Integral effects of brassinosteroids and timber waste biochar enhances the drought tolerance capacity of wheat plant

Lalarukh, Irfana

2022-07-27

Lalarukh , I , Amjad , S F , Mansoora , N , Al-Dhumri , S A , Alshahri , A H , Almutari , M M , Alhusayni , F S , Al-Shammari , W B , Poczai , P , Abbas , M H H , Elghareeb , D , Kubra , K T & Abdelhafez , A A 2022 , ' Integral effects of brassinosteroids and timber waste biochar enhances the drought tolerance capacity of wheat plant ' , Scientific Reports , vol. 12 , no. 1 , 12842 . https://doi.org/10.1038/s41598-022-16866-0

http://hdl.handle.net/10138/347777
https://doi.org/10.1038/s41598-022-16866-0

scientific reports

OPEN

Integral effects of brassinosteroids and timber waste biochar enhances the drought tolerance capacity of wheat plant

Irfana Lalarukh[1], Syeda F. Amjad[2]✉, Nida Mansoora[2], Sami A. Al-Dhumri[3], Abdullah H. Alshahri[4], Mohammad M. Almutari[5], Fatimah S. Alhusayni[6], Wasimah B. Al-Shammari[7], Peter Poczai[8]✉, Mohamed H. H. Abbas[9], Doaa Elghareeb[10,11], Khadija tul Kubra[12] & Ahmed A. Abdelhafez[13,14]✉

Drought stress is among the major threats that affect negatively crop productivity in arid and semi-arid regions. Probably, application of some additives such as biochar and/or brassinosteroids could mitigate this stress; however, the mechanism beyond the interaction of these two applications is not well inspected. Accordingly, a greenhouse experiment was conducted on wheat (a strategic crop) grown under deficit irrigation levels (factor A) i.e., 35% of the water holding capacity (WHC) versus 75% of WHC for 35 days while considering the following additives, i.e., (1) biochar [BC, factor B, 0, 2%] and (2) the foliar application of 24-epibrassinolide [BR, factor C, 0 (control treatment, C), 1 (BR1) or 3 (BR2) µmol)]. All treatments were replicated trice and the obtained results were statistically analyzed via the analyses of variance. Also, heat-map conceits between measured variables were calculated using the Python software. Key results indicate that drought stress led to significant reductions in all studied vegetative growth parameters (root and shoot biomasses) and photosynthetic pigments (chlorophyll a, b and total contents) while raised the levels of oxidative stress indicators. However, with the application of BC and/or BR, significance increases occurred in the growth attributes of wheat plants, its photosynthetic pigments, especially the combined additions. They also upraised the levels of enzymatic and non-enzymatic antioxidants while decreased stress indicators. Furthermore, they increased calcium (Ca), phosphorus (P) and potassium (K) content within plants. It can therefore be deduced that the integral application of BR and BC is essential to mitigate drought stress in plants.

Drought stress possesses a potential threat that hampers plant growth and productivity in arid and semiarid regions[1,2]. This stress has adverse effects on photosynthetic machinery, particularly disrupting thylakoid electron transport, stomatal conductance, CO_2 assimilation and Calvin cycle[3–5]. Moreover, drought stress interrupts the balance between antioxidative defense mechanisms and production of reactive oxygen species (ROS) causing ROS accumulation and this, consequently, disorganize cell membrane lipids, protein and DNA strands[6]. Accordingly,

[1]Department of Botany, Government College Women University Faisalabad, Faisalabad 38000, Pakistan. [2]Department of Botany, University of Agriculture Faisalabad, Faisalabad 38000, Pakistan. [3]Department of Biology, Al Khurma University College, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia. [4]Civil Engineering Department, College of Engineering, Taif University, P.O. Box 888, Taif 21974, Saudi Arabia. [5]Ministry of Environment, Water and Agriculture, Riyadh 11442, Saudi Arabia. [6]Ministry of Environment, Water and Agriculture, Saudi Arabia, Ministry of Environment, P.O. Box 7878, Riyadh 11195, Saudi Arabia. [7]Department of Biology, College of Science, University of Hail, P.O. Box 2440, Hail 55476, Saudi Arabia. [8]Botany Unit, Finnish Museum of Natural History, University of Helsinki, 00014 Helsinki, Finland. [9]Department of Soils and Water, Faculty of Agriculture, Benha University, Benha, Egypt. [10]Department of Biology, Jumum College University, Umm Al-Qura University, P.O Box 7388, Makkah 21955, Saudi Arabia. [11]Agriculture Genetic Engineering Research Institute (AGERI), Agriculture Research Centre, Giza, Egypt. [12]Department of Agronomy, Faculty of Sciences, University of Agriculture Faisalabad, Faisalabad, Pakistan. [13]Department of Soils and Water, Faculty of Agriculture, New Valley University, Kharga, Egypt. [14]National Committee of Soil Sciences, Academy of Scientific Research and Technology, Cairo, Egypt. ✉email: fasihamushadi75@gmail.com; peter.poczai@helsinki.fi; ahmed.aziz@agr.nvu.edu.eg

Components	Values
pH*	7.5
EC**	7.82 dS m^{-1}
Organic matter content	14.2 g kg^{-1}
Available- P	6.133 mg kg^{-1}
Available-N	190 mg kg^{-1}
Available- K	33.4 mg kg^{-1}

Table 1. Soil component analysis. *pH was determined in 1:2.5 (soil:water) suspension, **EC was determined in soil paste extract.

plants have developed a wide range of adaptive processes during their evolution, including physiological, morphological and biochemical mechanisms allowing a proper response to water scarcity, e.g., increasing the phytohormonal signaling pathways in response of external abiotic stress[7].

Different safe approaches have been introduced for increasing plant tolerance to drought stress. For example, brassinosteroids (BR) are polyhydroxylated steroidal phytohormones, which regulates numerous processes of plant physiology and morphogenesis starting from seed germination up to the regulation of flowering and senescence[8]. Moreover, BR are involved in controlling abiotic stress responses[9] via: (1) increasing activities of antioxidative enzymes[10]; hence, lessening the production rate of superoxide anion[11], (2) reducing abscisic acid (ABA) accumulation[12] in spite of that these hormones increase stomatal closure under drought conditions[10,13] and (3) increasing the osmotic permeability of root cells to take up more water from soil.

