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Effect of Inoculation of PGPR Consortia on N Supply, Growth and Grain Yield of Wheat Crop

Diksha Vishwakarma[1*], J.K. Thakur[2] and S.C. Gupta[1]

[1]Rajmata Vijayaraje Scindia Krishi Vishwa Vidayalaya, Gwalior, RAK College of Agriculture, Sehore, (Madhya Pradesh) 466 001, India
[2]Division of Soil Biology, ICAR-Indian Institute of Soil Science, Bhopal - 462 038, Madhya Pradesh, India
**Corresponding author*

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A B S T R A C T

The aim of present investigation was to study the inoculation effect of PGPR consortia on N supply, growth and grain yield of wheat. Results revealed the highest nitrate reductase activity with inoculation of PGPR at recommended dose of N fertilizer compared to all other treatments, indicating bioinoculant on availability of nitrogen. The highest root length was recorded in T4 (50%N+ Consortium of PGPR) which was statistically at par with T6 and T5. The root length measured for T2 (Consortium of PGPR only) ($p<0.05$; 14.4cm) was at par with T3 (Recommended Dose of Fertilizers). The straw yield of wheat was found significantly higher when PGPR consortia was applied along with recommended dose of inorganic fertilizers ($p<0.05$; 13.27g/pot) which was equal to recommended dose of chemical fertilizers ($p<0.05$; 13.27g/pot) and at par with T5 ($p<0.05$; 12.22g/pot). The grain yield of wheat was found numerically higher in T6 ($p<0.05$; 11.68g/pot) but was statistically at par with T3 *i.e.* RDF treatment ($p<0.05$; 10.28g/pot). Study suggested that after confirmation these isolates can be utilized as potential bioinoculants for enhancing wheat productivity.

Introduction

Wheat, together with rice, represent the most important staple food crops used to sustain humanity, as they provide more calories and proteins in the diet than any other crop. Many a time the response of effective inoculums gets diluted compared to the efficiency observed in lab study primarily because the interaction between microbes and plants in soil environments are complex and difficult to manage. To achieve the maximum growth promoting activity, it is important to understand the interaction between PGPR and associated plants and how the beneficial attribute of PGPR are altered by various biotic and abiotic soil factors. Therefore, it is necessary to develop efficient strains for real conditions.

Some PGPR release a blend of volatile components like 2, 3-butanediol and acetoin that promote growth of *Arabidopsis thaliana* (Ryu *et al.,* 2003). The diazotroph bacterial inoculation significantly increases the seed cotton yield, plant height and microbial population in soil (Anjum *et al.*, 2007).The plant growth stimulating efficiency of bacterial inoculants is affected by soil nutritional condition. The bacterial inoculation has a much better stimulatory effect on plant growth in nutrient deficient soil than in nutrient rich soil (Egamberdiyeva, 2007).

Plant growth-promoting bacteria (PGPB) influence the growth, yield, and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth. The isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically (Joseph and Yasmin *et al.*, 2007). The plant growth-promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, as potentially useful for increased growth and yields of potato, sugar beet, radish and sweet potato (Farzana *et al.*, 2009). Therefore, present investigation conducted to study the inoculation effect of PGPR consortia on N supply, growth and grain yield of wheat.

Materials and Methods

The present investigation conducted during *rabi* season of 2016-17 at Research Farm of ICAR-IISS, Bhopal to study the “Effect of inoculation of PGPR consortia on N supply, Growth and grain yield of wheat crop”. The material used and methods adopted during the course of experiment in the field and laboratory are described in brief under following heads.

Media and glasswares sterilization

At 15 psi (1.06 kg/cm^2) pressure all media were autoclaved for 20 minutes. The glasswares used were sterilized in hot air oven at 180^{0}C for 2 hours.

Pot study

Chemical characteristics of soil

Soil under study had pH 7.9, Available N 215kg/ha, Phosphorus-110 kg/ha, Potash-390 kg/ha and organic carbon- 0.75%.

