JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 1627-1632

Effect of Biochar Along with Plant Growth Promoting Rhizobacteria (PGPR) on Growth and Total Dry Matter Yield of Rice

Awtar Singh, A. P. Singh, S.K. Singh and C.M. Singh

Department of Soil Science & Agricultural Chemistry, Institute of Agricultural Sciences Banaras Hindu University, Varanasi - 221005, India.

(Received: 09 October 2014; accepted: 12 December 2014)

A pot experiment was conducted to investigate the effect of rice husk biochar and PGPR on growth and total dry matter yield of rice, in alluvial soil at Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences B.H.U., Varanasi, India during *kharif* season of 2012. The soil used in pot experiment was sandy loam with bulk density 1.63 Mgm^{-3}, pH 7.6, E.C. 0.21 dsm^{-1}, CEC 11.63 cmol(P^+) kg^{-1}, organic carbon 0.34%, available soil N 135 kg ha^{-1}, available P 22.7 kg ha^{-1} and available K 183.5 kg ha^{-1}. The characteristics of biochar were bulk density 0.40 Mgm^{-3}, particle density 1.40 Mgm^{-3} , pH (1:5) 10, porosity 71.42%, water holding capacity 218%, total carbon 45.60%, organic carbon 4.80%, N 0.1%, P 0.15%, K 0.20%, Ca + Mg 0.21% and Na 0.35%. The PGPR used for experimentation included *Pseudomonas aeruginosa*, *Azotobacter chroococcum* and *Azospirillum brasilense*. The experiment was designed as a factorial completely randomized design (FCRD) with three replicates. The treatments comprises four levels of biochar (i.e. 0, 1.8, 3.6 and 7.2 g kg^{-1} soil), two levels of PGPR (uninoculated and inoculated) and two levels of N, P, K and Zn fertilizers (100% and 75% of RDF). Application of full doses of fertilizers (100% RDF) resulted in significantly ($P < 0.05$) higher growth and total dry matter yield of rice over 75% RDF. Sole and combined application of biochar along with RDF resulted in non significant effect on growth and yield of rice. Combined application of biochar 3.6 g kg^{-1} soil along with PGPR (biochar×PGPR) was produced significantly highest growth and total dry matter yield of rice. Inoculation with PGPR also resulted in significantly higher growth and total dry matter yield of rice over uninoculated level.

Key words: Biochar, Total dry matter yield, rice.

Rice (*Oryza sativa*) is one of the most important staple food crop for more than half of the world population, especially for south-eastern Asia, where 90% of the world production of rice is grown and consumed. In India, it occupies 44 million ha of land and produces about 103.41 million tonnes of grain with the productivity of 2.35 tonnes ha^{-1} (Anonymous, 2012). However, this is not enough to feed the ever-increasing population, and there is need to increase the production to keep pace with population growth. On the contrary there is very limited scope for further expansion of area under rice and the only alternative left is vertical increase in yield.

Biochar is a fine grained and porous carbon rich substance that is produce by combustion of biomass under oxygen limited condition. The effect of biochar on crop productivity has been observed to vary, but is generally positive. Majority of the experiments were carried out in soils of low fertility, including acidic tropical soils and in general, large yield improvements were obtained when biochar was applied on such soils, up to 300% over adequate, unamended controls (reviewed by Zwieten *et al*., 2010). Long-term positive effects of biochar applications were observed in a few studies which were monitored over several years (Blackwell *et*

* To whom all correspondence should be addressed.
E-mail: singhawtar91@gmail.com

al., 2009; Major *et al.*, 2010). Some studies documented lower yields when biochar was applied compared to unamended controls. This could be attributed to N immobilization with biochar (Blackwell *et al.*, 2010; Rondon *et al.*, 2007), and this phenomenon is expected to be of relatively short duration while the "unstable" fraction of biochar is decomposed.

