RESEARCH ARTICLE

CHARACTERIZATION OF POTENTIAL PGPR'S ISOLATED FROM RHIZOSPHERE OF WHEAT FROM TRANS-HIMALAYAS AND THEIR EFFICACY ON SEED GERMINATION AND GROWTH PROMOTION OF WHEAT UNDER NET HOUSE CONDITIONS

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Abstract: In the present study, the diversity of rhizobacterial isolates from rhizosperic soils under wheat cultivation in districts of Solan and Sirmour of Himachal Pradesh a Himalayan belt of India. Phenotypic and genotypic characteristics of the PGPR isolates were recorded to categorize and identify the bacteria. In total seventy three rhizobacterial isolates were isolated from different locations of both the districts of which some sites were rainfed and some sites were irrigated. The characteristics of the bacterial isolates were determined using the colony morphology, gram staining as well as biochemical properties. After screening for PGP attributes *in-vitro* conditions. Three isolates (Kn-7, De-21 and Dh-7) were found hyperpotential for PGP attributes such as production of siderophore, P-solubilization, ammonia, HCN and growth regulators. These three isolates had shown maximum PGP potential *in- vitro* conditions and thus were selected to construct bioformulations for the wheat crop under net house conditions.

Keywords: Wheat, PGPR, Rhizosphere, PGP Attributes, Growth Promotion of wheat

INTRODUCTION

Wheat is a commercially important crop belonging to gramineae family. At present India is the second largest producer of wheat after China. The use of microorganisms in agriculture is at a low level despite the investment in scientific work. Microbial inoculants can be used as an alternative to chemical fertilizers in view of the damaging effect of pesticides, fungicides and insecticides. Plant Growth Promoting Rhizobacteria (PGPR) is such groups of bacteria that colonize the rhizosphere and improve plant growth. The use of PGPR can be used in the future to enhance agricultural production. PGPR's also played an important role in enhancing the root and shoot growth, and act as efficient microbial competitors in the root zone. Significant effects have been observed in wheat. The use of PGPR reduces soil borne pathogens and thus enhances plant growth. Himachal Pradesh is an important Himalayan state for wheat cultivation the state has unique pattern of terraced cropping system.

Agriculture status of Himachal Pradesh

Himachal Pradesh is situated in the north-western part of Himalaya. Most of the geographical area of the state comes under forest, pasture, and grazing land, agriculture is possible only on less than ten percent of the state's net area. The physiography and climatic condition in the state favours diversified potential for farming and allied activities. Due to the undulating terrain condition ranging from plains to high hills, mixed farming is predominant. Most of the farming activities are concentrated along the channels of major rivers and their tributaries. Different crops are being cultivated in the state. Among the cereals, wheat, rice, maize, and barley are important. The state also produces pulses and oilseeds. Cash crops are also becoming important, since fair amounts of potatoes, ginger, tea, and peas come from the state. Fruits, dry fruits, and a variety of vegetables are grown in the state Kant S (1995). Mechanisms that can promote plant growth include production of phytohormones, biological nitrogen fixation and increased solubility of insoluble elements in soil (Rovera *et al*., 2008). Interest in the beneficial rhizobacteria associated with cereals has increased recently and several studies clearly demonstrated the positive and beneficial effects of PGPR on growth and yield of different crops especially wheat at different environment under variable ecological conditions (Mehnaz *et al.*, 2010, Zhang *et al.*, 2012).

PGPR

PGPR are free living bacteria that resides in the rhizosphere region in the soil. They either directly or indirectly assist rooting. They play different roles in the soil which proves beneficial for plant health and productivity.

The mechanism by which PGPR exerts their beneficial effect on plants can be very diverse. They can establish themselves on root surface or inside the roots. PGPR can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) that may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex. The bacterial general such as *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Flavobacterium,*

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Pseudomonas and *Serratia* belong to ePGPR. The other category is intracellular plant growth promoting rhizobacteria (iPGPR) that locates generally inside the specialized nodular structures of root cells (Figueiredo *et al.*, 2011). It belongs to the family of *Rhizobiaceae* includes *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*, endophytes and *Frankia* species both of which can symbiotically fix atmospheric nitrogen with the higher plants.

PGPR are free living or symbiotic associated bacteria that reside in rhizospheric soil or intracellularly as endophytes. They play very important roles in the soil which proves beneficial for plant health and productivity. They colonize the rhizosphere and protect plants from its pathogens, by producing secondary metabolites such as antibiotics, volatile compounds that suppress harmful pathogenic bacteria and fungi by different mechanisms. PGPR's also produce siderophores (iron chelating compounds), and phytohormones (Auxins, Gibberellins and Cyatokinins), can fix atmospheric nitrogen, and help in providing nutrition uptake by solubilizing phosphate and produce biologically active substances which influence the plant growth and development (Mayak *et al.*,1999).

Keeping in mind the present study was planned to isolate the native strains from rhizosphere of wheat grown on different soils of Solan and Sirmour districts of Himachal Pradesh. These bacteria were characterized and screened *in vitro* for PGP potentials. Furthermore to evaluate the efficacy of selected strains of PGPR in seed germination and growth promotion of wheat under net house conditions.

MATERIALS AND METHODS

Sample Collection, isolation and purification of PGPR: Soil samples were collected from wheat rhizosphere from different locations of Solan (Kandaghat, Deothi, Dharja) and Sirmour (Rajgarh, Habban, Pulwahal) districts of H.P. Nitrogen-free medium (Jensen medium), Luria Bertani agar, King's B agar and Nutrient agar medium were used for isolation of PGPR by serial dilutions method followed by purification on the same solid media with a repeated plating method.

Colony morphology and pigment production

Colony morphology (form, elevation, and margin, and opacity, surface) and the production of pigment was checked on Nutrient agar medium at 28±2 ^{0} C.

