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Enhancing physio biochemical traits and yield of common buckwheat *Fagopyrum esculentum* with rice husk biochar and nano iron oxide under water stress

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Climate change is making droughts more frequent, which is a major problem for crop yield, especially for crops that are vulnerable to drought, such as common buckwheat (*Fagopyrum esculentum*). Drought stress affects negatively on physiological and biochemical processes of plants, leading to reduced yields. This study addresses the knowledge gap regarding effective strategies to mitigate drought-induced damage and enhance productivity in buckwheat. We hypothesized that iron oxide nanoparticles (Fe_3O_4 NPs) and rice husk biochar could improve drought tolerance in buckwheat by modulating its physiological and biochemical responses. To test this, buckwheat plants were grown under well-watered (80% of field capacity, FC) and drought (40% of FC) conditions following a completely randomized design (CRD) with three replications. Results showed that the application of 50 g/kg rice husk biochar and 400 ppm Fe_3O_4 NPs, either separately or in combination, significantly enhanced the yield and improved key physiological and biochemical traits, including relative water content, photosynthetic rate, stomatal conductance, chlorophyll content, and antioxidant activity. The combination of Fe_3O_4 NPs and rice husk biochar led to improvements the plants' relative water content, photosynthetic rate, chlorophyll levels, membrane stability index, proline, antioxidant activity (DPPH), and seed yield by 22.37, 17.11, 43.05, 16.07, 43.75, 8.59, and 50.87%, respectively compared to untreated drought plants. Moreover, this treatment reduced oxidative stress indicators such as hydrogen peroxide and malondialdehyde by 31.09 and 38.19%, respectively. These results show that Fe_3O_4 NPs, when combined with rice husk biochar, significantly improve drought tolerance in common buckwheat, providing a viable strategy to increase crop yields in water-limited environments. In view of climate change, this study emphasises the potential of combining biochar with nanomaterials for sustainable agricultural practices.

Keywords Buckwheat, Biochar, Nanoparticles, Physio-biochemical, Production, Drought

Buckwheat is a pseudocereal originating from China, and it became a pan-Eurasian crop by 3000 BCE[1]. This crop has gained considerable interest worldwide due to its nutritional, economic, and pharmaceutical values[2–10]. Two buckwheat species are cultivated, common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn), although the first accounts for 90% of the production[11]. Although Tartary buckwheat was reported to be widely adaptable to hostile environments[12], common buckwheat is more susceptible to drought stress concerning physiological and biochemical characteristics. Compared to Tartary buckwheat common buckwheat generally exhibits lower water deficit tolerance[13].

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A carbon-rich substance called biochar (BC) can be applied as a soil conditioner to enhance soil quality and soil carbon sequestration. Biochar is produced by pyrolyzing organic materials at a temperature between 250 and 700 °C[14]. It has several special qualities that make it an effective, affordable, and environmentally friendly source of soil conditioner[15], and it's also regarded as a potential solution for drought-related issues[16]. Additionally, BC has been shown to improve soil and increase water holding capacity[17,18] and the amount of water accessible to plants[19,20]. Biochar improves the growth and yield of sunflower[21], rapeseed[22], and wheat[23] under drought conditions. Biochar enrichment significantly improved the stomatal conductance, water use efficiency, and photosynthesis in tomato crops[24], osmotic potential, photosynthetic rate, transpiration rate, relative water contents, leaf water potential, and leaf turgor potential in maize[25], wheat[26] and quinoa[27] as well as improved the electron transfer and protective enzymes activity in crops[28] under water-scarce conditions. Biochar is the most feasible alternative to cope with drought conditions for sustainable agriculture production due to its long-term carbon sink in the soil, high porosity, cation exchange capacity, and ability to serve as a home for beneficial microbes[20,21,29]. Soil application of biochar improves the water and nutrient holding capacity of the soil, seed germination, seedling emergence, productivity, microbial activity, and other chemical processes in soil[30–32]. Therefore, applying biochar could be a step forward in alleviating the adverse effects of drought on the growth, physiology, biochemical traits, and yield of buckwheat.

Nanotechnology (NT) has emerged as a promising field commonly used in the agricultural, food, and medical industries[33]. Various nanoparticles (NPs), including titanium dioxide (TiO_2), iron oxide (Fe_3O_4), zinc oxide (ZnO), silicon oxide (SiO_2), copper (Cu-NPS), and selenium (Se-NPS), have received significant attention recently owing to their non-threatening use in the agriculture sector[34,35]. NPs protects plants from oxidative damage by increasing the activities of antioxidants[36], can reduce drought-induced toxic effects by decreasing hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) accumulation, and maintaining the efficiency of the photosynthetic apparatus[36,37] which improves plant tolerance against abiotic stresses. NPs can also penetrate the plant chloroplast reach the photo system-II (PS-II) reaction center, and increase the transmission of electrons and light absorption in chloroplasts under stress, thereby improving photosynthetic efficiency and plant growth[38]. NPs enter the plant body through the roots and leaves, and after entering the plant body, induce biochemical, morphological, molecular, and physiological changes in the crops[39]. The primary response of drought stress is stomata closing, which affects CO_2diffusion, reduces photosynthesis and diminishes plant growth[40]. The application of NPs improves plant growth by triggering hormonal signaling, root activity, water uptake, and antioxidant activities[41], improves photosynthetic efficiency, synthesis of secondary metabolites and chlorophyll, and antioxidant activity, thereby improving plant growth under drought[42–44]. Biochar and NPs alone can effectively manage abiotic stress like drought by raising levels of photosynthetic pigments, antioxidant enzyme activity, and total soluble sugars while lowering stress markers like MDA content and electrolytic leakage in plant leaves. The mitigating effects of Fe_3O_4 NPs in the combination of rice husk biochar on drought-stressed buckwheat, however, has not been studied yet. Thus, the goal of the current study was to assess the effects of Fe_3O_4 NPs as a foliar spray, either alone or in combination with rice husk biochar, on the physio-biochemical characteristics and yield of common buckwheat in water-scarce environments.

