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RESEARCH ARTICLE

Comparative Study of PGPR Isolated from Crop Plants (Mustard and Maize) and Wild Medicinal Plant (Lantana) and their Potency for Enhancement of Wheat Plant

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are those beneficial bacteria which colonize the rhizosphere region of the root and increase the plant growth activity by the various mechanisms. PGPR induced the production of plant hormones (IAA), ammonia, siderophore, HCN and phosphate solubilisation to enhance the plant growth and development. The aim of this study was to isolate the microorganisms from rhizosphere soil of crop plant (Mustard and maize) and wild medicinal plants (Lantana) of different areas of Lucknow and Kanpur (UP, India). Out of thirty strains, three were giving best PGPR result in which two isolates from wild plant (VY_1 and RC_2) and one from crop plant (PM_1) were selected for the pot experiment. Subsequently, an experiment was conducted in plastic cups containing soil in which seeds of wheat were sown in each cup and treated with selected PGPR to analyze the effect of PGPR on the growth of wheat (*Triticum* sp.) plant. Present study results that PGPR of wild plant give the significant result with increasing the shoot length, root length and dry weight than crop's PGPR. Hence, it is expected that in future PGPRs of wild plant is also very effective as other PGPR and are used as bio-fertilizer to enhance the growth and yield of plants.

Key words: PGPR, IAA, Soil microorganisms, Phosphate Solubilisation, Lantana, Wheat.

INTRODUCTION

Lanatana camara (Lantana) is a type of an ornamental plant which is used in traditional medicine for the treatment of various diseases (Banik, 2007). All the parts of lantana (root, stem and leaves) have various medicinal value and they contain several compounds like allelopathic, antimicrobial, nematicidal and insecticidal activities (Achhireddy & Singh, 1984; Begum *et al*., 2000; Abdel-Hady *et al*., 2005; Marongiu *et al*., 2007; Sharma *et al*., 2007). In agricultural field various studies has been done and some are in working condition on the side effects of chemical fertilizer. Hence, on the basis of present literature and data we can say that intensive introduction of chemical fertilizers in agricultural field causes the reduction of crop productivity and yield. This destructive effect is exhibit through the changing of physicochemical properties of soil and other biological changing (Adediran *et al*., 2004). For controlling these destruction microorganisms work as relevant agent by promoting agricultural yield and productivity and minimize the use of chemical fertilizer and pesticides.

Microbes affect the plant growth in various ways some microbes cause diseases and inhibit plant growth; whereas others can directly or indirectly promote the plant growth through a various mechanisms such as Nitrogen fixation, Phosphate solubilisation, Production of siderophore, phytohormone and ACC deaminase (Glick 2003; Bais *et al*., 2006). A large array of bacterium including species of Pseudomonas, Azospirillum, Azotobacter, Burkholderia, Bacillus, Klebsiella, Enterobacter, Alcaligens, Arthrobacter, and Serratia have reported as plant growth promoting rhizobacteria to enhance plant growth (Kloepper *et al*., 1989). Theses microbes inhabiting in and around the root and enhance the soil qualities also

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(Dastager *et al.*, 2011) and various research work shows that only 1-2% of bacteria work as PGPR (Antoun and Kloepper, 2001).

The exact mechanism of PGPR by which it promote plant growth are not fully understood, but it is thought that PGPRs control the growth of plant either directly or indirectly and includes (i)- the ability to produce or change the concentration of plant growth regulators like indoleacetic acid, gibberellic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993; Glick, 1995), (ii)- a symbiotic N2 fixation (Boddey and Dobereiner, 1995), (iii)- antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), antibiotics (Shanahan *et al*., 1992) and cyanide (Flaishman *et al*., 1996), (iv)- solubilisation of mineral phosphates and other nutrients (De Freitas *et al*., 1997; Gaur, 1990).