The ash in biochar contains plant nutrients, mostly bases such as Ca, Mg, K and also P and micronutrients including zinc (Zn) and manganese (Mn). The mineral elements contained in biomass will mostly be found in biochar ash, with the notable exception of N. During the pyrolysis process, significant proportions of biomass N are lost by volatilization (Chan and Xu, 2009). The N remaining in the biochar tends to remain poorly available to plants (Gaskin *et al.*, 2010), since a fraction of it is found inside aromatic C structures (Chan and Xu, 2009).

The PGPR like Azotobacter, *Azospirillum* and *Pseudomonas* have ability to fix atmospheric nitrogen or to solubilise phosphorus such as unavailable form of phosphorus into available forms for plant uptake, besides other advantages of phytohormone secretion and disease suppression (Chung *et al.*, 2005). The continuous application of agrochemicals such as fertilizers and pesticides adversely affected biological composition of the soil biota and their activities. Over the past few decades the organic content of soil and nutrient use efficiency of various cropping systems have been declining. Besides that 15-20 % of the applied phosphorus is utilized by crop and the remaining is fixed in soil. Under these conditions, application of *pseudomonas* as PGPR, having property of P-solubilisation can play vital role in phosphorus use efficiency, production of growth hormones and disease suppression through induced systemic resistance. The basic problem, now a days is inconsistent performance of PGPR. Biofertilizers of single strain may be effective for one crop in one soil and may not be effective for other crop in other soil. Hence, to overcome such type of problems, use of mixed inoculants of one or more microorganisms, having synergistic relationship, may provide am ambient microbial environment in rhizosphere to achieve consistent performance of PGPR inoculants.

MATERIALS AND METHODS

Pot experiment

The experiment was conducted in net house of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India to evaluate the effect of rice husk biochar and PGPR on growth and total dry matter yield of rice. The experiment was arranged in factorial completely randomized design with three replications. The treatment consisted of inorganic fertilizers viz., 100% of recommended doses of fertilizers (RDF) (60:30:30:12.5 mg N, P_2O_5, K_2O and Zn kg^{-1} soil) and 75% of RDF (Factor 1) and 0, 1.8, 3.6 and 7.2 g kg^{-1} biochar (Factor 2) and two levels (inoculated and uninoculated) of PGPR (Factor 3). Bulk soil sample (0-15cm) was collected from research farm of institute of Agricultural Sciences, Banaras Hindu University. Varanasi, India. After collecting, it was ground and passed through 5.0-mm sieve and 10 kg of soil filled in each polythene lined pot. Soil in each pot was puddled manually and 5 seedling of rice (variety- BPT-5204) transplanted. After establishment, four plants were maintained. The pots were irrigated and 2 cm of standing water was maintained by daily addition of water. The height of plants was measured from the surface of soil to the tip of plant at 30 and 60 days after transplanting to the with the help of a meter scale. Chlorophyll content in the leaves of rice plants was measured by using of spadometer in SPAD units at 30 and 60 days after transplanting. Numbers of tillers pot^{-1} were counted at 30 and 60 days after transplanting of rice seedling. Numbers of effective tillers pot^{-1} were counted after emergence of ears. The total dry matter production is obtained by summation of grain yield (pot^{-1}) and straw yield (pot^{-1}) was done.

RESULTS AND DISCUSSION

Effect on plant height of rice

A critical perusal of the data presented in table 1 revealed that a significant increase in plant height (30 DAT) was recorded with the application of RDF_{100} (74.16cm) than the plant height obtained from the treatment of RDF_{75} (71.76cm). Application of biochar resulted non significant effect on plant height (30 DAT) when compared to the treatments comprising of no biochar. The inoculation with PGPR showed significantly higher plant height (74.33cm) at 30 DAT than uninoculated treatment (71.58cm). Almost similar trend was noticed with the plant height recorded at 60 DAT. Mathivanan *et al.* (2005) reported that application of PGPR significantly increased the plant height over control. The increase in plant height may be attributed due to adequate supply of nutrients by the PGPR. Abbasi *et al.* (2011) have also reported that inoculation of PGPR in wheat increase shoot length by 25% over the uninoculated control.

