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Full Length Article

Biochar Application Improves the Wheat Productivity under Different Irrigation Water-Regimes

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Abstract

Under soil water-deficit conditions, the production of toxic reactive oxygen species (ROS) at cellular levels adversely affects plant growth, development and ultimately final yield. Use of biochar, a soil conditioner, is considered a newly emerging tool to increase the tolerance by antioxidant defence system in plants and to conserve moisture. Therefore, a field trial on wheat crop was conducted during 2015–2016 and 2016–2017. In this field investigation, biochar application (cotton sticks @ 4 t ha^{-1}) was compared with no application under four levels of irrigation water-regimes at critical growth stages *i.e.*, tillering (T), booting (B), heading (H) and milking (M) were maintained as $I_{(T+B+H+M)}$, $I_{(T+B+H)}$, $I_{(T+B)}$ and $I_{(T+M)}$. The results depicted that maximum grain yield, due to significant improvement in entire yield related traits, was harvested by BC application with water-regimes at $I_{(T+B+H+M)}$ followed by $I_{(T+M)}$ during both years of trial. Soil-applied BC triggered wheat plant antioxidant defense system and improved performance of gas exchange behavior under irrigation-regimes at tillering and booting stages. Overall results indicated that biochar application is effective under limited water conditions. In conclusion, biochar application with only two irrigations at tillering and booting stages of wheat seemed a viable technique to get better yield and water productivity of wheat particularly under water limited conditions.

Keywords: Biochar; Irrigation water-regimes; Antioxidants; Gas exchange parameters; *Triticum aestivum*

Introduction

Currently water scarcity has become the leading menace to curtail crop productivity around the globe (Hussain *et al*., 2018). The severity of drought depends on the availability of moisture, water-holding capacity of soil and soil composition with reference to aggregates of organic carbon compounds. However, prolong imposition of drought on agricultural lands negatively affects the food security, which ultimately exerts a serious threat to the economy of various countries (Lal, 2009).

All the metabolic activities of plants are badly influenced due to prolonged drought conditions. Lowering of stomatal conductance during transpiration process is the most prominent negative responses of plants in curtailing the crop yield (Yordanov *et al*., 2000; Dennison *et al*., 2001). Under the limited water supply, efficiency of plants is also decreased like destructive performance of sub-stomatal conductance causing inhibition in the pathway of photosynthetic electron transport rate (Chakir and Jensen, 1999). To tackle with these drastic effects influencing physiological processes in plants, among various other approaches, application of some organic products as soil amendments may improve the soil-plant relationships in terms of providing better moisture conditions under water scarcity periods (Flexas *et al*., 2006).

Biochar (BC) is known as "*Black Gold*" for the agriculture sector and produced by the process called pyrolysis, which is anoxic decomposition of organic substances, thermally converted into the charred solid material (Joseph *et al*., 2010). Biochar is considered as a soil conditioner for improving soil fertility, best mitigating agent against adverse effects of climate changes by carbon (C) sequestration and also increases water-holding capacity for enhancing the crop yield (Lehmann and Joseph, 2015; Hussain *et al*., 2017). Soil amended with BC results in enhanced crop productivity by retaining more rain-water and by conserving irrigation water in arid and irrigated regions (Basso *et al*., 2013). Soil treated with BC prepared from crop residues increased water-holding capacity and porosity which maintained the soil health in terms of organic fertility and decreased carbon dioxide emission (Sohi *et al*., 2010). Beyond these approaches, BC may have a potential to provide better defense system against reactive

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oxygen species (ROS) in plants under water stress conditions through enhancing antioxidants enzymatic activities and increasing non-enzymatic antioxidants contents. Deficit irrigation triggers production of ROS molecules in plants such as free radical species {superoxide anion ($^{\bullet}O_2^{-}$), singlet oxygen ($^1O_2$), per-hydroxyl radical (HO_2)} and non-radical species {hydrogen peroxide (H_2O_2), reactive hydroxyl radical ($^{\bullet}OH$) which cause cellular oxidative damage (Nawaz *et al*., 2015). Plants show tolerant mechanism in the response of ROS production through the antioxidant defense system. Enzymatic {superoxide dismutase (SOD), peroxidase (POD), catalase (CAT)} and non-enzymatic {total soluble protein (TSP), Ascorbic Acid (AsA)} antioxidants mitigate the injurious effect of ROS at cellular level under water stress conditions (Cheng *et al*., 2015).

