

Optimizing soil health through activated acacia biochar under varying irrigation regimes and cultivars for sustainable wheat cultivation

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Abstract

Wheat, a staple food crop globally, faces the challenges of limited water resources and sustainable soil management practices. The pivotal elements of the current study include the integration of activated acacia biochar (AAB) in wheat cultivation under varying irrigation regimes (IR). A field trial was conducted in Botanical Garden, University of the Punjab, Lahore during 2023-2024, designed as split-split-plot arrangement with RCBD comprising three AAB levels (0T, 5T, and 10T, T= Tons per hectare), three wheat cultivars (Dilkash-2020, Akbar-2019, and FSD-08) receiving five IR levels (100%, 80%, 70%, 60%, and 50% Field capacity). Biochar amended soil showed improved BET surface area, pore size and volume. Carbon recovery and carbon sequestration capacity of AAB amended soil was better than non-amended soil. Significant improvement in micro-porosity and water retention capacity of soil was observed with 10T-AAB amendment with 1.1-folds and 2.24-folds higher, respectively. Statistical analysis showed that reduction in IR negatively affected plant growth and yield. The 10T-AAB levels significantly increased sugar contents, relative water content, membrane stability index and photosynthetic pigments, of wheat leaves under deficit irrigation among all the cultivars. Maximum stress markers (catalase, proline, peroxidase, and superoxide dismutase) were observed from Akbar under 50% irrigation with 0T-AAB and least were observed from 50% irrigated Dilkash-2020 with 10T-AAB amended soil. Among cultivars, Dilkash-2020 was observed to be the best for maximum yield followed by FSD-08 and Akbar, respectively. Maximum yield enhancement (11.8, 10.9, and 9.2

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times for Dilkash-2020, FSD-08, and Akbar, respectively) was recorded with 10T-AAB amended soil in 70% IR as compared to other IR levels. Hence, AAB enhanced wheat production under water deficit conditions by improving soil properties, drought tolerance, and yield attributes.

Keywords: Activated biochar, soil porosity, organic matter, water scarcity, antioxidants, yield.

Introduction

Deficit water resources because of climate change are extremely challenging to address. The shortage of water alters evapotranspiration patterns, soil moisture level and plant rhizosphere (Albacete, Martínez-Andújar & Pérez-Alfocea, 2014). These factors highlight the cruciality of specific crop management strategy that ensures water availability during stress intervals as well as increase crop productivity (El Chami et al., 2019). This strategy can increase crop productivity by improving crop water use efficiency, minimizing surface runoff, and reducing deep percolation (Capraro et al., 2018). In water scarce areas with less fertile soils, increasing wheat production is very critical (Huang et al., 2022). Besides being a major consumed crop around the globe, wheat faces the challenges of deficit water resources and sustainable soil management practices (Yu et al., 2020).

Biochar in this regard has gained popularity for improving physicochemical properties and increasing moisture retention potential of soil for plant growth during dry spells (Shakeel et al., 2022; Jahan et al., 2022a). Biochar is produced through pyrolysis of organic substances at a very high temperature. It provides soil with a high surface area and micropore volume that helps to maintain proper moisture content in soil and reduce water shortage (Lehmann & Joseph, 2015). Impact of biochar for improving soil health can be boosted by its activation. Researchers are focusing on organic activation of biochar using substances like vermicompost which is an effective plant growth promotor (Sanchez-Hernandez, Ro & Díaz, 2019). Perlite as well has been reported to activate biochar together with vermicompost, this activation imposes a combined effect of hydrated volcanic glass and decomposing organic waste to enhance soil water retention capacity (Jahan et al., 2022b).

Activated biochar is reported to improve soil quality and fertility with enhanced water stress resilience (Lehmann & Joseph, 2015). The multifaceted benefits of biochar range from its impact on soil structure and water retention to its influence on morpho-physiological and biochemical responses in plants. Improved soil health and water retention capacity directly improves plant water use efficiency which in turn supports plant growth and development. Biochar have also been reported to improve carbon recovery because it remains in soil for a longer period and plays role in carbon sequestration (Daer et al., 2024). These ameliorations help recover soil fertility, health, and productivity. These improvements in soil physicochemical properties ultimately help in plant growth attributes and establish a sustainable food production.

Researchers reported that biochar amended soil mitigates the negative effects of water scarcity and leads to reduced production of reactive oxygen species (ROS) in plants (Wu et al., 2023). As it provides plant rhizosphere with increased moisture for absorption from water stored in its capillary pores. Hence, biochar leads to increased water use efficiency helping plant growth and development even under water deficit conditions. Ultimately the plant doesn't go under deficit

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water stress and enzymatic antioxidant production including peroxidases, superoxide dismutase, etc., is reduced (Shakeel et al., 2022). Moreover, soils amended with activated biochar have been reported to improve the photosynthetic pigments and sugar contents in plants under abiotic stress. These ameliorations position biochar as a valuable tool in enhancing wheat resilience to imbalanced irrigation regimes and improving overall crop productivity. However, plant responses towards diverse biochar materials vary according to the nature, activation process, and physicochemical properties of biomass (Jahan et al., 2024).

