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Phosphorus solubilizing bacteria and rice straw biochar consequence on maize pigments synthesis

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Abstract

To determine the effect of phosphorus solubilizing bacteria and biochar various application rates on fresh and dry weight, photosynthetic and accessory pigments production in maize plants a pot experiment was conducted. There were 4 doses of Rice straw biochar (0, 1, 2 and 3%) with and without phosphorus solubilizing bacteria (PSB) making 8 treatments with 4 replications (CRD). After 40, days of seeds sowing plants were harvested and pigments (chlorophyll a, chlorophyll b, total chlorophyll, lycopene, carotenoids and anthocyanin) analysis was done in maize shoot as well as pH, EC (µS/cm) and OM (%) in post harvested soil. Results indicated a significant increase in soil pH, EC and OM due to addition of 1%, 2% and 3% rice straw biochar (RSB) which helps in enhancing the activity of photosynthesis by providing nutrients. PSB significantly reduced the pH of soil through organic secretions and OM by decomposition. Interactive effect of PSB and biochar was significant for chlorophyll (a, b, total) while it changed the lycopene, carotenoids and anthocyanin synthesis non significantly. In case of lycopene the maximum synthesis was noted in control (B_oP_o) treatment plants. The plants cultivated in a soil having treatment 3% rice straw biochar + PSB showed maximum production of pigments as compared to the control (B_oP_o) treatment plants. The main and interactive effects of RSB as well as PSB were significant on maize plants fresh and dry weight. An increasing rate of biochar enhanced the fresh and dry weight gain in combination with PSB.

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Introduction

In all over the world Maize (*Zea mays* L.) is considered the 3rd largest cereal that contributes in fulfilling the requirement of food for population. It is reported that 100 million hectares land is under the maize crop cultivation in 125 countries that provide a huge amount of about 829 Metric tons / annum maize yield for the world population feeding. According to crop modulation the world population will increase from 7.7 billion people (2020) to 9.3 billion (2050) which increased population food demand as well as *Zea mays* L. (Rosegrant *et al.,* 2009). The consumption of maize among humans and animals as fodder is 30% and 70% respectively. Pakistan as part of developing countries provides 67% maize that is a major contribution in food (Rosegrant *et al.,* 2009). However the productivity of many crops are directly associated with the fertility of soil that affect the nutrients and water bioavailability (Warkentin, 1995). The growth of plants is always associated with the nutrients and water retention capability of soil in various environmental conditions that affect their availability to roots (Hodge, 2004). In saline soils especially in Pakistan conditions after nitrogen bioavailability of phosphorus is a serious problem that promotes root growth (Srinivasan *et al.,* 2012). Due to high immobilization of applied phosphorus in the form of inorganic fertilizers scientist recommend inoculation of PSB (*Azospirillum, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Pseudomonas, Enterobacter, Erwinia, Rhizobium, Flavobacterium*) (Kloepper and Schroth, 1978) that make phosphorus mobile (Glick, 1995). However the addition of organic amendments is preferred that not only enhance the nutrients bioavailability but also improved soil physical, chemical and biological attributes. Activated black carbon organic nature compound biochar is one of such amendment that not only reduced the loss of NPK but also a source of nutrition especially phosphorus (Verheijen *et al.,* 2010). It is produced by the pyrolysis of waste biomass (Sohi *et al.,* 2009) at very high temperature (450-650 °C) in the partial presence or absence of oxygen (Tagoe *et al.,* 2008). The relatively small particle size of biochar provides it the capability to modify the physiochemical behavior of which makes it a good soil organic conditioner (Amonette and Joseph, 2009). Due to less decomposition rate of biochar it becomes quite stable in the soil as compared to organic matter that have high decomposition rate carried by microbes of soil (Thies and Rillig, 2009). The application of biochar also enhanced the interaction of microbes with the roots of plants that is usually related with the increasing pool of C in the soil. The increasing C pool supports the microbes and then they increased in their population (Thompson and Troeh, 1978). Such improvement in the soil microbial population also mobilized the immobilized nutrients especially PSB that play a key role in the improvement of photosynthesis and productivity of crops (Stevenson, 1994). Water is an essential requirement for plants to carry out the photosynthesis that enhanced the plants growth. Biochar mixing in the soil increase the water holding capacity of soil (Lorenz, 2007) thus making it more available for roots of plants to uptake that is consumed in photosynthesis for production of pigments (Danish *et al.,* 2014). In that way the nutrients and water use efficiency are improved that support the plants to grow well (Steiner *et al.,* 2008; Lehmann *et al.,* 2003; Glaser *et al.,* 2002). In the past studies only the relationship of organic matter and PSB was studied by various scientists. But high degradation rate of biochar decrease the life span of soil organic matter that is main center of microbial activities. Needs of time is to replace organic matter with such organic amendment that is resistant against fast decomposition. One of such amendment is activated biochar that not only enhanced the soil organic matter stabilization but also improved action of microbes in the soil and nutrients uptake which directly involved in the promotion of pigments synthesis (Younis *et al.,* 2014). The aim of this study was to evaluate the interactive and main effects of biochar and phosphorus solubilizing bacteria on the maize pigments (photosynthetic and accessory) synthesis that promote the fresh and dry weight gain. Also to determined the consequences of rice straw biochar and its interactive impacts with phosphorus solubilizing bacteria on soil pH, EC (µS/cm) and OM

