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Native PGPMs as Bioinoculants to Promote Plant Growth: Response to PGPM Inoculation in Principal Grain and Pulse Crops

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Abstract

Soil organisms interact intrinsically with plant roots in rhizosphere and directly influence plant development by provision of important growth promoting metabolites that affect nutrient availability, uptake and overall growth in host plants. Plant growth promoting microbes (PGPMs) can be used as viable alternative to chemical fertilizers as well as to improve fertility (health) of degraded and marginal soils. Isolation of native microbes was done from native soils of catchment areas of Yamuna river bed, NOIDA, UP. Reference MTCC culture with known PGPM properties (designated as PGPR1) was used in the study along with native isolates. The PGPMs isolated from different host plant rhizosphere were designated as PGPR 2, PGPR 3, PGPR 4 and PGPR 5 respectively. Isolated PGPMs were tested for plant growth promoting properties through various *in vitro* biochemical assays. When tested for production of plant growth regulator hormone Indole acetic acid, all PGPMs except for PGPM 5 tested positive. Siderophores involved in mobilizing minerals and other metal ions as well as defense against phytopathogenic microorganisms were assayed for. Both hydroxymate and cathechol type siderophore production was assayed and PGPMs 1, 2, 3 and 5 were found to produce catechol type siderophore that is particularly implicated with plant growth promoting abilities. Lipase, an important plant growth indicator that facilitates nutrient availability (by contributing to breakdown of complex compounds and emulsification of phospholipids) was studied. PGPR 1, 2, 3 and 5 tested positive for lipase production and for emulsification.
After establishing plant growth promoting properties of isolates through *in vitro* biochemical assys, the plant growth promoting effects were confirmed through *in vivo* plant inoculation assay. Parameters

recorded as a measure of plant's growth promoting abilities are: root and shoot elongation, impact on percent germination and biomass changes. Experiment was conducted with and without PGPM inoculation in 3 principal hosts: wheat, moong and gram. While all the PGPMs reflected plant growth promoting properties in *in vitro* assays, the results of *in vivo* tests reflected a clear preference of some PGPM to certain hosts as compared to others. The percent germination varied from 84.4% to 96.3% in case of un-inoculated and inoculated host plants and none of the isolates showed a very pronounced impact on germination rate. Significant increase in biomass was recorded up to 67.65% in moong inoculated with PGPR 5. PGPR 1 and PGPR 2 showed consistent improvement in plant growth parameters in all hosts indicating no host specific bias. PGPR 3 and PGPR 4 exhibited preference for moong and wheat while PGPR 5 showed very strong preference for moong only. Results from in vitro plant bioassays clearly support the hypothesis that host preferences play an important role in PGPM inoculation. The study furthers the importance of such prior testing and screening before selecting appropriate host-microbe combinations as bioinoculants for agricultural applications.

Keywords: PGPM, bioinoculants, IAA, Siderophore, in vitro plant bioassays.

1. Introduction

Plant growth promoting organisms (PGPMs) promote plant growth affecting nutrient availability and uptake from rhizosphere. They also help to counter pathogen invasion and/or assist in fighting various stresses thus improving overall growth of host plant. Huge amount of scientific literature is available documenting the successful use of many such symbiotic and non-symbiotic rhizobacteria that improve growth, development, and yield of agriculturally important crop plants. Growth promoting properties of these PGPMs are brought about due to various mechanisms that include production of plant hormones as IAA, GA, Phosphate solubilization, nutrient mobilization, siderophore production, release of various enzymes like lipase, protease and biocontrol of pathogens etc., is well documented [Cassan et al, 2009].

