RESEARCH ARTICLE

PLANT GROWTH PROMOTING *PSEUDOMONAS* STRAINS EFFECTIVELY ENHANCE PLANT GROWTH OF *ORYZA SATIVA*.

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Abstract: Aim of present study is to evaluate the effect of PGPR *Pseudomonas* strains on plant growth activity of paddy crop. All *Pseudomonas* strains were isolated from rhizosphere of paddy crop. *Pseudomonas* strains were isolated on King's B medium and fluorescent *Pseudomonas* strains were characterized by biochemical tests. Further, three *Pseudomonas* strains which were IAA positive, HCN positive and Phosphorous solubilize strains named as *Pseudomonas* PS1, PS2, PS3. Total 04 treatments were prepared and these were *Pseudomonas* PS-1 + Paddy seed, *Pseudomonas* PS-2 + Paddy seed, *Pseudomonas* PS-3 + Paddy seed and uninoculated seed (control). Few plant growth parameters such as seed germination, plant height, fresh weight and dry weight of paddy crop were recorded. *Pseudomonas* PS1 showed highest seed germination which was 58.33% more as compared to control. These isolated plant growth promoting *Pseudomonas* strains increased root and shoot length by at least 100 and 50 % more respectively as compared to control. Highest root length has been observed in *Pseudomonas* PS2 treatment but highest shoot recorded in *Pseudomonas* PS1. Further, all strains increased fresh weight and dry weight of root by at least 354 and 202 % more respectively as compared to control but *Pseudomonas* PS1 enhanced 379.27 and 218.57 % more fresh weight and dry weight of root respectively as compared to control. *Pseudomonas* PS1 treatment showed highest fresh and dry weight of shoot by 207.3 and 459.46 % respectively. All results suggested that *Pseudomonas* strains effectively increase plant growth in Paddy crop.

Keywords: *Pseudomonas*, IAA, HCN, Phosphorous solubilization, Paddy crop

INTRODUCTION

Plant growth promoting rhizobacteria are bacteria that colonize plant roots, and they promote plant growth and/or reduce disease or insect damage. In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is a potentially important issue (Deshwal *et al.*, 2003, Deshwal and Kumar, 2013).

These PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized (Dey *et al.*, 2004, Herman *et al.*, 2008). A PGPR *Pseudomonas* fluorescens isolated from the roots of graminaceous plants has been shown to colonize the roots of various plants, and to increase the height, flower number, fruit number and total fruit weight of wheat plant. Under salt stress, PGPR have shown positive effects in plants on such parameters as germination rate, tolerance to drought, weight of shoots and roots, yield, and plant growth (Kloepper *et al.*, 2004, Kokalis-Burelle *et al.*, 2006). Another major benefit of PGPR is to produce antibacterial compounds that are effective against certain plant pathogens and pests (Dey *et al.*, 2004, Herman *et al.*, 2008). Moreover, PGPR mediate biological control indirectly by eliciting induced systemic resistance against a number of plant diseases (Jetiyanon and Kloepper, 2002). Application of some PGPR strains to seeds or seedlings has also been found to lead to a state of induced systemic resistance in the treated plant (Kloepper *et al.*, 1999). PGPR have also been reported in cereal crops including paddy. In addition to improvement of plant growth, PGPR are directly involved in increased uptake of nitrogen, synthesis of phytohormones, solubilization of minerals such as phosphorus, and production of siderophores that chelate iron and make it available to the plant root (Lalande *et al.*,1989, Glick, 1995, Bowen and Rovira, 1999). It has also been reported that PGPR is able to solubilize inorganic and/or organic phosphates in soil (Liu *et al*., 1992). Paddy is the most important staple food in several developing countries, and chemical fertilizer is the most important input required for rice cultivation. In Bangladesh, 70% of the total cropped land and 82% of the irrigated land are used for paddy cultivation (Bangladesh Bureau of Statistics, 2002). The high-yielding rice variety has resulted in an increase in rice production but requires large amounts of chemical fertilizers, leading to health hazards and environmental pollution. In order to make rice cultivation sustainable and less dependent on chemical fertilizers, it is important to know how to use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that can contribute to

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the improvement of paddy growth. There is a growing interest in PGPR due to their efficacy as biological control and growth promoting agents in many crops (Deshwal *et al.*, 2003). So aim of present study is to evaluate the effect of Plant Growth Promoting *Pseudomonas* strains on seed germination, plant height, fresh weight and dry weight of paddy crop.

