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SCREENING OF PLANT GROWTH PROMOTING RHIZOBACTERIA FOR GROWTH OF PIGEON PEA PLANT

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Abstract:

Plant growth promoting rhizobacteria (PGPR) play an important role to increase soil fertility, plant growth promotion, and suppression of phytopathogens for development of ecofriendly sustainable agriculture. The use of PGPR inoculants as a biofertilizer and antagonistic of plant pathogens provide a promising alternative to the chemical fertilizers and pesticides. Therefore the current study was carried out to enhance the growth of Pigeon pea plant by using PGPR. *Bacillus* spp. and *Pseudomonas* spp. were isolated from Pigeon pea rhizospheric soil. Isolated PGPR were characterized and used as consortium for growth of Pigeon pea plant. It was observed that Pigeon pea plants treated with the consortium of PGPR *Bacillus* spp. and *Pseudomonas* spp. were found to be more in height with luxuriant foliage and more dry mass. The height and dry mass weight of the plants in consortium treated one was more as compared with that of the control and the single rhizobacterial treated one.

Keywords: Plant Growth Promoting Rhizobacteria, Pigeon Pea

INTRODUCTION

Soil is used in agriculture, where it serves as the anchor and primary nutrient base for plant. Pathogenic microorganisms affecting plant health are a major and chronic threat to sustainable agriculture and ecosystem stability worldwide. The various chemical fertilizers were used in the agriculture to increase yields, they kill pathogens, pests, and weeds, and they also have a big harmful impact on the ecosystem. Because of current public concerns about the side effects of agrochemicals, there is an increasing interest in improving the understanding of cooperative activities among plants and rhizosphere microbial populations.

Microbes that indirectly or directly promote plant growth are referred to as plant growth promoting rhizobacteria (PGPR). The use of plant growth promoting rhizobacteria can be the better alternative for this problem. They play an important role to increase soil fertility, plant growth promotion, and suppression of phytopathogens for development of ecofriendly sustainable agriculture. They provides environment to increase crop production and crop health, development of sustainable agriculture and commercialization with global applicability. Thus soil PGPR play important role in determining plant productivity. For successful functioning of introduced microbial bio-inoculants, exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behavior in soil habitats. PGPR are directly responsible for re-cycling of nutrients. In the era of the sustainable agricultural production, the interaction of PGPR play a pivotal role in the transformation, mobilization and solubilization of nutrients from a limited nutrient pool in the soil and ensure uptake of essential nutrients by the crop plant (Bolton et al., 1993; Mantelin and Touraine, 2004). Bacteria belonging to genera *Arthrobacter, Azospirillum, Bacillus, Enterobacter, Serratia and Pseudomonas* have been found to have enormous potential as plant growth promoting rhizobacteria and are now used in agriculture as bio-inoculants (Cattelan et al., 1999; Gupta et al., 2015). *Pseudomonas* comprises a large portion of bacterial population in the rhizosphere. Due to the ubiquity and versatility, there is a considerable interest in exploiting these bacteria for diverse agricultural applications such as plant growth promotion and pest management (Praveen et al., 2012). Many *Bacillus* species are able to secrete large quantities of enzymes. *Bacillus*-based biocontrol agents play a fundamental role in the field of biopesticides. They have been reported as plant growth promoters and competitors for growth factors (space and nutrients) with other pathogenic microorganisms through colonization.

Pigeon pea is mainly cultivated and consumed in developing countries of the world. It is the most important pulse crop in India. It is also known as Red gram or Arhar or Tur. Though pigeon pea is grown in a wide range of agro-ecological situations, its deep rooting and drought tolerant characters make it especially useful crop in the area of low and uncertain rainfall and on the lighter soils. This crop is widely grown in India and India is the largest producer and consumer of Pigeon pea in the world. Pigeon pea is a protein rich staple food (Rajasekhar et al., 2016). Pathogenic microorganisms affecting plant health is a major problem to sustainable agriculture and ecosystem stability worldwide. The chemical fertilizers used in the agriculture also have a harmful impact on the ecosystem. So, there is an urgent need of biological agents is accepted worldwide (Goldstein, 1995). Furthermore plant growth promoting rhizobacteria can reduce chemical fertilizers application and economically, environmentally beneficial for lower production cost as well as recognize the best soil and crop management practices to achieve more sustainable agriculture as well as fertility of soil Therefore the current study was carried out to enhance the growth of Pigeon pea plant using plant growth promoting rhizobacteria.

MATERIALS AND METHODS

Collection of rhizospheric soil sample:

The rhizospheric soil samples around the roots of the healthy pigeon pea crop plants were collected from the field area of Chandrapur (Soil A) and Yavatmal (Soil B), Maharashtra, India. Each sample was collected from the different sites in the same field. In this way two soil samples were collected from the different fields in 1cm depth. The sample was collected from each field in the sterile polythene bags and processed within 24 hours.