Even though literature is there since many years, we still do not have a chosen biofertilizer that could be applied across different crop plants across different ecological zones. This is because PGPMs that are known to be helping in plant growth promotion are very specific with respect to the soil type, host, nativity of strain, soil physiochemical properties and dosage of application. Even though many of the PGPMs are non endophytic and non symbiotic, they are known to have preferential host choice among different host plants [Santa et al, 2004]. Hence it is important that we should have specific isolates from particular geographical zone to be devoted as bioinoculants so as to offer maximum benefit to crop plants in same or similar geographical location. With this intention we have isolated native soil inhabiting Plant Growth Promoting Microbes (PGPMs) from rhizosphere of different crop plants and

studied their promise to be used as bioinoculants for agriculture. In order to determine if there is any choice of host and PGPM both pulse and grain crops were tested in combination with different PGPMs.

2. Material and Methods

2.1 Isolation of Microbes from Field Soil

Samples were collected from Potato (*Solanum tuberosum*), banana (*Musa musa*), sorghum (*Sorghum bicolor*) and Grass sp. from adjoining fields near NOIDA (Western U.P, India). Soil temperature was recorded at the sites of sample collection and soil pH was measured immediately after carrying soil samples to lab. Collected soil samples were homogenized by sieving and a representative sample of soil was added to sterilized distilled water (1:10W/V).From this soil solution,$10^{5\text{-}6}$ times diluted soil samples were plated on Kings' B Selective Agar with antibiotics and incubated at 30^0C for 24 hrs. Based on fluorescence exhibited in UV, Isolated colonies were picked and re-plated on Kings'B selective plates in order to obtain distinct CFUs (colony forming units). Visibly different colonies based on morphology were selected for further categorization following standard microbial procedures for gram's staining, catalase, amylase and urease activities. The PGPMs isolated from different host plant rhizosphere were designated as PGPR 2, PGPR 3, PGPR 4 and PGPR 5 respectively. Reference MTCC culture with known PGPM properties used in the current study was designated as PGPR1. Favorable growth conditions as pH and temperature optima were standardized using standard microbiology protocols. All selected isolates were grown on Kings'B media for routine experiments and stocks were stored at -80^0C in 20% Kings'B -glycerol broth.

Table 1: Sources of native soil isolates.

Nomenclatures	PGPM isolated from rhizosphere of
PGPR1	MTCC 2421
PGPR2	Solanum tuberosum
PGPR3	Musa musa
PGPR4	Sorghum bicolor
PGPR5	Grass Sp.

2.2 Screening for Plant Growth Promotion Properties

Various *in vitro* biochemical assays were performed to test plant growth promoting properties of selected isolates.

2.2.1 IAA Production

IAA producing activity was measured following the protocol given by Akbari et al., [2007] with all PGPMs. The measurement of IAA was done by spectrophotometry at 570 nm using Salkowsky reagent (2% 0.5M FeCl in 35% perchloric acid).

2.2.2 Siderophore Production

Arnow Assay was performed for detection of Catechol-type siderophores. One ml of actively growing bacterial culture was hydrolyzed with 1 ml of 1 N HCl. Thereafter, 1ml of a nitrite molybdate reagent (10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of milli Q water) was added to the hydrolyzed culture supernatant. The presence of a catechol type of siderophore would be indicated by the development of a yellow color and confirmation will be obtained if the yellow color changes to red within 5 min of the addition of 1 ml of 0.5 N NaOH [Zamin et al, 2011]. $FeCl_3$ test was performed for Hydroxymate type siderophore detection. One ml of culture filtrate was mixed with 5 ml of 1 mM ferric chloride solution. The presence of hydroxamate type of siderophores would be indicated by the development of green color. If no color appears it would indicate absence of hydroxamate type siderophores [Logeshwaran et al, 2009].

2.2.3 Lipase Production

Olive oil was added to the growth medium @ 30ml/l and plates were incubated for 12hr - 24hr after inoculation. Appearance of blue color of oil globules after flooding the plate with copper sulfate should be recorded as positive reaction for lipase production.

2.2.4 Emulsification assay

20 ml of distilled water with 10 µl of crude oil was added to a Petri dish (10cm diameter) followed by the addition of 10 µl of culture broth to the oil surface. Observations recorded after 15 minutes for appearance of clearing zones on the oil surface [Cipinyte et al., 2011].