(%).

Materials and methods

For experimental purpose soil was collected at the depth of 0-15 cm from research area of Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan. The latitude and longitude of soil taken site were 30.26° N and 71.49° E. The physiochemical properties of soil are provided in Table 4.

Biochar

Biochar was manufactured from waste material dry rice straw which was collected from agricultural research area. Straws of rice were initially chopped into small pieces. After that chopped straw was pyrolyzer at 470 °C for 75 min in the absence of air by using specially designed pyrolyzer followed by cooling, grinding (5 mm) and packing in air tight packets as described by Danish *et al* (2014a). The physiochemical properties of biochar are provided in Table 4.

Phosphorus Solubilizing Bacteria

The Phosphorus solubilizing bacteria was taken from the Ayub Research institute Faisalabad. The bacterium was *Bacillus megaterium* which was isolated from the cotton crop field through dilution plate technique. Pikovskaya medium was used for the purification and screening of *Bacillus* (Pikovskaya, 1948).

Experimental Plan

There were 8 treatments including four different application rates of rice straw biochar (0, 1, 2 and 3%) in the presence and absence of phosphorus solubilizing bacteria (PSB) with 4 replications following complete randomized design (CRD).

Treatments

The treatments include control having no biochar and no PSB (B0P0), no biochar + PSB (B0P1), 1% Biochar + no PSB (B1P0), 1% Biochar + PSB (B1P1), 2% biochar + no PSB (B2P0), 2% biochar + PSB (B2P1), 3% biochar + no PSB (B3P0), 3% biochar + PSB (B3P1).

Pots Preparation and Seeds sowing

RSB was applied at the rate of 1%, 2% and 3% w/w (50, 100 and 150 g / 5 kg soil). In the pre weighted 5 kg soil RSB were mixed manually and finally soil was filled in plastic pots. Fertilizers were applied in solution form for fulfilling the macro and micro nutrients according to the crop requirements. All the treatments were mixed by hand and then 5 seeds per pot were sown that was inoculated when the PSB by making sticky concentrated solution of sugar. Later on 2 healthy plants were maintained in each pot.

Harvesting and Analysis

After 40 days of seeds sowing harvesting was done and fresh as well as dry weight (after heating at 65 °C for 24 h) was noted. For the determination of chl. a, chl. b, total chl., carotenoids, anthocyanin and lycopene fresh leaves of plants were used (Arnon, 1949) while Lycopene was calculated by following Ravelo-Pérez *et al* (2008) methodology.