Analysis of physical properties of collected soil:

The collected soil was air dried and sieved. Color and texture of the soil was observed.

Isolation and identification of plant growth promoting rhizobacteria (*Bacillus* species and *Pseudomonas* species):

The rhizospheric soil samples were transferred to the microbiology laboratory under the sterile condition. Each soil samples (1gm) was serially diluted in sterile distilled water for isolating rhizobacteria. A 0.1ml of soil suspension from 10^{-5} was spreaded on *Bacillus* differentiation agar and *Pseudomonas* isolation agar by spread plate method. The plates were incubated at 37^{0}C for 24 hours. The isolated organisms were identified on the basis of their morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

Characterization of Plant Growth Promoting Rhizobacteria:

- **Nitrogen fixation test:**

Nitrogen free malate media, containing Bromothymol blue (BTB) as an indicator, was used for the preliminary screening of nitrogen fixation by PGPR. Inoculated plates were incubated at 37^{0}C up to 24 hrs, the blue colored zone forming rhizobacteria were marked as nitrogen fixers (Gothwal et al., 2007).

- **Phosphate solubilization test:**

For estimation of phosphate solubilization, the plate of Pikovskayas agar medium was used. Each PGPR culture was streaked on the center of the media plate containing the tricalcium phosphate as an insoluble phosphate source. Then the inoculated plates were incubated at 28^{0}C for 5 days and observed for the development of the clear zone around the bacterial colony (Rosas et al., 2006).

- **Potassium solubilization test:**

The potassium solubilization activity was analysed by inoculating PGPR culture on the Aleksandrov medium. Each bacterial culture was spot inoculated on these medium plates. The plates were incubated at 37^{0}C for 7 days and were examined for the appearance of the clear zone around the bacterial growth (Sugumaran and Janarthan, 2007).

- **Zinc solubilization test:**

For estimation of zinc solubilization, the mineral salt medium was used. Each PGPR culture was spot inoculated on the center of the media containing zinc compound. Then the inoculated plates were incubated at 28^{0}C for 2 days and observed for the clear zone around the bacterial colony (Goteti et al., 2013).

- **Cellulase production test:**

The Cellulase production was evaluated by inoculating PGPR culture on the carboxymethyl cellulose (CMC) sodium salt medium. Each bacterial culture was inoculated on the media and then inoculated plates were incubated at 28^{0}C for 2 days and after incubation the plates were flooded with the Gram's iodine for 3 to 5 minutes. The plates were observed for clear halos around the bacterial colony (Kasana et al., 2008).

- **Protease production test:**

The protease production was evaluated by inoculating the PGPR culture on the Skim milk agar medium. Each bacterial culture was spot inoculated on the center of the media plates and was incubated at 28^{0}C for 2 days and observed for the clear zone around the bacterial colony (Smibert and Krieg, 1994).

- **Ammonia production:**

The ammonia production was tested by inoculating PGPR culture into peptone water and incubated at 25^{0}C for 7 days. After the incubation added 0.5ml of Nessler's reagent in each tube and observed the color development from yellow to brown (Cappuccino and Sherman, 1992).

- **Indole acetic acid (IAA) production:**

For IAA production the PGPR culture were spot inoculated on the tryptic soya agar (TSA) medium. After inoculation, the plates were incubated at 30^{0}C for 24 hours. The plates were flooded with the Salkowski's reagent for the development of pink color on the spot of inoculation (Bric et al., 1991).

- **Salt tolerance test:**

For the salt tolerance test each PGPR culture was inoculated in nutrient broth medium containing 3%, 5% and 7% NaCl concentration. After inoculation the tubes were incubated at 30^{0}C for 2 days and observed for the presence or absence of the growth.

Plantation of pigeon pea crops:

Out of the 9 bacterial isolates, 3 bacterial isolates were selected as the PGPR (plant growth promoting rhizobacteria) i.e., PGPB4 (Plant growth Promoting *Bacillus* spp.4), PGPB6 (Plant growth Promoting *Bacillus* spp.6), and PGPP2 (Plant growth Promoting *Pseudomonas* spp.2). A loopful culture of the selected PGPR was inoculated in 100 ml sterile nutrient broth and incubated at 37^{0}C for 7 days on a rotary shaker. These inoculums were used for Pigeon pea plants. An amount of 0.3 gm sterile soil was taken in the plastic pots. Pigeon pea seeds were washed with sterile water seven times. Seeds were then treated with PGPR isolates for 30 min. No treated seeds with any isolate were designated as control. Ten seeds were sown at 4 to 5 cm depth of soil in each plastic pot. A total of 10 pots were planted for Pigeon pea seeds by using 2 types of soil samples i.e. Soil A and Soil B (Ashrafuzzaman et al., 2009). The pots were planted as follows.