MATERIALS AND METHODS

Isolation and characterization of *Pseudomonas*: Rhizospheric soil was collected from healthy Rice plant. 0.1gm rhizospheric soil was suspended into 10ml sterilized distilled water (SDW) and vortexed for 10 seconds to prepare homogenous suspension. Serial dilution method was used for reduction of number of microorganisms. 0.1ml soil suspension from each tube was spreaded on individual plates containing King's B medium. All plates were incubated at 28 ± 1^0C for 48hrs. After incubation colonies appeared on King's B plates. Fluorescent colonies were selected for purification of fluorescent *Pseudomonas* and streaked on King's B media plates.

Characterization of *Pseudomonas*: These isolated strains were characterized on the basis of biochemical tests as mentioned in Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).

Plant Growth Promoting Activity: These isolated strains were characterized on the basis of IAA, HCN and Phosphorous solubilizing activity of isolated strains.

(a) **Indole production test:** Tryptone broth was prepared and transferred into test tubes. After sterilization, these test tubes were then inoculated with the culture and one tube was kept uninoculated as control. These inoculated tubes incubated at 28^oC for 24 h. After 24 h of incubation, 1 ml of kovac's reagent was added to each tube including control. Shaked the tubes gently after intervals for 10-15 min and allowed tubes in standing position. Development of cherry red color in the top layer of the tube indicated a positive result.

(b) HCN production: *Pseudomonas* strains were streaked on TSM medium plates supplemented with 4.4 g per litre glycine with simultaneously supplemented filter paper soaked in a 0.5% picric acid in 1% Na_2CO_3 in the upper lid of petri plate. The plates were sealed with paraffin and control plates did not receive any *Pseudomonas* inoculum. Plates were incubated at 28^oC for 1-2 days. Change in color of the filter paper from yellow to brown.

(c) P-solubilization test: Characterized strains were transferred on Pikovskya's agar medium and inoculated at 28 ± 1^oC for 3-5 d and clear zone around the colony showed P-solubilization.

Plant Growth Activity: Seed bacterization technique is used for evaluation of PGPR activity of *Pseudomonas* strains.

Seed bacterization: Only three PGPR strains of *Pseudomonas* were selected and recoded as *Pseudomonas* PS1, *Pseudomonas* PS2, *Pseudomonas* PS3. Paddy seeds were surface-sterilized with 0.5% NaOCl solution for 1-2 min, rinsed in sterilized distilled water and dried under a sterile air stream. Cells of PGPR strains were grown under continuous shaking condition (150 rpm) on King B broth for *Pseudomonas* at 28 ± 1°C for 24h. Each culture was separately centrifuged at 7000 rpm for 15 min at 4°C. The culture supernatant was discarded and the pellets were washed with sterile distilled water (SDW) and resuspended in SDW to obtain a population density of 10^8 cfu ml^{-1}. The cell suspension was mixed with 1% carboxymethylcellulose (CMC) solution. The slurry was coated separately on the surface of rice seeds and allowed to air-dry overnight in aseptic condition. The seeds coated with 1% CMC slurry without bacterial strains served as control.

Pot size and soil: Sterile earthen pots (24 cm × 12 cm × 12 cm) were filled with sterilized sandy loam soil (0.25% total organic matter, 0.096% total organic C, 38% water-holding capacity, pH 6.8).