Additionally, there is a gap of on-field studies to determine the efficacy and level of activated biochar under deficit irrigation for wheat cultivation. Integrating biochar especially its activated form in soil for crop cultivation with precision irrigation may help to sustain crop productivity and ensure food security. Further it is need of the time to reconcile health and fertility of degraded soil to improve crop growth. Hence, it was hypothesized that activated biochar amended soil may help wheat plants survive deficit irrigation leading to improved crop growth and productivity. Farther, this amendment may play a crucial role in improving soil physicochemical properties as well. Primarily, this study aims to investigate the synergistic effects of activated biochar with three commercial wheat cultivars (Dilkash-2020, Akbar-2019, FSD-08) under varying irrigation regimes on soil quality, plant physiology, growth and yield indices. This research will help to screen stress resilient wheat cultivar, optimum level of activated biochar with specific irrigation regime. This study will validate the integration of activated biochar in areas prone to water scarcity and leads to sustainable agriculture with enhanced food security.

Materials and Methods

Production of Activated biochar

Wood twigs of *Acacia nilotica* were utilized for biochar production as optimized by (Jahan et al., 2022b). Before pyrolysis, raw biomass was air-dried to reduce its moisture content. Production of biochar was carried out by slow pyrolysis technique at 450 °C for three-hour duration using batch pyrolysis temperature-controlled unit. After the cooling, physico-chemical properties of biochar were analyzed by (Jahan et al., 2023, p. 23). For activation purposes, biochar, vermicompost, and perlite were mixed in 1:1:1 ratio along with molasses to speed up the process and incubated for thirty days. Mixing and turning of the material was done daily to maintain proper aeration. After incubation, samples of the activated acacia biochar were assessed to determine its physicochemical characteristics (Jahan et al., 2023).

Experimental Design and Area

A field trial was executed at Botanical Garden, University of the Punjab, Lahore, Pakistan (N 31° 30' 4.3236", E 74° 18' 5.4684), during 2023-2024. The experiment comprised of split-split plot arrangement with randomized complete block design (RCBD). Factors under observation comprised mainly of activated acacia biochar (0T-AAB, 5T-AAB, and 10T-AAB), Cultivars (Dilkash-2020, Akbar-2019, FSD-08) and irrigation regimes (100%, 80%, 70%, 60%, and 50% field capacity). Activated biochar (AB) was applied manually to the topsoil (15 cm) and thoroughly mixed. Cultivars were selected as per recommended cultivars for irrigated soils from Ayub

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Agricultural Research Institute (AARI), Faisalabad. Basal fertilizer dose for N, P, and K were applied in the form of urea, SOP, and DAP but urea was applied in two splits with second dose in subsequent irrigation.

Meteorological data

Meteorological data was obtained from National Aeronautics and Space Administration (NASA) (<https://power.larc.nasa.gov/data-access-viewer/>). Parameters of meteorological data specifically included UV index (Wm^2), minimum and maximum temperature ($^{\circ}C$), wind speed (km/day), relative humidity (%), specific humidity (g/Kg), and precipitation (mm) (Fig.1).

Strategy for maintaining Irrigation Regimes

Water requirements of crop was calculated by the given Equation (1) presented by Food and Agriculture Organization for United States.

$$IN = ETc - Pe \quad (1)$$

Where IN presents net water requirement, Etc stands for evapotranspiration of crop and Pe shows effective rainfall. Moreover, evapotranspiration was estimated by using the expression (Eq. 2) as given by (Mehta & Pandey, 2015),

$$ETc = ETo \times Kc \quad (2)$$

Where Eto is reference evapotranspiration, and Kc is crop coefficient. Reference evapotranspiration was determined by using Penman Monteith Equation 3 (Mehta & Pandey, 2015),

$$Eto = \frac{0.14\Delta(Rn-G) + \gamma \left[\frac{900}{T+273} \right] U_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34 U_2)} \quad (3)$$

Where, T is mean daily temperature at height of 2 meters ($^{\circ}C$), Rn symbolizes net radiation, G presents the soil heat flux in MJm^2/day , Δ presents Gradient of the vapor pressure-temperature curve in $KPa/^{\circ}C$, γ is psychometric constant ($KPa/^{\circ}C$), U_2 shows the wind rate per day at 2 meter elevation in meters per second, e_s and e_a present average and real saturation vapor pressure respectively. Reference evapotranspiration (Eto) was calculated by CROPWAT 8.0 (Soomro et al., 2023). Whereas the effective rainfall was observed as given below (Eq. 4)

$$Pe = (0.6 \times P) - 3.33 \quad (4)$$

If $P \leq 70$ mm, Pe and P show effective rainfall and total precipitation respectively. Irrigation regimes (100% to 50%) were calculated in the field through the entire cropping period. Soil moisture content was observed using Lutron PMS-714 moisture meter at regular intervals before each irrigation.

