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The pivotal role of biochar in enhancement soil properties, morphophysiological and yield characters of barley plants under drought stress

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Abstract

Drought is one of the most harmful abiotic stresses in arid and semiarid regions, so, field experiments were performed to examine biochar impact (15 or 20 t ha^{-1}) on soil properties, physiological, morphological, and yield of barley under drought conditions. Our results displayed that drought caused a remarkable decrease in stem height and leaf area. Additionally, relative water contents (RWC%), chlorophyll a and b concentrations, as well as yield parameters were significantly reduced under drought. Conversely, lipid peroxidation (MDA), electrolyte leakage (EL%), and enzymatic activity were significantly augmented in the stressed plants during both seasons. Application of biochar led to improve leaves number (15.3), stem height (57%) and leaf area. Also, physiological characters like chlorophyll (72%) and RWC (33%), as well as yield, were increased considerably. Contrariwise, MDA and EL were reduced significantly (47 and 54%) under biochar application; furthermore, biochar led to regulate peroxidase and catalase activity in the stressed plants. It is concluded that biochar treatment can significantly improve soil properties, particularly soil EC (dSm^{-1}), soil organic matter % and soil pH as well as increase yield characters via improving stress tolerance of barley under drought conditions; the best treatment was 20 t biochar ha^{-1} in the plants irrigated twice.

Keywords: antioxidants; barley; biochar; drought; electrolyte leakage; lipid peroxidation

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Introduction

Barley (*Hordeum vulgare* L.) is one of the most important crops belonging to Poaceae family. There are numerous environmental stress factors that affect the growth characters and yield, such as biotic (Hafez *et al.*, 2014; 2016; Abdelaal *et al.*, 2018; Esmail *et al.*, 2019; Omara *et al.*, 2019; El-Nashaar *et al.*, 2020) and abiotic factors (EL-Sabagh *et al.*, 2017; Abdelaal *et al.*, 2021a; Arafa *et al.*, 2021). Among the abiotic, chilling, heat, salinity, and drought are the most common factors (EL-Sabagh *et al.*, 2019; Elkelish *et al.*, 2020; Abdelaal *et al.*, 2020a and b). Drought is one of the maximum problematic stresses, particularly in semi-arid or arid environments (IPCC, 2014). Under water deficit, many changes were shown in physiological, biochemical, and anatomical characters linked with oxidative damages in plants. Morphological characters; leaves number, stem height, and leaf area were decreased (Samarah, 2005; Abdelaal, 2015), also physiological characteristics such as relative water content, chlorophyll a and b were reduced under water deficit conditions (Abdelaal *et al.*, 2017; Rashwan and Abdelaal, 2019). Reactive oxygen species (ROS) are very important signals that accumulate under stress (Abdelaal *et al.*, 2017). They are very effective molecules in the plant defence system under various stresses. However, the high concentration and accumulation of ROS can cause oxidative stress to plant cells and, finally, cell death (Abdelaal *et al.*, 2021b). Stem height and yield components were reduced (Abdelaal, 2015), however, electrolyte leakage (EL%) was increased under drought (Arafa *et al.*, 2021). In order to improve the growth and production of plants, some compounds can be used like biochar, it is a safe and natural compound, it is a significant soil improvement that contains various elements, mainly nitrogen, carbon, and sulfur (Hafez *et al.*, 2020). In agricultural production, biochar plays a pivotal role in enhancing composting processes (Sanchez-Monedero *et al.*, 2018), alleviating water and salt stress (Ali *et a*l., 2017), improving plant growth and production (Agegnehu *et al.*, 2017; El Nahhas *et al.*, 2021). Also, chlorophylls and relative water contents (RWC%) were improved with biochar treatment under drought (Hafez *et al.*, 2020) and salinity (El Nahhas *et al.*, 2021). Nutrient absorption in kiwi plants was enhanced (Sorrenti *et al.*, 2016), and a considerable increase of nitrogen, phosphorus, and magnesium was observed in maize with biochar application (Chng *et al.*, 2015). Under drought conditions, biochar treatments led to increased chlorophyll concentrations and improved plant growth characters in maize (Chng *et al.*, 2015) and barley (Hafez et al., 2020). Application of biochar + jasmonic acid led to improve growth characters of faba bean plant under salinity stress conditions (El Nahhas *et al.,* 2021) mitigated the negative impacts of salinity, and improved the plant height, relative water content, and chlorophyll concentration. Furthermore, biochar + jasmonic acid gave the best results of seeds number and weight of 100 seeds in the two seasons (El Nahhas *et al.*, 2021). Soil pH is one of the main soil properties to manage plant phosphorus (P) availability (Penn and Camberato, 2019) and it is an essential factor in regulation P availability in plants (Cerozi and Fitzsimmons, 2016). So, the decrease in soil pH may cause decrease P adsorption and increase bioavailability in alkaline soils (Naeem *et al.*, 2013). Likewise, biochar is an important source for nutrients and could release large amounts of N (23-635 mg kg^{-1}) and P (46-166 mg kg^{-1}) (Zheng *et al.*, 2013). So, the biochar has great potential as available nutrients.

Previous studies indicated that organic compounds could alleviate the adverse effects of many stresses and improve yield production (Ali *et al.*, 2017; 2019). Recently, there is a universal trend to use natural products of agricultural output to protect human health and the ecological system. Hence, the objective of this study was to evaluate the role of biochar in improving the soil properties, physiological, morphological, and yield parameters of barley under drought conditions.

