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

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Abstract

Drought stress is among the major constraints that threat agricultural productivity within the arid and semi-arid regions, worldwide. In this study, wheat (a strategic crop) was selected to test its growth under drought stress and the mechanisms beyond this adaptation while considering two factors, i.e., (1) deficit irrigation at 35% of the water holding capacity (WHC) versus 75% of WHC (Factor A) and (2) the following safe treatments: the control treatment (C), amending soil with biochar (BC) at a rate of 2%, foliar application of 24-epibrassinolide at two different levels (1 (BR1) or 3 (BR2) µmol) and the combination between BC and BR treatments. The obtained results were statistically analyzed, and the heat-map conceits between measured variables were also calculated by using the Python software. This investigation took place under the greenhouse conditions for 35 days following a complete randomized design and all treatments were replicated trice. Results obtained herein revealed that drought stress decreased all studied vegetative growth parameters (root and shoot biomasses) and photosynthetic pigments (chlorophyll a, b and total contents while increased oxidative stress indicators. All additives, specifically the combined ones BR1 + BC and BR2 + BC, were effective in increasing growth attributes, photosynthetic pigments and ion assimilation by wheat plants. They also upraised the levels of enzymatic and non-enzymatic antioxidants while decreased stress indicators. Furthermore, they increased Ca, P and 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.

Introduction

Abiotic stresses pose potential threats that hamper plant growth and productivity [1], especially drought [2,3] in arid and semiarid regions [4,5] which has adverse effects on photosynthetic machinery, particularly disrupting thylakoid electron transport, stomatal conductance, CO_2 assimilation and Calvin cycle [6–8]. Moreover, this stress interrupts the balance between antioxidative defense mechanisms and production of reactive oxygen species (ROS) causing ROS accumulation, and this may lead to oxidative stress to cell membrane lipids, protein and disorganized DNA strands [9].

Superoxide (O^{2-}), hydroxyl ions (OH^-), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) are well known ROS that are accumulated during drought stress [10]. 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 activities in response of external abiotic stress[2].

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 [11]. Apart from their role in plant growth regulation and cell development, BR are involved in controlling abiotic stress responses [12] via: (1) increasing activities of antioxidative enzymes [13]; hence, lessening the production rate of superoxide anion [14], (2) reducing abscisic acid (ABA) accumulation [15] in spite of that these hormones increase stomatal closure under drought conditions [13,16] and (3) increasing the osmotic permeability of root cells to take up more water from soil. It can; therefore, be deduced that supplying plants with exogenous BR could enhance plant stress tolerance under drought conditions [12,17,18] via induction of various physiological responses [17,19].

Biochar is another promising additive that can mitigate the adverse effects of drought on plant growth and productivity, e.g., cucumber [20], fenugreek [21], maize [22] and sorghum [23]. This might take place via (I) increasing soil water retention [24,25], hence reducing soil irrigation demands [26,27], (II) increasing nutrient use efficiency [28], (III) stimulating auxin, gibberellin and brassinosteroids regulation [29] and (IV) increasing plant chlorophyll content, stomatal conductance, cytotoxicity and leaf K^+ content [30]. This cost-effective carbon rich product is formed through the pyrolysis of organic residues under limited oxygen supply [31] to give a product of high porous aromatic carbon content [32]. 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 [33].

In the next years, significant increases in global crop products will be required to fulfill rising human and animal food demands [34]. Wheat is among the most important crops for global food security [35] which contributes in 40% to the world food demand. Its global production is estimated by 757.4 million tons in the year of 2019 [36]. Although, the modern high yielding wheat cultivars are introduced to increase wheat productivity [37] and these cultivars consume massive amounts of mineral fertilizers [38]; 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 [36] and others nutrients mostly do not exceed 50%. Under drought conditions, nutrient use efficiency by the grown plants decreases considerably [39] and this may cause further reductions in plant productivity. In this investigation, we tested the feasibility of using the combined application between 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 conducted 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 for mitigating 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. [40] and the results are presented in Table 1.

Table 1.

Wheat seeds (*Triticum aestivum* L., cv. Lasani-2008) were obtained from certified seed dealer of the Government of Punjab, Pakistan. All the seeds were disinfected with 95% ethanol followed by 70% sodium hypochlorite solution washing. Finally, the seeds were rinsed 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 the production of timber waste biochar, timber waste was collected from regional timber market. The timber waste was first sun-dried for a week and then pyrolyzed at 390°C for 80 min in a pyrolizer. Then, the timber waste biochar (BC) was crushed in a grinder and sieved through a 2 mm sieve and the fine powder was stored. Some major timber waste biochar properties were (Table 2).