Wheat (*Triticum aestivum* L.) crop subjected to drought stress either at vegetative or reproductive phase undergo heavy yield penalty due to poorly developed entire yield related traits (Farooq *et al*., 2015; Hussain *et al*., 2016, 2017). Some researchers have revealed the healthy effect of BC as a soil conditioner in term of enhancing the growth and development in maize (*Zea mays*), rice (*Oryza sativa*) and wheat (Yamato *et al*., 2006; Noguera *et al*., 2010; Vaccari *et al*., 2011). However, the role of biochar under irrigation water-stress for ameliorating the wheat productivity is still less explored. Therefore, this field experiment was designed to explore the role of BC application to improve physiological and biochemical activities leading to better wheat productivity under various irrigation regimes.

Materials and Methods

Biochar (BC) Preparation and Soil Characteristics

Harvested waste cotton stalks were collected after the last picking from the cotton field at the Agricultural Farm, Bahauddin Zakariya University Multan (Pakistan) and chopped into 5 mm small pieces. To prepare BC, a modified method described by Qayyum *et al*. (2015) was used. The equipment comprised of a laboratory scale designed stainless steel incinerator (having 10 kg capacity) with a gas burner for pyrolysis of the plant material under a limited supply of oxygen. The temperature of the incinerator was kept constant at 450°C for 2 h in order to furnish decomposition of the plant material. After cooling, the BC was stored in a furnace for further analysis. The physicochemical values of the prepared BC were as colour; black amorphous carbon, structure; porous, moisture contents; <13.5%, ash contents; 15.46%, pH; 9.5, EC; 1.54 dS m^{-1}, volatile matter; 20% and total carbon; 47.1%. For soil analysis, representative samples were collected with stainless steel spade from three different locations of experimental site. Soil characterization and its physicochemical properties were measured according to Nawaz *et al*. (2016). Experimental soil belonged to Sindhlianwali soil series (fine silt + mixed + hyperthermic + sodic haplocambids) and according to FAO classification in USDA Hap-lic Yermosols. Soil contained physicochemical properties included texture; clay loam, ECe; 2.43 dS m^{-1}, pH; 8.67, organic matter; 0.84–0.89%, total nitrogen; 0.06–0.07%, available phosphorus; 5.56–5.59 mg kg^{-1}, available potassium; 103–105 mg kg^{-1} and zinc; 0.37–0.38 mg kg^{-1} during both years of study.

Experimental Layout and Crop Husbandry

This field experiment was conducted during Rabi season of 2015–2016 and 2016–2017 at Agronomic Research Area, Department of Agronomy, Faculty of Agricultural Sciences & Technology, Bahauddin Zakariya University Multan, (71.4°E, 30.2°N and 122 m above sea level with semi-arid to sub-tropical climate) Pakistan. Experiment was laid out by using randomized complete block design (RCBD) with factorial arrangements having net plot size 1.8 m × 6 m and replicated three times. Plant rows were spaced at 21.5 cm distance. Irrigation water-regimes and biochar were randomized in main and sub-plot respectively. Wheat variety Galaxy-2013 was used as test specie. Weather data of mean maximum and minimum monthly temperatures, average rainfall and relative humidity during the year-I and year-II of crop growing period is presented in Fig. 1.

The study involved four irrigation water-regimes at critical growth stages of wheat crop *i.e*., Tillering (T), Booting (B), Heading (H) and Milking (M). The set treatments included $I_{(T+B+H+M)}$ (control), $I_{(T+B+H)}$, $I_{(T+B)}$ & $I_{(T+M)}$, with applied Biochar (BC) @ 4 t ha^{-1} and without BC (control). A well-prepared BC was incorporated in the topsoil before sowing. Irrigation was applied at the depth of 10 cm and the field was observed continuously for moisture level to achieve the workable conditions. The field was then ploughed twice followed by planking to prepare a seedbed. The recommended seed rate of wheat crop (125 kg ha^{-1}) was used and the crop was sown during the first fortnight of November of both the years. NPK fertilizers were applied @ 120-100-62.5 kg ha^{-1} by using fertilizer sources urea, single super phosphate and potassium sulphate fertilizers respectively. All phosphorus and potassium along with 1/3 of nitrogen were applied at the time of sowing. The remaining N was applied into two equal splits, one at first irrigation and the second as required during the growing period of the wheat crop. The meteorological data (Fig. 1) depicts that mild showers were received by the crop; however, the intensity did not affect soil moisture under the applied irrigation water-regimes at various critical growth stages in both the years. Uniform plant protection measures and intercultural practices were performed as per the need of the crop. Twenty number of healthy spike were collected carefully. Number of grains per spike was counting randomly from each spike. For 1000-grains weight, five random sample having 1000 grains from seed lot were