Materials and Methods

Experimental site, plant materials and biochar preparation

The experiments were carried out in a private farm (El-Mahalla El-Kubra, Egypt) under field conditions during 2019/2020 and 2020/2021 growing seasons. The biochemical and physio-logical characters were recorded at PPBL Lab. and Excellence Centre (EPCRS), Kafrelsheikh University. The grains of barley (*Hordeum vulgare* L. var. 'Giza123') (120 kg grains ha^{-1}) were sown on 4th and 7th Nov. in 2019/2020 and 2020/2021, respectively. The design of experiments was in a randomized complete block design (five replicates), with the experimental unit (4 × 5m). The irrigation system was surface irrigation, and the amount of water was 650 m^3 ha^{-1} for each irrigation. The experimental soil was analysed to study the physical and chemical characteristics according to A.O.A.C (2015) as follows: electrical conductivity 1.7 dS m^{-1}, available P 10.3 ppm, available K 286 ppm, available N 33.1 ppm, sand 17.6%, silt 35.8%, clay 47.6%, pH (water) 8.1, and texture clay. The chemical fertilizers were added as recommended of the ministry of agriculture and land reclamation. The super phosphate fertilizer was used as phosphorus source (15.5 kg phosphor) with the soil preparation while, ammonium nitrate was used as nitrogen source (45 kg nitrogen) divided into two doses before the irrigation. The biochar was produced during the pyrolysis process at 500 °C for 20 min from rice-straw, the analysis of biochar indicated that it is a rich source of some vital elements such as carbon 463.6 g kg^{-1}, nitrogen 9.7 g kg^{-1}, phosphorus 1.7 g kg^{-1}, potassium 19.7 g kg^{-1}, magnesium 3.9 g kg^{-1}. The irrigation was one, twice and three times after germination, the treatments were done as follow:

1. Plants irrigated three times (control), well-watered (650 m^3 water every one irrigation).
2. Plants irrigated one irrigation only after germination (D1).
3. Plants irrigated two irrigations after germination (D2).
4. Plants irrigated one irrigation and treated with 15 t biochar ha^{-1} (D1 + biochar 1).
5. Plants irrigated one irrigation and treated with 20 t biochar ha^{-1} (D1 + biochar 2).
6. Plants irrigated two irrigations and treated with 15 t biochar ha^{-1} (D2 + biochar 1).
7. Plants irrigated two irrigations and treated with 20 t biochar ha^{-1} (D2 + biochar 2). The harvest date was at 5th and 3rd Apr. 2020 and 2021 respectively.

Morphological characters

At the anthesis stage, ten plants were randomly selected from each plot to study leaves number, stem height, and leaf area/plant.

Physio-biochemical characters

Concentrations of chlorophyll a and b

To estimate chlorophyll a and b, one g fresh leaves (flag) were placed in 5 ml N,N-Dimethyl formamide overnight. The absorbance was measured according to Moran and Porath (1982) at 663 and 647 nm using UV/VIS spectrophotometer (Japan).

Proline content and relative water content (RWC %)

For proline determination, 0.5 g fresh leaves from each treatment were ground in liquid nitrogen and homogenized with 10 mL of 3% sulphosalicylic acid. Proline was recorded as μg g^{-1} FW at 520 nm (Bates *et al.*, 1973).

To determine the relative water content (RWC%), fresh weight (FW) of the fully expanded leaves was taken and turgid weight was recorded (TW), after drying at room temperature and in oven at 70 °C for 48 hours, dry weight was measured (DW). Relative water content was recorded as follow (Levitt, 1980):

$$RWC\% = (FW - DW)/(TW - DW) \times 100$$

Estimation of lipid peroxidation as MDA and electrolyte leakage (EL%)

According to *Hodges et al.* (1999), lipid peroxidation was determined as MDA by estimating thiobarbituric acid reactive substances (TBARS). 500 g barley fresh leaves were ground in a cold mortar, with 15 ml of ethanol: water (95:5 v/v) and 0.1% butylated hydroxyl toluene, the supernatant was centrifuged (3000 x g for 10 min). Later the supernatant (1 ml) was added to 20% TCA (1 ml) with 0.65% thiobarbituric acid (TBA) in a clean tube and mixed vigorously for 10 min. Another 1 ml of supernatant was treated with 1 ml of 20% TCA and mixed strongly. The mixture was heated for 30 min at 95 ˚C, then cooled immediately by ice and centrifuged for 10 min at 3000 × g. MDA content was measured at 450, 532, and 600 nm and determine as follow:

MDA (nmol g^{-1} fw) = [6.45 × (A532 – A600) – (0.56 × A450)] × V-1 W

Electrolyte leakage (EL %) was recorded in fresh leaves with taken twenty discs (1 cm2) and shaken in deionized water at ambient temperature to assist electrolyte leakage. Electrolyte leakage (%) was recorded as follows: Initial conductivity/final conductivity × 100 (Szalai *et al.*, 1996).

