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FULL-LENGTH RESEARCH ARTICLE

Impact of Seed Applied Rhizobacterial Inoculants on Growth of Wheat (*Triticum aestivum*) and Cowpea [*Vigna unguiculata*] and their Influence on Rhizospheric Microbial Diversity

Anand Kumar[1] · Dinesh Chandra[1,2] · Pallavi[1,3] · Anil Kumar Sharma[1]

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Abstract Current study was planned to demonstrate (Experiment I), the impact of seed bacterization (inoculation dose—10^6, 10^8, 10^{10}, 10^{12} CFU/ml) with two bacterial isolates *Variovorax paradoxus* RAA3 and M11 (Unidentified) on growth and foliar nutrient content (NPK) of wheat (var. H1105 and PBW660) and cowpea (var. PL-1 and PL-2) cultivars under glass house conditions. Strain RAA3 treated plants exhibited most promising results for shoot fresh weight (26.7%, 30.2%), shoot dry weight (44.4%, 63.3%) and chlorophyll content (66.8%, 66.9%) as compared to the control plants of respective varieties. This inoculant also caused significant changes in the foliar nitrogen (14.5%, 14.2%), phosphorus (36.4%, 46.7%) and potassium (20.1%, 65.9%) content in wheat and cowpea, respectively, as compared to the non-inoculated plants. Moreover, the inoculum dose of 10^8 of bacterial inoculum was found to be most effective and thus, considered as an optimum dose for the plant growth promotion. In another study (Experiment II), seed bacterization with RAA3 (10^8 CFU/ml) on nine different varieties of wheat was performed, and significant varietal and treatment effect were observed for many of growth parameters as compared to untreated control plants. Overall results showed maximum response at inoculum dose of 10^8, therefore this dose was taken to assess the influence of PGPR inoculation on rhizospheric microbial diversity of wheat and cowpea. We observed that RAA3 inoculation has led to a shift in microbial population in both wheat and cowpea varieties. Irrespective of varieties, RAA3 (inoculum dose of 10^8 CFU/ml) treated plants of wheat showed dominant microbial groups of siderophores producers, nitrogen fixers and actinomycetes, whereas, in RAA3 treated plants of cowpea the dominant microbial population of only siderophores producers was recorded.

Keywords PGPR · *Variovorax paradoxus* · Seed bacterization · Siderophores · Nitrogen fixers · Actinomycetes · Inoculum dose · Nutrient content

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✉ Anand Kumar
anand9167@gmail.com

Dinesh Chandra
dchandra.009@gmail.com

Pallavi
pallavi.mp08@gmail.com

Anil Kumar Sharma
anilksharma_99@yahoo.com

[1] Department of Biological Sciences, Govind Ballabh Pant University of Agriculture and Technology, U.S. Nagar, Pantnagar-263 145, CBS&H Pantnagar, Uttarakhand, India

[2] GIC Chamtola, Almora, Uttarakhand 263 622, India

[3] J.N.L. College Khagaul Patna, Bihar 801105, India

Introduction

A steady increase in the world population provides new challenges to assure food security. Availability of arable land, poorly managed production factors like water resources and long-term effects are brought up by climatic changes, could all make to possible disastrous consequences [1, 10, 15]. Moreover, the soil is the main prerequisite for crop production. The perpetual application of chemical fertilizers, insecticides, fungicides and herbicides disturb natural soil ecosystem; deteriorate soil condition making it deprive of essential nutrients. Thus, need of the

hour to generate alternative strategies that can promise better crop yields and also provides environmental safety. For this reason, research has to be centered on the novel concept of rhizo-engineering that permits improvement of plant and soil health based on the favorable partitioning of the exotic biomolecules, which makes the unique interaction between plants and microbes [4, 29]. In the recent years, the growing interest in the development of sustainable agriculture has led producers to reduce the use of chemical fertilizers by increasing crop inoculation with plant beneficial microorganisms [11, 12, 27].

Soil hosts an overwhelming diversity of microbes, and these microbes are known to involve in multifaceted interaction with each other, and a small subset is known as root microbiota that is capable of colonizing the plants rhizosphere [23]. In natural environment, plant associate with plentiful microbes that play a significant role in nourishing their growth and health [16, 46]. Plant-microbes interactions can be beneficial, detrimental or neutral [28, 47]. Rhizosphere has a bacterial population with beneficial activities for the plant. These bacteria are commonly defined as plant growth promoting rhizobacteria (PGPR) that stimulates the growth of host plant. PGPR improves plant growth and development. Growth enhancement could be provided by phytohormone production [22, 52], soluble phosphate [2, 14], fixing nitrogen [25, 38], iron chelators [26, 35], 1-aminocyclopropane-1-carboxylate (ACC) deaminase [12, 43]. Bacteria with ACC deaminase activity helps plant to overcome the effect of stress generated by ethylene [29, 30].

The plenty of microbes and their functions are needed during formation of soil, maintenance of fertility by complex cycle and interaction. The microorganisms are responsible for cycling of nutrients like N, P, K and S, for plants availability [34]. The application dose of the inoculum is one of the important elements which govern the survivability; establishment of introduced bacteria, in turn, performs a function to promote plant growth. The inoculation dose of other PGPB has been investigated, and their effects have been demonstrated [6]. To the best of our knowledge, this is the first report on impact of bacterial inoculation doses (10^6, 10^8, 10^{10} and 10^{12}) on wheat and cowpea growth response. Therefore, keeping in view that PGPRs enhance plant growth, nutrient content the present work was carried with the following objectives: (i) to see the varietal response of wheat (*Triticum aestivum* L.) and cowpea (*Vigna unguiculata* (L.) Walp.) on inoculation with PGPR, (ii) standardization of PGPR inoculum dose for optimum growth response and (iii) evaluation of the effect of PGPR inoculation on native soil microbial diversity.

Materials and Methods

Bacterial Culture and Cultivation

Strain *Variovorax paradoxus* RAA3 previously described by Chandra et al. [12], and a new bacterial isolate M11 (unidentified) was used for inoculation experiments. Both these bacterial isolates isolated from the dryland agriculture soils of Kumaun Himalaya, Uttarakhand, India. Bacterial isolates were maintained in the nutrient agar (NA) medium throughout the investigation.

Functional Properties of Plant Growth Promoting Bacteria (PGPB)

Phosphorus solubilizing activity was determined according to qualitative method of Pikovskaya [45]. The IAA production was tested as per Gordon and Weber [32], siderophore as per Schwyn and Neilands [49], ACC deaminase activity as per Penrose and Glick [44] and ammonia production using method outlined by Dey et al. [20].

