Combined application of biochar and PGPR consortia for sustainable production of wheat under semiarid conditions with a reduced dose of synthetic fertilizer

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Abstract

This study investigates the combined effect of locally adopted plant growth promoting rhizobacteria (PGPR), biochar, and synthetic fertilizer on the wheat crop for the production and economic returns. A total of 20 PGPR strains were isolated from three different ecological zones of Pakistan and were evaluated. Of them, three isolates were selected for further studies. The treatments included (i) control with a full dose of the recommended fertilizer, (ii) control with half a dose of the fertilizer, (iii) PGPR consortia with half a dose of the fertilizer, (iv) biochar with half a dose of the fertilizer, and (v) PGPR + biochar with half a dose of the fertilizer. The study was repeated at three different locations. The data collected for leaf area index (LAI), grain yield, biological yield, straw yield, and harvest index (HI) revealed significant differences ($P \leq 0.05$) for the locations and treatments, but the interaction of location and treatments was not significant. Based on the productivity and economic returns, the treatment with PGPR + biochar with half a dose of the fertilizer proved to be the best. Thus, the use of the PGPR consortia and biochar can improve the yield and profit of wheat crop with reduced synthetic fertilization.

Keywords Biochar · PGPR · Nitrogen fixation · Grain yield · Economic returns

Introduction

Bread wheat (*Triticum aestivum* L.) feeds at least one third of the world's population [1]. Its current level of productivity represents the achievements of a century or more of agronomic and breeding research. A substantial gap still remains between its actual and theoretically attainable productivity; this can be bridged by improvements in agronomy [2, 3], seed quality [4], the supply of moisture [5], and the quality of the soil [6]. With respect to the soil quality, the content of the organic matter is known to be highly influential [7], but in many developing countries, including Pakistan, cropping soils are known for their notoriously low organic matter content. As a result, substantial improvements in productivity should be possible through the use of organic manure, through the nurturing of soil microfauna and/or through the adoption of some type of organic farming procedures [8, 9].

The soil microfauna has a positive effect on soil fertility. Many microbial species are able to fix atmospheric nitrogen (N); others can decompose organic waste, solubilize phosphorus (P), detoxify pesticides, and produce stimulants of plant

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growth [6, 9, 10]. A number of plant growth promoting rhizobacteria (PGPR) secrete organic acids that are able to convert insoluble P into a soluble form [6, 11, 12]. Biological N₂ fixation converts large quantities of atmospheric nitrogen into ammonia and/or nitrate [13]; while much of this process is carried out by the legume-*Rhizobium* symbiosis, some free-living bacteria are also capable of fixing nitrogen [14]. The production of the phytohormone indole-3-acetic acid (IAA) by the microfauna is an important promoter of lateral root growth, which aids the crop in taking up moisture and nutrients [15, 16]. Such microbes are effective only when provided with soil conditions amenable for their growth.

Crop production has become highly dependent on synthetic fertilizers [17], but their continuous use has proven to be environmentally destructive in a number of ways, which include the suppression of beneficial microbes [18] and pollution of the underground water [19]. Combining PGPR with biochar has been documented as a possible means of reducing the requirement for synthetic fertilizers, as well as for its likely positive effect on soil health, soil organic matter content, and microbial growth [8, 10, 20]. Suitable combinations of biochar and a synthetic fertilizer could, in principle, maintain crop productivity by replacing the loss of nutrients provided by a conventional application of synthetic fertilizers with those released by a better-supported soil microfauna that not only improves soil health but also reduces underground water contamination. There is insufficient knowledge about the types of PGPR in subtropical semiarid soils and their ability to improve plant nutrient availability. Keeping in view the abovementioned facts, this study aimed to (i) explore the most efficient bacterial types with respect to soil improvement, (ii) investigate the potential of aforementioned types for supplementing a wheat crop with a combination of biochar and low levels of NPK fertilization, and (iii) assess the economic returns of the combination of biochar + PGPR and with varying levels of NPK application. The outcome of this study may be helpful in sustainable wheat production to ensure food security and environmental health.

Materials and methods

Soil sampling and bacterial isolates

The soil samples were collected from three locations of major wheat growing areas. This included Mailsi (Central Punjab: 23°59'N, 72°15'E 156 m a.s.l.), Multan (South Punjab: 30°12'N 71°26'E, 154 m a.s.l.), and Bahawalpur (South Punjab: 29°59'N, 73°15'E 159 m a.s.l.), prior to the 2014–2015 cropping season. A 0.1 g aliquot of soil sampled from the root zone was combined with 1.0 mL semisolid nitrogen-free malate medium (NFM) [21] and left for 48 h. A 50 µL sample was transferred to a fresh tube containing 1.0 mL

NFM and cultured for 48 h at 30 ± 2 °C. The procedure was repeated further five times and then the cultures were streaked onto an NFM-containing solid plate to obtain bacterial colonies. These colonies were incubated at 30 °C on a Pikovskaya medium for a week and colonies displaying a halo-type growth (indicative of the ability to solubilize tricalcium phosphate [TCP]) were collected and purified on a fresh Pikovskaya medium [22].