Determination of enzymes activity

Fresh flag leaves samples were taken (0.5 g), homogenized in 3 mL of 50 mM TRIS buffer at 0-4 °C and centrifuged (12,000 rpm, 20 min). The activity of total soluble enzymes was rec-orded using UV/VIS spectrophotometer (model 180+, S/N:23-18885-01-0145). Catalase (CAT) activity was determined according to Aebi (1983), Peroxidase activity (POX) was determined according to Hammerschmidt *et al.* (1982).

Yield parameters

Twenty plants were taken from each plot to estimate yield parameters at harvesting date to determine grains yield (t ha^{-1}), biological yield [total dry matter (t ha^{-1})] and 1000 grain weight.

Soil analyses at harvest

Soil samples were taken from the experimental sites after the harvest of the experiment for chemical analysis. Samples were taken from the surface soil (0-30 cm), 5 subsamples were taken from each plot and mixed together to give a composite sample. The composite sample air-dried and sieved by 2 mm sieve. The soil parameters such as EC (dSm^{-1}), soil organic matter %, and soil pH were conducted according to Jackson (1967), Richards (1954), and Ohno and Zibilske (1991).

Statistical analysis

Statistical analysis for data was done using analysis of variance (One-way ANOVA) according to Gomez and Gomez (1984) using the MSTAT-C Statistical Software package, USA. The means were compared using Duncan (1955) when the differences were significant ($p \leq 0.05$).

Results

Impact of biochar on leaves number, stem height, and leaf area plant^{-1} in barley under water deficit

Our results in Figure 1 reveal that the growth characteristics of plants are negatively affected under drought (D1 and D2). Leaves number, leaf area, and stem height are considerably reduced in the treatments comparing to control in both seasons. Our findings indicated that adding biochar at two levels (15 and 20 t ha^{-1}) caused significant increases in leaves number, leaf area, and stem height in the treated plants compared to the untreated plants with biochar (stressed plants; D1 and D2) during both seasons. The lowest values of the abovementioned characters were obtained in the plants irrigated one time; conversely, the best findings of stem height (74.6 and 72 cm), leaves number (14.5 and 14.8), and leaf area (275.6 and 284.5 cm^2) were obtained in the stressed plants (D2) and treated with 20 t ha^{-1} biochar (Biochar2) during two seasons, respectively compared with stressed untreated plants.

Impact of biochar on chlorophyll, proline, and relative water content (RWC %)

Our findings in Figure 2 showed that the physiological features of barley are negatively affected by the water deficit (one or two irrigations). Chlorophyll a and b concentrations as well as RWC% considerably decreased in the stressed plants (D1 or D2) compared with control during two seasons. Additionally, a significant increase in proline was shown in the D1 treatment followed by the D2 treatment. Conversely, adding biochar at two levels (Biochar1 or Biochar2) considerably increased concentrations of chlorophyll a and b as well as RWC% in the stressed plants compared with untreated plants during the two seasons. The best results of chlorophyll a (2.23 and 2.24 mg g^{-1} FW), chlorophyll b (0.86 and 0.888 mg g^{-1} FW), and RWC% (76 and 74%) were obtained from the stressed plants (D2) and treated with 20 t/ha biochar in both seasons, respectively comparing with stressed plants without biochar treatment. However, proline was considerably reduced with biochar treatments (Biochar1 or Biochar2) compared with untreated plants (Figure 2).