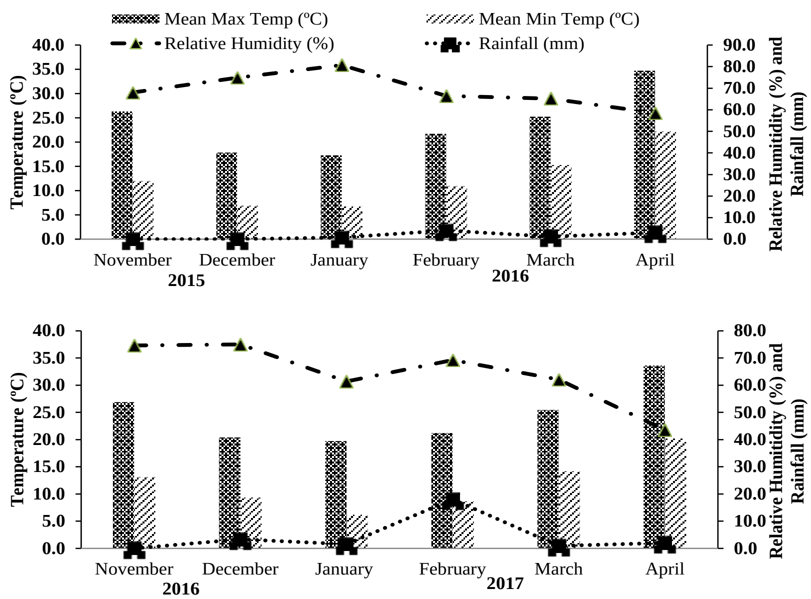

Fig. 1: Weather data during wheat growing period of 2015-2016 and 2016-2017
Metrological department, Central cotton research institute (CCRI) Multan, Pakistan

weighed and recorded. The crop was harvested in the second fortnight of April in both the years. Harvest material of each experimental unit was cut manually, sun dried for 7 days and tied into bundles. These bundles were threshed with hand to measure grains yield, and biological yield.

Antioxidants and Physiological Gas Exchange Biochemical Analysis

To monitor the physiological attributes, flag leaves from the standing wheat crop were randomly collected after the last irrigation at milking stage (90 days) from each experimental unit for biochemical analysis in the morning time (temperature 20 ± 2ºC) and stored in the zip polythene bags at -80ºC. Total soluble proteins (TSP) were determined by the modified procedure of Bradford (1976), Bovine serum albumin was used as standard. For enzymatic antioxidants, leaf samples were extracted in 50 m*M* phosphate buffer (pH 7.8) and after centrifugation at 15,000×g for 20 min the supernatant used for further assay of Superoxide dismutase (SOD) (Giannopolitis and Ries, 1997), catalase (CAT) and Peroxidase (POD) activity (Chance and Maehly, 1955) by recoding absorbance at 560, 240 and 470 nm, respectively. As non-enzymatic antioxidants, total phenolic content (TPC) (mg g^{-1}) of fresh leaf samples were determined at 765 nm using gallic acid as reference standard (Waterhouse, 2001). Ascorbic acid (AsA) (m*M* g^{-1}) of fresh leaf samples were measured following Ainsworth and Gillespie (2007) using AsA as a reference standard and recording absorbance at 525 nm. All the biochemical analyses were performed in the Genomics Laboratory, Department of Plant Breeding & Genetics Bahauddin Zakariya University, Multan Pakistan.

A portable infrared gas analyzer (IRGA (LCA-4) Germany) was used to quantify the physiological attributes related to gas exchange (photosynthetic rate, transpiration rate, stomatal conductance, sub-stomatal conductance, water use efficiency, and intrinsic water use efficiency) between 10:00 a.m. and 02:00 p.m. using photosynthetic photon flux density at 1200–1400 μmol m^{-2} s^{-1}).

Statistical Analysis

The effects of two applied factors *i.e*., biochar and irrigation water-regimes on the wheat crop were measured by two way Fisher’s analysis of variance (ANOVA) technique in Statistics 8.1 software and treatments means were separated by LSD test at 5% probability level (Steel *et al*., 1997). Meteorological data were presented graphically by using Microsoft Excel Program 2013.

