliha et al., 2004; Ponmurugan and Gopi, 2006).

Numerous studies have shown a substantial increase in plant growth and seed yield following inoculation with PGPR strains including PSMs and N_2 fixers on legumes (Perveen et al., 2002; Wani et al., 2007). However, where P is scarce, it was found that plants inoculated with arbuscular mycorrhizal (AM) fungi either alone or in combination with PSMs increased the P uptake in wheat (Raja et al., 2002) or maize (*Zea mays* L.) (Evans and Miller, 1990). The dual inoculation of asymbiotic N_2 fixer *A. chroococcum* and AM fungus *G. fasciculatum* resulted in enhanced root infection, which stimulated plant growth, and increased N and P uptake by greengram (*Vigna radiata* L. Wilczek) (Zaidi et al., 2004). Similarly, a significant increase in the dry matter yield of wheat plants with co-inoculation of rock-phosphate solubilizing fungi *Aspergillus niger* and *Penicillium citrinum*, and *G. constrictum* is reported (Omar, 1998). In P deficient soils or soils amended with rock-phosphate, PSMs interact

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well with the AM fungi and release some phosphate ions from sparingly soluble P sources, which could be tapped by the AM fungi, and is translocated to the plants (Poi et al., 1989). Zaidi and Khan (2005) have suggested a synergistic relationship between PSMs (*Pseudomonas striata* and *Penicillium*) and *A. chroococcum*, allowing a better use of poorly soluble P sources and increased dry matter accumulation, grain yield, and P uptake of wheat plants. During interaction, the PSMs increase the availability of P and efficiency of N_2 fixation by phosphate solubilizing activity and releasing the plant growth promoting substances (Kucey et al., 1989).

Wheat (*Triticum aestivum* L.) is a widely cultivated cereal crop and is grown under various climatic conditions between 47°S and 57°N latitudes on diverse soils ranging from sandy to clayey soil. In India, wheat is cultivated in an area of about 26,121.8 thousand-hectares with production of about 2493 kg ha^{-1} (Sharma, 2000). Most of the studies on the effect of N_2 fixing, PGPR, or AM fungus have been performed in either sterilized soil or in quite small amounts of nonsterile soil, using these organisms either alone or in combination. However, the effects of multiple inoculations of N_2 fixing bacteria with PSMs and AM fungus have rarely been tested. There is little information available on the possible synergistic effects of these organisms on wheat under field conditions. Therefore, this study was conducted to evaluate the effect of N_2 fixing (*Azotobacter chroococcum*), a phosphate solubilizing bacterium (*Bacillus* sp. 8), phosphate solubilizing fungus (*Penicillium variabile*), and AM fungus (*Glomus fasciculatum*), used either alone or in combination, on growth, seed yield, grain protein, and nutrient uptake of wheat plants. The establishment of these organisms in the wheat rhizosphere was also investigated.

Materials and Methods

The inoculants were prepared by growing *Azotobacter chroococcum* (Institute of Microbial Technology, Chandigarh, India) in Jensen broth (Jensen, 1942) for 10 days and *Bacillus* sp. 8 and *Penicillium variabile* (our own culture collections) for 5 days in National Botanical Research Institute Phosphate (NBRIP) growth medium (Nautiyal, 1999) in flasks shaken at 130 rpm at 28 ± 2 °C. The NBRIP growth medium contained (g l^{-1}): glucose, 10; Ca_3 $(PO_4)_2$ 5; $MgCl_2.6H_2O$, 5; $MgSO_4.7H_2O$, 0.25; KCl, 0.2 and $(NH_4)_2$ SO_4, 0.1. During these periods, the populations of cultures reached a density of 5×10^6 (*A. chroococcum*), 2.3×10^9 (*Bacillus* sp. 8) and 4.5×10^5 (*P. variabile*) colony forming unit per milliliter.