Biochemical characterization

Biochemical characteristics of the purified PGPR's isolates like Gram reaction, catalase reactions, methyl red, Voges–Proskauer test, citrate utilization, casein hydrolysis (Subba Rao, 1977).

Bio assays for Plant Growth Promoting Attributes

P-Solubilization

For estimation of phosphate solubilizing capacity of PGPR isolates. Pikovskaya agar plates (Pikovskaya's, 1948) with known amount of inert phosphorus ($Ca_3(PO_4)_2$). Phosphate solubilization expressed in terms of mm diameter of yellow colored zone produced around well/bit at 28^0C after 72h.

Siderophore production

Siderophores production was detected by chrome azurol-S (CAS) plate assay method (Schwyn and Neilands, 1987). 25 ml of CAS dye was mixed with 250 ml of nutrient agar and mixed well before pouring. 100µl of 72 h old culture supernatant of each test bacteria was placed on prepoured chrome azurol-S agar (CAS) plates. Plates were incubated at 28^0 C for 72 h. Production of siderophore was expressed in terms of mm diameter of pinkish/orange halo zone produced around the well at 28^0 C in 72h.

Ammonia production

Ammonia production was checked according to Lata and Saxena (2003). PGPR's isolates were grown in peptone water (5 ml) in tubes. Tubes were incubated at 28^0 C for 4 days. 1ml of Nessler's reagent was added to each culture tube. Presence of faint yellowish to brown color (+) indicated small amount of ammonia and deep yellow (++) to brown color (++++) indicated large amount of ammonia production.

HCN production

PGPR's isolates were screened out for the production of hydrogen cyanide (HCN). (Bakker and Schippers, 1987) bacterial cultures were streaked on prepoured plates of nutrient agar medium amended with 1.4 g/l glycine. Whatman No.1 filter paper strip were soaked in 0.5 per cent picric acid followed by 2 per cent sodium carbonate and were placed in the lid of each petriplates. Petriplates were sealed with parafilm and were incubated at 28^0 C for four days. Uninoculated control with picric acid paper strips was kept for comparison of results. Plates observed for change of color of filter paper from yellow (-) to brown (+++) to dark brown (+++++). Intensity of color developed indicated as high production of volatile HCN.

Plant Inoculation and Root Colonization

Pot experiment in net house conditions

Pot experiment was conducted under the net house conditions. Plastic pots having (20 cm diameter, 20 cm deep) were used for this experiment containing 3 kg of sterilized soil. Fresh culture of each isolates was used for the each treatment. Total twenty five treatments were used for the study in which individual, consortia of each isolate and recommended dose of fertilizer were used in each treatment. Seeds were surface sterilized with 0.1% $HgCl_2$ for the prevention of surface fungal/bacterial contamination. Sterilized seeds were coated with bacteria by dipping the seeds of wheat in liquid bioformulation. The pot experiment contained control (no bacterial inoculation) and inoculation with bacterial culture in the form of individual and

consortia of isolates. Pots were incubated in net house conditions at temperature 16-20^{0}C (day/night) for 50 days and after a week germination percentage and shoot length were calculated as seedling growth parameters (Meena *et al.*, 2016). Three individual isolates (Kn-7, Dh-7 and De-23) and their consortia (Kn-7+ Dh-7 + De-23) along with different doses of fertilizer.

Estimation of chlorophyll content of leaves (Withem *et al.*, 1971)

Fresh leaves of wheat were collected from the field and were weighed 1gm of each treatment. Dip the leaves in 80% acetone in a test tube, keep it overnight as such and record the optical density on next day at two wavelengths i.e. A_{663} and A_{645}. Chlorophyll a, chlorophyll b and total chlorophyll in mg/g of tissue was calculated by formula,

mg of chlorophyll a/g tissue =12.70 (A_{663})-2.69(A_{645}) ×V/ w×1000

mg of chlorophyll b/g tissue = 22.9 (A_{645})- 4.68(A_{663}) ×V/w×1000

mg total chlorophyll /g tissue=20.0 (A_{645})+8.02 (A663)×V/w×100

Where, A= optical density

V=final volume of 80% acetone chlorophyll extract (10ml)

W= fresh weight in gm of tissue extract (1gm)

RESULTS

Isolates from soil samples

Total seventy three PGPR's isolates were isolated from different rhizosperic soil samples of wheat from different locations of Solan and Sirmour districts of Himachal Pradesh on specific nutrient media (Nitrogen-free medium (Jensen media), Luria Bertani agar, King's B agar and Nutrient agar medium). Out of total seventy three isolates of PGPR, twenty nine hyperpotential PGP rhizobacteria were selected for further studies after primary (qualitative) screening *in-vitro* conditions.

Table 1. Characterisation of isolates isolated from rhizospheric soil of wheat from different sites (Deothi , Kandaghat, Dharja, Habban, Rajgarh and Pulwahal) of Solan and Sirmour districts of Himachal Pradesh