Biochar is another promising additive that can mitigate the adverse effects of drought on plant growth and productivity, e.g., sorghum[14], maize[15] and wheat[2,16]. This might take place via (I) increasing soil water retention, hence reducing soil irrigation demands[17], (II) increasing nutrient use efficiency[18], (III) stimulating auxin, gibberellin and brassinosteroids regulation[19] and (IV) increasing plant chlorophyll content[20], stomatal conductance, cytotoxicity and leaf K^+ content[21]. This cost-effective carbon rich product is formed through the pyrolysis of organic residues under limited oxygen supply[22,23] to give a product of high porous aromatic carbon content[24]. It is worth mentioning that this product retains longer in soil versus other organic residues such as compost; hence mitigate climate changes through carbon sequestration[20,25].

In the next years, significant increases in global crop products will be required to fulfill the increasing human and animal food demands[26]. Wheat is among the most important crops for global food security[27] which contributes up to 40% to the world food demand. Its global production is estimated by 757.4 million tons in the year of 2019[28]. Although, the modern high yielding wheat cultivars are introduced to increase wheat productivity[29] and these cultivars consume massive amounts of mineral fertilizers[30]; yet, appropriate techniques are also needed to maximize nutrient utilization by plants in order to sustain the available limited resources. For example, nitrogen use efficiency by wheat does not exceed 33% worldwide[28] and others nutrients mostly do not exceed 50%. Under drought conditions, nutrient use efficiency decreases considerably[31] and this may cause further reductions in plant productivity. In this investigation, we tested the feasibility of using the combined application of exogenous BR and BC as potential safe approaches to mitigate drought stress via increasing plant capability to utilize soil nutrients as well as the enzymatic and non-enzymatic antioxidants; hence this combined application might increase considerably crop yield productivity under drought conditions. Although, many studies highlighted the positive effects of the sole application of each of exogenous BR and BC on plant growth; yet, the implications of this combined application on increasing plant tolerance to stress conditions is not so far investigated.

The current study is a trial to investigate the potential co-application ameliorative impacts of timber waste biochar as a soil amendment and exogenous brassinosteroids as foliar application to alleviate the stress conditions on wheat plants subjected to deficit irrigations. Specifically, we hypothesized that this combined application could effectively mitigate drought stress effects on wheat than applying each solely; consequently, the productivity of treated plants increased considerably under such adverse conditions. Wheat was selected as an experimental model plant in this study, following guidelines for testing chemicals by the Organization for Economic Cooperation and Development (OECD) guideline 208.

Materials and methods

Materials of study. Surface soil samples (0–30 cm) were collected from the experimental farm of Government College Women University Faisalabad, then air dried, crashed and sieved to pass through a 2-mm sieve. Soil characters were investigated according to Sparks et al.[32] and the results are presented in Table 1.

Wheat seeds (*Triticum aestivum* L., cv. Lasani-2008) were obtained from certified seed dealer of the Government of Punjab, Pakistan. All seeds were disinfected with 95% ethanol followed by 70% sodium hypochlorite solution washing, then washed three times with distilled water. The use of plants in the present study complies with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

For production of timber waste biochar, timber wastes were collected from the regional timber market. These wastes were sun-dried for a week and then pyrolyzed at 390 °C for 80 min in a pyrolizer. Afterwards, timber waste biochar (BC) was crushed in a grinder and sieved through a 2 mm sieve and the fine residue was kept for the experimental procedure. Some major timber waste biochar properties are presented in Table 2.

Component	Value
pH (1:10) suspension	7.01
Ash contents	17.0 g kg^{-1}
C	863.0 g kg^{-1}
H	26.9 g kg^{-1}
N	3.8 g kg^{-1}
K	16.0 g kg^{-1}
P	6.0 g kg^{-1}

Table 2. Characterization of timber waste biochar.

Plant material, experimental design and growth conditions. A pot experiment was conducted at the Botanical Garden, Government College Women University Faisalabad, Pakistan to test the hypothesis of the study. In this experiment, soil portions equivalent to 8 kg soil were mixed thoroughly with one of the following treatments: 0% (control, no timber waste biochar) and 2% biochar (equivalent to 160 g biochar per 8 kg of soil) were packed uniformly in plastic pots (28 cm diameter × 20 cm height). These pots were arranged in a complete randomized block design, and each treatment was replicated trice. Seven seeds were sown in each pot, and five healthy seedlings were left by thinning at 15 days after planting till the end of the incubation period (35 days after planting). All plants were watered optimally at 75% WHC (75% of the water holding capacity) until three weeks of sowing; thereafter one group of pots (drought-stressed) was watered at only 35% WHC (35% of the water holding capacity), while the other group was watered at 75% of WHC. Plants were sprayed trice with 24-epi-brassinolide ($C_{28}H_{48}O_6$ having Mw = 480.7 sourced from Sigma-Aldrich) at either 0 (distilled water), 1 (BR1) or 3 (BR2) µmol concentrations per pot starting from the third week after sowing with one day interval between foliar applications using a hand sprayer at the rate of 50 mL per plant.

Plant harvesting and growth attributes. Plants were harvested at 35 days of sowing and the fresh weights of their roots and shoots were determined immediately using a digital weighing balance. Root and shoot lengths were recorded using the measuring tape. Three plant samples were selected randomly from each pot, washed with tape water then deionized water to remove the stunted dirt afterwards, oven-dried at 65 °C for 72 h to determine their dry weights. Other fresh materials were stored at − 30 °C for fresh analysis.

Chlorophyll contents. The chlorophyll a, b and total contents as well as carotenoids pigments were estimated in leaves following the Arnon protocol[33] i.e. 0.1 g fresh leaf sample was placed in 8 mL of 95% acetone then incubated overnight at 4 °C. Color intensity was recorded at 646, 663 and 450 nm using spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan).

H_2O_2 content. Estimation of H_2O_2 contents was achieved following Mukherjee and Choudhari technique[34]. In this method, 0.1 g leaf sample was extracted in 10 mL cold acetone, centrifuged at 10,000 rpm, then 4 mL titanium reagent and 5 mL of concentrated ammonium solution were added to the reaction mixture. The mixture was then centrifuged at 10,000 rpm for 5 min and the precipitate was dissolved in 10 mL of 2 N H_2SO_4. The residue was again centrifuged to remove suspended particles. Optical density was recorded at 415 nm against blank by spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan).

Measurement of malondialdehyde and electrolyte leakage. Chloroplast's lipid peroxidation was determined by estimating malondialdehyde (MDA) contents following thiobarbituric acid (TBA) reaction by Heath and Packer method[35]. The electrolyte leakage (EL) was determined following Anjum et al.[36] protocol.

Estimation of proline and non-enzymatic antioxidants. To estimate osmolytes, i.e., proline and other non-enzymatic antioxidants, 50 mg dried plant samples were extracted in 10 mL ethanol (80%), then filtered followed by re-extraction in ethanol (10 mL). A final volume of 20 mL was maintained by mixing the two samples. The obtained extracted solution was used to estimate proline[37], flavonoids and anthocyanin[38], phenolics[39], ascorbic acid[40], proteins[41] and glycine betaine[42] contents.