Undamaged and clean seeds of wheat var. HD 2204 were surface sterilized (alcohol 70%, 3 min; sodium hypochlorite 3%, 3 min), rinsed 6 times with sterile water, and shade dried. Surface sterilized seeds were inoculated by soaking the seeds in liquid culture medium for 1 h using 10% gum arabic as adhesive agent. For combined inoculations, the liquid cultures of each organism were mixed in equal proportion to soak 100 g of seeds in 200 ml of broth solution for 1 h. The microbial population at the time of inoculation were 3×10^5, 2×10^8, and 2×10^5 per seed for *A. chroococcum*, *Bacillus* sp., and *P. variabile*, respectively. The AM fungus *G fasciculatum* (Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India) was multiplied on rhodes grass (*Chloris gayana* Kunth) following the open pot culture method (Gilmore, 1968). In combined treatments with *G. fasciculatum*, inoculated seeds were sown in soils that received 125 g of the mycorrhizal inoculum (infected roots and spores) and were covered with a soil layer of 2 cm over which the inoculated seeds were sown. The inoculated seeds were sown by seed drill at a soil depth of 5-6 cm in 2 m × 2 m plots at 0.2 m row distance and 0.15-0.18 m plant distance within the rows.

The study was conducted during the winter at the experimental fields of Agricultural Sciences, Aligarh Muslim University, Aligarh. The experimental soil was an alluvial sandy clay loam with P 16 mg kg^{-1}, N 0.2 g kg^{-1}, organic C 0.4%, pH 7.2, WHC 44%, CEC 11.7 cmol kg^{-1}, and anion exchange capacity 5.1 cmol kg^{-1}. Uninoculated control plants received 60 and 40 kg ha^{-1} of N and P fertilizers, 40 days after sowing (DAS) the wheat. Nitrogen was applied as urea and P as diammonium phosphate. The plots receiving inoculation treatments remained unfertilized. The treatments were as follows: T1 *A. chroococcum;* T2 *Bacillus* sp. 8; T3 *Penicillium variabile*; T4 *Glomus fasciculatum*; T5 *A. chroococcum* + *Bacillus* sp.; T6 *A. chroococcum* + *P. variabile;* T7 *A. chroococcum* + *G. fasciculatum;* T8 *Bacillus* sp. + *P. variabile;* T9 *Bacillus* sp. + *G. fasciculatum;* T10 *A. chroococcum* + *Bacillus* sp. + *G. fasciculatum*; T11 *A. chroococcum* + *P. variabile* + *G.*

fasciculatum; T12 *A. chroococcum* + *G. fasciculatum* + *Bacillus* sp + *P. variabile*; T13 uninoculated control ($N_{60}P_{40}$). The bio-primed seeds (100 kg ha^{-1}) were sown (5 cm deep) on 15 November 2002 and were repeated with the same treatments on 20 November 2003. The plots were arranged in a randomized complete block design with 13 treatments and 6 replications. The plants were watered when required.

All plants from each treatment were harvested 135 DAS and the adhering soil particles were carefully removed by gentle shaking under running tap water. To estimate shoot dry weight, the shoots were severed at the soil level, oven dried (80 °C) for 24 h, and weighed. The roots were washed in distilled water, oven dried (80 °C), and weighed. The total plant biomass, seed yield, and grain protein (Sadasivam and Manikam, 1992) were recorded at harvest (135 DAS). The N and P contents in wheat plants were measured at harvest using the modified micro-Kjeldahl (Iswaran and Marwah, 1980) and molybdate blue method (Jackson, 1967). Soil samples collected from each inoculation treatment at 135 DAS were used for total residual N and available P analysis.

Rhizosphere soils were collected from each experimental plot at 80 DAS and populations of *A. chroococcum* were quantified by dilution plating using Jensen N free medium. Plates were incubated at 28 ± 2 °C for 7 days and colonies showing brown to black pigments indicating melanin were recorded. The population counts of PSMs in the rhizospheric soils of each treatment were determined at 80 DAS by enrichment culture technique using NBRIP growth medium. Each plate was replicated 3 times, and incubated for 5 days (for phosphate solubilizing bacteria) and 3 days (for phosphate solubilizing fungus) at 28 ± 2 °C. Colonies showing a clear halo around growth indicating P solubilization were counted. The mycorrhizal colonization in the roots was estimated by the root clearing and staining method (Phillips and Hayman, 1970). The wet sieving and decanting method described by Gerdemann and Nicolson (1963) was used for enumeration of AM spores in soils. Since wheat was grown for 2 consecutive years and the data obtained were homogeneous, the data of the 2 year trials were pooled together and subjected to analysis of variance and the difference among treatment means was compared by high range statistical domain (HSD) using Tukey's test (Meyers and Grossen, 1974) at $P \leq 0.05$.