Materials and methods

Experimental location, soil, treatments and design

The experiment was conducted in a semi-controlled vinyl house from February to April. The experimental site is 8.4 m above mean sea level at latitude 24° 5′ 23′′ N and longitude 90° 15′ 36′′ E. During the trial, the mean highest temperature was 37.29 °C, while the mean lowest temperature was 16.7 °C. The average maximum and minimum relative humidity throughout the growing season were 62.67 and 54.47%, respectively at 2.00 pm. The experimental soil had a pH of 6.31 and a sandy loam texture, consisting of 52.99% sand, 33.00% silt, and 13.21% clay. Soil organic carbon, available P, total N, exchangeable K, CEC, and EC values were, in that order, 0.55%, 0.06 mg /100 g, 0.07%, 0.73 cmol /kg dry soil, 12.75 cmol /kg dry soil, and 0.02 dS /m. The soil retains around 30% of its moisture content at field capacity (FC). Each plastic pot (30 cm in length and 24 cm in diameter) contained a 4:1 blend of soil and cow excrement. It held six kilograms of blended soil that had been air-dried. The experiment was made up of two factors. Factor A: water regimes- (i) well water (80% of field capacity, FC) and (ii) drought (40% of FC). Factor B: the four treatments of (i) control (no treatment), (ii) rice husk biochar (BC) at 50 g/kg soil, (iii) foliar application of Fe_3O_4 NPs at 400 ppm concentration (Fe_3O_4 NPs), and (iv) combination of biochar application and the foliar application of Fe_3O_4 NPs (BC + Fe_3O_4 NPs). The experiment was laid out a completely randomized design (CRD) with 3 replications.

Rice husk biochar

The rice husk biochar has been produced in a biochar stove following the method described by Islam et al.[45]. The chemical composition of rice husk biochar is pH 7.10, N 2.51%, P 0.23%, K 0.235%, Ca 1.012%, Mg 0.446%, S 0.326% and EC 1.23 mS/cm.

Fe_3O_4 nanoparticle solution preparation

Iron (II, III) oxide- Nanopowder (50–100 nm particle size (SEM), 97% trace metal basis was used to create nanoparticle solutions. Table 1 shown the chemical makeup of the Fe_3O_4nanoparticles[46].

To prepare 400 ppm nano-Fe_3O_4solutions, 400 mg of this powder was added to 1 L of distilled water. For sixteen hours, the solution was heated to 60 °C using a hot plate and a magnetic stirrer. After that, a sonication bath was given to the solution with continuous vibration to mix all the particles into the water homogenously, so that the solution could penetrate through the plant leaves effortlessly during the application[47]. These solutions were then kept at room temperature in a plastic bottle. When applying the solution to the plant, the necessary volume was filled in a hand sprayer.

Elements	Concentration	Percent
Aluminium (Al)	227.2 ppm	0.02272%
Boron (B)	0.8 ppm	0.00008%
Barium (Ba)	1.6 ppm	0.00016%
Calcium (Ca)	241.4 ppm	0.02414%
Cadmium (Cd)	10.3 ppm	0.00103%
Chromium (Cr)	43.5 ppm	0.00435%
Copper (Cu)	18.3 ppm	0.00183%
Potassium (K)	11.7 ppm	0.00117%
Magnesium (Mg)	76.1 ppm	0.00761%
Manganese (Mn)	732.3 ppm	0.07323%
Molybdenum (Mo)	1.8 ppm	0.00018%
Sodium (Na)	58.9 ppm	0.00589%
Nickel (Ni)	34.9 ppm	0.00349%

Table 1. Elements of trace metal of iron nanoparticle.

Treatments and cultural practices

The rice husk biochar was evenly mixed with soil at a rate of 50 g/kg soil in pots treated with biochar. The buckwheat seeds were repeatedly cleaned with distilled water after being sterilized with 1% sodium hypochloride. After sterilization, the seeds were left to dry overnight on a sanitized bench. Each pot contained ten seeds, spaced equally apart. To ensure steady germination, a tiny bit of water was added to the pots. Seeds started germinating in 5 days after sowing. Twelve pots were well-watered (Control) in the fourth leaf stage and received frequent watering to maintain moisture at 80% of FC throughout the growing season. The remaining 12 pots were maintained in a water-stressed environment with a 40% of FC (drought) for the duration of the growing season. A portable digital moisture meter (POGO Soil Sensor II, Stevens, USA) was regularly used to check the moisture content of the soil in each pot. After seven days of the fourth leaf stage, a 400 ppm concentration of nano-Fe_3O_4 solution was sprayed on the leaves of the control and drought-treated plants. Every plant received two sprayings spaced seven days apart.

Data collection

Data on physio-biochemical parameters were made on both control and drought-treated leaves during the flowering stage. Yield-related data were recorded at maturity.

Relative water content (RWC), Water saturation deficit (WSD), and water use efficiency (WUE) measurement
To measure the RWC, five fully grown topmost fresh leaves from each treatment were collected randomly, placed in polyethylene bags, and brought to the lab. To prevent any dehydration, first get the samples' fresh weight as soon as possible. To find the sample's turgid weight, it was submerged in distilled water. The sample was taken out of the distilled water and its turgid weight was measured a day later. After that, the sample was oven-dried for three days at 65 °C to dry it out. Lastly, each treatment's relative water content (RWC) and water saturation deficit (WSD) were calculated using the formula below[48]:

i) RWC % = [(FW-DW)/(TW-DW)] ×100
ii) WSD % = [(TW - FW) / (TW - DW)] × 100

where, RWC, FW, DW, and TW are relative water contents, fresh weight, dry weight, and turgid weight of the leaf samples.