Table 2.

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. 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 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) were watered at only 35 WHC (35% of the water holding capacity), while the other group was watered at 75 of WHC. On the other hand, a foliar application of 24-epibrassinolide was sprayed on stressed plants at either 0 (distilled water), 1 (BR1) or 3 (BR2) µmol concentrations per pot three times with one day interval after the three weeks period of sowing.

Plant harvesting and growth attributes

Plants were harvested after 35 days of sowing and their root and shoot fresh weights 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 treatment then oven-dried at 65°C for 72 h to determine their dry weights. Other fresh materials were stored at -30°C for further fresh analysis.

Chlorophyll contents

The chlorophyll a, b and total contents as well as carotenoids pigments were estimated in fresh leaf samples following the Arnon protocol [41]. To determine photosynthetic pigments, a 0.1 g 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 done following Mukherjee and Choudhari technique [42]. In this method, 0.1 g leaf sample was extracted in 10 mL cold acetone, centrifuged at 10,000 rpm and then 4 mL titanium reagent and 5 mL of concentrated ammonium solution was 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 [43]. The electrolyte leakage (EL) was determined following Anjum et al. [44] 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 [45], flavonoids and anthocyanin [46], phenolics [47], ascorbic acid [48], proteins [49] and glycine betaine [50] contents.

Ca, Na, P and K ion concentrations

Molybdate/ascorbic acid blue method was used for P determination [51] 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 two-way analysis of variance to find significance of applied treatments in drought stress. All the treatment means were compared by LSD test at 5% level of significance ($P < 0.05$). Logarithmic transformations for data normalization were carried out before analysis, 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 at 35 WHC decreased significantly versus the ones that received 75% WHC (Fig. 1). Likewise, drought stresses affected significantly and negatively root and shoot lengths. On the other hand, the investigated treatments improved significantly the studied plant growth parameters versus the control. In this concern, foliar application of BR raised significantly plant growth parameters exceeding those attained for the application of biochar (BC). Also, increasing the level of BR application resulted in additional increases in the investigated plant growth parameters. The combination between BR and BC caused additional significant increases in plant growth parameters, especially in the presence of the higher application level of BR (3µmol). It is worth mentioning that the usage of BR+BC improved root and shoot fresh weights, dry weights and lengths for those subjected to 35 WHC beyond 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.

Fig 1.

Photosynthetic pigments

Deficit irrigations negatively affected chlorophyll a, b and 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 with those irrigated with 75% of WHC. In contrast, all investigated treatments raised significantly plant contents of photosynthetic pigments versus the control, especially for the foliar application of BR versus soil application of BC. The combination between these two amendments (BR+BC) furtherly improved these photosynthetic pigments, specifically when using the higher application level of BR (3µmol). These results also highlight the significance of these amendments as effective safe approaches to mitigate the drought stress conditions.

Fig. 2.

Oxidative stress indicators, proteins and ionic contents

Nutrient (N, 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 effects 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 sole applications of either BR or BC were better in decreasing these oxidative stress indicators that applying them both. 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.

Table 3.

Pearson correlation

A person correlation analysis was performed to highlight the relation between reductions in plant oxidative stress and the improvements that took place in plant growth parameters. 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). Likewise, drought stress affected significantly and negatively root and shoot lengths. On the other hand, the investigated treatments improved significantly the studied plant growth parameters versus the control. In this concern, foliar application of BR raised significantly plant growth parameters exceeding those attained for the application of biochar (BC). Also, increasing the level of BR application resulted in additional increases in the investigated plant growth parameters. The combination between BR and BC caused additional significant increases in plant growth parameters, especially in the presence of the higher application level of BR (3µmol). It is worth mentioning that the usage of BR+BC improved root and shoot fresh weights, dry weights and lengths for those subjected to 35 WHC beyond 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.

Pearson correlations showed that root and shoot lengths as well as their fresh and dry weights were correlated significantly and positively with the pigment (chlorophyll A, chloro B, total chlorophyll and carotenoids) contents in plants. In addition, these growth parameters were also correlated with nutrient contents of plants (Ca, K and P). However, these growth parameters were correlated significantly and negatively with the stress indicators and antioxidents in plant leaves (MDA, H2O2, glycine betaine, proline, phenolics, ascorbic acid, anthocyanin and flavonoids) (Fig. 3.).