Quantification of N fixation and P solubilization

The capacity of the isolated bacterial strains to fix N₂ and solubilize P was determined separately. In order to determine N₂ fixation capacity, the acetylene reduction method [23] was used. In this method, cultures were grown on both semisolid NFM and combined carbon medium (CCM) [24]. Acetylene reduction activity (ARA) was measured using a model gas chromatograph (Gasukuro Kogyo, Model 370, Tokyo, Japan) equipped with a Porapak N column (Supelco Inc., Bellefonte, PA, USA). The quantification of P solubilization was achieved using the phosphomolybdate blue color method. The content of the solubilized P (primary orthophosphate and secondary orthophosphate) was measured by a spectrophotometer (CamspecM350-Double Beam UV-Visible Spectrophotometer, UK) at 882 nm.

Estimation of IAA production

Single bacterial colonies from LB agar medium plates were grown overnight in LB broth (50 mL) medium. From the overnight grown bacterial culture, 1.0 mL aliquot was added to 150 mL LB supplemented with 100 mg L⁻¹ tryptophan, and the culture was allowed to grow for 15 days. A cell-free supernatant was obtained by centrifugation (6000×g, 15 min). The pH of the supernatant was adjusted to 2.8 with 1 M HCl and then extracted with an equal volume of ethyl acetate following Tahir et al. [25]. The extract was evaporated to the point of dryness and resuspended in 1.0 mL ethanol, then subjected to HPLC using a Series 200 device equipped with a Techsphere 5-ODS C-18 column and a UV detector (Perkin-Elmer, Waltham, MA, USA). Pure IAA (Sigma-Aldrich, St. Louis, MO, USA) was used as the identification and quantification standard. The mobile phase was a mixture of methanol:acetic acid:water in the ratio of 30:1:70, provided at a flow rate of 1.2 mL min⁻¹.

On the basis of ARA, P solubilization, and IAA production, three isolates (BTH-11, MTH-1, and THM-5) were selected for further studies (explained in the [Results](#) section).

Compatibility test

The selected isolates to be used in the consortium were investigated for their compatibility with each other by the line-

streak assay. In the line-streak assay, the isolates were streaked as straight lines parallel to each other with equal distance on LB agar plates (Sigma-Aldrich, USA) with three sets of replications. The strains to be assayed were streaked vertically onto the initial cultures and incubated at 28 °C for 96 h. None or minimal rhizobial growth at the intersection of streaks was observed to have occurred due to antagonism of the isolates, whereas the isolates with normal growth in the close vicinity were compatible with each other.

16S rRNA gene sequencing for isolate identification

The total genomic DNA of the three isolates BTH–11, MTH–1, and THM–5 was extracted using the alkaline lysis method and used as a template for the amplification of the 16S rRNA gene obtained by using the primer pair fd1/rD1 [26]. Each reaction mixture contained 5 µL 10× Taq buffer (Fermentas, www.thermofisher.com), 3 µL 25 mM MgCl₂, 5 µL 2 mM dNTP, 0.5 µL DMSO, 1.5 µL each of each primer (10 µM), 0.75 µL 5 U µL⁻¹ Taq DNA polymerase (Fermentas), 40 ng template DNA, and 32.75 µL nuclease-free water to make up the total volume of 50 µL. After an initial denaturation step of 95 °C/5 min, the reactions were cycled 30 times through 95 °C/60 s, 55 °C/30 s, and 72 °C/60 s, after which they were subjected to a final extension step of 72 °C/10 min. Amplicon purification was achieved with a QIAquick PCR purification kit (Qiagen, Valencia USA), and sequencing in both directions was performed commercially (Eurofins, Hamburg, Germany). The obtained nucleotide sequences were analyzed using the Sequence scanner software package (<http://sequence-scanner-software.software.informer.com/2.0/>); both ends were joined by Cap3 assembly software and compared with the sequences deposited in GenBank using BLASTN. The three 16S rRNA sequences have been deposited in GenBank under the accession number KT963030–32.

