

EFFECTS OF PLANT GROWTH PROMOTING RHIZOBACTERIA AND BIOCHAR ON THE GROWTH AND YIELD OF WHEAT

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Abstract

Wheat (*Triticum aestivum* L.), next to rice, is the most vital cereal crop in the context of meeting the food demands of the country's growing population, in Bangladesh. A pot experiment was conducted following completely randomized design with six treatment combinations and three replications. The objective was to find out the effects of plant growth-promoting rhizobacteria (PGPR) and biochar on the growth and yield of wheat (cv. BARI gom28). Results showed that application of GAU PGPR2 (*Priestia megaterium*) either alone or combined with biochar significantly improved plant height (cm), tiller number per plant, 100-grain weight (g), grain yield (g/pot) and straw yield (g/pot) compared to control (where no PGPR or biochar was added). The findings reveal that the growth and yield of wheat is possible to be enhanced by using biochar or GAU PGPR2 (*Priestia megaterium*) along with the required amount of chemical fertilizers. The findings from this study are crucial that in order to enhance crop productivity it is very important to follow judicious fertilizer management practice.

Keywords: Biochar, BARI gom, PGPR, *Priestia megaterium*, Wheat

1. Introduction

In Bangladesh, wheat (*Triticum aestivum* L.) is the second most significant cereal crop after rice. It is essential to the country's cropping system's diversification and ensuring the national food security. Compared to other cereal crops, wheat provides a balanced diet that is rich in protein, carbs, vitamins, and more calories (Desoky *et al.*, 2020). Although wheat has low potential yield it covers 35% of total grain production. Sustainable agriculture practices are essential to meet up the demand of increasing populations with limited arable land (BBS, 2023). Usually, farmers are intended to use excessive chemical fertilizers to maximize crop yields but use of organic fertilizer is very poor. Unbalanced fertilizer application has resulted in decreased soil fertility, imbalances in soil nutrients, and issues with the environment and consequently leads to harmful effects on human health (Das *et al.*, 2023). In this context, adding biochar and biofertilizers to soils can be a good and sustainable way to increase crop yields by maintaining the natural fertility of soil. Biochar can serve as a soil conditioner,

which improves the physical and biological characteristics of the soil, increases its capacity to store water, and supplies and, more significantly, retains nutrients to promote the growth of plants (Rahman *et al.*, 2020). Plant growth-promoting rhizobacteria (PGPR) are a diverse group of soil bacteria that, when cultivated together with a host plant, stimulate the host's growth by improving the plant's mobility, uptake, and nutrient enrichment (Khanam *et al.*, 2022). Beneficial soil microorganisms PGPR improve plant development through a number of processes, such as phosphate solubilization, nitrogen fixation, phytohormone synthesis (such as indole acetic acid), and plant disease inhibition (Hasan *et al.*, 2024). Microorganisms reduce the demand for chemical fertilizers by promoting and improving the availability of plant nutrients. Direct application of plant growth-promoting rhizobacteria to seeds or plants can improve the growth of crops (Cakmakci *et al.*, 2007). Rhizobacteria that promote plant growth not only produce compounds that promote plant growth but also play a crucial role in the cycling of macro- and micronutrients by altering the morphology of the roots, which increases the surface area of the roots for nutrient uptake in the soil and protects crops from disease (Kumar *et al.*, 2019). Integrated use of PGPR and biochar with chemical fertilizers could enhance nutrient availability, lower the need for chemical inputs, and encourage sustainable wheat production in Bangladesh (Zou *et al.*, 2024). However, the data on the use of PGPR and biochar with chemical fertilizers for the growth and yield of wheat in Bangladesh is scarce. Therefore, this study aims to evaluate the effects of PGPR and biochar on the growth and yield performance of wheat.

2. Materials and Methods

2.1 Isolation and characterization of PGPR

Rhizospheric soils were collected from wheat-fallow- T. aman rice cropping pattern of Baliadangi upazila. Then the PGPR were isolated by serial dilution and drop plate count method (Reed and Reed 1984). The biochemical characteristics such as indole acidic acid (IAA), P solubilization, catalase and Zn solubilization were identified (Table 1). Based on the Zn solubilization characteristics and higher IAA production capacity the GAU PGPR1 and GAU PGPR2 were selected for pot experiment. The molecular characterization of GAU PGPR1(*Metabacillus* sp) and GAU PGPR2 (*priestia megaterium*) were done by 16S rDNA partial gene sequencing. For future usage, the bacterial cultures were kept on nutrient agar slant medium and stored at 4°C.