- Pot 1(Control) : Soil Sample A + Seeds
- Pot 2 (*Bacillus* 4) : Soil Sample A + PGPB 4 treated Seeds
- Pot 3 (*Bacillus* 6) : Soil Sample A + PGPB 6 treated Seeds
- Pot 4 (*Pseudomonas* 2) : Soil Sample A + PGPP 2 treated Seeds
- Pot 5 (*Bacillus* 4 + *Bacillus* 6 + *Pseudomonas* 2) : Soil Sample A+ PGPB 4, PGPB 6 and PGPP 2 treated Seeds
- Pot 6 (Control) : Soil Sample B + Seeds
- Pot 7 (*Bacillus* 4) : Soil Sample B + PGPB 4 treated Seeds
- Pot 8 (*Bacillus* 6) : Soil Sample B + PGPB 6 treated Seeds
- Pot 9 (*Pseudomonas* 2) : Soil Sample B + PGPP 2 treated Seeds
- Pot 10 (*Bacillus* 4 + *Bacillus* 6 + *Pseudomonas* 2) : Soil Sample B + PGPB 4, PGPB 6 and PGPP 2 treated Seeds

Harvesting of the plant and analysis:

Pigeon pea plants were harvested after one and half months. The plants were washed through dipping into a vessel. Plant height (cm) of each plant was recorded and dry weight (gm) of each plant was recorded.

RESULTS AND DISCUSSION

In the current study, two soil samples were collected from the Pigeon pea plants field from the selected field regions. The physical characteristics of the soil samples showed that soil A was brown in color with rough texture while soil B was black in color with smooth texture (Table 1). These samples were further processed for the isolation and presence of the plant growth promoting rhizobacteria i.e. *Bacillus* species and *Pseudomonas* species. From the two soil samples 9 rhizobacteria including 7 *Bacillus* species and 2 *Pseudomonas* species were isolated (Table 2).

Further, the isolates were tested for Nitrogen, Phosphate, Potassium and Zinc solubilization activity. In nitrogen fixation activity, 2 isolates each of *Bacillus* species and *Pseudomonas* species were found to fix nitrogen. In Phosphate and Potassium solubilization activity, 2 *Bacillus* species and 1 *Pseudomonas* species solubilize the phosphate as well as potassium each. However, 4 *Bacillus* species were able to solubilize zinc (Table 2). Plant growth promoting rhizobacteria exert the beneficial effect on the plant growth and the development of the wide variety of mechanism. The exact mechanism by which the PGPR stimulate the plant growth was not clearly established, although the several hypothesis such as the production of phytohormones, suppression of deleterious organism, activation of phosphate solubilization, and promotion of mineral nutrient uptake are usually believed to be involved (Lalande et al., 1989). Metals such as Zn, Cu, Mo, Mn, Co, and Ni are essential for crucial biological processes and developmental pathways (Salla et al., 2011).

Enzyme production test for Protease and Cellulase was performed. In protease production 2 *Bacillus* species and 2 *Pseudomonas* species showed the protease production. On the other hand, 4 *Bacillus* species and 1 *Pseudomonas* species showed the cellulase production. When the isolates were analyzed for ammonia production, it was found that 5 *Bacillus* species and 2 *Pseudomonas* species produced ammonia. Indole acetic acid production test for the isolates showed that 5 *Bacillus* species were able to produce Indole acetic acid (Table 2). Plant growth hormones indole acetic acid induces and accelerates growth in plant via different mechanism for instance IAA which is an endogenous or natural hormone serves to promote root formation (El-al and Faten, 2009). After performing salt tolerance test at 3%, 5% and 7%, it was observed that 7 PGPR were found to tolerate 3% salt concentration followed by 6 PGPR to 5% salt concentration and 1 PGPR to 7% salt concentration in nutrient broth (Graph 1).

When plant growth parameters were evaluated for two soil samples, it was found that the pot, in which the consortium of all the Rhizobacteria was added, has shown very good shoot length with healthy leaves in Soil A (20 cm) and Soil B (22 cm). However, the pots with *Bacillus* spp.6 inoculum followed by the *Pseudomonas* spp.2 inoculum and *Bacillus* spp.4 inoculum was less in shoot length for soil A (18 cm, 16 cm and 15 cm respectively) and for soil sample B (19 cm, 18 cm, and 16 cm respectively). It was noticed that in control plants the plant height is less i.e. soil A (13 cm) and soil B (15 cm) with less foliage compared to consortia treated plants where the plants look green with luxuriant foliage (Table 3).

