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Exploring the potential soil bacteria for sustainable wheat (*Triticum aestivum* L.) production

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Abstract

Plant growth promoting rhizobacteria (PGPR) are capable to reduce the use of chemical fertilizers input cost of farmer. Keeping in view the study was designed to investigate and evaluate inoculation effect of indigenous rhizospheric bacteria on growth and yield of wheat (*Triticum aestivum* L.) under in vitro and in vivo conditions using different treatments. Ten potential strains were selected on the basis of their ACC deaminase activity, siderophore production, P-solubilization and production of indole acetic acid (IAA). Further these strains were tested in three different experiments (growth chamber, pot and field). We found significant increase in crop growth response to the inoculants in comparison with un-inoculated control. In pot and field trial we tested PGPR with recommended dose of inorganic fertilizers. The results of present study revealed that inoculation of bacterial strains with wheat seeds significantly increased plant growth and improved crop yield. Results of present study reveal that these strains could be employed in different combinations and can get higher yield in case of half recommended doses of inorganic fertilizers along with consortium of strains in comparison with sole application of recommended dose of fertilizer and with consortium of strains. These strains were further identified by 16Sr RNA gene sequencing, fatty acid profile and biolog. It can be concluded that inoculated bacteria have more potential and contributes in good crop quality, increased yield when they are applied in combination, thus have potential to minimize use of chemical fertilizers.

Keywords: inoculation, PGPR, soil bacteria, wheat,

Introduction:

The importance of soil-plant-microbe interaction in recent decades has increased to a large extent. Plant growth promoting rhizobacteria (PGPR) are a group of free living bacteria in the soil or in association with plants, enhance plant growth and yield by different mechanisms of action. They may produce different hormones which stimulate plant growth, solubilize nutrients including phosphorous and iron, fix atmospheric nitrogen, act as bio-control agents and improve soil structure (Hayat et al., 2010). Numerous types of bacteria are identified in soil, particularly from rhizosphere thus playing important role in growth of plant. Soil bacteria produce special type of organic acids like carboxylic acid (Deubel & Merbach, 2005) thus decrease rhizosphere soil pH and dissociate the bound forms of calcium phosphate in calcareous soil. Soil bacteria help to increase the uptake and availability of nutrients for the plants (Vessey, 2003). Some potential bacterial candidates for biofertilizer include the genera such as *Azospirillium*, *Pseudomonas*, *Bacillus*, *Azotobacter*, *Enterobacter*, *Burkholderia*, *Acinetobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* and *Jeotgalicoccus* etc. (Rodriguez and Fraga, 1999, hayat et al., 2010).

Wheat is used as the main staple food in Pakistan but the average yield in Pakistan is below the potential yield and the major reasons for low productivity are low soil fertility, shortage of irrigation water and inefficient fertilizer use. Soils are low in organic matter contents which affect soil fertility and soil structure badly (Ullah et al., 2007). Beneficial effects of PGPR on growth and yield of different crops are well documented has been correlated to the production of phytohormones and increased nutrient supply. Similar results were obtained when barley seed was inoculated with different PGPRs (Canbolat et al., 2006). Root weight was increased by 9 to 17% and shoot weight was increased by 29 to 35% over the control. According to Wu et al.,

(2005) microbial inoculum of two *Bacillus* species *(Bacillus megaterium* and *Bacillus mucilaginous*) increased the growth of plant as well as nutritional assimilation of plant improved (total N, P, and K). Egamberdiyeva (2007) inoculated maize with bacterial strains *Bacillus polymyxa*, *Pseudomonas alcaligenes*, and *Mycobacterium phlei* and reported a significant increase in root dry weight (19–52%) and maize total dry matter was also increased up to 38 percent. Keeping in view the importance of PGPR, a lab study was conducted in which bacterial strains were isolated from wheat rhizosphere, identified them and their efficiency as PGPR were evaluated on growth of wheat in growth pouches under controlled conditions and in field experiment with full and half recommended dose of fertilizers. The objectives of this study to determine, the new combination of reduce rate of inorganic fertilizer fixed with indigenous PGPR inoculants for wheat crop will produce equivalent to the full recommended dose of fertilizer in growth and yield parameter.

Results:

Plant growth promoting (PGB) activity of soil bacteria:

All ten strains possess four PGP traits i.e. production of indole-3-acetic acid (IAA), solubilization of insoluble tricalcium phosphate, ACC deaminase activity and siderophore production (Table 1). All the strains produce IAA with tryptophan (1.84 to 12.02 µg mL^{-1}) and without addition of tryptophan (1.24 to 2.42 µg mL^{-1}). All strains used in this study solubilized insoluble mineral phosphate ranged from 84 to 212 µg mL^{-1} along drop in medium pH. The maximum drop in pH was observed in case of strain RA-7 upto 4.38 from an initial pH of 7 during seven days of incubation. For plant growth promoting bacteria, ACC deaminase activity was considered as an efficient marker because these strains have potential to lowering the level of ethylene inhibition in plants. All ten strains utilize ACC as a sole source of nitrogen and results revealed that

different strains differed in ACC activity as shown in Table 1. Maximum ACC activity was observed in case of strain RA-8 (782 nmol h^{-1}) (*Pseudomonas brassicacearum subsp. neoaurantiaca*) and minimum was observed in RA-4 (475 nmol h^{-1}) (*Pseudomonas corrugata*). Siderophore production of all the strains were confirmed by quantitative CAS assay and maximum activity was observed in case of RA-10 (*Pseudomonas azotoformans*). On the basis of absorbance value siderophore activity were categories in three levels high (+++) moderate (++) and lower (+).