According to Yuping et al.[49], instantaneous water use efficiency (WUE) was calculated as the ratio of the rates of transpiration and photosynthesis. WUE = PN / E; PN-photosynthesis, E-transpiration rate.

Gas exchange characteristics measurement
Each measurement of the gas exchange parameters, such as transpiration rate (Tr), photosynthetic rate (Pn), stomata conductance (Gs), and intercellular CO_2 concentration, was performed using a portable photosynthetic system (Li-COR, 6400, Lincon, NE, USA). On a sunny day between 11:00 am and 1 pm, all gas exchange characteristics were measured on the uppermost leaves of each pot that had fully developed.

Leaf chlorophyll content measurement
One completely developed leaf at the apex of the plant was sampled in accordance with Mannan et al.[50] to determine the amount of chlorophyll in each replication. After 20 mg fresh leaf samples were collected, vials holding twenty ml of 80% acetone were put in the dark for 72 h, covered with aluminum foil, and left. Double-beam Thermo Fisher Scientific spectrometer (model 20020) was used to measure the wavelengths at 663 nm and 645 nm. The concentrations of chlorophyll a, b, and total chlorophyll were determined using the following formula:

$$\text{Chlorophyll a (mg/g fresh weight)} = [12.7(\text{D}663) - 2.69(\text{D}645)] \times [\text{V}/100\times\text{W}]$$

$$\text{Chlorophyll b (mg/g fresh weight)} = [22.9(\text{D645}) - 4.68(\text{D663})] \times [\text{V}/100 \times \text{W}]$$

$$\text{Total chlorophyll (mg/g fresh weight)} = [20.2(\text{D645}) - 8.02(\text{D663})] \times [\text{V}/100 \times \text{W}]$$

where, D (663, 645) = Optical density of the chlorophyll extract at a wavelength of 663 and 645 nm, respectively.
V = Final volume (ml) of 80% acetone with chlorophyll extract.
W = Weight of fresh leaf sample in g.

Cell membrane stability measurement
We computed the cell membrane stability using the procedure outlined by Rady[51]. Ten (10) discs were produced in each of the two sets of samples. One set was incubated in a water bath at 40 °C for 30 min before its electrical conductivity (EC1) was measured. After incubation at 100 °C for 10 min, the other group's electrical conductivity (EC2) was assessed. To calculate MSI, the following formula was used:

$$\text{MSI(Cell membrane stability \%)} = [1 - (\text{EC1}/\text{EC2})] \times 100$$

Lipid peroxidation (Malondialdehyde) measurement
The methodology outlined by Rao and Sresty[52] was followed while measuring malondialdehyde (MDA) content in buckwheat leaves. The MDA level was measured under stress and control conditions using a reaction mixture containing 0.5 g of fresh leaf, 0.1% tri-chloroacetic acid (TCA), and 20% thiobarbituric acid (TBA). Ultimately, a spectrophotometer (Shimadzu, UV-1201; 1, Nishinokyo Kuwabara-cho, Nak-agyo-ku, Kyoto 604–8511, Japan) was used to assess the absorbance of colored supernatants at 530 and 600 nm.

Proline estimation
The proline content was measured by following the procedure described by Bates et al.[53]. The proline content was measured using 2.0 ml of proline extract, 2.0 ml of acid ninhydrin, and 2.0 ml of glacial acetic acid. The absorbance was measured at 520 nm. Real proline at a specific concentration was used to create a standard curve.

Total sugar measurement
Using the technique outlined by Somogyi[54], the total sugar content of the buckwheat leaves was measured. For 10 to 15 min, a test tube holding 0.1 g of fresh leaf and 80% ethanol was submerged in the water bath. An additional 5 ml of 80% ethanol was used for at least 2 extractions. The leaf extract solution was made with 20 ml of distilled water. Then, a test tube was filled with 0.5 ml of phenol solution (5%), and 0.5 ml of leaf extract. Three ml of concentrated H_2SO_4 were added, and the mixture started to heat up and become colored. The optical density at 490 nm waves was measured using a spectrophotometer (Systronics UV-VIS 118) after the sample was cooled to room temperature. The result was expressed in mg/g of fresh leaf weight.

Hydrogen peroxide (H_2O_2) measurement
The hydrogen peroxide (H_2O_2) content was measured using a modified Velikova et al.[55] method. Two ml of ice-cold 0.1% trichloroacetic acid (TCA) (w/v) was mixed well with 300 mg of frozen leaf powder, and the mixture was centrifuged (12,000 × g) for 15 min at 4 °C. In every replication, this method was used three times. One ml of 1 M potassium iodide and 5 ml of 10 mM potassium phosphate buffer (pH 7.0) were added to the 0.5 ml supernatant. The blank was treated with 0.1% TCA. With a Cary 100 Bio, Varian, Australia, an absorbance measurement at 390 nm was made. The same set of conditions applied to the H_2O_2 standard.

Estimation of total antioxidant (2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity)
Using the protocol outlined by Okonogi et al.[56], spectrophotometric analysis was utilized to evaluate the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical's scavenging activity. The DPPH solution's color changing from purple to yellow when the radical was neutralized by antioxidants served as the fundamental concept for the assay. The extracts were mixed with methanol to create a stock solution for each leaf extract (10 mg/ml). One thousand aliquots of different leaf extract dilutions were mixed with 5000 aliquots of DPPH (150 µM) that had been dissolved in methanol. Following a vigorous shake, the mixture was left to stand at room temperature in the dark for half an hour. The absorbance at 517 nm was recorded in order to calculate the quantity of residual DPPH. Three measurements were made for each. The radical-scavenging activity as a percentage was calculated using the following equation:

$$\text{DPPH radical} - \text{scavenging}\,(\%) = \text{A0} - \text{A1}/\text{A0} \times 100$$

where A1 is the sample's absorbance and A0 is the control's absorbance. A sample's IC50 value indicates the concentration needed to scavenge 50% of the DPPH free radicals. The reaction mixture's lower absorbance suggests a higher degree of free radical scavenging activity.