Soil and Biochar physicochemical analysis

Soil pH and electrical conductivity were estimated using pH and **Ec** meter by following the standard procedure of (Rayment & Lyons, 2011). Standard procedures by (Estefan, Sommer & Ryan, 2013) were followed for estimation of water holding capacity, soil porosity and pore size. The yield of activated biochar was assessed using Eqs (5). Brunauer-Emmett-Teller (BET) Surface Area Analysis and Barrett-Joyner-Halenda (BJH) pore Size and volume Analysis were performed using Quantachrome Instruments version 11.04 with nitrogen gas media. Carbon recovery (CR)

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was estimated using Eq. (6) (Li et al., 2022). Mean residence time (MRT) and percent carbon remaining in soil over 100 years (HC_{+100}), were calculated according to Eqs. (7) and (8) respectively (Venkatesh et al., 2022). Where H/C shows atomic ratio of activated biochar and amended soils. The letter 'e' represents exponential term. R_{50} presents an indicator of carbon's recalcitrance in amended soil as well as activated biochar, defined by Eqs. (9) (Harvey et al., 2012; Li et al., 2022). where in Eqs. (9), $T_{50\text{Biochar}}$ and $T_{50\text{Graphite}}$ are temperatures required for 50% weight loss of activated biochar and graphite respectively. Graphite was used as reference substance with purity $\geq 99.85\%$ and 100 mesh. To assess the effect of temperature on carbon sequestration potential of activated biochar, Equation (10) was used (Nan et al., 2020).

$$Yield = \frac{\text{Biochar weight}}{\text{Raw weight}} \times 100 \quad (5)$$

$$\text{Carbon Recovery (CR)} = \frac{C_{\text{Biochar}}}{C_{\text{Biomass}}} \times Yield \quad (6)$$

$$MRT = 4501 \times e^{-3.2 \times \frac{H}{C}} \quad (7)$$

$$HC_{+100} = 1.05 - 0.616 \times \frac{H}{C} \quad (8)$$

$$R_{50} = \frac{T_{50\text{ Biochar}}}{T_{50\text{ Graphite}}} \quad (9)$$

$$\text{Carbon sequestration} = R_{50} \times CR \quad (10)$$

Plant physiological and biochemical analysis

Wheat leaves were analyzed for leaf proline content profiling at the grain filling stage using the method of (Bates, Waldren & Teare, 1973). The method of (DuBois et al., 1956) was used to evaluate the sugar contents in the leaf sample. Lipid peroxidation in the leaf sample was analyzed by the method of (Procházková, Boušová & Wilhelmová, 2011) where malondialdehyde (MDA) was the indicator of lipid peroxidation. Membrane stability index (MSI) of the leaf was assessed by method given by (Sairam, 1994). The method of (Mullan & Pietragalla, 2012) was followed for relative water content (RWC). Arnon method was used to find the chlorophyll content (Arnon, 1949) while carotenoid content was assessed by the method of (Lichtenthaler & Wellburn, 1983). Protein content was estimated by the method of (Bradford, 1976). (Beauchamp & Fridovich, 1971) method was used to observe the activity of superoxide dismutase (SOD). The peroxidase (POD) level was analyzed following the method of (Gorin & Heidema, 1976) and the method of (Iwase et al., 2013) was used to analyze the catalase activity in the leaf sample.

Plant growth and yield analysis

Plant growth and yield parameters were analyzed at grain filling stage and a digital analytical balance (Model FA2204E, China) was used to measure the fresh and dry weights. The method of (Usman, Liedl & Shahid, 2014) was used to determine apparent water productivity as follows:

$$\text{Apparent water productivity (Kg. m}^{-3}\text{)} = \frac{\text{Seed Yield (kg.ha}^{-1}\text{)}}{\text{Irrigation Water (m}^{-3}\text{)}} \quad (16)$$

190 **Statistical analysis**

191 Experimental data was statistically analyzed utilizing IBM SPSS Statistics 23.0 software for
192 analysis of variance (ANOVA) and post-hoc comparisons including Duncan's test for alphabetic
193 arrangement of data ranges. Descriptive statistics were used for generating graphs based on means
194 and standard deviation. Pearson correlation was generated through Origin (2024) software. The
195 PCA analysis and heatmap were constructed to predict the correlation of treatments with growth
196 variables of wheat grown under varying irrigation regimes using R Studio (R-4.3.1-x86 64.pkg).

197 **Results**

198 **Soil and Biochar physicochemical analysis**

199 The physicochemical soil analysis is presented in Table 1. The organic matter (OM) was highest
200 in soil with 10T-AAB amendment, reaching 5.23%. There was 1.25-folds higher organic carbon
201 in soils amended with 10T-AAB as compared to 0T-AAB amendment. The highest percent carbon
202 value was observed by 10T amended soil with 2.9% carbon. Nitrogen content peaked in 5T-AAB
203 treatment with 1.07%. Soil water holding capacity (WHC) was maximum in 10T-AAB amended
204 soil with 27.93%, which is 36.71% higher than non-amended soil. Macropore space was highest
205 at 117.58% under 0T-AAB treatment. Porosity was highest in 10T-AAB amended soil, at 269.38
206 (1.1-Folds higher). The pH levels remained relatively stable, peaking at 6.7 i.e., near to neutral pH
207 and effective for wheat growth in 10T-AAB amended soil. Electrical conductivity (EC) was
208 slightly increased in 10T-AAB amended soil. Other attributes including hydrogen, oxygen, carbon
209 recovery, carbon sequestration capacity and mean residence time were increased in 10T-AAB
210 amended soil.