Sr.no.	Isolate	Isolated from	Colony size and shape	Colony color	Cell shape	Gram reaction	Catalase
1	**De-1**	**Deothi**	Medium, Round	White	Small rods	+	+
2	**De-2**	**Deothi**	Medium, Round	Milky white	Small rods	+	+
3	**De-3**	**Deothi**	Medium, Round	White	Small rods	+	+
4	**De-4**	**Deothi**	Medium, Round	White	Small rods	+	+
5	**De-5**	**Deothi**	Large, Round	Dark yellow	Cocci	+	+
6	**De-6**	**Deothi**	Medium, Round	White	Small rods	+	+
7	**De-7**	**Deothi**	Medium, Round	White	Small rods	+	+
8	**De-8**	**Deothi**	Large, Round	Off-white	Small rods	+	+
9	**De-9**	**Deothi**	Medium, Round	Milky white	Small rods	+	+
10	**De-10**	**Deothi**	Medium, Round	White	Small rods	+	+
11	**De-11**	**Deothi**	Large, Round	Off-white	Small rods	+	+
12	**De-12**	**Deothi**	Medium, Round	Milky white	Small rods	+	+
13	**De-13**	**Deothi**	Medium, Round	White	Small rods	+	+
14	**De-14**	**Deothi**	Small, Round	Milky white	Small rods	+	+
15	**De-15**	**Deothi**	Medium, Round	White	Cocci	-	+
16	**De-16**	**Deothi**	Medium, Round	Milky white	Small rods	+	+
17	**De-17**	**Deothi**	Medium, Round	Off-white	Small rods	-	+
18	**De-18**	**Deothi**	Medium, Round	White	Cocci	+	+
19	**De-19**	**Deothi**	Small, Round	Off-white	Small rods	-	+
20	**De-20**	**Deothi**	Medium, Round	Off-white	Small rods	+	+
21	**De-21**	**Deothi**	Medium, Round	White	Small rods	-	+
22	**De-22**	**Deothi**	Medium, Round	Milky white	Small rods	-	+
23	**De-23**	**Deothi**	Medium, Round	White	Cocci	+	+
24	**De-24**	**Deothi**	Medium, Round	Milky white	Small rods	-	+
25	**Kn-1**	**Kandaghat**	Small, Round	Yellowish green	Small rods	-	+
26	**Kn-2**	**Kandaghat**	Medium, Round	Greenish yellow	Small rods	-	+
27	**Kn-3**	**Kandaghat**	Medium, Round	Dark yellow	Medium rods	-	-
28	**Kn-4**	**Kandaghat**	Medium, Round	Yellowish green	Small rods	-	-
29	**Kn-5**	**Kandaghat**	Small, Round	Greenish	Small rods	-	-

				yellow			
30	**Kn-6**	**Kandaghat**	Medium, Round	Greenish yellow	Cocci	-	+
31	**Kn-7**	**Kandaghat**	Medium, Round	Yellowish green	Small rods	-	-
32	**Kn-8**	**Kandaghat**	Small, Round	Greenish yellow	Small rods	+	-
33	**Kn-9**	**Kandaghat**	Medium, Round	Dark yellow	Thin rods	-	-
34	**Kn-10**	**Kandaghat**	Medium, Round	White	Small rods	-	+
35	**Kn-11**	**Kandaghat**	Small, Round	Yellowish green	Small rods	-	-
36	**Kn-12**	**Kandaghat**	Medium, Round	Yellowish green	Cocci	-	-
37	**Kn-13**	**Kandaghat**	Medium, Round	Greenish yellow	Oval	-	-
38	**Kn-14**	**Kandaghat**	Medium, Round	White	Small rods	-	-
39	**Kn-15**	**Kandaghat**	Small, Round	Greenish yellow	Medium rods	-	+
40	**Kn-16**	**Kandaghat**	Medium, Round	Greenish yellow	Oval	+	-
41	**Kn-17**	**Kandaghat**	Medium, Round	Yellowish green	Small rods	-	-
42	**Kn-18**	**Kandaghat**	Medium, Round	Greenish yellow	Small rods	-	-
43	**Kn-19**	**Kandaghat**	Small, Round	Yellowish green	Cocci	-	-
44	**Kn-20**	**Kandaghat**	Medium, Round	White	Small rods	-	+
45	**Kn-21**	**Kandaghat**	Small, Round	yellowish	Small rods	-	-
46	**Kn-22**	**Kandaghat**	Small, Round	Greenish yellow	Small rods	+	-
47	**Kn-23**	**Kandaghat**	Small, Round	Yellowish green	Small rods	-	-
48	**Kn-24**	**Kandaghat**	Small, Round	Yellowish green	Small rods	-	-
49	**Pul-1**	**Pulbahal**	Large, Slimy	White	Small rods	-	-
50	**Pul-2**	**Pulbahal**	Large, Slimy	Creamish	Small rods	-	-
51	**Pul-3**	**Pulbahal**	Large, Slimy	White	Small rods	+	-
52	**Pul-4**	**Pulbahal**	Large, Slimy	White	Small rods	+	-
53	**Hb-1**	**Habban**	Medium, Round	Creamish	Small rods	+	-
54	**Hb-2**	**Habban**	Small, Round	yellowish	Small rods	-	-
55	**Hb-3**	**Habban**	Medium, Round	yellowish	Cocci	+	+
56	**Hb-4**	**Habban**	Small, Round	Creamish	Small rods	-	+
57	**Hb-5**	**Habban**	Medium, Round	yellowish	Small rods	+	+
58	**Hb-6**	**Habban**	Medium, Round	Creamish	Small rods	-	+
59	**Rj-1**	**Rajgarh**	Medium, Round	White	Small rods	+	+
60	**Rj-2**	**Rajgarh**	Small, Round	Cream	Small rods	-	-
61	**Rj-3**	**Rajgarh**	Medium, Round	Cream	Small rods	+	+
62	**Dh-1**	**Dharja**	Medium, Round	Creamish	Thin rods	-	+
63	**Dh-2**	**Dharja**	Large, Round	Creamish	Small rods	-	-
64	**Dh-3**	**Dharja**	Large, Slimy	Creamish	Medium rods	-	-
65	**Dh-4**	**Dharja**	Medium, Round	Creamish	Small rods	-	-
66	**Dh-5**	**Dharja**	Large, Round	Creamish	Small rods	-	-
67	**Dh-6**	**Dharja**	Large, Round	Creamish	Cocci	-	-
68	**Dh-7**	**Dharja**	Large, Round	Creamish	Thin rods	+	+
69	**Dh-8**	**Dharja**	Medium, Round	Creamish	Small rods	-	-
70	**Dh-9**	**Dharja**	Large, Round	Creamish	Small rods	-	-
71	**Dh-10**	**Dharja**	Large, Round	Creamish	Small rods	-	-