The species of bacteria capable of producing IAA include Pseudomonas sp. Azospirillum sp. Bacillus sp. Klebsiella sp. Enterobacter sp. and Serratia sp. (Martens and Frankenberger, 1991; Frankenberger and Arshad, 1995). Many phosphate solubilising bacteria (PSB) belongs to Pseudomonas, Bacillus, Enterobacter, Serratia, Pantoea, Azospirillum, Azotobacter, Rhizobium, Burkholderia, Flavobacterium and to the fungal genera Aspergillus and Penicillium (Deepa *et al*., 2010). A successful example of PGPR is "YIB" (Yield Increasing Bacteria) which was used on a large scale on China (Yan Li, 2011). Hence, there is an important need to explore the area of PGPR from different region of world.

MATERIAL AND METHODS

Isolation of PGPR:

In this study comparative assay were performed between randomly collected soil samples from rhizosphere region of wild plant (Lantana) and crop plant (Mustard and Maize) from different areas of Lucknow and Kanpur (UP, India) to see the potentiality of plant growth promoting rhizobacteria. All soil samples were collected at winter season in the first week of January 2015 in polythene bag from the rhizosphere region at 5 cm depth and stored at 4^oC for further analysis. Bacteria isolated on nutrient agar plates and pure cultures were maintained in glycerol solution and stored at 4^oC for further study.

Characterization of Isolates:

Bacterial strains were biochemically and morphologically characterized as per standard method of Aneja (2003). Pure cultures of the strains were streaked on nutrient agar plates separately for colony development. The individual colonies were examined for shape, type, colour, growth, margin, elevation, size, texture, appearance and optical property. The isolates were biochemically characterized by Citrate agar test, Amylase production test, Dextrose metabolism test, Mac Conkey agar test, catalase test, urease test, MR test, indole production, VP test, Citrate utilization test etc.

Screening of isolates for best plant growth promoting activities:

Production of Indole Acetic Acid

Brick *et al*., (1991) described the production of Indole acetic acid. In this test nutrient broth amended with 1% typtophan were used. After sterilization of broth inoculate the test tubes and incubate it for 3-4 days. After incubation add few drops of orthophosphoric acid then Salkowaski reagent in filtrate. Development of pink colour indicates the presence of IAA in the tubes and absorbance of was recorded at 530 nm wavelength.

Ammonia Production

Bacterial isolates was tested for the production of ammonia in sterilized peptone water broth. Inoculate the tubes and incubate it for 3-4 days. After incubation add few drops of Nessler's reagent in the test tubes. Development of brown colour indicate (+++), faint yellow colour (++) and light yellow colour (+) in tested broth tubes (Cappucino and Sherman, 1992).

Production of HCN

All isolates were screened for the production of hydrogen cyanide by following the method of Lorck, 1948. In this test King's B media amended with 4.4g glycine/l was used, bacteria were streaked on these plates. A Whatman filter paper soaked in 2% sodium carbonate and 0.5% picric acid solution and placed in lid of the plate. Plates were properly sealed with parafilm and incubated at $\pm 28^oC$ for 3-4 days. Development of orange colour indicates the strong production; dark brown indicates moderate and light brown indicates low production of HCN.

Determination of Phosphate Solubilisation

For qualitative analysis of Phosphate solubilisation modified Pikovaskya agar was used (Gupta, 1994). In this media bromophenol blue dye was added for measurement of halo zone developed by phosphate solubilizing bacteria. The

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modified Pikovaskya agar plates were inoculated and observed for inhibition zone around the spot inoculums for three to six days. The inhibition zone was measured and used for calculation of Phosphate solubilization index (De Freitas *et al.*, 1997).