16S rRNA gene sequence identification of bacterial strains:

All bacterial isolates were identified using 16S rRNA gene sequencing. The 16S rRNA gene sequence of all isolates were obtained and compared with available 16S rRNA gene sequences of bacteria from GenBank databases as describe in Table 2. Diversity of rhizosphere bacteria with varying physiological and biochemical traits were identified to the species level of all isolates. Out of 10 bacterial strains, five strains were identified from genus *Pseudomonas* as RA-1 (*Pseudomonas fragi*), RA-4 (*Pseudomonas corrugata*), RA-6 (*Pseudomonas arsenicoxydans*), RA-8 (*Pseudomonas brassicacearum subsp. neoaurantiaca*), RA-10 (*Pseudomonas azotoformans*), four strains were belong to genus *Bacillus* as RA-3 (*Bacillus sefensis*), RA-5 (*Bacillus cereus*), RA-7 (*Bacillus aryabhattai*), RA-9 (*Bacillus thuringiensis*) and one strain belong to genus *Alcaligenes* as RA-2 (*Alcaligenes faecalis subsp. faecalis)*. For phylogenetic tree construction (Fig 1) we obtained closely related taxa of our strains from BLAST search using eztaxon server (http://eztaxon-e.ezbiocloud.net). All strains were also identified by analyzing through Sherlock microbial identification system (MIDI) (Library RTSA6 6.0, MIDI Sherlock software package, version 6.0) for cellular fatty acid profile composition. The major fatty acid observed in bacterial strains were $C_{16:0}$ 30.19 ± 0.01,summed feature 3 25.87 ± 0.01 in RA-1, $C_{16:0}$ 30.19 ± 0.01,summed feature 3 25.87 ± 0.02, summed feature 8 25.1 ± 0.03 in RA -2,

anteiso $C_{15:0}$ 23.46 ± 0.01, iso-$C_{15:0}$ 27.42 ± 0.01 in RA-3, $C_{16:0}$ 28.11 ± 0.01, summed feature 3 21.68 ± 0.01, summed feature 8 22.97 ± 0.01 in RA-4, iso-$C_{15:0}$ 28.5 ± 0.01, iso-$C_{17:0}$ 9.62 ± 0.01 in RA-5, $C_{16:0}$ 27.16 ± 0.05, summed feature 3 24.31 ± 0.01, summed feature 8 24.26 ± 0.01 in RA-6, iso-$C_{15:0}$ 25.88 ± 0.05, anteiso-$C_{15:0}$ 29.72 ± 0.04 in RA-7, $C_{16:0}$ 29.95 ± 0.01, summed feature 3 28.05 ± 0.01 in RA-8, $C_{16:0}$ 17.73 ± 0.02, $C_{18:0}$ 11.56 ± 0.01 in RA-9 and anteiso-$C_{15:0}$ 33.49 ± 0.01, summed feature 3 10.31 ± 0.01, $C_{16:0}$ 15.96 ± 0.01 in RA-10. Other minor components detail was given in Table 6. The biolog of all 10 strains were performed and results were given in Table 7.

Response of wheat to soil bacteria under controlled and field condition:

The first experiment was carried out in growth chamber under controlled conditions for 1 month and 10 strains were inoculated with seed before sowing. All the parameters taken showed the positive results with increase in shoot length, root length, fresh and dry weight of plants. The result of growth chamber experiment was shown in Table 3. Significant increase in shoot length was observed in all the treatments over control. Maximum increase was observed in T 8 (*Pseudomonas brassicacearum subsp. neoaurantiaca*), which gave 82% increase in shoot length followed by 77%, 75%, 74%, 62% by T 7, T 3, T 11 and T 5, respectively. An increase of 161% in root length was observed by T 7 followed by 141%, 119%, 108% and 93% by T 8, T 9, T 3 and T 11, respectively when compared to control. Increase in fresh and dry weight of the plants was observed in all the treatments over control. The maximum increase in fresh weight was 335% by T 8 followed by 309%, 287%, 258% by T 7, T 3 and T 11. Six potential bacterial strains were screened on the basis of their performance under growth chamber for further investigation under pot and field trials. Inorganic fertilizers were applied in these experiments for comparison with individual strains and consortium of strain with full and half recommended

dose of fertilizer for wheat crop. However, the inoculants efficacy decreases in higher dose of inorganic fertilizer. The data taken at harvest stage in pot and field trial, showed positive results in every parameter by all bacterial strains application over control. All the strains significantly improve shoot length over uninoculated control. The results showed that T 5 (*Bacillus cereus*) had an increase of 25% in shoot length over control followed by T 2, T 4 and T 7 which showed an increase of 20%, 19% and 17% respectively. The significant negative correlation ($R^2=0.91$) was observed in percentage increase in crop parameters over various doses of inorganic fertilizers was shown in (Table 4). Similar trend was observed to field trial with respect to efficacy of inoculants at different doses of inorganic fertilizer. All the treatments applied in pot experiment were repeated again in field experiment by dividing consortium in 2 different groups. Total 15 treatments were applied in field experiment including control, with full and half recommended dose of fertilizers individually and along with 2 different consortiums group shown in (Table 5). The maximum yield was observed in case of consortium 1 and consortium 2 along with half recommended dose of fertilizer.