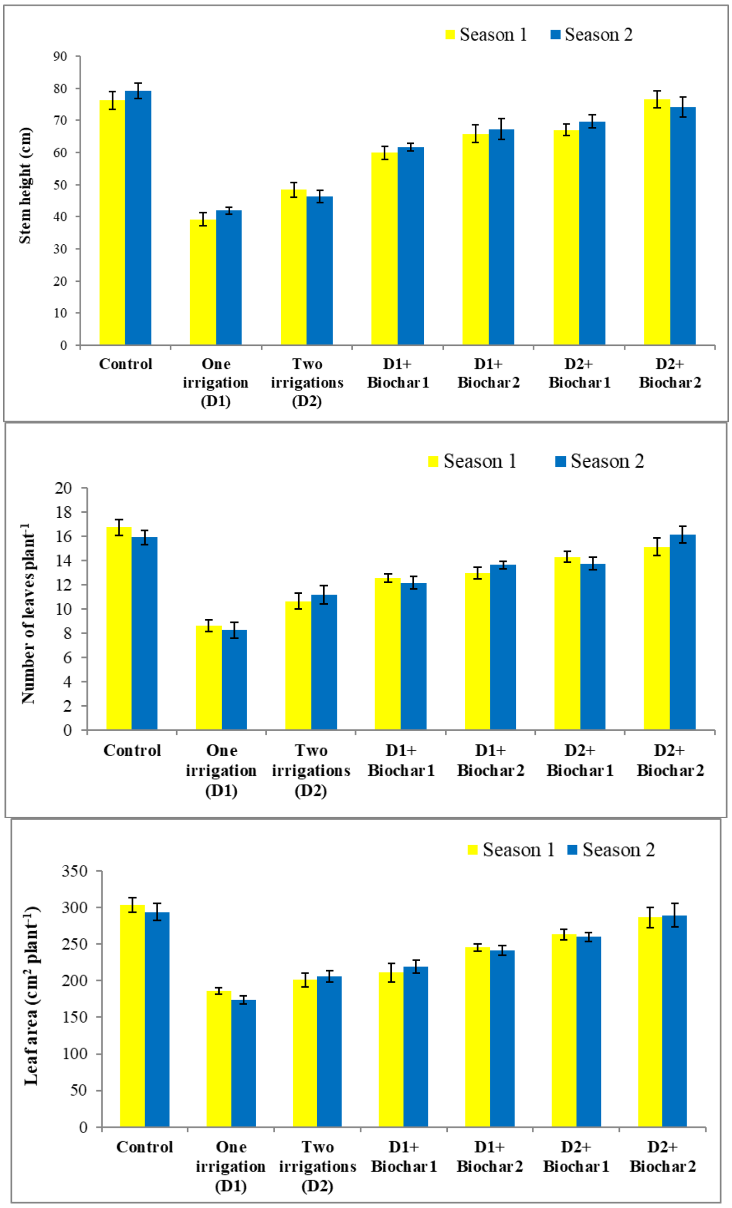

Figure 1. Impact of biochar on stem height (a), leaves number (b) and leaf area (c) in barley under drought during 2019/2020 and 2020/2021 seasons. (D1). One irrigation (D2). Two irrigations

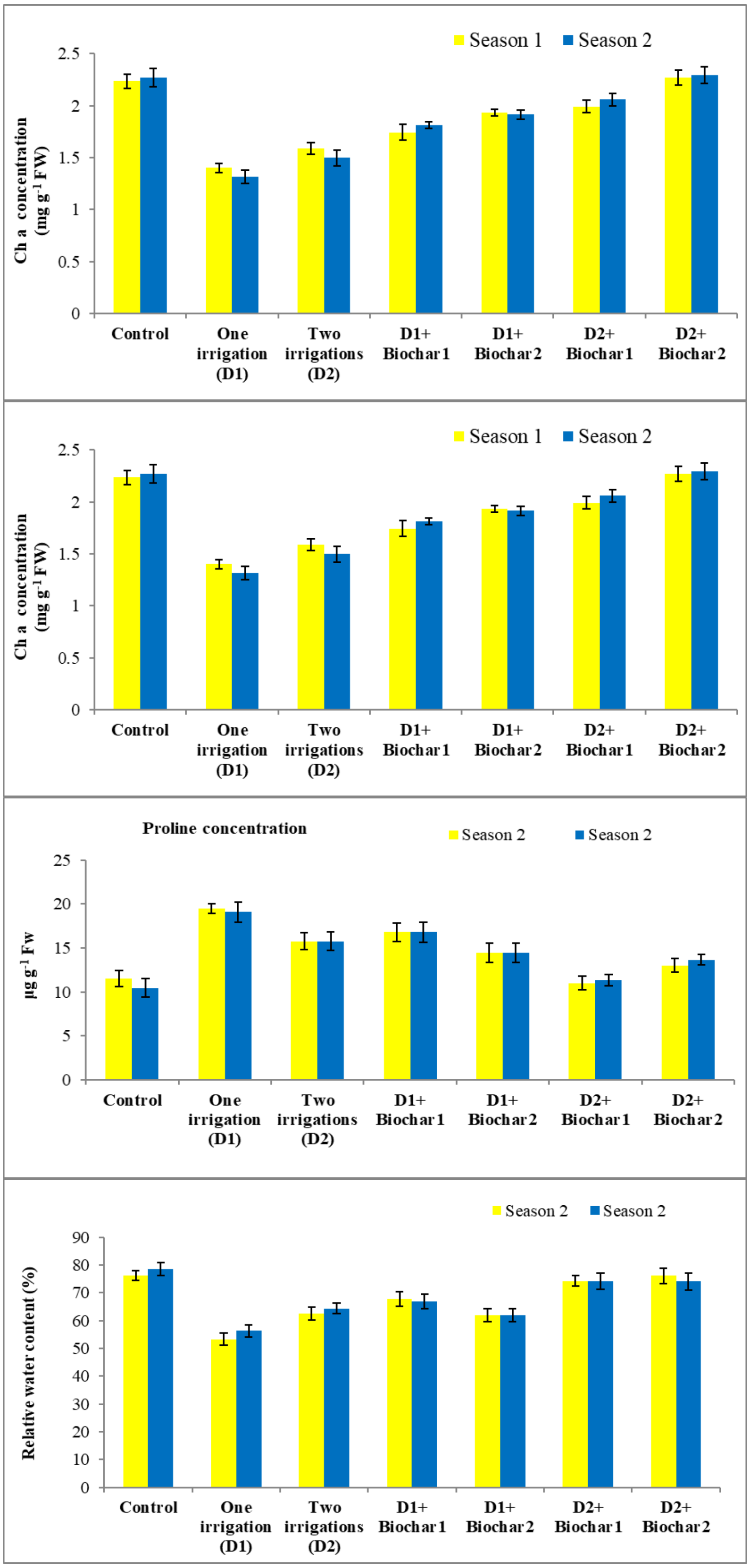

Figure 2. Impact of biochar on chlorophyll a (a), chlorophyll b, (b), proline content (c), and RWC (d) in barley under drought during both seasons (2019/2020 and 2020/2021). (D1). One irrigation (D2). Two irrigations

Effect of biochar on lipid peroxidation under water deficit

The stressed plants indicated that lipid peroxidation was significantly augmented (Figure 3). Water deficit (D1 or D2) led to a significant increase in MDA as a response to oxidative damage in barley, the increase in MDA was more significant in the D1 treatment (9.8 and 10.1µ mol g-1 fresh weight) than the D2 treatment (9.0 and 9.2 µ mol g^{-1} fresh weight) in both seasons, respectively. Conversely, biochar treatments at both levels caused an improvement in the growth characters of barley and decreased MDA in barley under water deficit comparing with the untreated stressed plants. The best effects were shown in the plants (D2+Biochar2) compared with the untreated plants and control.