Glasshouse Experiment

Experiment I: Impact of bacterial inoculum doses (CFU/seed) on growth promotion and nutrient content (NPK) of wheat (WH1105, PBW660) and cowpea (PL-1 and PL-2).

Experiment II: Effect of potent bacterial strain *Variovorax paradoxus* RAA3 on growth promotion and nutrient content of wheat (HD2967, PBW343, PDW233, UP262, UP2338, UP2526, UP2565, UP2844 and UP2855) varieties under glasshouse conditions.

Plant Materials and Seed Sterilization

In experiment I, two varieties each of wheat (WH1105, PBW660) and cowpea (PL-1 and PL-2) and in experiment II, nine different varieties of wheat (HD2967, PBW343, PDW233, UP262, UP2338, UP2526, UP2565, UP2844 and UP2855) were taken to see the response of bacterial inoculants on growth promotion. These varieties of wheat and cowpea are procured from the two research station Crop Research Center, Pantnagar and Breeder Seed production center, Pantnagar, respectively. For both experiments, seeds were surface treated using 3% sodium hypochlorite for 3 min followed by washing with autoclaved distilled water 3–4 times and then 70% ethanol for 1 min followed by rinsing again with autoclaved distilled water 5–6 times to remove the residual alcohol. The seeds were then germinated in sterilized Petri dishes containing one sheet of moistened sterilized paper and placed in an incubator at 30 °C for 2 days.

Soil Physicochemical Properties

The soil was collected in the month of October, 2016 from the upper 0–15 cm soil layer from agriculture field of Pantnagar. Unsterilized soil with the sand (1:3) ratio was used in the experiment. The potting mixture was analyzed for various physiochemical properties- pH (6.98), OC- (0.74%), N- (0.19%), P- (10.97 mg/kg) and K (138.88 mg/kg). The soil was filled into the pots of 500 g capacity. The methodology of Walkley and Black [56] and Olsen [42] were adopted for estimation of organic carbon and available soil phosphorus, respectively. The nitrogen content was estimated by Kjeldahl digestion and K content by flame photometry.

Preparation of Bacterial Inoculum

Each bacterial isolate was grown in 150 ml Erlenmeyer flask containing 50 ml of nutrient broth (pH 7.2), incubated in rotary shaker (120 rpm) at 28 °C. After certain periods of incubation, cells were centrifuged at 8000 rpm for 10 min at 4 °C, and pellets were washed with sterile saline solution (0.85%) and resuspended to obtain a population density of 10^6, 10^8, 10^{10} and 10^{12} CFU/ml. Each suspension was mixed with 1 g of charcoal and coated onto the wheat and cowpea seeds. The seeds were allowed to air dry overnight under aseptic conditions. Seeds coated with charcoal suspension (without inoculants) served as the control. Subsamples of inoculated seeds were analyzed for the abundances of applied bacteria (colony forming units, CFU) using dilution plate technique and nutrient agar plates. The populations of each bacterial isolate per seed are shown in Table 1 (Experiment I). For the Experiment II, inoculum dose 10^8 of *V. paradoxus* was assessed to see the response of wheat varieties.

Experimental Details

The experiment was carried out in a glasshouse under following growth conditions: Temperature 28 ± 2 °C, photo period: 16/8 day/night cycle, light intensity: 400 Em $^{-2}$ s^{-1}(400-700 nm), relative humidity: 60%. The experiment was performed in a completely randomized design with 4 replicates, 3 treatments- Control, RAA3 and M11 (in experiment I, three way ANOVA- 3 × 4 × 2 (3 inoculations (2 strains + uninoculated control; 4 inoculum doses and 2 cultivars)), and 2 treatments- Control and RAA3 (in experiment II, two way ANOVA). The moisture level of the soil and sand was maintained at 90% of water holding capacity throughout the experiment. Eight bacterized seeds of each variety for each treatment were sown (4.11.2016) in a single pot. After two weeks, only four seedlings of same vigor were allowed to grow in each pot.

Plant Sample Collection and Analysis

Plant samples were harvested after 45 days of plant growth. Wheat roots, in case of experiment I, were left in pot itself for further experimental use. Roots of cowpea and nine wheat varieties (experiment II) were rinsed delicately with tap water to remove soil particles. After air drying, root/shoot fresh weight, length were recorded. Then, the plant samples (root and shoot) were kept in paper bags, dried at 37 °C in an electric oven till the dry weight became constant to determine the dry matter. For both the experiments, similar growth parameters were recorded.

Foliar NPK Analysis of Wheat and Cowpea

The oven-dried leaves of were taken for NPK analysis. P content of leaves was estimated as per the method described by Jackson [33]. The N content was determined by Kjeldahl digestion, and K content was estimated by Flame photometry.

Statistical Analysis

The collected data were analyzed statistically using SPSS (IBM SPSS statistics 20). Following the analysis of variance (ANOVA), differences among treatment means (wherever applicable) were determined using the Duncan's New Multiple Range Test (DMRT) comparison method at 5% level of significance.

Results

Functional Properties of PGPB

The isolate RAA3 and M11 both exhibited different plant growth promoting traits as described in Table 2 such as IAA production, phosphate solubilization, siderophores, ACC deaminase and ammonia production. These traits also differed in terms of their extent as well, for example RAA3 was found to be strong ACC deaminase producer while M11 was weak.

Glasshouse experiment I: Impact of bacterial inoculum doses (CFU/seed) on growth promotion and nutrient content (NPK) of wheat and cowpea.

Plant Growth Characteristics

The results of three factor analysis of variance showed that irrespective of treatments, both the wheat varieties showed significant differences only for shoot dry weight. Irrespective of varieties, both the treatments RAA3 and M11 significantly increased shoot length by 8.3% and 6.6%,

Table 1 Bacterial inoculum doses on per seed of wheat and cow pea

Bacterial isolates					
Wheat					
RAA3			M11		
Optical Density (600 nm)	Dilution taken	CFU/seed	Optical Density (600 nm)	Dilution taken	CFU/seed
0.723	10^6	4.28×10^7	0.513	10^6	5.44×10^7
0.931	10^8	2.74×10^8	0.723	10^8	5.55×10^7
1.118	10^{10}	3.38×10^8	0.873	10^{10}	6.34×10^7
1.225	10^{12}	4.48×10^8	1.005	10^{12}	2.76×10^8
Cowpea					
RAA3			M11		
Optical density (600 nm)	Dilution taken	CFU/seed	Optical density (600 nm)	Dilution taken	CFU/seed
0.723	10^6	5.36×10^7	0.513	10^6	6.12×10^7
0.931	10^8	4.32×10^8	0.723	10^8	4.97×10^8
1.118	10^{10}	5.51×10^8	0.873	10^{10}	5.44×10^8
1.225	10^{12}	6.53×10^8	1.005	10^{12}	6.63×10^8