211 **Plant stress markers**

212 Analysis of variance showed a significant effect of activated acacia biochar (AAB) on proline
213 content of wheat cultivars under varying irrigation regimes. The mean comparison showed that
214 proline contents were increased 10T-AAB reduced proline contents by 48.39%, 58.38%, and
215 39.32% in Dilkash-2020, Akbar-2019, and FSD-08 respectively in 50% IR when compared to 0T-
216 AAB (Fig. 2a). The MDA content was significantly reduced by AB under deficit IR (Fig. 2c).
217 Catalases showed the highest level in Akbar-2019 at 50% IR and 0T-AAB, and the lowest in
218 Dilkash-2020 in 100% IR with 10T-AAB (Fig. 4b). For Peroxidase, the peak level was in Akbar-
219 2019 at 50% IR and 0T-AAB (Fig. 4c). Superoxide Dismutase had the highest value in Akbar-
220 2019 at 50% IR (Fig. 4d). The AB amendment in deficit irrigation reduced the antioxidant activity
221 by decreasing these enzyme's levels by 17-57% in all cultivars.

222 **Plant Physiological and Biochemical attributes**

223 Results showed that AAB amendment significantly improved RWC, MSI and other physiological
224 attributes of wheat under varying irrigation regimes. Compared to control (0T-AAB), 5T-AAB
225 and 10T-AAB increased RWC by 10% and 28% respectively (Fig. 2d). Deficit IR reduced MSI
226 by 20-50% with 0T-AAB. Whereas 5T-AAB and 10T-AAB improved MSI by 27% and 55%

respectively (Fig. 3a). Sugar contents were reduced with a reduction in IR by 7.05%, 8.03%, and 14.14% in Dilkash-2020, Akbar-2019, and FSD-08 respectively in 50% IR as compared to 100% irrigated plants in 0T-AAB (Fig. 2b). The significant effect of biochar on photosynthetic pigments was observed under deficit IR. Compared to control (0T-AAB), 5T-AAB and 10T-AAB increased Chl a content by 17.95 and 25.98 times respectively (Fig. 3b). whereas 5T-AAB and 10T-AAB produced Chl b content 17.8 and 26.57 times higher respectively (Fig. 3c) and maximum carotenoids content was observed from 10T-AAB with 100% IR in Dilkash-2020 (Fig. 3d). Biochar amendment in low IR was observed to increase the protein contents in all cultivars but the major increase (17.9 times higher) was observed in Dilkash-2020 with 10T-AAB in 70% IR as compared to its counterpart with 0T-AAB followed by 50% IR with 10T-AAB (Fig. 4a).

Plant Growth and yield attributes

There was a significant ($p \leq 0.05$) effect of AB on plant indices where irrigation regimes significantly affected plant growth except for number of tillers and leaves. The difference among cultivars for these traits was non-significant for number of tillers and root length. Plant morphological traits including LFW, SFW, RFW, LDW, SDR, and RDW showed significant increase with biochar (5T-AAB and 10T-AAB) and percent increases ranged from 16.24% to 80.95% respectively compared to the control (0T-AAB) under deficit IR conditions (Table 2 & 3). It was observed that reduction in IR significantly decreased plant yield by 77.13, 80.58%, and 81.48% in Dilkash-2020, FSD-08, and Akbar-2019 respectively (Table 4). But when these cultivars were grown in amendment with AB, increased yield attributes were observed with both 5T-AAB (114.30%, 112.30%, and 87.75) and 10T-AAB (118.57%, 109.45, and 91.94 respectively). The highest yield was observed from 100% IR with 10T-AAB in Dilkash-2020 and Akbar-2019 cultivars but 5T-AAB in 70% IR gave best grain yield for FSD-08. For spike length, spike weight, number of spikes per plant, spikelet per spike, and grains per spike, 10T-AAB in 70% IR proved to be the best (Table 4). Maximum yield per hectare and highest apparent water productivity were observed from Dilkash-2020 with 10T-AAB in 100% followed by 70% IR (Fig. 5a and 5b).

Pearson Correlation

Pearson correlation was performed on all the observed traits to understand their correlations with the most relevant traits (Fig. 6). In terms of wheat morphological attributes as well as yield attributes were positively correlated. As red color in the plot presents a positive association between two traits and blue shows negative correlation whereas white color shows no correlation among traits. It was observed that carotenoids, MDA, proline, POD and SOD significantly had a negative correlation with all other traits.

Multivariate Analysis

This study utilizes principal component analysis (PCA) heatmap and biplot to explore the relationship between activated acacia biochar amended soil under varying irrigation regimes cultivated with different wheat cultivars and physiological, biochemical, and yield variables (Fig.

7). The analysis effectively distinguished plants exposed to deficit irrigation with activated biochar amended and non-amended soil. The heatmap clearly showed that the changes made by 10T-AAB had the most significant impacts on morphological, physiological and yield attributes (Fig. 8). However, 0T-AAB had the highest levels of peroxidase, catalase, superoxide dismutase, and proline content, indicating the severity of stress. Biplot presented two main clusters showing groups of applied treatments (Fig. 9).

Discussion

Among several types of biochar, the one produced using wood biomass exhibits large surface area due to higher lignin content of the wood. Farther, its activation with organic wastes adds valuable and promising properties (Jahan et al., 2023). Current study showed that increased water holding capacity with applied activated biochar is consistent with ameliorating soil health and fertility. Biochar improved soil water retention by improving soil's micropore structure and reduced macropore surface (Abel et al., 2013). This reduction was attributed to biochar's ability to fill macropore surfaces in soil influencing pore size distribution leading to higher soil density and increased micropore proportion. Moreover, biochar itself has a porous nature which adds to the total soil pore volume, improving aerations and water infiltration (Karhu et al., 2011). Current study revealed that under biochar amendment, soil pH was increased from acidic to neutral which can be a consequence of liming effect of biochar due to the presence of basic cations such as magnesium, calcium, and potassium (Yuan & Xu, 2011). Whereas variation in electrical conduction of soil with AB treatment can be associated with nutrient content and mineral's interactions among soil and biochar. Biochar initially releases soluble salts thereby increasing electrical conductivity of soil but over time, these salts are utilized by plants or leached away (Major et al., 2010). Moreover, carbon recovery, mean residence time and carbon sequestration are a consequence of recalcitrant carbon components on acacia activated biochar that remains in soil over a long period.