Impact of biochar on electrolyte leakage (EL%), catalase, and peroxidase activity under water deficit

The presented results in Figure 3 indicated that EL%, catalase, and peroxidase activity were noticeably increased in the stressed plants. EL% considerably increased in the D1 treatment (55.9 and 52.4%) than the D2 treatment (36.4 and 38.1%) in both seasons, respectively. Likewise, catalase and peroxidase activity were considerably increased, mainly in the D1 treatment followed by the D2 treatment compared with control. Catalase activity was higher (114.8 and 112.1 mM g^{-1} H_2O_2 FW min^{-1}) with D1 treatment than the activity with D2 treatment (106.8 and 104.2 mM g–1 H2O2 FW min^{-1}) in both seasons, respectively. Addition-ally, peroxidase activity was higher (0.72 and 0.70 µmol tetra-guaiacol g^{-1} FW min^{-1}) with D1 treatment than the activity with D2 treatment (0.55 and 0.57 µmol tetra-guaiacol g^{-1} FW min^{-1}) in both seasons, respectively. Nevertheless, biochar treatments enhance the barley growth status, decrease EL%, regulate catalase and peroxidase activity under water deficit comparing with untreated plants during both seasons. The plants irrigated twice and treated with 20 t/ha biochar (D2+Biochar2) gave the best results compared with other treatments.

Effect of biochar on yield parameters

The obtained results in Figure 4 showed that the stressed barley plants were negatively affected and showed a significant reduction in yield parameters under water deficit during two seasons. 1000 grain weight, biological yield, and grain yield significantly decreased in the stressed plants compared with control during both seasons. However, adding biochar at two levels (Biochar1 or Biochar2) increased yield parameters in the stressed plants. 1000 grain weight was noticeably increased in the plants treated with 15 t ha^{-1} biochar and irrigated twice (54.3 and 56.2 g), followed by the plants irrigated twice and treated with 20 t ha^{-1} biochar (53.9 and 53.8 g) compared with stressed plants without biochar (D1) (45 and 44.6 g) and D2 (50.8 and 51.3 g) during two seasons, respectively. Also, the best results of biological and grain yield/ha were obtained in the D2 treatment and treated with biochar 20 t ha^{-1} (D2+Biochar2) during both seasons.