The interaction between fertilizer and PGPR was found to be significant (Table 3). At 30 DAT the highest plant height was obtained with the application of RDF_{100} (75.68cm) in

Table 1. Rice growth and yield affected by different levels of fertilizer, biochar and PGPR

Treatment	Plant height (cm)		Chlorophyll content (SPAD value)		No of tillers pot^{-1}		No of effective tillers pot^{-1}	Length of panicle (cm/ plant/pot)	Total dry matter yield (g pot^{-1})
	30 DAT	60 DAT	30 DAT	60 DAT	30 DAT	60 DAT			
RDF_{75}	71.76a	89.10a	33.29a	28.48a	20.25a	21.75a	21.25a	22.19a	76.88a
RDF_{100}	74.16b	90.19b	33.97b	28.88b	20.83b	22.87b	22.46b	22.92b	82.72b
SEm±	0.096	0.105	0.096	0.070	0.131	0.159	0.107	0.100	0.193
CD (0.05)	0.395	0.305	0.278	0.202	0.380	0.459	0.309	0.290	0.557
BC_0	72.90	89.39	33.84abc	28.81abc	20.33	22.25	21.58	22.36	79.30
$BC_{1.8}$	72.78	89.56	33.84bac	28.76bac	20.66	22.42	21.92	22.41	79.64
$BC_{3.6}$	73.20	89.96	33.53cabd	28.75cab	20.66	22.50	21.92	22.71	80.27
$BC_{7.2}$	72.94	89.68	33.29dc	28.40d	20.50	22.08	22	22.74	79.99
SEm +	0.136	0.149	0.136	0.099	0.186	0.224	0.151	0.142	0.273
CD (0.05)	NS	NS	0.393	0.286	NS	NS	NS	NS	NS
$PGPR_0$	71.58a	88.95ab	33.11ab	28.29ab	19.04ab	20.37a	20.17a	22.15ab	76.80a
$PGPR_1$	74.33b	90.35ba	34.16ba	29.06ba	22.04ba	24.25b	23.54b	22.96ba	82.80b
SEm +	0.096	0.105	0.096	0.070	0.131	0.159	0.107	0.100	0.193
CD (0.05)	2.237	2.446	2.226	1.620	3.04	3.67	2.479	2.321	4.46
Interaction									
(BC×PGPR)	S	S	S	S	S	S	NS	NS	S
(RDF×PGPR)	S	NS	S	S	S	NS	NS	NS	NS

For each parameter, different letters within the same column indicate that treatment means are significantly different at P<0.05 according to Duncan's Multiple Range Test for separation of means

Table 2. Interaction effect between biochar and PGPR on plant height, chlorophyll content and No of tillers

	Plant height (cm) at 60 DAT		Chlorophyll content (SPAD value) at 30 DAT		Chlorophyll content (SPAD value) at 60 DAT		No of tillers pot^{-1} at 30 DAT		No of tillers pot^{-1} at 60 DAT		Total dry matter yield (g pot^{-1})	
PR_1	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$
90efh	89.02abd	89.77ef	33.79abeh	33.89eagh	28.68abeh	28.93cafh	19.33abc	21.33e	20.83abc	23.67efh	77.34abcd	81.26e
35fegh	88.90bacd	90.23feh	33.29ba	34.85fg	28.37bacd	29.15fegh	19.17bacd	22.16fgh	20.67bacd	24.17fegh	76.31bacd	82.98fgh
66gfh	88.96cbd	90.95gh	32.68cd	34.36gefh	28.14cbd	29.36gf	18.83cabd	22.50gfh	20.16cabd	24.83gfh	76.76cabd	83.79gfh
40hefg	88.92dabc	90.45hfg	32.66ac	33.90haeg	27.98dbc	28.81haef	18.83dbca	22.16hfg	19.83dbc	24.33hefg	76.79dabc	83.19hfg
	0.211		0.192		0.140		0.263		0.318		0.386	
	0.611		0.556		0.405		0.761		0.918		1.115	