Table 2 Functional properties of selected plant growth promoting bacteria

Bacterial isolate	RAA3	M11
Phosphate solubilization	–	+
Siderophore production	+ +	–
Ammonia production	–	+
ACC deaminase	+ + +	+
N—fixer	+	+
IAA production	–	–

The presence of activity is indicated by " + " absence by "–"

shoot fresh weight by 26.7% and 20.0% and shoot dry weight by 44.4% and 33.3% as compared to non-inoculated control. The inoculum dose (CFU/seed) of 10^8 exhibited maximum increases in these studied parameters as compared to other doses of bacterial inoculum (Table 3). When we compared both the varieties, RAA3 and its inoculum dose of 10^8 treated plants of PBW660 showed higher shoot length and shoot dry weight than the WH1105, while maximum shoot fresh weight was observed in WH1105.

Similarly, in cowpea, irrespective of treatments, both varieties did not exhibit significant differences in the shoot length, however, show significant differences in shoot fresh and dry weight. PL-2 showed higher shoot length, shoot fresh/dry biomass than PL-1. Irrespective of varieties, both treatments RAA3 and M11 significantly increased shoot length by 12.1% and 9.9%, shoot fresh weight by 30.2% and 24.0%, and shoot dry weight by 63.3% and 43.3%, respectively, as compared to non-inoculated control. Inoculum dose of 10^8 exhibited maximum increase in shoot length, shoot fresh/dry weight followed by 10^{10}, 10^{12} and 10^6 (Table 3). When we compared the effect of both treatments and their inoculum doses within varieties, RAA3 and its inoculum dose of 10^8 treated plants of PL-2 was more responsive with higher shoot length, shoot fresh/dry weight than PL-1.

Moreover, when comparing both the varieties, irrespective of treatments, PL-2 showed significant differences for the root length, root fresh/dry biomass than PL-1. Irrespective of varieties, both the treatments, RAA3 and M11 significantly increased root length by 27.1% and 22.8%, shoot fresh weight by 41.7% and 25.0% and root dry weight by 128.6% and 57.1%, respectively, as compared to non-inoculated control. Inoculum dose of 10^8 was able to incite maximum response in terms of increased root length, root fresh/dry biomass as compared to other doses of inoculum (Table 3). When we compared varieties, RAA3 and its inoculum dose of 10^8 treated plants of PL-2 exhibited higher root length and root fresh biomass than PL-1, whereas variety PL-1 showed higher root dry weight than PL-2. The interaction effect of varieties x treatments, varieties x inoculum doses and treatment x inoculum doses showed significant differences for the root length (Table S1).

Table 3 Effect of bacterial inoculants on wheat and cowpea growth parameters. (A) Varietal effect, irrespective of PGPR (B) PGPR effect, irrespective of varieties and (C) Inoculum doses effect, irrespective of varieties and PGPR. Values with different letters (a-c) are significantly different at $p < 0.05$

Varieties (A)	Wheat			Cowpea					
	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
WH1105/PL-1	27.82 [a]	0.35 [a]	0.11 [a]	20.47 [a]	1.43 [a]	0.38 [a]	12.12 [a]	0.26 [a]	0.12 [b]
PBW660/PL-2	28.09 [a]	0.35 [a]	0.12 [b]	20.70 [a]	1.62 [b]	0.43 [b]	14.10 [b]	0.33 [b]	0.10 [a]
Mean	27.96	0.35	0.11	20.59	1.52	0.41	13.11	0.29	0.11
LSD ($P \leq 0.05$)	0.63	0.01	0.01	0.28	0.04	0.02	0.25	0.03	0.01
SEm ±	0.22	0.00	0.00	0.10	0.02	0.01	0.09	0.01	0.00
Treatments (B)									
Control	26.63 [a]	0.30 [a]	0.09 [a]	19.18 [a]	1.29 [a]	0.30 [a]	11.24 [a]	0.24 [a]	0.07 [a]
RAA3	28.85 [b]	0.38 [b]	0.13 [b]	21.51 [c]	1.68 [c]	0.49 [c]	14.29 [c]	0.34 [c]	0.16 [c]
M11	28.39 [b]	0.36 [b]	0.12 [b]	21.07 [b]	1.60 [b]	0.43 [b]	13.80 [b]	0.30 [bc]	0.11 [b]
Mean	27.96	0.35	0.11	20.59	1.52	0.41	13.11	0.29	0.11
LSD ($P \leq 0.05$)	0.77	0.01	0.01	0.34	0.05	0.02	0.30	0.03	0.01
SEm ±	0.27	0.00	0.01	0.12	0.02	0.01	0.11	0.01	0.00
Inoculum doses (C)									
10^6	27.35 [a]	0.32 [a]	0.11 [a]	20.04 [a]	1.44 [a]	0.36 [a]	12.03 [a]	0.27 [a]	0.09 [a]
10^8	28.63 [b]	0.38 [c]	0.13 [b]	21.54 [c]	1.65 [c]	0.46 [c]	13.85 [c]	0.33 [b]	0.14 [b]
10^{10}	28.40 [b]	0.35 [b]	0.11 [a]	20.55 [b]	1.52 [b]	0.42 [b]	13.56 [c]	0.30 [ab]	0.12 [b]
10^{12}	27.45 [a]	0.33 [a]	0.11 [a]	20.21 [ab]	1.47 [ab]	0.38 [a]	13.00 [b]	0.27 [a]	0.10 [ab]
Mean	27.96	0.35	0.11	20.59	1.52	0.41	13.11	0.29	0.11
LSD ($P \leq 0.05$)	0.89	0.01	0.02	0.39	0.06	0.03	0.35	0.04	0.02
SEm ±	0.31	0.01	0.01	0.14	0.02	0.01	0.12	0.01	0.00

Chlorophyll Content

The result of analysis of variance showed that irrespective of treatment, variety PBW660 significantly showed higher total chlorophyll content as compared to WH1105. Irrespective of varieties, treatments RAA3 and M11 showed significantly higher total chlorophyll by 66.8% and 58.4%, respectively, as compared to non-inoculated control. The inoculum dose of 10^8 showed significantly higher total chlorophyll content as compared to other doses (Table 4). When we compared both the treatments within varieties, RAA3 and its inoculum dose of 10^8 treated plants of PBW660 showed higher total chlorophyll content than WH1105. The effect of interaction between treatments x inoculum doses showed significant differences for the total chlorophyll, (Table S2).