Statistical Analysis

Soil pH, soil EC, soil OM, plant fresh weight, plant dry weight, Chlorophyll a, Chlorophyll b, Total Chlorophyll, Carotenoids, Lycopene and Anthocyanin were analyzed using by two way ANOVA. Tukey-HSD test was applied for differentiation at $P \leq 0.05$. For statistical analyses statistical computer software package SPSS was used (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago).

Results and discussions

Soil characteristics

The main effect of PSB and various biochar application rates were significant ($P \leq 0.05$) on the soil pH, EC (µS/cm) and OM (%) but the interactive effect was significant ($P \leq 0.05$) only for EC (µS/cm) and OM (%). Increasing rate of biochar enhanced the soil pH but PSB decreased it in comparison with non PSB inoculated soils (Table 1). Among biochar various application rates Highest biochar application rate (3%) enhanced the soil pH up to 1.81% in the absence of PSB while 1.95% in the presence of PSB as compared to control (Table 1). Similarly at 3% biochar amended soil an increment of 1.44 and 1.33 folds were observed in the non inoculated PSB and inoculated PSB soils respectively in comparison with control (0% biochar). In case of soil organic matter (%) 150% increased was observed in the post harvested non PSB inoculated soils while 133% in PSB inoculated soils (Table 1). According to Beesley and Marmiroli (2011) application of biochar enhanced the soil pH EC and water holding capacity of soil as well as it improves the soil carbon contents. They attributed this increase in the soil pH as the presence of hydroxyl ions in the biochar. In another experiment Keiluweit *et al* (2009) and Fellet *et al* (2011) amended also reported similar sort of results on pH and increase in CEC of soil when they applied the biochar as soil conditioner. They noted that when biochar is applied in the soil they retention of metals ions are resulted due to increase in the soil pH.

Table 1. Effects of biochar (B) various application rates and phosphorus solubilizing bacteria (PSB) on post harvested soil pH, EC (µS/cm) and OM (%).

Biochar (B)	Phosphorus solubilizing bacteria (PSB)					
	No PSB (P0)	PSB (P1)	Mean	No PSB (P0)	PSB (P1)	Mean
	Soil pH (1:5)			EC (µS/cm)		
B (0g/5kg soil)	8.26^{d}	8.20^{e}	8.23D	735^{g}	800^{f}	768D
B (50g/5kg soil)	8.32^{bc}	8.28^{cd}	8.30C	851^{e}	884^{d}	867C
B (100g/5kg soil)	8.36^{b}	8.30^{cd}	8.33B	956^{c}	1036^{b}	996B
B (150g/5kg soil)	8.41^{a}	8.36^{b}	8.38A	1062^{ab}	1064^{a}	1063A
	8.34A	8.28B		901B	946A	
	Soil organic matter (%)					
B (0g/5kg soil)	0.4d^{e}	0.3^{e}	0.4C			
B (50g/5kg soil)	0.7^{bc}	0.5^{cd}	0.6B			
B (100g/5kg soil)	0.8^{ab}	0.7^{bc}	0.7A			
B (150g/5kg soil)	1.0^{a}	0.7^{bc}	0.8A			
	0.7A	0.5B				

The values followed by different letters along column are significantly different at $P \leq 0.05$.

Table 2. Effects of biochar (B) various application rates and phosphorus solubilizing bacteria (PSB) on plants fresh and dry weight (g) of maize.

Biochar (B)	Phosphorus solubilizing bacteria (PSB)					
	No PSB(P0)	PSB(P1)	Mean	No PSB(P0)	PSB(P1)	Mean
	Fresh weight (g)			Dry weight (g)		
B (0g/5kg soil)	13.8^{e}	15.4^{e}	14.6D	3.4^{e}	3.8^{e}	3.6D
B (50g/5kg soil)	24.1^{d}	30.3^{cd}	27.2C	5.9^{d}	7.5^{cd}	6.7C
B (100g/5kg soil)	36.8^{bc}	39.5^{ab}	38.2B	9.1^{bc}	9.8^{ab}	9.4B
B (150g/5kg soil)	42.9^{ab}	45.0^{a}	43.9A	10.6^{ab}	11.1^{a}	10.8A
	29.4B	32.6A		7.3B	8.0A	

The values followed by different letters along column are significantly different at $P \leq 0.05$.

