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Influence of plant growth promoting rhizobacteria on seed germination and seedling vigour of green gram

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Abstract
In recent decades world population is increasing rapidly and food demand is a global upcoming conundrum. Chemical fertilizers are using forcibly to execute food demand it causes negative impact on soil health and environment and ultimately to human being. In this view, present study counsel promising approach to apply plant growth promoting rhizobacteria as an alternative to chemical fertilizers. Furthermore, total ten rhizospheric bacterial isolates were selected on the basis of siderophore production potential and zinc solubilization efficiency. The biochemical characteristics of the isolates confirmed their abilities to show extracellular enzymatic activity for urease, cellulase, catalase, lipase, casienase, gelatinase and amylase enzymes. Bacterial isolates also exhibited other plant growth promoting traits such as phosphate solubilization, indole acetic acid, ammonia, hydrogen cyanide and exopolysaccharide production. Afterwards, on the basis of plant growth promoting traits best six potential bacterial isolates were further evaluated for their plant growth promotory properties through seed vigour assay with green gram (*Vigna radiata* var. sweta). Obtained results confirmed significant bacterial influence on seed germination and other contributing characters. Among all, bacterial isolates WRPA 4 and WRPA 13 showed better plant growth promotion and could be used as premier bioinoculant to enhance crop yield and productivity by maintaining sustainable ecosystem.

Keywords: Plant growth promoting rhizobacteria, extracellular enzymes, green gram, seedling vigour

Introduction
In current era, nutrient availability in plants, low productivity, food safety and imbalanced ecosystem are great issues of threat. Rampant use of chemical fertilizers and pesticides are used as solution of these problems. These solutions are able to increase plant productivity but reduced soil fertility and imprinted a drastic effect on integrity of ecosystem (Pahari *et al*., 2017)[13]. Therefore since past few decades, use of plant growth promoting rhizobacteria gain a huge attraction to enhance plant productivity and sustainable ecosystem and used as an alternative strategy. Hiltner (1904) [10] defined rhizosphere as a narrow zone of soil around the root which consist high nutrient availability because of chemical secretion which are secreted by roots called as root exudates such as amino acids, organic acids, enzymes and sugars, which provide a rich source of energy and nutrients for microbial growth and promotion (Singh *et al*., 2017; Singh and Singh, 2017)[36 37]. Bacteria resides in such region are called rhizobacteria, they have capability to enhance plant growth by various direct and indirect mechanisms therefore known as plant growth promoting rhizobacteria (Glick, 1995; Upadhayay *et al*., 2018)[7, 39]. These rhizobacteria play a very crucial role to maintain plant health, nutrient uptake and environment stress (Bowen and Rovira, 1999)[2]. They are not only beneficial for plants but also play an important role to develop sustainable agriculture and maintain ecosystem integrity. During last few decades a large number of studies have been illustrated the plant growth promoting ability of PGPRs (Singh *et al*., 2013; Singh and Goel, 2015; Joshi *et al*., 2018; Singh *et al*. 2018) [28, 26, 11, 29]. They concluded that the plant growth promoting rhizobacteria (PGPR) are the part of the protective micro flora which are useful for plants by enhancing root functions, suppression of disease, acceleration of growth and development (Glick, 1995)[7]. The bioprotective and plant growth promoting activity of PGPR have been reported by many researchers worldwide on a wide range of crops like cereals, pulses, vegetables, oil seeds and plantation crops. A wide array of bacteria including species of *Alcaligenes, Burkholderia, Aeromonas, Azotobacter Arthrobacter, Gluconacetobacter, Pseudomonas, Serratia, Azospirrilium, Acinetobacter, Klebsiella, Bacillus, enterobacter*

and *Clostridium* are considered as most important PGPRs. These PGPR enhances plants growth directly and indirectly by enhancing soil fertility via increasing the amount of available nitrogen, soluble phosphorous, zinc, iron and other plant micro and macronutrients by synthesizing and secreting many different hormones and enzymes such as IAA, phosphatases, nitrogenases, ACC deaminases or by lowering the pH. These rhizobacteria are able to suppress many pathogenic fungi and bacteria due to the production of HCN, siderophore and antibiotics (Upadhayay *et al*., 2018)[39]. These microorganisms also reported to consign the increased iron concentration in food crops besides improving or enhancing the soil fertility and crop yield by iron sequestration via siderophore production (Singh and Shah, 2013; Singh *et al*., 2017)[34, 36]. Therefore, by utilization of potential plant growth promoting rhizobacteria in agriculture practices can significantly enhance plant growth and reduce the dependency of chemical fertilizers through eco-friendly crop production as well as support sustainable agriculture. The aim of the present study was to find out potential plant growth promoting rhizobacteria from wheat rhizosphere of hill regions of Uttarakhand and evaluated their effect on seed germination and seedling vigour of green gram to develop a potential bioinoculant for crop production enhancement.