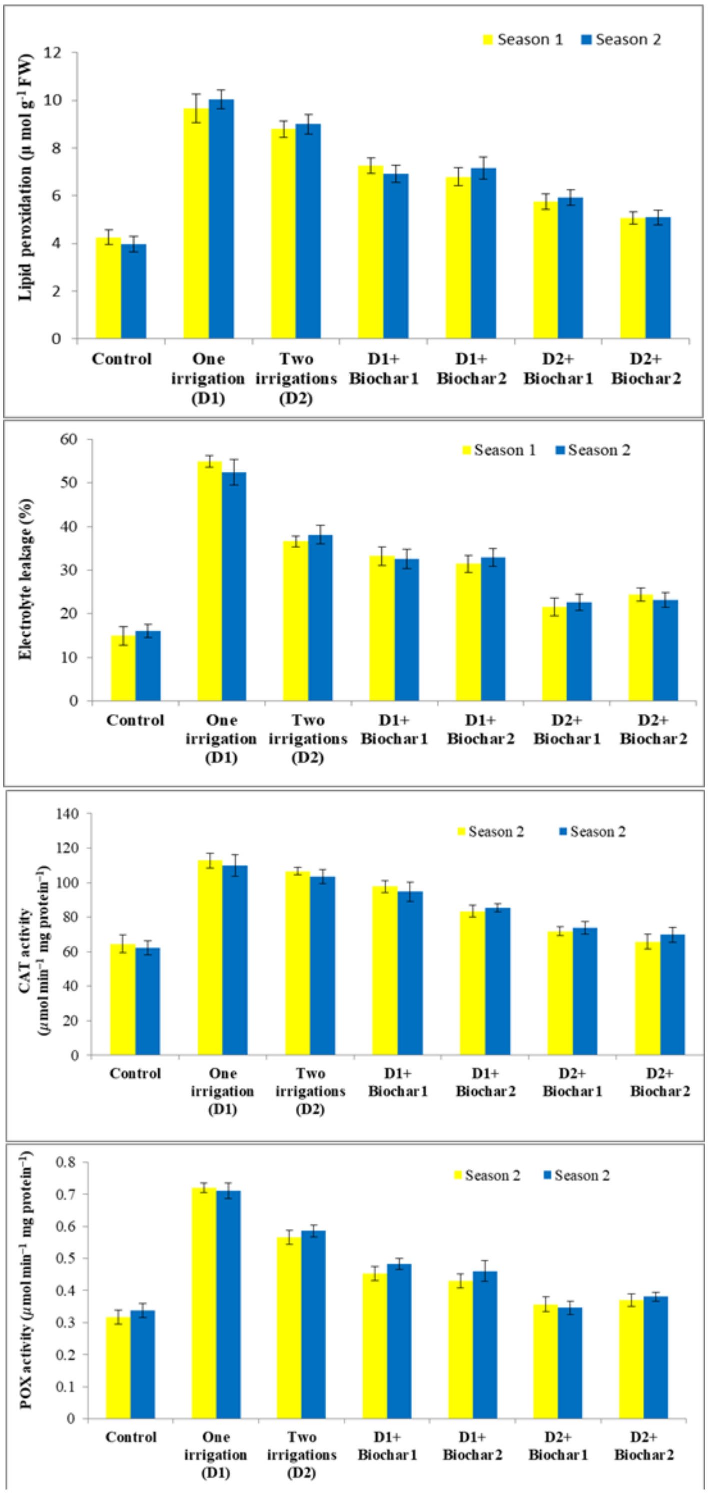

Figure 3. Effect of biochar on lipid peroxidation, electrolyte leakage, catalase, and peroxidase activity in barley under drought during two seasons (2019/2020 and 2020/2021)
(D1). One irrigation (D2). Two irrigations.

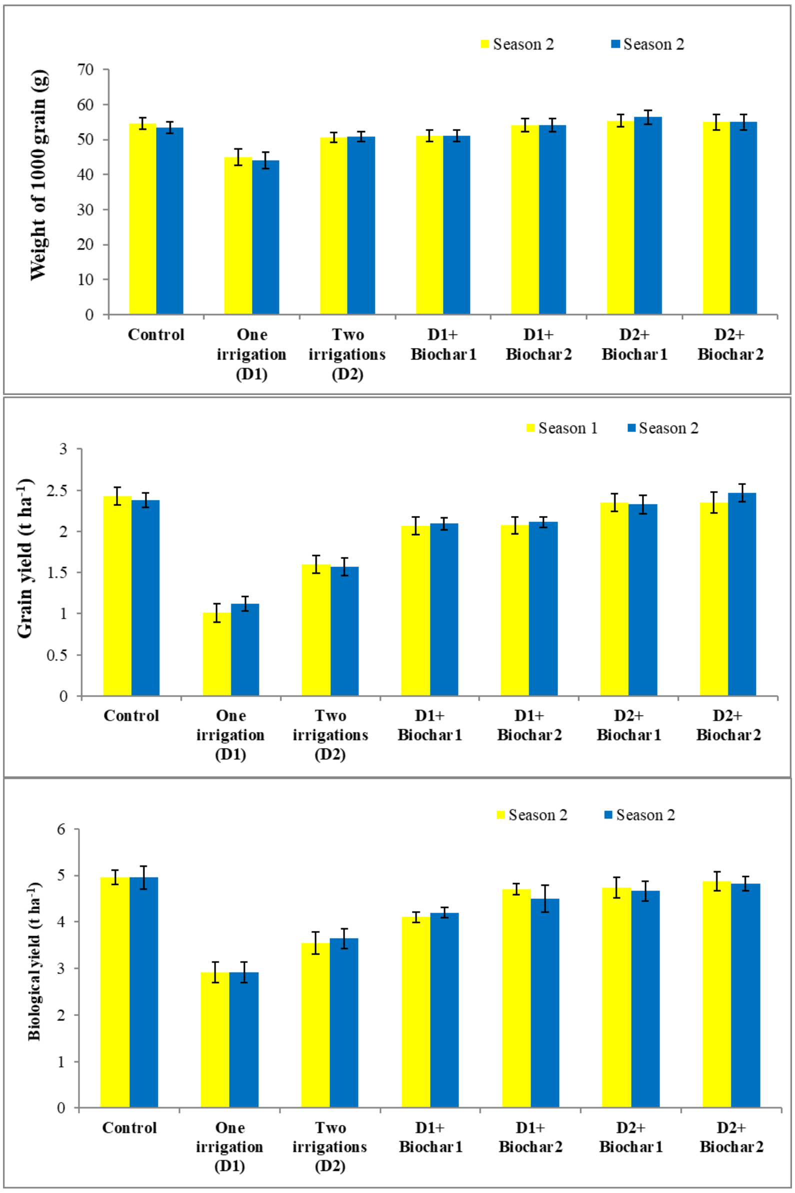

Figure 4. Impact of biochar on 1000 grain weight (a), grain yield t ha-1 (b) and biological yield t ha-1 (c) in barley under drought during two seasons (2019/2020 and 2020/2021). (D1). One irrigation (D2). Two irrigations

Effect of biochar on soil analysis

Soil chemical properties mainly, EC (dSm^{-1}), soil organic matter %, and soil pH, are essential factors for nutrient status in soil. The obtained results in Figure 5A showed that the soil EC was decreased significantly ($P \leq 0.05$) under drought stress, especially the soil irrigated one time; nevertheless, biochar application led to improve soil EC, and the best results were recorded with the soil of D2+Biochar2 treatment (0.19) compared with control (0.14). The observed changes in soil organic matter have been presented in Fig. 5B and showed a significant reduction in soil organic matter due to drought stress in the soil with D1 and D2 treatments compared with control.