As an indicator of stress, the levels of proline and lipid peroxidation increased in stress conditions, but biochar reduced the proline accumulation by improving water retention in the soil and mitigating the negative effects of deficit irrigation (Gharred et al., 2022). Under drought stress conditions, various physiological parameters such as leaf chlorophyll content, gas-exchange characteristics, water use efficiency (WUE), and transpiration rate are adversely affected (Liu et al., 2019). Plants usually accelerate their antioxidant activity to cope up with reactive oxygen species (ROS) produced under abiotic stress (Mu et al., 2021). Current study observed an increased level of protein content but reduced antioxidants (CAT, POD, and SOD) with biochar application under low irrigation regime especially 60% and 50% as compared to 0T-AAB. This effect was attributed to biochar's ability to enhance ROS scavenging mechanisms, reducing oxidative stress (Nawaz et al., 2023). Biochar improved plant growth and metabolic rates by enhancing water retention in soil, hence reducing antioxidant enzyme activities and protecting against lipid damage (Farhangi-Abriz & Torabian, 2017).

Sugar content, RWC, and MSI were enhanced with 10T-AAB even under low irrigation level. Improved sugar contents were associated with reduced osmotic stress and enhanced soil fertility

by increasing soil organic carbon (SOC). Higher SOC level leads to improved nutrient availability and better plant growth (Jahan et al., 2023). Tanure et al. (2019) stated that biochar's porous structure enhances water retention that helps maintain higher RWC in plant tissues thereby improving cellular metabolism. Drought stress directly affects photosynthetic ability of plants which can be ameliorated using biochar (Sattar et al., 2019). Photosynthetic pigments were observed to be increased with increasing level of AB and played role in mitigating negative effects of low irrigation regime. These increased levels with biochar were attributed to enhanced nutrient use efficiency, promoting chlorophyll biosynthesis (Abideen et al., 2020). Additionally, the study observes that biochar alters soil pH, influencing nutrient absorption and availability in the rhizosphere (Ayaz et al., 2021). The observed improved soil structure contributes to better plant development (Manolikaki & Diamadopoulos, 2019).

Plants treated with activated biochar showed enhanced root growth which can be attributed to improved soil structure and increased nutrient availability (Zhao et al., 2019). According to (Jahan et al., 2023), porous structure of biochar is crucial for helping crops withstand drought stress. As previously indicated, biochar can improve root growth in plants, which is essential for absorbing water and surviving during dry spells (Zulfiqar et al., 2022). The resistance of root biomass to drought stress is determined by its size and morphology. The fine roots explore soil moisture and enhance water absorption. Previous research demonstrated that under drought stress, biochar can increase crops' fine root development and length, hence improving their ability to absorb more water (Tanure et al., 2019).

This study demonstrated that biochar amendments contribute to the reduction of losses in wheat growth and yield due to stress by retaining water in soil pores and gradually releasing it under dry spells like the findings of (Ali et al., 2017). Activated biochar positively influenced spike development which can be attributed to enhanced nutrient uptake, especially phosphorus and potassium, essential for spike growth. Furthermore, improved water retention in soil due to biochar can contribute to larger spikes. Number of spikelet per spike along with grain filling in spikes was higher in 10T-AAB treated plants with low irrigation regimes as compared to 0T-AAB which is according to the findings of (Haider et al., 2020). Activated biochar can lead to heavier grains by improving nutrient uptake and water availability during grain filling. A higher 1000-grain weight indicates better grain quality and yield (Zulfiqar et al., 2022). Yield attributes with applied biochar under drought stress. Slow crop growth rates, poor source-sink relationships, and malfunctioning metabolic systems all contribute to low grain weight under drought stress. But biochar was observed to mitigate these effects. Biochar improved nutrient availability resulting in higher metabolic functions in plants. Hence, AB improved soil properties, nutrient availability, and water retention, ultimately benefiting wheat growth and yield under water deficit conditions.

Conclusion

Current study illustrates the potential of activated acacia biochar (AB) to enhance wheat crop productivity under deficit water conditions as can be evidenced by improved apparent water productivity and overall crop yield. The application of AAB resulted in significant improvements in soil physicochemical properties. Furthermore, AAB enhanced drought tolerance in wheat plants

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by improving biochemical contents, promoting root growth, and enhancing photosynthetic efficiency as can be witnessed by enhanced photosynthetic pigments. These positive effects ultimately translated into improved yield attributes, such as increased grain yield. Overall, the findings highlight the promising role of AAB as a sustainable agricultural practice for mitigating the adverse impacts of water scarcity on wheat cultivation. Further investigations are necessary to optimize application rates and assess long-term effects on soil-plant-water interactions and crop productivity.

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Competing Interests

Authors have no competing interests.