Antibiotic Resistant Test of Selected PGPR:

Antibiotics sensitivity test of the selected bacterial isolates (VY_1, RC_2 and PM_1) was done on MHA plates with antibiotics disc of streptomycin, ampicillin, and cefepime. After inoculation plates were incubated at $\pm 28^{o}C$ for 24 to 48hrs and observe for the inhibition zone. This test is done by following standard method the Kirby- Bauer disk diffusion method (Bauer *et al.*, 1996)

Pot Experiment:

For pot experiment wheat (Triticum sp.) was selected for determination of plant growth ability of selected isolates in plastic pots (6.5 cm diameter). In this pot trails firstly the seeds of wheat were surface sterilized by using ethanol for 2 minutes and $HgCl_2$ for 5 minutes. After sterilization seeds were washed with tap water by ten minutes. Inoculum was prepared in nutrient broth and diluted for obtaining 10^8 cfu/ml. The sterilized seeds were immersed in this suspension for coating of PGPR and incubate for 45 minutes on rotary shaker (Bhatt and Vyas, 2014). After this removed the seeds from broth, air dried and sown on autoclavable Petri plates for germination .Untreated seeds were treated with distilled water and worked as control. Take sterilized wet filter paper with sterilized Petri plates and put ten seeds in each Petri plate. Covered the seeds with other wet filter paper and incubate for 3 days. During these days properly add distilled water so that wet condition will be maintained. Three replicates were maintained for each treatment. The total germination percentage was calculated by using the following formula (Mathivanan, 2014)

$$\text{Germination\%} = \frac{\text{Total number of seeds}}{\text{Total number of seed sown}}$$

After three days number of germinated seedlings of each treatment recorded for calculation of germination percentage and five seedling of each treatment were maintained in plastic posts containing 60 gm sterilized soil and fertilizer. Pot experiment was done in laboratory under the favorable condition for wheat in triplicates (Bhatt and Vyas, 2014). After 15 days removed the plants carefully and washed with tap water. Plant height, root length, dry shoot and root weights were measured and recorded for analysis. Plant growth promoting activity of the antagonists was assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1985). The seedling vigour index was calculated by using formula as described by Adbul Baki and Anderson (1973).

Vigour index (VI) = (Mean root length+ Mean shoot length) × Germination (%)

RESULTS AND DISCUSSION

Thirty bacteria were isolated from agricultural and non agricultural soil samples collected from Lucknow and Kanpur district of Uttar Pradesh, India in which seventeen isolates are of crop plant and thirteen isolates are of wild plant. From seven different soil samples the total viable count and types of cell are given in (**Table 1**). The isolates were characterised for PGPR activity such as production of ammonia, IAA, HCN and phosphate solubilisation. Out of thirty isolates three were selected for the pot experiment on the basis of PGPR screening in which two strains from wild plant (VY_1 and RC_2) and one from crop plant (PM_1) shown in (**Table 2 & 3**). These isolates show three PGPR activities like production of NH3, IAA and phosphate solubilisation (**Fig 1**). HCN production was not reported for any isolates. IAA production in RC_2 is higher than both the isolates VY_1 and PM_1 and ammonia production is recorded as same. Phosphate solubilisation in all the isolates was recorded in very high amount and isolate PM_1 is more solubilising than RC_2 and VY_1. The best isolates were characterized biochemically on the basis of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) were shown in (**Table 4**). These three isolates have resistant properties against the antibiotics namely ampicillin (A), cefepime (cpm) and streptomycin (S) (**Table 5**).

Three effective strains PM_1, VY_1, RC_2 were selected for the pot experiment which gives the best plant growth promoting activity. Results of pot experiment shown, that efficient PGPRs enhance the growth of seedling at significant level than control (**Table 6**). Dry weight of plants is increased by PM_1 (109%), RC_2 (29%), and VY_1 (138%) at efficient level in respect to control and VI is also enhanced as 138%, 135% and 125% by VY_1, RC_2 and PM_1 respectively. This result illustrated that isolates VY_1 is more efficient than other two. Plant treatment shows that isolate RC_2 is not effective for growth but the data shows that

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root dry weight and length is significantly higher than control (**Fig 2**). On the basis of such result we can say that root elongation is enhanced by PGPR and same result is reported by Bertrand *et al*. (2001).