Estimation of yield and yield contributing parameters
At maturity, 3 plants per pot were harvested and the number of grains/plants, 1000-grain weight, and grain yield/plant was calculated.

Statistical analysis
The obtained data were statistically analyzed for each parameter using analysis of variance (ANOVA). Using the Duncan technique, the treatments were compared using the least significant difference (LSD) at a confidence

Treatments	Relative water content (%)		Water saturation deficit (%)		Water use efficiency	
	Well water	Drought	Well water	Drought	Well water	Drought
Control	83.36 ± 0.52*b	67.79 ± 1.08d	16.64 ± 0.52*c	32.21 ± 1.08a	1.44 ± 0.061b	1.75 ± 0.079a
BC	86.08 ± 1.01a	75.56 ± 3.69c	13.92 ± 1.01c	24.44 ± 3.69b	1.42 ± 0.088b	1.46 ± 0.149b
Fe_3O_4 NPs	88.16 ± 1.15a	80.12 ± 2.34c	11.84 ± 1.15c	19.88 ± 2.34b	1.42 ± 0.086b	1.42 ± 0.060b
BC + Fe_3O_4 NPs	89.27 ± 1.64a	82.96 ± 2.11c	10.73 ± 1.64c	17.04 ± 2.11c	1.43 ± 0.093b	1.35 ± 0.027b
CV (%)	4.1		18.3		10.3	

Table 2. Effects of rice husk biochar and Fe_3O_4 nanoparticle on relative water content, water saturation deficit, and water use efficiency of buckwheat leaf under limited water conditions. * SE, Different letters in each column indicated that the means of the treatments were statistically varied at $p = 0.05$.

Treatments	Photosynthesis rate (μmol CO_2 /m² /s)		Stomatal conductance (mol H_2O /m² /s)		Transpiration rate (mol H_2O/ m² /s)	
	Well water	Drought	Well water	Drought	Well water	Drought
Control	4.93 ± 0.24*a	3.74 ± 0.09c	0.26 ± 0.033NS	0.18 ± 0.024NS	3.45 ± 0.31a	2.15 ± 0.05c
BC	5.08 ± 0.24a	3.98 ± 0.08b	0.27 ± 0.025NS	0.21 ± 0.011NS	3.59 ± 0.14a	2.79 ± 0.27b
Fe_3O_4 NPs	5.17 ± 0.23a	4.24 ± 0.11b	0.29 ± 0.027NS	0.24 ± 0.008NS	3.65 ± 0.14a	3.00 ± 0.16b
BC + Fe_3O_4 NPs	5.26 ± 0.22a	4.38 ± 0.07b	0.32 ± 0.032NS	0.25 ± 0.009NS	3.71 ± 0.15a	3.26 ± 0.12a
CV (%)	6.7		16.0		2.5	

Table 3. Effects of rice husk biochar and Fe_3O_4 nanoparticle on photosynthetic rate, stomatal conductance, and transpiration rate of buckwheat leaf under limited water conditions. * SE, Different letters in each column indicated that the means of the treatments were statistically varied at $p = 0.05$, NS-Non significant.

level of $p = 0.05$[57]. For this investigation, CropStat 7.2 was utilized as the program, and Microsoft Office Excel 2016 was used to create the graphs. Principal component analysis (PCA) was performed by R software (R Core Team, 2019) for data analysis to check the association among different studied morpho-physiological attributes and treatments.

Results

Relative water content, water saturation deficit, and water use efficiency

The effects of biochar and NPs treatments on relative water content, water saturation deficit, and water use efficiency in both drought and well water conditions are presented in Table 2. The application of BC, Fe_3O_4 NPs, and BC + Fe_3O_4 NPs significantly improved water relation features under well water (80% of FC) and drought (40% of FC) conditions as compared to control treatments. Drought stress dramatically reduced the relative water content by 19.39% as compared to well water conditions. Additionally, water saturation deficit and water use efficiency rose by 93.56% and 21.52%, respectively. In both well water and drought conditions, the application of BC and Fe_3O_4 NPs, either separately or in combination, improved the water relation features. Comparing to untreated drought conditions, BC, Fe_3O_4 NPs, and BC + Fe_3O_4 NPs increased the relative water content by 11.46, 18.18, and 22.37%, respectively. Comparing these treatments to untreated drought circumstances, however, the water saturation deficit efficiency was decreased by 24.12, 38.28, and 47.09%, respectively. Additionally, it was found that, in comparison to untreated drought conditions, BC, Fe_3O_4 NPs, and BC + Fe_3O_4 NPs decreased the water use efficiency by 16.57%, 18.55%, and 22.85%, respectively. Compared to other treatments, it was shown that BC + Fe_3O_4 NPs was the most successful choice for treatment in both well water and water-stressed situations.

Photosynthesis rate, stomatal conductance, and transpiration rate

The effects of biochar and Fe_3O_4 NPs as a foliar spray on the photosynthetic rate, stomatal conductance, and transpiration rate of buckwheat under well water and drought conditions are shown in Table 3. Comparing the photosynthetic rate to the control, the drought stress reduced it to 24.13%. The positive was observed when buckwheat under drought stress was treated with biochar and Fe_3O_4 NPs single or combined. By comparison, the photosynthetic rate rose to 17.11% when combined biochar and Fe_3O_4 NPs were applied as compared to plants exposed to drought without treatment. However, it was enhanced by 6.42% when only the biochar was applied in the soil. The photosynthetic rate increased by 13.37% when 400 ppm Fe_3O_4 NPs was applied as foliar spray alone. When compared to untreated buckwheat leaves, the combination of biochar and Fe_3O_4 NPs increased stomatal conductance and transpiration rate (Table 3). In comparison to untreated drought-exposed plants, the plants that received only biochar and 400 ppm Fe_3O_4 NPs showed a 16.67% and 29.77% increase in stomatal conductance and transpiration rate, respectively. When plants were treated with only 400 ppm Fe_3O_4 NPs, these were 33.33% for stomatal conductance and 39.53% for transpiration rate throughout treatment. Buckwheat leaves' stomatal conductance and transpiration rate increased by 38.89% and 51.63%, respectively, when 400 ppm Fe_3O_4 NPs and rice husk biochar were applied together, compared to untreated drought-stressed plants.