Table 1. Biochemical characteristics of PGPR

Isolates	3% KOH	PSB	ZSB	Catalase	IAA (ppm)
GAU PGPR1(<i>Metabacillus</i> sp)	(+)ve	(+)ve	(+)ve	(+)ve	56.1
GAU PGPR2 (<i>Priestia megaterium</i>)	(+)ve	(-)ve	(+)ve	(+)ve	62.1

2.2 Inoculation of seed

The PGPR strains were cultured in liquid broth media on shaking incubator (110 rpm) at $28\pm 2^{\circ}\text{C}$ for 48 h. The wheat seeds were cleaned and inoculated in broth cultures contains PGPR strains in the sameday of the transplanting. To inoculate the wheat seeds properly with PGPR strains, the appropriate amount of PGPR strains were used. The rest of the treatments other than PGPR the wheat seed were inoculated with control broth media (where no PGPR strains were added).

2.3 Experiment description

A pot experiment was conducted during growing season of 2022-2023 at Gazipur Agricultural University, Gazipur to find out the effect of PGPR and biochar on growth and yield of wheat. The winter wheat variety BARI gom28 was used as the test crop. Earthen clay pots were used in the experiment and each pot was filled with 8 kg surface soil (0-20 cm). The surface area of pot was 0.06 m^2 . Before sowing the seeds were treated with provax 200 WP and inoculated with plant growth promoting rhizobacteria (PGPR). The seeds of wheat were sown in the pot at late afternoon in the month of December, 2022. All the intercultural operations were performed as per standard methods and procedures. Harvesting was done by following standard procedure.

2.4 Experimental design and treatments

The experiment was carried out in a completely randomized design with six (06) treatments and three (03) replications. The treatment combinations were as: T1: control; T2: T1 + Biochar; T3: T1 + GAU PGPR1; T4: T1 + GAU PGPR2; T5: Biochar + GAU PGPR1; T6: Biochar + GAU PGPR2. Biochar was used @ 3-ton ha^{-1} and it was prepared from rice husk using the modified biochar preparation stove developed by the Department of Soil Science, GAU. Characterization of biochar was performed using standard methods. The characteristics of biochar are shown in Table 2. According to the Fertilizer Recommendation Guide (FRG, 2018), fertilizers including triple superphosphate (TSP) for P, muriate of potash (MoP) for K, and gypsum for S were applied as the basal dosage in the soil of pot. The urea for N was applied as top dressing in three equal splits at 10, 30, and 50 days after sowing. The PGPR were applied @ 1ml in the root surface of the per plant and this process was repeated every 15 days interval. And at the same process the control broth media (no PGPR strain) was applied for T1 and T2 treatments.

2.5 Data collection and analysis

Four wheat plants per pot were used to obtain various data including plant height (cm), number of tillers per plant, 100 grain weight (g), grain yield (g/pot) and straw yield (g/pot). The initial soil was collected and after collection, the soil samples were air dried and cleaned by removal of any remaining undesirable materials. Then the soil samples were grinded and sieved with a 2-mm sized sieve before chemical analysis (Walkley and Black, 1934). The sieved soil samples were stored in labeled plastic container in laboratory for analysis. The soil pH (Jackson, 1958), soil texture (Bouyoucos, 1962) organic carbon (OC) (Walkley and Black

1934), total nitrogen (N) (Bremmer, 2018), available phosphorus (P) (Bray and Kurtz, 1945), exchangeable potassium (K), sulphur (S) iron (Fe) and zinc (Zn) (Page et al., 1982) contents of the soils were analyzed by following the standard laboratory procedure. The initial physical and chemical characteristics of the soil of pot experiment are shown in Table 2.