Materials and methods

Collection of soil sample and bacterial isolation

Soil samples of wheat rhizosphere were collected in sterile plastic bags aseptically from the different regions of Uttarakhand i.e. Almora, Mavakot and Pauri. The soil samples were air dried then bacterial isolates were isolated by serial dilution method on nutrient agar plates and incubated at 28±2˚C for 24 hrs. Different bacterial colonies with different morphological appearance were selected and purified. Pure culture of the bacterial isolates were maintained on nutrient agar slants at 4˚C for regular use and in 20% glycerol stocks for long-time preservation at −20˚C.

Morphological characterization of bacterial Isolates

Colony morphology of the each bacterial isolates was examined after 24 hr of inoculation of each bacterial isolate on nutrient agar plate and stereoscopic microscope (Olympus, SZH 10) was used for better resolution of bacterial colony morphological characteristics such as shape, pigment, edge, elevation and surface. Gram staining was done to identify cell morphology.

Biochemical characterization of bacterial isolates

All bacterial isolates were further characterized on the basis of biochemical property. Enzymes such as amylase, caseinase, catalase, urease, gelatinase, cellulose, lipase and pectinase production were determined through standard protocols (Hankin and Anagnostakis, 1975; Cappuccino and Sherman, 2002)[8, 4]. Identification of fermentation pathways and ability to utilize various carbon sources were determined by standard IMVIC kit (Hi-Media, India), which contains four biochemical tests for identification of fermentation pathway and eight carbohydrate utilization tests to determine the carbon source utilization ability.

Plant growth promotory traits

1. Siderophore production

Siderophore productions of bacterial isolates were examined on chrome azurol S (CAS) agar medium as described by Schwyn and Neilands, 1987[23]. Log phase bacterial cultures were spot inoculated on CAS agar medium and incubated at 28±2˚C for 72h (Schwyn and Neilands, 1987)[23]. Appearance of yellow or orange zone around the bacterial colony represents siderophore production potential. Solubilization efficiency of iron was measured as per Ramesh *et al*. (2014) [19].

2. Zinc solubilization

All bacterial isolates were screened for zinc solubilization efficiency on two insoluble zinc compounds viz. zinc oxide (ZnO) and zinc carbonate ($ZnCO_3$). Overnight grown bacterial cultures were transferred aseptically by spot inoculation on basal agar medium supplemented with 1% ZnO and $ZnCO_3$, separately. Plates were incubated at 28±2˚C for 4 to 5 days. Appearance of clear halo zone around the bacterial colony indicated as positive results and diameter of zone was measured to calculate percent (%) zinc solubilization efficiency as per Ramesh *et al*. (2014)[19].

3. Phosphate solubilization

Ability of strains to solubilize phosphorous were determined on Pikovskaya's agar medium containing tricalcium phosphate as insoluble source of phosphate (Pikovskaya, 1948)[15]. Each overnight grown bacterial culture was spot inoculated on pikovskaya agar medium and incubated 28±2˚C for 3 to 4 days. Appearance of clear halo zone around bacterial colony indicates phosphate solubilization. Size of the zone diameter seen around the bacterial colonies provides a semi quantitative potential of phosphate solubilization.

4. HCN production

Production of HCN by each bacterial culture was determined by inoculating bacterial culture on nutrient agar medium amended with glycine (4.4g/l) (Miller and Higgins, 1970)[12]. After inoculation, the sterile filter paper soaked in 2% sodium carbonate and 0.5% picric acid solutions were placed in upper lid of each petri plates. The plates were sealed by parafilm and incubated at 28±2˚C for 3 to 4 days. Change in the colour of filter paper from yellow to brown was used as indicator for positive results.