Ca, Na, P and K ion concentrations. In the same extract, Ca, Na, P and K ion concentrations were estimated as follows: molybdate/ascorbic acid blue method was used for P determination[43] then measured by spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan). K ion concentrations in plant extracts were measured by flame photometer while Ca and Na concentrations in these extracts were estimated using Atomic Absorption Spectrum (AAS; Shimadzu instruments, Inc., Spectra AA-220, Kyoto, Japan).

Statistical analysis. Statistical analysis was conducted by using the analysis of variance to find significance of applied treatments in drought stress. All the treatment means were compared by Dunken's test at 5% level of significance ($P < 0.05$). Logarithmic transformations for data normalization were carried out before analysis,

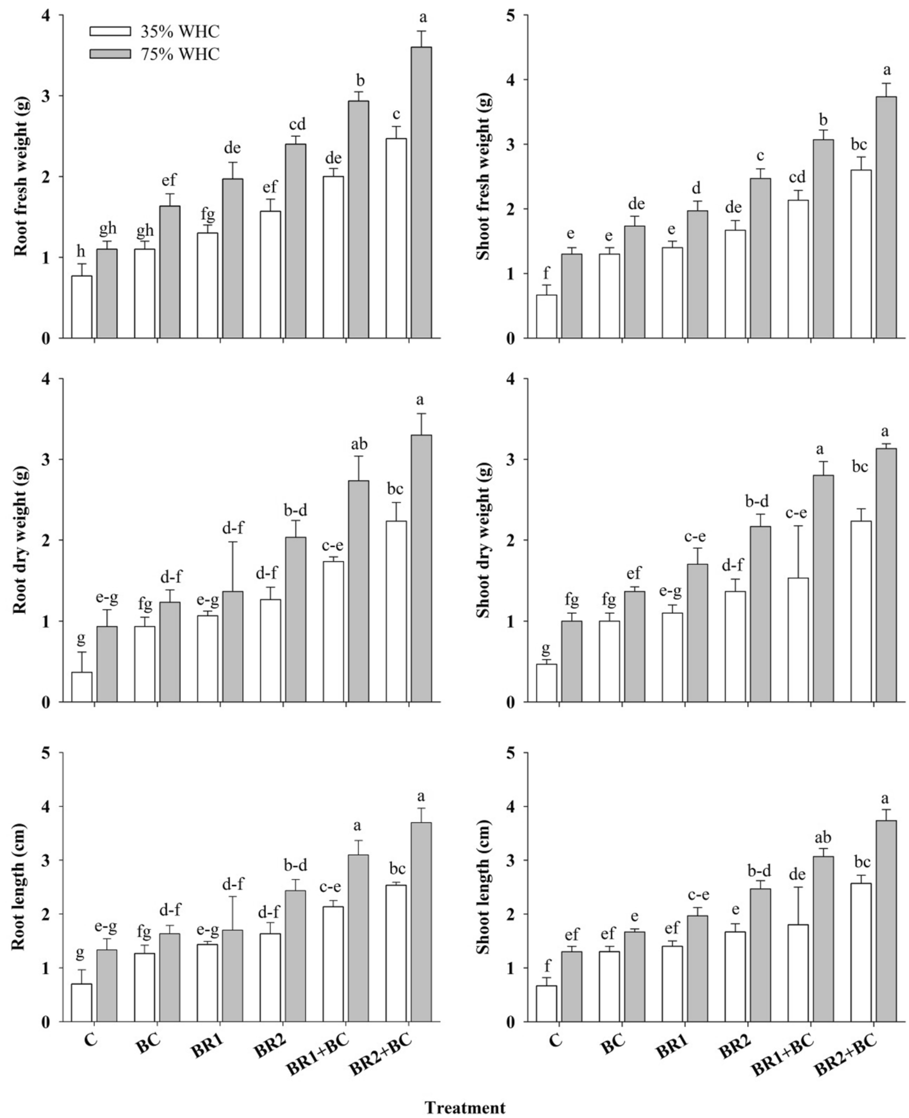

Figure 1. Efficacy of sole and combined application of BR and BC on enhancing different growth parameters of wheat under 35% WHC and 75% WHC. Bars show means of three replicates. Different error bars represent SD. Different letters indicate significant difference at $p \leq 0.05$; LSD Test (*75 WHC* well-watered, *35 WHC* drought stress, *C* control, *BR1* 1 µmol foliar applied 24-epibrassinolide, *BR2* 3 µmol foliar applied 24-epibrassinolide, *BC* timber waste biochar).

where necessary. Pearson's correlation analysis was performed to compute associations among various analyzed variables. The heat-map conceits between measured variables were also calculated by using the Origin software.

Results

Growth parameters. Root and shoot (fresh and dry) weights of wheat plants subjected to deficient irrigations (35%) WHC were significantly lower than the corresponding ones that received 75% WHC (Fig. 1). Likewise, drought stresses affected significantly and negatively root and shoot lengths.

The abovementioned growth parameters were improved significantly owing to BR and/or BC applications versus the control. In this concern, the foliar application of BR raised significantly plant growth parameters exceeding those attained for the application of biochar (BC), especially with increasing the level of BR application. On the other hand, the application of biochar did not noticeably improved plant growth parameters under deficit irrigation (35% WHC) versus the control (except for root fresh weight).

The combination of BR and BC caused further significant increases in plant growth parameters, especially in presence of the higher application dose of BR (3 µmol). It is worth mentioning that the usage of BR + BC with 35% WHC considerably enhanced root and shoot fresh weights, dry weights and lengths of wheat plants exceeding those recorded for the control plants that were irrigated with 75% WHC. These results probably signify the value of these amendments in increasing the efficiency of water use by the grown plants.