Results

The effects of inoculation with N_2 fixing and PSMs and AM fungus on growth, grain yield, and N contents of wheat plant differed significantly (Table 1). Among the single inoculation treatments, only *G. fasciculatum* increased the dry matter accumulation in shoots and whole plants significantly ($P \leq 0.05$), by 78% and 73%, respectively, more than the control, at 135 DAS. In contrast, the combined inoculation of *A. chroococcum* and *Bacillus* sp., *A. chroococcum* and *G. fasciculatum* and *Bacillus* sp. with *G. fasciculatum* increased the dry matter accumulation in roots, shoots, and total biomass significantly at harvest, compared with the uninoculated control. The co-inoculation treatment of *Bacillus* sp. 8 and *G. fasciculatum* enhanced the dry matter accumulation in roots, shoots, and whole plants by 1.7-, 1.5-, and 1.6-fold, respectively, compared with the control, and was superior to other single or dual inoculation treatments. The addition of *G. fasciculatum* to a combination of *A. chroococcum* and *Bacillus* sp. resulted in the largest increase in the whole plant biomass and increased the total dry matter by 2-fold more than the control. The increase in shoot dry mass was accompanied by an increase in root biomass, and consequently root:shoot ratios did not vary much among treatments. Increases in dry matter accumulation in roots and shoots and roots and total biomass were highly correlated ($r = 0.94$).

Generally, the number of tillers per plant increased significantly ($P \leq 0.05$) following microbial inoculations, except in the plots receiving a single inoculation of *P. variabile* and *G. fasciculatum* (Table 1). A trend similar to those observed for total plant dry matter was observed for tiller formation on wheat plants. A single inoculation of *A. chroococcum*, and co-inoculation of *A. chroococcum* with *Bacillus*, *A. chroococcum* with *G. fasciculatum*, and *Bacillus* with *G. fasciculatum* increased the grain yield by 0.7-, 1.1-, 1.3-, and 1.3-fold, respectively, compared with the control. Inoculation of plants with *G. fasciculatum* coupled with *A. chroococcum* and *Bacillus* sp. was superior to all treatments and doubled the grain yield relative to the control. Inoculation of plants with triple cultures of *A. chroococcum*, *P. variabile*, and *G. fasciculatum* significantly increased the grain yields but showed an inferior effect compared to *A. chroococcum* inoculated with *G. fasciculatum* or *Bacillus* sp. with *G. fasciculatum* (Table 1). Among the single treatments, a maximum increase in straw production was observed

Table 1. Co-inoculation effects of plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on growth, seed yield, grain protein, and N concentration at harvest in field grown wheat.

