

# Nano-Biochar Enhances Wheat Crop Productivity by Vindicating the Effects of Drought: In Relation to Physiological and Phenological Stages

Muhammad Aown Sammar Raza,<sup>†</sup> Adnan Noor Shah,<sup>†</sup> Muhammad Asghar Shahid,<sup>\*</sup> Muhammad Nawaz,<sup>\*</sup> Muhammad Arif Ibrahim, Rashid Iqbal, Muhammad Usman Aslam, Sezai Ercisli,<sup>\*</sup> and Qurban Ali

Cite This: *ACS Omega* 2023, 8, 37808–37819

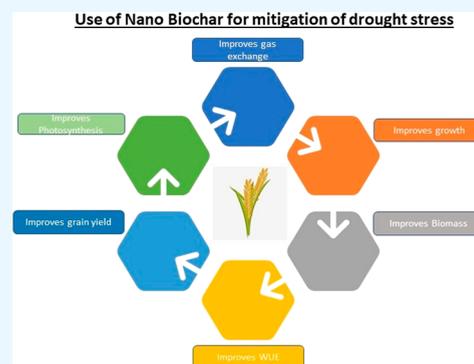
Read Online

ACCESS |

Metrics & More

Article Recommendations

**ABSTRACT:** Climatic changes are major hindrances to crop productivity. Likewise, water scarcity is the major obstacle during different physiological and phenological stages, which ultimately reduces the wheat crop yield. So, there is a dire need to adopt modern approaches such as soil amendments, i.e., using nano-biochar (NBC) to boost soil health and wheat crop productivity. Therefore, a case study was performed in the wire house of the Agronomy Department, Faculty of Agriculture and Environmental Sciences, Islamia University of Bahawalpur. CRD (completely randomized design) with four treatments of NBC, i.e., NBC0 (control), NBC1 (0.5%), NBC2 (1.00%), NBC3 (1.5%), and four drought levels D0 = control, D1 = drought at tillering, D2 = drought at flowering, and D3 = drought at grain filling was used. The hypothesis for the case study was to investigate if the NBC increases crop productivity by boosting physiological and chemical attributes under different drought conditions at different phenological stages. Results showed that among NBC treatments, NBC2 (1.00%) showed 37.10% increase in peroxidase activity, 28.60% in superoxide dismutase, 63.33% in catalase, 22.03% in ascorbate peroxidase, and 6.66% in plant height as compared to other NBC treatments, whereas among drought treatments, D0 = control stood out in comparison to water deficit treatments at critical growth and development stages, statistically analyzed data revealed that D0 was able to generate plant height 6.17 times more, 12.76% in the number of grains per spike, 4.60% in osmotic potential, and 2.96% in stomatal conductance activities of wheat crop. D3 and NBC0 were identified as treatment levels with the statistically lowest growth and yield returns, respectively. It showed a decrease of 4.69% in leaf relative water contents, 12.33% in water potential, and 23.64% in fertile tillers. The use of organic substances (fertilizers) must be promoted as they possess soil and crop health-promoting properties and also reduce different management expenses (fertilizer cost). Using NBC helps boost crop growth in the presence of a limited water supply. However, extensive research is needed to find out the impact of these organic substances (humic acid, farmyard manure, and NBC) on different crops, particularly on wheat, under stress conditions.



## INTRODUCTION

A lot of challenges are there to the growth and productivity of agriculture, like climate change, shortage of water, changes in the pattern of rainfall, shrinking arable lands, fluctuating temperature, population transfer from rural to urban areas, and increasing prices of inputs. Hence, there is a dire need for the adoption of modern agriculture tactics to increase per unit area of crop production<sup>1,2</sup> Food production should be doubled until 2050 as the world's population is growing so fast.<sup>3</sup> It was suggested by<sup>4</sup> that optimal production practices for increasing wheat yield should be adopted rather than increasing the production area. Water scarcity is a severe issue in this regard, which substantially affects wheat production.<sup>5,6</sup> Total area affected by drought is 42% compared to area affected by heat

stress which makes 58%.<sup>7</sup> In rain-fed areas, wheat ranked first due to its high cultivation, and in irrigated areas, it ranked second after rice. In rain-fed areas, wheat crop faced heat stress and drought at the grain-filling stage, resulting in poor yields.<sup>8–10</sup>

This condition substantially challenges food security and supply chain globally, especially in Asian countries, as half of

Received: March 15, 2023

Accepted: August 7, 2023

Published: October 2, 2023



wheat is cultivated in this region. Heat stress and drought conditions are major components for lowering the yield in the Indian peninsula, especially during the grain-filling stage. Biochar can be used for soil amendment, for boosting the efficiency of chemical fertilizers, and for enhancing the grain yield of the crop. High rates of biochar are often applied, as biochar may be lost by runoff, so this practice may be impractical for optimizing the fertilizer strategy successfully. However, some researchers investigated that this biochar can be converted after some special treatments into nano-biochar (NBC), which is extensively present in the atmosphere and vegetation. Due to its surface and particle size, this NBC can easily be coupled with other elements to become a high-efficiency fertilizer.<sup>11–14</sup>

NBC application to sunflower crops enhanced crop yield and growth,<sup>15</sup> rapeseed and wheat<sup>16,17</sup> along drought conditions. It was proposed<sup>18</sup> that NBC application as a soil amendment can lower the soil erosion caused by rainfall, and this application also positively affected the soil moisture in plateau. It was reported to increase the grain yield by 10–20% and reduce fertilizer usage to 30–50%.<sup>19</sup> It was investigated that NBC application hampers the negative impact of drought stress by enhancing the protective enzymes activity and electron transfer in crops.<sup>20</sup> Drought conditions lead to the closure of stomata, turgor loss, and reduction in water contents, even sometimes it causes the death of plants by disturbing plant metabolism.<sup>21</sup>

Biochar is the best alternative for coping with the stress situations like drought in a sustainable agriculture system due to its long-lasting availability as a carbon sink in the soil; cation exchange capacity, soil porosity, and it also act as a shelter for beneficial microbes.<sup>15,22,23</sup> Soil application of biochar brushes up the seed germination, soil water holding capacity, seedling emergence, crop yield, and other chemical functions of soil.<sup>19,24,25</sup> So, this biochar application hampers the adverse effect of drought on different critical situations of wheat.

Humic acids play several important roles, such as increasing soil physical and biochemical activities by improving the structure, texture, water holding capacity (WHC), and microbial population;<sup>26</sup> increasing soil nutrients availability, especially micronutrients, by chelating and cotransporting micronutrients to plants,<sup>27</sup> reducing the transportation of toxic heavy metals by precipitating them, thus reducing toxic heavy metals intake by plants.<sup>28</sup> Humic acids also increase crop growth by increasing plant growth-promoting hormones such as auxin and cytokinin, which aid in stress resistance, nutrient metabolism, and photosynthesis.<sup>26,29,30</sup> Some studies have reported no effects on crop growth and soil health following humic acid application.<sup>31–33</sup>

Although high humic acid doses are associated with enhanced soil physical characteristics,<sup>34</sup> their effects on soil chemical characteristics and crops are still uncertain.<sup>35</sup> Among the factors analyzed in mostly greenhouse experiments, the humic acid source significantly affected both root and shoot growth, while the application rate only affected shoot growth significantly. De Melo et al.,<sup>36</sup> highlighted carboxylic (COOH) and phenolic (OH) groups as predominant humic acid feature largely responsible for their functions in the soil. Humic acid chemical and molecular structures, sources, and application rates are critical for determining their effects on crops and soil. Importantly, humic acid application can have inconsistent results on yield, possibly due to the different humic acid's biological origins.<sup>37</sup>

So, we hypothesize that NBC application hampers the adverse effect of drought and boosts the plant physiologically and phenologically.

## MATERIALS AND METHODS

The experiment was conducted in an agronomic field area (latitude: 29° 23' 60.00"N, longitude: 71° 40' 59.99"E), faculty of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur. Significant drought levels and NBC dose levels were tested in the field experiment. The experiment was replicated three times under a completely randomized design (CRD) with four treatments of NBC0 (control), NBC1 (0.5%), NBC2 (1.00%), and NBC3 (1.5%) and four drought levels D0 = control, D1 = drought at tillering, D2 = drought at flowering, and D3 = drought at grain filling.

Akbar 2019 variety of wheat was used for experimentation purpose. Seeds were obtained from the RARI (Regional Agricultural Research Institute). Seeds were planted in plastic bags (26 × 29 cm) filled with biochar mixed soil. Physiochemical analysis of the soil is given below in Table 1.

**Table 1. Physiochemical Analysis of Soil**

parameters	soil profile
sand	56%
silt	33%
clay	11%
texture class	sandy loam soil
pH	7.23
electric conductivity (dS m <sup>-1</sup> )	2.55
ammoniac N (mg g <sup>-1</sup> )	1.58
organic matter (%)	0.92%
available phosphorus (ppm)	6.75 ppm
available potassium (ppm)	112 ppm

Wire house was covered with plastic sheets to avoid plants from direct rain exposure. Pots were watered evenly until full emergence, and four plants per pot were maintained by uprooting the extra plants.