Plants weigh gain

The main and interactive effect of PSB and biochar was significant ($P \leq 0.05$) on the gain of maize plants fresh and dry weigh (g) gain. It was noted that the plants cultivated in soils which were inoculated with the PSB showed more gain in weight (fresh and dry) as compared to the non inoculated PSB soils (Table 2). In case of biochar increasing biochar rates in the soil also significantly ($P \leq 0.05$) enhanced the fresh and dry weight of maize plants. As compared with the control biochar (B0P0 and B0P1) treatments a net increase in the fresh weighs was 210% in B3P0 and 192% in B3P1. The dry weight was increased up to 3.11 in B3P0 and 2.92 folds B3P1 in comparison with control B0P0 and B0P1 (Table 2). As compared to control PSB (B0P0) an increase of 3.26 folds was noted in highest biochar application rate along with PSB inoculation in plants fresh as well as dry weights. Park *et al* (2011) also reported similar results in his experiment by using waste water sludge's biochar for the production of tomato. He found that addition of biochar gives 74% more biomass than control one. Similar increase in the plants fresh and dry weights was also reported by Danish *et al* (2014) when they applied biochar in the sandy soils for the cultivation of wheat under drought stress condition. They argued that the decrease in the stress of water due to application of biochar enhanced the fresh and dry weight gain in the wheat especially in case of sandy soils where water holding capacity is very low. According to Lehmann *et al* (2006) such improvement in the yield of crops was due to more and easily availability of nutrients in balance concentration. Blackwell *et al* (2010) also reported an increase in the growth of plants due to addition of biochar. He found that addition of biochar improves the phosphorous fertilizer use efficiency (FUE) and positive plant-Mycorrhizal interaction in wheat.

Table 3. Effects of biochar (B) various application rates and phosphorus solubilizing bacteria (PSB) on pigment production in maize leaves.

Biochar (B)	Phosphorus solubilizing bacteria (PSB)					
	No PSB(P0)	PSB(P1)	Mean	No PSB(P0)	PSB(P1)	Mean
	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)		
B (0g/5kg soil)	0.12^{f}	0.23^{e}	0.17D	0.09^{g}	0.16^{f}	0.12D
B (50g/5kg soil)	0.32^{d}	0.43^{c}	0.38C	0.22^{e}	0.29^{d}	0.25C
B (100g/5kg soil)	0.51^{c}	0.60^{b}	0.56B	0.37^{c}	0.43^{bc}	0.40B
B (150g/5kg soil)	0.64^{ab}	0.70^{a}	0.67A	0.48^{ab}	0.52^{a}	0.50A
	0.40B	0.49A		0.29B	0.35A	
	Total chlorophyll (mg/g)			Carotenoids (mg/g)		
B (0g/5kg soil)	0.24^{g}	0.42^{f}	0.33D	0.17^{b}	0.28^{ab}	0.22A
B (50g/5kg soil)	0.58^{e}	0.75^{d}	0.67C	0.23^{ab}	0.25^{ab}	0.24A
B (100g/5kg soil)	0.92^{c}	1.07^{b}	0.99B	0.28^{ab}	0.23^{ab}	0.26A
B (150g/5kg soil)	1.15^{ab}	1.25^{a}	1.20A	0.28^{ab}	0.30^{a}	0.29A
	0.72B	0.87A		0.24A	0.27A	
	Anthocyanin (µmol/ml)			Lycopene (µg/g)		
B (0g/5kg soil)	0.017^{b}	0.030^{ab}	0.024B	40.37^{a}	34.18^{ab}	37.27C
B (50g/5kg soil)	0.027^{ab}	0.032^{ab}	0.029AB	33.66^{ab}	28.17^{ab}	30.92BC
B (100g/5kg soil)	0.035^{ab}	0.027^{ab}	0.031AB	19.35^{b}	18.42^{b}	18.89AB
B (150g/5kg soil)	0.036^{ab}	0.043^{a}	0.040A	15.74^{b}	14.42^{b}	15.08A
	0.029A	0.033A		27.28A	23.80A	