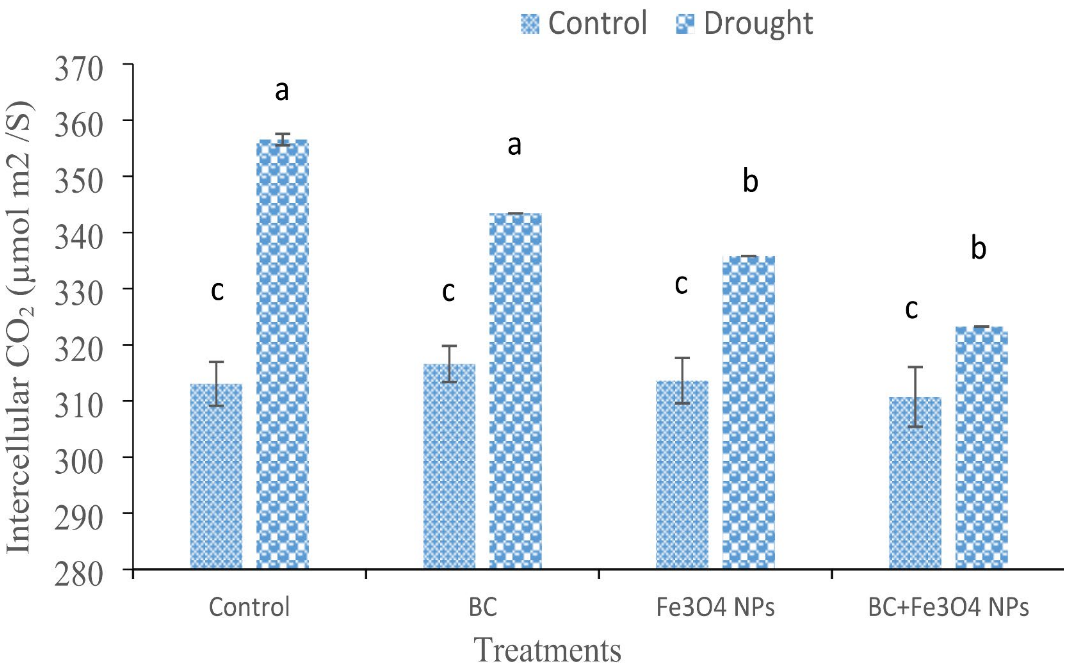

Fig. 1. Effects of rice husk biochar and Fe_3O_4 nanoparticle on intercellular CO_2 of buckwheat leaf under limited water conditions. Bars indicate ± SE, Different letters in each column indicated that the means of the treatments were statistically varied at $p = 0.05$.

Treatments	Chlorophyll a (mg/g fresh wt. of leaf)		Chlorophyll b (mg/g fresh wt. of leaf)		Total chlorophyll (mg/g fresh wt. of leaf)	
	Well water	Drought	Well water	Drought	Well water	Drought
Control	1.68 ± 0.06*b	1.07 ± 0.06e	0.58 ± 0.02NS	0.44 ± 0.02NS	2.26 ± 0.05c	1.51 ± 0.08f
BC	1.74 ± 0.07b	1.10 ± 0.03e	0.63 ± 0.01NS	0.48 ± 0.03NS	2.37 ± 0.07c	1.58 ± 0.06f
Fe_3O_4 NPs	1.99 ± 0.03a	1.31 ± 0.06d	0.71 ± 0.03NS	0.53 ± 0.02NS	2.70 ± 0.03b	1.84 ± 0.08e
BC + Fe_3O_4 NPs	2.13 ± 0.05a	1.55 ± 0.06c	0.80 ± 0.02NS	0.61 ± 0.04NS	2.93 ± 0.07a	2.16 ± 0.03d
CV (%)	6.1		7.1		4.9	

Table 4. Effects of rice husk biochar and Fe_3O_4 nanoparticle on chlorophyll a, chlorophyll b, and total chlorophyll in buckwheat leaf under limited water conditions. * SE, Different letters in each column indicated that the means of the treatments were statistically varied at $p = 0.05$, NS-Non significant.

Intercellular CO_2 concentration

The application of rice husk biochar and Fe_3O_4 NPs had a substantial impact on the intercellular CO_2 content in buckwheat leaves (Fig. 1). When compared to plants that were not grown under well water, it was found that drought stress elevated the internal CO_2 content of buckwheat leaves. Under drought stress when buckwheat was treated with biochar and Fe_3O_4 NPs singly or combined, intercellular CO_2 concentrations decreased, and the decreasing rate was higher in combined treatment. In comparison to untreated drought-exposed plants, the plants that received biochar and 400 ppm Fe_3O_4 NPs showed a 9.35% decrease in intercellular CO_2 concentrations.