Seed Inoculation with potential PGPR isolates

About 50g wheat seeds were surface sterilized by dipping seed for 30 sec in 70% ethanol solution followed by 2 minutes in $HgCl_2$ (0. 1%). The seeds were washed thoroughly with sterile distilled water for three times. Inoculums of potential isolate JS1 and MER4 was built in Jensen’s broth and Nutrient broth respectively and incubated at 28±2°C, 200 rpm in an incubator shaker for 4 days. Enumeration of viable cell count was done by dilution plating technique. Equal volume of two cultures was mixed in a sterile flask and carboxymethyl cellulose (2%) was added for seed adhesion. The surface sterilized seed were added to this flask and shaken vigorously for seed coating. After seed coating 10 seed were put in 9 ml water blank and the enumeration of organisms adhered to the seed surface was done on Jensen’s N free media and nutrient agar media and number of PGPR per seed was calculated.

Experimental set up

In vivo study of effect inoculation of PGPR on growth and yield of wheat (*Variety: Malwa Shakti*) was conducted in pot culture. The treatment consisted of:

T_1: Control (no fertilizer or manure)

T_2: Consortium of PGPR

T_3: Recommended Dose of Fertilizers (RDF) on Wheat

T_4: 50%N+full PK + Consortium of PGPR

T_5: 75% N +full PK+ Consortium of PGPR

T_6: RDF+ Consortium of PGPR

Excepting T1 (control) and T2 (Consortium of PGPR only), graded basal dose of N and full dose of P and K fertilizer calculated for 12 kg soil based on recommended dose of fertilizers for the variety(100:60:40), was added to respective treatment pots. The pots were irrigated to 60% of moisture holding capacity for uniform distribution of fertilizers added and left for one day. Five seeds previously coated with PGPR isolates were sown in each pot. Control and RDF treatment pot were sown with uninoculated surface sterilized seeds.

Five replications per treatment were maintained and the pots were kept in net house. A separate set of same treatment structure was also maintained for destructive plant sampling and measurement of agronomic parameters. Moisture was maintained with regular addition of measured and equal volume of water to each pot.

Second split dose of N was added at CRI stage and third at flowering stage. Root length; shoot length; root fresh weight and shoot fresh weight was measured by uprooting five randomly selected plants from each treatment at 80DAS from the pots meant for destructive sampling.

Statistical analysis

Statistical analyses were carried out through one-way analysis of variance (ANOVA) and the mean of treatments were compared according to Fisher's multiple comparison tests. Least significant difference (LSD) was calculated at $p<0.05$ using statistical package of SAS.

Results and Discussion

Effect of inoculation of PGPR consortia on N supply, growth and yield of Wheat

Three potential isolates (JS1, JS4 and MER4) after evaluation of Nitrogen fixing ability and other PGP attributes, found most promising and hence used for seed inoculation. MER4 was grown in nutrient agar to viable cell count of 3.4×10^8 and JS1 and JS2 in Jensen's N-free broth to viable cell density of 5.6×10^8, 6.9×10^8 respectively. The wheat seed was treated with PGPR @ 10^3 viable cells/seed of each isolate was sown in pot with graded dose of nitrogen fertilizers as per the defined treatment structure. Plant height, root length, root fresh and dry weight, and shoots fresh and dry weight was recorded at 80 days after sowing.

The highest root length was recorded in T4 (50%N+full PK + Consortium of PGPR) which was statistically at par with T6 and T5. The root length measured for T2 (Consortium of PGPR only) ($p<0.05$; 14.4cm) was at par with T3 (Recommended Dose of Fertilizers) on Wheat. Lowest root length was recorded in Control treatment ($p<0.05$; 11.3cm) where no fertilizers, manure or bioinoculum was applied. Shoot length was recorded highest for the treatment where consortia of PGPR were applied along with recommended dose of fertilizers which was statistically at par with RDF without PGPR. No significant difference in shoot length was observed between T3&T5, T5&T4 and T1&T2 but the shoot length was significantly higher in T3 compared to T4, T2 and T1. The lowest shoot length was observed in control treatment (Table 1).