Preparation of the inoculum

The three selected isolates were grown separately overnight in 150 mL LB broth with shaking (150 rpm) at 30 ± 2 °C. The cell density of the inoculum was determined by serial dilution. A series of tenfold dilutions were made in 0.85% (w/v) NaCl, and a 100 µL aliquot from each dilution was spread on an LB agar plate, which was incubated overnight at 30 °C. The number of bacterial colonies was thus formed counted, and the inoculum concentration was adjusted to 10 [27] cells mL⁻¹. A combined inoculum was prepared by mixing equal volumes of the three isolates.

Soil sampling and analyses

Soil samples were collected from each site before the experiment. After the crop harvest, plant samples were collected

again from each treatment/replication to test the effect of the treatment. The samples were air dried and ground to pass through a 2-mm sieve prior to analyses. Soil pH was recorded with a 2.5:1 water-soil suspension. The methods described earlier were used for the determination of exchangeable K [27], N content [28], and available P [29]. Soil texture was estimated by the hydrometer method [30]. Soil characteristics are presented in Table S1.

Preparation of biochar and its characterization

The source material for the biochar was cotton stems, which were oven dried and pyrolyzed using a Muffle furnace (Thermolyne BenchTop Muffle Furnace, Model no. 1230F28, Thomas Scientific, Inc.) [3]. In each cycle, 2 kg of crushed feedstock was used for pyrolysis. A bent outlet composed of a glass rod was used for the removal of vapors and gases from the working area. High-temperature-resistant silicon grease was used to seal the junction of the Pyrex flask and a glass rod to avoid the entry of oxygen in the reaction chamber. The increase in temperature (per unit time) of the muffle furnace was adjusted at 8–9 °C min⁻¹. Residence time was maintained at 20 min after attaining the final temperature (300 °C) in the reaction chamber. After the residence time, the muffle furnace was allowed to cool down and the biochar thus formed was collected. The collected biochar was ground to pass through a 2-mm sieve. It was heated in a muffle furnace to 450 °C to determine the volatile matter, which was 20 and to 550 °C to determine the ash content, which was 15.2%. The electrical conductivity and pH of the biochar, determined in an aqueous suspension of the biochar in distilled water (1:20, w/v), were found to be 1.70 and 9.60 dS m⁻¹, respectively. Its total carbon, nitrogen, and hydrogen contents, as measured using an Elementar elemental analyzer (Vario EL III Element Analyzer, Elementar Analysensysteme GmbH, Germany) were respectively 46.30%, 1.70%, and 3.60%, and its oxygen percentage (determined by the difference method) was 48.40. The biochar material was digested in a mixture of HNO₃:HClO₄ to determine the concentrations of P, K, and Na. The P concentration was measured spectrophotometrically (CamspecM350-Double Beam, UV-Visible Spectrophotometer, UK) and was found to be 0.40%, whereas those of K and Na were determined by flame photometry (Flame photometer, BWB XP, UK) and were found to be 1.60 and 1.10%, respectively.

Field experiment

A field experiment was conducted to investigate the potential of the selected PGPR consortia for supplementing a wheat crop with a combination of biochar and low levels of synthetic fertilization during the winter season of 2014–2015 at three locations, namely Bahawalpur, Mailsi, and Multan from where the isolates were collected. At each site, the experiment was laid out as a set of randomized complete blocks with three

replications. Each plot measured 1.5 m × 5 m and comprised six rows separated from one another by 25 cm. The seeds (at the rate of 120 kg ha⁻¹) of the wheat cultivar cv. Punjab–2011 were pelleted with a mixture containing PGPR consortial inoculum (~10⁹ cells mL⁻¹) and sterilized filter-mud, that is, the waste of sugar industry (at the rate of 0.02 g mud per 1.0 g seed). The following treatments were applied: (1) T₁: untreated control with a full dose of synthetic fertilizer (NPK at 130–115–62 kg ha⁻¹)—neither PGPR consortia nor biochar; (2) T₂: untreated control with half a dose of synthetic fertilizer (NPK at 65–57–30 kg ha⁻¹)—neither PGPR consortia nor biochar; (3) T₃: PGPR consortia + half a dose of synthetic fertilizer; (4) T₄: biochar at 500 kg ha⁻¹ + half a dose of synthetic fertilizer; and (5) T₅: PGPR consortia + biochar at 500 kg ha⁻¹ + half a dose of synthetic fertilizer. A full dose of P and K, along with one third of N was applied at sowing, and a further one third of N at each of the two subsequent irrigation times when the plants had reached Zadoks stages Z21 (start of tillering) and Z31 (the first node visible) was applied. The soil physiochemical characteristics were determined both prior to sowing and after harvesting (Table S1). Leaf area index (LAI) was recorded at the vegetative stage, whereas the grain yield, straw yield, biological yield, and harvest index (HI) were recorded at maturity. The samples were taken from a randomly selected 0.25 m² quadrat within each treatment. The leaves of each plant were removed and weighed. A subsample of 5 g was taken from each leaf lot. To measure the LAI, leaves were harvested from a randomly selected 0.25 m² quadrat, and their surface area quantified using a digital leaf area meter (DT Area Meter, Model MK2, Delta T Devices, Cambridge). The postharvest traits (grain yield, straw yield, and biological yield) were estimated from manually threshed 1 m² area. HI was given by the ratio between the grain yield and aboveground biomass.