For each parameter, different letters indicate that treatment means are significantly different at P<0.05 according to Duncan's Multiple Range Test for separation of means combination with $PGPR_1$, while the lowest being observed in the treatment where application of RDF_{75} (70.54cm) in combination with $PGPR_0$. The data pertaining to plant height at this stage pointed out towards a significant positive interaction between biochar and PGPR (table 2). Application of $BC_{3.6}$ in combination with $PGPR_1$ gave highest plant height (74.66cm) whereas lowest plant height (71.22cm) was recorded in the treatment $BC_{1.8}$ in combination with $PGPR_0$. It was probably due to the biochar, which favours the activity of PGPR. Solaiman *et al.* (2010) observed that biochar applied in a wheat field encouraged mycorrhizal root colonization in the crop.

Effect on chlorophyll content

Data pertaining to chlorophyll content (leaf SPAD value) were presented in table 1. There was significant increase in chlorophyll content (leaf SPAD value, 33.97) at 30 DAT was recorded with the application of RDF_{100} than the chlorophyll content (leaf SPAD value, 33.29) obtained from the treatment of $RDF_{75.}$ Effect of application of biochar resulted significant decrease in chlorophyll content (leaf SPAD value) at 30 DAT when compared to the control. Application of $BC_{7.2}$ showed lower chlorophyll content in leaves by 1.65% compared to $BC_{0.}$ Decrease in chlorophyll content (leaf SPAD value) with biochar has been reported by Asai *et al*. (2009) in rice, possibly due to reduction in the availability of soil nitrogen to the plant because of its high C:N ratio. The inoculation with PGPR show significantly higher chlorophyll content (leaf SPAD value, 34.16) than, without its inoculation (33.11). The increase in chlorophyll content may be attributed to adequate supply of nitrogen by PGPR. Almost similar trend was observed in chlorophyll content recorded at 60 DAT.

The data pertaining to chlorophyll content (Table 2) revealed a significant positive interaction between biochar and PGPR on chlorophyll content (leaf SPAD value). Application of $BC_{1.8}$ in combination with $PGPR_1$ gave highest chlorophyll content (leaf SPAD value, 34.85) where as lowest chlorophyll content (leaf SPAD value, 32.66) was obtained in the treatment of $BC_{7.2}$ in combination to $PGPR_0$. The interaction between fertilizer and PGPR was found to be significantly positive (table 3). The highest chlorophyll content (leaf SPAD value, 34.31) at 30 DAT was obtained with the application of RDF_{100} in combination with $PGPR_1$, while the lowest being observed in the treatment of RDF_{75} (leaf SPAD value, 32.58) in combination with $PGPR_0$. Almost similar trend was noticed with the chlorophyll content (leaf SPAD value) recorded at 60 DAT.

Effect on number of tillers

Data pertaining to number of tillers (30 DAT) were presented in table 1 revealed that a significant increase in number of tillers (20.83) was recorded with the application of RDF_{100} than the number of tillers (20.25) obtained from the treatment of $RDF_{75.}$ Application of biochar resulted non significant effect on number of tillers (30 DAT) when compared to the treatments comprising of no biochar. The inoculation with PGPR showed significantly higher number of tillers (22.04) at 30 DAT than without its inoculation (19.04). Almost similar trend was noticed with the number of tillers recorded at 60 DAT. Inoculation with PGPR increase the number of tillers in wheat was reported by Zahir *et al.* (2004).

The interaction between fertilizer and

Table 3. Interaction effect between Fertilizer and PGPR on plant height, chlorophyll content and No of effective tillers

	Plant height (cm) at 30 DAT		Chlorophyll content (SPAD value) at 30 DAT		Chlorophyll content (SPAD value) at 60 DAT		No of tillers pot^{-1} at 30 DAT	
(RDF×PGPR)	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$	$PGPR_0$	$PGPR_1$
RDF_{75}	70.54a	72.97c	32.58a	34.00cbd	28.21ab	28.75c	18.50a	22cd
RDF_{100}	72.62b	75.68d	33.63bc	34.31dc	28.38ba	29.38d	19.58b	22.10dc
SEm ±	0.009	0.136	0.099	0.186				
CD (0.05)	0.025	0.393	0.286	0.538				