Treatments: Total 04 treatments were prepared and these were *Pseudomonas* PS-1 + Paddy seed, *Pseudomonas* PS-2 + Paddy seed, *Pseudomonas* PS-3 + Paddy seed and uninoculated seed (control). Four seed seeds of each treatment were sown in each pot. After 15 days, thinning was done to raise only single healthy plant in each pot. The plants were irrigated with sterilized water when ever required. Few parameter such as seed germination rate, root and shoot length, fresh and dry weight of paddy crop were analyzed after 30 days of sowing.

RESULTS AND DISCUSSION

All characterized *Pseudomonas* strains were evaluated for Plant Growth Activity i.e. IAA, HCN and Phosphorous solubility. The cherry red colour was developed in tubes when 1ml of kovac's reagent was added in 24 hours old culture which confirmed that isolated characterized *Pseudomonas* strains were Indole positive. After 24 hours, filter paper soaked in a 0.5% picric acid in 1% Na_2CO_3 showed change in colour of the filter paper from yellow to brown confirmed that isolated characterized *Pseudomonas* strains were HCN positive. These *Pseudomonas* strains showed clear zone around colony on Pikovskya's agar medium confirmed that strains have ability to solubilize phosphorous. Three *Pseudomonas* strains were selected which showed all three PGPR activity and rename it i.e. *Pseudomonas* PS1, *Pseudomonas* PS2 and *Pseudomonas* PS3.

All *Pseudomonas* strains increased seed germination by at least 33.33 % and *Pseudomonas* PS1 showed highest seed germination which was 58.33% more as compared to control. These isolated plant growth promoting *Pseudomonas* strains increased root and shoot length by at least 100 % and 50 % more

respectively as compared to control. Highest root length has been observed in *Pseudomonas* PS2 treatment but highest shoot recorded in *Pseudomonas* PS1. Further, all strains increased fresh weight and dry weight of root by at least 354 and 202 % more respectively as compared to control but *Pseudomonas* PS1 enhanced 379.27 and 218.57 % more fresh weight and dry weight of root respectively as compared to control. *Pseudomonas* PS1 treatment showed highest fresh and dry weight of shoot by 207.3 and 459.46 % respectively (Table 1). Similarly, *Pseudomonas* GN 1201 increased significantly the dry shoot weight, root length and root dry weight in soybean crop as compared to control (Cattelan *et al.*, 1999). Similarly, Plant growth promoting Pseudomonas strains increased 27.6% productivity in *Pelargonium graveolens* L. (Mishra *et al*. (2010). Field trials of a pseudomonad strain (GRP3) lead to a great increase in yield of legumes (Johri, 2001). Yazdani *et al*. (2009) reported that phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR). Our finding confirmed that Plant growth promoting *Pseudomonas* significantly increased plant growth in paddy crop. These isolated strains would be better PGPR for paddy crop.

Table 1. Evaluation of plant growth activity of *Pseudomonas* strains in paddy crop.

S. No.	Treatment	Seed germination (%)	Length (Inch)		Weight (g)			
					Root		Shoot	
			Root	Shoot	Fresh	Dry	Fresh	Dry
1	*Pseudomonas* PS1	95	3.0±0.1	18.6±0.2	3.93±0.2	2.23±0.1	4.21±0.2	2.07±0.2
2	*Pseudomonas* PS2	80	3.2±0.1	18.1±0.4	3.76±0.2	2.22±0.1	3.9±0.2	2.1±0.2
3	*Pseudomonas* PS3	90	3.1±0.1	18.0±0.4	3.73±0.2	2.12±0.1	4.01±0.1	2.2±0.2
4	Control	60	1.5±0.1	12±0.2	0.82±0.1	0.70±0.1	1.37±0.2	0.37±0.2

REFERENCES

Deshwal, V.K., Dubey, R.C. and Maheshwari, D.K. (2003). Isolation of plant growth promoting strains of Bradyrhizobium Arachis sp. with biocontrol potential against Macrophomina phaseolina causing charcoal rot of peanut. Current Science, 84(3): 443-448.