The statistical analysis reflected that irrespective of treatments, variety PL-2 significantly showed higher total chlorophyll content as compared to PL-1. Irrespective of varieties, treatments RAA3 significantly increased total chlorophyll by 66.9% as compared to non-inoculated control. Irrespective of varieties and treatments, inoculum dose of 10^8 showed higher total chlorophyll content significantly as compared to other doses (Table 4). When we compared both treatments within varieties, RAA3 and its inoculum dose of 10^8 treated plants of PL-2 showed higher total chlorophyll content than PL-1. The effect of interaction between treatments x inoculum doses showed significant differences for total chlorophyll content (Table S2).

Foliar Nutrient (NPK) Content

Irrespective of treatments, variety WH1105 showed significantly higher foliar NPK content than PBW660. However, the maximum NPK in the response to bacterial application was observed with the treatment RAA3 (14.5%, 36.4%, 20.1%) followed by M11 (4.8%, 21.2%, 17.6%) as compared to control where no inoculants were applied. Also observed that inoculum dose of 10^8 showed significantly higher NPK content compared to other doses

Table 4 Effect of bacterial inoculants on total Chl and NPK content of wheat and cowpea. (A) Varietal effect, irrespective of PGPR (B) PGPR effect, irrespective of varieties and (C) Inoculum doses effect, irrespective of varieties and PGPR. Values with different letters (a-d) are significantly different at $p < 0.05$

Varieties (A)	Wheat				Cowpea			
	Chl (mg/g)	N (%)	P (%)	K (%)	Chl (mg/g)	N (%)	P (%)	K (%)
WH1105/PL-1	2.72 [a]	2.28 [b]	0.42 [b]	1.80 [a]	2.19 [a]	2.78 [b]	0.64 [a]	1.10 [a]
PBW660/PL-2	3.01 [b]	2.13 [a]	0.36 [a]	1.78 [a]	2.43 [b]	2.60 [a]	0.85 [b]	1.22 [b]
Mean	2.86	2.21	0.39	1.79	2.31	2.69	0.75	1.16
LSD ($P \leq 0.05$)	0.09	0.02	0.04	0.08	0.07	0.05	0.03	0.04
SEm ±	0.03	0.02	0.01	0.03	0.03	0.02	0.01	0.02
Treatments (B)								
Control	2.02 [a]	2.07 [a]	0.33 [a]	1.59 [a]	1.63 [ab]	2.53 [a]	0.60 [a]	0.82 [a]
RAA3	3.37 [c]	2.37 [c]	0.45 [c]	1.91 [b]	2.72 [c]	2.89 [c]	0.88 [c]	1.36 [c]
M11	3.20 [b]	2.17 [b]	0.40 [b]	1.87 [b]	2.59 [b]	2.65 [b]	0.76 [b]	1.29 [b]
Mean	2.86	2.21	0.39	1.79	2.31	2.69	0.75	1.16
LSD ($P \leq 0.05$)	0.11	0.02	0.05	0.10	0.09	0.06	0.04	0.04
SEm ±	0.04	0.02	0.01	0.03	0.03	0.02	0.01	0.02
Inoculum doses (C)								
10^6	2.29 [a]	2.12 [a]	0.37 [a]	1.69 [a]	1.85 [a]	2.59 [a]	0.66 [a]	0.93 [a]
10^8	3.46 [d]	2.34 [c]	0.44 [b]	1.90 [b]	2.79 [d]	2.85 [c]	0.87 [c]	1.40 [d]
10^{10}	3.00 [c]	2.21 [b]	0.39 [a]	1.81 [b]	2.42 [c]	2.70 [b]	0.75 [b]	1.21 [c]
10^{12}	2.71 [b]	2.15 [ab]	0.37 [a]	1.76 [ab]	2.19 [b]	2.62 [ab]	0.70 [a]	1.09 [b]
Mean	2.86	2.21	0.39	1.79	2.31	2.69	0.75	1.16
LSD ($P \leq 0.05$)	0.13	0.03	0.09	0.11	0.10	0.07	0.04	0.05
SEm ±	0.04	0.02	0.01	0.04	0.04	0.03	0.02	0.02

[#]Chl- Chlorophyll, N- Nitrogen, P- Phosphorus, K- Potassium

(Table 4). When we compared both the varieties, RAA3 and its inoculum dose of 10^8 treated plants of WH1105 showed higher NPK content than PBW660. Interaction effect of treatments x inoculum doses showed significant differences in nitrogen and phosphorus content (Table S3).

Irrespective of treatment, variety PL-1 significantly showed higher NPK content than PL-2. Irrespective of varieties, treatments RAA3 and M11 showed significantly higher nitrogen content by 14.2%, 46.7%, 65.9% and 4.7%, 26.7%, 57.3%, respectively, as compared to non-inoculated control. Irrespective of treatment, inoculum dose 10^8 showed significantly higher NPK content as compared to other doses (Table 4). When we compared varieties, RAA3 and its inoculum dose of 10^8 treated plants of PL-1 exhibited higher N than the PL-2, whereas variety PL-2 displayed higher PK than PL-1. The interaction effect of treatments x inoculum doses only showed significant differences for nitrogen and potassium content (Table S4). In case of phosphorus, interaction effect of varieties x inoculum doses, treatments x inoculum doses and varieties x treatments x inoculum doses showed significant differences (Table S5).

Glasshouse experiment II: Effect of potent bacterial strain *V. paradoxus* RAA3 on growth promotion and nutrient content of wheat varieties under glasshouse conditions.

Growth Characteristics of Wheat

On the basis of results obtained in Experiment I, we found that strain *V. paradoxus* RAA3 showed promising response at the inoculum dose of 10^8, therefore, another experiment was carried with 9 different varieties of wheat (HD2967, PBW343, PDW233, UP262, UP2338, UP2526, UP2565, UP2844 and UP285) to observe the varietal response toward seed bacterization with RAA3.

Strain *V. paradoxu*s RAA3 treated plants showed higher shoot/root length, shoot and root fresh/dry weight when comparing to non-inoculated plants. Among the varieties, irrespective of treatment, HD2967 showed maximum shoot/root length, shoot fresh biomass, root fresh/dry weight, however, variety UP2855 showed higher shoot dry weight, as compared to other varieties. Irrespective of varieties, RAA3 treated plant significantly increased shoot

length by 14.6%, shoot fresh weight by 16.3% and shoot dry weight by 19.4% (Fig. 1), root length by 18.5%, root fresh weight by 34.8%, root dry weight by 34.6% (Fig. 2) as compared to control. Within varieties, the RAA treated plants of HD2967 showed higher shoot/root length, shoot/root fresh weight, root dry weight, whereas, UP2855 showed higher shoot dry weight as compared to other varieties.