72	**Dh-11**	**Dharja**	Large, Round	Creamish	Thin rods	-	-
73	**Dh-12**	**Dharja**	Large, Round	Creamish	Small rods	-	-

Plant growth promoting attributes

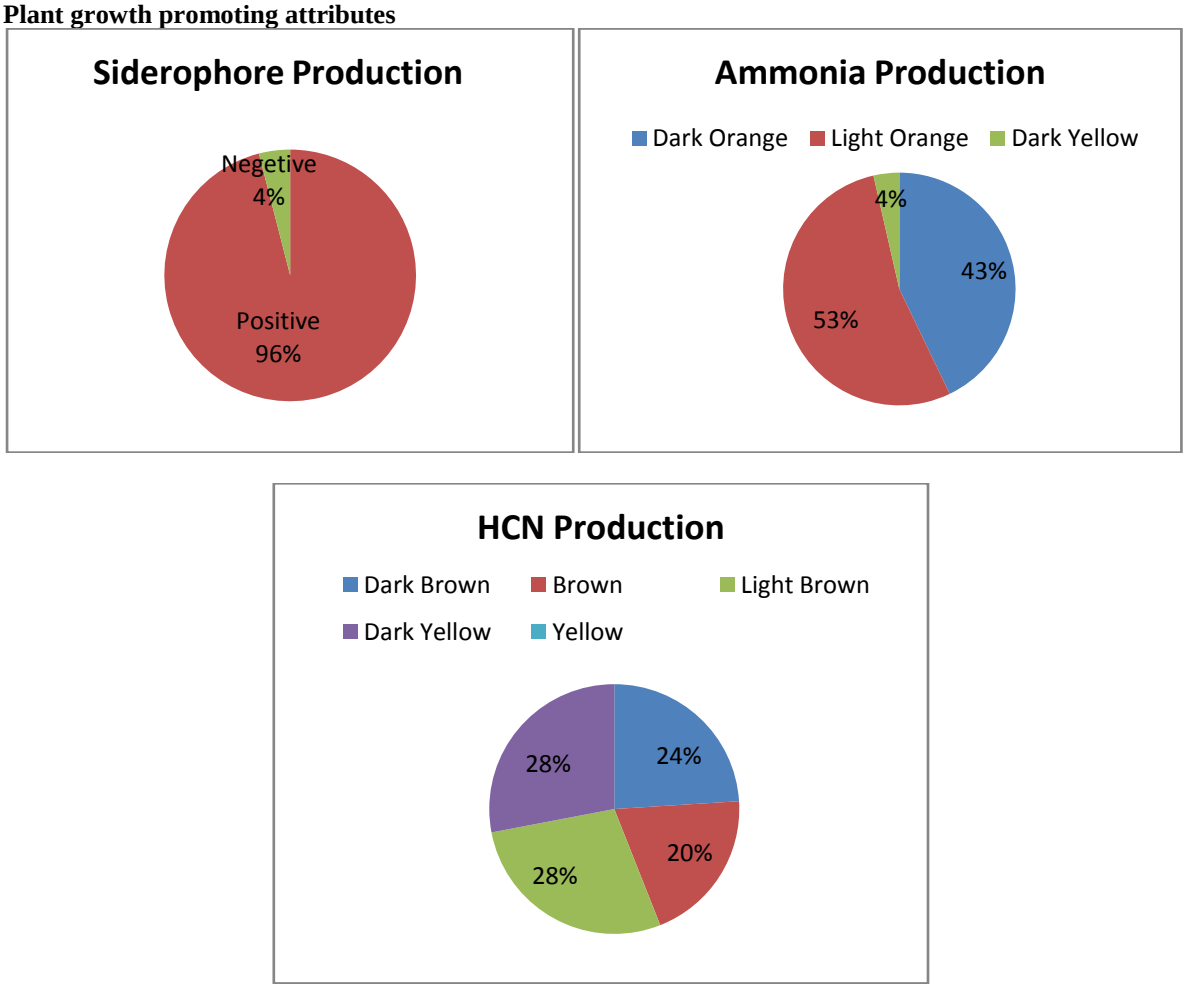


Fig 1. Plant Growth Promoting attributes of bacterial isolates from the wheat rhizosphere in the mid Himalayan zone of Himachal Pradesh

P-Solubilization

All the twenty nine isolates of PGPR showed positive for inorganic P-Solubilization *in vitro* conditions. Phosphorus (P) is one of the major essential macronutrients for biological growth and development. Rhizobacteria offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. The ability of some rhizobacteria to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant growth and yield (Saharan and Nehra 2011).

Siderophore production

Out of twenty nine isolates 96% isolates showed positive and 4% showed negative results for siderophore production. Siderophores are small iron carriers, chemically high-affinity iron chelating compounds secreted by PGPR's and are among the strongest soluble Fe^{3+} binding agents known. Comprehensive information on the role of siderophores in increasing iron oxide solubility and promoting dissolution in soils requires the consideration of the rates of various processes such as siderophore exudation, the uptake, and the degradation rates (Scavino and Pedraza, 2013).

Ammonia production

Out of twenty nine isolates 43% isolates showed dark orange, 53% light orange and 4% showed dark yellow color in case of ammonia production. Ammonia production is also a important traits of PGPR because huge amount of free nitrogen is present in the environment but this form of nitrogen is unavailable for the plants, so in ammonia production the free nitrogen is converted to ammonia by the enzyme nitrogenase present in the bacteria, which is the suitable absorbable form of nitrogen for the plant growth.

HCN

Out of twenty nine isolates 24% showed dark brown, 20% brown, 28% light brown and 28% dark yellow coloration of filter paper strip. A secondary

metabolite produced commonly by rhizospheric bacteria is Hydrogen Cyanide (HCN), a gas known to compatible mechanism for biological control of major plant pathogens (Heydari *et al.*, 2009). Hydrogen Cyanide is a poisonous gas produced by rhizobacteria as it has toxic properties.