## BIOCHAR APPLICATION AND IMPOSITION

Vertical kiln was used for pyrolyzing the wheat straw at 500 °C as described by ref 38 with the following properties: particle size of 3 mm, bulk density of 0.53 g cm<sup>-3</sup>, micropore surface area of 73.6 m<sup>2</sup> g<sup>-1</sup>, micropore volume of 0.024 cc g<sup>-1</sup>, cation exchange capacity of 13.4–14.8 c mol kg<sup>-1</sup>, ash content of 20.7%, and pH 9.1.<sup>17</sup>

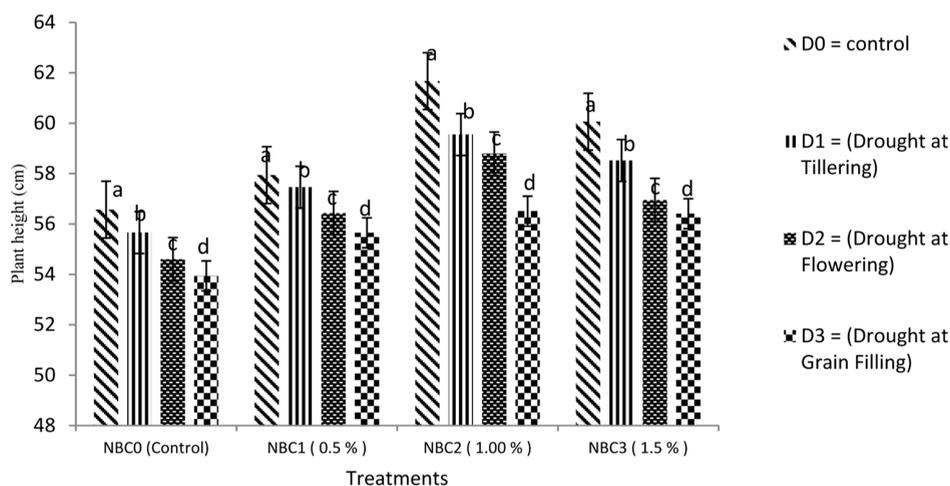
Every pot was watered evenly before drought initiation. Then, 30% pot water holding capacity was maintained at tillering, flowering, and grain filling stages. While 80% water holding capacity was declared as the control condition.

## RECORDED PARAMETERS

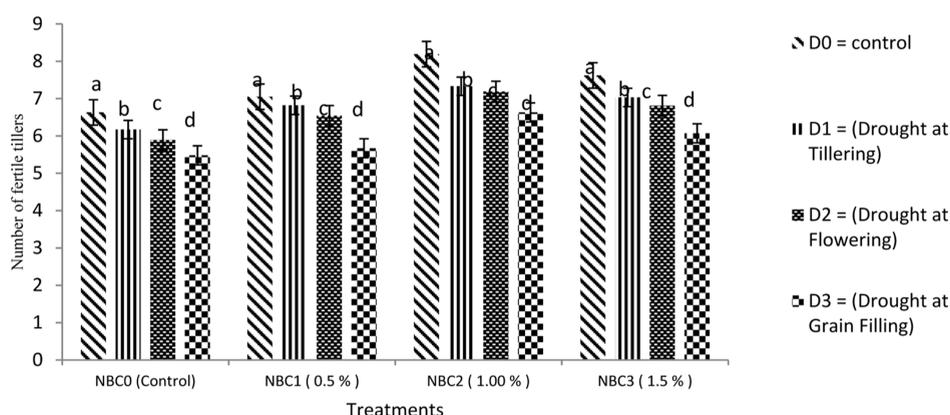
**Growth and Yield Parameters.** Growth and yield-related parameters like plant height (cm), grain per spike, spike length (cm), 1000 grain weight (g), biological yield/plant, and grain yield/plant were recorded per standard procedure.

The following formula recorded harvest index

$$HI = \frac{\text{grain yield}}{\text{biological yield}} \times 100$$



**Figure 1.** Effect of NBC treatments on wheat plant height under drought stress



**Figure 2.** Effect of NBC treatments on wheat plant NFT under drought stress.

**Determination of Physiological Parameters.** Photosynthetic pigments like chlorophyll *a* and chlorophyll *b* were extracted through a spectrophotometer process in 80% acetone solution.<sup>39</sup> A pulse amplitude modulation fluorometer was used to detect the fluorescence of chlorophyll in leaves (Handy PEA, Hansatech, Norfolk, UK). Leaves were adapted to darkness for 30 min. Total 30 measurements were collected from each treatment. The water use efficiency (WUE) was calculated as described by ref 25.

$$\text{WUE} = \text{grain yield}/\text{total water applied}$$

Stomatal conductance was calculated using a porometer MK-3, Delta-T Devices, Burwell, England.

**Determination of Electrolyte Leakage and Hydrogen Peroxide Concentration.** Antioxidant enzyme activity and oxidative stress were measured after 65 days of planting. For correct determination of electrolyte leakage % (EL) in shoots of plants, the samples were kept vertically in tubes. Heating was done in distilled water for a period of 2 h and temperature of 32 °C. The recorded reading was mentioned as EC1. Another sample was given the same treatment but at a temperature of (121 °C) and a period of 20 min and the reading was mentioned as EC2. Finally, the EL (%) was calculated by following formulas.

$$\text{EL} = \frac{\text{EC1}}{\text{EC2}} \times 100$$

While for the calculation and estimation of H<sub>2</sub>O<sub>2</sub>, 3.0 mL buffer solution of phosphate was added to 50 mg of sample, and this solution was centrifuged for 30 min at a temperature of 4 °C. Then, the supernatant was added with 1.0 mL of titanium sulfate (0.1%), and this solution was centrifuged at 6000 rpm for 20 min at a temperature of 4 °C; then, the absorbance of supernatant was determined. H<sub>2</sub>O<sub>2</sub> determination and calculation were done by an extinction coefficient of 0.28 mol<sup>-1</sup> cm<sup>-1</sup>.

**Estimation of Antioxidant Enzymes.** The activity of superoxide dismutase (SOD) was determined by the method described by ref 40. 50 mM phosphate buffer (with pH of 7.8), 13 mM methionine, 0.1 μM EDTA, 0–100 μL of enzyme extract, and 75 μM nitro blue tetrazolium in a 3 mL mixture were added to 2 μM riboflavin. A 15 W fluorescent tube was used to illuminate the test tubes, which were shaken continuously.

The reaction was allowed to continue for 10 min, the fluorescence tube was switched off, and an absorbance reading was done at 560 nm. One unit of SOD was described as the amount of enzyme required for 50% inhibition in the reduction rate of nitroblue tetrazolium.

Hwang et al.,<sup>41</sup> purposed a procedure for measuring the CAT activity by determining the decomposition rate of H<sub>2</sub>O<sub>2</sub> at 240 nm. POD activity was determined by the guaiacol oxidation method as described by Maehly and Chance 1954 with minor modifications, i.e., 50 mM potassium phosphate

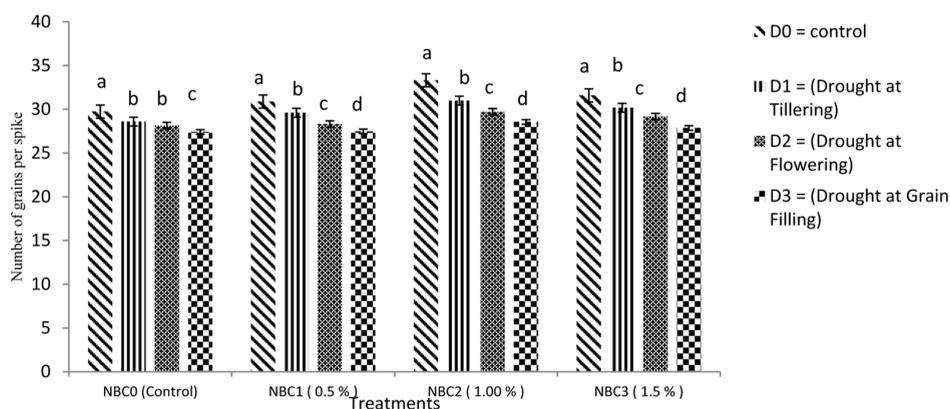


Figure 3. Effect of NBC treatments on wheat plant NGS under drought stress.