After harvesting the plants, dry mass weight of the plants, was taken in which the weight of consortium treated plants in both soil samples was more (3.39 gm and 3.6 gm respectively for soil A and soil B) than those which treated by single bacterial inoculum i.e. *Bacillus* spp. 6 (2.79 gm), *Pseudomonas* spp. 2 (2.58 gm) and *Bacillus* spp.4 (2.08 gm) for soil A while *Bacillus* spp.6 (3.11 gm) *Pseudomonas* spp. 2 (2.95 gm) and *Bacillus* spp.4 (2.18 gm) for soil B. On the other hand control plants dry mass weight was less as compared to other treated plants (Table 3).

CONCLUSION:

Plant growth parameter in the present study showed that the Pigeon pea plants treated with the consortium of the isolated plant growth promoting rhizobacteria *Bacillus* spp. and *Pseudomonas* spp. were found to be more in height with luxuriant foliage and more dry mass as compared with that of the control and the single rhizobacterial inoculum treated one. Therefore it was concluded that plant growth promoting rhizobacteria facilitates nutrients availability and its use could form sustainable solutions for present and future agricultural practices.

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Table 1: Physical properties of soil

Soil Samples	Color	Texture
A	Brownish	Rough
B	Black	Smooth

Table 2: Characterization of plant growth promoting rhizobacteria

PGPR Isolates	Soil Sample	Nitrogen fixing activity	Phosphate solubilization activity	Potassium solubilization activity	Zinc solubilization activity	Protease activity	Cellulase activity	Ammonia production	Indole acetic acid production
PGPB1	A	–	–	–	+	–	+	+	+
PGPB2	A	–	–	–	+	+	–	+	–
PGPB3	B	–	–	–	+	–	–	–	–
PGPB4	A	+	+	+	+	–	+	+	+
PGPB5	B	–	–	–	–	–	–	–	+
PGPB6	B	+	+	+	–	–	+	+	+
PGPB7	B	–	–	–	–	+	+	+	+
PGPP1	A	+	–	–	–	+	–	+	–
PGPP2	B	+	+	+	–	+	+	+	–

Where, PGPR = Plant growth promoting Rhizobacteria

PGPB = Plant Growth Promoting *Bacillus* spp.

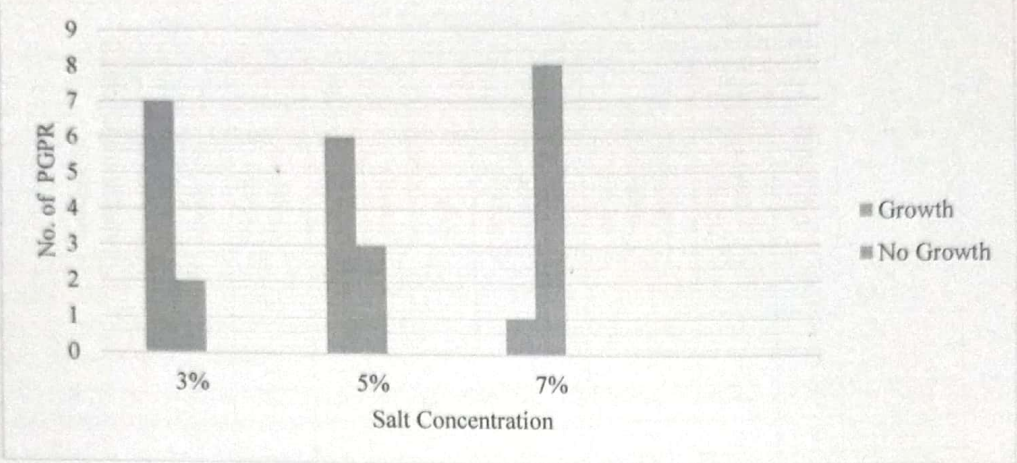
PGPP = Plant Growth Promoting *Pseudomonas* spp.

– = Negative test

\+ = Positive test

Table 3: Pigeon Pea Plant Growth Parameters

Soil samples	Pots	Rhizobacterial Inoculum	Plant Height	Dry Mass
A	1	Soil Sample A + Seeds	13 cm	0.99 gm
	2	Soil Sample A + PGPB4	15 cm	2.08 gm
	3	Soil Sample A + PGPB6	18 cm	2.79 gm
	4	Soil Sample A + PGPP2	16 cm	2.08 gm
	5	Soil Sample A + PGPB4 + PGPB6 + PGPP2	20 cm	3.39 gm
B	6	Soil Sample B + Seeds	15 cm	1.1 gm
	7	Soil sample B + PGPB4	16 cm	2.18 gm
	8	Soil Sample B + PGPB6	19 cm	3.11 gm
	9	Soil Sample B + PGPP2	18 cm	2.95 gm
	10	Soil Sample B + PGPB4 + PGPB6 + PGPP2	22 cm	3.6 gm



Graph 1: Effect of Salt concentration on plant growth promoting rhizobacteria