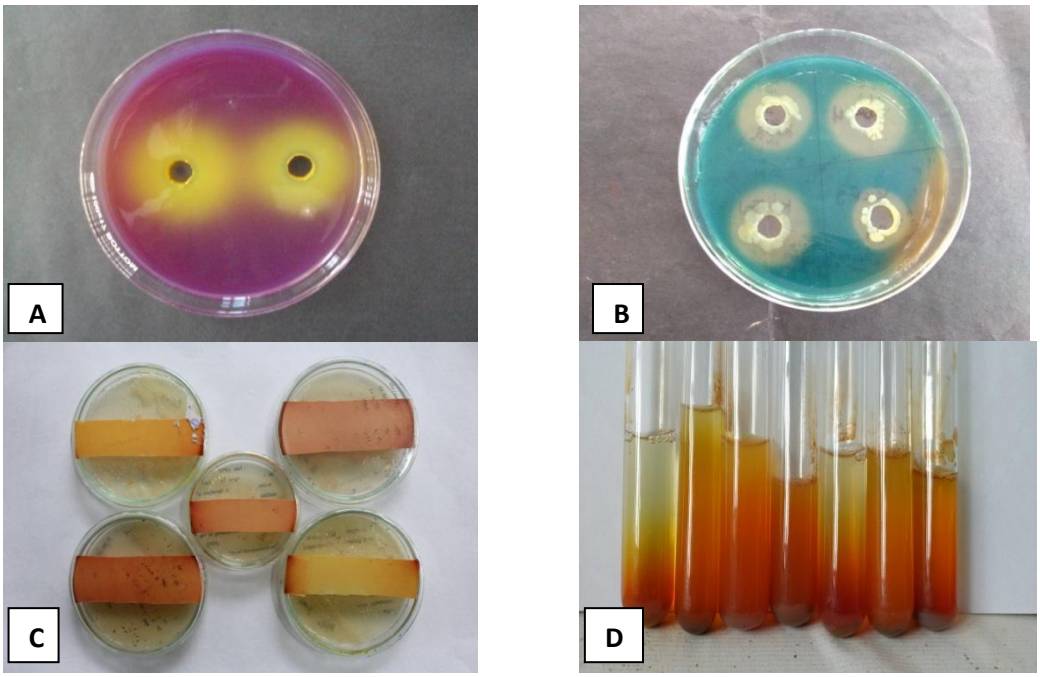


Plate 1. Production of Siderophore (A), Phosphate solubilization (B), HCN and Ammonia (C,D) shown by PGPR's isolates on PVK, CAS –agar, peptone water and nutrient agar supplemented with glycine at 28± 2^{0}C respectively.

Pot experiment

Out of twenty nine isolates of PGPR, three hyperpotential isolates Dh-7 (*Bacillus pumilus*), Kn-7 (*Pseudomonas putida*) and De-21 (*Stenotrophomonas maltophilia*) were selected for pot experiment because of their hyperpotential among PGP attributes. The results indicated that the percentage of the seed germination and shoot elongation of wheat was significantly increased under the influence of the consortia of the PGPR along with different doses of fertilizer in comparison with the control.

Seed Germination percentage

The germination percentage of seeds was 100% in each treatment except control plants with 50% NPK, Kn-7+De-21 with 50% NPK and Dh-7+De-21 with 50% NPK showed 93.33% germination. All the seeds were germinated after one week of sowing. Survival percentage of wheat plants in each treatment was 100 % along with control plants (figure 2).