Discussion:

Bacterial inoculants are nature gifted machinery for integrated management of agro-environmental problems because bacteria possess the ability to improve plant growth, boost nutrient availability or uptake, and support plant health directly and indirectly (Adesemoye et al., 2009). Mostly in many studies, effect of inoculants on crop yields were only detected in pot experiments and very few examples were found when these inoculants were test in field trials (Kaschuk et al., 2010). Our investigation was based on three experiments including in vitro and in vivo mainly focus on quantitative effects of inoculants on wheat crop individually, consortium of inoculants and with full and half recommended doses of chemical fertilizers. The increase in

the crop shoot length can be due to release of metabolites by bacteria (van Loon, 2007) and mineralization of nutrients which are easily available for plants. Increase in dry weight of wheat plants by application of PGPRs were also reported by (Prashant et al., 2009). The PGPRs also had positive effect on the number of tillers, an increase upto 25% in number of tillers in wheat by the application of PGPRs as reported by (Afzal et al., 2005) much less as compare to our results. The production of IAA by the rhizobacteria has been discussed as the cause of increase in tillers of the plant but still this factor cannot be the only one reason. Negative correlation ($R^2=0.91$) was observed in efficacy of PGP strains with increasing rate of inorganic fertilizers our finding was similar to the finding of (Shaharoona et al., 2008).The results correlate significantly with findings of (Saber et al., 2012). Results also showed that when PGPR inoculants were applied with full recommended dose of fertilizer the crop growth parameter and yield were lower than the half dose rate of recommended fertilizer with PGPR inoculants. They reported that under green house conditions dry weight of tomato with 75% fertilizers and two PGPR inoculants was significantly greater than from the full recommended dose of fertilizers without PGPR inoculants also reported that there is significant increase in root length due to application of PGPRs; they also state that phyto-hormones production by PGPRs can be major cause of increase in root length of plants (Shaharoona et al., 2008). There was significant increase in shoot length of wheat plants due to application of PGPRs (Akhtar et al., 2009). The results correlate significantly with findings of (Saber et al., 2012).

Numerous studies were conducted and PGPR used as inoculants for improvement of crop growth and yield. The selection of inoculants was very vital and critical step the inoculants used in our study were native and specific to the wheat crop. Effective biofertilzer/biocontrol agent against soil-born plant phytopathogen strains isolated from one region may not perform better in other

soil and climatic conditions due to the variability and inconsistency of soil and climate affects the benefit influence of PGPR (Duffy et al., 1997, Khalid et al., 2004). This study is based on our objective to reduce the chemical fertilizers by utilizing potential wheat rhizospheric bacteria as inoculants and all results showed that PGPR play important role and useful to reduce the rate of inorganic fertilizers. In recent decades investigation on PGPR revealed that it can promote plant growth directly or indirectly producing ACC deaminase it reduces the level of ethylene in the root of plant developing plants (Dey et al., 2004) by producing plant growth hormones like IAA (Mishra et al., 2010), exhibit antagonistic activity against phytopathogenic soil-borne pathogens by producing siderophore (Pathma et al., 2011) and mineral phosphates solubilization along other nutrients (Hayat et al., 2010). ACC deaminase activity was considered as an efficient marker for plant associated bacteria to improve plant growth by lowering the level of ethylene reserved in plants under stress conditions (Li et al., 2011). ACC deaminase activity producing PGPR significantly improve root growth under control conditions (Shaharoona et al., 2006a). Siderophore production by rhizospheric bacteria improve strains colonization and also important for iron nutrition of plant (Vansuy et al., 2007) antagonistic action against phylopathogen (Chincholkar et al., 2007b). Siderophore produced by *Pseudomonas sp.* efficiently used against soil born plant pathogen as biocontrol agent (Bholay et al., 2012). Indole acidic acid produced by bacterial strains promotes plant growth induces positive effect on crop yield (Swain et al., 2007). While inoculation was effective with inorganic fertilizer doses, its positive impact decreased with increasing rates of fertilizer application.

Conclusions:

We concluded that the application of PGPR in consortium and alone improves wheat yield and growth. The indigenous PGPR have more potential and useful to reduce the rate of inorganic

fertilizers. Also the non significant effects of inorganic fertilizers on soil health were conquering to some extent by application of PGPR with less dose of NPK. Moreover we utilize these PGPR with lower dose of fertilizer in environment friendly way and application of PGPR with suitable combination of chemical fertilizers were useful to get maximum benefit in term of growth and saving fertilizer. Native crop specific PGPR play vital role and enhance soil health even in short-term.

Material and methods:

Isolation and screening of soil bacteria:

Bacterial strains were isolated from wheat sandy loam rhizospheric soil (33º14'26.38" N and 72º23'10.29" E). Isolation of the stains was carried out by dilution plate technique using phosphate buffer saline as a saline solution and grown Tryptic Soya Agar (TSA; Difco) medium at 28 ºC for 48 hrs. Then single bacterial colonies were picked and streaked on TSA medium plates with the aim to achieve single colonies.

Plant growth promoting assay and biochemical characterization of soil bacteria:

Plant growth promotion activities like IAA production, phosphorus solubalization and presence of ACC deaminase activity, siderophore and biolog of strains were performed following standard procedures. For IAA production, bacterial cultures were grown in Tryptic Soya Broth (TSB). Supernatant was then mixed with 2 drops of Orthophosphoric acid and 4 mL of the Salkowski reagents and the optical density was determined at 530 nm using spectrophotometer. Development of pink color was an indicator of IAA production. IAA production by strains was measured by standard curve graph where standards range was up to 10 µg mL^{-1} (Brick et al., 1991). P- solublization was determined quantitatively as described by Pikovskaya (1948). The supernatant was measured for available phosphorus by the protocol given by Watanabe & Olsen

(1965). The optical density of the supernatant was determined at 700 nm using spectrophotometer and the values were determined by standard curve graph and standards range was up to 1 µg mL^{-1}. The estimation of quantitative siderophore produced by all ten strains was done through chrome azurol-S (CAS) assay. The color obtained was measured by using spectrophotometer at 630 nm. The siderophore unit was estimated by using proportion of CAS color shifted using the equation A/Ar where A is the absorbance of the sample (supernatant + CAS solution) and Ar is the absorbance of reference (uncultured medium + CAS solution). (Payne 1994). ACC deaminase activity of all strains was measured following procedure of Penrose and Glick (2003). The calibration curve was determined according to (Bradford, 1976). And for protein calibration curve, we used bovine serum albumin (BSA) (Penrose and Glick, 2003). The absorbance value was measured at 540 nm wavelength.