2.3 Host-microbe interaction studies

In vivo plant growth promoting traits of all PGPMs were tested via plant inoculation assay. Surface sterilized seeds were coated with PGPM culture broth containing 10^9 cfu of PGPMs. PGPM coated seeds were placed for germination in petriplates on sterilized cotton. 25 seeds per plate per host (wheat, moong and gram) were inoculated in triplicate for comparing germination percent. PGPM-Inoculated and un-inoculated seeds with healthy radical and plumule were selected and grown for an extended period of ten days using tube-in-tube method developed in our laboratory. Impact on seed germination, root and shoot elongation and biomass changes were recorded as a measure of plant's growth promoting abilities upon PGPM inoculation. Statistical analyses were done using one-way ANOVA including all replicate values. For root length and shoot length data presented are mean of selected twelve replicates. XLstat Microsoft software was used for all analysis. All comparisons of means were calculated at P value 0.01.

3. Results and Discussion

Many rhizosphere bacteria are known to have beneficial effect upon plant growth (Glick, 1995). An increasing number of PGPR are successfully used as commercial biofertilizers for agricultural improvement but still no chosen PGPMs as biofertilizer have broad range applicability that could be applied across different types of hosts. To

understand these specific PGPM- host interactions, native microbes were Isolated from rhizospheric soils of Potato (*Solanum tuberosum*), banana (*Musa musa*), sorghum (*Sorghum bicolor*) and grass fields. The pH of the soil samples from where soil samples were collected was observed in range of 7.5-7.8 pH and the soil temperature (at a depth of 10 inch approximately) was in the range of 28^0C-31^0C.The PGPMs obtained from representative host rhizosphere were named as listed in Table: 1 below. The temperature tolerance range for all the PGPMs found to be in range of 30°C to 40°C. The pH optimum, for all the PGPMs was observed to be in near neutral range. Hence native rhizospheric conditions having subsurface temperature between 28^0C-31^0C and pH ranging from 7.5-7.8 were perfectly suitable to flourish these PGPMs. Results of in vitro biochemical assays to determine plant growth promoting properties and agronomic utility of these PGPMs are tabulated as Table: 2 below.

One of the principal mechanisms of promoting plant growth is related to the capability of PGPMs to produce plant-growth-promoting substances as IAA [Cassan et al, 2009, Akbari et al, 2007]. All the five PGPMs were tested for IAA production and all found to be positive except PGPR 5.Presence of another growth promoting substances, hydroxymate and cathechol type siderophores was assayed and PGPR 1, PGPR 2, PGPR 3 and PGPR 5 were found to produce catechol type siderophore frequently reported in plants growth promoting organisms. In biochemical assays, PGPR 1, PGPR 2, PGPR 3 and PGPR 5 were tested positive for lipase production and for emulsification. All these test results confirmed that these PGPMs isolated from native soils process promising plant growth promoting properties.

Table 2: Results of biochemical analysis.

Isolates	IAA Production	Siderophore catechol type	Siderophore hydroxymate type	Lipase production	Emulsification
PGPR1	+ve	+ve	-ve	+ve	+ve
PGPR2	+ve	+ve	-ve	+ve	+ve
PGPR3	+ve	+ve	-ve	+ve	+ve
PGPR4	+ve	ND	-ve	ND	ND
PGPR5	ND	+ve	ND	+ve	+ve

Further to confirm effect of these PGPMs isolated from native soils on plant growth promotion and to understand host-microbe interactions, *in vivo* plant inoculation studies were performed. Inoculated and un-inoculated seeds were tested for germination and uniformity of growth. The percent germination in un-inoculated moong and gram was 96.3% .Impact of PGPM inoculation on germination ranged from 84.4% (with PGPR 2) to 95.6% (with PGPR 5) in moong and 85.4% (with PGPR 5) to 93% (with PGPR 1) in gram. Un-inoculated percent germination for wheat was 89.7% which showed maximum 1.5% increase with PGPR 3 and PGPR 4 inoculation. However these differences observed were not significant statistically as evident from one way ANOVA analysis conducted.