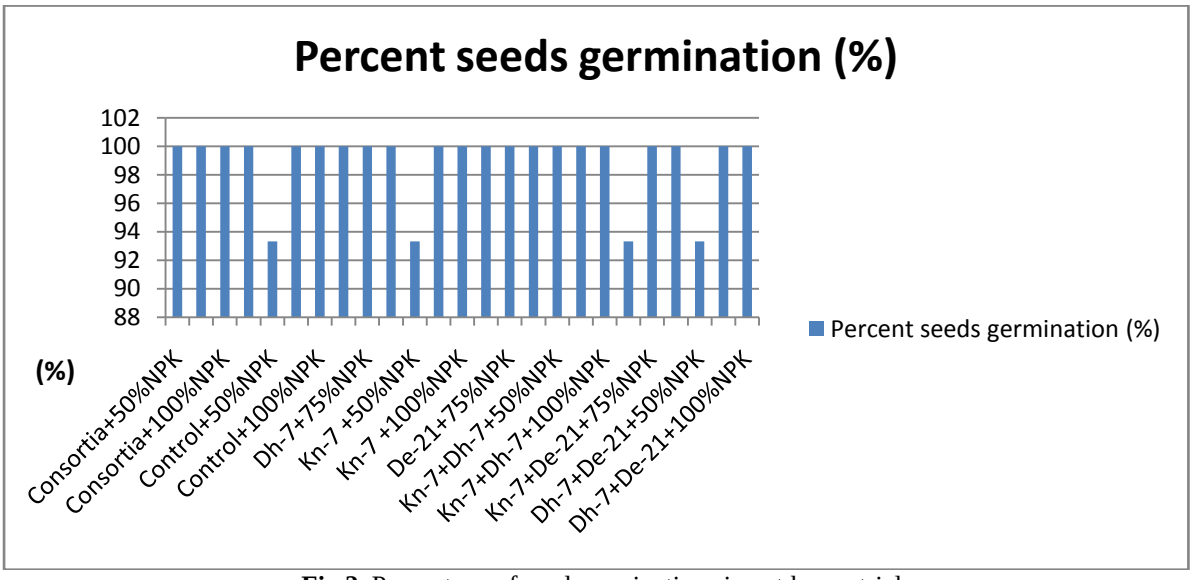


Fig 2. Percentage of seed germinations in net house trial

Shoot growth

On an average, in all the treatments with bioformulation of individual and consortia of isolates Dh-7, Kn-7 and De-21, there was an increase in plant height as compared to control after 45 days of seed sowing of *Triticum aestivum*. All the formulations showed significant increase in plant height after regular interval of time. The maximum height (19.83 cm) was recorded in consortia of three isolates with 100% NPK dose followed by (19.24 cm) consortia of three isolates with 50% NPK dose after 45 days of seed sowing. Minimum plant height was recorded in individual treatment of Kn-7 with 50% NPK dose i.e. 14.58 cm. It was found that shoot growth enhancement was observed in the treatments of consortia of three isolates (Kn-7, Dh-7 and De-21) with 100% NPK dose and consortia of three isolates without NPK dose this is due to that these strains were effective when applied collectively instead individually they were found to solubilize inorganic phosphate in the rhizosphere of wheat plants so they provide available form of phosphorus to the plants, production of siderophore which chelate the iron from the rhizospheric environment and made available to the plants so no iron deficiency was there for the PGR treated plants.

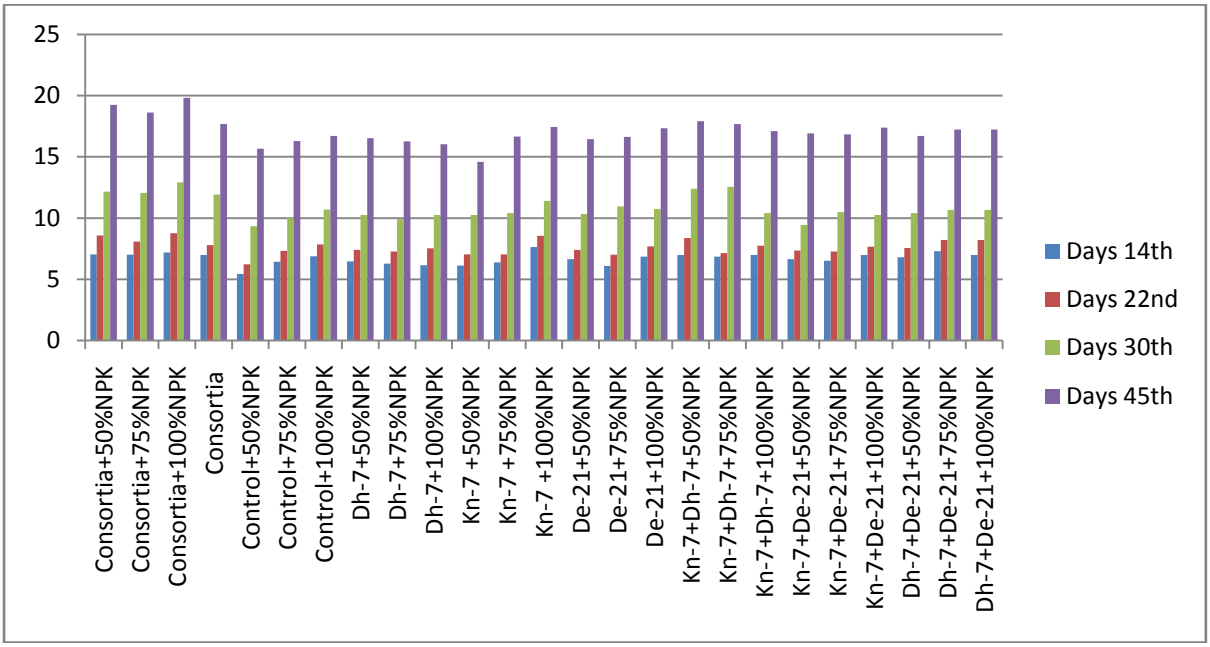
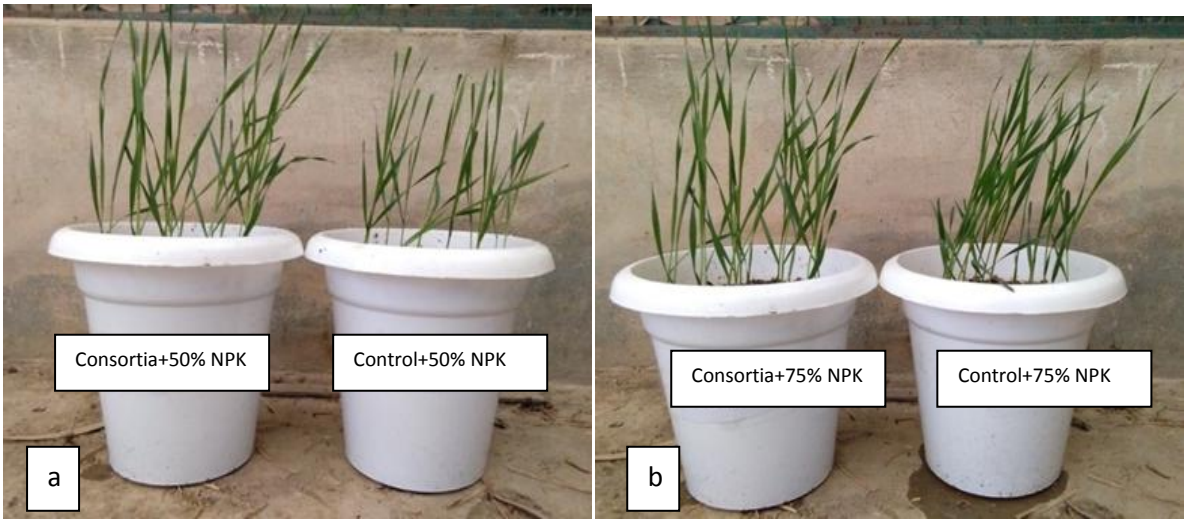