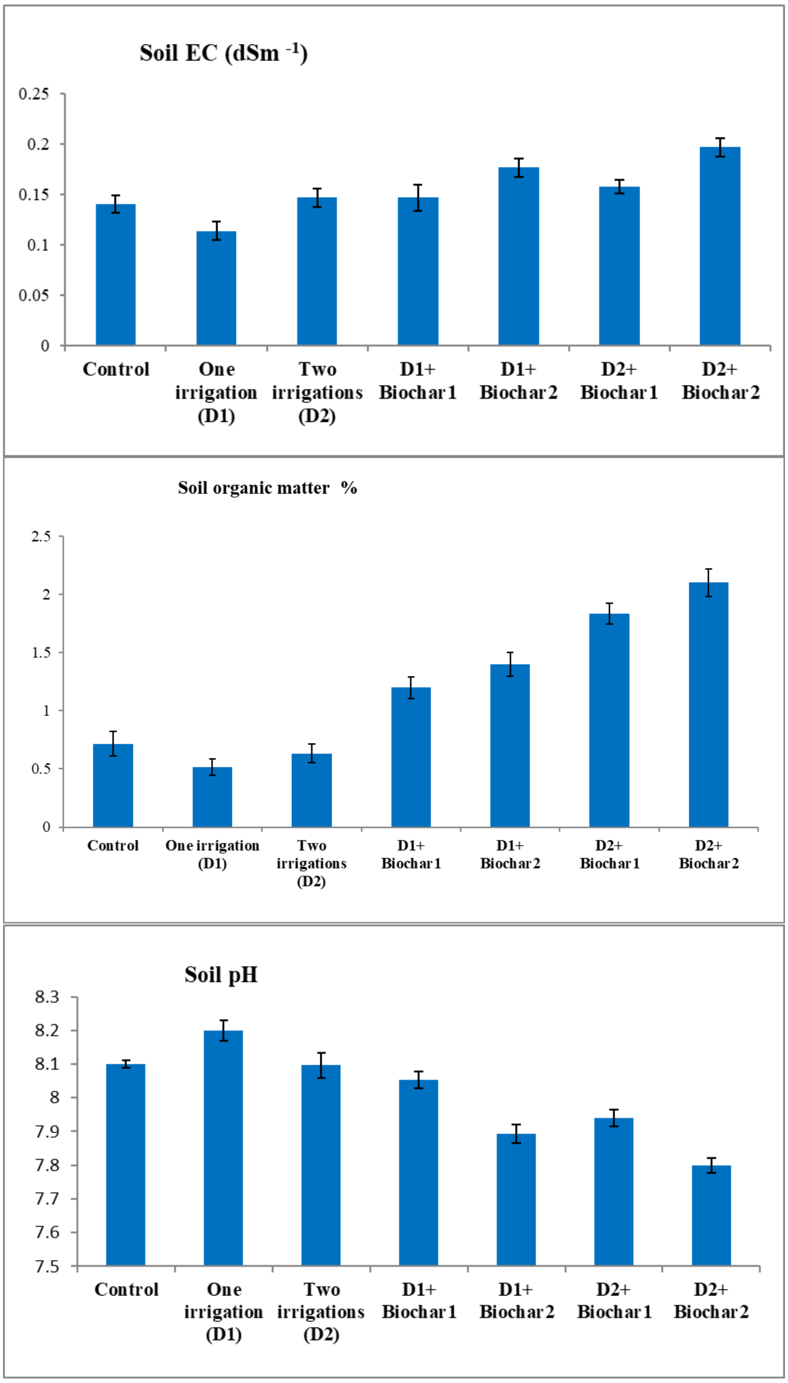

Figure 5. Impact of biochar on soil EC (a), soil organic matter (b), and soil pH (c) during 2020/2021 season. (D1). One irrigation (D2). Two irrigations

Additionally, soil organic matter was improved due to biochar application in all treatments, and the best one was D2+Biochar 2 (2.15%). Interestingly, soil pH was significantly increased ($P \leq 0.05$) under drought conditions (8.2) in comparison to control treatment (8.1). However, a significant decrease in soil pH was observed according to all biochar treatments (Figure 5C). Application of biochar led to mitigate the negative effect of drought by decreasing soil pH in stressed treated plants with D2+Biochar2 treatment (7.8) and D1+Biochar2 (7.9).