Identification of bacterial strains using 16S rRNA gene sequencing:

Standard method of 16S rRNA gene sequencing was used to identify the strains. Universal primers 9F and 1510R were used for PCR amplification (Yamamoto and Harayama 1995). The PCR product samples were sequenced using DNA sequencing service of MACROGEN, Korea. The sequence results were blast through NCBI/Eztaxon (Kim et al., 2012) and sequence of all related species were retrieved to get the exact nomenclature of the isolates. Phylogenetic analyses were performed using bioinformatics software MEGA-5 (Tamura et al., 2007). Other software used for sequence alignment and comparisons were CLUSTAL X and BioEdit. DNA accession numbers of each strain were obtained from National Center for biotechnology information (NCBI). The accession number allotted by NCBI for strains from RA-1 to RA-10 was KF848983 to KF848992 respectively.

Response of wheat to potential soil bacteria under controlled and field conditions:

Wheat experiments were conducted at Department of soil science and soil water conservation, PMAS-Arid Agriculture University Rawalpindi Pakistan. One month growth chamber experiment (GC) was conducted in rhizo beg trays using sterilized soil. In growth chamber experiment all isolated strains were tested along control with four replications under CRD as the experimental design. In pot trial, plastic pots were used with 4 kg sterilized soil. Six potential strains were shortlisted on the bases of growth chamber experiment results and inoculated to wheat seeds. Similarly same six strains were tested in field trial. For all experiments wheat cultivar Chakwal 50 was used. The field soil texture was sandy loam(clay14%, silt 16%, sand 70%). Soil was alkaline with a pH of 7.2 along Available P (7.2 µg g^{-1}), exchangeable K (119 µg g^{-1}), NO_3-N (3.04 µg g^{-1}) and total organic carbon (TOC) (0.47 g 100 g^{-1}). Plant parameters like shoot length, root length, fresh and dry weight were recorded after a month of germination in GC experiment and at harvesting stage in pot and field trail. The full recommended dose of NPK for wheat was used 100-80-60 kg h^{-1} respectively. The inorganic source of NPK used were urea, DAP and MOP respectively was applied at the time of sowing along different combination with potential bacterial strains.

Whole-cell fatty acid analysis

All ten strains were grown on TSA plates and incubated at 30 ºC for 2 days. Sherlock microbial identification system (MIDI) (Library RTSA6 6.0, MIDI Sherlock software package, version 6.0) was used for determination of cellular fatty acid composition. Strains were harvested and fatty acid methyl esters were prepared as described by Sasser (1990).

Statistics:

The obtained crop data was analyzed statistically by using statistix 8.1 through ANOVA and the means were compared using LSD test with significance level of ≤ 0.05 (Steel et al., 1997).

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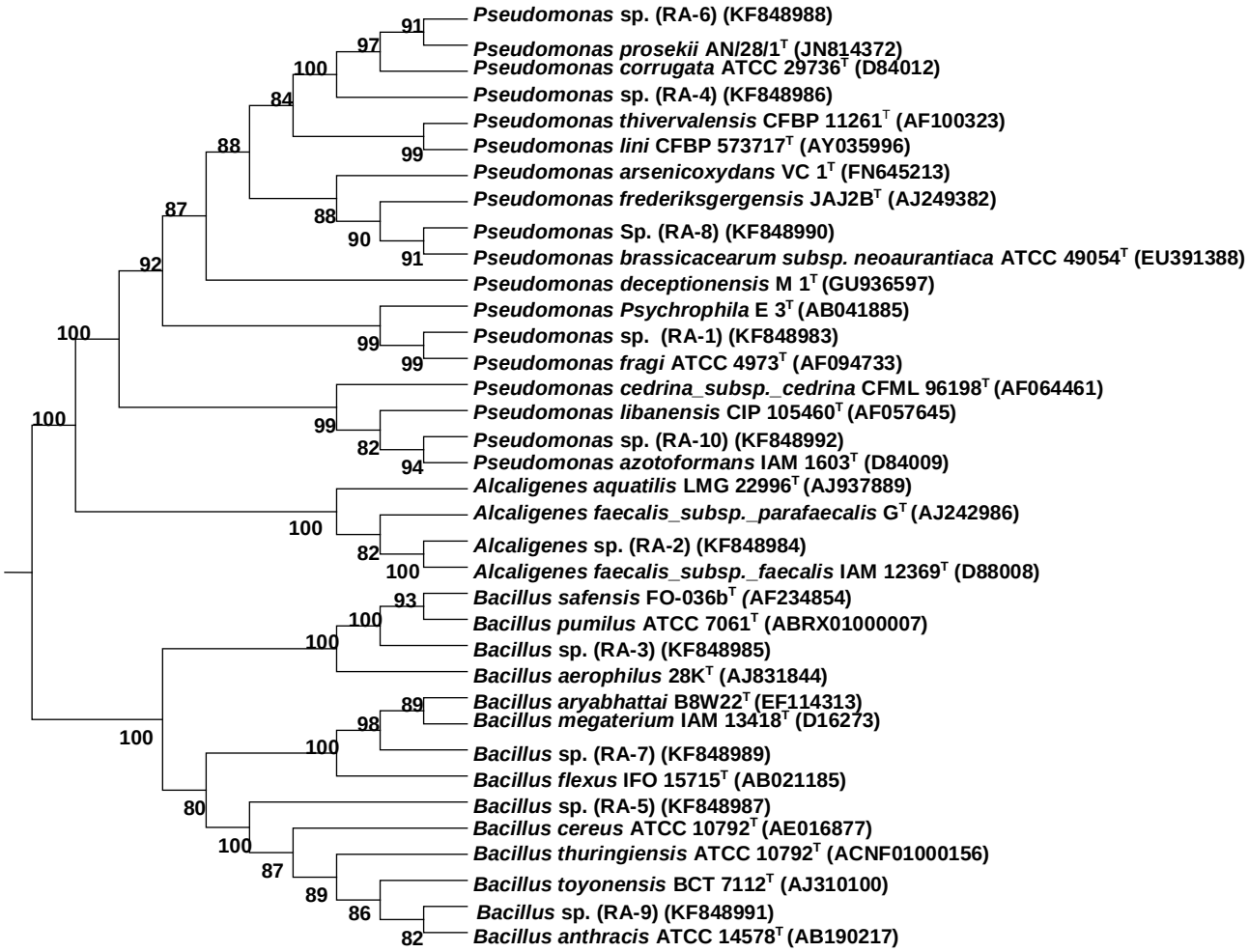