Results

Yield Components

The data given in Table 1 showed that a maximum number of grains per spike were counted in the plants grown on BC treated soil as compared to those grown on untreated soil under applied irrigation regime at $I_{(T+B+H+M)}$ during both the years of study. A significant interaction was observed between BC application and irrigation water-regimes for 1000-grain weight. Regarding individual effects, the soil application of BC resulted in higher grain weight as compared to control treatment (without BC) and irrigation regime at $I_{(T+M)}$ gave the least grain weight during both the years of study. It was observed that plants under BC treated soil resulted in statistically similar values of 1000-grains

Table 1: Effect of biochar on yield components of wheat under various irrigation regimes

Irrigation regimes	Biochar levels (t ha^{-1})							
	2015–2016		2016–2017		2015–2016		2016–2017	
	No. of grains spike^{-1}				1000-grain weight (g)			
	0	4	0	4	0	4	0	4
$I_{(T+B+H+M)}$	35.00bc	40.00a	35.85c	41.82a	36.00cd	43.66a	35.59cd	43.65a
$I_{(T+B+H)}$	33.33cd	35.00bc	34.67c	36.54c	33.33e	37.33bc	33.76d	37.66c
$I_{(T+B)}$	34.00c	37.00b	35.10c	38.98b	35.33d	38.66b	37.11c	40.09b
$I_{(T+M)}$	29.00e	31.00de	29.68d	31.00d	29.66f	33.00e	30.32e	33.77d
Year	34.93A		35.45A		35.87		36.49	
LSD@5%	2.40		2.10		1.79		2.07	
	Grain yield (t ha^{-1})				Biological yield (t ha^{-1})			
$I_{(T+B+H+M)}$	5.12c	6.23a	5.67c	6.81a	11.75de	16.58a	12.56d	17.83a
$I_{(T+B+H)}$	4.13e	4.51d	4.13f	4.63d	12.58cd	14.83b	13.17cd	15.51b
$I_{(T+B)}$	4.42d	5.55b	4.42e	6.23b	13.75bc	14.33b	14.34bc	14.91b
$I_{(T+M)}$	3.47g	3.87f	3.71g	4.55de	11.25e	12.33de	12.41d	13.49cd
Year	4.66B		5.02A		13.42B		14.28A	
LSD@5%	0.14		0.15		1.19		1.20	

Means sharing the same letter(s), within a row or column, for each trait do not differ significantly at $p \leq 0.05$
T = Tillering; B = Booting; H = Heading; M = Milking

weight under both irrigation regimes at $I_{(T+B+H)}$ and $I_{(T+B)}$. Results indicated that irrigation regimes and BC treatment caused a significantly positive effect on the grain yield. Plants on BC treated soil had improved grain yield under applied irrigations at $I_{(T+B)}$ after $I_{(T+B+H+M)}$, as compared to remaining irrigation regimes during both the years of experiment. Observations illustrated that greater reduction in biological yield by $I_{(T+B)}$ irrigation treatment under both the treatments, control and BC during both the years of study. The biological yield was significantly enhanced by soil amendment with BC and irrigation application at $I_{(T+B+H+M)}$ as well as $I_{(T+B)}$ shown in Table 1.

Physiological Gas Exchange Attributes

The interactive effect of BC with various irrigation regimes on physiological parameters of wheat is presented in Table 2. Water regimes applied at $I_{(T+B+H)}$ and $I_{(T+M)}$ significantly reduced the leaf photosynthetic rate during both years of the trial. Soil application of BC increased the plant's photosynthetic rate under irrigations applied at $I_{(T+B+H+M)}$ during both the years of trial. The significant impact of BC application under applied irrigation at $I_{(T+B)}$, followed by $I_{(T+M)}$ was observed resulting in the maximum transpiration rate as compared to control treatment during the years I and II of the trial. Stomatal conductance increased significantly in the plants under applied irrigation water regimes at $I_{(T+B+H+M)}$. During both the years of study, stomatal conductance under the various irrigation water regimes was significantly greater in the BC soil-applied plants as compared to control (without BC). The combined effect of BC application and irrigation water-regimes on sub-stomatal conductance was statistically significant being the maximum in the treatment combination of BC application and irrigations applied at $I_{(T+B+H+M)}$ and $I_{(T+B)}$. A significant interaction was observed among BC treatment and irrigation water regimes on water use efficiency of wheat plants. Data depicted that BC application to soil resulted in the maximum in values of water use efficiency than control plants in case of irrigation water regimes at $I_{(T+B)}$ compared with other irrigation regimes during both the years of study (Table 2). During the two years of trial, plants on BC treated soil performed better for of intrinsic water use efficiency under the applied irrigation water regimes at $I_{(T+B+H+M)}$, followed by $I_{(T+B)}$ and $I_{(T+M)}$.