For each parameter, different letters indicate that treatment means are significantly different at P<0.05 according to Duncan's Multiple Range Test for separation of mean

PGPR was found to be significant (table 3).The highest number of tillers (22.10) at 30 DAT was obtained with the application of RDF_{100} in combination with $PGPR_1$, while the lowest being observed in the treatment where RDF_{75} was applied in combination with $PGPR_0$ (18.50). The data pertaining to number of tillers (30 DAT) point toward a significant (table 4.2.2) positive interaction between biochar and PGPR. Application of $BC_{3.6}$ in combination with $PGPR_1$ gave highest number of tillers (22.50). The lowest number of tillers (18.83) was recorded in treatments $BC_{7.2}$ and $BC_{3.6}$ in combination with $PGPR_0$. The similar trend was noticed with the number of tillers recorded at 60 DAT.

Effect on number of effective tillers pot^{-1}

Data pertaining to number of effective tillers has been presented in table 1, revealed that a significant increase in number of effective tillers (22.46) was recorded with the application of RDF_{100} than the number of effective tillers (21.25) obtained from the treatment of $RDF_{75.}$ Application of biochar resulted non significant effect on number of effective tillers when compared to the treatments comprising of no biochar. The inoculation with PGPR showed significantly higher number of effective tillers (23.54) than without its inoculation (20.17). The increase in number of effective tillers was due to their ability of plant growth promotion activities like increased solubilization of phosphorus, nitrogen fixation and its availability to plants, production of plant growth hormones and reduction of diseases in the crop as also reported by Klopper *et al.* (1992). None of the interaction effects were found to be significant among RDF, BC and PGPR on number of effective tillers.

Effect on Length of panicle

It is obvious from the data (Table 1) that the ear length of rice significantly increased with RDF_{100} as compared to $RDF_{75.}$ Application of biochar resulted non significant effect on length of panicle when compared to the treatments comprising of no biochar. The inoculation with PGPR significantly showed increase in length of panicle as compared to uninoculated. The result showed that application of RDF_{100} and PGPR helped in increasing panicle length which could be attributed to adequate supply of nutrients by the PGPR and $RDF_{100.}$ Mathivanan *et al.* (2005) reported that application of PGPR significantly increased the panicle length over control. None of the interaction effects were found to be significant among RDF, BC and PGPR on panicle length.

Effect on total dry matter yield of rice

Data presented in Table 1 revealed that total dry matter yield obtained with application of RDF_{100} was found to be significantly higher by 7.59% than the total dry matter yield obtained from RDF_{75}. This shows that total dry matter yield of rice can be increased with application of full dose of fertilizer. Application of BC(biochar) has been resulted a non significant effect on total dry matter yield of rice when compared to the treatments comprising of no biochar. The inoculation with PGPR showed significantly higher total dry matter yield (82.80 g pot $^{-1}$) than the uninoculated treatments (76.80 g pot $^{-1}$).

Data presented in Table 2 reflect the interaction effect between biochar and PGPR on total dry matter yield. Application of $BC_{3.6}$ in combination with $PGPR_1$ recorded the highest dry matter yield (83.79 g pot $^{-1}$)) followed by the combination of $BC_{7.2}$ and $PGPR_1$ (83.19g pot $^{-1}$). The lowest dry matter yield was recorded in treatments which had $BC_{1.8}$ and $PGPR_0$ (76.31g pot $^{-1}$).

CONCLUSION

Based on the results of present investigation following conclusions could be drawn

A significant increase in growth and total dry matter yield of rice could be achieved by application of fertilizers and PGPR. Sole application of biochar has no immediate significant effect on growth and total dry matter yield of rice. However, when combined with PGPR, biochar application resulted in significantly improved growth and total dry matter yield of rice. Among the various treatments tested, the highest growth and total dry matter yield was obtained with combined application of 3.6 g kg^{-1} biochar and PGPR.