FIG 1: Neighbor-joining phylogenetic dendrogram based on a comparison of the 16S rRNA gene sequences of the wheat rhizospheric representative isolates and some of their closest phylogenetic taxa.

	P- solubilization (μgmL^{-1})±S.E	**pH** (7.0)	**ACC-deaminase activity (nmol h^{-1})**	**Siderophore activity level**	***A/Ar***	**IAA mg L^{-1} with tryptophan** ($\mu g\ mL^{-1}$)±S.E	**IAA mg L^{-1} without tryptophan** ($\mu g\ mL^{-1}$)±S.E
RA-1	84.41±1.66	5.74	654± 54	+++	0.478±0.025	2.08±0.085	1.36±0.13
RA-2	117.73±2.41	5.12	754±121	+++	0.514±0.021	1.84±0.060	1.24±0.091
RA-3	93.34±1.80	4.82	541±47	+++	0.361±0.017	2.04±0.11	2.42±0.40
RA-4	88.18±1.77	5.02	475±69	++	0.723±0.029	3.50±0.27	1.30±0.17
RA-5	162.16±1.46	4.58	589±79	++	0.651±0.031	2.3±0.098	1.06±0.067
RA-6	127.84±1.59	4.75	671±56	++	0.715±0.042	2.61±0.28	1.097±0.035
RA-7	212.47±2.72	4.38	621±98	+++	0.586±0.015	12.02±0.61	2.408±0.31
RA-8	104.34±0.98	4.55	782±79	+++	0.681±0.034	9.51±0.73	2.17±0.28
RA-9	105.81±1.80	5.12	480±56	++	0.814±0.044	1.91±0.13	1.32±0.32
RA-10	110.73±2.82	4.98	590±74	+	0.976±0.036	2.12±0.086	1.36±0.16

Table 1: Plant growth promoting traits of strains isolated from wheat rhizosphere:

All values are average of three replicates

Table 2: identification of soil bacteria on the bases of 16S rRNA gene sequencing

	16S rRNA gene (bp)	DDBJ Accession number for 16S rRNA gene sequence	Closely related Taxa (Species)	Type Strain (gene bank ID)	DDBJ Accession of 16S rRNA gene sequence	Similarity (%)
RA-1	1330	KF848983	*Pseudomonas fragi*	ATCC 4973$^{(T)}$	AF094733	99.47
RA-2	1332	KF848984	*Alcaligenes faecalis subsp. faecalis*	IAM12369$^{(T)}$	D88008	99.1
RA-3	1321	KF848985	*Bacillus safensis*	FO-036b$^{(T)}$	AF234854	100
RA-4	1467	KF848986	*Pseudomonas corrugate*	ATCC 29736$^{(T)}$	D84012	99.23
RA-5	1335	KF848987	*Bacillus cereus*	ATCC 14579$^{(T)}$	AE016877	100
RA-6	1302	KF848988	*Pseudomonas arsenicoxydans*	VC-1$^{(T)}$	FN645213	99.31
RA-7	1323	KF848989	*Bacillus aryabhattai*	B8W22$^{(T)}$	EF114313	100
RA-8	1280	KF848990	*Pseudomonas brassicacearum subsp. neoaurantiaca*	ATCC 49054$^{(T)}$	EU391388	99.92
RA-9	1327	KF848991	*Bacillus thuringiensis*	ATCC 10792$^{(T)}$	ACNF01000156	100
RA-10	1311	KF848992	*Pseudomonas azotoformans*	IAM1603$^{(T)}$	D84009	99.62

All values are average of three replicates

Table 3: Effect of inoculation on shoot, root and plant biomass under growth chamber condition