Treatment	Mean dry mass (g plant^{-1})			R/S	Mean yield			Grain protein (mg g^{-1})	N concentration (mg plant^{-1})
	Root	Shoot	Total biomass		No. of tillers plant^{-1}	Grain (g plant^{-1})	Straw (g plant^{-1})		
Control	1.4	10.4	11.8	0.13	5.7	1.8	2.8	126.2	18.6
A. chroococcum	2.3cd	15.8de	18.1h	0.15	9.5b	3.2e	3.9cd	303.7d	25.2e
Bacillus sp. 8	1.8ef	11.5gh	13.3j	0.17	11.7b	2.2g	3.9cd	167.5e	23.4f
Penicillium variabile	1.0g	10.3g	11.3k	0.09	7.3d	1.7h	4.6a	141.2ef	20.5i
G. fasciculatum	1.9de	18.5d	20.4g	0.10	5.6e	2.8e	3.9cd	113.7g	22.6g
A. chroococcum + *Bacillus* sp.	2.5cd	22.5c	25.0f	0.11	11.7b	3.8bc	3.9c	241.5a	21.2h
A. chroococcum + *P. variabile*	1.8de	14.6f	16.4e	0.12	7.5d	1.9h	2.8e	115.6g	26.4d
A. chroococcum + *G. fasciculatum*	2.8	25.6bc	28.4e	0.11	11.8b	4.2b	4.2bc	180.6d	18.9j
Bacillus sp. + *P. variabile*	1.9de	17.8de	19.7g	0.11	7.9d	2.2ef	2.9e	115g	24.8e
Bacillus sp. + *G. fasciculatum*	3.8b	26.5b	30.3d	0.14	9.4c	4.2b	4.4b	203.7c	26.2d
A. chroococcum + *Bacillus* sp. + *G. fasciculatum*	4.6a	31.1a	36.1a	0.15	14.7a	5.4a	4.4b	251.2a	33.6a
A. chroococcum + *P. variabile* + G. *fasciculatum*	2.6b	28.9a	32.5c	0.12	9.7c	3.8cd	3.7d	228.8b	27.2c
A. chroococcum + *G. fasciculatum* + *Bacillus* sp. + *P. variabile*	4.0a	30.5a	34.5b	0.13	11.2b	3.2cd	3.1de	255.2a	29.2b
F value (df = 12)	52.3*	106.6*	1100.7*	-	183.8*	63.5	22.9*	236.9*	1202.8*

Values are mean of 6 replicates. Mean values followed by different letters in the same column are different from each other at $P \leq 0.05$ according to Tukey's test. * Significant at $P \leq 0.05$; R/S indicates root:shoot

with *P. variabile* (4.6 g plant^{-1}) while straw production with dual inoculation increased up to 57% (*Bacillus* with *G. fasciculatum*), as did the triple inoculation of *A. chroococcum*, *Baccillus*, and *G. fasciculatum,* compared with the control. The dry mass and seed yield were highly correlated (r = 0.83).

Single inoculation of *A. chroococcum* and dual inoculation of *A. chroococcum* with *Bacillus* significantly increased the grain protein (GP) by 61% and 91%, respectively, more than the control. The single inoculation treatment, in general, did not differ significantly in GP. The highest GP (255 mg g^{-1}) was observed with *A. chroococcum* inoculated with *G. fasciculatum, Baccillus*, and *P. variabile*, which was followed by the combination of *A. chroococcum*, *Bacillus*, and *G. fasciculatum* (251.2 mg g^{-1}). The N concentration in plants differed significantly at 135 DAS among the treatments. The single inoculation of *A. chroococcum* increased the N concentration by 35% compared with the control. Among the co-inoculation treatments, *A. chroococcum* with *P. variabile* showed the largest increase in N contents (26.4 mg plant^{-1}) above the control. The highest N content in wheat plants measured at 135 DAS was recorded in the treatment having *A. chroococcum*, *Bacillus,* and *G. fasciculatum* (33.6 mg plant^{-1}). The combined application of *A. chroococcum, Bacillus, G. fasciculatum,* and *P. variabile* demonstrated a better N accumulation by the wheat plants compared to other treatments but was less than those obtained for triple inoculation of *A. chroococcum*, *Baccillus,* and *G. fasciculatum*.

The P concentration in roots, shoots, and whole plants differed among the microbial treatments (Table 2). Among the single inoculation treatments, *P. variabile* significantly increased the P concentration of wheat plants by 94% above the control at 135 DAS. Inoculation of *Bacillus* sp. with *P. variabile* or *G. fasciculatum* significantly increased the P contents in roots, shoots, and whole plants relative to the control. *A. chroococcum* inoculated with either *G. fasciculatum* or *P. variabile* significantly increased the P concentration by 2.6- and 1.6-fold more than the control. The highest P contents were recorded as 2.48 mg g^{-1} and 67.8 mg $plant^{-1}$ for roots and whole plants, respectively, in triple inoculation of *A. chroococcum*, *Bacillus* sp., and *G. fasciculatum*, and was significantly greater than the combined application of *A. chroococcum, P. variabile,* and *G. fasciculatum* (48.7 mg $plant^{-1}$) or when 4 cultures were used together (44.5 mg $plant^{-1}$). The triple inoculation of *A. chroococcum*, *Bacillus* sp., and *G. fasciculatum* increased the P concentration of roots, shoots, and whole plants by 0.9-,