Thus, biochar, when applied with PGPR, was capable of increasing growth as well as total dry matter yield of rice.

ACKNOWLEDGEMENTS

The authors wish to thank ICAR, New Delhi for financial assistance in form of Junior Research Fellowship and Head Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India for providing necessary facilities during the course of investigation.

REFERENCES

1. Abbasi, M.K., Sharif, S., Kajmi, M., Sultan, T. and Aslam, M., Isolation of plant growth promoting rhizobacteria from wheat rhizosphere and their effect on improving growth, yield and nutrient uptake of plant. *Plant Biosystems,* 2011; **145** (1): 159-168.
2. Anonymous., Agriculture statistics at a glance. Department of Agriculture and Co-operation, Ministry of Agriculture, Government of India, New Delhi, 2012.
3. Asai, H., Samson, B.K., Stephan, H.M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shiraiwa, T. and Horie, T. (2009) Biochar amendment techniques for uplandrice production in northern Laos. *Field Crops Res*, **111**, 81–84.
4. Blackwell, P., Evelyn, K., Butler, G., Herbert, A., and Solaiman, Z., Effect of Banded Biochar on Dryland Wheat Production and Fertiliser Use in South-Western Australia: An Agronomic and Economic Perspective. *Australian Journal of Soil Research* 2010; **48**: 7 531-45.
5. Blackwell, P., Riethmuller G. and Collins, M., Biochar application to soil. In: Biochar for Environmental Man-agement: Science and Technology. (Lehmann J., S. Jo-seph, ed.), *Earthscan*, London, UK, 2009; 207-226.
6. Chan, K.Y. and Xu., Z., Biochar: Nutrient properties and their enhancement. In: Biochar for Environmental Management: Science and Technology. (Lehmann, J. and Joseph, S. eds.), *Earthscan*, London, UK, 2009; 67–84.
7. Chung, H., Park, M., Seshadri, S., Song, J., Cho, H. and Sa, T., Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of korea. *Soil Biol. Biochem*, 2005; **37**: 1970-1974.
8. Gaskin, J.W., Speir, R.A., Harris, K., Das, K.C., Lee, R.D., Morris, L.A and Fisher, D.S., Effect of Peanut Hull and Pine Chip Biochar on Soil Nutrients, Corn Nutrient Status, and Yield. *Agronomy Journal,* 2010; **102**: 623-633.
9. Klopper, J. W. and Baeauchamp, C.J., A review of issues related to measuring of plant roots by bacteria. *Canadian J. Microbio,* 1992; **38**: 1219-1232.
10. Major, J., Rondon, M., Molina, D., Riha, S.J. and Lehmann, J., Maize yield and nutrition during 4 years after biochar application to a colombian savanna oxisol. *Plant and Soil,* 2010; **333**:117-128.
11. Mathivanan, N., Prabavathy, V.R. and Vijayanandraj, V.R., Application of talc formulations of *Pseudomonas fluoresens* Migula and *Trichoderma Viride* pers. Ex S.F. Gray disease the sheath blight disease and enhance the plant growth and yield in rice. *J. Phytopathology*, 2005; **153**: 679-701.
12. Rondon, M.A., Lehmann, J., Ramirez, J. and Hurtado, M., Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char, 2007.
13. Solaiman, Z. M., Blackwell, P., Abbott, L. K. and Storer, P., Direct and Residual Effect of Biochar Application on Mycorrhizal Root Colonisation, Growth and Nutrition of Wheat." *Australian Journal of Soil Research* 2010; **48**(7): 546-54.
14. Zahir, Z.A., Arshad, M. and Frankenberger, W.T., Plant growth promoting rhizobacteria: application and prospective in agriculture. *Advances in agronomy*, 2004; **81**: 97-168.
15. Zwieten, V.L., Kimber, S., Morris, S., Chan, K.Y., Downie, A., Rust, J., Joseph, S. and Cowie, A., Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and Soil,* 2010; **327**: 235-246.