Table 2. Initial properties of soil and biochar

Properties	Soil	Biochar
Texture	Silt loam	
Soil pH	6.99	7.50
OC (%)	1.58%	41.7%
N (%)	0.20%	2.1%
P *	25.4 (mg kg ⁻¹)	0.15%
K *	0.12 (meq /100g soil)	1.6 meq 100 g ⁻¹
S *	14.5 (mg kg ⁻¹)	0.65%
Fe*	13.93 (mg kg ⁻¹)	0.12 %
Zn *	1.92 (mg kg ⁻¹)	0.02%

*indicates available status of nutrients in soil

2.6 Statistical analysis

The analysis of variance (ANOVA) plant height (cm), number of tillers per plant, 100 grain weight (g), grain yield (g/pot) and straw yield (g/pot) were calculated using the statistical program Statistix 10. Tukey's honest significant difference (HSD) test was applied to compare the treatment means at a 5% significance level.

3. Results and Discussion

3.1 Effects of PGPR and biochar on growth and yield of wheat

The plant height, number of tillers per plant, 100 grain weight grain yield and straw yield of the wheat was significantly influenced by the application of PGPR and biochar (Table 3). The highest plant height was observed in T6 (67.5 cm) whereas the lowest plant height was observed for T1 (61.2 cm). In compared to controlled treatment the combined application of biochar and PGPR significantly increased the plant height. As the GAU PGPR1 and GAU PGPR2 are zinc solubilizing bacteria and can produce higher IAA (Table 1) and biochar is enriched with organic matter (Table 2) acts as suitable and promising carrier of PGPR, which helps to increase the plant height significantly (Zou et al., 2024). Khoso et al., (2024) reported that the enhanced plant height might be due to production of growth hormones including indole-3-acetic acid and solubilization of nutrients like P, K, Zn and Fe through PGPR like *Bacillus sp* *Pseudomonads sp* *Rhizobium sp*, *Burkholderia sp* and *Thiobacillus*. The highest number of tiller was recorded for T4 (3.50) while the lowest was recorded in T5 (2.75). The application of PGPR significantly increases the number of tiller production compared to

control and PGPR2 showed the better results compared to PGPR1. The sole application of biochar increases the tiller numbers but the combined application biochar and PGPR decreases the tiller numbers which are very much similar to control. The enhancement of the number of tillers in wheat was shown to significantly influence by the inoculation with PGPR (Khalid *et al.*, 2004). The 100-grain weight was highest for T3 (4.02 g) and T4 (4.03 g) [whereas the lowest 100-grain weight was recorded for T1 (3.48 g). The sole application of PGPR significantly influenced the grain weight of wheat which are mostly related to grain yield. The application of biochar alone or combined PGPR positively influenced the 100-grain weight compared to T1 (3.48 g). The inoculation of wheat seed with PGPR enhanced the 100-grain weight by 17% in comparison to the non-inoculated wheat seed (Raza *et al.*, 2024). The PGPR synthesizes different types of plant growth hormone mainly Indole-3-acetic acid (IAA) and biochar enhance the soil organic matter, colonization of microbes and beneficial microorganisms which aids to increase the grain weight of wheat (Naveed *et al.*, 2021; Zou *et al.*, 2024). The mean value of grain yield ranged from 11.86 to 17.03 g/pot. The treatment T4 (17.03 g/pot) showed the highest grain yield of wheat, while T1 (11.86 g/pot) showed the lowest grain yield. The application of PGPR significantly enhanced the grain yield of wheat while the individual or combined application of biochar with PGPR also positively influenced the grain yield compared to control. The superior grain yield was recorded in the PGPR inoculated treatment compared to non-inoculated treatment (Riaz *et al.*, 2021). Sharafzadeh *et al.* (2018) observed that inoculation of PGPR may alter the morphology of root which helps to utilize more water and nutrients resulting in increased grain yield. It is well known that PGPRs produce growth regulators, which improve the structure of the soil, creating an environment that is more favorable for plant growth and development may improve the yield of wheat crop. From this study the highest straw yield was recorded for T2 (12.80 g/pot) while lowest yield was for T1 (9.91 g/pot). The sole application of biochar and PGPR significantly enhanced the straw yield of wheat which are statistically almost similar. However, the integrated application of biochar and PGPR enhanced the straw yield compared to control but decreased the straw yield compared to sole application of biochar and PGPR. The author Gebremedhin *et al.* (2015) reported that the yield of wheat grain and straw was substantially increased through biochar by 15.7% and 16.5%, respectively.