Table 2. Co-inoculation effects of rhizotrophic microorganisms on P concentration at 135 days after sowing and root infection, AM fungal spores, populations of PSMs at 80 DAS, and available P and N contents of soils at 135 DAS in field grown wheat.

	P content			Root infection (%)	No. of AM fungus spore (g^{-1} soil)	Population of *Azotobacter* of soil ($\times 10^4$ cells g^{-1})	Population of PSMs of soil ($\times 10^4$ cells g^{-1})		Available P of soil (mg kg^{-1})	Total N of soil (mg kg^{-1})
	Root (mg g^{-1})	Shoot (mg g^{-1})	Whole plant (mg $plant^{-1}$)				PSB	PSF		
Control	1.25	1.02	12.7	-	-	0.4	0.2	0.3	19.2	26
A. chroococcum	0.95i	1.01e	17.6h	-	-	45.5c	0.2g	0.8f	20.5ef	36a
Bacillus sp.	1.36f	1.02e	22.5g	-	-	0.3g	44.3c	22.6d	25.2ab	30bc
Penicillium variabile	1.27g	1.16c	24.7g	-	-	0.2g	-	44.7c	24.7b	29bc
G. fasciculatum	1.36f	1.10de	22.9g	40.5	108.2	22.6e	12.6f	8.5e	26.2ab	29bc
A. chroococcum + *Bacillus* sp.	1.47e	1.14de	21.5g	-	-	55.8b	54.8b	10.5e	27.5ab	32ab
A. chroococcum + *P. variabile*	1.02h	1.10e	33.6f	-	-	21.8e	-	-	25.5cd	32ab
A. chroococcum + *G. fasciculatum*	1.37f	1.15de	45.7c	48.5	120.4	58.5b	30.6d	46.3bc	27.2ab	34ab
Bacillus sp. + *P. variabile*	2.26b	1.75b	38.8c	-	-	0.8g	21.5e	48.5b	18.7g	21d
Bacillus sp. + *G. fasciculatum*	2.07d	1.89a	50.6b	66.6	138.6	12.4f	53.7b	-	25.8ab	32ab
A. chroococcum + *Bacillus* sp. + *G. fasciculatum*	2.48a	1.76a	67.8a	90.2	322.5	90.6a	77.7a	0.3f	28a	34b
A. chroococcum + *P. variabile* + *G. fasciculatum*	2.13c	1.56b	48.7bc	64.7	164.5	35.8d	-	45.2c	23.8cd	31bc
A. chroococcum + *G. fasciculatum* + *Bacillus* sp. + *P. variabile*	2.06d	1.57b	44.5d	52.2	122.8	45.8c	33.5d	56.7a	21.6e	32ab
F value (df = 12)	2285*	34.3*	610.4*	-	-	2080*	4589*	1895*	24.3*	12.6*

Values are mean of 6 replicates. Mean values followed by different letters in the same column are different from each other at $P \leq 0.05$ according to Tukey's test. * Significant at $P \leq 0.05$; PSB and PSF indicate phosphate solubilizing bacteria and fungus, respectively

0.7-, and 0.4-fold, respectively, relative to the control. Microscopic examination of stained roots showed that the roots of all treatments of wheat plants grown in plots receiving AM fungus alone or AM fungus when used in co-inoculation treatments became infected with *G. fasciculatum*. The AM fungus in combination maintained a high percentage of root infection and had a greater number of AM spores when compared with *G. fasciculatum* alone. Number of mycorrhizal spores and percentage root infections were significantly greater in the triple inoculation of *A. chroococcum*, *Bacillus,* and *G. fasciculatum* (322.5 spores g^{-1} soil) compared with the other treatments.