Chlorophyll Content

Irrespective of treatment, within varieties no significant differences were observed for total chlorophyll content. Irrespective of varieties, treatment RAA3 treated plant significantly increased total chlorophyll content by 22.8% as compared to non-inoculated control (Fig. 3). Among varieties, the RAA3 treated plants of which UP2855 showed higher total chlorophyll content as compared to other varieties.

Foliar nutrient (NPK) Content of Wheat

The variety PDW233, UP2855 and PBW343 exhibited significantly higher accumulation of nitrogen, phosphorus and potassium content, respectively, as compared to other varieties. Irrespective of varieties, treatment RAA3 significantly increased N by 19.7%, P by 43.8% and K content by 28.6% as compared to non-inoculated control (Fig. 4). Among varieties, the RAA3 treated plants of PBW233 exhibited higher N, UP2338 exhibited higher phosphorus and PBW343 exhibited higher potassium as compared to other varieties. The interaction effect of varieties and treatment showed a significant difference for the NPK content (Table S6).

Functional Diversity of Culturable Microorganisms in Potting Mixture

The rhizosphere populating microorganisms were enumerated for their functionality by dilution plating on specific medium. The microbial population is enumerated in soil as a parameter to study soil health status, variable results were observed among the population of different microbial groups between uninoculated and treated pots of wheat and cowpea experiment.

Functional Diversity of Wheat and Cowpea Soil Samples

Irrespective of treatments, wheat variety WH1105 showed enhanced population of siderophore compared to PBW660. However, no significant difference in nitrogen fixer and phosphate solubilizers population was observed among the varieties. Irrespective of varieties, treatment RAA3 resulted in increased number of siderophore producers and nitrogen fixers as compared to control. Irrespective of treatments, variety PBW660 showed higher fungal and actinomycetes population compared to WH1105. Irrespective of varieties, treatment RAA3 resulted in less number of fungal population and higher population of actinomycetes as compared to control (Table 5). Similarly, irrespective of treatment, PL-2 had significantly higher population of siderophores and phosphate solubilizers, whereas PL-1 exhibited higher population of nitrogen fixers. Irrespective of varieties, treatment RAA3 significantly increased siderophores and showed non-significant differences for P-solubilization as compared to control (Table 5). The varietal and treatments effect showed non-significant differences for fungal and actinomycetes population count.

Discussion

The use of PGPR as biofertilizer is being considered as an alternative or a supplemental way to reduce usage of chemical fertilizer in agriculture. PGPR are reported to promote plant growth through different mechanisms including direct and indirect or a combination of both which can be correlated with their ability to provide plants with phytohormones, fixed nitrogen, a soluble phosphate, iron through production of bacterial siderophore, or ACC deaminase [11, 39, 41]. In this study, an increase in plant growth of wheat and cowpea by seed bacterization with different inoculum doses has been demonstrated. Both the inoculants significantly increased shoot/root length (root parameter was not observed in wheat), shoot and root fresh/dry weight, chlorophyll content and also enhanced the NPK contents of inoculated wheat (WH1105 and PBW660) and cowpea (PL-1 and PL-2) seedlings as compared to their respective untreated control. The plant growth promotion could be the result of beneficial functions of applied PGPR isolates, like IAA, nitrogen fixation, ACC deaminase and P-solubilization. As inoculated plants were not supplied with any additional source of NPK, a higher amount of NPK detected in leaves of inoculated plants as well as growth promotion may be attributed to bacterial-assisted growth enhancement phenomenon. Moreover, also noticed that inoculum dose of 10^8 showed the better response to plant growth in both wheat and cowpea cultivars in comparison to other doses (10^6, 10^{10} and 10^{12}) of bacterial inoculum and considered as an optimum dose for the plant growth promotion. Majeed et al. [37] reported that *Stenotrophomonas rhizophila* AJK-3 and *Acetobacter pasteurianus* AJK-7 treated plants significantly increased shoot and root length, shoot and root biomass of wheat. Numerous studies reported that an increase in chlorophyll content was