5. Ammonia production

All the bacterial isolates were evaluated for the production of ammonia in peptone water as described by Cappuccino and Sherman (2002)[4]. In brief, each freshly grown bacterial culture was inoculated in 10 ml peptone water and incubated for 48 -72 h at 28 ± 2˚C. After incubation Nessler's reagent (0.5 ml) was added in each test tube and leaved as such for 5 minutes. Development of brown to yellow colour was a positive test for ammonia production.

6. Indole acetic acid production

Indole-3-acetic acid production was qualitatively determined by the method as described by Patten and Glick (2002)[14]. All bacterial cultures were inoculated in luria broth supplemented with L-tryptophan (100µg/ml) and incubated at 28±2˚C for 3 days followed by centrifuged at 10,000 rpm for 10 minutes then supernatant was collected and Salkowsky reagent was added in 1ml of supernatant and left for 30 minutes at room temperature. Development of pink colour shows the presence of IAA.

7. Exopolysaccharide production

To determine EPS production by all bacterial isolates, all bacterial cultures were inoculated in 50ml basal medium and incubated at 28±2˚C for 3 days. After incubation, medium suspension was centrifuged at 10,000 rpm for 10 min and

pellets were collected followed by the addition of two volume ice cold isopropanol and stored at 4°C for 24 hrs. Afterwards, the suspension was centrifuged again at 10,000 rpm for 20 minutes and pellets were collected and dried at 65°C (Vijayabhaskar *et al*., 2011)[40]. Presence of pellet showed the exopolysaccharide (EPS) production.

Assessment of plant growth promotion

On the basis of *In vitro* plant growth promoting traits, selected potential bacterial isolates were examined for plant growth promotion through seed vigour assay under lab conditions on green gram (*Vigna radiata* var. Sweta). Seeds were collected from breeder seed production centre (BSPC), Pantnagar, Uttarakhand, India. To compile this assay the seeds of green gram (*Vigna radiata* var. Sweta) were selected, which were free from obvious damage and surface sterilized as described by Singh *et al*. (2010, 2013)[33, 28]. In brief, the seed were soaked in ethanol (95% v: v) for 30 sec and then a sodium hypochlorite solution (1.2% w: v) for 5 min, followed by 10 rinses with sterile tap water. Afterwards, surface sterilized seeds were transferred in overnight grown bacterial suspension for 4 hr at 28± 2°C in an incubator shaker at 100rpm. Germination tests were carried out by paper towel assay. Hundred bacterized seeds were placed aseptically in between paper (B.P.) then kept in seed germinator in tilled position at 25 ± 2°C. Towel paper were regularly watered and daily analyzed for germination of seeds up to 8 days of incubation. This experiment had designed with 3 replicates per treatment. One un-inoculated, surface sterilised disinfected seed treatment was used as a control.

Observation recorded

1. **First count:** The study was carried out on hundred seeds with three replications. All seeds were placed in between paper (BP) then treatments were kept in germinator at 25±2°C. Normal seedlings were counted only at 5th day of incubation.
2. **Standard germination:** Hundred seeds of green gram (var. Sweta) bacterized with potential bacterial isolates were placed in between paper (BP) and incubated in germinator at 25±2°C with three replications. The normal seedlings were counted on 8th day of incubation as final count.
3. **Seedling root length:** On 8th day of the start of germination test ten normal seedlings were randomly selected from each replication. The length of radicals (in cm) was measured and means root length was calculated.
4. **Seedling shoot length:** Ten normal seedlings were randomly selected on 8th day of the start of germination test from each replication. The shoot length (in cm) was measured with the help of a scale and mean value was obtained by calculating shoot length of 10 seedlings from each replication.
5. **Seedling length:** Shoot and root length was added to obtain total length of seedlings (in cm).
6. **Seedling fresh weight:** fresh weights of earlier selected randomly ten seedlings were recorded with the help of an electronic balance in milligram at the end of seed germination test on 8th day.
7. **Seedling dry weight:** Seedlings selected at 8th day were subjected to oven at 80°C for 24 hrs to remove the moisture from seedlings. Weigh of dried seedlings were measure on an electronic balance.
8. **Seedling vigour index:** Seedling vigour index was calculated by two different formulas.
   A. Seedling vigour index-I: Calculated as-
   Seed Vigour Index I = Standard germination (%) × Seedling Length (cm)
   B. (b) Seedling vigour index-II: Calculated as-
   Seed Vigour Index II = Standard germination (%) × Seedling dry weight (mg)
9. **Speed of germination:** To carry out the assay 100 seeds were placed in between paper with three replications and kept in germinator at 25±2°C for germination. After commencing seed germination, all treatments were checked daily for germination at approximately same time. When normal seedlings attained a predetermined size then they were removed from the test. This procedure was followed until all seeds had germinated to form a normal seedling. For each treatment an index was calculated by dividing number of removed seedlings each day by corresponding day of counting.
10. **Relative growth index (RGI):** Relative growth index was calculated by the formula as described by Brown and Mayer (1986)[3].