Chlorophyll a, chlorophyll b, and total chlorophyll

The application of biochar, along with Fe_3O_4 NPs resulted in a significant ($p < 0.05$) increase in total chlorophylls and chlorophyll a. On the other hand, chlorophyll b in buckwheat leaves was increased but the difference was non-significant (Table 4). In comparison to the well-water non-treated plants, the single application of biochar raised the levels of chlorophyll a, chlorophyll b, and total chlorophyll content by 3.57% (1.74 mg/g FW), 8.62% (0.63 mg/g FW), and 4.86% (2.37 mg/g FW), respectively. Chlorophyll a, b, and total chlorophyll content increased by 18.45% (1.99 mg/g FW), 22.41% (0.71 mg/g FW), and 19.47% (2.70 mg/g FW), respectively, upon the application of 400 ppm Fe_3O_4 NPs alone. When 400 ppm Fe_3O_4 NPs were combined with biochar, there was an enhancement of 26.79% (2.13 mg/g FW), 37.93% (0.80 mg/g FW), and 29.65% (2.93 mg/g FW) in chlorophyll a, b, and total chlorophyll content, respectively, compared to untreated well water samples. Under drought stress, there was a reduction of 36.31%, 24.14%, and 33.18% in chlorophyll a, b, and total chlorophyll in buckwheat leaves, respectively, when compared to untreated well-water conditions. The exogenous application of Fe_3O_4 NPs and soil application of biochar either individually or in combination exhibited a positive synergy with the plant system, resulting in an augmentation of chlorophyll a, b, and total chlorophyll synthesis under conditions of water stress (Table 4). Notably, it was observed that the application of 400 ppm Fe_3O_4 NPs in conjunction with biochar yielded maximum outcomes in comparison to other experimental groups. This particular treatment led to a substantial increase in chlorophyll a, b, and total chlorophyll levels by 44.86% (1.55 mg/g FW), 38.64% (0.61 mg/g FW), and 43.05% (2.16 mg/g FW), respectively, as opposed to their corresponding to drought-stressed plants.

Membrane stability index, malondialdehyde, and proline

The enhancement of membrane stability is crucial for the proper functioning of plants. The findings, as presented in Table 5, indicated that the application of 400 ppm Fe_3O_4 NPs + biochar yielded the most favorable outcomes in non-stress plants, resulting in a remarkable increase of 9.65% in membrane stability compared to the non-treated group. However, under drought stress, the membrane stability decreased significantly by 14.31% compared to the well-water plants. Interestingly, the use of biochar as soil amendment singly led to a notable improvement in membrane stability, with an increase of 11.84%. Additionally, the application of 400 ppm Fe_3O_4 NPs resulted in respective increases of 13.53% in membrane stability. Notably, the most significant improvement was observed when 400 ppm Fe_3O_4 NPs were combined with bioch*a*r resulting in a remarkable increase of 16.07% in the membrane stability index compared to untreated drought-stressed plants. These findings highlight the potential of Fe_3O_4 NPs and biochar in enhancing membrane stability, particularly in water deficit conditions.

The malondialdehyde content in buckwheat leaves significantly increased by 68.25% under drought-stress conditions compared to well-watered plants (Table 5). Interestingly, the application of Fe_3O_4 and rice husk biochar alone and combined resulted in a decrease in malondialdehyde content. Application of biochar in soil led to a reduction in MDA levels to 10.24%, while the use of 400 ppm Fe_3O_4 NPs decreased it by 27.54%. Furthermore, the combined application of 400 ppm Fe_3O_4 NPs + biochar resulted in a decrease in MDA to 38.19% compared to untreated drought-stressed plants.

Drought stress led to a rise in proline production under drought conditions (Table 5). The soil application of rice husk biochar resulted in a 33.33% increase in proline production (0.18 mg/g FW) in plants experiencing drought. The application of 400 ppm Fe_3O_4 NPs led to proline increases of 31.25% (0.21 mg/g FW) compared to the plants exposed to drought. Furthermore, the combined use of 400 ppm Fe_3O_4 NPs + biochar led to a 43.75% increase (0.23 mg/g FW) compared to untreated plants experiencing drought stress.

Total sugar content

The sugar content decreased due to drought by 56.52% in buckwheat leaves compared to well-water plants. Applying biochar and Fe_3O_4 NPs alone or combined increased the total sugar under well-water and drought stress, but the increasing rate was higher in drought-stressed plants compared to well-water plants (Fig. 2). Under drought conditions biochar and Fe_3O_4 NPs it was increased the sugar by 25% and 56.25%, respectively compared to non-treated plants. Notably, when Fe_3O_4 NPs were combined with biochar, even better results were observed. The application of 400 ppm Fe_3O_4 NPs + biochar led to sugar content increases of 68% (0.27 µg/g FW), in comparison to the control group exposed to drought conditions.

Hydrogen peroxide (H_2O_2) content

The utilization of Fe_3O_4 nanoparticles and rice husk biochar resulted in a reduction in H_2O_2 content, with a more significant decrease observed when Fe_3O_4 and biochar were used in combination. Specifically, the application of biochar and 400 ppm Fe3O4 NPs led to reductions in H_2O_2 levels by 11.26% (4.74 µmol/g FW) and 26.44% (3.93 µmol/g FW), respectively, compared to untreated drought conditions. Conversely, the joint application of biochar + 400 ppm Fe_3O_4 NPs further decreased the H_2O_2 content to 31.09% (3.68 µmol/g FW) in comparison to the non-treated drought-exposed plants (Fig. 3).

Total antioxidant contents

The soil amendment with rice husk biochar and combined with foliar spray of Fe_3O_4 NPs at 400 ppm concentration significantly influenced the activity of total antioxidants of buckwheat plants grown under well water and drought conditions (Fig. 4). The biochar application alone increased the total antioxidant by 3.99% and it was 7.99% when Fe_3O_4 NPs at 400 ppm concentration was applied singly in drought-treated plants compared

Treatments	Membrane stability index (%)		Malondialdehyde (MDA) (nano mole/g fresh wt. of leaf)		Proline (mg/g fresh wt. of leaf)	
	Well water	Drought	Well water	Drought	Well water	Drought
Control	72.96 ± 2.39*b	62.52 ± 1.57c	33.67 ± 0.88d	56.65 ± 2.88a	0.12 ± 0.004NS	0.16 ± 0.012NS
BC	77.64 ± 1.06a	69.92 ± 0.80b	32.51 ± 0.36d	50.82 ± 2.89b	0.11 ± 0.004NS	0.18 ± 0.006NS
Fe3O4 NPs	78.38 ± 0.83a	70.98 ± 0.94b	33.81 ± 0.39d	41.05 ± 2.98c	0.11 ± 0.008NS	0.21 ± 0.006NS
BC + Fe3O4 NPs	80.00 ± 0.09a	72.57 ± 0.59b	31.69 ± 0.88d	35.05 ± 1.65d	0.12 ± 0.005NS	0.23 ± 0.006NS
CV (%)	2.1		3.3		0.11	

Table 5. Effects of rice husk biochar and Fe_3O_4 nanoparticle on membrane stability index, malondialdehyde, and proline in buckwheat leaf under limited water conditions. * SE, Different letters in each column indicated that the means of the treatments were statistically varied at $p = 0.05$, NS-Non significant.