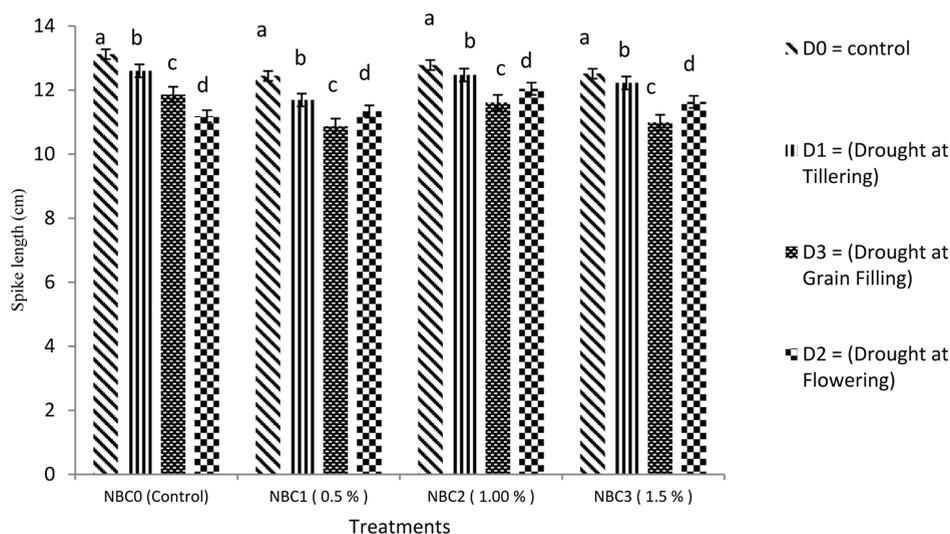


Figure 4. Effect of NBBC treatments on wheat plant spike length under drought stress.

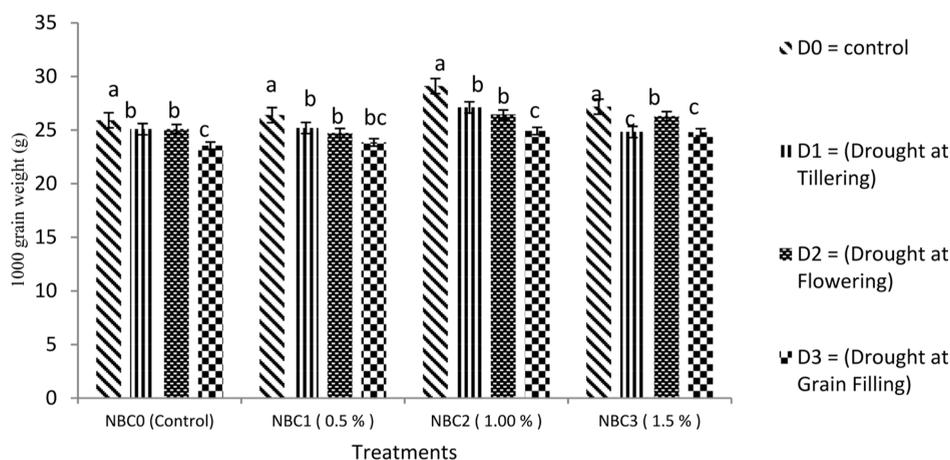


Figure 5. Effect of NBC treatments on wheat plant 1000 GW under drought stress

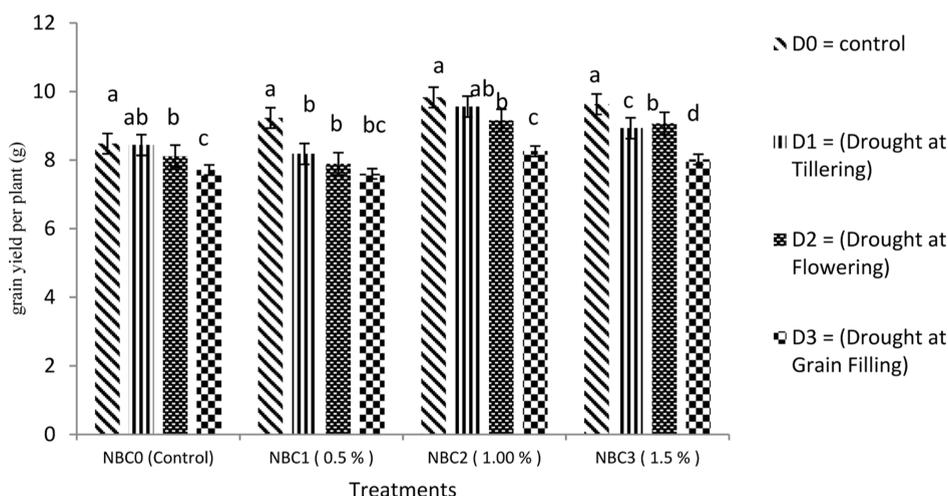
mixed with 0.4%  $H_2O_2$ , 1% guaiacol, and enzyme extracts were mixed with 3 mL solution. The absorption rate increased as guaiacol oxidation increased ( $E = 25.5 \text{ mM}^{-1} \text{ cm}^{-1}$ ) at 470 nm.

**Statistical Analysis.** STATISTIX 8.1 was used on raw data to compute variance analysis (ANOVA) and least significant difference (LSD) at 5% probability level for mean data comparison.<sup>31</sup> Then, the data were subjected to PCA (principal component analysis), and biplot figures were

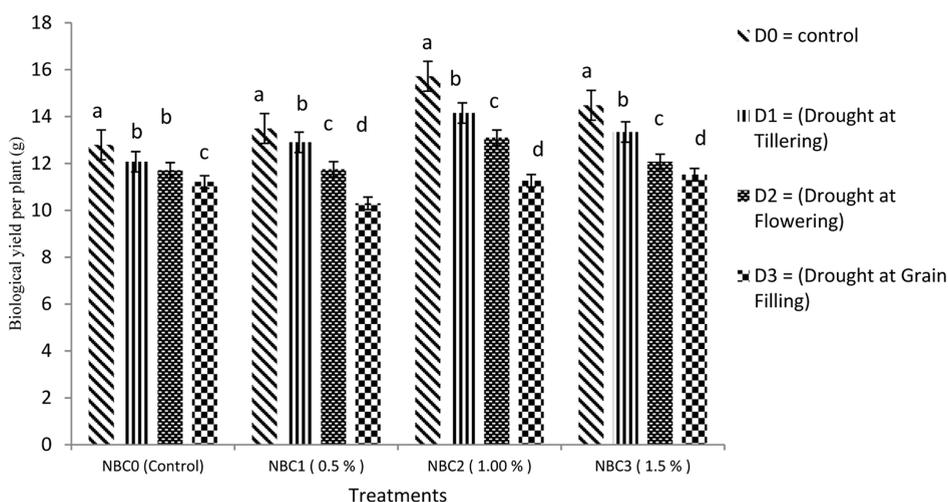
developed through origin pro 9.1 software to determine the results.

## RESULTS

**Plant Height.** Statistically analyzed data of plant height in Figure 1 show that wheat plant height was significantly affected by different drought treatments at critical growth stages and changes with NBC treatments. Wheat maximum plant height



**Figure 6.** Effect of NBC treatments on wheat plant grain yield under drought stress.



**Figure 7.** Effect of NBC treatments on wheat plant biological yield under drought stress

was reported under control treatment (61.67) and lowest in drought at grain filling (53.94). Interaction relation was statistically nonsignificant between drought and NBC treatments

**Number of Fertile Tillers.** Statistically analyzed data of no. of fertile tillers (NFT) presented in Figure 2 show that fertile tillers were significantly affected by different drought treatments at critical growth stages and NBC treatments. Wheat plant with the highest population of productive tillers was reported under control treatment (8.19) and lowest in drought at grain filling (5.48). Interaction relation was statistically nonsignificant between drought and NBC.

**No. of Grains per Spike.** The analytically arranged data of the number of grain spike-1 in Figure 3 indicates that different drought treatments at critical growth stages and multiple doses of NBC had a significant impact on wheat grains per spike (NGS). Wheat plant spikes with the highest grain population were present in the control treatment (33.31), and statistically, minimum wheat plant NGS (no. of grains per spike) were in drought at grain filling (27.41). Interaction relation was statistically nonsignificant between drought and NBC.

**Spike Length (cm).** Analytically arranged data of spike length shown in Figure 4 indicate that different drought treatments at critical growth stages and NBC treatments had a

significant impact on wheat spike length (SL). Wheat plant spikes with the highest length were present in control treatment (13.12), and a statistically minimum length of wheat plant spikes was in drought at grain filling (10.87). Interaction relation was statistically nonsignificant between drought and NBC (nano biochar).

**1000 Grain Weight (g).** Statistically analyzed data regarding 1000 grain weight represented by Figure 5 show that different levels of drought significantly impacted the weight of 1000 grains at crucial growth and development stages and NBC treatments. Statistically maximum 1000 grain weight of wheat plants were recorded in the control treatment (29.10) and the lowest in drought at grain filling (23.54). Interaction relation was statistically nonsignificant between drought and NBC.

**Grain Yield ( $\text{g pot}^{-1}$ ).** Statistically analyzed data regarding grain yield per pot represented by Figure 6 show that different levels of drought significantly impacted grain yield at crucial growth and development stages and NBC treatments. Statistically maximum grain yield of wheat plants was recorded in the control treatment (9.83) and the lowest in drought at grain filling (7.60). Interaction relation was statistically nonsignificant.