$$\text{RGI} = \frac{\text{No. of Seeds germinated at first count}}{\text{No. of Seeds germinated at final count}} X\ 100$$

11. **Germination index (GI):** Germination index was calculated according to the evaluation of association of official seed analysts by using following formula-

$$Germination\ Index = \frac{No.\ of\ germinated\ seeds}{Days\ of\ first\ count} + .. + \frac{No.\ of\ germinated\ seeds}{Days\ of\ final\ count}$$

12. **Mean germination time (MGT):** Mean germination time (MGT) was calculated by following equation (Eills and Robert, 1981)[6].

$$\text{MGT} = \frac{\sum Dn}{\sum n}$$

Where, D = Number of days counted from beginning of germination; n = Number of seeds germinated on day D.

13. **Time to 50% germination (T50):** The time taken for 50% seed germination (T50) was calculated according to the following formula of Coolbear *et al*. (1984)[5].

$$(T_{50}) = t_i + \frac{\left[\frac{N}{2} - n_i\right]\left[t_j - t_i\right]}{n_j - n_i}$$

Where, N = Final number of germination; ni, nj = Cumulative number of seed germinated by adjacent counts at time ti and tj, when ni < N/2 < nj.

14. **Germination value:** Calculated as = Peak value × Mean daily germination
15. **Mean daily germination:** Mean daily germination of seeds was calculated according to following formula:

$$\text{Mean daily germination} = \frac{Final\ germination\ percentage}{Total\ No.\ of\ days}$$

16. **Peak value:** Peak value of germination was calculated according to formula under:

$$\text{Peak Value} = \frac{Final\ germination\ percentage}{No.\ of\ days\ to\ reach\ maximum\ germination}$$

Statistical analysis

Statistical analysis was done by completely randomized design (CRD) using STPR3 program. All experiments were performed in four replications. The critical difference at 5 percent level of significance was calculated to compare the mean of different treatments.

Results and discussion

Morphological and biochemical characterization

A total 10 bacterial isolates having different impression were characterized on the basis of colony characteristics such as shape, edge, elevation, surface and pigmentation (Table 1) and cell morphology such as gram positive or negative bacteria, cell shape and cell arrangement (Table 1). Among all ten, five bacterial isolates were either gram positive rods or cocci and remaining five were gram negative rods.

Furthermore, all bacteria were evaluated and characterized for extracellular enzyme production and carbon source utilization. During biochemical characterization some of the bacterial isolates showed extracellular enzymatic activity and exhibited amylase, caseinase, cellulose, lipase and urease activity by producing clear halo zone on respective agar plates (Table 2). None of the bacterial isolates were positive for pectinase and laccase activity (Table 2). While, nine bacterial isolates were able to produce catalase by placing H_2O_2 over the bacterial colony on a clean glass slide (Table 2). Gelatinase and caseinase activity were confirmed by seven bacterial isolates, while six isolates were positive for cellulase enzyme production (Table 2). During investigation four isolates showed their potential for lipolytic and urease activity, while two isolates were positive for amylase enzyme production (Table 2). These results were agreement with Singh *et al*. (2012)[35] and Sharma *et al*. (2013)[25] who observed extracellular enzymatic activity of bacteria and described the functional status of extracellular enzymes through solid state fermentation. In other hand Yadav *et al*. (2015)[41] and Joshi *et al*. (2018)[11] described the extracellular enzymatic activity of the endophytic fungi and bacteria, respectively, isolated from *O. sanctum* and *Aloe vera*. During IMVIC test, only two isolates were positive for Indole, five were positive for methyl red, four isolates showed positive results for voges prauskeur and five isolates were able to utilize citrate as a carbon source. In carbohydrate utilization test varying level of sugars utilized by the different bacterial isolates. During study of carbon source utilization, adonitol, sorbitol, mannitol, arabinose and rhamnose were limited for very few bacterial isolates, whereas glucose and sucrose were utilized by nine and six bacterial isolates, respectively (Table 3).