Fig 3. Effect of PGPR formulation on the shoot growth promotion of wheat seedlings in net house experiment



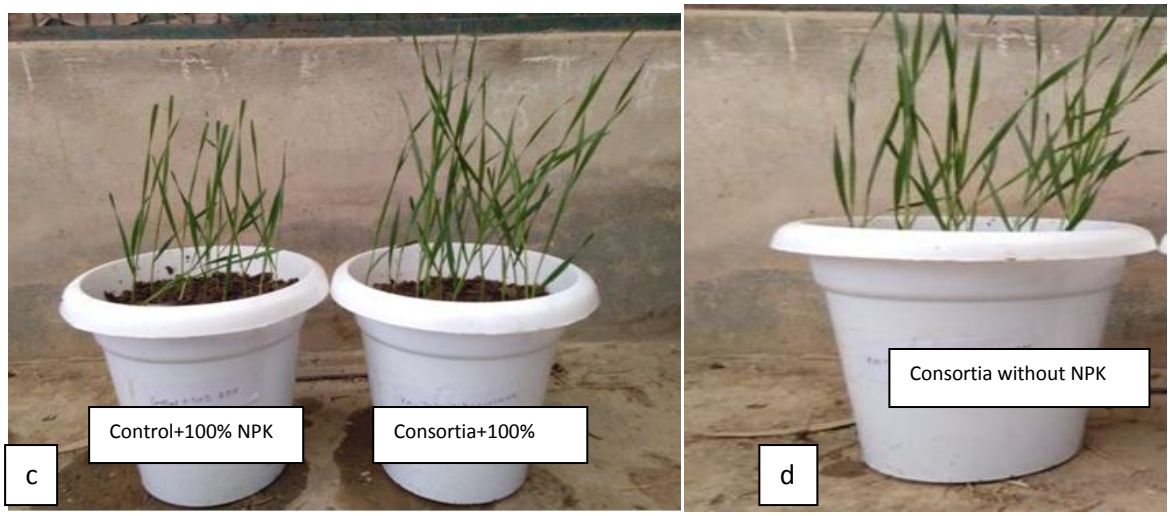


Plate 2. Effect of PGPR formulation on the shoot growth promotion of wheat seedlings in net house experiment

Plate 3. Overview of net house experiment at UHF Nauni- Solan (H.P)

Chlorophyll content of leaf

All the treatments comprised of PGPR significantly increased the chlorophyll content of leaves (a, b and total) over uninoculated control plants after 30 days of plantation (Fig 4). The chlorophyll content 'a' ranged from 0.11 to 0.13 mg/g fresh weight, whereas maximum chlorophyll content (0.138 mg/ml fresh weight) was noted in treatment consortia of three isolates with 100% NPK and minimum (0.115 mg/g fresh weight) was found in treatment Dh-7 with 50% NPK dose. Overall the chlorophyll content 'b' of leaves ranged from 0.21 to 0.26 mg/g fresh weight where the maximum chlorophyll content (0.264 mg/g fresh weight) was noted in treatment De-21 with 100% NPK dose and minimum was recorded for treatment Dh-7 with 50% NPK dose (0.210 mg/g fresh weight). The increase in total chlorophyll content of leaves was found in the range of 0.33 to 0.38 mg/g fresh weight. While the maximum chlorophyll content (0.386 mg/g fresh weight)) was noted for consortia with 100% NPK dose and minimum (0.336 mg/g fresh weight) was noted for treatment Dh-7 with 50% NPK dose and De-21 with 50% NPK dose.

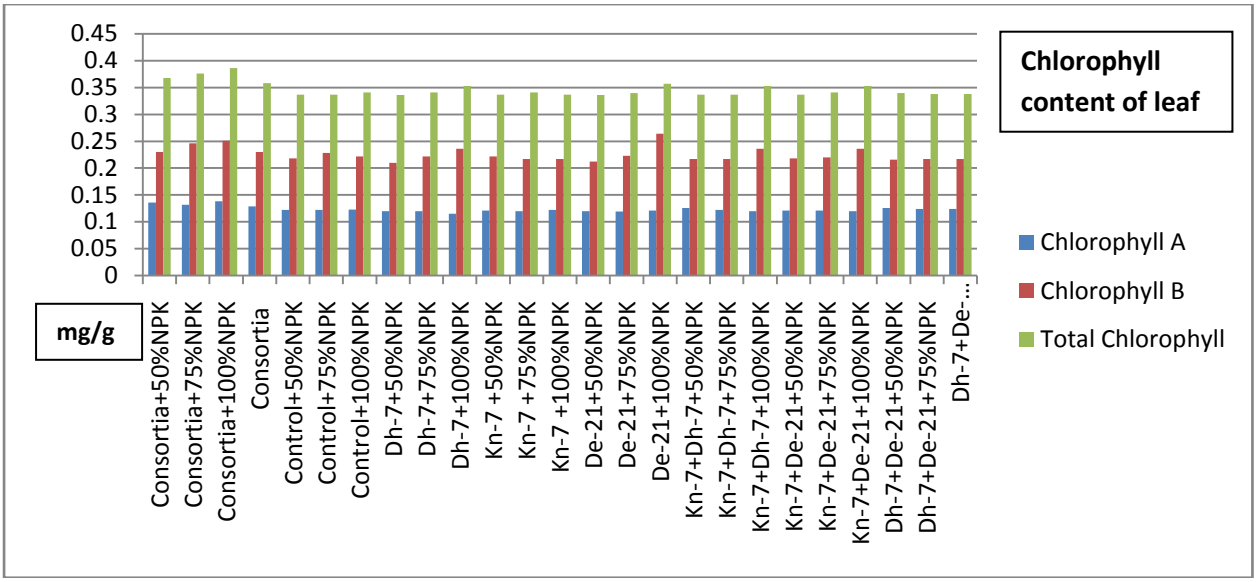


Fig 4. Effect of PGPR formulation on the chlorophyll content of leaves of wheat plants in net house experiment