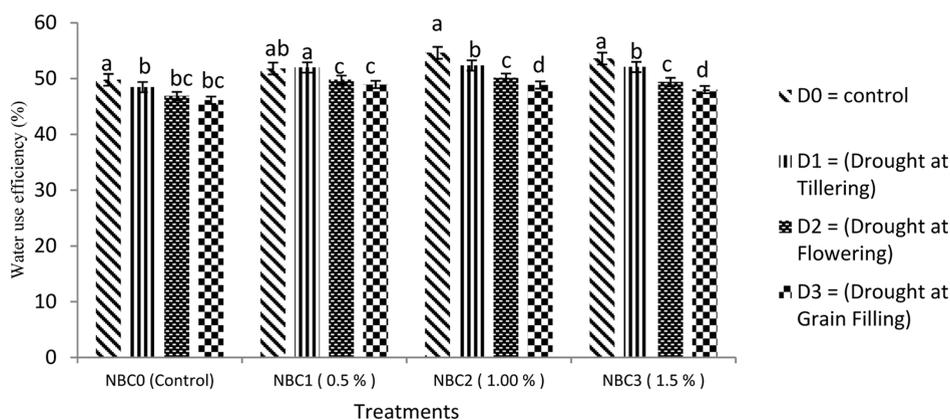


Figure 8. Effect of NBC treatments on wheat plant WUE under drought stress.

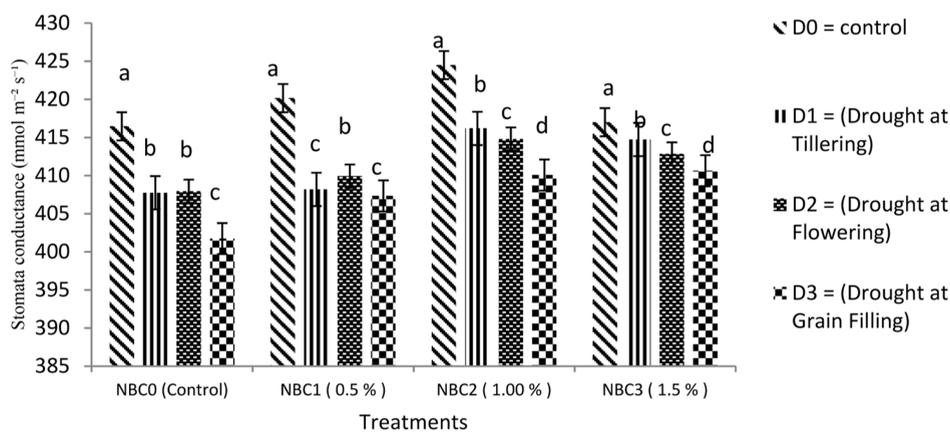


Figure 9. Effect of NBC treatments on wheat plant SC under drought stress.

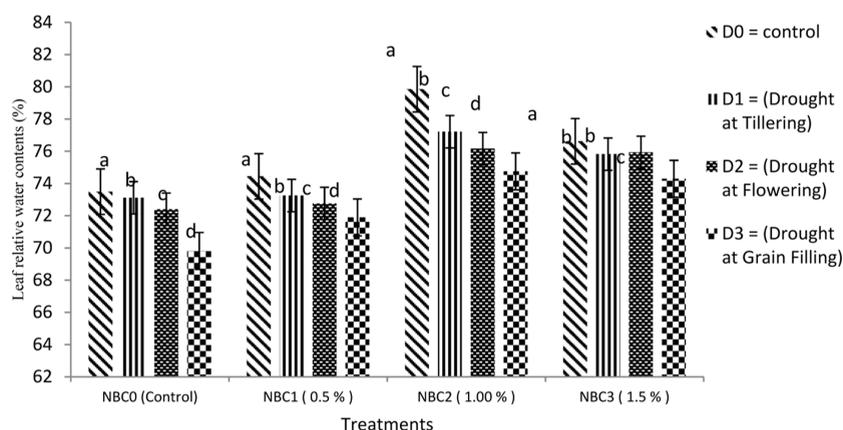


Figure 10. Effect of NBC treatments on wheat plant LRWC under drought stress.

**Biological Yield (g pot<sup>-1</sup>).** Statistically analyzed data regarding biological yield per pot represented by Figure 7 show that grain yield has significantly fluctuated with different levels of drought at significant growth and development stages and NBC treatments. Statistically maximum biological yield of wheat plants was recorded in the control treatment (15.72) and the lowest in drought at grain filling (10.30).

**Water Use Efficiency.** Statistically analyzed data regarding WUE represented by Figure 8 show that WUE fluctuated significantly with different levels of drought at important wheat crop stages and NBC treatments. Statistically maximum WUE of wheat plants was recorded in the control treatment (54.62)

and the lowest in drought at grain filling (46.12). Statistically, nonsignificant interaction was observed between drought and NBC.

**Stomatal Conductance.** The wheat leaf's stomatal conductance (SC) activity is represented by Figure 9. Emergence of drought stress at different critical intervals during wheat crop life cycle and different treatment levels of NBC significantly impacted the wheat crop's SC activity. Statistical analysis revealed that maximum values of SC were found in the control treatment (424.48), and activity of wheat crop leaf SC was reported to be lowest by D3 = drought at

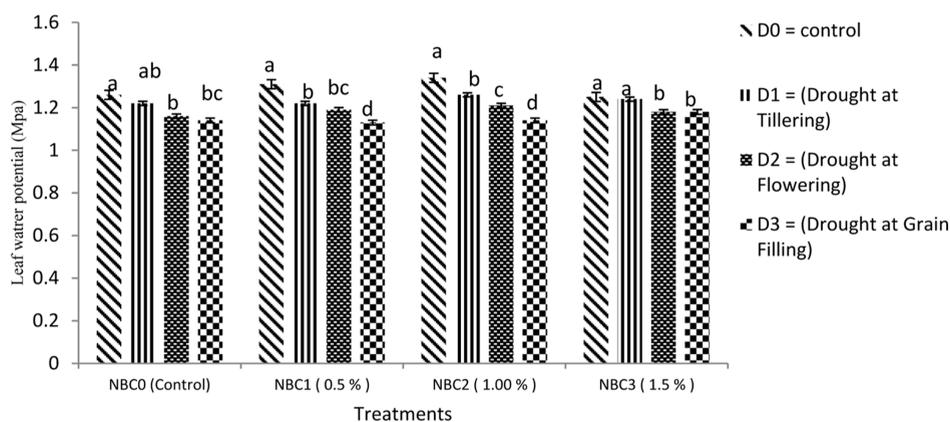


Figure 11. Effect of NBC treatments on wheat plant WP under drought stress.

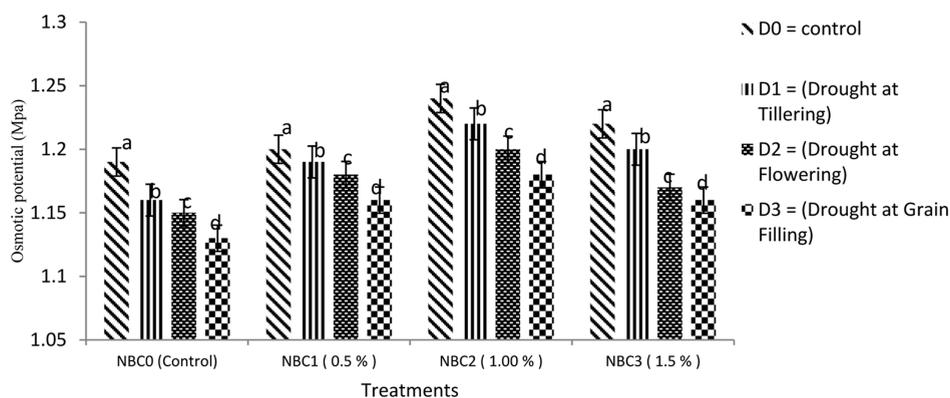


Figure 12. Effect of NBC treatments on wheat plant LOP under drought stress.

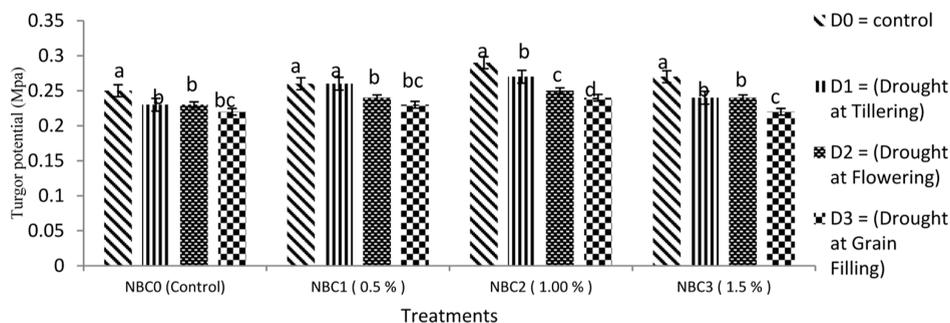


Figure 13. Effect of NBC treatments on wheat plant TP under drought stress.

grain filling (401.73). Statistically, nonsignificant interaction activity was observed between drought and NBC treatments.