Majority of PGPM recorded increased root length with hosts moong and wheat. Highest percent increase in root length was 28.3% in case of PGPR 1-wheat combination. This was closely followed by combination PGPR 3-moong showing 26.5% increase (Graph-1.a and 2.a). Least effects were observed when PGPMs were combined with gram host. Gram plant showed only 2% and 1% increase with PGPR 1 and PGPR 2 respectively which are once again, statistically insignificant. PGPR 3, 4 and 5 combinations showed reduction in root length with gram.Overall, on an average, wheat and moong showed 20% increase in root length upon PGPM inoculation.

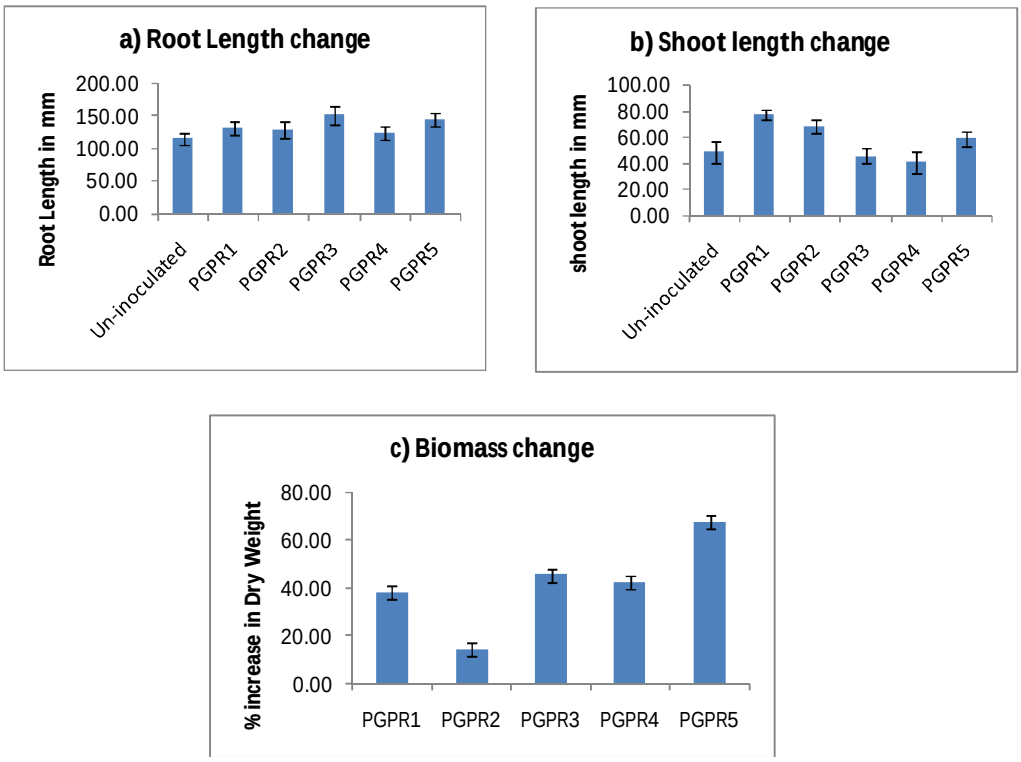

Figure 1: Effect of PGPR inoculation on Moong.