The lowest root fresh weight per plant was noticed in control (T1) treatment followed by T2 and T3. No significant difference of root fresh weight per plant was observed between

T2, T3 and T4 but T4 was significantly higher than T1. The highest root fresh weight was noticed in T6 (RDF+ Consortium of PGPR) treatment which was statistically at par with T5 (75% N +full PK+ Consortium of PGPR) and T4 (50%N+full PK + Consortium of PGPR). Similarly, the highest shoot fresh weight was recorded in T6 *i.e.* RDF+ Consortium of PGPR which was at par with T3 *i.e*. Recommended Dose of Fertilizers. No significant difference was observed between T3, T5 and T4. The shoot fresh weight per plant in T4 and T2 was par with each other and was significantly higher than control (T1) (Table 1) (Fig. 1). Shoot dry weight per plant was recorded significantly higher in T6 compared to T3, T4 and T5 which was statistically at par with each other. No significant difference was recorded in shoot dry weight of T2 and T1 and these recorded the lowest shoot weight per plant.

Similarly root dry weight per plant was recorded significantly higher in T6 followed by T5and T3. No significant difference in root dry weight per plant was noticed between 50% N (T4) and only PGPR (T2) however; T2 was statistically at par with T1 also which recorded lowest root dry weight per plant (Table 1).

Table.1 Along with PGPR inoculants the effect of different levels of N-fertilizers on agronomic parameters of wheat

Treatment	Straw wt. (g/pot)	Grain wt. (g/pot)	Root length (cm)	Shoot length (cm)	Root fresh wt. (g/plant)	Shoot fresh wt. (g/plant)	Root dry wt. (g/plant)	Shoot dry wt. (g/plant)
T1	6.74d	5.47d	11.3c	24.4d	1.64c	3.56d	0.32e	1.27c
T2	8.05c	6.58cd	14.4b	27.9d	1.84bc	4.12c	0.36de	1.26c
T3	13.27a	10.28a	16.8b	40.1ab	1.84bc	5.04ab	0.75c	1.85b
T4	10.13b	7.56bc	20.7a	35.0c	2.07ab	4.57bc	0.47d	1.80b
T5	12.22a	8.04b	19.7a	36.6bc	2.29a	4.75b	0.91b	1.65b
T6	13.27a	11.68a	20.5a	41.8a	2.36a	5.34a	1.32a	2.33a
LSD(0.05)	1.29	1.42	2.69	4.26	0.29	0.5	0.11	0.3