All the isolates which is selected as PGPR are able to shown the multiple plant growth properties, due to this its have ability to enhance the plant growth. Same result is reported by other researcher like Joseph *et al*., 2007; Yasmin *et al*., 2007. The PGPR which is used in this experiment for improvement of wheat growth until it is isolated from rhizosphere of lantana, mustard and maize plant. Hence, we can say that PGPR have ability to enhance the growth of different crops and plants. Wu *et al*., 2005 and Bhattacharyya and Jha, 2012 also reported same findings. Phosphate solubilization improves the growth of plant by providing inorganic soluble phosphorus as nutrients other workers also reported these findings (Whitelaw, 2000).

All the tested PGPRs show higher degree of phosphate solubilization index (more than 15 mm) and have good ability to solubilize phosphate (De Freiteas, 1997) and PM_1, VY_1 and RC_2 have high value of phosphate solubilization index. The result of PGPR tests shows that IAA production is done by PM_1, VY_1 and RC_2 in the presence of precursor L-tryptophan (Tsavkelova *et al*., 2007). In the environment precursor is provided via exudates of root. IAA production in presence of precursor is reported in several genera such as Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Pantoea, Pseudomonas, and Serratia (Bhatt and Vyas, 2014). In pot growth of wheat plant is improved as dry weight and vigour index (Khalid *et al*., 2004) other researcher is also reported that PGPR enhanced the growth and germination in pot in natural condition (Yilmaz, 2003; Amellal *et al*., 1998). This study demonstrates the positive impact of PGPR either it is isolated from wild plant or crop under in vitro conditions. On the basis of these findings we can assumes that in future PGPR is the best alternative of chemical fertilizers and pesticides.

Today, in agriculture, chemical fertilizers are used for high yield of plant, but the deposition of chemicals in the environment potentially disturbs the ecosystem, and finally affects the agricultural ecosystem. Such type of problem is controlled by the use of other alternatives such as plant growth promoting rhizobacteria (PGPR), which have not any harmful effect to the environment. In all over world there is an emergent need to discover various ecological niches in agriculture for the beneficial microorganisms. Several researchers are involved in this field but it is an important to search potential plant growth promoter to achieve desired product and are specific from region to region. Findings of this experiment express that the significant PGPR of wild plant also shows the effective result than other. Therefore, on the basis of this pot trail it is expected that in future PGPR of wild plant is also used as biofertilizer, biocontrol, bioremediators etc. as PGPR inhabited in crop rhizosphere.

Fig 1: Showing the Phosphate solubilisation index (PSI) of all the selected PGPR

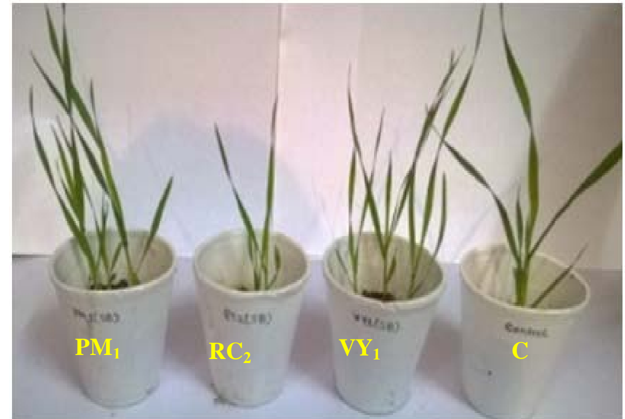
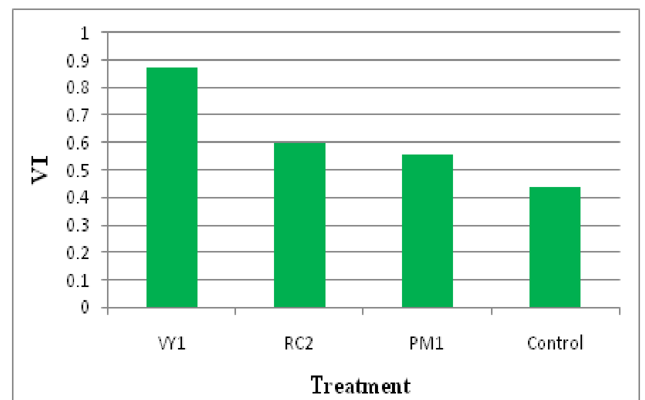


