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Nutrient enhancement and growth promotion of wheat cultivars by native plant growth promoting bacteria from Punjab state, India

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Abstract
Soil bacteria are a rich source for several useful biological attributes. The property of plant growth promotion is one such attribute which has practical applications in agriculture and remediation. Here, we employed bacteria (S2, PC and RA6) isolated from local soil for growth promotion and nutrient enhancement in three wheat cultivars, HD3086, HD2967 and WH1105. One non-local bacterial isolate (CDP13) and a commercial formulation (Symbion - K) were used for comparison. All these bacterial isolates were used alone and in combination with vesicular arbuscular mycorrhiza (VAM). HD3086 plants inoculated with RA6 gave the best results with reference to shoot and root length and plant weight. The levels of macro and micronutrients estimated using inductively coupled plasma mass spectrometry (ICP-MS) showed the same trend with the combination of RA6 and HD3086 displaying the best results. The local plant growth promoting bacteria (PGPB) characterized for promoting growth in wheat in this study can be used for future studies for biofortification of wheat grains.

Keywords: Inductively coupled plasma mass spectrometry, macronutrients, micronutrients, plant growth promoting bacteria, wheat

Introduction
Wheat is a dominant staple food crop which makes up for 50% of the diet. At present, the world population is around 7 billion people and is predicted to rise to around 9.5 billion by 2050. In just 100 years, world population has increased around fourfold. Global warming has resulted in a significant reduction in food production (IPCC 2014) [13]. Intensive agricultural and farming practices including the use of chemical fertilizers to increase crop productivity had an adverse effect on soil quality, plant health, food safety and soil microbiome (He *et al.*, 2019) [12]. The soil is extensively contaminated with Cd, Cu, Zn and Ni. It is important to remove these contaminants through various remediation approaches from the soil as they could be a potential risk for human, soil and plant health.
Plant-associated soil microbial communities influence plant growth, health, productivity and development. These microbes also play an important role in regulating soil fertility, nutrient cycling and maintaining plant diversity (Fitzsimons and Miller, 2010) [11]. The above traits paved way for the use of microbes in agriculture and biotechnological applications. Understanding the molecular mechanism involved in plant-microbe interactions can be employed to harness the beneficial bacteria for agriculture with a direct effect on food security. The use of naturally occurring PGPB in sustainable agriculture has gained importance in the past decade due to their beneficial effects on soil and crop productivity.
Our previous studies have shown the utility of selected soil bacteria in enhancing plant growth and regulating biomacromolecules including metabolites in maize, sorghum and foxtail millet (Li and Ramakrishna, 2011; Li *et al.*, 2014; Dhawi *et al.*, 2015; Dhawi *et al.*, 2016; Dhawi *et al.*, 2017; Dhawi *et al.*, 2018) [17, 18, 5-8]. In the present study, the emphasis is on how the native microbial strains isolated from local soil can be utilized for wheat growth and nutrient content. This information can be utilized to deploy these bacteria in the field thereby reducing the extensive use of chemical fertilizers.

Materials and Methods
Plant growth promoting bacteria and their characterization
Six bacterial strains were used in the present study. Two of these bacteria were native and previously collected from three areas of Bathinda region and characterized as *Pseudomonas sp. RA6* and *Pseudomonas citronellis* (PC) based on 16S ribosomal DNA sequence analysis (Adhikary *et al.*, 2019; Accession numbers KM594398 and KM594397) [1]. The third strain is also a native bacterium classified as *Serratia marcescens* S2 based on DNA sequence analysis

(Dogra *et al.*, 2019; accession number GU046543) [9]. These bacteria harbor plant growth promotion traits such as production of IAA, phosphate solubilization and siderophore production, based on previous studies. The fourth bacterium, *Serratia marcescens* CDP13 is a PGPR associated with *Capparis decidua* plant (Singh and Jha, 2016; Accession number KJ950714) [23]. The last two strains, Symbion-K and Symbion-VAM PlusTM are commercial bio-fertilizers selected as positive controls. Symbion-K consists of potash solubilizing bacteria, *Frateuria aurantia* (http://www.tstanes.com/products-symbion-k.htmL) and Symbion-VAM PlusTM vesicular arbuscular mycorrhiza (http://www.tstanes.com/products- symbion-vam.htmL). In this study, wheat seeds were subjected to three treatments: VAM plus (My), VAM plus + plant growth promoting bacteria (My + B) and PGPB alone (B). These strains were employed in combination alone and in combination (PC+VAM, RA6+VAM, S2+VAM, CDP13+VAM and Symbion-K+VAM).