	Shoot length (cm)	(%)	Root length (cm)	(%)	Fresh weight (gm)	Dry weight (gm)
Control	15.9±2.23 D	100	5.13±0.93 F	100	1.47±0.46 D	0.76±0.15 E
RA-1	22.8±3.18 BC	143	7.44±1.01DEF	145	2.96±1.70 CD	1.24±0.44 CDE
RA-2	27.9±3.16 A	175	10.69±2.63ABCD	208	5.69±1.94 AB	2.79±1.39 AB
RA-3	22.1±3.09 C	139	6.85±1.21EF	134	2.75±1.34 CD	1.14±0.81 DE
RA-4	26.7±3.41AB	168	8.81±2.06 CDE	172	2.99±1.14 CD	1.68±1.34 BCDE
RA-5	22.3±3.06 C	140	7.96±1.04 CDEF	155	2.64±1.19 D	1.37±1.22 CDE
RA-6	28.2±3.45 A	177	13.37±3.17 A	261	6.01±1.54 AB	3.98±1.06 A
RA-7	28.9±3.55 A	182	12.34±2.03 AB	241	6.40±1.09 A	3.96±1.26 A
RA-8	25.7±3.97 ABC	162	11.25±2.45 ABC	219	4.53±1.57 BC	2.35±1.17 BC
RA-9	22.9±3.19 BC	144	7.68±1.24 DEF	150	3.13±1.36 CD	1.71±1.31 BCDE
RA-10	27.7±4.09 A	174	9.91±2.19 BCDE	193	5.26±2.19 AB	2.39±1.09 BCD
CV	10.44		21.42		26.40	37.16
P-value	0.0001		0.001		0.0000	0.0004

All values are average of three replicate

Table 5: Effect of inoculation with PGP traits on plant height, grain and yield under field condition

Treatments	plant height		1000 grain wt.		Total biomass	
	(cm)	(%)	(g)	(%)	(kg ha^{-1})	(%)
Control	57.8 I	100	32.53 J	100	3902 I	100
Half dose	61.7 I	108	37.09 I	114	4700 G	120
Full dose	100.8 BC	177	61.08 D	188	5709 C	146
RA-2	79.3 E	139	55.86 E	172	4977 F	128
RA-4	73.8 FG	129	48.78 H	150	4439 H	114
RA-6	76.9 EF	135	54.22 EF	167	5206 E	133
RA-7	92.9 D	163	53.54 F	165	5452 D	140
RA-8	84.5 EF	148	56.28 G	173	5657 C	145
RA-10	86.1 E	151	48.92 H	150	4728 G	121
RA-2+ RA-4+RA-6	73.0 H	128	54.91 F	169	5157 E	132
RA-7, RA-8, RA-10	77.8 G	136	53.43 F	164	5127 E	131
Consortium 1 + half dose	103.8 AB	182	69.76 BC	214	6337 B	162
Consortium 1 + Full dose	97.1CD	170	68.58 C	211	6256 B	160
Consortium 2 + half dose	107.1 A	188	75.94 A	233	6597 A	169
Consortium 2 + Full dose	99.7 BC	175	70.64 B	217	6283 B	161
CV	**1.36**		**1.80**		**1.26**	
P-value	**0.0000**		**0.0000**		**0.0000**	

Consortium 1(RA-2,4,6)
Consortium 2(RA-7,8,10)

Table 6: Cellular Fatty Acid Profiles (%) of bacterial strain:

Characteristics	RA-1	RA-2	RA-3	RA-4	RA-5	RA-6	RA-7	RA-8	RA-9	RA-10
C $_{10:0}$ 3OH	3.78	-----		3.65	0.06	2.6	-----	3.27	-----	-----
$C_{12:0}$	2.75	2.41	-----	5.66	0.44	5.18	0.19	5.21	-----	1.46
C $_{12:0}$ 2OH	4.9	-----	-----	1.95	-----	3.11	-----	2.68	-----	1.35
C $_{12:0}$ 3OH	4.85	-----	-----	3.7	-----	3.69	-----	4.16	------	1.53
$C_{14:0}$	1.37	5.82	-----	-----	3.19	-----	1.59	0.55	3.55	1.51
$C_{16:0}$	30.19	25.84	7.41	28.11	5.52	27.16	9.18	29.95	17.73	15.96
$C_{17:0}$	-----	-----	-----	-----	0.36	-----	0.23	0.14	1.01	-----
$C_{18:0}$	-----	2.31	4.34	1.29	2.99	1.57	-------	2.69	11.56	2.63
Anteiso-$C_{13:0}$	-----	-----	-----	-----	1.21	-----	0.41	--------	-----	-----
Anteiso-$C_{14:0}$	1.04	-----	-----	-----	1.08	-----	0.4	--------	-----	-----
Anteiso-$C_{15:0}$	-----	-----	23.46	-----	4.89	-----	29.72	0.1	3.48	33.49
Anteiso-$C_{17:0}$	-----	-----	8.82	-----	1.98	-----	5.62	0.06	1.59	3.32
Anteiso 17:1 A	-----	-----	-----	-----	1.88	-----	------	------	-----	-----
Cyclo 17:0	7.61	5.61	-----	5.05	-----	3.86	-------	4.15	-----	2.07
iso-$C_{13:0}$	-----	-----	-----	-----	5.23	-----	0.21	--------	3.68	-----
iso-$C_{14:0}$	-----	-----	1.43	-----	3.58	-----	5.98	---------	4.86	2.19
iso-$C_{15:0}$	-----	-----	27.42	-----	28.5	-----	25.88	0.15	8.51	2.64
iso-$C_{16:0}$	-----	-----	5.62	-----	5.51	-----	4.64	-------	6.09	4.28
iso-$C_{17:0}$	-----	-----	10.31	-----	9.62	-----	4.04	0.04	5.44	-----
Iso C $_{17:1}$ w5c	-----	-----	-----	-----	4.16	-----	------	-------	2.64	-----
iso $C_{17:1}$ ω10c	-----	-----	-----	-----	1.54	-----	0.29	--------	1.34	-----
Cyclo $_{19:0}$ ω8c	-----	1.35	-----	-----	-----	-----	------	0.4	-----	-----
$C_{15:1}$ ω5*c*	-----	-----	-----	-----	1.03	-----	0.09	-------	-----	-----
$C_{16:1}$ ω11*c*	-----	-----	-----	-----	0.25	-----	1.28	-------	-----	-----
$C_{18:1}$ ω9*c*	-----	-----	1.73	-----	-----	-----	1.48	0.29	4.24	1.56
Summed Feature 1	-----	-----	-----	-----	-----	-----	------	0.21	1.12	-----
Summed Feature 2	-----	7.34	-----	-----	3.01	-----	------	0.11	2.69	-----
Summed features 3	25.87	16.59	-----	21.68	6.99	24.31	0.22	28.05	6.08	10.31
Summed features 8	7.37	25.1	3.52	22.97	-------	24.26	1.39	15.27	2.8	9.8