Antioxidant Contents Activities

Total soluble proteins (TSP) content significantly increased in the leaves of the plants under BC treatment as compared to the control during both the years of study. Performance of BC treated plants in the production of TSP was significantly greater under irrigation applied water regimes at $I_{(T+B)}$ and lesser at $I_{(T+B+H+M)}$ during both years of study (Table 3). Antioxidant enzymatic activities under different irrigation water-regimes (at tillering, booting, heading and milking) were observed significantly different as a result of soil application of BC as compared to the control (without BC) during both the years of trial. Superoxide dismutase, peroxidase and catalase activities were significantly greater in the plants grown on BC treated soil and the least in control treatment during both years. The plants under both BC treatments showed significantly greater SOD activity under the irrigation water regimes at $I_{(T+M)}$ during both the years of study. However, POD activity of significantly greater in the treatment of irrigation water regimes at $I_{(T+B)}$ for both years of study as well. Catalase (CAT) activity was higher in the plants on soil treated with BC as compared to control plants under the irrigation water regimes at $I_{(T+B)}$ as compared to control ($I_{(T+B+H+M)}$) during both the years of study. BC application to soil also improved the production of ascorbic acid (AsA) in wheat plants under the applied irrigation water-regime at $I_{(T+B)}$. Total phenolic contents (TPC) under water deficit regimes at $I_{(T+B)}$ with the

Table 2: Effect of biochar on the physiology of wheat under various irrigation regimes

Irrigation regimes	Biochar levels (t ha^{-1})							
	2015–2016		2016–2017		2015–2016		2016–2017	
	Photosynthetic rate (mmol m^{-2} s^{-1})				Transpiration rate (E) (mmol m^{-2} s^{-1})			
	0	4	0	4	0	4	0	4
$I_{(T+B+H+M)}$	14.43d	23.69a	15.96c	24.62a	5.43c	5.37c	5.50c	5.71bc
$I_{(T+B+H)}$	15.29c	17.32b	11.76f	15.40d	4.30d	4.40d	4.37d	4.74d
$I_{(T+B)}$	12.35f	15.23c	16.27c	17.60b	6.17b	7.33a	6.24b	7.67a
$I_{(T+M)}$	9.57g	13.32e	8.86g	13.14e	6.24b	6.67b	6.31b	7.01a
Year	15.15		15.45		5.74		5.94	
LSD@5%	0.27		0.31		0.35		0.48	
	Stomatal conductance (g) (mmol m^{-2} s^{-1})				Sub-stomatal conductance (Ci) (vpm)			
$I_{(T+B+H+M)}$	0.45b	0.65a	0.59b	0.84a	281.67b	294.33a	282.80b	295.43a
$I_{(T+B+H)}$	0.24d	0.24d	0.38de	0.43d	256.67d	263.67c	257.55d	264.11c
$I_{(T+B)}$	0.35c	0.36c	0.51c	0.57bc	284.67b	286.33b	285.10b	286.70b
$I_{(T+M)}$	0.14e	0.15e	0.30f	0.36ef	223.33f	245.33e	223.74f	245.65e
Year	0.32B		0.50A		267.00		267.64	
LSD@5%	0.04		0.05		3.89		3.83	
	Water use efficiency (WUE=A/E)				Intrinsic water use efficiency (I_{WUE}=A/g_s)			
$I_{(T+B+H+M)}$	2.30g	2.44f	2.35g	2.48f	130.88b	140.14a	131.86b	140.95a
$I_{(T+B+H)}$	2.53e	2.68d	2.57e	2.72d	102.16d	105.56d	102.91d	106.30d
$I_{(T+B)}$	3.14b	3.27a	3.19b	3.32a	123.69c	136.69a	124.55c	137.41a
$I_{(T+M)}$	2.72d	3.03c	2.77d	3.09c	125.78c	136.24a	126.42c	136.92a
Year	2.76B		2.81A		125.14		125.91	
LSD@5%	0.05		0.05		2.94		3.03	

Means sharing the same letter(s), within a row or column, for each trait do not differ significantly at $p \leq 0.05$
T = Tillering; B = Booting; H = Heading; M = Milking

Table 3: Effects of biochar application on antioxidants of wheat grown under varying irrigation regimes