Preparation of bacterial inoculum

An overnight bacterial culture from a single colony was grown in 1 mL LB media and was inoculated in 250 mL liquid culture. The culture grown was grown at 37oC for 24 hours followed by pelleting at 6000rpm for 10min. The pellet was resuspended in saline (0.85% NaCl solution). 108 cfu mL-1 culture was used for wheat seed coating. Each of My or My + B group had approximately ~1000 propagules (1 gram) of mycorrhiza suspended in saline.

Plant material, inoculation and growth conditions

Three wheat varieties, HD-3086, HD-2967 and WH-1105 procured from Punjab Agricultural University, Ludhiana were used in this study. Plastic pots were used to grow plants in the soil collected from the Central University of Punjab, Bathinda city campus. Wheat seeds were subject to surface sterilization with 70% ethanol for 2 minutes followed by rinsing three times with sterile water. The seeds were then treated with 1% sodium hypochlorite solution for 3 minutes followed by rinsing with sterile water. Seeds were soaked in PGPB suspended in the saline buffer for one hour for bacterial colonization. Five seeds from each treatment and control were sown in pots. The bacterial culture was also added into rhizosphere during transplantation. The pots were kept in the greenhouse and allowed to grow for 45 days.

Plant growth measurements

Shoot lengths of plants were measured after the 30th day and 45th day after germination. At the end of the experiment, the plants were washed with deionized water followed by the separation of roots and shoots. Root lengths of treated plants were measured and compared with the control plants. The biomass of plants before drying (fresh weight) and after drying at 60ºC (dry weight) was recorded.

Nutrient quantification using inductively coupled plasma mass spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) and combustion based elemental analysis were employed to detect the macro and micronutrients in plant tissues. For ICP-MS, gram of plant tissue per plant was mixed with 10 mL digestion solution (8 mL 70% HNO3 and 2 mL H2O2) in heat resistant vessels for microwave digestion for 4-5 hours using Ethos UP Microwave Digestion System-Milestone® (Krachler *et al.*, 2002) [16]. After digestion, samples were filtered through Whatman filter paper followed by 0.22 µm syringe filter. Samples were diluted 10,000 times using sterile water (Becker *et al.*, 2008) [2]. The samples were given for ICP-MS analysis to the Central Instrumentation Laboratory, Central University of Punjab.

Nitrogen and phosphorus quantification

Nitrogen estimation in shoot samples (percent by weight) was determined using the Kjeldahl method (IS 5194, 1969) [14]. Shoot samples (1 gm) per plant each were digested for 3 hours in Kjeldahl flask with 10 mL sulfuric acid and a mixture of 3.5 gram of cupric sulfate and potassium sulfate (1:10 ratio). Ammonia released during distillation was absorbed by boric acid. Nitrogen estimation was done as per the Bureau of Indian Standards (IS 5194, 1969) [14]. Total phosphorus of wheat shoot samples was estimated using Allen's method (Jackson, 1973) [15]. Briefly, 1 gram shoot tissue from each sample was digested in 10 mL of nitric acid and perchloric acid mixture (1:2 v/v). A fixed volume of water (25 mL) was added. The sample was filtered with Whatman number 1 filter paper and diluted to 100 mL with sterile water. In a 50 mL volumetric flask, 2.5 mL each of diluted filtrate and ammonium molybdate were added followed by 4-5 drops of stannous chloride in glycerol and final volume was made up to 50 mL with sterile water. The mixture was incubated for 10 minutes at room temperature till the appearance of blue color whose absorbance was measured at 660 nm. Total P content of the sample was estimated as per Jackson (1973) [15].

Statistical analysis

Values of the mean and standard deviation (mean ± standard deviation) were calculated and a pairwise t-test was performed to identify statistically significant differences ($p<0.05$) between each treated and control groups.