Table 3. Effects of PGPR and biochar on growth and yield of wheat

Treatment	Plant height (cm)	No. of tiller/plant	100 grain weight (g)	Grain Yield (g/pot)	Straw Yield (g/pot)
T1	61.17 c	2.83 b	3.48 c	11.86 c	9.91 d
T2	62.58 bc	3.25 ab	3.80 ab	13.52 bc	12.80 a
T3	64.00 abc	3.00 ab	4.02 a	14.33 abc	12.40 abc
T4	64.25 abc	3.50 a	4.03 a	17.03 a	12.54 ab
T5	67.33 ab	2.75 b	3.67 bc	13.55 bc	10.72 cd
T6	67.50 a	2.92 ab	3.68 bc	16.55 ab	10.90 bcd
P value	0.0053	0.0110	0.0006	0.0009	0.0006

There is no significant difference between the means that have the same letter (s) in each column

3.2 Relationship among growth and yield parameter of wheat

The Pearson correlation coefficient was calculated to observe the relationship among growth and yield parameter of wheat (Table 4). The positive and significant correlations were observed between grain yield and plant height ($p < 0.05$), grain yield and 100-grain weight ($p < 0.05$), 100 grain weight and tiller per plant ($p < 0.01$), straw yield and 100-grain weight ($p < 0.001$) and tiller/plant and straw yield ($p < 0.01$). This relationship indicates that grain yield of wheat is significantly influenced by plant height and 100-grain weight while straw yield is significantly influenced by the tiller/plant and 100 grain weight. The negative correlation was observed between plant height and tiller/plant. This suggests that the number of tillers per plant decreases as plant height increases. The study results are very much consistent with the findings of Fageria *et al.* (2013).

Table 4. Pearson correlation matrix of growth and yield parameters of wheat

	Plant height	Tiller/plant	100-grain weight	Grain yield	Straw yield
Plant height	1.000				
Tiller/plant	-0.262	1.000			
100-grain weight	0.024	0.709**	1.000		
Grain yield	0.528*	0.353	0.519*	1.000	
Straw yield	0.001	0.617**	0.762***	0.427	1.000

*Significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$

4. Conclusions

Applying PGPR either alone or in combination with biochar significantly increased wheat production. Plant height (cm), number of tillers per plant, 100-grain weight (g), grain yield (g/pot), and straw yield (g/pot) showed significant improvements compared to control. The highest results for growth and yield of wheat were obtained through PGPR2 (*Priestia megaterium*). However, further investigations are needed to expand on and confirm these results in field conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding publication of this paper.

References

- BBS. 2023. Yearbook of Agricultural Statistics 2022. Bangladesh Bureau of Statistics, Statistics and Informatics Division, Ministry of Planning, Government of Bangladesh.
- Bouyoucos, G.J. 1962. Hydrometer Method Improved for Making Particle Size Analyses of Soils *J. Agron.* 54: 464–465.
- Bray, R.H. and Kurtz, L.T. 1945. Determination of total, organic, and available forms of phosphorus in soils, *Soil Sci.* 59: 39–46 PGPR and biochar effect on wheat