Table 1: Morphological characteristics of bacterial isolates

S.No.	Isolates	Gram reaction	Cell morphology	Arrangement	Colony morphology				
					Shape	Chromogenesis	Edge	Elevation	Surface
1.	WRA5	Positive	Small rod	Scattered	Circular	Off white	Entire	Umbonate	Smooth
2.	WRM6	Negative	Long rod	Scattered	Irregular	Yellowish	Entire	Flat	Smooth
3.	WRPA4	Positive	Coccus	Scattered	Irregular	Off white	Entire	Convex	Smooth
4.	WRPA6	Negative	Small rod	Scattered	Circular	Pale orange	Entire	Flat	Rough
5.	WRPA12	Positive	Small rod	Scattered	Irregular	Creamy white	Entire	Flat	Dry
6.	WRPA13	Negative	Short rod	Scattered	Irregular	Pale yellow	Entire	Flat	Shiny
7.	WRPA23	Negative	Small rod	Scattered	Circular	Yellowish	Entire	Umbonate	Smooth
8.	WRPA26	Positive	Coccus	Chain	Circular	Creamy	Entire	Raised	Shiny
9.	WRPA28	Positive	Long rod	Scattered	Circular	Off white	Entire	Flat	Smooth
10.	WRPA32	Negative	Small rod	Bunch	Irregular	Off white	Entire	Flat	Dry

Table 2: Extracellular enzymatic activity of bacterial isolates

S.No.	Bacterial isolates	Catalase test	Amylase test	Gelatine test	Caseinase test	Cellulase test	Pectinolytic test	Lipolytic test	Urease test	Laccase test
1.	WRA5	-	-	+	+	+	-	+	-	-
2.	WRM6	+	+	-	+	+	-	-	_	-
3.	WRPA4	+	-	+	+	-	-	-	+	-
4.	WRPA6	+	-	+	-	-	-	+	+	-
5.	WRPA12	+	-	+	+	+	-	-	_	-
6.	WRPA13	+	-	+	+	-	-	+	+	-
7.	WRPA23	+	-	+	-	+	-	-	-	-
8.	WRPA26	+	+	-	+	-	-	+	+	-
9.	WRPA28	+	-	+	+	+	-	-	-	-
10.	WRPA32	+	-	-	+	+	-	_-	+	-

− No activity, + activity available

Table 3: IMVIC and carbon source utilization characteristics of bacterial isolates

S. No.	Bacterial isolates	Indole	Methyl red	Voges proskauer's	Citrate	Glucose	Adonitol	Arabinose	Lactose	Sorbitol	Mannitol	Rhammnose	Sucrose
1	WRA5	-	-	-	-	+	-	-	-	-	-	-	-
2.	WRM6	-	+	-	-	+	-	-	-	-	-	-	+
3.	WRPA4	-	-	+	-	+	-	-	-	-	-	-	+
4.	WRPA6	-	+	-	+	-	-	+	+	+	+	+	+
5.	WRPA12	-	+	+	_	+	-	-	-	-	-	-	+
6.	WRPA13	+	+	+	+	+	-	-	+	+	+	+	-
7.	WRPA23	-	-	-	+	+	-	-	-	-	-	-	-
8.	WRPA26	+	-	-	+	+	+	+	+	+	+	+	+
9.	WRPA28	-	-	-	+	+	-	+	+	-	-	-	+
10.	WRPA32	-	+	+	-	+	-	-	-	-	-	-	-