Author contributions

- Lubaba Komal analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article
- Summera Jahan devised and designed the review outline, performed the literature survey, analyzed the data, prepared figures and/or tables, authored and reviewed drafts of the article, and approved the final draft.
- Atif Kamran authored and reviewed drafts of the article and approved the final draft.
- Abeer Hashem performed literature reviews, analyzing database search results qualifies as analyzing the data.
- Graciela Dolores Avila-Quezada performed literature reviews, analyzing database search results qualifies as analyzing the data.
- Elsayed Fathi Abd_Allah performed literature reviews, analyzing database search results qualifies as analyzing the data.

Data Availability

All relevant data are presented in the manuscript.

References

Abel S, Peters A, Trinks S, Schonsky H, Facklam M, Wessolek G. 2013. Impact of biochar and hydrochar addition on water retention and water repellency of sandy soil. *Geoderma* 202–203:183–191. DOI: 10.1016/j.geoderma.2013.03.003.

Abideen Z, Koyro H -W., Huchzermeyer B, Ansari R, Zulfikar F, Gul B. 2020. Ameliorating effects of biochar on photosynthetic efficiency and antioxidant defence of *Phragmites karka* under drought stress. *Plant Biology* 22:259–266. DOI: 10.1111/plb.13054.

Albacete AA, Martínez-Andújar C, Pérez-Alfocea F. 2014. Hormonal and metabolic regulation of source–sink relations under salinity and drought: From plant survival to crop yield stability. *Biotechnology Advances* 32:12–30. DOI: 10.1016/j.biotechadv.2013.10.005.

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383 Ali S, Rizwan M, Qayyum MF, Ok YS, Ibrahim M, Riaz M, Arif MS, Hafeez F, Al-Wabel MI,
 384 Shahzad AN. 2017. Biochar soil amendment on alleviation of drought and salt stress in
 385 plants: a critical review. *Environmental Science and Pollution Research* 24:12700–12712.
 386 DOI: 10.1007/s11356-017-8904-x.
 387 Arnon DI. 1949. COPPER ENZYMES IN ISOLATED CHLOROPLASTS.
 388 POLYPHENOLOXIDASE IN *BETA VULGARIS*. *Plant Physiology* 24:1–15. DOI:
 389 10.1104/pp.24.1.1.
 390 Ayaz M, Feizienė D, Tilvikienė V, Akhtar K, Stulpinaitė U, Iqbal R. 2021. Biochar Role in the
 391 Sustainability of Agriculture and Environment. *Sustainability* 13:1330. DOI:
 392 10.3390/su13031330.
 393 Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress
 394 studies. *Plant and Soil* 39:205–207. DOI: 10.1007/BF00018060.
 395 Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable
 396 to acrylamide gels. *Analytical biochemistry* 44:276–287.
 397 Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of
 398 protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72:248–254.
 399 DOI: 10.1016/0003-2697(76)90527-3.
 400 Capraro F, Tosetti S, Rossomando F, Mut V, Vita Serman F. 2018. Web-Based System for the
 401 Remote Monitoring and Management of Precision Irrigation: A Case Study in an Arid
 402 Region of Argentina. *Sensors* 18:3847. DOI: 10.3390/s18113847.
 403 Daer D, Luo L, Shang Y, Wang J, Wu C, Liu Z. 2024. Co-hydrothermal carbonization of waste
 404 biomass and phosphate rock: promoted carbon sequestration and enhanced phosphorus
 405 bioavailability. *Biochar* 6:70. DOI: 10.1007/s42773-024-00356-9.
 406 DuBois Michel, Gilles KA, Hamilton JK, Rebers PA, Smith Fred. 1956. Colorimetric Method for
 407 Determination of Sugars and Related Substances. *Analytical Chemistry* 28:350–356. DOI:
 408 10.1021/ac60111a017.
 409 El Chami D, Knox JW, Daccache A, Weatherhead EK. 2019. Assessing the financial and
 410 environmental impacts of precision irrigation in a humid climate. *Horticultural Science*
 411 46:43–52. DOI: 10.17221/116/2017-HORTSCI.
 412 Estefan G, Sommer R, Ryan J. 2013. Methods of soil, plant, and water analysis. *A manual for the*
 413 *West Asia and North Africa region* 3:65–119.
 414 Farhangi-Abriz S, Torabian S. 2017. Antioxidant enzyme and osmotic adjustment changes in bean
 415 seedlings as affected by biochar under salt stress. *Ecotoxicology and environmental safety*
 416 137:64–70.
 417 Gharred J, Derbali W, Derbali I, Badri M, Abdelly C, Slama I, Koyro H-W. 2022. Impact of
 418 Biochar Application at Water Shortage on Biochemical and Physiological Processes in
 419 *Medicago ciliaris*. *Plants* 11:2411. DOI: 10.3390/plants11182411.
 420 Gorin N, Heidema FT. 1976. Peroxidase activity in Golden Delicious apples as a possible
 421 parameter of ripening and senescence. *Journal of Agricultural and Food Chemistry* 24:200–
 422 201. DOI: 10.1021/jf60203a043.

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Haider I, Raza MAS, Iqbal R, Aslam MU, Habib-ur-Rahman M, Raja S, Khan MT, Aslam MM, Waqas M, Ahmad S. 2020. Potential effects of biochar application on mitigating the drought stress implications on wheat (*Triticum aestivum* L.) under various growth stages. *Journal of Saudi Chemical Society* 24:974–981. DOI: 10.1016/j.jscs.2020.10.005.