Results

Wheat varieties HD3086 and HD2967 showed best growth response to PGPB (RA6) native isolate from local soil

HD3086 variety plants treated with the bacterial strains RA6, S2, CDP13 and CDP13+VAM showed a significant increase in shoot growth (Table 1). In the case of HD2967, a significant increase in shoot length was observed in PC, RA6, CDP13, VAM, S2+VAM and RA6+VAM treated plants. However, WH1105 showed a significant increase in shoot length only in PC+VAM treatment. CDP13 treated HD3086 showed maximal increase in shoot length of 4.8% compared to control. RA6 treated HD2967 showed the highest shoot length with 9.3% increase compared to the control plants. We can conclude from the results that wheat variety HD3086 and HD2967 perform better to native bacterial inoculation compared to WH1105 in terms of shoot length. The root length of wheat variety HD3086 treated with only RA6 showed a significant increase whereas in the case of HD2967, RA6, S2+VAM, PC+VAM and RA6+VAM treated plants showed a significant increase compared to the control. In contrast to HD3086 and HD2967, a significant increase in root length of WH1105 was seen only in S2 treated plants. RA6 inoculated plants of HD3086 and HD2967 showed 8% and 4% increase respectively, compared to control plants. The highest increase (approximately 10%) in root length was seen in PC+VAM inoculated plants in HD2967.

HD 3086 variety plants showed significant fresh weight increase in most of the treatments except S2, PC and S2+VAM (Table 1). In the case of HD2967, a significant

increase in fresh weight was observed in RA6 and CDP13 treated plants. S2, CDP13 and PC when inoculated in combination with VAM increased fresh weight in wheat variety WH1105. The fresh weight of RA6 treated plants in HD3086 and HD2967 showed 20% and 19% increase, respectively whereas the highest increase (21%) was seen in CDP13 treated plants in HD3086 compared to the control plants. In the case of wheat variety WH1105, the highest increase in fresh weight was only 8% in CDP13 treated plants. Dry weight of all the treated plants increased significantly with highest increase (76%) in RA6 inoculated plants compared to control in HD2967 whereas in the case of HD3086, 72% increase was seen in RA6 treated plants. The bacterial strains, S2, CDP13, VAM and CDP13+VAM enhanced the dry weight in WH1105 with a maximum of 35% highest increase in the case of S2 treated plants. Shoot, root and biomass results indicated that the native RA6 strain consistently increased shoot, root and biomass in HD3086 and HD2967.

Macro and micronutrient enhancement in PGPB treated wheat varieties HD3086 and HD2967

Major or Macronutrients (N, K, Ca, Mg, P, and S) are required in comparatively larger amounts and minor or micronutrients (Cl, Fe, B, Mn, Zn, Cu, Mo, and Ni) are required in a lower amount for optimal vegetative and reproductive growth of plants. Macro and micronutrients were analyzed in the cultivars HD-3086 and HD-2967 as they showed superior performance compared to WH-1105 with reference to plant growth and weight. The level of the macronutrient, nitrogen in HD-2967 showed marked increase compared to the control in RA6, PC, CDP13, PC+VAM and RA6+VAM (Table 2). In comparison, level of nitrogen increased significantly in HD-3086 treated with RA6 and PC+VAM. RA6 was the common treatment where a significant increase in nitrogen was observed in both HD-3086 and HD2967. Similarly, phosphorus level in HD-3086 inoculated with RA6 showed 43% increase whereas 36% and 30% increase was seen in RA6+VAM and CDP13 inoculated plants compared to control. The increase in phosphorus level was less pronounced in the case of HD-2967 with maximum increase of about 27% in RA6 inoculated plants whereas CDP13 and RA6+VAM showed 24% increase compared to control. Based on our results, it can be stated that wheat variety HD3086 was more responsive to RA6, a native bacterial strain to enhance phosphate uptake compared to HD2967. Increase in magnesium level in HD3086 was significant in all treatments except CDP13 and RA6 in combination with VAM. The best response was shown by RA6 with 70% whereas S2 and S2+VAM treated plants showed about 48% increase in magnesium compared to the control plants. In the case of HD-2967 inoculated with RA6, SYM-K, CDP13, S2+VAM, CDP13+VAM and RA6+VAM, a significant increase in magnesium levels with the highest increase of 29% was observed in RA6 treated plants. These results indicate that magnesium increase was not profound in the case of HD2967 plants. Calcium levels in HD-3086 treated with Symbion K, PC and RA6 increased by 56%, 47% and 34%, respectively, compared to the control plants. In the case of HD-2967, RA6 treated plants showed the maximum calcium levels with 83% increase compared to the control plants. The levels of all the macronutrients estimated in this study were much higher in the control plants of HD-3086 than HD-2967.