− No activity, + activity available

Plant growth promotory traits

All bacterial isolates were further screened for various plant growth promoting traits like siderophore production, zinc solubilization, phosphate solubilization, ammonia, HCN, indole acetic acid and exopolysaccharide production. During investigation all cultures were positive for siderophore production and zinc solubilization by forming halo orange zone around the bacterial colony. The bacterial isolates WRPA4, WRPA26 and WRPA6 showed best potential of siderophore production with solubilization efficiency of 233.3%, 240.0% and 250.0%, respectively (Table 4). This finding agreed with the finding of Joshi *et al*. (2018)[11] who confirmed the siderophore production ability of endophytic bacterial isolates. On the other hand Sayyed *et al*. (2005)[22] confirmed the potential of two bacterial strains namely *Pseudomonas putida* and *Pseudomonas flourescens* for maximum siderophore yield i.e. 83% and 87% unit for each bacterial strain, respectively. During investigation of zinc solubilization, the bacterial isolate WRPA13 showed highest efficiency of zinc solubilization on basal agar medium supplemented with $ZnCO_3$ i.e. 600%, while highest efficiency on ZnO supplemented medium was recorded for WRPA32 bacterial isolates i.e. 666.0 % (Table 4). Saravanan *et al*. (2004)[21] reported that the ZSB-O-1 (*Bacillus* sp.) was producing a clearing zone of 2.8cm with a zone diameter of 14.50cm^2, while the isolate ZSB-S-2 (*Pseudomonas sp*.) showed zinc solubilization ability with clearing zone of 3.30cm and zone of diameter 20.43 cm in basal medium supplemented with ZnO and showed lower efficiency in $ZnCO_3$ with a clear zone of 2.00cm and zone area of 7.00cm^2. Phosphorous (P) is second most essential plant macronutrient after nitrogen, their availability in soil through microbial solubilization is one of the alternative environment friendly approach (Singh et *al*., 2011, 2013, 2018; Singh and Prasad, 2014) [30, 28, 29, 32]. During current investigation six bacterial isolates were able to solubilize phosphate, out of which three isolates i.e. WRPA12, WRPA13 and WRPA 32 showed better efficiency compare to other (Table 5). The present finding of P solubilization agreed with the finding of Singh *et* al. (2010, 2010a, 2010b) [33, 27, 31] who observed similar observations for the rhizospheric bacterial isolates and confirmed their potential in plant growth promotion. Furthermore, all the isolates were analyzed for IAA producing ability and seven isolates i.e. WRA5, WRM6, WRPA6, WRPA23, WRPA26, WRPA28 and WRPA32 were able to produced IAA (Table 5). This finding similar to Ullah *et al*. (2018)[38] who confirmed IAA production ability of bacterial endophytes recovered from two medicinal plants of Pakistan. Ammonia producing ability confirmed by four bacterial isolates namely WRA5, WRM6, WRPA6 and WRPA12 (Table 5). Hayat *et al.* (2010)[9] confirmed the ability of ammonia producing bacteria in plant growth promotion and enhance crop production. EPS production is a common trait of plant growth promotory rhizobacteria and capable to improve soil structures through water holding and cementing properties. In present study, only seven bacterial isolates WRA5, WRPA4, WRPA6, WRPA12, WRPA13, WRPA26 and WRPA28 were able to produce exopolysaccharide (Table 5). Qurashi and Sabri (2012)[17] quantified the EPS production from bacterial isolates and determine their stimulatory effect on chickpea growth and soil aggregation under salt stress. All the bacterial isolates were found negative for the HCN production, due to no colour change was observed on filter paper during investigation (Table 5).

Table 4: Siderophore production and zinc solubilization efficiency of bacterial isolates

SN	Isolates	Zinc solubilisation efficiency						Siderophore production efficiency		
		ZnCo3			ZnO					
		Colony diameter (cm)	Zone of inhibition (cm)	Solubilization efficiency (%)	Colony diameter (cm)	Zone of inhibition (cm)	Solubilization efficiency (%)	Colony diameter (cm)	Zone of inhibition (cm)	Solubilization efficiency (%)
1	WRA5	0.4	1.2	200.0	0.5	1.3	160.0	0.7	1.6	128.5
2	WRM6	0.4	1.1	175.0	0.4	1.1	175.0	0.6	1.4	133.3
3	WRPA4	0.4	1.8	350.0	0.2	0.5	150.0	0.6	2.0	233.3
4	WRPA6	0.1	0.3	200.0	0.2	0.6	200.0	0.4	1.4	250.0
5	WRPA12	0.4	1.1	175.0	0.2	0.5	150.0	1.1	1.9	72.7
6	WRPA13	0.2	1.4	600.0	0.4	2.4	500.0	0.8	1.8	125.0
7	WRPA23	0.7	2.3	228.5	0.2	0.6	200.0	0.6	1.4	133.3
8	WRPA26	0.4	1.4	250.0	0.4	2.2	450.0	0.5	1.7	240.0
9	WRPA28	0.3	1.3	333.3	0.4	2.1	425.0	0.6	1.2	100.0
10	WRPA32	0.4	2.7	575.0	0.3	2.3	666.0	0.8	1.4	75.00