The values followed by different letters along column are significantly different at $P \leq 0.05$.

Pigments synthesis in maize

The main and interactive effect of PSB and various application rates of biochar was significant ($P \leq 0.05$) on the synthesis of chlorophyll a, chlorophyll b, total chlorophyll. However, synthesis of carotenoids was not significantly changed in the leaves of maize plants through PSB and biochar application. In case of anthocyanin and lycopene the main effect of PSB was non-significant while 3% biochar application significantly ($P \leq 0.05$) affects anthocyanin as well as lycopene production in maize plants as compared to control biochar (Table 3). Similarly the interactive effect of PSB and biochar was not significant on the anthocyanin and lycopene synthesis. Increasing application rates of biochar increase the synthesis of pigments (except carotenoids) while lycopene (antioxidant) production was decreased (Table 3). Maximum production of chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin was noted in those plants that were cultivated in 3% biochar amended soils in the presence of PSB (with minimum lycopene production) while minimum was noted in such plants cultivated in control biochar having no inoculation of PSB (with maximum lycopene production).

Application of PSB and 3% biochar (B3P1) enhanced 5.83 folds chlorophyll a, 5.77 folds chlorophyll b, 5.20 folds total chlorophyll, 1.76 folds carotenoids, 2.52 folds anthocyanin while reduced 2.80 folds lycopene as compared to control (B0P0) (Table 3). According to Singh *et al* (2010) more bioavailability of nutrients by less losses increase the growth of plants as a result of proper photosynthesis (Ding *et al.,* 2010; Laird *et al.,* 2010; Lehmann *et al.,* 2003; Major *et al.,* 2009; Novak *et al.,* 2009a). Caris-Veyrat *et al* (2004) also observed more production of antioxidants like lycopene, anthocyanin and carotenoids where organic amendments were applied. Danish *et al* (2014) suggested that the easy uptake of nitrogen and phosphorus are the key factors that are involved in high chlorophyll synthesis as well as carotenoids and anthocyanin but the shifting of nutrients stress in the biochar plants decrease the antioxidant lycopene production.

Table 4. The physicochemical characteristics of the pre experimentation soil and biochar.

Characteristics	Unit	Value
Soil		
Texture	-	Clay loam
pH_s	-	8.28
EC_e	µS/cm	740
Organic matter	%	0.45
Soluble Carbonates	meq./L	0.00
Soluble Bicarbonates	meq./L	5.04
Soluble Ca^{2+} + Mg^{2+}	meq./L	4.13
Soluble Na^{+}	meq./L	3.27
SAR	meq./L	2.29
RSC	meq./L	0.91
Biochar		
pH (1:10)	-	8.48
EC (1:10)	(µS/m)	1390
Volatile matter	%	10.3
Ash	%	46.5
Total C	%	27.6
Total N	%	0.21
Total P	%	2.29
Total K	µg/g	66.0
Total Na	µg/g	35.0

Conclusions

Additions of biochar promote the photosynthesis which leads to more production of photosynthetic pigments and other accessory pigments. The maize plants fresh and dry weight gain is increased by inoculation of PSB and Biochar. Biochar and PSB modified the soil pH, EC and SOM which play a vital role in improvement of maize plant growth. Combine application of biochar and PSB is better than individual as both interactive effects promotes more growth in maize plants as compared to individual one.

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