- Bremner, J.M. 2018. Nitrogen-Total. In: D.L. Sparks, A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnston, M.E. Sumner (Eds.), *SSSA Book Series, Soil Science Society of America, American Society of Agronomy*, Madison, WI, USA. 1085–1121.
- [Cakmakci, R., Dönmez, M.F. and Erdogan., U. 2007. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *J. Agric. For.* 31(3): 189-199.
- Das, H., Devi, N., Venu, N. and Borah, A. 2023. Chemical fertilizer and its effects on the soil environment. *Res. Rev. Agr. Sci.* 7: 31-51.
- Desoky, E.S.M., Saad, A.M., El-Saadony, M.T., Merwad, A.R.M. and Rady, M.M. 2020. Plant growth-promoting rhizobacteria: Potential improvement in antioxidant defense system and suppression of oxidative stress for alleviating salinity stress in *Triticum aestivum* (L.) plants. *Biocatal. Agric. Biotechnol.* 30: 101878.
- Fageria, N.K., Moreira, A., Ferreira, E.P.B. and Knupp, A.M. 2013. Potassium-use efficiency in upland rice genotypes. *Commun. Soil Sci. Plant Anal.* 44(18): 2656-2665.
- Gebremedhin, G H., Bereket, H., Daniel, B. and Tesfaye, B. 2015. Effect of biochar on yield and yield components of wheat and post-harvest soil properties in Tigray, Ethiopia. *J. Ferti. Pest.* 6(2): 2-5.
- Hasan, A., Tabassum, B., Hashim, M. and Khan, N. 2024. Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: A review. *Bacteria.* 3(2): 59-75.
- Jackson, M. 1958. *Soil chemical analysis* prentice Hall, Inc., Englewood Cliffs, NJ 498: 183-204.
- Khalid, A., Arshad, M. and Zahir, Z. A. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbio.* 96(3): 473-480.
- Khanam, M., Solaiman, A.R.M., Rahman, G.K.M.M., Haque, M.M. and Alam, M.S. 2022. Effect of plant growth promoting bacteria on growth and nutrient content of rice. *Asian J. Adv. Agric. Res.* 19: 20-29.
- Khoso, M.A., Wagan, S., Alam, I., Hussain, A., Ali, Q., Saha, S., Poudel, T.R., Manghwar, H. and Liu, F. 2024. Impact of plant growth-promoting rhizobacteria (PGPR) on plant nutrition and root characteristics: Current perspective. *Plant Stress.* 11: p.100341.
- Kumar, A. and Verma, J.P. 2019. The role of microbes to improve crop productivity and soil health. *Eco Wis. Ins. Res. Eng.* 249-265.
- Naveed, M., Ditta, A., Ahmad, M., Mustafa, A., Ahmad, Z., Conde-Cid, M., Tahir, S., Shah, S.A.A., Abrar, M.M. and Fahad, S. 2021. Processed animal manure improves morpho-physiological and biochemical characteristics of *Brassica napus* L. under nickel and salinity stress. *Environ. Sci. Pollut. Res.* 28: 45629-45645.
- Page, A., Miller, R. and Keeney, D. 1982. *Methods of soil analysis. Part 2.* American Society of Agronomy, Soil Science Society of America, Madison, WI, USA. 4: 167–179.

- Rahman, G.M., Rahman, M.M., Alam, M.S., Kamal, M.Z., Mashuk, H.A., Datta, R. and Meena, R.S. 2020. Biochar and organic amendments for sustainable soil carbon and soil health. *Carb. Nitr. Cycl. Soil.* 45-85.
- Raza, M. A. S., Saleem, A., Khan, I. H., Tahir, M.A., Iqbal, R., Aslam, M.U., Harsonowati, W., Dawoud, T.M., Alarjani, K.M. and Ditta, A. 2024. Enhancing Wheat Growth: Impact of PGPR Co-Inoculation with *Azospirillum lipoferum* and *Agrobacterium fabrum*. *Pol. J. Environ. Stud.* 1-11.
- Reed, R. W., and Reed, G. B. 1948. "Drop plate" method of counting viable bacteria. *Canadian J. Res.* 26(6): 317-326.
- Riaz, A., Qureshi, M. A., Aftab, M., Afzal, I., Akhtar, N., Javed, H., Ali, M.A. and Akhtar, S. 2021. Microbes mediated zinc biofortification for promoting growth and yield of wheat. *J. Agric. Res.*, 59(1): 35-42.
- Sharafzadeh, A., Shaposhnikov, A., Belimov, A.A., Dodd, I.C., Ali, B. 2018. Auxin production by rhizobacteria was associated with improved yield of wheat (*Triticum aestivum* L.) under drought stress. *Arch. Agron. Soil Sci.* 4:574.
- Walkley, A. and Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method, *Soil Sci.* 37: 29-38.
- Zou, Q., Zhao, L., Guan, L., Chen, P., Zhao, J., Zhao, Y., Du, Y. and Xie, Y., 2024. The synergistic interaction effect between biochar and plant growth-promoting rhizobacteria on beneficial microbial communities in soil. *Front. Plant Sci.* 15: p.1501400.