Economic analysis

The potential economic return of cropping wheat with or without various biochar and PGPR was calculated considering both the production costs (purchase of inputs) and the income (sale of grain and straw). The net return was given by the value of increased yield obtained—the total cost and the percent increase in the income from the ratio between the additional income accrued by using ameliorants and the total income. Rupee values were converted into US\$ based on the exchange rate prevailing during 2015–2016.

Statistical analyses

The obtained data were statistically analyzed considering a randomized complete block design with the location as a random factor. Differences in the mean performance were statistically tested for significance using ANOVA followed by the least

significant difference (LSD) test implemented in the SAS software package [31].

Results

PGPR strain isolation, physiological characterization, and identification

Of the 20 bacterial strains isolated from the rhizosphere of wheat crops, 4 were obtained from Mailsi, 5 from Multan, and 11 from Bahawalpur. The isolates included 12 Gram-negative and 8 Gram-positive ones; 17 of the isolates were motile and 3 non-motile. All the isolates produced considerable quantities of IAA in the culture medium supplemented with tryptophan. However, the amount of the IAA produced by these isolates was low when grown in the growth medium without tryptophan. The most effective IAA producer was BTH–11 (from Bahawalpur); it was able to produce 720 ± 21 mg L⁻¹ in the presence of tryptophan and 2.6 mg L⁻¹ in its absence. Nine of the isolates were able to fix N, and these were all ARA positive in both the media used. The most effective strain was MTH–1 (from Mailsi), which had an acetylene reduction potential of 1987 ± 143 μ mol C₂H₂ h⁻¹ mg⁻¹ protein. Eleven of the strains were able to reduce the pH of the medium and thus solubilize P; the most efficient isolate was THM–5 (from Multan), which generated 336 ± 8 μ g mL⁻¹ solubilized P (Table 1).

The results of the line-streak assay showed that all three selected isolates (MTH–1, THM–5, and BTH–11) were compatible to be used as a consortium as no growth inhibition zone was seen on intersection areas. Therefore, the three isolates MTH–1, THM–5, and BTH–11 were used as consortia designed to maximize the N₂-fixation, the solubilization of P, and the production of IAA. Based on their 16S rRNA gene sequences, both MTH–1 and THM–5 were identified as belonging to the genus *Pseudomonas*, whereas BTH–11 was classified as a *Bacillus* species.

The effect of PGPR and biochar on wheat yield

At each of the three field sites, the performance with respect to LAI was as good in T₅ (biochar and PGPR with a 50% NPK) as in T₁ (100% NPK), and these two treatments were consistently superior to the other three treatments (Table 2), but the differences were not significant in all the cases. With respect to both grain and straw yields, the combined biochar and PGPR plus the 50% rate of synthetic NPK fertilizer also performed as well as the 100% NPK fertilizer treatment. However, there was no treatment effect on HI. The effect of combining PGPR alone or biochar alone with the 50% NPK fertilizer increased the LAI, grain yield, straw yield, and HI over those recorded for plots which received only 50% dressing of NPK fertilizer, but the advantage was not as great as that when biochar and PGPR were combined. On an

Table 1 Morphological characteristics of the bacterial isolates from rhizosphere of the wheat plants grown at three different locations