Fig. 3.

Discussion

Wheat is a strategic crop in global food security [52,53], yet the climatic changes threat its production in many countries around the world including Pakistan [54]. Drought stress is among the severe environmental conditions that adversely affect wheat production [55]. Accordingly, many countries import wheat grains [56] to lessen the gap between actual production and local market needs [57]; nevertheless, wheat prices are rising continuously [58]. Accordingly, untraditional water management practices should be taken into account to alleviate drought stress in order to increase the productivity of wheat under such adverse conditions. The current study is a trial to investigate the consequences of amending wheat plants grown under drought conditions with brassinosteroids (BR) and timber waste biochar (BC), either solely or in combination to improve wheat growth parameters. Results obtained herein indicate that drought stress affected negatively plant growth parameters (fresh and dry weights of plants as well as the plant heights). These results are in consistent with the findings observed in previous studies, e.g., Sankar et al. [59] and Hussain et al. [60]. Probably, leaf turgor potential and photosynthetic assimilation were adversely affected causing significant reductions in plant biomass [61]. Under such conditions, plants suffer from drought stress exhibited higher oxidative stress indicators such as increases in malondialdehyde, hydrogen peroxide, electrolyte leakage. Increases in several active oxygen species like H_2O_2 in plant cells were also reported in drought stress conditions [55]. Although, ROS works as signaling molecules under low concentrations; yet they are toxic in higher concentrations [62]. The major outcome of drought stress is the reduction in photosynthetic activity that is associated with the concurrent reductions that took place in Phyto-assimilates. Application of BR increased photosynthetic activities and hence yield under drought stress in tomato plants under drought stress [2]. To cope from drought stress, wheat plants adopt several defensive mechanisms such as increasing the activities of the antioxidant enzymes and non-enzymes. Further changes takes place at both cellular and whole-organism levels, making drought stress tolerance a complex physiological phenomenon [63].

In this study the possible adaptations of wheat towards drought stress were thought to be via direct and indirect pathways. The direct approach was through supplying plants with BR that decreased the oxidative stress indicators in plant [64]. This consequently improved plant growth parameters. The indirect approach is through amending soil with biochar which might increase soil water retention [65] and also increasing Ca, P and K utilization by plants; hence, promote plant biomass [66]. Moreover, biochar can stimulate gibberellic acid pathway within biochar treated plants grown under stress conditions; hence improve their growth [67]. The improvements that took place in plant growth were mostly attributed to the concurrent increases that occurred in both enzymatic and non-enzymatic antioxidants which, in turn, decreased oxidative stress indicators. It is worthy to mention that the BR and BC additives may antagonize each other; to some extent, as the activities of the enzymatic and non-enzymatic antioxidants were higher in case of their sole applications compared with those attained for the combined applications. Nevertheless, the overall effect of the combined treatment (BR + BC) on plant growth parameters was more detectable versus the sole applications of any of these additives. The above results; therefore, support the main hypothesis of the study which signify that the combined application of BR + BC could be more effective for mitigating drought stress effects on wheat versus their sole applications.

The combination between BR and BC and decreased significantly the drought stress for plants grown under deficient water treatment (35% WHC). In addition, this combined application improved substantially plant growth parameters. In this case, photosynthetic pigments improved significantly in plants versus the drought ones. Probably, the combination between BR and BC maximizes the benefits of both amendments in drought stress on and enhanced nutrients uptake by the growing plants. The obtained results of correlation study emphasized the success of used amendments for enhancing plant growth under drought stress condition.

Conclusion

Application of either of brassinosteroids (BR) or timber waste biochar can successfully ameliorate drought stress and positively affect wheat growth parameters. However, the combined application of these two additives could have further significant effects on increasing plant growth parameters via their integral modes of action especially in decreasing oxidative stress indicators in plants. There is a need for more investigation in field level along with other climatic conditions to further validate the results with the application of timber waste biochar and brassinosteroids as an effective amendment against drought stress for wheat.

Declarations

Author contribution statement

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. 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. and S.F.A. revised the manuscript and funding.