Deshwal, V.K. and Kumar, P. (2013). Plant growth promoting activity of Pseudomonads in Rice crop, International Journal of Current Microbiology & Applied Science, 2(11): 152-157.

Dey, R., Pal, K.K., Bhatt, D.M. and Chauhan, S.M. (2004). Growth promotion an yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiology Research, 159: 371-394.

Herman, M.A.B., Nault, B.A. and Smart, C.D. (2008). Effects of plant growthpromoting rhizobacteria on bell pepper production and green peach aphid infestations in New York. Crop Protection, 27: 996-1002.

Kloepper, J.W., Reddy, M.S., Rodríguez-Kabana, R., Kenney, D.S., Kokalis-Burelle, N., Martinez-Ochoa, N. and Vavrina, C.S. (2004). Application of Rhizobacteria in transplant production and yield enhancement. Acta Horticulturae, 631: 217-229.

Kokalis-Burelle, N., Kloepper, J.W. and Reddy, M.S. (2006). Plant growthpromoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Applied Soil Ecology, 31: 91- 100.

Jetiyanon, K. and Kloepper, J.W. (2002). Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases Biological Control, 24: 285–291

Kloepper, J.W., Rodriguez-Ubana, R., Zehnder, G.W., Murphy, J.F., Sikora, E. and Fernandez, C. (1999). Plant root bacterial interactions in biological control of soil borne diseases and potential extension to systemic and foliar diseases. Austrian Plant Pathology, 28:21- 26.

Lalande, R., Bissonnette, N., Coutlée, D. and Antoun, H. (1989). Identification of rhizobacteria from maize and determination of their plant-growth promoting potential. Plant Soil. 115: 7-11.

Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology, 41: 109-117.

Bowen, G.D. and Rovira, A.D. (1999). The rhizosphere and its management to improve plant growth. Advances in Agronomy, 66:1-102.

Liu, S.T., Lee, L.Y., Tai, C.Y., Hung, C.H., Chang, Y.S., Wolfram, J.H., Rogers, R. and Goldstein, A.H. (1992). Cloning of an *Erwinia herbicola* gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in *Escherichia coli* HB101. Journal of Bacteriology, 174: 5814-5819.

Bangladesh Bureau of Statistics (2002). Statistical Yearbook of Bangladesh. Statistics Division, Ministry of Planning, Govt. of the People's Republic of Bangladesh, Dhaka.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994). Bergey's manual of determinative bacteriology, 9th edn. Baltimore: Williams and Wilkins press.

Thakuria, D., Talukdar, N.C., Goswami, C., Hazarika, S., Boro, R.C. and Khan, M.R. (2004). Characterization and screening of bacteria from the rhizosphere of rice grown in acidic soils of Assam. Current Science, 86: 978-985.

Cattelan, A.J., Hartel, P.G. and Fuhrmann, J.J. (1999). Screening for plant promoting rhizobacteria to promote early soybean growth. Soil Science Society American Journal, 63:1670-1679.

Mishra, R.K., Prakash, O., Alam, M. and Dikshit, A. (2010). Influence of plant growth promoting rhizobacteria (pgpr) on the productivity of *Pelargonium graveolens* L. Herit Recent Research in Science and Technology, 2(5): 53-57.

Johri, B.N. (2001). Technology development and demonstration of a new bacterial inoculant (GRP3) for improved legume production. Uttar Pradesh Govternment, Project report.

Yazdani, M., Bahmanyar, M.A., Pirdashti, H and Ali, M. (2009). Effect of Phosphate Solubilization Microorganisms (PSM) and Plant Growth Promoting Rhizobacteria (PGPR) on Yield and Yield Components of Corn (Zea mays L.) World Academy of Science, Engineering and Technology 49.