Isolate	Gram staining	Cell shape and motility	IAA production (mg L ⁻¹)		ARA (n mol C ₂ H ₄ h ⁻¹ mg ⁻¹ protein)		pH of the medium	P-solubilization (μg mL ⁻¹)
			Without tryptophan	With tryptophan	NFM	CCM		
MTH-1	–	Spirilla-shaped, motile	0.5 ± 0.2	221 ± 10	921 ± 121	1987 ± 143	7.0 ± 0.2	ND*
MTH-2	+	Motile rods	1.4 ± 0.1	180 ± 8	ND*	ND	5.2 ± 0.1	58 ± 4
MTH-3	+	Nonmotile rods	1.2 ± 0.2	130 ± 15	ND	ND	5.5 ± 0.1	12 ± 2
MTH-4	–	Spherical-shaped, motile	0.4 ± 0.1	145 ± 14	23 ± 2	1221 ± 63	7.0 ± 0.2	ND
THM-5	–	Motile rods	2.5 ± 0.3	510 ± 19	ND	ND	4.1 ± 0.1	336 ± 8
THM-6	–	Oval-shaped, motile	1.5 ± 0.2	418 ± 17	12 ± 1	125 ± 12	7.0 ± 0.2	ND
THM-7	–	Spherical-shaped, motile	2.0 ± 0.4	505 ± 12	14 ± 1.2	118 ± 15	7.0 ± 0.2	ND
THM-8	+	Motile rods	1.8 ± 0.1	221 ± 14	ND	ND	5.1 ± 0.2	21 ± 3
THM-9	–	Rod-shaped, motile	0.8 ± 0.1	185 ± 15	ND	ND	5.8 ± 0.2	10 ± 1
THM-10	–	Oval-shaped, motile	0.5 ± 0.1	192 ± 14	11 ± 2	75 ± 5	7.1 ± 0.1	ND
BTH-11	+	Motile rods	2.6 ± 0.2	720 ± 21	ND	ND	4.4 ± 0.1	320 ± 9
BTH-12	–	Spirilla-shaped motile	2.0 ± 0.2	75 ± 10	11 ± 1	241 ± 11	6.8 ± 0.2	ND
BTH-13	–	Spherical-shaped, motile	1.8 ± 0.1	121 ± 8	10 ± 1	193 ± 10	6.9 ± 0.1	ND
BTH-14	–	Spirilla-shaped, motile	1.8 ± 0.3	85 ± 5	10 ± 1	211 ± 11	6.8 ± 0.2	ND
BTH-15	–	Rod-shaped, motile	0.9 ± 0.1	15 ± 2	ND	ND	5.5 ± 0.3	27 ± 4
BTH-16	+	Rod-shaped, motile	2.1 ± 0.2	20 ± 4	ND	ND	4.5 ± 0.2	115 ± 9
BTH-17	–	Oval-shaped, motile	0.8 ± 0.1	22 ± 4	5 ± 0.5	75 ± 5	6.9 ± 0.1	ND
BTH-18	+	Round, nonmotile	1.0 ± 0.2	14 ± 2	ND	ND	5.4 ± 0.1	21 ± 2
BTH-19	+	Rod, motile	1.3 ± 0.3	225 ± 12	ND	ND	4.7 ± 0.2	110 ± 7
BTH-20	–	Round, nonmotile					6.0 ± 0.2	12 ± 2

MTH, isolates from Mailsi; THM, isolates from Multan; BTH, isolates from Bahawalpur; ND, not detectable; IAA, indole acetic acid; ARA, acetylene reduction activity; NFM, nitrogen-free malate medium; CCM, combined carbon medium

average, over all the locations, biochar application along with half a dose of NPK fertilizer with PGPR consortia increased the grain yield by 39.97% over half a dose of NPK fertilizer (Table 2). With respect to the N and P contents of the dry matter, the effect of treatments was nonsignificant. However, N and P contents were slightly higher in the treatments, wherein PGPR consortia and biochar were combined with the 50% rate of synthetic NPK fertilizer as compared with all other treatments (Table S2). With respect to the location, yield was always superior at Multan followed by Mailsi and Bahawalpur, but the difference was not always significant (Table 2). Differences were due to the soil quality and weather conditions of the respective sites (Fig. 1, Table S1).

Effect on soil quality

From Table S1, it is very clear that the use of biochar and PGPR consortia improved the soil physiochemical properties. The replacement of 50% of the synthetic fertilizer requirement has a significant positive effect on the soil quality, in addition to increasing the grain yield and farmer's net income.

Economic gains obtained from the PGPR consortia plus biochar supplementation combined with a reduced dressing of synthetic NPK fertilizer

At all three locations, the treatment involving both the PGPR consortia and biochar along with the 50% rate of synthetic fertilizer increased the potential financial return of the wheat crop. The net return (per hectare) of this treatment was estimated to be US\$438 at Multan, US\$348 at Bahawalpur, and US\$421 at Mailsi, representing a percentage gain of 50%, 64%, and 44%, respectively (Tables 3, 4, and 5). Supplementation with only biochar or only PGPR, along with the 50% rate of synthetic fertilizer was also beneficial as compared with the recommended dose of the fertilizer.