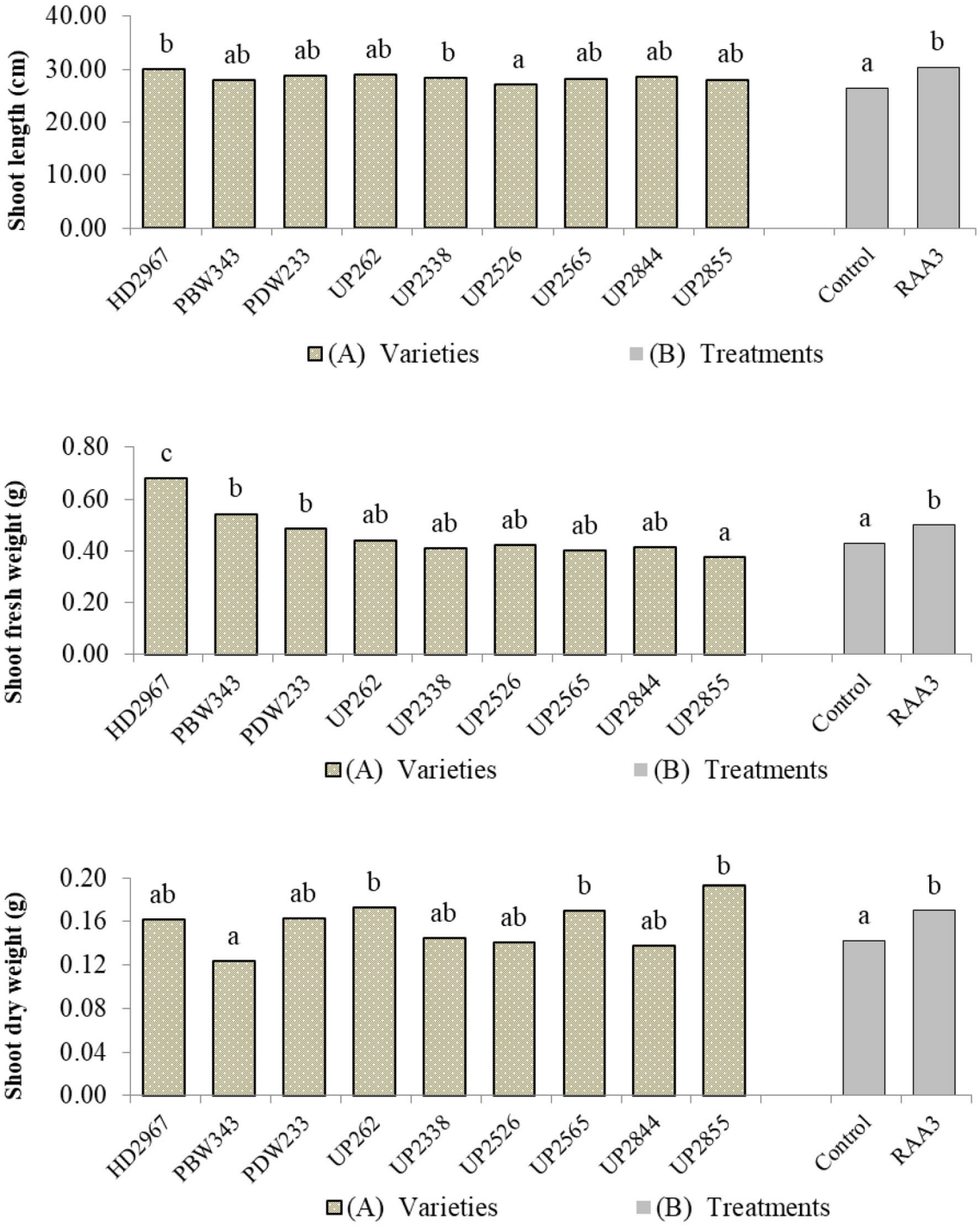

Fig. 1 Effect of bacterial inoculation on shoot length, shoot fresh weight and shoot dry weight of wheat under glasshouse conditions. (**A**) Varietal effect, irrespective of PGPR (**B**) PGPR effect, irrespective of varieties Values with different letters (**a–c**) are significantly different at $p < 0.05$

found in plants treated with PGPR either alone or in combination as compared to untreated control plants [48, 51].

Several studies also demonstrated that inoculation of PGPR significantly increased growth parameters and nutrient uptake of plants [13, 19]. In the present study, both bacterial strain exhibited growth in DF (Dworkin and Foster) medium with ACC as a sole source of nitrogen and positively influences root growth and development, thereby enhancing foliar nutrient content in wheat and cowpea

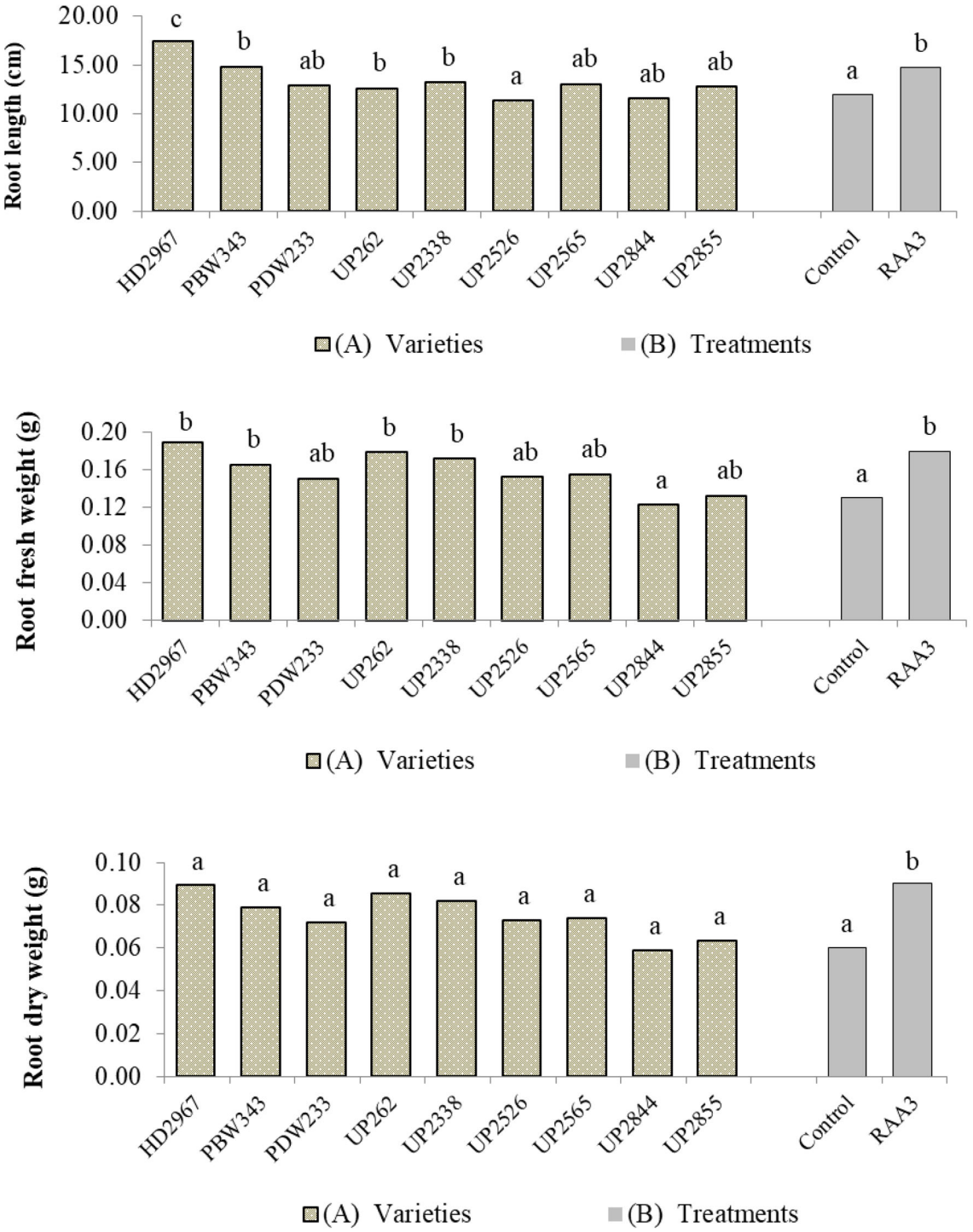

Fig. 2 Effect of bacterial inoculation on root length, fresh root weight and root dry weight of wheat under glasshouse conditions. (**A**) varietal effect, irrespective of PGPR (**B**) PGPR effect, irrespective of varieties Values with different letters (**a–c**) are significantly different at $p < 0.05$

compared with their respective uninoculated control under glasshouse conditions. In this regard, our results are strongly supported by results of Dastager et al. [17] who observed that cowpea seedlings bacterized with *Pontibacter niistensis* NII-0905 yielded significantly higher root and shoot lengths compared to untreated control.