Discussion

Drought can damage plant development and production because of its negative effect on morphological and physiological characters. Our results displayed a significant reduction in leaves number, leaf area, stem height, chlorophyll a, b and RWC% in stressed barley plants during both seasons. This reduction could be due to the decrease in soil solution and the sup-pression of root nutrient uptake, which resulted in decrease cell elongation and performance, accordingly decrease leaves number, leaf area, and RWC% (Abdelaal, 2015). Also, chlorophyll concentrations reduction could be due to the detrimental effect of water deficit on chloroplast structure, thylakoids, and specific enzymes in the photosynthetic process, which decrease assimilation of carbon, inhibit the photosynthetic process, and decrease chlorophyll concentrations (Boyer, 1988; Yang *et al.,* 2012). Contrariwise, proline content was increased significantly in D1 and D2 treatment because of proline is a signal for many stresses like water deficit. Additionally, proline plays a vigorous role against various stresses; this result was in agreement with those recorded by Abdelaal *et al.* (2020a). Nevertheless, the application of Biochar1 or Biochar2 was shown to improve the root osmoregulatory capacities, which led to increased water absorption. Also, biochar treatments can play an important role in increasing the elements in the soil and improve soil structure and quality consequently, increase mor-phological parameters like leaf area and RWC (El Nahhas *et al.*, 2021). This result was similar to the result of Sanchez-Monedero *et al.* (2018) and Wei *et al.* (2020). Furthermore, concentrations of chlorophyll a and b were noticeably increased with biochar treatments in stressed plants; this result may be due to the beneficial effect of biochar in improving soil structure, water absorption, stem height, leave number, and RWC%, consequently increase chlorophyll a and b. Contrary, proline content was reduced significantly in stressed plants under biochar treatment; this reduction in proline could be due to the defensive role of biochar in plants and improved growth characteristics against water deficit (Hafez *et al.,* 2020). Drought consider-ably increased MDA and EL; this increase was more noticeable in the D1 treatment followed by D2 treatment compared to the control (Figure 3). This detrimental effect of water deficit in increasing MDA and EL could be due to the impact of water deficit in increasing ROS formation, which impairs plant macromolecules such as lipids and proteins and causes metabolism disorder, consequently increases MDA. Likewise, EL was increased noticeably under water deficit because of the deleterious effect on membrane stability which led to increased EL% in barley (Ghorbanpour *et al.*, 2020). Furthermore, catalase and peroxidase activity were significantly increased as a result of water deficit in D1 and D2 treatments, this increment in enzymes activity is a normal defence system to cope with the ROS, which accumulated under stress. However, the stressed-treated plants with biochar showed moderate levels of peroxidase and catalase activity compared to control; these results are in agreement with the results of Hafez *et al.* (2020) and Wang *et al.* (2016).

In the current study, drought caused a significant reduction in yield parameters mainly, grain yield, biological yield, and 1000 grain weight in barley during two seasons (Figure 4); this injurious impact may be due to that water deficit impair plant growth such as leaves number, leaf area and chlorophyll a and b concentrations, consequently decrease 1000 grain weight, biological yield, and grain yield. This damaging effect of water deficit was regular with the findings of Abdelaal (2015) and Abdelaal *et al.* (2017). Conversely, biochar treatments led to a significant increase in grain yield, biological yield, and 1000 grain weight in stressed plants, this improvement effect of biochar may be due to the effective role in enhancement growth characteristics and photosynthetic process because it is a rich source of essential elements such as nitrogen and carbon resulted in increase the aforementioned yield parameters in barley (Hafez *et al.*, 2020). Our findings indicated that soil EC (dSm^{-1}), soil organic matter %, and soil pH showed a different response to biochar treatments under drought conditions. Drought stress caused a significant decrease in the soil EC and soil organic matter; however, soil pH was increased significantly due to drought conditions. This decrease in soil EC and soil organic matter may be due to the effect of drought on water availability in soil, also, drought caused a change in the composition of Soil organic carbon (SOC), reducing the non-hydrolyzed carbon and carbon sources. Interestingly enough, drought hurts bacterial community structure, and carbon application forms consequently cause a contrary effect on soil EC, soil organic matter (Su *et al.*, 2020). On the other hand, biochar application can alleviate drought stress by increasing the water holding capacity of soil through improving soil physio-chemical characters which, increase water availability in soil. Also, biochar plays a significant role in the availability of P solubilizing bacteria (Qayyum *et al.*, 2021), keep and stabilize the soil under abiotic stresses conditions (Rafique *et al.*, 2020), enhancing nutrient uptake (Rehman *et al.,* 2021) and reduced nutrient leaching in some soils (Haider *et al.,* 2016). The positive role of biochar may be due to that biochar can induce indigenous jasmonic acid biosynthesis, which results in improved plant status under different stresses (Waqas *et al.,* 2018). Biochar + JA application led to enhance the faba bean plant performance through several mechanisms (El Nahhas *et al.,* 2021). They decrease chlorophyll degradation and enhance photosynthesis and carbon fixation as well as regulate the mineral hemostasis in soil (Jaiswal *et al.*, 2020; Wang *et al.*, 2020).

Conclusions

It can be concluded that application of biochar caused a significant increase in growth and yield parameters of drought stressed barley plants. The results showed that leaf area and leaves number significantly reduced; also, chlorophyll, RWC%, and grain yield, as well as yield parameters considerably decreased in stressed barley plants. Nevertheless, MDA and EL% as a negative indicator of drought noticeably increased in the stressed plants during both seasons. Contrariwise, biochar treatment positively affected growth parameters of barley such as leaves number, RWC%, chlorophyll concentration, and yield parameters during both seasons. Moreover, biochar treatments led to enhance and regulate catalase and peroxidase activity in the stressed plants, enhances the soil EC (dSm^{-1}) and soil organic matter %. It could be concluded that biochar treatment may alleviate the injurious impact of drought on barley plants and on soil properties.

Authors' Contributions

Conceptualization, Kh.A.A, K.At. methodology, Kh.A.A, M.E, N.El., A.Ab.; software, Kh.A.A, Y.H., S.Al., N.El.; validation, Kh.A.A, Y.H.; investigation, Kh.A.A.; resources, Kh.A.A, Y.M., N.El.; data curation Kh.A.A, Y.M.; writing—original draft preparation, Kh.A.A, M.E., K.At., N.El.; writing—review and editing, Kh.A.A., M.El., S.Al., N.El., A.Ab.; funding acquisition, S.Al., Kh.A.A., K.At., A., Ab.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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