**Leaf Relative Water Content.** Figure 10 represents the relative water contents of wheat leaf. Prevalence of drought stress at different sensitive stages during wheat crop life cycle and different treatment levels of NBC significantly impacted the leaf relative water contents of wheat crop. Statistical analysis revealed that maximum readings of RWC were found in the control treatment (79.85), and water contents of wheat crop leaf were yielded lowest by D3 = drought at grain filling (69.81). Statistically, nonsignificant interaction activity was observed between drought and NBC treatments.

**Leaf Water Potential.** The water potential (WP) contents of the wheat leaf are represented in Figure 11. The emergence of drought stress at different critical intervals during the wheat crop life cycle and different treatment levels of NBC significantly impacted the leaf water potential (LWP) of the

wheat crop. Statistical analysis revealed that maximum values of LWP were found in the control treatment (1.34), and the water potential of wheat crop leaf was reported to be lowest by D3 = drought at grain filling (1.13). Statistically nonsignificant interaction activity was observed between drought and NBC treatments.

**Osmotic Potential.** Osmotic potential (OP) in Figure 12 represents the contents of the wheat leaf. Emergence of drought stress at different critical intervals during the wheat crop life cycle and different treatment levels of NBC significantly impacted OP of wheat crop. Statistical analysis revealed that maximum values of OP (osmotic potential) were found in the control treatment (1.24), and OP of wheat crop leaf was reported lowest by D3 = drought at grain filling (1.13). Statistically, nonsignificant interaction activity was observed between drought and NBC treatments.

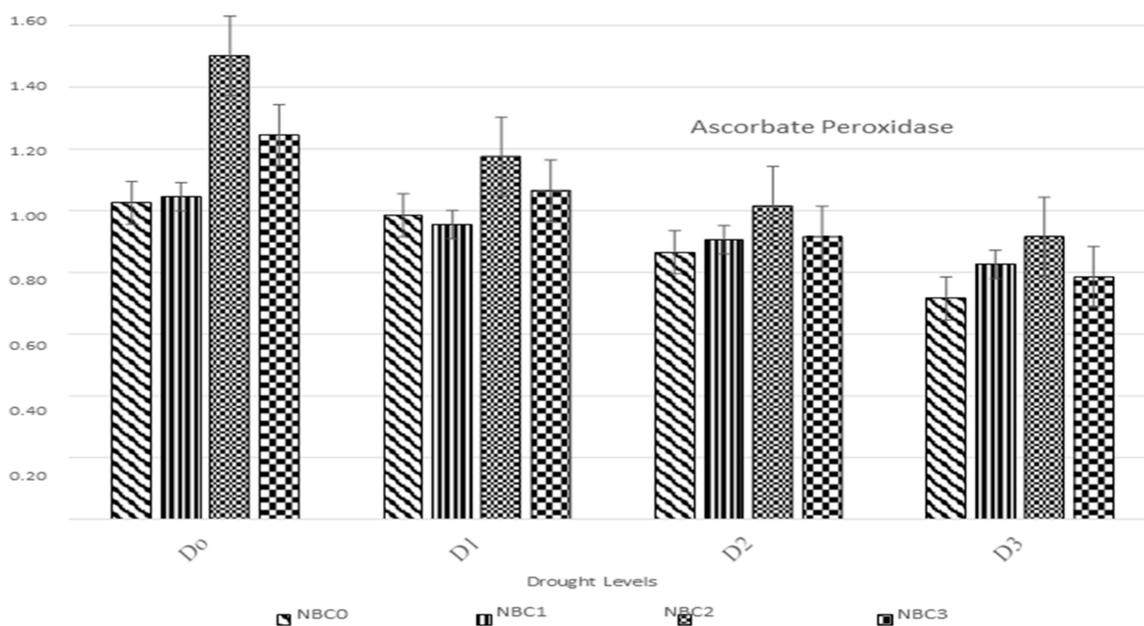


Figure 14. Effect of NBC treatments on wheat plant APX under drought stress

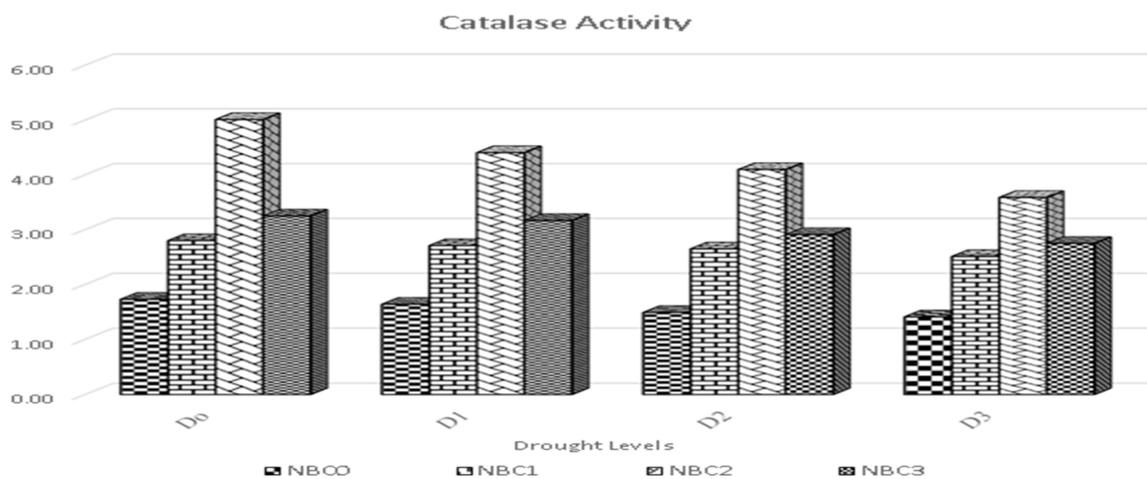


Figure 15. Effect of NBC treatments on wheat plant CAT under drought stress.

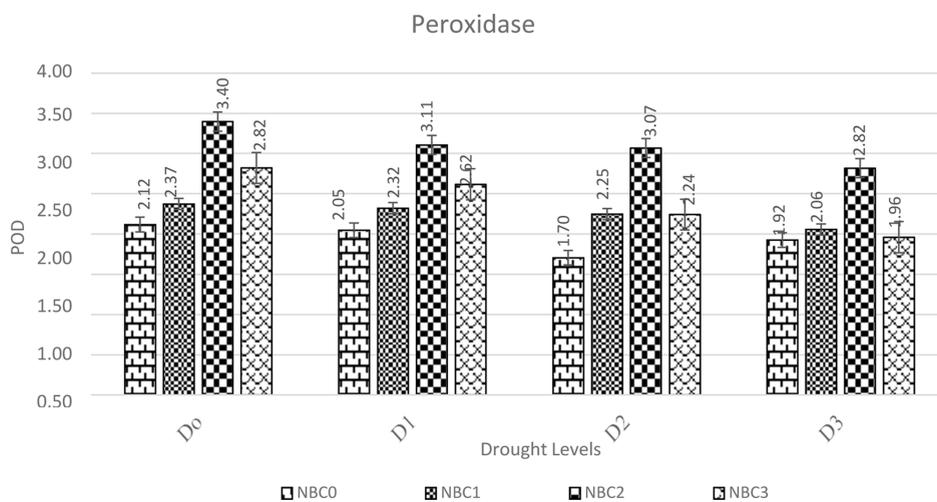


Figure 16. Effect of NBC treatments on wheat plant POD under drought stress.

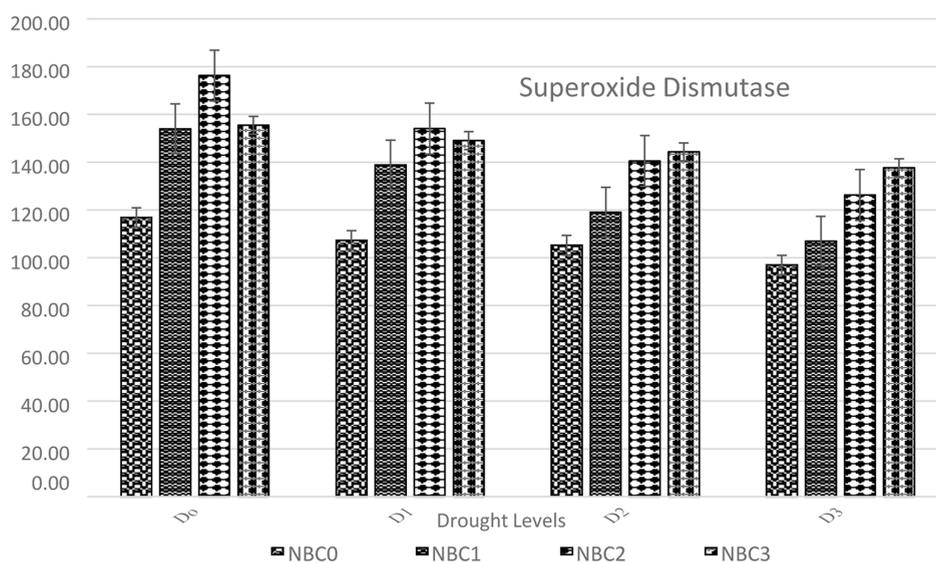


Figure 17. Effect of NBC treatments on wheat plant SOD under drought stress.