Fig.1 Effect of PGPR inoculation on wheat growth over control

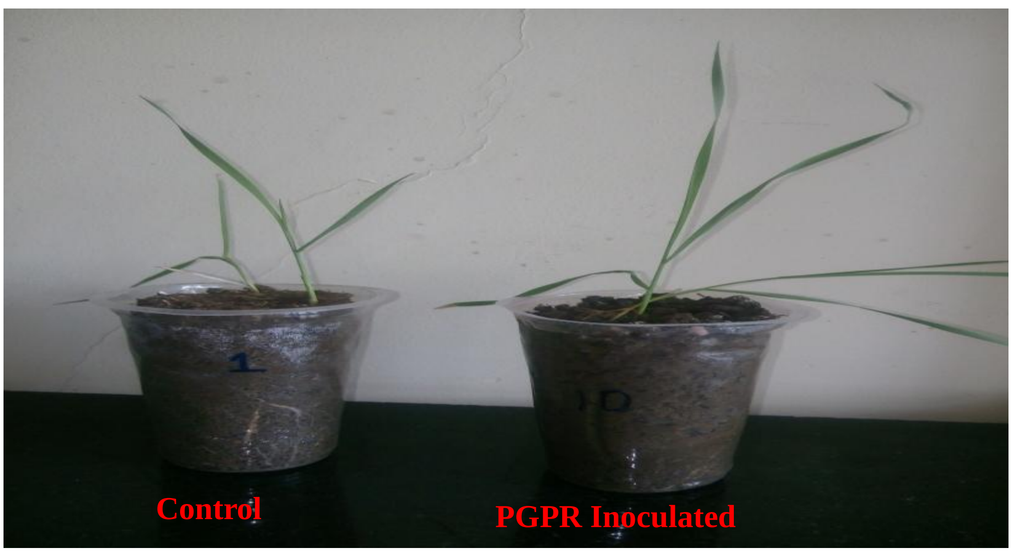


Fig.2 Root and shoot length at 80 das

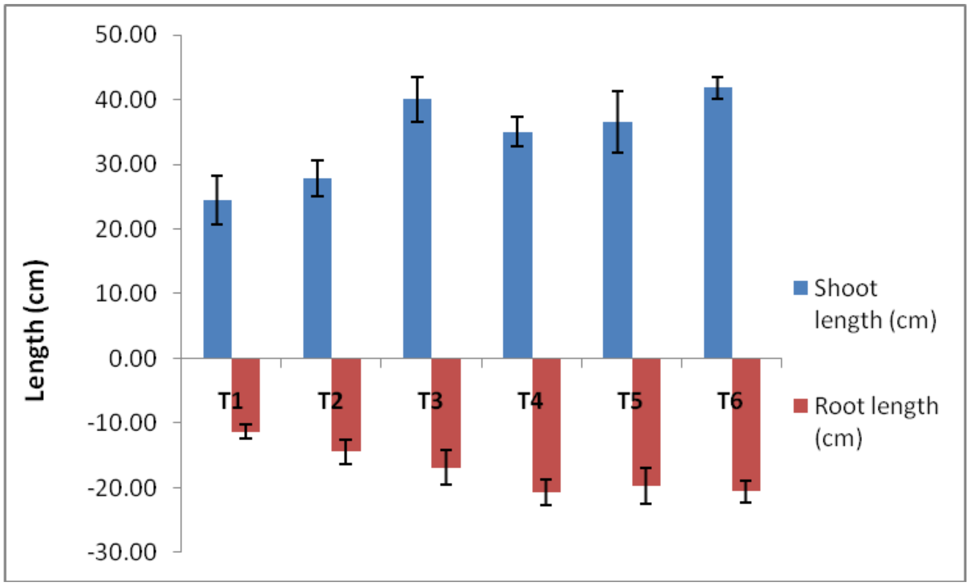
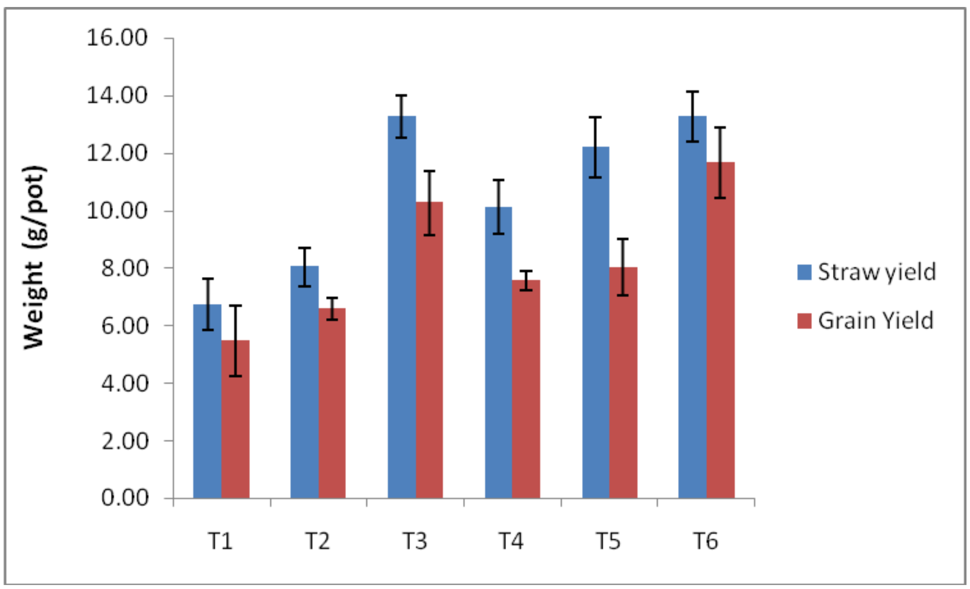