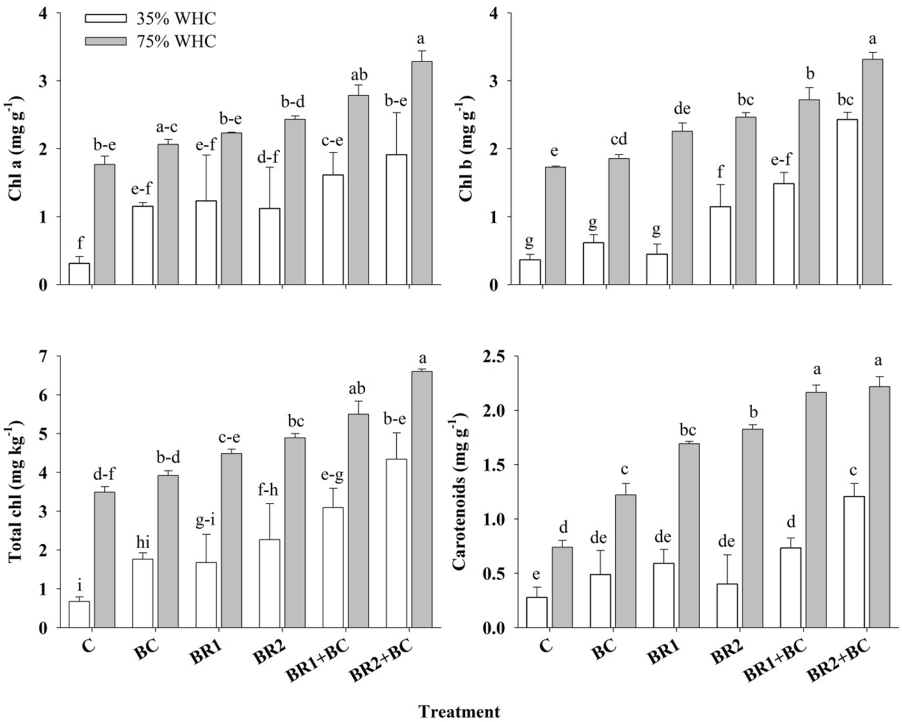

Figure 2. Role of sole and combined application of BR and BC on photosynthetic pigments contents of wheat under 35 WHC and 75 WHC. Bars show means of three replicates. Different error bars represent SD. Different letters indicate significant difference at p ≤ 0.05; LSD Test. (*75 WHC* well-watered, *35 WHC* drought stress, *C* control, *BR1* 1 µmol foliar applied 24-epibrassinolide, *BR2* 3 µmol foliar applied 24-epibrassinolide, *BC* timber waste biochar).

Photosynthetic pigments. Deficit irrigations negatively affected chlorophyll a, b and the total contents as well as plant carotenes (Fig. 2) i.e., all these parameters were significantly lower in plants subjected to drought stress at 35% WHC in comparison to those irrigated with 75% of WHC. Although, BC and BR1 treatments raised these pigments in plants subjected to drought versus the control; yet such increases seemed to be insignificant. In contrast, BR2 treatments raised significantly plant contents of photosynthetic pigments versus the control,. The combination of these two treatments (BR + BC) furtherly improved these photosynthetic pigments, specifically when using the highest application level of BR (3 µmol). These results also highlight the significance of these amendments as effective safe approaches to mitigate the drought stress conditions.

Oxidative stress indicators, proteins and ionic contents. Nutrient (K, P and Ca) contents increased significantly in plants tissues owing to irrigation with water to achieve 75% of WHC comparable with the ones that were irrigated to reach only 35% of WHC (Table 3). On the other hand, oxidative stress indicators increased significantly in plants subjected to drought stress, i.e., MDA, antioxidant enzyme activities, H_2O_2, electrolyte leakage and Na content increased under such conditions. Table 3 also revealed significant increases in glycine betaine, proline, phenolics, ascorbic acid, anthocyanins and flavonoids contents in plants subjected to drought stress compared with the corresponding ones in non-stressed ones.

All additives (BR and BC) showed positively significant improvements in ameliorating drought stress on plants via decreasing MDA and oxidative stress indicators. Such improvements were more detectable with increasing the dose of BR foliar application. Also, these additives decreased considerably H_2O_2 and Na (sodium) contents beside of the electrolyte leakage, while increased proteins, Ca (calcium), K (potassium) and P (phosphorous) in plant tissues. It seems that the sole applications of either BR or BC were superior in decreasing these oxidative stress indicators than their integral application. Moreover, all applied treatments decreased significantly all enzymatic and non-enzymatic antioxidants under investigation (glycine betaine, proline, phenolics, ascorbic acid, anthocyanin, flavonoids) and this probably signify the success of these treatments in ameliorating drought stress effects. In this concern, BR2 + BC recorded the least enzymatic and non-enzymatic enzymes antioxidants activities.

Pearson correlation. Pearson correlation analyses were conducted to highlight the relationships between plant growth parameters and the increases that took place in plant oxidative stress indicators grown under drought conditions. As mentioned above, significant improvement in plant growth parameters, i.e., root and shot weights and lengths was observed due to the applications of BR and BC. Root and shoot (fresh and dry) weights of wheat plants subjected to deficient irrigations at 35% WHC decreased significantly versus the ones that received 75% WHC (Fig. 1).

Treatment	Na	Ca	K	P	MDA	H_2O_2	EL	Antho	TSP	Flav	Phen	AsA	GB	Pro
Main effect of treatments														
C	18.42 ± 5.26a	3 ± 0.71c	3.33 ± 1.08b	8.5 ± 5.24a	3.11 ± 0.35a	3.15 ± 0.3a	24.5 ± 3.62a	3.2 ± 0.27a	1.27 ± 0.58d	3.21 ± 0.18a	3.19 ± 0.28a	26 ± 1.79a	3.18 ± 0.28a	3.17 ± 0.26a
BC	17.08 ± 4.44a	4.25 ± 1.54bc	4.58 ± 2.31b	9.83 ± 5.12a	2.57 ± 0.39b	2.56 ± 0.45b	19.67 ± 1.37ab	2.52 ± 0.39bc	1.64 ± 0.39 cd	2.59 ± 0.42b	2.52 ± 0.37b	20.17 ± 2.48bc	2.52 ± 0.38b	2.54 ± 0.44b
BR1	15.83 ± 3.53a	3.97 ± 1.85bc	5.33 ± 1.08ab	11.33 ± 7.23a	2.6 ± 0.25b	2.6 ± 0.2b	17.17 ± 3.97b	2.65 ± 0.23b	2.09 ± 0.41c	2.63 ± 0.22b	2.67 ± 0.23ab	21.5 ± 5.47b	2.66 ± 0.21ab	2.62 ± 0.19b
BR2	16.5 ± 3.66a	6.17 ± 2.29ab	6.17 ± 2.23a	12.17 ± 6.79a	2.2 ± 0.5b	2.17 ± 0.59b	19 ± 7.46ab	2.11 ± 0.62 cd	2.21 ± 0.36c	2.14 ± 0.63bc	2.11 ± 0.62bc	16.67 ± 4.41 cd	2.11 ± 0.61bc	2.13 ± 0.62bc
BR1 + BC	14.17 ± 4.96a	5.25 ± 2.21abc	6.17 ± 2.71a	18.17 ± 14.34a	1.63 ± 0.46c	1.64 ± 0.46c	15.33 ± 2.73b	1.95 ± 0.48d	2.93 ± 0.58b	1.76 ± 0.35c	1.68 ± 0.5 cd	15.67 ± 4.08d	1.66 ± 0.48 cd	1.62 ± 0.45 cd
BR2 + BC	14.83 ± 5.42a	8 ± 4.11a	5.67 ± 1.99ab	21.17 ± 13.8a	1.21 ± 0.49c	1.22 ± 0.53c	16 ± 5.44b	1.11 ± 0.45e	3.57 ± 0.71a	1.23 ± 0.51d	1.19 ± 0.57d	15.5 ± 1.87d	1.21 ± 0.56d	1.22 ± 0.5d
Main effect of stress														
35 WHC	19.75 ± 2.96a	3.43 ± 1.11b	4.58 ± 1.62a	6.06 ± 2.53b	2.45 ± 0.71a	2.47 ± 0.72a	20.28 ± 4.5a	2.53 ± 0.65a	1.85 ± 0.74b	2.51 ± 0.64a	2.47 ± 0.72a	18.72 ± 5.62a	2.46 ± 0.7a	2.45 ± 0.71a
75 WHC	12.53 ± 2.28b	6.78 ± 2.88a	5.83 ± 2.39a	21 ± 1.90a	1.99 ± 0.74a	1.98 ± 0.75a	16.94 ± 5.46a	1.98 ± 0.8b	2.72 ± 0.88a	2.01 ± 0.8b	1.98 ± 0.8a	19.78 ± 4.58a	1.98 ± 0.8a	1.98 ± 0.78a
ANOVA														
F-values	16.14	5.11	5.21	13.53	2.22	2.22	18.61	2.26	2.29	2.26	2.22	19.25	2.22	2.22
CV	27.85	53.64	40.49	73.87	33.93	34.44	28.00	34.12	40.07	33.39	35.42	26.41	35.04	34.82