**Turgor Potential.** Turgor potential (TP) contents of the wheat leaf are represented in Figure 13. The emergence of drought stress at different critical intervals during the wheat crop life cycle and different treatment levels of NBC significantly impacted the turgor potential (TP) of wheat crop. Statistical analysis revealed that maximum values of TP were found in the control treatment (0.29), and the TP of wheat crop leaf was reported to be lowest by D3 = drought at grain filling (0.22). Statistically, nonsignificant interaction activity was observed between drought and NBC treatments.

**Ascorbate Peroxidase.** APX (Ascorbate peroxidase) activity data are represented by Figure 14, which shows that drought application at different critical stages and different NBC treatments had a statistically significant effect on APX activity of wheat crop. APX activity was higher (1.50) in plots made up of control treatment; however, minimum activity of APX was recorded in plots receiving drought at grain filling (0.72). Statistically significant interaction activity was observed between drought and treatments of NBC.

**Catalase Activity.** CAT (catalase) activity data are represented by Figure 15, which shows that drought application at different critical stages and different NBC treatments had a statistically significant effect on CAT activity of wheat crop. CAT activity was higher (5.01) in plots made up of the control treatment; however, minimum activity of catalase was recorded in plots receiving drought at grain filling (1.57). Statistically, nonsignificant interaction activity was observed between drought and NBC treatment.

**Peroxidase Activity.** POD (peroxidase) activity (PA) data are represented by Figure 16, which shows that drought application at different critical stages and different NBC treatments had a statistically significant effect on PA of wheat crop. PA was higher (3.40) in plots of the control treatment; however, minimum activity of peroxidase was recorded in plots receiving drought at grain filling (1.70). Statistically nonsignificant interaction activity was observed between drought and NBC treatments.

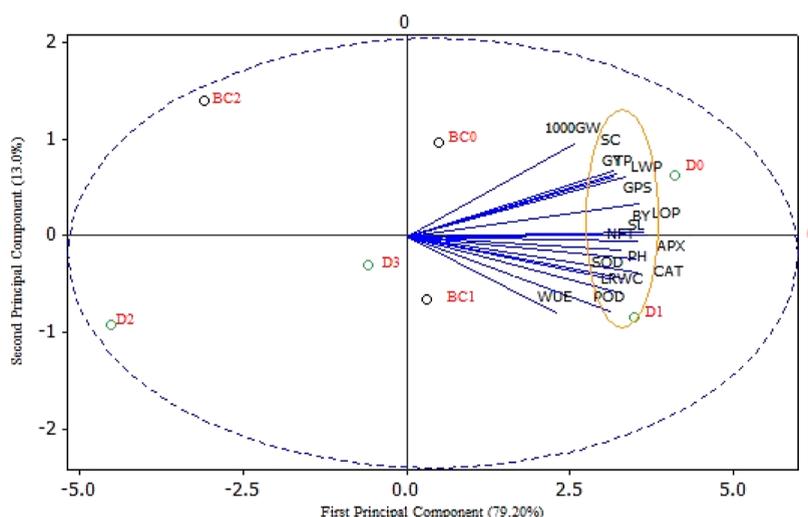
**Superoxide Dismutase.** SOD activity (SDA) data are represented by Figure 17, which shows that drought application at different critical stages and different NBC treatments had statistically significant effects on SDA of wheat

crop. SDA was higher (176.28) in plots of the control treatment; however, minimum activity of SOD was recorded in plots receiving drought at grain filling (96.96). Statistically significant interaction activity was observed between drought and levels of NBC.

## DISCUSSION

Crop growth and yield parameters play an important part in determining various responses of plant attributes to different field and environmental conditions, having a significant impact. Plant height is also among those attributes that are important in crop growth and development. An increase in soil moisture and fertility increases plant height too. A similar record was observed in a wire house experiment showing that normal irrigation yielded a 6.17% increase in plant height, whereas nutritional values of NBC also showed significant results in increasing plant length. The application of NBC (1.00%) yielded the highest height with a 6.66% increase. This study is similar to Ahmad et al.,<sup>44</sup> who reported that biochar had a significant effect on wheat crop growth. Number of fertile tillers contributes directly toward the final grain by producing maximum number of tillers with the ability to produce spikes with healthier and heavier seeds. Under normal conditions with abundant irrigation levels, a maximum number of tillers was produced, and an increase in fertile tillers by 23.64% was reported under normal irrigation, whereas NBC (1.00%) influenced the production of productive tillers by 17.63%.<sup>42</sup> stated that NBC application had a positive impact on wheat growth and yield.

Different yield contributing factors (spike length, number of grains per spike, 1000 grain weight, grain yield, and biological yield) are interlinked in such a way that any fluctuation in one yields changes in all other dependent attributes. The absence of proper moisture level at critical growth stages results in smaller spikes, leading to lower grain yield. Under normal irrigation and growth enhancing factor (NBC 1%), spike length was enhanced by 12.17 and 1.74%. Grain count per spike was also boosted by increasing the above-mentioned yield-determining components as higher grains were produced under enhanced growth due to the prevalence of desired moisture and nutrient levels. Under normal irrigation, grain



**Figure 18.** Biplot description of experiment showing 92.20% data variability: for example, acute angles showed the positive and negative relations among different parameters such as 1000 grain weight (1000GW), SC, grain yield per plant (GYP), LWP, grains per spike (GPS), spike length (SL), NPT, APX, plant height (PH), SOD, catalase activity (CAT), leaf relative water contents (LRWC), POD, WUE, biochar levels BC<sub>0</sub>, BC<sub>1</sub>, BC<sub>2</sub>, and BC<sub>3</sub>, and different drought levels, i.e., D<sub>0</sub>, D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub>.

count per spike was increased by 12.76%, and providing soil and crop promoting supplements, NBC (1.00%) produced 7.09 times more grains per spike than stress or non NBC application.

A 1000 grain weight phenomenon is quite familiar with these components as it also points out the crop response to photosynthates produced as a result of the photosynthesis process. Maximum utilization of these photosynthates is facilitated under optimum moisture levels, and the presence of extra nutritional sources also had a positive impact. Under normal irrigation level, the 1000 grain weight was increased by 11.38%, and NBC treatment (1.00%) increased the 1000 grain weight by 7.38%. All these findings regarding yield components align with the studies of<sup>42,43</sup> who reported that drought at any stage decreases wheat crop growth and yield.

Crop management practices aim to attain maximum grain yield by using all of the available resources. The absence of water at any stage of crop growth directly affects the grain yield as reduction in any growth stage ultimately results in reduced grain production and lower grain mass as lighter and weaker grains are produced. Maximum grain yield is obtained under normal irrigation in combination with providing nutritional support to crop. Under normal irrigation, grain yield showed boost of 17.66% and NBC (1.00%) provided maximum positivity toward crop grain yield by showing an increase of 11.03%. Biological yields comprise the total dry matter consumed during the crop life cycle in a particular season. Maximum activity of TDM accumulation was reported under normal irrigation, with a 27.48% increase. NBC (1.00%) also contributed effectively toward TDM accumulation, resulting in enhanced biological yield by 11.88 times. All these findings regarding yield components align with the studies of refs 43 and 44 who reported that drought at any stage decreases wheat crop growth and yield.