Fig.3 Wheat grain and straw yield



Effect of microbial consortia on biomass and grain yield of wheat

The straw yield of wheat was found significantly higher in T6 ($p<0.05$; 13.27g/pot) which was equal to T3 ($p<0.05$; 13.27g/pot) and at par with T5 ($p<0.05$; 12.22g/pot). The straw yield T2 ($p<0.05$; 8.05g/pot) was significantly higher than control ($p<0.05$; 6.74g/pot) but was statistically lower than T4 ($p<0.05$; 10.13g/pot). The grain yield wheat was found numerically higher in T6 ($p<0.05$; 11.68g/pot) but was statistically at par with T3 *i.e.* RDF treatment ($p<0.05$; 10.28g/pot). The second best performing treatment was T5 ($p<0.05$; 8.04g/pot) which was statistically at par with T4 ($p<0.05$; 7.56g/pot). No significant difference was recorded in wheat grain yield of T4 and T2 ($p<0.05$; 6.58g/pot). The lowest grain yield was recorded in control treatment ($p<0.05$; 5.47g/pot) which was statistically at par with T2 but significantly lower than other treatments (Table 1). Overall the biomass and grain yield was found numerically highest in T6 treatment but was statistically at par with T3. At reduced dose of N fertilizers, compensation in the biomass and grain yield was recorded with application of PGPR bioinoculum.

Three potential isolates (JS1, JS4 and MER4) after evaluation of Nitrogen fixing ability and other PGP attributes used for seed inoculation. These three isolates were evaluated for effect of co-inoculation on wheat at reduced dose of N fertilizers. Root length of the wheat was found to be significantly higher than control with inoculation of PGPR. The effect on root elongation was more pronounced when the PGPR was applied along with chemical fertilizers. However, the effect of inoculants on shoot elongation was not conspicuous when compared with control and recommended dose of fertilizers. Nevertheless, seed inoculation with PGPR along with recommended dose of fertilizers recorded highest shoot elongation (Fig. 2) Root and shoot weight (both fresh and dry) were found higher when PGPR were applied along with chemical fertilizers. Production of phytohormones especially auxin (IAA) helps in root development (Takahashi, 2013) and thus helps for better establishment of plant but the plant height is mainly governed by genetic factors. Production of phytohormones (IAA) by microbes and improvement in root elongation by microbial inoculants is well documented (Etesami *et al.,* 2015), Kumar *et al.,* (2014), Masciarelli *et al.,* (2014). Plant biomass (straw yield) was found to be highest where PGPR inoculation was done along with recommended dose of fertilizers, RDF and it was at par with 75% N with PGPR inoculation. There was reduction in grain yield of wheat with reduce dose of N fertilizer compared to RDF but grain yield of wheat was found highest where PGPR inoculation was done along with recommended dose of fertilizers followed by75% N with PGPR inoculation. No significant difference in yield was recorded at 50%N and 75% N application along with seed inoculation with PGPR (Fig. 3). It is possible that, at reduced dose of nutrients, soil microbes are stress-compelled for synthesis, fixation or mobilization of nutrient from soil and atmosphere to meet its own and associated crop demand. Rathi*et al.,* (2014) reported improved growth of wheat with co-inoculation of PGPR with AM Fungi. Amongst PGPRs, *Flavobacterium* gave best response both singly and in combination with AMF. Inoculation with most of the PGPRs gave better response than 60 kg N ha^{-1} compared to 120N kg ha^{-1}. Swarnalakshmi *et al.,* (2013) revealed that combined inoculation of mixtures and biofilmed bio-inoculants (*Anabaena torulosa* + *Pseudomonas striata* and/or *Anabaena torulosa* + *Azotobacter chroococcum*) were superior over single inoculation and chemical fertilizer control in term of plant growth and nutrient uptake. The pot culture study overall revealed improvement in growth and yield of wheat with inoculation of PGPR at recommended dose of fertilizers. Reduction in yield was recorded with limited supply of nitrogen but maximum benefit of PGPR inoculant was observed at lower dose of N fertilizers.

The pot culture study with wheat revealed improvement in growth and yield of wheat with inoculation of PGPR at recommended dose of fertilizers. Reduction in yield was recorded at lower dose of nitrogen compared to recommended dose but no significant difference in yield was recorded at 50%N and 75% N application along with seed inoculation with PGPR indicating maximum benefit of PGPR inoculants at lower dose of nitrogen fertilizers. Improvement in nitrate reductase activity was noticed with PGPR inoculation. The highest nitrate reductase activity was observed with inoculation of PGPR at recommended dose of N fertilizer compared to all other treatments, indicating favourable effect of bioinoculant on availability of nitrogen.

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