Harvey OR, Kuo L-J, Zimmerman AR, Louchouart P, Amonette JE, Herbert BE. 2012. An Index-Based Approach to Assessing Recalcitrance and Soil Carbon Sequestration Potential of Engineered Black Carbons (Biochars). *Environmental Science & Technology* 46:1415–1421. DOI: 10.1021/es2040398.

Huang B, Yuan Z, Zheng M, Liao Y, Nguyen KL, Nguyen TH, Sombatpanit S, Li D. 2022. Soil and water conservation techniques in tropical and subtropical Asia: A Review. *Sustainability* 14:5035.

Iwase T, Tajima A, Sugimoto S, Okuda K, Hironaka I, Kamata Y, Takada K, Mizunoe Y. 2013. A Simple Assay for Measuring Catalase Activity: A Visual Approach. *Scientific Reports* 3:3081. DOI: 10.1038/srep03081.

Jahan S, Ahmad F, Rasul F, Amir R, Shahzad S. 2023. Physicochemical Analysis of Vermicompost-Perlite Based Activated Biochar and its Influence on Wheat (*Triticum aestivum* L.) Growth Under Water Stress. *Journal of Soil Science and Plant Nutrition* 23:3034–3050. DOI: 10.1007/s42729-023-01258-8.

Jahan S, Habiba UE, Akbar M, Zafar M, Shah AA, Alomrani SO. 2024. Chitosan Beads-Infused Biochar for Enhancing Physio-Chemical and Yield Attributes of Sunflower (*Helianthus Annus* L.) Grown Under Wastewater Irrigation. *Journal of Soil Science and Plant Nutrition*. DOI: 10.1007/s42729-024-01869-9.

Jahan S, Iqbal S, Rasul F, Jabeen K. 2022a. Evaluating biochar amendments for drought tolerance in soybean (*Glycine max* L.) using relative growth indicators. *Pakistan Journal of Botany* 54. DOI: 10.30848/PJB2022-5(10).

Jahan S, Iqbal S, Rasul F, Jabeen K. 2022b. Evaluating biochar amendments for drought tolerance in soybean (*Glycine max* L.) using relative growth indicators. *Pakistan Journal of Botany* 54. DOI: 10.30848/PJB2022-5(10).

Karhu K, Mattila T, Bergström I, Regina K. 2011. Biochar addition to agricultural soil increased CH₄ uptake and water holding capacity – Results from a short-term pilot field study. *Agriculture, Ecosystems & Environment* 140:309–313. DOI: 10.1016/j.agee.2010.12.005.

Lehmann J, Joseph S. 2015. *Biochar for Environmental Management: Science, Technology and Implementation*. Routledge.

Li F, Jiang Z, Ji W, Chen Y, Ma J, Gui X, Zhao J, Zhou C, 1. College of Resources and Environment Science, Anhui Science and Technology University, Fengyang 233100, Anhui, China, 2. School of Resources and Environmental Engineering, Hefei University of Technology, Hefei 230009, China, 3. College of Land Resources and Environment, Jiangxi Agricultural University, Nanchang 330045, China. 2022. Effects of hydrothermal carbonization temperature on carbon retention, stability, and properties of animal manure-

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462 derived hydrochar. *International Journal of Agricultural and Biological Engineering*
 463 15:124–131. DOI: 10.25165/j.ijabe.20221501.6758.
 464 Lichtenthaler HK, Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls *a* and
 465 *b* of leaf extracts in different solvents. *Biochemical Society Transactions* 11:591–592. DOI:
 466 10.1042/bst0110591.
 467 Liu B, Liang J, Tang G, Wang X, Liu F, Zhao D. 2019. Drought stress affects on growth, water
 468 use efficiency, gas exchange and chlorophyll fluorescence of *Juglans* rootstocks. *Scientia*
 469 *Horticulturae* 250:230–235.
 470 Major J, Rondon M, Molina D, Riha SJ, Lehmann J. 2010. Maize yield and nutrition during 4
 471 years after biochar application to a Colombian savanna oxisol. *Plant and Soil* 333:117–128.
 472 DOI: 10.1007/s11104-010-0327-0.
 473 Manolikaki I, Diamadopoulos E. 2019. Positive Effects of Biochar and Biochar-Compost on Maize
 474 Growth and Nutrient Availability in Two Agricultural Soils. *Communications in Soil Science*
 475 *and Plant Analysis* 50:512–526. DOI: 10.1080/00103624.2019.1566468.
 476 Mehta R, Pandey V. 2015. Reference evapotranspiration (ET_o) and crop water requirement (ET_c)
 477 of wheat and maize in Gujarat. *Journal of Agrometeorology* 17:107–113.
 478 Mu Q, Cai H, Sun S, Wen S, Xu J, Dong M, Saddique Q. 2021. The physiological response of
 479 winter wheat under short-term drought conditions and the sensitivity of different indices to
 480 soil water changes. *Agricultural Water Management* 243:106475. DOI:
 481 10.1016/j.agwat.2020.106475.
 482 Mullan D, Pietragalla J. 2012. Leaf relative water content. *Physiological breeding II: A field guide*
 483 *to wheat phenotyping* 25:25–35.
 484 Nan S, WANG P, ZHU Q, SUN J, ZHANG H, LIU X, CAO T, Xin C, HUANG Y, ZHOU Q.
 485 2020. Comprehensive characterization of yam tuber nutrition and medicinal quality of
 486 *Dioscorea opposita* and *D. alata* from different geographic groups in China. *Journal of*
 487 *Integrative Agriculture* 19:2839–2848.
 488 Nawaz F, Rafeeq R, Majeed S, Ismail MS, Ahsan M, Ahmad KS, Akram A, Haider G. 2023.
 489 Biochar Amendment in Combination with Endophytic Bacteria Stimulates Photosynthetic
 490 Activity and Antioxidant Enzymes to Improve Soybean Yield Under Drought Stress. *Journal*
 491 *of Soil Science and Plant Nutrition* 23:746–760. DOI: 10.1007/s42729-022-01079-1.
 492 Procházková D, Boušová I, Wilhelmová N. 2011. Antioxidant and prooxidant properties of
 493 flavonoids. *Fitoterapia* 82:513–523.
 494 Rayment GE, Lyons DJ. 2011. *Soil chemical methods: Australasia*. CSIRO publishing.
 495 Sairam RK. 1994. Effects of homobrassinolide application on plant metabolism and grain yield
 496 under irrigated and moisture-stress conditions of two wheat varieties. *Plant Growth*
 497 *Regulation* 14:173–181. DOI: 10.1007/BF00025220.
 498 Sanchez-Hernandez JC, Ro KS, Díaz FJ. 2019. Biochar and earthworms working in tandem:
 499 Research opportunities for soil bioremediation. *Science of The Total Environment* 688:574–
 500 583. DOI: 10.1016/j.scitotenv.2019.06.212.