Roots of all treatments of wheat plants grown in field plots were colonized by *A. chroococcum.* The seeds inoculated with *A. chroococcum* either singly or in combination resulted in higher number of *A. chroococcum*, compared with the control. Addition of *P. variabile* to dual inoculation or triple inoculation treatments, in general, markedly reduced the population of *A. chroococcum* in the root zones compared with the single inoculation of *A. chroococcum* (45.5×10^4 cells g^{-1} soil). The population of *A. chroococcum* at 80 DAS increased by 99% due to inoculation with mixed cultures of *A. chroococcum*, *G. fasciculatum,* and *Bacillus* sp. compared with the single inoculation of *A. chroococcum* (Table 2). The number of phosphate solubilizing bacteria was significantly higher in the inoculation of *A. chroococcum* inoculated with *Bacillus* sp. (1.26×10^5 cells g^{-1} soil) and *Bacillus* sp. applied with *G. fasciculatum* (5.37×10^5 cells g^{-1} soil) compared to the single inoculation of *Bacillus* sp. (4.43×10^5 cells g^{-1} soil). The population of phosphate solubilizing bacteria increased even further in the presence of *G. fasciculatum*. Populations of both *A. chroococcum* and phosphate solubilizing bacteria were significantly higher when *A. chroococcum*, *G. fasciculatum,* and *Bacillus* sp. were inoculated together. A trend similar to those observed for phosphate solubilizing bacteria was observed for phosphate solubilizing fungus. The maximum number of phosphate solubilizing bacteria (77.7×10^4 cfu g^{-1} soil) was observed in the treatment having *A. chroococcum*, *Bacillus* sp., and *G. fasciculatum* followed by *A. chroococcum* inoculated with *Bacillus* sp. The populations of phosphate solubilizing fungus (56.7×10^4 cfu g^{-1} soil) were higher in combined treatments of *A. chroococcum*, *G. fasciculatum*, *Bacillus* sp., and *P. variabile.* The single inoculation of *Bacillus* and *P. variabile* increased the P contents of soil by 31% and 29%, respectively, above the control. A co-inoculation treatment of *A. chroococcum* with *Bacillus* sp. or with *G. fasciculatum* significantly ($P \leq 0.05$) increased the P content of soils by 43% and 42%, respectively, above the control. The highest P contents (28 mg kg^{-1}) in soil were recorded with triple inoculation of *A. chroococcum*, *Bacillus,* and *G. fasciculatum*, compared to the control. The total N contents in soil at harvest, however, did not change appreciably except for the single inoculation of *A. chroococcum*, dual inoculation of *A. chroococcum* with G. *fasciculatum,* and triple inoculation of *A. chroococcum*, *Bacillus* sp., and *G. fasciculatum*, which significantly increased the N contents of soil.

Discussion

The plant growth promoting rhizobacteria play an active role in soil through their natural ability to provide important but scarce nutrients to the plants. Among the plant nutrients, N and P are the 2 key plant nutrients provided by these organisms under natural field conditions. In this context, the inoculation effects of PGPR including N_2 fixers, PSMs, and AM fungus are receiving increased attention for their use to develop microbial inoculants in order to improve crop productivity. The synergistic effects of N_2 fixer and PSMs on plant vigor, nutrient uptake, and yields of various crops have been reported (Rai and Gaur, 1988). Further, AM fungus has been shown to possess the ability to increase nutrient uptake of plants by developing an association with roots (Schreiner et al., 1997). Generally, the inoculation effects of PGPR used in this study were more profound compared with those of the control plants, suggesting a synergism among the tested organisms, which together increased the wheat growth. Inoculation of wheat with PSMs and AM fungus considerably increased the P accumulation and augmented the growth under field conditions. These results could be attributed to the ability of PSMs to solubilize inorganic P of soils. Furthermore, during interaction, the phytostimulator, *A. chroococcum*, in addition to providing N, produces considerable amounts of plant growth promoting substances in the