Discussion

The rhizosphere is inhabited by a variety of bacteria, some of which support the health of soil and plants. Determining the microorganisms that are beneficial has thus long been a focus

Table 2 Effect of PGPR and biochar on leaf area index, grain yield, straw yield, biological yield, and harvest index of wheat at three locations

Treatments	Leaf area index	Grain yield (t ha ⁻¹)	Straw yield (t ha ⁻¹)	Biological yield (t ha ⁻¹)	Harvest index (%)
Multan site					
T ₁	2.66ab	4.54ab	5.45a	9.99a	45.4a
T ₂	2.20c	3.16c	4.10b	7.26c	43.5a
T ₃	2.50b	4.31b	5.29a	9.60ab	44.9a
T ₄	2.48b	4.36ab	5.29a	9.65ab	45.2a
T ₅	2.79a	4.70a	5.52a	10.22a	46.0a
Average	2.52A	4.21A	5.13A	9.34A	45.26A
Mailsi site					
T ₁	2.58a	4.50ab	5.30a	9.80a	45.9a
T ₂	2.10b	3.32c	4.16b	7.48c	44.4a
T ₃	2.21b	4.30b	5.21a	9.51ab	45.2a
T ₄	2.11b	4.26b	5.24a	9.50ab	44.8a
T ₅	2.66a	4.63a	5.32a	9.95a	46.5a
Average	2.33B	4.20A	5.05B	9.24A	45.36A
Bahawalpur site					
T ₁	2.38ab	4.21a	5.20a	9.41ab	44.7a
T ₂	1.90c	3.28b	4.18b	7.46d	43.8a
T ₃	2.05bc	4.07a	5.12a	9.19c	44.3a
T ₄	2.16bc	4.19a	5.21a	9.40ab	44.6a
T ₅	2.59a	4.33a	5.25a	9.58a	45.2a
Average	2.21C	4.01B	4.99B	9.01B	44.5B

The values sharing the same alphabetic letter do not have any statistical difference. Small letters are used to describe differences within treatments and capital letters describe differences in locations; T₁, non-inoculated control with full recommended NPK fertilizer; T₂, half dose of recommended NPK fertilizer; T₃, PGPR with half dose of recommended NPK fertilizer; T₄, biochar with half dose of recommended NPK fertilizer; T₅, biochar and PGPR with half dose of recommended NPK fertilizer

of crop research [18, 32]. Normally, soil contains numerous species of microorganisms, of which only a few are favorable for crop growth, whereas others are either neutral or harmful.

One of the major criteria to identify their usefulness for crop growth includes the efficiency of microorganisms to provide/produce nutrients for the plants. This usefulness is either

Fig. 1 Weather data of the study locations during experimentation as obtained in 2014 and 2015

Table 3 Cost and net return of wheat production at Multan site using PGPR consortia and biochar in combination with the synthetic fertilizer

Treatments	T ₁	T ₂	T ₃	T ₄	T ₅	Remarks
Grain yield	4.54	3.16	4.31	4.36	4.70	t ha ⁻¹
Adjusted grain yield	4.09	2.84	3.88	3.92	4.23	10% less than actual to bring at farmer's level
Gross benefit (a)	1097	764	1042	1054	1136	268.5 US\$ tone ⁻¹
Straw yield	5.45	4.1	5.29	5.29	5.52	t ha ⁻¹
Adjusted straw yield	4.91	3.69	4.76	4.76	4.97	10% less than actual to bring at farmer's level
Gross benefit (b)	17.3	13.0	16.8	16.8	17.5	3.53 US\$ tone ⁻¹
Gross benefit (c)	1114	777	1058	1070	1153	Gross benefit (a + b)
Cost of SOP (A)	15.7	7.9	7.9	7.9	7.9	3.94 US\$ kg ⁻¹
Cost of DAP (B)	215	106	106	106	106	42.97 US\$ bag ⁻¹
Cost of urea (C)	41.0	21.0	21.0	20.8	20.8	18.4 US\$ bag ⁻¹
Cost of biochar (D)	0.0	0.0	0.0	25.6	25.6	25.6 US\$ ha ⁻¹
Cost of PGPR (E)	0.0	0.0	4.91	0.0	4.91	4.91 US\$ ha ⁻¹
Fixed cost (F)	550	550	550	550	550	US\$ ha ⁻¹ (seed bed preparation, irrigation, weeding)
Cost that vary (G)	271	135	140	160	165	(A + B + C + D + E) US\$ ha ⁻¹
Total cost (H)	821	685	690	710	715	(F + G) US\$ ha ⁻¹
Net benefit	293	92	369	360	438	(c-H) US\$ ha ⁻¹
% increase		-68.6	25.9	22.9	49.5	(Treated control)/control × 100

T₁, non-inoculated control with full recommended NPK fertilizer; T₂, half dose of recommended NPK fertilizer; T₃, PGPR with half dose of recommended NPK fertilizer; T₄, biochar with half dose of recommended NPK fertilizer; T₅, biochar and PGPR with half dose of recommended NPK fertilizer; SOP, sulfate of potash; DAP, di-ammonium phosphate