Table 7: Biolog of bacterial strains

	24 hour									
	RA1	RA2	RA3	RA4	RA5	RA 6	RA7	RA 8	RA9	RA10
Water	-	-	-	-	-	-	-	-	-	-
α-Cyclodextrin	-	+	-	-	-	-	-	-	+	-
β-Cyclodextrin	-	+	-	W+	W+	+	-	W+	+	W+
Dextrin	W+	+	-	-	-	-	W+	-	+	-
Glycogen	+	+	-	+	-	+	+	+	+	+
Inulin	+	+	-	+	-	+	-	+	+	+
Mannan	-	+	-	-	-	-	W+	-	+	-
Tween 40	-	+	-	+	+	+	+	W+	+	+
Tween 80	-	+	-	-	+	-	+	-	+	+
N-Acetyl-D-Glucosamine	W+	+	W+	+	-	+	+	+	+	+
N-Acetyl-β-D-Mannosamine	-	+	-	+	-	+	+	+	+	+
Amygdalin	-	+	-	-	-	W+	-	-	-	-
L-Arabinose	-	+	-	-	-	-	W+	-	+	+
D-Arabitol	-	+	+	+	-	+	-	+	W+	+
Arbutin	-	+	-	-	-	-	-	-	-	-
D-Cellobiose	-	+	-	+	W+	+	W+	+	-	+
D-Fructose	-	+	-	-	-	-	+	-	W+	-
L-Fucose	-	+	-	+	-	+	-	+	-	+
D-Galactose	-	+	-	+	-	+	W+	+	-	+
D-Galacturonic Acid	-	+	-	-	-	-	-	-	-	-
Gentiobiose	-	+	-	-	-	-	+	-	-	-
D-Gluconic Acid	-	+	-	-	-	-	W+	-	-	-
α-D-Glucose	-	+	-	+	+	+	+	+	+	+
m-Inositol	-	+	-	+	-	+	-	+	+	+
α-D-Lactose	-	+	-	-	-	-	+	-	-	-
Lactulose	-	+	-	-	-	-	+	-	-	-
Maltose	-	+	-	-	+	-	+	-	+	-
Maltotriose	-	+	-	-	+	-	+	-	+	-
D-Mannitol	-	+	-	-	-	-	W+	-	-	-
D-Mannose	-	+	-	-	-	-	+	+	-	+
D-Melezitose	-	+	-	+	-	+	+	-	-	+
D-Melibiose	-	+	-	+	-	+	W+	+	-	+
α-Methyl-D-Galactoside	-	+	-	-	-	-	+	-	-	-
β-Methyl-D-Galactoside	-	+	-	-	-	-	W+	-	-	-
3-Methyl-D-Glucose	+	+	-	+	W+	+	+	+	+	+
α-Methyl-D-Glucoside	+	+	-	W+	-	+	+	+	-	W+
β-Methyl-D-Glucoside	+	+	-	+	-	+	+	+	+	+
α-Methyl-D-Mannoside	+	+	-	+	-	+	W+	+	-	+
Palatinose	+	+	-	+	-	+	+	+	-	+
D-Psicose	+	+	-	+	-	+	+	-	-	W+
D-Raffinose	-	+	-	-	W+	+	+	+	W+	+
L-Rhamnose	-	+	-	-	-	+	-	+	-	+
D-Ribose	-	+	+	+	W+	+	+	+	+	+
Salicin	-	+	-	+	W+	+	+	+	+	+
Sedoheptulosan	-	+	-	-	-	+	W+	+	-	+
D-Sorbitol	+	-	-	W+	W+	+	+	-	-	W+
Stachyose	+	+	-	+	-	+	W+	+	-	+