Table 5: Plant growth promoting activities of bacterial isolates

S.No.	Isolates	Phosphate solubilization	HCN production	Ammonia production	IAA production	EPS production
1.	WRA5	-	-	+++	+	+
2	WRM6	-	-	++	+	-
3	WRPA4	++	-	-	-	+
4	WRPA6	++	-	+	+	+
5	WRPA12	+++	-	++	-	+
6	WRPA13	+++	-	-	+	+
7	WRPA23	-	-	-	++	-
8	WRPA26	++	-	-	-	+
9	WRPA28	-	-	-	++	+
10	WRPA32	+++	-	-	+	-

− No activity, + lower activity, ++ moderate activity, +++ maximum activity

Plant growth promotion assessment

Plant growth promotory effects of bacterial isolates were examined through seed vigour assay on green gram (*Vigna radiata* var. sweta) seeds by using in between paper (BP) method. Seed vigour is a very important part of seed value and significantly higher levels are requisite in addition to conventional quality norms of moisture, purity, germination and seed health to acquire maximum plant stand and high productivity from crops. After evaluation of plant growth promotory traits and functional characterization six best potential bacterial isolates i.e. WRA5, WRPA4, WRPA6, WRPA12, WRPA13 and WRPA26 were selected for seed vigour assay. The seed vigour assay for bacterial inoculated green gram seeds was incubated for 8 days. After completion of incubation period, the count of germinated seeds was determined in each treatment, represents the vigour potential with respect to first count (%) and standard germination (%) for different bacterial isolates (Table 6). The maximum germination percentage for first count and standard germination were recorded for WRPA4 inoculated seeds i.e. 89%, 91.66%, respectively, followed by WRPA13, WRPA23 inoculated seeds, while un-inoculated control was least influenced i.e. 73 and 77%, respectively (Table 6). The numbers of normal seedlings recorded in the first count express the fast germination ability of seeds and thus function as a vigour measurement. The seed lot showing the higher standard germination is considered to be more vigorous (Abdul-Baki and Anderson, 1973) [1]. Highest root length, shoot length and seedling length was found in seeds inoculated with WRPA4 i.e. 14.99cm, 26.00cm, 41.00 cm, respectively and un-inoculated control showed least value (Table 6). However, the effects of all bacteria on fresh and dry weight of seedling were almost at par and enhanced significantly compare to control (Table 6).Vigour index reflects the health of the seedling produced and also shows the ability seeds to endure various stress. The maximum seedling vigour index I was reported in seeds inoculated with WRPA4 (3757.37) while minimum seedling vigour index was obtained for un-inoculated control (2471.88) (Table 6). Whereas, seedling vigour index II was maximum for treatment WRPA13 i.e. 19.94, while minimum value recorded for un-inoculated control i.e. 13.84 (Table 6). Seed germination and their development as normal seedling are formative features of a seed variety, valuable for both economic and ecologic implements. During this germination period seeds are very susceptible for injury, disease, therefore seed germination consider as most critical phenomenon in plant life cycle (Rajjou *et al*., 2012)[18]. Germination is a composite phenomenon during which the swill full bloom seed must quickly shift to a germination driven program of development and prepare for seedling growth (Rawat *et al*., 2010; Prasad *et al*., 2016)[20, 16]. The bacterium WRPA4 was more effective in rest of the parameters of seed vigour assay except relative growth index, fresh and dry weight parameters. Speed of germination, germination index, germination value, mean daily germination and peak value were highly influenced by WRPA4 and achieve value 30.11, 18.22, 349.87, 11.45 and 30.53, respectively, while the least value were recorded with un-inoculated control i.e. 22.71, 15.18, 247.08, 9.62, and 25.66, respectively (Table 7). Relative growth index was highest for seedling inoculated with WRPA13 i.e. 97.42 followed by all other treatments and control having minimum relative growth index i.e. 94.83 (Table 7). Seeds inoculated with WRPA6, WRPA12 and WRPA26 were showed highest fresh and dry weight and values were at par with each other, while un-inoculated control showed least value (Table 7). Mean germination time and time for 50% germination (T_{50}) was highest for un-inoculated treatments, while the least value for these parameters were recorded for WRPA4, which showed the capability of the WRPA4 bacterium in seed emergence compare to un-inoculated control (Table 7). Seeds and seedling quality are the phenomenon that describe on the basis of germination parameters such as degree of germination, rapidity and peak value of germination. The results confirmed the beneficial effects of all selected plant growth promoting rhizobacteria on germination and growth of green gram over un-inoculated control. Similarly Joshi *et al*. (2018)[11] also reported significant increase in germination percentage and other parameters during seed vigour assay with potential plant growth promoting bacterial endophytes over un-inoculated control. The present finding also agreed with the finding of Yadav *et al*. (2016)[42] who investigated the influenced of endophytic fungi on plant growth, seed vigour and germination potential of green gram seeds. In other hand Singh *et al*. (2010b)[31] also reported the improved seed germination and seedling vigour upon inoculation with plant growth promoting rhizobacteria on lentil seeds. In addition, Shah *et al*. (2018)[24] demonstrated the beneficial effects of potential plant growth promoting rhizobaccteria on lentil.