Table 3. Oxidative stress indicators, proteins and ionic contents of wheat plant grown under drought stress conditions after 35 days of seedlings. All values are the means of three replicates ± standard error (SD). Different labels showed significant different alphabets using LSD test. *75 WHC* well-watered, *35 WHC* drought stress, *C* control, *BR1* 1 μmol foliar applied 24-epibrassinolide, *BR2* 3 μmol foliar applied 24-epibrassinolide, *BC* timber waste biochar, *Na* sodium, *Ca* calcium, *K* potassium, *P* phosphorous, *MDA* malondialdehyde, H_2O_2 hydrogen peroxide, *EL* electrolyte leakage, *Anth* anthocyanin, *TSP* total soluble proteins, *Flav* flavonoids, *Phen* phenolics, *AsA* ascorbic acid, *GB* glycine betaine, *Pro* proline.

Generally, the different growth parameters of wheat plants i.e. root and shoot (fresh and dry weights) and their lengths were significantly and positively correlated with each other (Fig. 3). Improvements in these growth parameters were positively correlated with plant nutritive status (Ca, K and P) and also with the leaf pigment contents (carotenoids, chlorophyll a, b and the total contents), while negatively correlated with the increases that took place in plant oxidative stress indicators i.e., MDA, antioxidant enzyme activities, H_2O_2, electrolyte leakage and Na content. Proline content in leaves also affected significantly and negatively all the studied growth parameters. It is worth noting that the oxidative stress indicators were correlated positively and significantly with proline in leaves and these stress parameters affected negatively pigment contents in plants (Fig. 3).

Discussion

The current study is a trial to alleviate drought stress conditions on wheat plants via the foliar application of brassinosteroids (BR) and/or timber waste biochar (BC) as a soil amendment.

Effect of drought on wheat plants. Wheat plants absorb and accumulate high concentrations of Na in their tissues; while contents of other nutrients such as Ca and P decreased considerably. Probably, this stress down-regulates genes encoding plasma membrane intrinsic proteins in plant roots in addition to plant hydraulic conductivity[44]. Potassium is a special case because its content did not vary significantly in tissues of stressed plants versus non-stressed ones. This is because its influx occurs via non-energetic transporters and gated channels which are highly specific for K ions[45]. Moreover, this nutrient is highly mobile in plant and acts directly in regulating the osmotic potential in compartments to increase their turgor[46]. Generally, the nutrient imbalance that occurred in drought-stressed plants may led to undesirable drop in plant pigments (chlorophyll A, chloro B, total chlorophyll and carotenoids) and photo-assimilates[16,47].

Significant increases in some oxidative stress indicators, namely anthocyanin and flavonoids occurred under drought conditions while total soluble protein contents diminished. Other mechanisms may be included to cope from drought stress such as regulating stomatal aperture to maintaining tissue hydration[44,48]. Overall, this stress affected negatively plant growth (fresh and dry weights of plants as well as the plant heights) and such a result was verified in many previous studies, e.g., Sankar et al.[49] and Hussain et al.[50].

Brassinosteroids and/or timber waste biochar as additives to alleviate drought stress. Application of either biochar or BR1 did not successfully increase nutrient contents within plant tissues and therefore the changes in plant pigments were not detectable versus the control. Likewise, the changes in root and shoot biomasses and heights owing to the sole application of these two treatments were not noticeable versus the control. Though, these additives lessened considerably all oxidative stress indicators such as malondialdehyde, hydrogen peroxide, electrolyte leakage via increasing the activities of antioxidant enzymes and non-enzymes. Such a result might indicate that plants synthesize these bioproducts to lessen the impacts of drought on plants prior to the synthesis of other metabolites needed for enhancing plant growth. Probably, radicals such as H_2O_2 works as signaling molecules under low concentrations; yet their accumulation within plant tissues in higher concentrations are toxic[51]. Further changes may takes place at both cellular and whole-organism levels, making drought stress tolerance a complex physiological phenomenon[52,53].