Irrigation regimes	Biochar levels (t ha^{-1})							
	2015–2016		2016–2017		2015–2016		2016–2017	
	Total soluble protein (mg g^{-1})				Superoxide dismutase (IU min^{-1}mg^{-1} protein)			
	0	4	0	4	0	4	0	4
$I_{(T+B+H+M)}$	1.24d	1.25d	1.61d	1.60d	32.64g	37.60f	33.63g	38.57f
$I_{(T+B+H)}$	1.23d	1.42bc	1.59d	1.79bc	37.98f	41.57e	38.95f	42.47e
$I_{(T+B)}$	1.53a	1.55a	1.90a	1.87ab	61.66d	67.10c	62.60d	67.85c
$I_{(T+M)}$	1.39c	1.45b	1.71c	1.78c	88.37b	98.19a	89.14b	97.30a
Year	1.38B		1.73A		58.14		58.81	
LSD@5%	0.03		0.05		1.71		1.75	
	Peroxidase (mmol min^{-1} mg protein^{-1})				Catalase (µmol min^{-1} mg protein^{-1})			
$I_{(T+B+H+M)}$	4.84g	5.72f	5.18g	6.03f	13.59h	18.82g	14.21h	19.95g
$I_{(T+B+H)}$	6.69e	7.66d	7.03e	8.00d	20.66f	21.58e	21.78f	22.69e
$I_{(T+B)}$	10.72b	13.47a	11.06b	13.81a	26.34b	27.50a	27.39b	28.59a
$I_{(T+M)}$	10.42b	9.36c	10.74b	9.70c	22.45d	24.84c	23.37d	25.75c
Year	8.61		8.94		21.97B		22.97A	
LSD@5%	0.42		0.39		0.53		0.52	
	Ascorbic acid (m mole g^{-1})				Total phenolic contents (mg g^{-1})			
$I_{(T+B+H+M)}$	64.35f	69.52e	72.92d	79.75c	11.23d	19.97b	13.56c	16.77bc
$I_{(T+B+H)}$	58.54h	59.42g	70.20e	65.05f	12.01cd	14.67c	13.58c	14.33c
$I_{(T+B)}$	80.66b	94.31a	81.09b	94.73a	17.97b	23.65a	22.33b	31.21a
$I_{(T+M)}$	72.50d	79.45c	59.86g	59.07h	10.87d	13.56cd	19.74bc	23.40b
Year	72.34		73.52		15.49B		19.37A	
LSD@5%	0.44		0.50		0.92		2.11	

Means sharing the same letter(s), within a row or column, for each trait do not differ significantly at $p \leq 0.05$
T = Tillering; B = Booting; H = Heading; M = Milking

treatment applied BC was depicted maximum as compared to other treatments during both years I & II (Table 3).

Discussion

The main theme of this project was to analyze the importance of BC in posing the favorable conditions for wheat crop productivity under deficit irrigation environment at $I_{(T+B)}$ and $I_{(T+M)}$. Enhancement in the number of grains spike^{-1} and 1000-grain weight at water-regimes $I_{(T+B)}$ after control $I_{(T+B+H+M)}$ might be due to the addition of BC to soil which enabled the plants to tolerate water deficit conditions by compensating the moisture availability for completion of phenological stages of wheat crop (Chan *et al.*, 2007). Grain yield is the final outcome of the crop and the highest production was observed under BC incorporated treatment with deficit irrigation condition at $I_{(T+B)}$, which might be due to the active antioxidant defense system and better gas exchange physiological behaviour in plants after control $I_{(T+B+H+M)}$ (Solaiman *et al*., 2010).

Oxidative damage at a cellular level caused under water deficit conditions poses a serious threat for plants through reduction of molecular oxygen, injurious ROS production and quenching behavior of antioxidants defense system (Laloi *et al*., 2004). The activity of ROS can be reduced by scavenging the toxic free reactive oxygen species ($O^{2\bullet-}$, H_2O_2, HO_2, $1O_2$) through enhanced enzymatic activities and accumulation of non-enzymatic antioxidants (Hernández *et al*., 2004). In present study, addition of BC in the soil with irrigation at tillering and booting (I_{T+B}) stages triggered antioxidants enzymatic activities to ameliorate the injurious effect of ROS (Abbas *et al*., 2017) by means of detoxification process as SOD convert $O^{2\bullet-}$ into O_2, CAT provides protection against H_2O_2 by converting into H_2O and O_2, POD acts as catalyzing agent against H_2O_2 and also AsA inhibits the production of HO_2 and $1O_2$ molecules (Mittler, 2002). The generation of maximum TPC at $I_{(T+B)}$ caused to mitigate the water deficit effect and to promote the photosynthetic activities in the case of treated BC wheat plants. Wheat plants showed tolerance against ROS through antioxidants defense system which might be due to soil-applied BC which helped in reduction of oxidative stress by increasing the enzymatic and non-enzymatic contents under irrigation water-regimes at $I_{(T+B)}$ and $I_{(T+M)}$ as compared with control ($I_{(T+B+H+M)}$) (Abbasi *et al*., 2016). Plants grown on BC applied to soil under deficit irrigation regimes at $I_{(T+B)}$ may favour to create ionic homeostasis and protected wheat crop from injurious effect of ROS at the cellular level. Overall observations proved that the addition of BC in the soil is very beneficial as it improved antioxidants contents in wheat plants, which compensated the yield losses under deficit irrigation-regimes at $I_{(T+B)}$.