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501 Sattar A, Sher A, Ijaz M, Irfan M, Butt M, Abbas T, Hussain S, Abbas A, Ullah MS, Cheema MA.
502 2019. Biochar application improves the drought tolerance in maize seedlings.
503 Shakeel H, Jahan S, Rafiq K, Iqbal S, Rasul F. 2022. Efficacy of Biochar-Supplemented Soil for
504 Modification of Physio-Biochemical Attributes of Canola (*Brassica napus* L.) Genotypes
505 under Different Moisture Regimes. *Journal of Soil Science and Plant Nutrition* 22:3667–
506 3684. DOI: 10.1007/s42729-022-00918-5.
507 Soomro S, Solangi GS, Siyal AA, Golo A, Bhatti NB, Soomro AG, Memon AH, Panhwar S,
508 Keerio HA. 2023. Estimation of irrigation water requirement and irrigation scheduling for
509 major crops using the CROPWAT model and climatic data. *Water Practice & Technology*
510 18:685–700.
511 Tanure MMC, Da Costa LM, Huiz HA, Fernandes RBA, Cecon PR, Pereira Junior JD, Da Luz
512 JMR. 2019. Soil water retention, physiological characteristics, and growth of maize plants in
513 response to biochar application to soil. *Soil and Tillage Research* 192:164–173. DOI:
514 10.1016/j.still.2019.05.007.
515 Usman M, Liedl R, Shahid MA. 2014. Managing Irrigation Water by Yield and Water Productivity
516 Assessment of a Rice-Wheat System Using Remote Sensing. *Journal of Irrigation and*
517 *Drainage Engineering* 140:04014022. DOI: 10.1061/(ASCE)IR.1943-4774.0000732.
518 Venkatesh G, Gopinath KA, Reddy KS, Reddy BS, Prabhakar M, Srinivasarao C, Visha Kumari
519 V, Singh VK. 2022. Characterization of Biochar Derived from Crop Residues for Soil
520 Amendment, Carbon Sequestration and Energy Use. *Sustainability* 14:2295. DOI:
521 10.3390/su14042295.
522 Wu Y, Wang X, Zhang L, Zheng Y, Liu X, Zhang Y. 2023. The critical role of biochar to mitigate
523 the adverse impacts of drought and salinity stress in plants. *Frontiers in Plant Science*
524 14:1163451.
525 Yu L, Zhao X, Gao X, Siddique KHM. 2020. Improving/maintaining water-use efficiency and
526 yield of wheat by deficit irrigation: A global meta-analysis. *Agricultural Water Management*
527 228:105906. DOI: 10.1016/j.agwat.2019.105906.
528 Yuan J -H., Xu R -K. 2011. The amelioration effects of low temperature biochar generated from
529 nine crop residues on an acidic Ultisol. *Soil Use and Management* 27:110–115. DOI:
530 10.1111/j.1475-2743.2010.00317.x.
531 Zhao L, Li L, Cai H, Fan J, Chau HW, Malone RW, Zhang C. 2019. Organic Amendments Improve
532 Wheat Root Growth and Yield through Regulating Soil Properties. *Agronomy Journal*
533 111:482–495. DOI: 10.2134/agronj2018.04.0247.
534 Zulfiqar B, Raza MAS, Saleem MF, Aslam MU, Iqbal R, Muhammad F, Amin J, Ibrahim MA,
535 Khan IH. 2022. Biochar enhances wheat crop productivity by mitigating the effects of
536 drought: Insights into physiological and antioxidant defense mechanisms. *PLOS ONE*
537 17:e0267819. DOI: 10.1371/journal.pone.0267819.

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