The combination between biochar and brassinosteroids (BR1 + BC and BR2 + BC) beside of the application of the higher dose of the latter additive i.e. BR2 recorded further significant further increases in both Ca and K uptake by plants (with superiority for the combined applications versus BR2); consequently their contents raised in plant tissues; hence plant pigments upgraded. In spite of that, these treatments were of no significant effect on

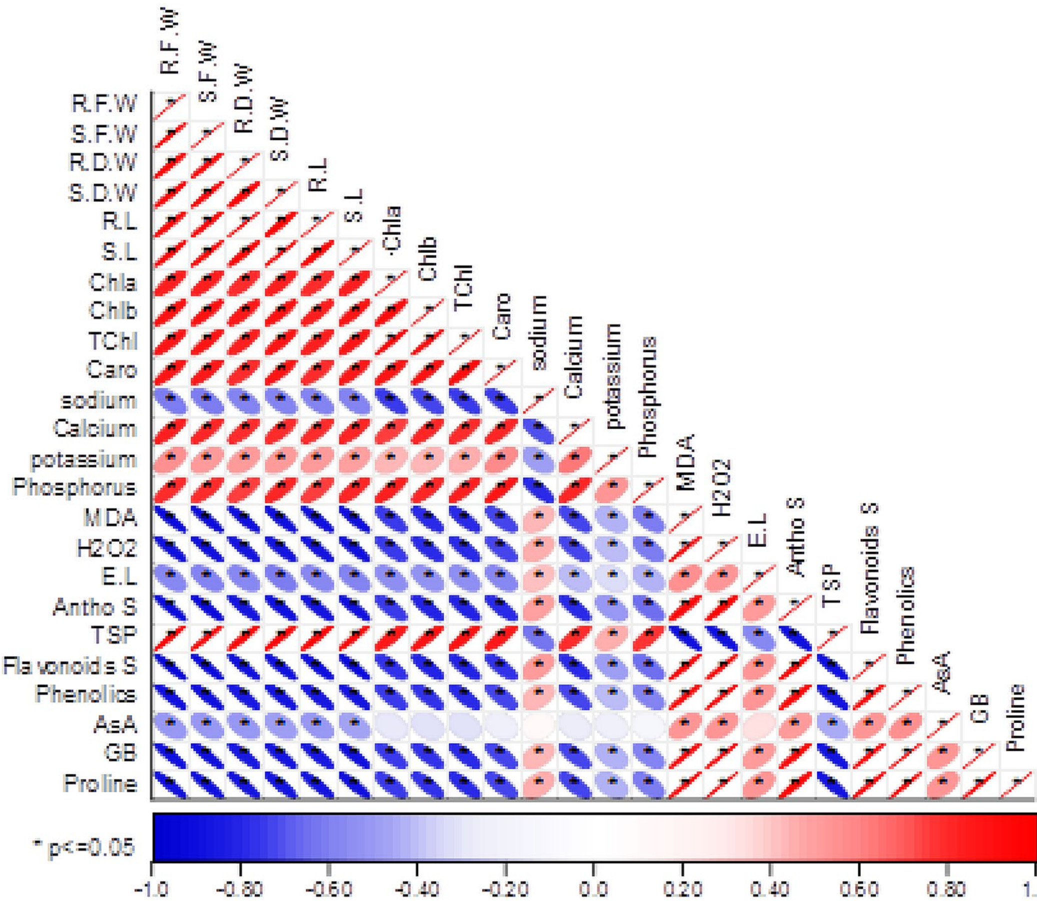

Figure 3. Role of sole and combined application of BR and BC on photosynthetic pigments contents of wheat under 35 WHC and 75 WHC. Bars show means of three replicates. Different error bars represent SE. Different letters indicate significant difference at $p \leq 0.05$; LSD Test (75 *WHC* well-watered, *35 WHC* drought stress, *C* control, *BR1* 1 μmol foliar applied 24-epibrassinolide, *BR2* 3 μmol foliar applied 24-epibrassinolide, *BC* timber waste biochar).

Na content within plant tissues; yet, these additives improved the cation balance in plant tissues, especially the Ca: Na ratio and therefore plant roots and shoots boosted notably. In this context, Ca is an integral component of cell wall[46] and also acts as a secondary messenger in cell osmoregulation[46].

The possible adaptations of wheat, in case of the combined applications of BC and BR, were either direct, as BR increase photosynthetic activities[7] and decrease the oxidative stress indicators in plant[54]. In case of biochar, it stimulates gibberellic acid synthesis in plants hence, improve their growth[55]. It may also be indirect through increasing soil moisture retention[17,56] that increases the efficiency of the grown plants to utilize soil nutrients and promote plant biomass[57].

It is worthy to mention that the activities of enzymatic and non-enzymatic antioxidants were higher in sole applications than the combined ones. This finding did not signify that BR and BC additives antagonize each other because of the superior effect of this combination on plant growth parameters comparable with the sole applications of any of these additives. It can therefore be deduced that the optimum strategy of improving plant growth under drought conditions is through enhancing more antioxidant activities as well as improving the nutritional status of the grown plants. The above results; therefore, support the main hypothesis of the study which signify the supremacy of the combined application of BR + BC for mitigating drought stress on wheat versus their sole applications.

Conclusion

Application of either of brassinosteroids (BR) or timber waste biochar (BC) can successfully alleviate drought stress and improve wheat growth under deficit irrigation system. Moreover, the combined application of these two additives had further positive impacts on increasing plant growth parameters via their integral role in decreasing oxidative stress indicators in plants. There is an actual need for more investigations under field level conditions to validate the results obtained herein using more deficit irrigation variations.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 16 April 2022; Accepted: 18 July 2022
Published online: 27 July 2022