Improvement in gas exchange physiological parameters (photosynthetic rate, transpiration rate, stomatal conductance, sub-stomatal conductance, water use efficiency and intrinsic water use efficiency) of wheat plants due to soil amendments with BC in the soil was observed in all applied irrigations treatments. However, plants performed better for transpiration rate, sub-stomatal conductance and water use efficiency under the applied BC in soil with irrigation treatment at $I_{(T+B)}$, probably due to increased water holding capacity and porosity of the soil (Atkinson *et al*., 2010). Another possible reason is that there is a synergistic relationship between soil conditioner BC and wheat plants that improve the photosynthetic rate, stomatal conductance and intrinsic water use efficiency which was prominently expressed in water-regimes $I_{(T+B+H+M)}$ and $I_{(T+B)}$ (Barrow, 2012). The improvement in the physiological gas exchange attributes in wheat plants might be due to the nutrient and moisture availability by adding BC in the soil even under deficit irrigation condition at (Younis *et al*., 2016).

Conclusion

The BC application has the potential to improve wheat productivity under limited water supply due to enlarged anti-oxidants activities and gas exchange parameters. Moreover, BC application with minimum two irrigations at tillering and booting stages resulted in higher yield than no biochar with all irrigations at tillering, booting, heading and milking stage. For future prospective, the active mechanism of soil-plant relationships after the application of biochar is required to be investigated.

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References

Abbas, T., M. Rizwan, S. Ali, M.Z. Rehman, M.F. Qayyum, F. Abbas, F. Hannan, J. Rinklebe and Y.S. Ok, 2017. Effect of biochar on cadmium bioavailability and uptake in wheat (*Triticum aestivum* L.) grown in a soil with aged contamination. *Ecotoxicol. Environ. Saf.*, 140: 37–47

Abbasi, G.H., M. Ijaz, J. Akhtar, M. Anwar-Ul-Haq, M. Jamil, S.A.R. Ali and H.N. Khan, 2016. Profiling of anti-oxidative enzymes and lipid peroxidation in leaves of salt tolerant and salt sensitive maize hybrids under NaCl and Cd stress. *Sains Malay,* 45: 177–184

Ainsworth, E.A. and K.M. Gillespie, 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.*, 2: 875–877

Atkinson, C.J., J.D. Fitzgerald and N. Hipps, 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant Soil,* 337: 1–18

Barrow, C.J., 2012. Biochar: Potential for countering land degradation and for improving agriculture. *Appl. Geogr.,* 34: 21–28

Basso, A.S., F.E. Miguez, D.A. Laird, R. Horton and M. Westgate, 2013. Assessing potential of biochar for increasing water-holding capacity of sandy soils. *Gcb Bioener.*, 5: 132–143

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann. Biochem.*, 72: 248–254

Chakir, S. and M. Jensen, 1999. How does *Lobaria pulmoria* regulate photosystem II during progressive desiccation and osmotic water stress? A chlorophyll fluorescence study at room temperature and at 77 K. *Physiol. Plantarum,* 105: 257–265

Chan, K.Y., Z.L. Van, I. Meszaros, A. Downie and S. Joseph, 2007. Agronomic values of greenwaste biochar as a soil amendment. *Aust. J. Soil Res.*, 45: 629–634

Chance, M. and A.C. Maehly, 1955. Assay of catalases and peroxidases. *Meth. Enzmol.*, 2: 764

Cheng, D., G. Wu and Y. Zheng, 2015. Positive correlation between potassium uptake and salt tolerance in wheat. *Photosynthetica*, 53: 447–454

Dennison, K.L., W.R. Robertson and B.D. Lewis, 2001. Functions of AKT1 and AKT2 potassium channels determined by studies of single and double mutants of *Arabidopsis*. *Plant Physiol*., 127: 1012–1019

Farooq, S., M. Shahid, M.B. Khan, M. Hussain and M. Farooq, 2015. Improving the productivity of bread wheat by good management practices under terminal drought. *J. Agron. Crop Sci*., 201: 173–188

Flexas, J., M. Ribas-Carbó, J. Bota, J. Galmés, M. Henkle, S. Martínez-Cañellas and H. Medrano, 2006. Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. *New Phytol.*, 172: 73–82

Giannopolitis, C.N. and S.K. Reis, 1997. Superoxide dismutase I. Occurrence in higher plants. *Plant Physiol.,* 59: 309–314

Hernández, I., L. Alegre and S. Munné-Bosch, 2004. Drought-induced changes in flavonoids and other low molecular weight antioxidants in Cistus clusii grown under Mediterranean field conditions. *Tree Physiol.,* 24: 1303–1311