Micronutrient analysis showed that manganese levels increased significantly in all treatments with RA6 showing the highest value in HD-3086 (Table 2). HD-2967 showed a significant increase in manganese in RA6, SYM-K and CDP-13 treatments and all PGPB combinations tested with VAM. Iron level was the highest in RA6+VAM treated HD3086 with significant increase in SYM-K, S2+VAM, CDP-13 and RA6 treatments. Iron level increase was significant in only RA6 and RA6+VAM treated HD-2967. Increase in nickel was significant in all treatments except S2 with RA6+VAM showing the highest accumulation in HD3086. Increase in nickel was significant in all treatments with RA6+VAM showing the highest accumulation in HD2967. Copper levels in HD3086 were significantly higher in three individual treatments (PC, RA6 and CDP13) and CDP13 and RA6 in combination with VAM. In the case of HD2967, RA6 alone and in combination with VAM showed a significant increase in copper compared to control. RA6 and CDP13 showed high levels of zinc in HD3086 whereas most of the treatments (except S2 and VAM) increased zinc levels in HD2967. Boron levels were significantly higher in HD3086 in all treatments with approximately 50% increase due to PC treatment. Similar trend was observed in HD2967 except for two differences: RA6 treatment resulted in the highest boron accumulation and S2+VAM treatment did not result in significant accumulation of boron compared to the control.

Our study showed that PGPB enhanced the macronutrients, nitrogen, phosphorus, magnesium and calcium levels in both wheat cultivars HD3086 and HD2967 but their levels were much higher in the wheat cultivar HD3086. Similar observation was made in the case of micronutrients. In single treatments, RA6 followed by PC showed the best results. RA6 was the only treatment, which showed significant increase in all macro and micronutrients evaluated in both HD3086 and HD2967. This is also true for RA6+VAM treated wheat variety HD2967 and six out of ten nutrients for HD3086. This study showed that native PGPB strains especially RA6 and RA6 in combination with VAM can be more effective in the wheat cultivar HD3086. These native PGPB can be utilized to enhance plant growth and macro and micronutrient in wheat.

Discussion

There is an urgent need to isolate and characterize native bacterial strains which are well suited to a particular regional soil and will be more effective than commercial bio-formulations and fertilizers. Our study indicated that RA6 gave the best results with reference to wheat growth. The use of native PGPB and cyanobacteria were shown to have many benefits in terms of reduced inputs of harmful chemical fertilizers, bio-fortification of food crops and enhanced plant health (Rana *et al.*, 2012) [21]. In addition, it is more environmental friendly and cost-effective (Prasanna *et al.*, 2016; Singh *et al.*, 2018) [20, 24]. The observed biomass enhancement and increased uptake of macro and micronutrients by wheat plants treated with PGPB reported in our study needs to be exploited for gains in agriculture. The production of IAA, phosphate solubilization and siderophore production are all responsible for enhancing plant growth and biofortification mediated by PGPB (Li and Ramakrishna, 2011) [17].

Table 1: Effect of potential PGPB on shoot and root length and fresh and dry weight of three wheat cultivars