Fig 2: Result of treatment of PGPR



Fi 3: Results of Vigour Index (VI)

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Table 1: Shows Microbial load and types of cells

Sampling sites	TVC (cfu/ml)	Types of cell
Sampling sites of Wild Plant (Lantana)		
RC	105×10^4	3
VY	73×10^4	5
BBAU	$\mathbf{328\times10^4}$	5
Sampling sites of Crop Plants (Mustard and Maize)		
PP	224×10^4	5
KM	$\mathbf{56\times10^4}$	3
PM	142×10^4	5
BL	260×10^4	4

Table 2: Result of PGPR tests of isolates of Agricultural soil

S. No	Isolates	IAA	NH_3	HCN	PSI
1	PP_1	-ve	++ve	-ve	-ve
2	PP_2	-ve	+ve	-ve	-ve
3	PP_3	+++ve	++ve	-ve	+ve
4	PP_4	-ve	+ve	-ve	-ve
5	PP_5	+ve	-ve	-ve	-ve
6	BL_1	-ve	-ve	-ve	-ve
7	BL_2	+ve	+++ve	-ve	-ve
8	BL_3	+ve	+++ve	-ve	+ve
9	BL_4	-ve	+++ve	-ve	+ve
10	PM_1	++ve	+++ve	-ve	+ve
11	PM_2	+ve	+++ve	-ve	-ve
12	PM_3	-ve	-ve	-ve	-ve
13	PM_4	-ve	-ve	-ve	-ve
14	PM_5	+++ve	+++ve	-ve	+ve
15	KM_1	-ve	++ve	-ve	-ve
16	KM_2	-ve	-ve	-ve	-ve
17	KM_3	-ve	+ve	-ve	+ve

Table 3: Result of PGPR tests of isolates of Non-Agricultural soil

S. No	Isolates	IAA	NH_3	PSI	HCN
1	VY 1	++ve	+++ve	+ve	-ve
2	VY_2	-ve	+++ve	-ve	-ve
3	VY_3	-ve	-ve	-ve	-ve
4	VY_4	-ve	-ve	-ve	-ve
5	VY_5	+ve	+ve	-ve	-ve
6	$BBAU_1$	-ve	-ve	-ve	-ve
7	$BBAU_2$	-ve	+ve	-ve	-ve
8	$BBAU_3$	-ve	-ve	-ve	-ve
9	$BBAU_4$	-ve	+ve	-ve	-ve
10	$BBAU_5$	+ve	+ve	-ve	-ve
11	RC_1	-ve	++ve	-ve	-ve
12	RC_2	+++ve	+++ve	+ve	-ve
13	RC_3	+ve	-ve	-ve	-ve

Table 4: Result of Biochemical tests of all the selected PGPR isolates

Strains	VY_1	RC_2	PM_1
Amylase Test	+ve	-ve	-ve
Protease Test	-ve	-ve	+ve
Citrate Utilization Test	+ve	+ve	+ve
Citrate Agar	+ve	+ve	+ve
Catalase Test	+ve	+ve	+ve
MRTest	-ve	-ve	+ve
Vp Test	-ve	-ve	-ve
Urease Test	+ve	+ve	+ve
Mac Conkey Agar Test	+ve	+ve	+ve
Dextrose Metabolism	+ve	+ve	+ve
Indole Production	-ve	-ve	-ve

Table 5: Antibiotic Resistant Test

Isolates	Antibiotic		
	A^{25}	S^{25}	Cpm^{30}
VY_1	R	R	R
RC_2	R	R	R
PM_1	R	R	R

Table 6: Results of Pot experiment

Treatment	Root length (cm)	Shoot length (cm)	Germination %	Enhancement of VI	Enhancement of dry weight
C	11.53	10.8	50		
VY_1	28.5	15.1	50	198%	138%
RC_2	19.5	10.4	50	135%	29%
PM_1	13.75	13.95	50	125%	109%

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