Hussain, M., S. Farooq, W. Hasan, S. Ul-Allah, M. Tanveer, M. Farooq and A. Nawaz, 2018. Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agric. Water Manage.,* 201: 152–167

Hussain, M., M. Farooq, A. Nawaz, A.M. Al-Sadi, Z.M. Solaiman, S.S. Alghamdi, U. Ammara, Y.S. Ok and K.H.M. Siddique, 2017. Biochar for crop production: potential benefits and risks. *J. Soils Sed.,* 17: 685–716

Hussain, M., M. Waqas-ul-Haq, S. Farooq, K. Jabran and M. Farooq, 2016. The impact of seed priming and row spacing on the productivity of different cultivars of irrigated wheat under early season drought. *Exp. Agric.,* 52: 477–490

Joseph, S.D., M. Camps-Arbestain, Y. Lin, P. Munroe, C.H. Chia, J. Hook, Z.L. Van, S. Kimber, A. Cowie, B.P. Singh, J. Lehmann, N. Foidl, R.J. Smernik and J.E. Amonette, 2010. An investigation into the reactions of biochar in soil. *Soil Res.,* 48: 501–515

Lal, R., 2009. Challenges and opportunities in soil organic matter research. *Eur. J. Soil Sci.,* 60: 158–169

Laloi, C., K. Apel and A. Danon, 2004. Reactive oxygen signalling: the latest news. *Curr. Opin. Plant Biol.,* 7: 323–328

Lehmann, J. and S. Joseph, 2015. *Biochar for Environmental Management: an Introduction, Biochar for Environmental Management: Science, Technology and Implematation*, 2nd edition, pp: 1–1214. Lehmann, J. and S. Joseph (eds.). Earthscan from Routledge, London, UK

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.,* 7: 405–410

Nawaz, H., A. Yasmeen, M.A. Anjum and N. Hussain, 2016. Exogenous application of growth enhancers mitigate water stress in wheat by antioxidant elevation. *Front. Plant Sci.,* 7: 1–11

Nawaz, H., N. Hussain and A. Yasmeen, 2015. Growth, yield and antioxidants status of wheat (*Triticum aestivum* L.) cultivars under water deficit conditions. *Pak. J. Agric. Sci.,* 52: 953–959

Noguera, D., M. Rondón, K.R. Laossi, V. Hoyos, P. Lavelle, M.H.C.D. Carvalho and S. Barot, 2010. Contrasted effect of biochar and earthworms on rice growth and resource allocation in different soils. *Soil Biol. Biochem.,* 42: 1017–1027

Qayyum, M.F., M. Abid, S. Danish, M.K. Saeed and M.A. Ali, 2015. Effects of various biochars on seed germination and carbon mineralization in an alkaline soil. *Pak. J. Agric. Sci.,* 51: 977–982

Sohi, S.P., E. Krull, E. Lopez-Capel and R. Bol, 2010. A review of biochar and its use and function in soil. *Adv. Agron.,* 105: 47–82

Solaiman, Z.M., P. Blackwell, L.K. Abbott and P. Storer, 2010. Direct and residual effect of biochar application on mycorrhizal colonization, growth and nutrition of wheat. *Aust. J. Soil Res.,* 48: 546–554

Steel, R.C.D., J.H. Torrie and D.A. Deekey, 1997. *Principles and Procedures of Statistics: a Biometric Approach*, 3rd edition, pp: 400–428. McGraw Hill Book Co. Inc., New York, USA

Vaccari, F.P., S. Baronti, E. Lugato, L. Genesio, S. Castaldi, F. Fornasier and F. Miglietta, 2011. Biochar as a strategy to sequester carbon and increase yield in durum wheat. *Eur. J. Agron.,* 34: 231–238

Waterhouse, A.L., 2001. "*Determination of Total Phenolics*", *Current Protocols in Food Analytical Chemistry*. Wrolstad, R.E. (ed.). John Wiley & Sons, Inc., New York

Yamato, M., Y. Okimori, I.F. Wibowo, S. Anshori and M. Ogawa, 2006. Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and peanut and soil chemical properties in south Sumatra, Indonesia. *Soil Sci. Plant Nutr.,* 52: 489–495

Yordanov, I., V. Velikova and T. Tsonev, 2000. Plant responses to drought, acclimation, and stress tolerance. *Photosynthesis,* 38: 171–186

Younis, U., S.A. Malik, M. Rizwan, M.F. Qayyum, Y.S. Ok, M.H.R. Shah, R.A. Rehman and N. Ahmad, 2016. Biochar enhances the cadmium tolerance in spinach (*Spinacia oleracea*) through modification of Cd uptake and physiological and biochemical attributes. *Environ. Sci. Pollut. Res.,* 23: 21385–21394

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