Similar to yield components, physiological processes are also linked with each other in different manners; different physiological processes contribute individually and in combination toward crop growth and metabolic processes. WUE points out economic production in terms of water units consumed. Maximum activity of WUE was recorded under

normal irrigation, which showed a 9.29% increase, whereas NBC treatments also produced a positive response from WUE activity, but a maximum response with 7.16 times higher activity of WUE was recorded under NBC (1.00%) treatment. This result agrees with refs 17 and 44 who reported that biochar application helped increase WUE. Leaf relative water content and potential water activity also showed significant activity variation under different drought treatments and NBC doses levels. Availability of the maximum moisture level speeds up the activity of these components, whereas under deficit conduction, activities of these components slow down. Maximum activity of LRWC with 4.69 increases and a 12.33% increase in water potential activity was recorded in normal irrigation. NBC also extracted positive responses from these physiological attributes having 6.22 and 3.21 times higher RWC and WP activity, respectively. It has been reported that biochar application helped increase physiological activities (RWC and water potential).<sup>17</sup>

TP and OP recorded a similar response pattern due to linkage with the above-discussed physiological (LRWC and water potential) components. TP activity was increased by 17.51% under normal irrigational conditions, whereas OP was increased by 4.60%. NBC treatments had an encouraging effect on both physiological activities, generating an 11.11 and 4.60% increase in turgor and osmotic potential, respectively. These results are similar to the findings of ref 17 who reported that biochar application helped increase WUE. SC relates to two environments (outside plant body and internal plant activities). Stomata conductivity is reduced whenever any stress condition is developed, which protects the plant's internal metabolic and physiological structure. Under normal irrigation, SC enhanced performance by 2.96 times, whereas NBC (1.00%) had a positive influence of 1.90 times higher than other treatments. It has been reported that biochar application reduced drought impact on SC.<sup>45</sup> The cocomposted biochar (CB) showed beneficial effects for eggplants to induce drought stress tolerance. Hence, CB might generate water stress tolerance in vegetable crop plants.<sup>46</sup> In addition, biochar may enhance the crop productive capacity of soils under various biotic and abiotic stresses, which may help to overcome the food shortage

worldwide.<sup>47</sup> It has been reported that using acidified biochar may also be a potential application for soil amendment for improving the growth and productivity of fava bean plants under drought conditions.<sup>48,49</sup>

Antioxidant enzymes are produced to reduce the effects of reactive oxygen species generated under stress, thus reducing the biochemical activity of crop plants. These antioxidant enzymes, including POD, SOD, CAT, and APX perform different complex activities and breakdown of macromolecules into micro molecules, ultimately eliminating the negative impact of ROS. NBC also had a positive influence on these antioxidant enzymes, thus enhancing their activity. NBC (1.00%) boosted POD performance by 37.10 times, SOD by 28.60 times, CAT by 63.33%, and APPX by 22.03 times. Whereas under normal irrigation conditions, increases of 48.56, 24.69, 22.26, and 28.78% are seen in APX, CCAT, POD, and SOD, respectively. The biochar application reduced drought impact on antioxidant enzymes.<sup>35,45</sup>

## CONCLUSIONS

Crop performs well under the availability of the optimum moisture level. Drought stress affected wheat crop productivity, but biochar application mitigated this effect. The whole experiment is described briefly in biplot design, as shown below in Figure 18.

The use of NBC positively impacted soil and crop health, specifically under drought conditions. Different growth, physiology, and biochemical attributes' efficiency was enhanced by NBC application under control conditions and NBC level. To economically, environmentally friendly, and maximize return from NBC application, it was recommended to avoid water stress and use NBC at a rate of 1.00%.

## AUTHOR INFORMATION

### Corresponding Authors

**Muhammad Asghar Shahid** – Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan; Email: [jamali1692@gmail.com](mailto:jamali1692@gmail.com)

**Muhammad Nawaz** – Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan 64200 Punjab, Pakistan; Email: [dmanawaz@kfueit.edu.pk](mailto:dmanawaz@kfueit.edu.pk)

**Sezai Ercisli** – Department of Horticulture, Faculty of Agriculture, Erzurum 25240, Turkey; Email: [sercisli@atauni.edu.tr](mailto:sercisli@atauni.edu.tr)

### Authors

**Muhammad Aown Sammar Raza** – Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

**Adnan Noor Shah** – Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan 64200 Punjab, Pakistan; [orcid.org/0000-0002-3722-8848](https://orcid.org/0000-0002-3722-8848)

**Muhammad Arif Ibrahim** – Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

**Rashid Iqbal** – Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

**Muhammad Usman Aslam** – Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan  
**Qurban Ali** – Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab, Lahore 54590, Pakistan

Complete contact information is available at: <https://pubs.acs.org/10.1021/acsomega.3c01629>

## Author Contributions

<sup>1</sup>M.A.S.R. and A.N.S. contributed equally to this work.

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This study was led by Professor Muhammad Aown Sammar Raza. The authors are thankful to HEC Pakistan.

## REFERENCES

- (1) Hassan, S. T.; Xia, E.; Huang, J.; Khan, N. H.; Iqbal, K. Natural resources, globalization, and economic growth: evidence from Pakistan. *Environ. Sci. Pollut. Res.* **2019**, *26*, 15527–15534.
- (2) GOP. *Economic Survey of Pakistan*: Islamabad, 2019.
- (3) Beddington, J. The future of food and farming: Challenges and choices for global sustainability *Final Proj Rep. UK Gov Foresight Glob Food Farming Future*, 2011.
- (4) Araus, J. L.; Slafer, G. A.; Royo, C.; Serret, D. M. Breeding for yield potential and stress adaptation in Cereals. *Crit. Rev. Plant Sci.* **2008**, *27*, 377–412.
- (5) Khan, I.; Awan, S. A.; Ikram, R.; Rizwan, M.; Akhtar, N.; Yasmin, H.; Sayyed, R. Z.; Ali, S.; Ilyas, N. Effects of 24-epibrassinolide on plant growth, antioxidants defense system, and endogenous hormones in two wheat varieties under drought stress. *Physiol. Plant.* **2021**, *172*, 696–706.
- (6) Jabborova, D.; Kannepalli, A.; Davranov, K.; Narimanov, A.; Enakiev, Y.; Syed, A.; Elgorban, A. M.; Bahkali, A. H.; Wirth, S.; Sayyed, R. Z.; Gafur, A. Co-inoculation of rhizobacteria promotes growth, yield, and nutrient contents in soybean and improves soil enzymes and nutrients under drought conditions. *Sci. Rep.* **2021**, *11*, 22081.
- (7) Crespo-Herrera, L. A.; Crossa, J.; Huerta-Espino, J.; Autrique, E.; Mondal, S.; Velu, G.; Vargas, M.; Braun, H. J.; Singh, R. P. Genetic yield gains in CIMMYT's International Elite Spring Wheat Yield Trials by modeling the genotype× environment interaction. *Crop Sci.* **2017**, *57*, 789–801.
- (8) Asseng, S.; Ewert, F.; Martre, P.; Rötter, R. P.; Lobell, D. B.; Cammarano, D.; Kimball, B. A.; Ottman, M. J.; Wall, G. W.; White, J. W.; Reynolds, M. P.; et al. Rising temperatures reduce global wheat production. *Nat. Clim. Change* **2015**, *5*, 143–147.
- (9) Bennett, D.; Izanloo, A.; Reynolds, M.; Kuchel, H.; Langridge, P.; Schnurbusch, T. Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments. *Theor. Appl. Genet.* **2012**, *125*, 255–271.
- (10) Pradhan, G. P.; Prasad, P. V.; Fritz, A. K.; Kirkham, M. B.; Gill, B. S. Effects of drought and high temperature stress on synthetic hexaploid wheat. *Funct. Plant Biol.* **2012**, *39*, 190–198.
- (11) Roy, J. L.; McGill, W. B. Flexible conformation in organic matter coatings: An hypothesis about soil water repellency. *Can. J. Soil Sci.* **2000**, *80*, 143–152.
- (12) Vacher, C. A.; Loch, R. J.; Raine, S. R. Effect of polyacrylamide additions on infiltration and erosion of disturbed lands. *Aust. J. Soil Res.* **2003**, *41*, 1509–1520.
- (13) Mesarič, T.; Baweja, L.; Drašler, B.; Drobne, D.; Makovec, D.; Dušak, P.; Dhawan, A.; Sepčić, K. Effects of surface curvature and surface characteristics of carbon-based nanomaterials on the