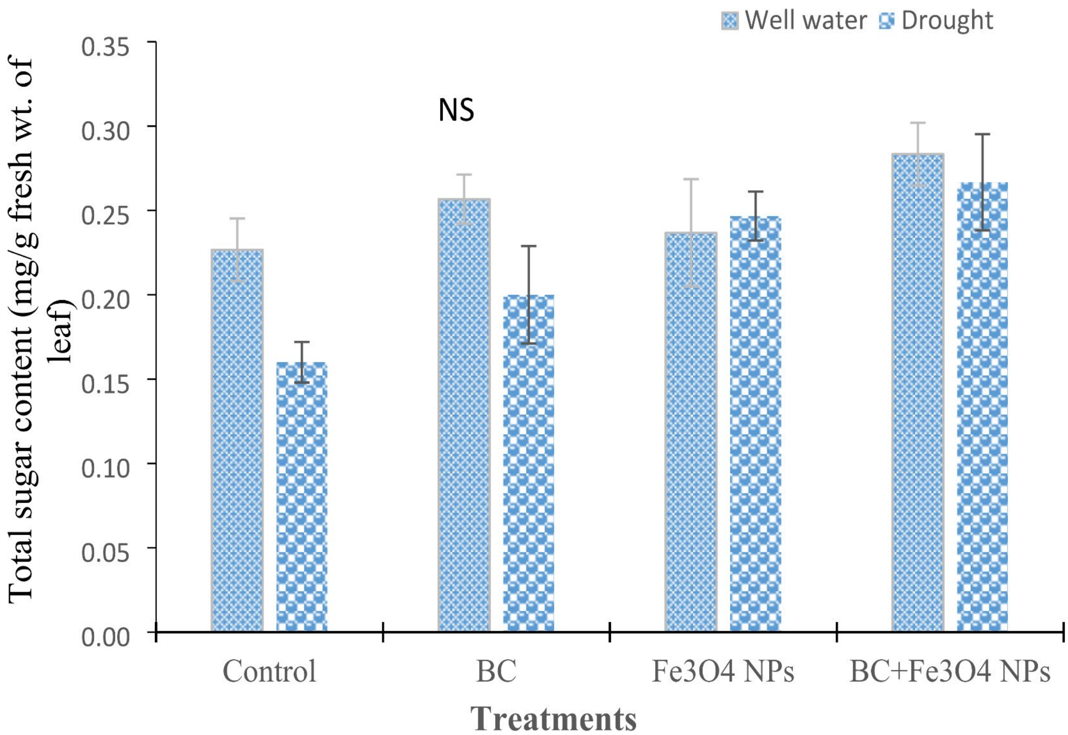

Fig. 2. Effects of rice husk biochar and Fe_3O_4 nanoparticle on total sugar content in buckwheat leaf under limited water conditions. Bars indicate ± SE.

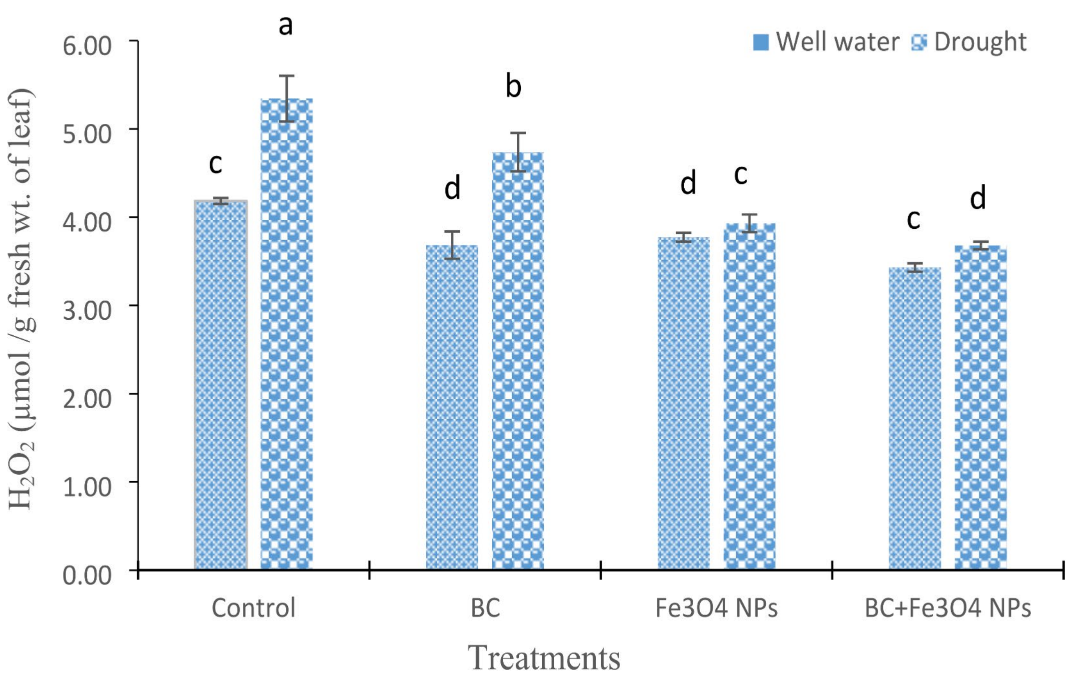

Fig. 3. Effects of rice husk biochar and Fe_3O_4 nanoparticle on H_2O_2 content in buckwheat leaf under limited water conditions. Bars indicates ± SE, Different letters in each column indicated that the means of the treatments were statistically varied at $p = 0.05$.