rhizospheres (Kucey et al., 1989). Combining an improved plant nutrient supply with N (*Azotobacter*) and P (PSMs and AM fungus) with plant growth promotion appears to have additive and possibly even multiplicative effects (factor interaction not calculated in this study). In comparison, some of the treatments (*Bacillus* with *P. variabile* and *A. chroococcum* with *P. variabile*) marginally augmented the measured parameters. The variations in the effectiveness of microbial combinations in this study are probably due to the differences in the functionality of the tested microbial strains, variations in their survivability and colonization efficiency of the inoculated cultures in the soil, or strong competition from the natural microbiota of field soils, leading possibly to the exclusion of the inoculated cultures from the rhizospheres.

The highest increase in yield of grain and concentrations of N and P was recorded with *A. chroococcum* while among the composite cultures triple inoculation of *A. chroococcum, Bacillus,* and *G. fasciculatum* showed the largest positive effects on yield and nutrient uptake. Higher yield and uptake of nutrients were correlated with a higher number of bacteria in rhizosphere soils. Moreover, the combined inoculation effects were greater than the sum of the individual inoculation effects, suggesting synergism beyond simple additive effects (positive multiplicative interaction). In contrast, the application of *P. variabile,* either alone or in combination with other organisms, caused a negative impact or stimulated the plant growth very poorly. These results, therefore, suggested a negative relationship between the fungi and other associative partners. Phosphate solubilizing fungi, in general, produce more organic acids (Venkateswarlu et al., 1984), which increase the chemical availability of P. However, the PGPR, on the other hand, require neutral or alkaline growth conditions for their active metabolism; the increased acidity, therefore, might have changed the environment, leading to an adverse effect on the colonization efficiency of the associative partners in the rhizospheres (Downey and Kessel, 1990).

The colonization of PGPR on root systems of plants or in soil via seed bacterization has long been of major interest to agronomists. This is primarily due to the secretion of nutrients by the organisms in the soil environment (or volume of soil), often termed rhizosphere, and release of signal compounds (root exudates) by the plants in the rhizosphere. These compounds in turn play an active role in bacterial composition of the rhizosphere and consequently affect the colonization. In the present study, phosphate solubilizing bacteria stimulated the population of *A. chroococcum* in the root zone of wheat but the effect of *A. chroococcum* on the number of phosphate solubilizing bacteria was comparatively less marked. However, the increase in the population of the 2 cultures when used together suggested that the introduced bacteria could survive, proliferate, and colonize the root zones. These bacteria were also recovered from other treatments, but their number was quite low compared to inoculated treatments. The populations of both associative N_2 fixer and phosphate solubilizing bacteria were further increased when AM fungus was added to the co-inoculation of *A. chroococcum* and phosphate solubilizing bacteria. The results indicated a possible synergism between the 3 groups of organisms used in this study.

The AM fungus, N_2 fixer, and phosphate solubilizing bacterium used in this study were found to be good competitors since growth of wheat plants was multiplied several fold after inoculation. Results from field experiments further revealed that root colonization and P contents in plants were greatest in plots receiving N_2 fixer, phosphate solubilizing bacteria, and AM fungus, together. These results strongly suggested that a relationship existed between root colonization, P uptake, and growth promotion. In the present study, inoculation of *Bacillus* sp. with or without *G. fasiculatum* increased the content of available P in the soil. However, with some treatments, the P contents in soil were comparatively poor, possibly due to the aggressive uptake of P by the plants or due to chemical fixation of P, as also reported by others (Islam et al., 1980; Kundu and Gaur, 1980).

In conclusion, this study revealed that the mixed inoculation of plant growth promoting rhizobacteria, especially the N_2 fixer, phosphate solubilizer, and AM fungus, improved the plant vitality, grain quality, and nutrient uptake and showed a dramatic increase in grain yield of wheat under filed conditions. Since wheat crop requires a greater amount of important but scarce plant nutrients, such as nitrogen and phosphorus, inoculation with favorably interacting PGPR strains can provide an alternative to chemical fertilizers.

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