Table 6 Effect of plant growth promoting rhizobacteria on germination percentage and agronomical parameters

Treatments	First count (%)*	Standard germination (%)*	Root length (cm) *	Shoot length (cm)	Seedling length (cm) *	Fresh weight (mg)	Dry weight (mg) *	Seedling vigour index I*	Seedling vigour index II*
Control	73.00	77.00	10.81	21.30	32.11	1380	180	2471.88	13.84
WRA5	80.66	84.33	13.64	24.29	37.94	1580	220	3199.46	18.55
WRPA4	89.00	91.66	14.99	26.00	41.00	1710	210	3757.37	19.26
WRPA6	86.33	89.00	14.50	24.65	39.15	1860	190	3484.45	16.90
WRPA12	85.33	89.66	14.04	24.61	38.65	1806	220	3467.17	19.72
WRPA13	88.33	90.66	12.78	24.49	37.27	1460	220	3379.83	19.94
WRPA26	81.66	85.66	12.08	25.02	37.11	1860	220	3179.35	18.84
SEm±	0.71	0.80	0.34	0.37	0.41	0.03	6.81	44.0	0.596

*Each value is the mean of three replicates.

Data were analyzed statistically at the 5% (p<0.05) level of significance.

Table 7: Effect of plant growth promoting rhizobacteria on other germination parameters

Treatments	Speed of germination*	Relative growth index*	Germination index*	Mean germination time (days) *	Time for 50% germination T_{50} (days) *	Germination value*	Mean daily germination*	Peak value*
Control	22.71	94.83	15.18	3.70	3.07	247.08	9.62	25.66
WRA5	25.93	95.64	16.70	3.52	2.83	296.28	10.54	28.10
WRPA4	30.11	97.09	18.22	3.32	2.70	349.87	11.45	30.53
WRPA6	28.13	97.00	17.69	3.42	2.82	329.98	11.12	29.66
WRPA12	29.48	95.18	17.74	3.36	2.70	335.01	11.20	29.88
WRPA13	29.68	97.42	18.04	3.33	2.71	342.41	11.33	30.21
WRPA26	26.68	95.32	16.95	3.52	2.84	305.68	10.70	28.50
SEm±	0.39	0.86	0.14	0.03	0.02	5.86	0.10	0.29

*Each value is the mean of three replicates.
Data were analyzed statistically at the 5% (p<0.05) level of significance.

Conclusion

Present study demonstrated the application of plant growth promoting rhizobacteria on plant growth promotion through seed vigour assay on green gram seeds. Results of the study illustrated that bacterial inoculated seeds were significantly increased seedling vigour in green gram seeds. Among all bacterial isolates, WRPA 4 and WRPA 13 showed various plant growth promoting activities such as siderophore production, zinc and phosphate solubilization, exopolysaccharide production which showed significant improvement on seed germination and agronomical parameters in green gram (*Vigna radiata* var. sweta) seedlings. The data collected from these isolates could offer the utilization their potential in field application as bioinoculants in green gram. Finally, the study concluded that selected bacteria have enough potential which directly or indirectly encourage plant growth. Therefore, WRPA 4 and WRPA 13 bacterium can be used as plant growth promoting bioinoculant after further evaluation and validation instead of chemical fertilizer to enhance crop production and sustainable agriculture practices.

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