Table 4 Cost and net return of wheat production at Bahawalpur site using PGPR consortia and biochar in combination with synthetic fertilizer

Treatments	T ₁	T ₂	T ₃	T ₄	T ₅	Remarks
Grain yield	4.21	3.28	4.07	4.19	4.33	t ha ⁻¹
Adjusted grain yield	3.79	2.95	3.66	3.77	3.90	10% less than actual to bring at farmer's level
Gross benefit (a)	1017	793	984	1013	1046	268.5 US\$ tone ⁻¹
Straw yield	5.2	4.18	5.12	5.21	5.25	t.ha ⁻¹
Adjusted straw yield	4.68	3.76	4.61	4.69	4.73	10% less than actual to bring at farmer's level
Gross benefit (b)	16.52	13.28	16.27	16.55	16.68	3.53 US\$ tone ⁻¹
Gross benefit (c)	1034	806	1000	1029	1063	Gross benefit (a + b)
Cost of SOP (A)	15.7	7.9	7.9	7.9	7.9	3.94 US\$ kg ⁻¹
Cost of DAP (B)	215	106	106	106	106	42.97 US\$ bag ⁻¹
Cost of urea (C)	41.0	21.0	21.0	20.8	20.8	18.4 US\$ bag ⁻¹
Cost of biochar (D)	0.0	0.0	0.0	25.6	25.6	25.6 US\$ ha ⁻¹
Cost of PGPR (E)	0.0	0.0	4.9	0.0	4.91	4.91 US\$ha ⁻¹
Fixed cost (F)	550	550	550	550	550	US\$. ha ⁻¹ (seed bed preparation, irrigation, weeding)
Cost that vary (G)	271	135	140	160	165	(A + B + C + D + E) US\$ ha ⁻¹
Total cost (H)	821	685	690	710	715	(F + G) US\$ ha ⁻¹
Net benefit	213	121	310	319	348	(c-H) US\$ ha ⁻¹
% increase		-42.9	46.0	50.0	63.7	(Treated control)/control × 100

T₁, non-inoculated control with full recommended NPK fertilizer; T₂, half dose of recommended NPK fertilizer; T₃, PGPR with half dose of recommended NPK fertilizer; T₄, biochar with half dose of recommended NPK fertilizer; T₅, biochar and PGPR with half dose of recommended NPK fertilizer; SOP, sulfate of potash; DAP, Di-ammonium phosphate

Table 5 Cost and net return of wheat production at Mailsi site using PGPR consortia and biochar in combination with synthetic fertilizer

Treatments	T ₁	T ₂	T ₃	T ₄	T ₅	Remarks
Grain yield	4.54	3.32	4.31	4.26	4.63	t ha ⁻¹
Adjusted grain yield	4.09	2.99	3.88	3.83	4.17	10% less than actual to bring at farmer's level
Gross benefit (a)	1097	802	1042	1029	1119	268.5 US\$ tone ⁻¹
Straw yield	5.30	4.16	5.21	5.24	5.32	t ha ⁻¹
Adjusted straw yield	4.77	3.74	4.69	4.72	4.79	10% less than actual to bring at farmer's level
Gross benefit (b)	16.84	13.22	16.55	16.65	16.90	3.53 US\$ tone ⁻¹
Gross benefit (c)	1114	816	1058	1046	1136	Gross benefit (a + b)
Cost of SOP (A)	15.70	7.86	7.86	7.86	7.86	3.94 US\$ kg ⁻¹
Cost of DAP (B)	215	106	106	106	106	42.97 US\$ bag ⁻¹
Cost of urea (C)	41.0	21.0	21.0	20.8	20.8	18.4 US\$ bag ⁻¹
Cost of biochar (D)	0.0	0.0	0.0	25.6	25.6	25.6 US\$ ha ⁻¹
Cost of PGPR (E)	0.0	0.0	4.9	0.0	4.9	4.91 US\$ ha ⁻¹
Fixed cost (F)	550	550	550	550	550	US\$/ha (seed bed preparation, irrigation, weeding)
Cost that vary (G)	271	135	140	160	165	(A + B + C + D + E) US\$ ha ⁻¹
Total cost (H)	821	685	690	710	715	(F + G) US\$ ha ⁻¹
Net benefit	293	130	369	336	421	(c-H) US\$ ha ⁻¹
% increase		-55.3	26.0	14.8	43.8	(Treated-control)/control × 100