DISCUSSION

The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatase play a major role in the mineralization of organic phosphorus in soil (Gaur *et al.,* 2005). Phosphorus (P) is one of the major plant nutrients, that promote shoot and root growth of plants. Chemical fertilizers are main source for phosphorus supplying in agricultural systems but about 75-90 % of added phosphorus to the soil is being fixed by Fe, Al^{3+} and Ca^{2+} complexes (Tarun *et al.*, 2006). The phosphate solubilization is the most common mode of action implicated by PGPR that increases nutrient availability to host plants (Thakur, 2014). Gupta (2012) also reported that the population of phosphate solubilizing microorganisms, in general, varied from 20-24% of the total population, however in some soils it may be 85% of the total population. In another studies conducted by Kundu *et al*., (2002) reported that about 16% of the total bacterial population in rhizosphere of wheat was P-solubilizer.

The organisms used were siderophoregenic pyoverdin-producing *Pseudomonas putida* and *Pseudomonas aeruginosa* strains from two diverse habitats. Inoculation with siderophoregenic PGPR increased percentage germination, shoot height, shoot and root length, weight of spikelets, chlorophyll content, grain yield and iron content in wheat crop (Sarode *et al.*, 2013, Mishra *et al.,* 2013). Similar study was demonstrated that *Acinetobacter calcoaceticus* isolated from wheat rhizosphere produces catechol type of siderophores during exponential phase, which is influenced by iron content of medium (Sarode *et al.,* 2009).

Previous studies Chaiharn *et al.,* (2008) reported the production of ammonia by phosphate solubilizing microorganisms, more than 64% of the isolates were found to produced ammonia. Another study by Ahmad *et al.,* (2006) reported that the several plant growth promoting rhizobacteria were found to produce ammonia in peptone water amended with Nesseler's reagent.

Although Hydrogen Cyanide acts as a general metabolic inhibitor, it is synthesized, excreted and metabolized by hundreds of organisms present in the rhizosphere mainly Plant Growth Promoting Rhizobacteria (PGPR) (Zeller *et al.,* 2009). The similar study demonstrated that the HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil of wheat a biocontrol metabolite in *Pseudomonas* species (Saharan and Nehra, 2011). Another previous study was demonstrated that the *Pseudomonas fragi* CS11RH1 (MTCC 8984), a psychrotolerant bacterium produces hydrogen cyanide (HCN) in the presence of glycine (Seval kumar *et al.*, 2009).

In this study seeds inoculation with the individual and consortia of bacterial culture has been found to improve the percentage seed germinations with different doses of fertilizer this was due to nutrient uptake of wheat seedlings via promotion of the plant growth and increased root surface area or the general root architecture of the treated seeds. Seeds inoculated with the bacterium has been found to improve the growth and nutrient uptake of wheat seedlings via promotion of the plant growth and increased root surface area or the general root architecture (Lucy *et al.,* 2004). (Laid *et al.,* 2016) Results analysis of PGPR effects of actinomycetes isolates on growth parameters show that the isolates have a significant effect on the germination rate of the treated and untreated seeds of the same degree by bioformulation. Analysis of variance revealed a very highly significant effect on germination rate for

foliage, and significant for shoot growth and root length.

Rana *et al.,* (2011) reported that number of other *Bacillus* spp. isolated from wheat rhizosphere have also been investigated for their growth-promoting property in wheat having similar effects on dry weight. All the nine selected PGPR significantly increase the root length (cm), dry root weight (g plant-1), shoot length (cm) and dry shoot weight (g plant-1) as compared to media (uninoculated) and control (uninoculated). Three PGPRs identified as *Bacillus anthracis* (A29), *Serratia proteamaculans* (A28) and *Psychrobacter maritimus* (A18) were performed best in growth chamber and selected as best potential strains the others (Amara *et al.,* 2015). Hayat *et al.,* (2012-13) also reported that *Bacillus, Enterobacter, Pseudomonas* and *Serratia* sp. were very good PGPRs with PGP traits like IAA production, phosphate solubilization and N_2-fixation and are also being used for crop production as bioinoculants. The same results were concluded by Adesemoye *et al.,* (2010) who reported that PGPR's applied along with fertilizers promote plant growth.

According to Zahra *et al.,* (2012), use of rhizobacterial inoculants as biofertilizer significantly improved the growth parameters of cereals. Various researchers reported that under controlled conditions, root and seed inoculation with PGPRs enhance root growth through PGP activity. Similar results are presented by Shaharoona *et al.,* (2008), who reported improved

efficiency of nutrients uptake by inoculation of PGPRs which resulted in increased root growth and hence efficient uptake of nutrients by plants.

Importance

Inoculations of wheat seeds with consortia of (Dh-7, Kn-7 and De-21) along with recommended dose of fertilizes has direct positive effect on increase in shoot growth, percentage of seed germination and chlorophyll content of the leaves under net house conditions.

CONCLUSION

This study that has provided an insight into the rhizobacterial community present in the rhizosphere of wheat in different locations of Solan and Sirmour district in the mid hill zone of Himachal Pradesh, India. We have demonstrated that efficient inorganic P-solubilizer, siderophore, ammonia and HCN producing rhizobacterial isolates were present among the natural population in the rhizosphere of wheat in this area. These characteristics are considered as important PGP attributes. In the present study we have been found that the consortia (*Pseudomonas putida*, *Stenotrophomonas maltophilia* and *Bacillus pumilus*) with the recommended doses of fertilizer (NPK) have been found effective in positively improving the seed germination percentage, chlorophyll content of leaf and increase in shoot growth of tested wheat plants. It is an environment friendly and cost effective technology.

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