Shoot length (cm)	**CONTROL**	**S2**	**PC**	**RA6**	**SYM-K**	**CDP13**	**VAM**	**S2+VAM**	**CDP13+VAM**	**PC+VAM**	**RA6+VAM**
HD3086	39.33 ± 1.09	40.77 ± 1.7*	39.11 ± 1.64	41.11 ± 2.03*	38.44 ± 1.01	41.22 ± 2.12*	39.55 ± 2.2	39.75 ± 1.5	40.55 ± 1.32*	40.11 ± 1.76	39.66 ± 1.58
HD2967	34.33 ± 2.64	36 ± 3.9	35.66 ± 2.39*	37.55 ± 4.55*	35.11 ± 1.61	36.33 ± 2.44*	36.88 ± 2.9*	36 ± 2.12*	35.22 ± 4.26	36.11 ± 1.9	36.89 ± 2*
WH1105	35.22 ± 4.94	35.33 ± 3.2	35.77 ± 3.07	35.66 ± 2.39	34.66 ± 2.34	35.88 ± 2.02	35.55 ± 1.74	36.11 ± 3.33	36.22 ± 4.63	39 ± 2.64*	35.44 ± 1
Root length (cm)											
HD3086	35.77 ± 2.43	34.88 ± 2.14	34.55 ± 1.87	38.77 ± 2.04*	36.11 ± 2.52	35.44 ± 2.83	36.00 ± 2.44	34.88 ± 2.14	35.66 ± 2.06	37.33 ± 2.23	36.33 ± 1.32
HD2967	32.44 ± 1.58	31.77 ± 0.97	32.22 ± 1.2	34 ± 2.95*	32.11 ± 1.53	33.44 ± 2.78	32.88 ± 1.45	35 ± 2.82*	33.33 ± 2.51	35.67 ± 5.36*	35.22 ± 3.5*
WH-1105	31.66 ± 1.5	33.33 ± 1.7*	32.44 ± 1.81	32.67 ± 1.32	31.11 ± 3.40	32 ± 2.29	32 ± 1.73	31.33 ± 1.32	32.22 ± 1.71	28.22 ± 2.53	32 ± 1.22
Fresh weight (gm)											
HD3086	3.37 ± 0.22	3.64 ± 0.44	3.66 ± 0.76	4.06 ± 0.24*	3.8 ± 0.49*	4.09 ± 0.21*	3.79 ± 0.33*	3.62 ± 0.44	3.97 ± 0.25*	3.73 ± 0.47*	3.98 ± 0.27*
HD2967	3.26 ± 0.52	3.56 ± 0.28	3.11 ± 0.19	3.9 ± 0.129*	3.06 ± 0.37	3.78 ± 0.29*	3.42 ± 0.46	3.05 ± 0.44	3.36 ± 0.169	3.46 ± 0.22	3.7 ± 0.25
WH1105	3.23 ± 0.13	3.18 ± 0.54	3.24 ± 0.7	2.23 ± 0.26	3.43 ± 0.5	3.36 ± 0.59	3.50 ± 0.6	3.51 ± 0.38*	3.42 ± 0.31*	3.54 ± 0.25*	3.47 ± 0.46
Dry weight (gm)											
HD3086	1.05 ± 0.28	1.46 ± 0.47*	1.60 ± 0.51	1.82 ± 0.32*	1.5 ± 0.39*	1.57 ± 0.48*	1.22 ± 0.47	1.65 ± 0.33*	1.59 ± 0.27*	1.65 ± 0.21*	1.73 ± 0.24*
HD2967	1.09 ± 0.25	1.78 ± 0.51*	1.64 ± 0.38*	1.92 ± 0.34*	1.74 ± 0.39*	1.88 ± 0.17*	1.59 ± 0.5*	1.78 ± 0.5*	1.69 ± 0.55*	1.8 ± 0.76*	1.66 ± 0.69*
WH1105	0.95 ± 0.19	1.29 ± 0.19*	0.99 ± 0.2	0.95 ± 0.14	1.05 ± 0.41	1.22 ± 0.28*	1.15 ± 0.25*	1.08 ± 0.28	1.22 ± 0.3*	1.15 ± 0.3	1.06 ± 0.24

All measurements were recorded after 45 days. Values shown are average ± standard deviation. *Denotes significance ($p<0.05$) compared to control using t-test.

Table 2: Macro and Micronutrient levels in shoots of two wheat cultivars using ICP-MS