References

1. Haider, S. A. *et al.* Drought stress alleviation by potassium-nitrate-containing chitosan/montmorillonite microparticles confers changes in *Spinacia oleracea* L. *Sustainability* **13**, 9903. https://doi.org/10.3390/su13179903 (2021).
2. Dianatmanesh, M. *et al.* Yield and yield components of common bean as influenced by wheat residue and nitrogen rates under water deficit conditions. *Environ. Technol. Innov.* **28**, 102549. https://doi.org/10.1016/j.eti.2022.102549 (2022).
3. Sharma, S. *et al.* Regulation of the Calvin cycle under abiotic stresses: An overview. *Plant Life Under Change Environ.* **681**, 717. https://doi.org/10.1016/B978-0-12-818204-8.00030-8 (2020).
4. Talaat, N. B. 24-Epibrassinolide and Spermine combined treatment sustains maize (*Zea mays* L.) drought tolerance by improving photosynthetic efficiency and altering phytohormones profile. *J. Soil Sci. Plant Nutr.* **20**, 516–529. https://doi.org/10.1007/s42729-019-00138-4 (2020).
5. Manaa, A. *et al.* Photosynthetic performance of quinoa (*Chenopodium quinoa* Willd.) after exposure to a gradual drought stress followed by a recovery period. *Biochim. Biophys. Acta Bioenerg.* **1862**, 148383. https://doi.org/10.1016/j.bbabio.2021.148383 (2021).
6. Arough, Y. K., Sharifi, R. S. & Sharifi, S. S. Bio fertilizers and zinc effects on some physiological parameters of triticale under water-limitation condition. *J. Plant Interact.* **11**, 167–177. https://doi.org/10.1080/17429145.2016.1262914 (2016).
7. Jangid, K. K. & Dwivedi, P. Physiological and biochemical changes by nitric oxide and brassinosteroid in tomato (*Lycopersicon esculentum* Mill.) under drought stress. *Acta Physiol. Plant.* **39**, 73. https://doi.org/10.1007/s11738-017-2373-1 (2017).
8. Gruszka, D. *et al.* Barley brassinosteroid mutants provide an insight into phytohormonal homeostasis in plant reaction to drought stress. *Front Plant Sci.* **7**, 1824. https://doi.org/10.3389/fpls.2016.01824 (2016).
9. Deb, A., Grewal, R. K. & Kundu, S. Regulatory cross-talks and cascades in rice hormone biosynthesis pathways contribute to stress signaling. *Front. Plant Sci.* **7**, 1303. https://doi.org/10.3389/fpls.2016.01303 (2016).
10. Vardhini, B. V. & Anjum, N. A. Brassinosteroids make plant life easier under abiotic stresses mainly by modulating major components of antioxidant defense system. *Front. Environ. Sci.* **2**, 67. https://doi.org/10.3389/fenvs.2014.00067 (2015).
11. Li, S., Zheng, H., Lin, L., Wang, F. & Sui, N. Roles of brassinosteroids in plant growth and abiotic stress response. *Plant Growth Regul.* **93**, 29–38. https://doi.org/10.1007/s10725-020-00672-7 (2021).
12. Vickers, N. J. Animal communication: When I'm calling you, will you answer too?. *Curr. Biol.* **27**, R713–R715. https://doi.org/10.1016/j.cub.2017.05.064 (2017).
13. Haubrick, L. L. & Assmann, S. M. Brassinosteroids and plant function: Some clues, more puzzles. *Plant. Cell Environ.* **29**, 446–457. https://doi.org/10.1111/J.1365-3040.2005.01481.X (2006).
14. Deng, B. *et al.* Drought stress and *Acacia seyal* biochar effects on sorghum gas exchange and yield: A greenhouse experiment. *Agric. Nat. Resour.* **53**, 573–580. https://doi.org/10.34044/j.anres.2019.53.6.03 (2019).
15. Alharby, H. F. & Fahad, S. Melatonin application enhances biochar efficiency for drought tolerance in maize varieties: Modifications in physio-biochemical machinery. *Agron. J.* **112**, 2826–2847. https://doi.org/10.1002/agj2.20263 (2020).
16. Lalarukh, I. *et al.* A combined use of rhizobacteria and moringa leaf extract mitigates the adverse effects of drought stress in wheat (*Triticum aestivum* L.). *Front. Microbiol.* **13**, 813–415. https://doi.org/10.3389/fmicb.2022.813415 (2022).
17. Bassouny, M. & Abbas, M. H. H. Role of biochar in managing the irrigation water requirements of maize plants: The pyramid model signifying the soil hydro-physical and environmental markers. *Egypt J. Soil Sci.* **59**, 99–115. https://doi.org/10.21608/EJSS.2019.9990.1252 (2019).
18. Elshony, M., Farid, I. M., Alkamar, F., Abbas, M. H. H. & Abbas, H. Ameliorating a sandy soil using biochar and compost amendments and their implications as slow release fertilizers on plant growth. *Egypt J. Soil Sci.* **59**, 305–322. https://doi.org/10.21608/EJSS.2019.12914.1276 (2019).
19. French, E. & Iyer-Pascuzzi, A. S. A. Role for the gibberellin pathway in biochar-mediated growth promotion. *Sci. Rep.* **8**, 5389. https://doi.org/10.1038/s41598-018-23677-9 (2018).
20. Farid, I. M. *et al.* Co-composted biochar derived from rice straw and sugarcane bagasse improved soil properties, carbon balance, and zucchini growth in a sandy soil: A trial for enhancing the health of low fertile arid soils. *Chemosphere* **292**, 133389. https://doi.org/10.1016/j.chemosphere.2021.133389 (2022).
21. Hafez, E. M., Omara, A. E. D., Alhumaydhi, F. A. & El-Esawi, M. A. Minimizing hazard impacts of soil salinity and water stress on wheat plants by soil application of vermicompost and biochar. *Physiol. Plant.* **172**, 587–602 (2021).
22. Abdelhafez, A. A. *et al.* Eco-friendly production of biochar via conventional pyrolysis: Application of biochar and liquefied smoke for plant productivity and seed germination. *Environ. Technol. Innov.* **22**, 101540. https://doi.org/10.1016/j.eti.2021.101540 (2021).
23. Tolba, M. *et al.* Integrated management of K-additives to improve the productivity of zucchini plants grown on a poor fertile sandy soil. *Egypt J. Soil Sci.* **61**, 355–365. https://doi.org/10.21608/ejss.2021.99643.1472 (2021).
24. Ali, S. *et al.* Biochar soil amendment on alleviation of drought and salt stress in plants: A critical review. *Environ. Sci. Pollut. Res. Int.* **24**, 12700–12712. https://doi.org/10.1007/s11356-017-8904-x (2017).
25. Abdelhafez, A. A., Abbas, M. H. H. & Li, J. B. in *Engineering Applications of Biochar* (ed W.-J. Huang) 7–27 (IntechOpen, 2017).
26. Wang, X. *et al.* Global irrigation contribution to wheat and maize yield. *Nat. Commun.* **12**, 1235. https://doi.org/10.1038/s41467-021-21498-5 (2021).
27. Dhillon, J. *et al.* Nitrogen management impact on winter wheat grain yield and estimated plant nitrogen loss. *Agron. J.* **112**, 564–577. https://doi.org/10.1002/agj2.20107 (2020).
28. Salim, N. & Raza, A. Nutrient use efficiency (NUE) for sustainable wheat production: A review. *J. Plant Nutr.* **43**, 297–315. https://doi.org/10.1080/01904167.2019.1676907 (2020).
29. Lu, Y., Yan, Z., Li, L., Gao, C. & Shao, L. Selecting traits to improve the yield and water use efficiency of winter wheat under limited water supply. *Agric. Water Manag.* **242**, 106410. https://doi.org/10.1016/j.agwat.2020.106410 (2020).
30. Zhang, L. *et al.* Tiller development affected by nitrogen fertilization in a high-yielding wheat production system. *Crop Sci.* **60**, 1034–1047. https://doi.org/10.1002/csc2.20140 (2020).
31. Wang, Y. *et al.* Efficient physiological and nutrient use efficiency responses of maize leaves to drought stress under different field nitrogen conditions. *Agronomy* **10**, 523. https://doi.org/10.3390/agronomy10040523 (2020).
32. Sparks, D. L. *et al.* (SSSA Book Series, 1996).
33. Arnon, D. I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant. Physiol.* **24**, 1–15. https://doi.org/10.1104/pp.24.1.1 (1949).
34. Mukherjee, S. P. & Choudhuri, M. A. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. *Physiol. Plant.* **58**, 166–170. https://doi.org/10.1111/j.1399-3054.1983.tb04162.x (1983).