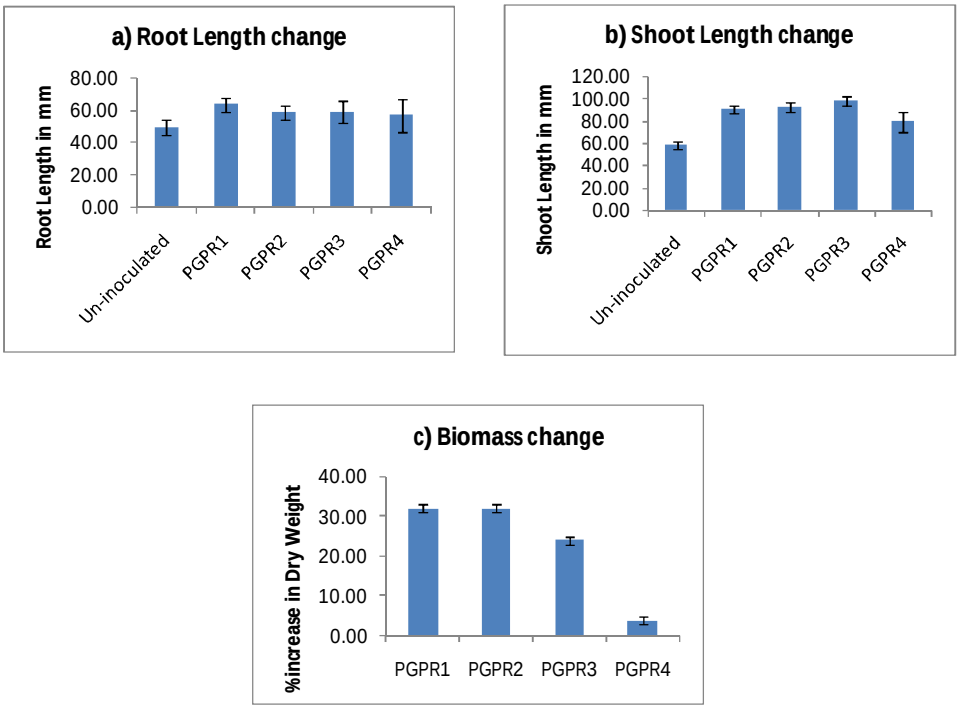

Figure 2: Effect of PGPR inoculation on Wheat

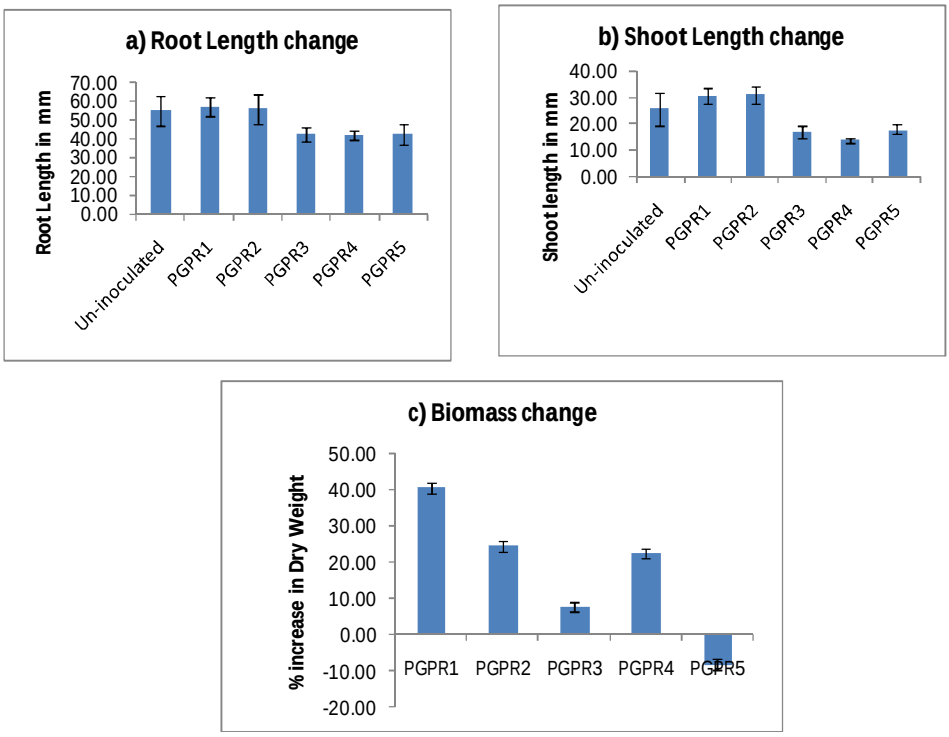

Figure 3: Effect of PGPR inoculation on Gram.