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Data Availability

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

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Tables

Table 1. Soil component analysis

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}

pH was determined in 1:2.5 (soil:water) suspension, EC was determined in soil paste extract

Table 2. Characterization of timber waste biochar

Component	**Value**
pH * (1:10) suspension	7.01
Ash contents	17.0 g kg^{-1}
C	8.63 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 3. Oxidative stress indicators, proteins and ionic contents of wheat plant grown under drought stress conditions after 35 days of seedlings

Treatment	**Main Effect of Treatments**									
	Na	Ca	K	P	MDA	H_2O_2	EL	Antho	TSP	Flav
C	18.42±5.26 a	3±0.71 c	3.33±1.08 b	8.5±5.24 a	3.11±0.35 a	3.15±0.3 a	24.5±3.62 a	3.2±0.27 a	1.27±0.58 d	3.21±0.18 a
BC	17.08±4.44 a	4.25±1.54 bc	4.58±2.31 b	9.83±5.12 a	2.57±0.39 b	2.56±0.45 b	19.67±1.37 ab	2.52±0.39 bc	1.64±0.39cd	2.59±0.42 b
BR1	15.83±3.53 a	3.97±1.85 bc	5.33±1.08 ab	11.33±7.23 a	2.6±0.25 b	2.6±0.2 b	17.17±3.97 b	2.65±0.23 b	2.09±0.41 c	2.63±0.22 b
BR2	16.5±3.66 a	6.17±2.29 ab	6.17±2.23 a	12.17±6.79 a	2.2±0.5 b	2.17±0.59 b	19±7.46 ab	2.11±0.62 cd	2.21±0.36 c	2.14±0.63 bc
BR1+BC	14.17±4.96 a	5.25±2.21 abc	6.17±2.71 a	18.17±14.34 a	1.63±0.46 c	1.64±0.46 c	15.33±2.73 b	1.95±0.48 d	2.93±0.58 b	1.76±0.35 c
BR2+BC	14.83±5.42 a	8±4.11 a	5.67±1.99 ab	21.17±13.8 a	1.21±0.49 c	1.22±0.53 c	16±5.44 b	1.11±0.45 e	3.57±0.71 a	1.23±0.51 d
	Main Effect of Stress									
35 WHC	19.75±2.96a	3.43±1.11 b	4.58±1.62a	6.06±2.53 b	2.45±0.71a	2.47±0.72 a	20.28±4.5 a	2.53±0.65 a	1.85±0.74b	2.51±0.64
75 WHC	12.53±2.28b	6.78±2.88 a	5.83±2.39a	21±1.90 a	1.99±0.74a	1.98±0.75 a	16.94±5.46 a	1.98±0.8 b	2.72±0.88a	2.01±0.8 b
ANOVA										
F-values	16.14	5.11	5.21	13.53	2.22	2.22	18.61	2.26	2.29	2.26
CV	27.85	53.64	40.49	73.87	33.93	34.44	28.00	34.12	40.07	33.39

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].

Figures

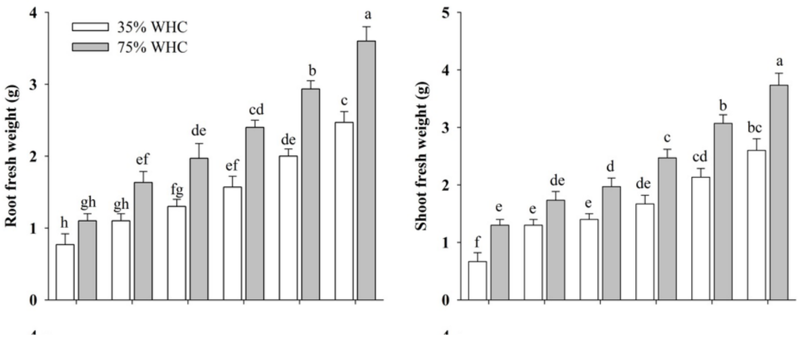

Figure 1

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]

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 $p \leq 0.05$; 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]

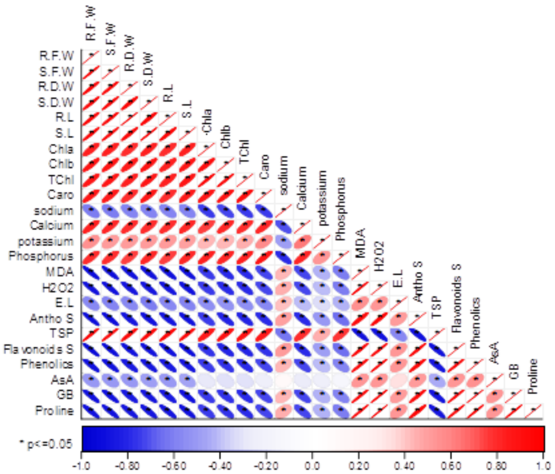

Figure 3

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]