35. Heath, R. & Packer, A. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125**, 189–198. https://doi.org/10.1016/0003-9861(68)90654-1 (1986).
36. Anjum, S. A. *et al.* Cadmium toxicity in Maize (*Zea mays* L.): Consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. *Environ. Sci. Pollut. Res.* **22**, 17022–17030. https://doi.org/10.1007/s11356-015-4882-z (2015).
37. Bates, L. S., Waldren, R. P. & Teare, I. D. Rapid determination of free proline for water-stress studies. *Plant Soil* **39**, 205–207. https://doi.org/10.1007/BF00018060 (1973).
38. Lewis, C. E., Walker, J. R. L., Lancaster, J. E. & Sutton, K. H. Determination of anthocyanins, flavonoids and phenolic acids in potatoes I: Coloured cultivars of *Solanum tuberosum* L. *J. Sci. Food Agric.* **77**, 45–57. https://doi.org/10.1002/(SICI)1097-0010(199805)77:1%3c45::AID-JSFA1%3e3.0.CO;2-S (1998).
39. Bray, H. G. & Thorpe, W. V. Analysis of phenolic compounds of interest in metabolism. *Methods Biochem. Anal.* **1**, 27–52. https://doi.org/10.1002/9780470110171.ch2 (1954).
40. Azuma, K. *et al.* Phenolic antioxidants from the leaves of *Corchorus olitorius* L. *J. Agric. Food Chem.* **47**, 3963–3966. https://doi.org/10.1021/JF990347P (1999).
41. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254. https://doi.org/10.1006/abio.1976.9999 (1976).
42. Holmström, K.-O., Somersalo, S., Mandal, A., Palva, T. E. & Welin, B. Improved tolerance to salinity and low temperature in transgenic tobacco producing glycine betaine. *J. Exp. Bot.* **51**, 177–185. https://doi.org/10.1093/jexbot/51.343.177 (2000).
43. Coutinho, J. The molybdate/ascorbic acid blue method for the phosphorus determination in very dilute and colored extracts by segmented flow analysis. *Commun. Soil Sci. Plant Anal.* **27**, 1363–1375. https://doi.org/10.1080/00103629609369638 (1996).
44. Li, X., Palta, J. A. & Liu, F. Editorial: Modulation of stomatal response by elevated CO_2 in plants under drought and heat stress. *Front. Plant Sci.* **13**, 843999. https://doi.org/10.3389/fpls.2022.843999 (2022).
45. White, P. in *Marschner's Mineral Nutrition of Higher Plants* (ed P. Marschner) Ch. 2, 7–47 (Elsevier, 2012).
46. Engels, C., Kirkby, E. & White, P. in *Marschner's Mineral Nutrition of Higher Plants.* (ed P. Marschner) Ch. 5, 85–134 (Elsevier, 2012).
47. Reddy, T. Y., Reddy, V. R. & Anbumozhi, V. Physiological responses of groundnut (*Arachis hypogea* L.) to drought stress and its amelioration: A critical review. *Plant Growth Regul.* **41**, 75–88. https://doi.org/10.1023/a:1027353430164 (2003).
48. Li, S., Li, X., Wei, Z. & Liu, F. ABA-mediated modulation of elevated CO_2 on stomatal response to drought. *Curr. Opin. Plant Biol.* **13**, 1–7. https://doi.org/10.1016/j.pbi.2019.12.002 (2019).
49. Sankar, B. *et al.* Drought-induced biochemical modifications and proline metabolism in *Abelmoschus esculentus* (L.) Moench. *Acta Botanica Croatica* **66**, 43–56 (2007).
50. Hussain, M., Malik, M. A., Farooq, M., Ashraf, M. Y. & Cheema, M. A. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* **194**, 193–199. https://doi.org/10.1111/j.1439-037X.2008.00305.x (2008).
51. Gill, S. S. & Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **48**, 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016 (2010).
52. Senapati, N., Stratonovitch, P., Paul, M. J. & Semenov, M. A. Drought tolerance during reproductive development is important for increasing wheat yield potential under climate change in Europe. *J. Exp. Bot.* **70**, 2549–2560. https://doi.org/10.1093/jxb/ery226 (2018).
53. Farooq, M., Hussain, M., Wahid, A. & Siddique, K. H. M. in *Plant Responses to Drought Stress: From Morphological to Molecular Features* (ed R. Aroca) 1–33 (Springer, 2012).
54. Naservafaei, S., Sohrabi, Y., Moradi, P., Mac Sweeney, E. & Mastinu, A. Biological response of *Lallemantia iberica* to brassinolide treatment under different watering conditions. *Plants* **10**, 496 (2021).
55. Vishal, B. & Kumar, P. P. Regulation of seed germination and abiotic stresses by gibberellins and abscisic acid. *Front. Plant Sci.* https://doi.org/10.3389/fpls.2018.00838 (2018).
56. Bornø, M. L., Müller-Stöver, D. S. & Liu, F. Biochar modifies the content of primary metabolites in the rhizosphere of well-watered and drought-stressed *Zea mays* L. (maize). *Biol. Fertil. Soils* **6**, 66. https://doi.org/10.1007/s00374-022-01649-6 (2022).
57. Mannan, M. A., Mia, S., Halder, E. & Dijkstra, F. A. Biochar application rate does not improve plant water availability in soybean under drought stress. *Agric. Water Manag.* **253**, 106940. https://doi.org/10.1016/j.agwat.2021.106940 (2021).

Acknowledgements

The authors extended their appreciation to the Government College Women University, Faisalabad to conduct the research. Also thanks to Taif University Research Supporting Project Number (TURSP-2020/315), Taid University, Taif Saudi Arabia for providing the financial support. Thanks for the support of the iASK Research Grand and Eötvös Research Grant (MAEO-00074-002-2021) to help publishing this research. This study was technically supported by the National Committee of Soil Science, Academy of Scientific Research and Technology, Egypt.

Author contributions

I.L., S.F.A., M.H.H.A. and A.A.A. conceptualization, S.F.A., N.M. and I.L. executed most of the experiments and analyzed the data, S.F.A., A.A.A., M.H.H.A., P.P., D.E, K.t.K. and S.A.A. wrote the manuscript, A.H.A., M.M.A., F.S.A., W.B.A. and P.P. formal analyses, I.L., A.A.A., M.H.H.A., P.P., D.E, K.t.K. and S.F.A. revised the manuscript and funding.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to S.F.A., P.P. or A.A.A.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.