T₁, non-inoculated control with full recommended NPK fertilizer; T₂, half dose of recommended NPK fertilizer; T₃, PGPR with half dose of recommended NPK fertilizer; T₄, biochar with half dose of recommended NPK fertilizer; T₅, biochar and PGPR with half dose of recommended NPK fertilizer; SOP, sulfate of potash; DAP, di-ammonium phosphate

measured by their ability to fix nitrogen, solubilize P, or to synthesize IAA [24, 33, 34]. All 20 isolates were characterized for the abovementioned parameters and the top performer isolated under each parameter was selected to be used as PGPR. Moreover, microbial consortia (BTH-11, MTH-1, and THm-5) were tested in this study. Many previous studies have reported the superiority of microbial consortia over single strain inoculation [35]. Higher improvement in the case of consortial inoculation might be due to the combined action of their complementary traits that might have enhanced their colonization and growth [36]. The choice of the three isolates to be trialed as soil ameliorants was aimed to maximize N₂ fixation, P solubilization, and IAA synthesis. Based on 16S rRNA profiling, the selected isolates were identified as belonging to either *Pseudomonas* or *Bacillus* species that harbor many potential PGPR strains [4, 25, 37].

Biochar is an emerging nonconventional fertilizer that can improve soil fertility, structure, and its interaction with the soil biota [38]. Biochar compared with other pyrolyzed carbonaceous materials such as activated carbon is suitable for agricultural use in alkaline soils. Activated carbon also has a high nutrient content and a porous nature [39], which make it a better metal adsorbent, and thus can be used in heavy metal-polluted soils [39]. As biochar has a high amount of plant nutrients and ash content and a high surface area due to its porous nature [38], it acts as a medium for beneficial bacteria and mycelium to grow and

proliferate, and this synergistic effect improves the soil fertility, as observed in this study.

When plant performance in a crop provided with a 50% NPK fertilizer treatment was compared with the same fertilizer level supplemented by the PGPR combination and biochar, LAI, for example, was boosted by 26.6–36.3%. The supplementation was able to replace the lost NPK fertilizer because the LAI of these plants was even slightly higher (3.0–8.8%) than that of plants given a 100% NPK fertilizer treatment, but no PGPR. The effect was transmitted to grain yield, which was 32.0–48.7% higher in the 50% NPK fertilizer supplemented by the PGPR combination and biochar treatment than in the nonsupplemented 50% NPK fertilizer treatment. Improvement with reduced fertilizer may be attributed to improved macro- and micronutrient availability by PGPR and biochar [13, 20, 24, 40]. Here, it was not evident that the supplementation increased the plants' N or P content (Table S2), instead, extra uptake contributed to the yield with a PGPR–biochar combination. A higher wheat yield could be due to the interactive effect of nutrients, the wheat variety, and tolerance to harsh environmental factors, which were negligible when acted alone but resulted in a higher yield collectively in the presence of PGPR and biochar. The present results relating to the yield are also consistent with the known benefit of bacterial IAA production for the root surface area, nutrient uptake, and ultimately, plant growth and economic yield [7, 12, 41, 42].

The benefit of biochar is thought to lie in its effect on the physical and chemical status of the soil. The effect can be direct, via the provision of mineral nutrients [13], or indirect, notably by promoting nutrient retention [43, 44], by increasing the soil's cation exchange capacity, by P and S transformations and turnover [4], by improving soil water's ability to retain moisture [45], and by altering the representation of the soil microfauna, and hence its effect on the plant growth and productivity [41]. Many of these effects probably act synergistically to improve crop performance. Adoption of any innovation on a commercial basis totally depends on its economic feasibility [46]. The economic analysis of data disclosed that inoculation with PGPR and biochar application (alone and in combination) resulted in higher net returns at all three experimental sites with half NPK application compared with that treated with half a dose of NPK application without PGPR and biochar (Tables 3, 4, and 5). Higher net income in the above-said treatments was due to substantial improvements in grain and straw yield of wheat under these respective treatments (Table 2). Recently, Hussain et al. [47] also reported higher net returns in wheat at half a dose of NPK with PGPR inoculation. Nonetheless, the use of biochar and PGPR not only warranted higher returns at half a dose of synthetic fertilizers but also has environmental benefits [38] due to curtailing of synthetic fertilizer dose up to 50% without compromising on the yield and net returns (Tables 2, 3, and 4).

Conclusion

An agricultural production system can only be sustainable if its outputs satisfy both the consumer and the producer, while minimizing (preferably abolishing) damage to the environment. Here, supplementation with PGPR consortia and biochar allowed a 50% cut in the synthetic NPK fertilizer requirement without compromising on the economic yield. This saving is of major interest to poorer producers, given the cost of synthetic fertilizers, while reducing an environmental load of synthetic fertilizer usage and improving the soil quality. Before the large-scale adoption of the strategy, further research would be needed to determine its feasibility across ecological zones within each of the major cropping areas.

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