With shoot length also majority of PGPMs recorded increase with wheat and moong (Graph-1.b and 2.b). Highest percent increase in shoot length was 60.3%

observed with combination PGPR1-moong, followed by combination PGPR1-wheat with 55.5% increase (Graph-2 and 5). Wheat was comparatively better performer where shoot length was reported with average 39.5% increase followed by average 20.2% increase with inoculation in moong. PGPM inoculation exhibited more pronounced affect on biomass production and recorded positive for all combinations. Minimum 4% increase in combination PGPR 4-wheat to maximum 67.65% increase in moong inoculated with PGPM 5 (Graph-1.c and 2.c). Only PGPR 5-gram combination showed slight reduction (8.2%) in biomass compared to un-inoculated (Graph-3.c). Further no correlation was observed between shoot growth and biomass production though total biomass values strongly correlated with root development.

While accessing suitability of PGPM isolates, it was observed that PGPR1, 2 had shown consistently better performance in all parameters studied including root length, shoot length increase and improvement in overall biomass as compared to other isolates. These two isolates did not show very high host bias amongst three hosts studied. PGPR1, 2 showed 15 to 40% increase in total biomass and 20-60% increase in shoot length in all hosts studied. Root length increase was 13-29% for moong, wheat whereas gram showed no affect. Such plant growth responses are well documented in literature [Mehnaz and Lazarovits, 2006]. PGPR 3 exhibited clear preference for wheat showing 19.0% increase in root length, 43.1% increase in shoot length and 24% increase in biomass production. Second favorite host for PGPR3 was moong with 32.1% increase in root length, and 45.6% increase in biomass production. Isolate PGPR3 did not favor gram host as observed in fig.3. PGPR3-gram combination show only marginal increase (7.8%) in biomass. However this may be but due to thick stunted malignant root growth not due to growth promotion. Similar development was observed with PGPR 4-gram combination where 22.36% increase in biomass was recorded despite 21.4 % and 69.4% decrease in root length and shoot length respectively. Many researchers reported similar nodule like tumorous growth at root tips of non-leguminous plants when inoculated with IAA producing bacteria [Akbari et al, 2007]. PGPR 4 showed most inconsistent performance with no clear preferences for any host. Though PGPR4 exhibited increase in moong root length and biomass by 8.0% and 42.7% respectively, shoot length decrease by 15.4% and thick stunted leaves develop which indicate malignancy and may be reason for unreasonable increase in biomass. With wheat host it show increase in all parameters, root length by 14.4%, shoot length by 21.4% but very marginal increase in biomass by 4% only renders it not very suitable candidate to be selected as prospective PGPR. PGPR5 showed strong preference for moong showing 26.5% increase in root length, 21.4% increase in shoot length and highest increase in biomass production of 67.7%.Many PGPM show host specific behavior as reported earlier also [Taurin et al, 2010]. PGPM 5 did not favor gram host as evidenced by the 23.2% decrease in root length, 55.8% decrease in shoot length and 8.2% decrease in biomass production as compared to the control. PGPR 5 also inhibited percent germination in wheat by 20.7% indicating no preference for wheat host as well.

4. Conclusion

Results of the present study clearly indicate that isolated native PGPMs stimulate some biological responses in host plants resulting into greater root length, shoot length and overall biomass in all host plants studied *ie*., moong, wheat and gram. Results from in vivo plant bioassays support the hypothesis of variable effects of PGPM inoculants having specific host preferences. PGPR1 and PGPR2 had emerged as better performer with no host specific bias amongst studied host plants thus can be most suitable candidates as a prospective PGPM. Inconsistent performance of PGPR 4 makes it less suitable as a prospective bioinoculant. PGPR5 showed strong preference for moong while PGPR 3 favored wheat and moong. These isolates can be selected as suitable bioinoculant with respect to their preferred hosts. As there is increasing trend of using PGPMs as commercial biofertilizers for agricultural improvement, our findings reiterate the importance of extensive prior testing and screening before selecting appropriate host-microbe combinations as bioinoculants for further agricultural use.

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