Sucrose	W+	+	-	-	W+	+	+	-	+	-
D-Tagatose	+	+	-	-	-	-	-	-	-	+
D-Trehalose	-	-	-	-	W+	-	+	-	+	+
Turanose	+	-	-	-	W+	+	+	-	-	+
Xylitol	-	-	-	+	-	+	-	+	-	+
D-Xylose	+	+	+	-	-	W+	+	-	-	W+
Acetic Acid	+	+	-	+	-	+	-	+	W+	+
α- Hydroxybutyric Acid	+	+	-	+	-	+	W+	+	-	+
β-Hydroxybutyric Acid	+	+	-	+	-	+	+	+	W+	+
γ-Hydroxybutyric Acid	-	-	-	+	W+	+	W+	+	+	+
p-Hydroxy-phenylacetic Acid	-	+	-	+	-	+	-	+	-	+
α-Ketoglutaric Acid	-	-	-	-	W+	-	W+	-	W+	-
α-Ketovaleric Acid	+	+	-	+	W+	+	+	+	+	+
Lactamide	+	+	+	+	-	+	+	+	-	+
D-Lactic AcidMethyl Ester	+	-	-	-	W+	W+	-	-	W+	-
L-Lactic Acid	-	+	-	W+	-	+	+	W+	-	W+
D-Malic Acid	+	+	-	+	-	+	-	+	-	+
L-Malic Acid	+	+	-	+	-	+	+	+	-	+
Pyruvic Acid Methyl Ester	+	+	-	+	-	+	-	+	-	+
Succinic AcidMono-MethylEster	-	+	-	+	-	+	-	+	-	+
Propionic Acid	+	+	-	+	-	+	-	+	W+	+
Pyruvic Acid	+	+	-	+	+	+	W+	+	+	+
Succinamic Acid	+	+	-	+	-	+	+	+	W+	+
Succinic Acid	-	+	-	-	-	-	+	-	+	-
N-Acetyl-L-GlutamicAcid	W+	+	-	+	-	+	W+	+	W+	+
L-Alaninamide	+	+	-	+	-	+	W+	+	+	+
D-Alanine	-	-	-	W+	-	+	+	+	-	+
L-Alanine	+	+	-	+	W+	+	+	+	-	+
L-Alanyl-Glycine	+	+	-	W+	W+	W+	+	-	+	+
L-Asparagine	+	+	-	-	-	-	+	-	-	-
L-Glutamic Acid	+	+	-	+	-	+	W+	+	+	+
Glycyl-L-Glutamic Acid	+	-	-	+	-	+	W+	+	+	+
L-Pyroglutamic Acid	W+	+	-	-	-	W+	W+	-	-	W+
L-Serine	W+	+	-	+	-	+	+	+	-	+
Putrescine	+	+	-	W+	W+	+	-	W+	-	+
2,3-Butanediol	-	-	-	+	W+	+	W+	+	-	W+
Glycerol	W+	-	-	+	-	+	+	+	+	+
Adenosine	+	+	-	+	-	+	+	+	+	+
2’-Deoxy Adenosine	-	+	-	+	W+	+	+	+	-	+
Inosine	-	+	-	+	W+	+	+	+	+	+
Thymidine	-	+	-	-	-	-	+	-	+	-
Uridine	W+	+	-	-	W+	-	-	-	+	-
Adenosine-5' Monophosphate	-	+	-	+	-	+	+	W+	+	-
Thymidine-5'-Monophosphate	-	-	-	+	-	+	W+	+	+	+
Uridine-5'-Monophosphate	-	-	-	-	W+	W+	+	-	-	-
D-Fructose-6-Phosphate	-	+	-	+	-	+	+	+	-	+
α-D-Glucose-1-Phosphate	-	+	-	+	-	+	W+	W+	W+	-
D-Glucose-6-Phosphate	-	+	-	-	-	-	W+	-	+	-
D-L-α-Glycerol Phosphate	-	+	-	-	-	-	+	-	+	-

Table 4: Effect of inoculation with PGP traits on wheat crop under pot trial

	Shoot length (cm)	(%)	Root length (cm)	(%)	No. of Tillers	Fresh weight (gm/plant)	Dry weight (gm/plant)	Spike length (cm)	Grain yield (Kg ha^{-1})	(%)
Control	66.61±5.16F	100	19.56±3.35F	100	4D	17.72±2.54GE	6.64±1.23E	6.70±0.99B	2900.43±203E	100
RA-2	88.39±8.1C	133	27.82±3.45BC	142	8C	26.98±3.19DE	8.63±2.65DE	7.55±1.27B	4119.82±249C	142
RA-4	84.01±8.6E	126	24.97±4.15CDE	128	6CD	21.87±2.15FG	7.92±1.19DE	6.99±1.09B	4158.98±293C	143
RA-6	6.96±8.4DE	131	25.87±4.58CD	132	6CD	22.52±2.19EF	8.32±2.19DE	7.19±2.14B	3612.36±353D	125
RA-7	90.02±8.9C	135	21.56±4.19EF	110	9BC	28.55±2.48CD	8.36±2.22DE	8.51±2.06B	4206.76±393C	145
RA-8	82.83±9.5E	124	22.21±5.16DEF	114	8C	24.27±3.76DEF	8.66±2.14DE	7.59±2.58B	4103.22±416C	141
RA-10	86.44±8.4DE	130	21.57±4.78EF	110	7CD	25.48±2.93DEF	9.87±3.09CD	7.85±2.77B	3805.54±347D	131
Full dose	94.42±9.8A	142	32.72±5.9A	167	12AB	36.41±2.41B	12.92±3.44AB	10.9±3.34A	4812.96±389A	166
Half dose	86.42±8.1D	130	24.52±4.9CDE	125	8C	28.55±2.55CD	9.96±2.34CD	7.41±2.06B	3706.56±338D	128
Consortium+ Half dose	97.83±8.7A	147	31.18±5.5AB	159	15A	43.27±5.97A	14.66±4.54A	12.9±2.67A	4903.92±416A	169
Consortium+ Full dose	94.56±8.9B	142	28.12±5.1BC	144	14A	32.48±4.18BC	11.87±4.15BC	10.8±2.97A	4605.84±347B	159
CV	**2.84**		**9.14**		**2.99**	**9.84**	**14.24**	**14.86**	**21.64**	
P-value	**0.0000**		**0.0000**		**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	

All values are average of three replicates and % column indicate change in percentage with reference to control