HD2967	**CONTROL**	**S2**	**PC**	**RA6**	**SYM-K**	**CDP13**	**VAM**	**S2+VAM**	**CDP13+VAM**	**PC+VAM**	**RA6+VAM**
Nitrogen (%)	0.14 ± 0.058	0.15 ± 0.058	0.17 ± 0.01*	0.19 ± 0.012*	0.13 ± 0.012	0.16 ± 0.021*	0.15 ± 0.01	0.15 ± 0.012	0.17 ± 0.012*	0.15 ± 0.058	0.18 ± 0.06*
Phosphorus (mg/kg)	485.64 ± 0.02	589.23 ± 0.01*	587.17 ± 0.04*	616.9 ± 0.03*	531.28 ± 0.02	601.02 ± 0.01*	584.6 ±0.01*	561.53 ± 0.03*	590.46 ± 0.03*	512.3 ± 0.02	603.1 ± 0.01*
Magnesium [ppb]	518.59 ± 39.3	612.1 ± 30.5*	605.88 ± 52.8	671.5 ± 19.8*	586.3 ± 19.64*	588.3 ± 12.2*	544.8 ± 15.5	638.2 ± 10.5*	600.06 ± 39.1*	554.99 ± 11.4	641.8 ± 2.1*
Calcium [ppb]	1422.6 ± 28.3	1600.6 ± 80.95*	1636 ± 86.5*	2615.6 ± 47*	1504.2 ± 19.2*	1382.5 ± 66.3	1607.7 ± 52.6*	1413.9 ± 53.3	1438.7 ± 34	1516.5 ± 5*	2457.3 ± 60.4*
Manganese [ppb]	18.26 ± 0.58	19.43 ± 0.932	19.89 ± 1.13	21.72 ± 1*	19.81 ± 0.8*	20.9 ± 0.7*	16.43 ± 2.11	21.4 ± 0.9*	19.96 ± 0.43*	20.6 ± 1.27*	20.93 ± 0.45*
Iron [ppb]	327 ±26.89	355.25 ± 44.47	343.75 ± 63.2	398.3 ± 13.7*	335.8 ± 76.4	338 ± 45.9	310.75 ± 76.1	339 ± 65.5	307 ± 30.77	341.3 ± 30.9	389 ± 55.9*
Nickel [ppb]	1.95 ± 0.322	3.07 ± 0.662*	3.2 ± 0.5*	3.63 ± 0.39*	2.81 ± 0.34*	2.73 ± 0.39*	2.81 ± 0.12*	3.04 ± 0.18*	2.52 ± 0.09*	3.4 ± 0.22*	3.84 ± 0.09*
Copper [ppb]	5.93 ± 0.157	5.92 ± 0.621	6.3 ± 0.46	6.61 ± 0.47*	5.28 ± 0.496	6.03 ± 0.29	5.43 ± 0.77	6 ± 0.4	5.99 ± 0.28	6.33 ± 0.38	6.33 ± 0.36*
Zinc [ppb]	42.59 ± 1.77	43.63 ± 1.16	45.4 ± 0.72*	46.57 ± 0.98*	45.24 ± 1.5*	45.7 ± 0.82*	41.92 ± 1.6	43.85 ± 2.3*	46.04 ± 0.67*	45.2 ± 1.05*	46.1 ± 0.89*
Boron [ppb]	1026.6 ± 58.7	1268.3 ± 78.8*	1434.2 ± 74.3*	1704 ± 51.3*	1525 ± 72.96*	1558.4 ± 73.1*	1566.6 ± 57*	1093 ± 62.8	1217.8 ± 77.2*	1465.5 ±72.7*	1643.1 ± 40.3*
HD3086											
Nitrogen (%)	0.19 ± 0.01	0.2 ± 0.01	0.2 ± 0.05	0.23 ± 0.05*	0.21 ± 0.025	0.21 ± 0.01	0.18 ± 0.057	0.20 ± 0.011	0.21 ± 0.058	0.22 ± 0.06*	0.21 ± 0.011
Phosphorus (mg/kg)	648.71 ± 0.01	794.87 ± 0.05*	774.4 ± 0.07*	928.7 ± 0.02*	647.17 ± 0.01	845.1 ± 0.05*	707.2 ± 0.03*	732.3 ± 0.01*	815.4 ± 0.01*	612.3 ± 0.01	883.6 ± 0.01*
Magnesium [ppb]	554.77 ± 72	821.95 ± 75.9*	782.2 ± 62.9*	946.1 ± 37.1*	752.2 ± 77.7*	735 ± 67.1*	771 ± 40.3*	836.5 ± 37.7*	658.5 ± 52.4	689.3 ± 62.3*	618.3 ± 35.8
Calcium [ppb]	2631.2 ± 61.2	1924.4 ± 71.8	3862.96 ± 74.7*	3512 ± 53.1*	4114.5 ± 70.8*	3297.6 ± 39.8*	1716.6 ± 69.7	1642.9 ± 57	2569.2 ± 84	1600.9 ± 72.9	2473.4 ± 63.9
Manganese [ppb]	16.05 ± 2.07	18.29 ± 0.86*	20.92 ± 0.43*	21.8 ± 0.98*	18.18 ± 0.69*	20.46 ± 0.9*	18.55 ± 0.43*	17.47 ± 1.08	13.6 ± 2.24	21.56 ± 0.47*	20.3 ± 1.3*
Iron [ppb]	305.9 ± 41.6	278.31 ± 53.7	291.37 ± 68.5	406.1 ± 9.5*	443.2 ± 25.1*	412.8 ± 47.4*	312.7 ± 28.1	420 ± 26.6*	355.8 ± 14.02*	332.8 ± 19.4	500.3 ± 28.2*
Nickel [ppb]	1.042 ± 0.37	1.78 ± 0.38	2.52 ± 0.43*	2.94 ± 0.33*	2.41 ± 0.5*	2.39 ± 0.54*	2.46 ± 0.58*	2.42 ± 0.33*	2.51 ± 0.12*	2.82 ± 0.12*	2.97 ± 0.12*
Copper [ppb]	4.5 ± 0.55	5.01 ± 0.27	5.88 ± 0.11*	6.1 ± 0.44*	3.69 ± 0.32	5.76 ± 0.3*	5.66 ± 0.67	5.3 ± 0.33	5.98 ± 0.116*	5.25 ± 0.77	6.3 ± 0.11*
Zinc [ppb]	36.13 ± 2.75	42.7 ± 1.47	44.28 ± 0.92	56.5 ± 1.93*	44.14 ± 3.1	55.71 ± 8.9*	42.5 ± 2.5	45.74 ± 1.2	48.14 ± 8.92	45.01 ± 0.5	45.85 ± 3.5
Boron [ppb]	1099.4 ± 49.4	1378.7 ± 55*	1693.2 ± 67.2*	1547 ± 79.3*	1229.8 ± 29*	1451.5 ± 34.6*	1263.4 ± 61.2*	1444.3 ± 51.5*	1503.7 ± 12.9*	1623.7 ± 36.7*	1321.5 ± 25.4*