In another experiment, influence of seed bacterization with *V. paradoxus* strain RAA3 on nine different varieties

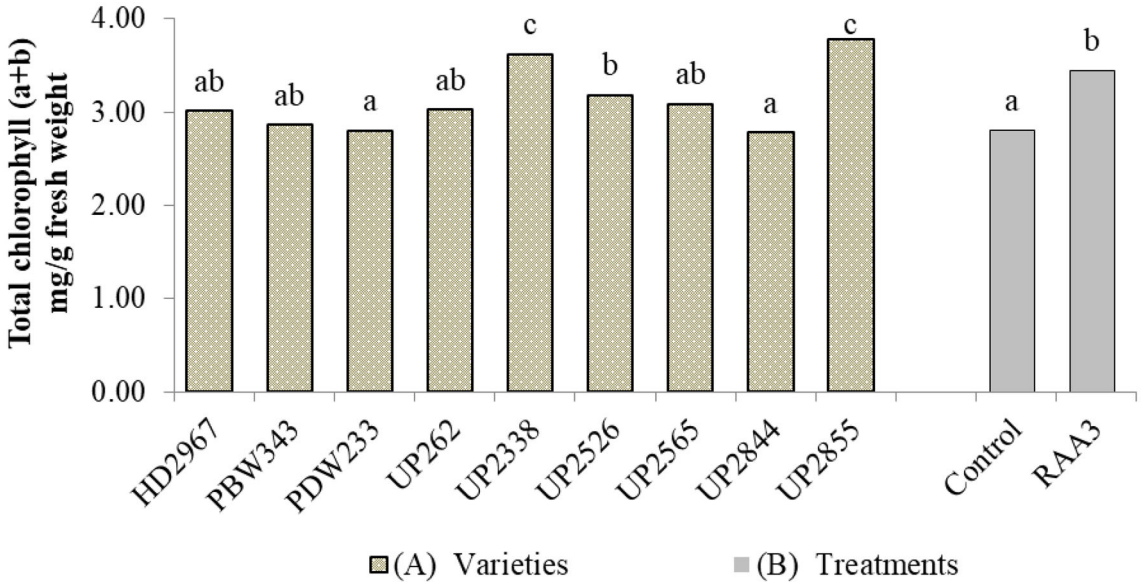

Fig. 3 Effect of bacterial inoculation on total chlorophyll content of wheat under glasshouse conditions. (**A**) Varietal effect, irrespective of PGPR (**B**) PGPR effect, irrespective of varieties Values with different letters (**a–c**) are significantly different at $p < 0.05$

of wheat have been demonstrated found that priming effect significantly increased shoot/root length, shoot/root fresh and dry weight, chlorophyll content, also enhanced NPK content. This premise is supported by the observation that seed bacterization significantly promoted the growth parameters of plants [9, 21]. Plant growth is very sensitive to the concentration of nutrients in the soil [7]. PGPR is more effective in plant growth promotion under a limited supply of nutrient [50]. Deficiency of nutrients results in ethylene production in plant tissues (nutritional stress) whose inhibitory effect can be countered with the ability of ACC deaminase activity of RAA3 thus, resulting in improved nutritional status of bacterized plants. Similarly, biomass of *Jatropha curcas* was increased over control after inoculation with culture MSA2 along with the other attributes of plant growth such as root length and shoot length [36]. Seed inoculation of common bean *(Phaseolus vulgaris)* by *Pseudomonas chlororaphis* TSAU13 and *P. extremorientalis* TSAU20 resulted improved root and shoot biomass in the nutrient deficient soil of Uzbekistan [24]. In another study, inoculation with *Azospirillum* resulted in root elongation and improved N, P, K and microelement uptake [21] which results in better mineral nutrition for a plant that is essential for the rhizobia-nodule formation and nitrogen fixing activities. PGPR has the ability to increase the availability of nutrient concentration for acquisition by plants by fixing nutrients in rhizosphere, and preventing it from leaching out. For example, nitrogen, which is required for synthesis of amino acid and proteins, is the most limiting nutrient for plants. The mechanisms through which atmospheric nitrogen is converted into ammonia that can be assimilated by plants are exclusive to prokaryotes [5, 54]. In our study, we found bacterized plants exhibited a significant increase in wheat growth parameters and nutrient content under glasshouse conditions.

Bacterial inoculants are becoming an attractive alternative to chemical fertilizers; however, it is of utmost importance to understand the impact of PGPR inoculation on the microbial community before attempting their usage as commercial inoculant. We noted that in wheat, siderophore producers, nitrogen fixers, phosphate solubilizers and actinomycetes population enhanced on inoculation with RAA3, while, fungal population was slightly reduced. As well in case of cowpea, no significant results were obtained in any microbial group except enhancement in siderophore producers and a slight reduction in nitrogen fixers. The results indicate that inoculated PGPR do not mainly interfere with other microbes in rhizosphere. The beneficial effect of inoculation on microbial population may be direct, due to an increased supply of available P and N, or indirect, through changes in the growth rate and metabolic activities of crop [3, 18]. The reason for a shift in microbial population can be because of changed carbon source (CS) utilization of the soil on inoculation [40, 53]. Another reason can be the change in plant root exudation, which consists of easily degradable organic compounds that govern the rhizospheric community by attracting and stimulating microbial growth [55]. The improved number of beneficial microbial population in soils may be considered as a positive indicator of using these microbes as biofertilizers for sustainable agriculture practices.