- adsorption and activity of acetylcholinesterase. *Carbon* **2013**, *62*, 222–232.
- (14) Guo, Y.; Li, Y.; Zhu, T.; Ye, M. Effects of concentration and adsorption product on the adsorption of SO<sub>2</sub> and NO on activated carbon. *Energy Fuels* **2013**, *27*, 360–366.
- (15) Paneque, M.; De la Rosa, J. M.; Franco-Navarro, J. D.; Colmenero-Flores, J. M.; Knicker, H. Effect of biochar amendment on morphology, productivity and water relations of sunflower plants under non-irrigation conditions. *Catena* **2016**, *147*, 280–287.
- (16) Bamminger, C.; Poll, C.; Sixt, C.; Högy, P.; Wüst, D.; Kandeler, E.; Marhan, S. Short-term response of soil microorganisms to biochar addition in a temperate agro-ecosystem under soil warming. *Agric. Ecosyst. Environ.* **2016**, *233*, 308–317.
- (17) Haider, I.; Raza, M. A.; Iqbal, R.; Aslam, M. U.; Habib-ur-Rahman, M.; Raja, S.; Khan, M. T.; Aslam, M. M.; Waqas, M.; Ahmad, S. Potential effects of biochar application on mitigating the drought stress implications on wheat (*Triticum aestivum* L.) under various growth stages. *J. Saudi Chem. Soc.* **2020**, *24*, 974–981.
- (18) Zhou, B.; Chen, X.; Wang, Q.; Wei, W.; Zhang, T. Effects of nano carbon on soil erosion and nutrient loss in a semi-arid loess region of Northwestern China. *Int. J. Agric. Biol. Eng.* **2018**, *11*, 138–145.
- (19) Wang, Y.; Wei, Y.; Sun, J. Biochar application promotes growth parameters of soybean and reduces the growth difference. *Commun. Soil Sci. Plant Anal.* **2016**, *47*, 1493–1502.
- (20) Lyu, S.; Du, G.; Liu, Z.; Zhao, L.; Lyu, D. Effects of biochar on photosystem function and activities of protective enzymes in *Pyrus ussuriensis* Maxim. under drought stress. *Acta Physiol. Plant.* **2016**, *38*, 220.
- (21) Jaleel, C. A.; Sankar, B.; Murali, P. V.; Gomathinayagam, M.; Lakshmanan, G. M.; Panneerselvam, R. Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation. *Colloids Surf., B* **2008**, *62*, 105–111.
- (22) Flexas, J.; Bota, J.; Loreto, F.; Cornic, G.; Sharkey, T. D. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol.* **2004**, *6*, 269–279.
- (23) Cornelissen, G.; Martinsen, V.; Shitumbanuma, V.; Alling, V.; Breedveld, G. D.; Rutherford, D. W.; Sparrevik, M.; Hale, S. E.; Obia, A.; Mulder, J. Biochar effect on maize yield and soil characteristics in five conservation farming sites in Zambia. *Agronomy* **2013**, *3*, 256–274.
- (24) Manolikaki, I.; Diamadopoulos, E. Positive effects of biochar and biochar-compost on maize growth and nutrient availability in two agricultural soils. *Commun. Soil Sci. Plant Anal.* **2019**, *50*, 512–526.
- (25) Raza, M. A.; Haider, I.; Farrukh Saleem, M.; Iqbal, R.; Usman Aslam, M.; Ahmad, S.; Abbasi, S. H. Integrating biochar, rhizobacteria and silicon for strenuous productivity of drought stressed wheat. *Commun. Soil Sci. Plant Anal.* **2021**, *52*, 338–352.
- (26) Nardi, S.; Schiavon, M.; Francioso, O. Chemical structure and biological activity of humic substances define their role as plant growth promoters. *Molecules* **2021**, *26* (8), 2256.
- (27) Yang, F.; Tang, C.; Antonietti, M. Natural and artificial humic substances to manage minerals, ions, water, and soil microorganisms. *Chem. Soc. Rev.* **2021**, *50*, 6221–6239.
- (28) Wu, S.; Li, R.; Peng, S.; Liu, Q.; Zhu, X. Effect of humic acid on transformation of soil heavy metals. *IOP Conf. Ser.: Mater. Sci. Eng.* **2017**, *207* (1), 012089.
- (29) Canellas, L. P.; Canellas, N. O. A.; da S Irineu, L. E. S.; Olivares, F. L.; Piccolo, A. Plant chemical priming by humic acids. *Chem. Biol. Technol. Agric.* **2020**, *7*, 12.
- (30) Melendrez, M. M. Humic acid: The science of humus and how it benefits soil; Eco Farming Daily, 2020; [Online] Available from: <https://www.Ecofarmingdaily.Com/build-soil/humus/humic-acid>.
- (31) Mukherjee, A.; Lal, R.; Zimmerman, A. R. Impacts of 1.5-year field aging on biochar, humic acid, and water treatment residual amended soil. *Soil Sci.* **2014**, *179* (7), 333–339.
- (32) Bar Bulent, T.; et al. Effects of humic substances on plant growth and mineral nutrients uptake of wheat under conditions of salinity. *Asian J. Crop Sci.* **2009**, *1*, 87.
- (33) Billingham, K. L. (2020). Humic Products—Potential or Presumption for Agriculture. Do Humic Products Have a Place in Australian Grazing Enterprises?.
- (34) Gollenbeek, L.; van der Weide, R. *Prospects for Humic Acid Products from Digestate in the Netherlands: quickscan* (No. WPR-867); Stichting Wageningen Research, Wageningen Plant Research, Business unit Open Teelten, 2020.
- (35) Zulfiqar, B.; Raza, M. A. S.; Saleem, M. F.; Aslam, M. U.; Iqbal, R.; Muhammad, F.; Amin, J.; Ibrahim, M. A.; Khan, I. H. Biochar enhances wheat crop productivity by mitigating the effects of drought: Insights into physiological and antioxidant defense mechanisms. *PLoS One* **2022**, *17*, 0267819.
- (36) De Melo, B. A. G.; Motta, F. L.; Santana, M. H. A. Humic acids: Structural properties and multiple functionalities for novel technological developments. *Mater. Sci. Eng. C* **2016**, *62*, 967–974.
- (37) Sible, C. N.; Seebauer, J. R.; Below, F. E. Plant biostimulants: A categorical review, their implications for row crop production, and relation to soil health indicators. *Agronomy* **2021**, *11*, 1297.
- (38) Zhou, J.; Chen, H.; Huang, W.; Arocena, J. M.; Ge, S. Sorption of atrazine, 17 $\alpha$ -estradiol, and phenanthrene on wheat straw and peanut shell biochars. *Water, Air, Soil Pollut.* **2016**, *227*, 7.
- (39) Hashem, H. A.; El-Sherif, N. A. Exogenous Jasmonic Acid Induces Lead Stress Tolerance in Kidney Bean (*Phaseolus vulgaris* L.) by Changing Amino Acid Profile and Stimulating Antioxidant Defense System. *Jordan J. Biol. Sci.* **2019**, *12*, 345.
- (40) Giannopolitis, C. N.; Ries, S. K. Superoxide dismutases: Occurrence in higher plants. *Plant Physiol.* **1977**, *59*, 309–314.
- (41) Hwang, S.-Y.; Lin, H.-W.; Chern, R.-H.; Lo, H. F.; Li, L. Reduced susceptibility to water logging together with high-light stress is related to increases in superoxide dismutase and catalase activities in sweet potato. *Plant Growth Regul.* **1999**, *27*, 167–172.
- (42) Steel, R. Analysis of variance I: The one-way classification. *Principles and Procedures of Statistics, A Biometrical Approach*; McGraw Hill, Inc., 1997; pp 139–203.
- (43) Naem, M. A.; Khalid, M.; Aon, M.; Abbas, G.; Tahir, M.; Amjad, M.; Murtaza, B.; Yang, A.; Akhtar, S. S. Effect of wheat and rice straw biochar produced at different temperatures on maize growth and nutrient dynamics of a calcareous soil. *Arch. Agron Soil Sci.* **2017**, *63*, 2048–2061.
- (44) Ahmad, H. M.; Fiaz, S.; Hafeez, S.; Zahra, S.; Shah, A. N.; Gul, B.; Aziz, O.; Fakhar, A.; Fakhar, A.; Rafique, M.; Chen, Y.; et al. Plant Growth-Promoting Rhizobacteria Eliminate the Effect of Drought Stress in Plants: A Review. *Front. Plant Sci.* **2022**, *13*, 875774.
- (45) Sattar, A.; Sher, A.; Abourehab, M. A.; Ijaz, M.; Nawaz, M.; Ul-Allah, S.; Abbas, T.; Shah, A. N.; Imam, M. S.; Abdelsalam, N. R.; Hasan, M. E.; et al. Application of silicon and biochar alleviates the adversities of arsenic stress in maize by triggering the morpho-physiological and antioxidant defense mechanisms. *Front. Environ. Sci.* **2022**, *10*, 979049.
- (46) Zulfiqar, F.; Nafees, M.; Darras, A.; Shaikat, N.; Chen, J.; Ferrante, A.; Zaid, A.; Latif, N.; Raza, A.; Siddique, K. H. Pre-harvest potassium foliar application improves yield, vase life and overall postharvest quality of cut gladiolus inflorescences. *Postharvest Biol. Technol.* **2022**, *192*, 112027.
- (47) Abd El-Mageed, T. A.; Abdelkhalik, A.; Abd El-Mageed, S. A.; Semida, W. M. Co-composted poultry litter biochar enhanced soil quality and eggplant productivity under different irrigation regimes. *J. Soil Sci. Plant Nutr.* **2021**, *21* (3), 1917–1933.
- (48) Semida, W. M.; Beheiry, H. R.; Sétamou, M.; Simpson, C. R.; Abd El-Mageed, T. A.; Rady, M. M.; Nelson, S. D. Biochar implications for sustainable agriculture and environment: A review. *South Afr. J. Bot.* **2019**, *127*, 333–347.
- (49) Abd El-Mageed, T. A.; Belal, E. E.; Rady, M. O.; Abd El-Mageed, S. A.; Mansour, E.; Awad, M. F.; Semida, W. M. Acidified Biochar as a Soil Amendment to Drought Stressed (*Vicia faba* L.) Plants: Influences on Growth and Productivity, Nutrient Status, and Water Use Efficiency. *Agronomy* **2021**, *11* (7), 1290.