All measurements were recorded after 45 days. Values shown are average ± standard deviation. *Denotes significance at $p<0.05$ compared to control using t-test.

Macronutrients are the major nutrients needed by plants for carrying out various functions. Nitrogen is one the nutrients which is part of chemical fertilizers. Nitrogen is crucial for plant growth as it is incorporated in amino acids which are part of proteins and contribute to the nutritional quality (Maheswari *et al.*, 2017) [19]. Phosphorus is the second most important macronutrient which is part of phospholipids and nucleic acids and has a key role in regulating many enzymatic reactions (Schachtman *et al.*, 1998) [22]. PGPB play an important role in converting insoluble P to soluble P which plants can use. Calcium is part of cell wall and has a role in intracellular signaling (White and Broadley, 2003) [25]. Micronutrients are needed in minute quantities. Iron is one of the key elements having important role in redox reactions as well other vital enzymatic reactions involved in nitrogen fixation, DNA synthesis and hormone synthesis (Connorton *et al.*, 2017) [3]. Nickel is present in active sites of many enzymes such as urease, glyoxalase-I, some superoxide dismutases, and carbon monoxide dehydrogenase (Fabiano *et al.*, 2015) [10]. Plastocyanin is a major copper-containing protein, which is involved in electron transport chain in chloroplast and mitochondria (Cohu and Pilon, 2010) [4]. In addition, copper is also involved in maintaining cellular homeostasis as it is an integral component of Cu/Zn superoxide dismutase. Enhancement of plant growth and nutrients of wheat by native PGPB reported in the present study is likely to enhance physiological and cellular functions.

Conclusion

The present study showed that local bacterial isolate, RA6 gave superior performance with the wheat cultivar, HD3086 compared to two other wheat cultivars tested under open greenhouse conditions with reference to plant growth and macro and micronutrient content. Future studies will be targeted towards comparison of the performance of the bacterial isolate RA6 with additional native bacteria and conducting field trials. The long term goal of this study is to employ these beneficial bacteria in agriculture thereby reducing the use of chemical fertilizers and biofortification to enhance the nutrient value of food crops.

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Conflict of Interest

All the authors declare that they have no conflict of interest.

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