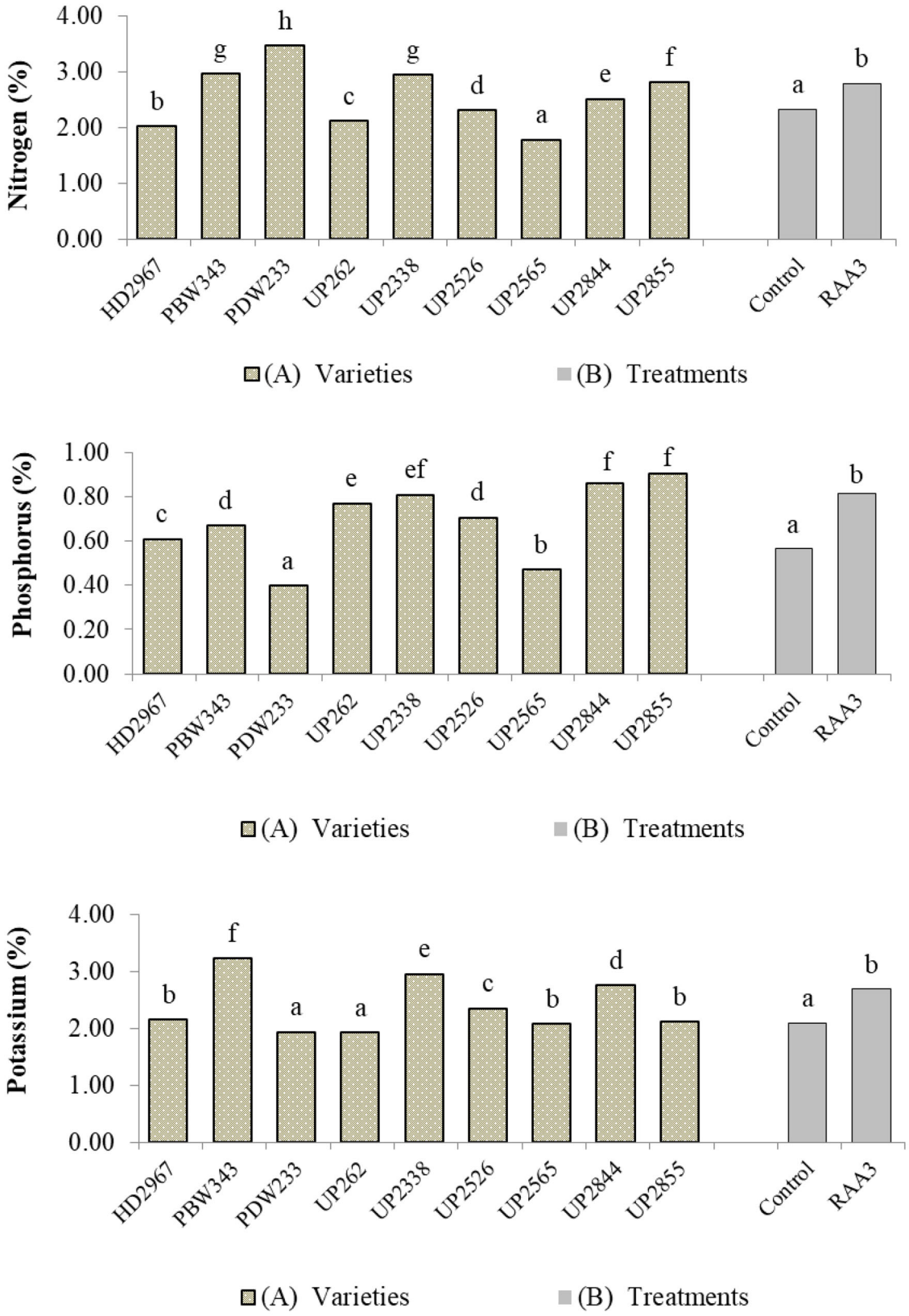

Fig. 4 Effect of bacterial inoculation on nitrogen, phosphorus and potassium content of wheat under glasshouse conditions. (**A**) Varietal effect, irrespective of PGPR (**B**) PGPR effect, irrespective of varieties Values with different letters (**a–f**) are significantly different at $p < 0.05$

Table 5 Effect of bacterial inoculation on functional diversity of rhizosphere of wheat and cowpea (Experiment I). Values with different letters (a-c) are significantly different at $p < 0.05$

	Siderophore producers					
	Wheat			Cowpea		
Treatments	WH1105	PBW660	Mean	PL-1	PL-2	Mean
Control	0.30 [b]	0.00 [a]	0.15 [a]	0.36 [a]	0.55 [b]	0.46 [a]
RAA3	0.42 [b]	0.36 [b]	0.39 [b]	1.10 [c]	1.22 [c]	1.16 [b]
Mean	0.36 [b]	0.18 [a]	0.27	0.73 [a]	0.89 [b]	0.81
LSD ($P \leq 0.05$)	0.14			0.17		
Treatments	*Nitrogen fixers*					
	WH1105	PBW660	Mean	PL-1	PL-2	Mean
Control	0.79 [a]	0.69 [a]	0.74 [a]	1.38 [b]	1.48 [c]	1.43 [b]
RAA3	1.10 [b]	1.13 [b]	1.12 [b]	1.33 [b]	1.05 [a]	1.19 [a]
Mean	0.95 [a]	0.91 [a]	0.93	1.36 [b]	1.27 [a]	1.31
LSD ($P \leq 0.05$)	0.23			0.11		
Treatments	*Phosphate solublizers*					
	WH1105	PBW660	Mean	PL-1	PL-2	Mean
Control	0.49 [a]	0.49 [a]	0.49 [a]	0.52 [a]	0.80 [b]	0.66 [a]
RAA3	0.66 [a]	0.66 [a]	0.66 [a]	0.55 [a]	0.84 [b]	0.69 [a]
Mean	0.58 [a]	0.58 [a]	0.58	0.53 [a]	0.82 [b]	0.68
LSD ($P \leq 0.05$)	0.25			0.08		
Treatments	*Fungal population*					
	WH1105	PBW660	Mean	PL-1	PL-2	Mean
Control	0.90 [a]	1.12 [c]	1.01 [b]	1.18 [a]	1.26 [a]	1.22 [a]
RAA3	0.86 [a]	1.04 [b]	0.95 [a]	1.25 [a]	1.18 [a]	1.21 [a]
Mean	0.88 [a]	1.08 [b]	0.98	1.22 [a]	1.22 [a]	1.22
LSD ($P \leq 0.05$)	0.07			0.87		
Treatments	*Actinomycetes population*					
	WH1105	PBW660	Mean	PL-1	PL-2	Mean
Control	1.37 [a]	1.21 [a]	1.29 [a]	1.49 [a]	1.31 [a]	1.40 [a]
RAA3	1.43 [b]	1.40 [b]	1.41 [b]	1.26 [a]	1.37 [a]	1.32 [a]
Mean	1.40 [b]	1.30 [a]	1.35	1.38 [a]	1.34 [a]	1.36
LSD ($P \leq 0.05$)	0.16			0.11		

Conclusions

This study revealed that bio-inoculants (RAA3 and M11) tested plants significantly improved the growth characteristics, chlorophyll content and foliar NPK contents of wheat and cowpea. The results also depicted that maximum response was observed at inoculum dose of 10^8, therefore this dose offered the best response in both wheat and cowpea thus can be considered as an optimum dose for the plant growth promotion. In addition, we observed that RAA3 inoculation has led to a shift in microbial population which was variable for different microbial groups in inoculated as compared to non-inoculated pots of wheat and cowpea. Hence, it is anticipated that inoculant RAA3 and M11 can be deployed as potential bio-inoculants for the sustainable agriculture.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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