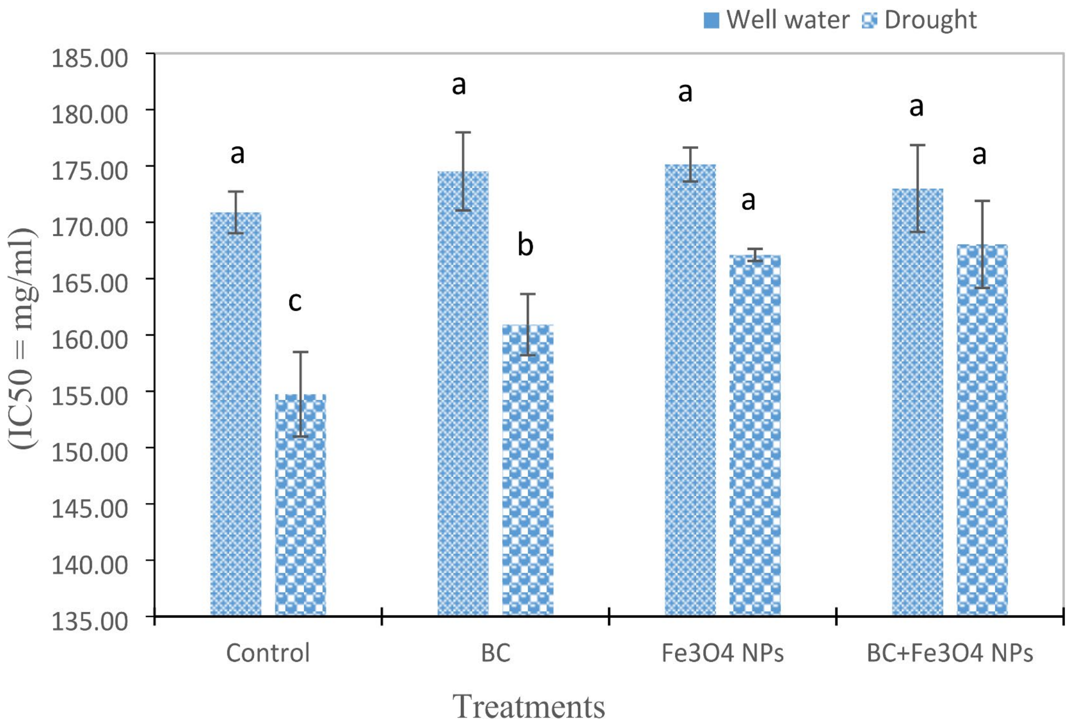

Fig. 4. Effects of rice husk biochar and Fe_3O_4 nanoparticle on total anti-oxidant (IC50) content in buckwheat leaf under limited water conditions. Bars indicates ± SE, Different letters in each column indicated that the means of the treatments were statistically varied at $p = 0.05$.

Treatments	Number of grains/plants		1000-grains weight (g)		Grain yield (g/plant)	
	Well water	Drought	Well water	Drought	Well water	Drought
Control	153.67 ± 2.03*a	116.67 ± 1.77c	15.47 ± 0.69b	9.53 ± 0.18e	2.32 ± 0.15b	1.14 ± 0.01e
BC	154.33 ± 2.97a	123.00 ± 2.65b	15.53 ± 0.52b	10.80 ± 0.35d	2.32 ± 0.09b	1.30 ± 0.01d
Fe_3O_4 NPs	155.00 ± 3.79a	126.33 ± 2.97b	16.40 ± 0.61a	11.37 ± 0.32d	2.62 ± 0.05a	1.45 ± 0.02d
BC + Fe_3O_4 NPs	156.33 ± 1.86a	130.67 ± 2.73b	17.37 ± 0.49a	13.67 ± 0.67c	2.73 ± 0.11a	1.72 ± 0.01c
CV (%)	3.3		6.4		6.6	

Table 6. Effects of rice husk biochar and Fe_3O_4 nanoparticle on the number of grains/plants, 1000-grain weight, and grain yield of buckwheat under limited water conditions. * SE, Different letters in each column indicated that the means of the treatments were statistically varied at $p = 0.05$.

to non-treated drought-stressed plants. On the other hand, combined biochar + NPs application increased total antioxidants by 8.59% in the same conditions.

Yield and its components of buckwheat

Under well water and drought conditions, the combination of foliar spraying Fe_3O_4 NPs and soil application biochar had a beneficial effect on buckwheat yield (Table 6). Compared to untreated plants, biochar and Fe_3O_4 NPs at 400 ppm concentrations, both alone and in combination, significantly increased buckwheat yield and its constituent parts. Furthermore, the most effective treatment was the combination of 400 ppm Fe_3O_4 and rice husk biochar this treatment increased the number of grains per plant by 11.99%, the weight of 1000- grains by 43.44, and the grain yield per plant by 50.87% when compared to the drought-treated plants that were not treated.

Pearson correlation coefficients

The heat map of Pearson correlation coefficients depicts the association among the 19 measured physiological, biochemical, and yield traits. The blue and red squares are negative and positive correlations, respectively (Fig. 5). Strong correlations were observed between grain yield and total chlorophyll (0.84), antioxidant (1.00), MSI (0.79), photosynthetic rate (0.99), proline (0.90), transpiration rate (0.91), relative water content (0.94), stomatal conductance (0.76), total sugar (0.88), and hydrogen peroxide (0.59). However, yield is negatively correlated with water saturation deficit (−0.94), water use efficiency (−0.86), MDA (−1.00), and intercellular CO_2 (−0.92). However, these four traits are positively correlated with each other.

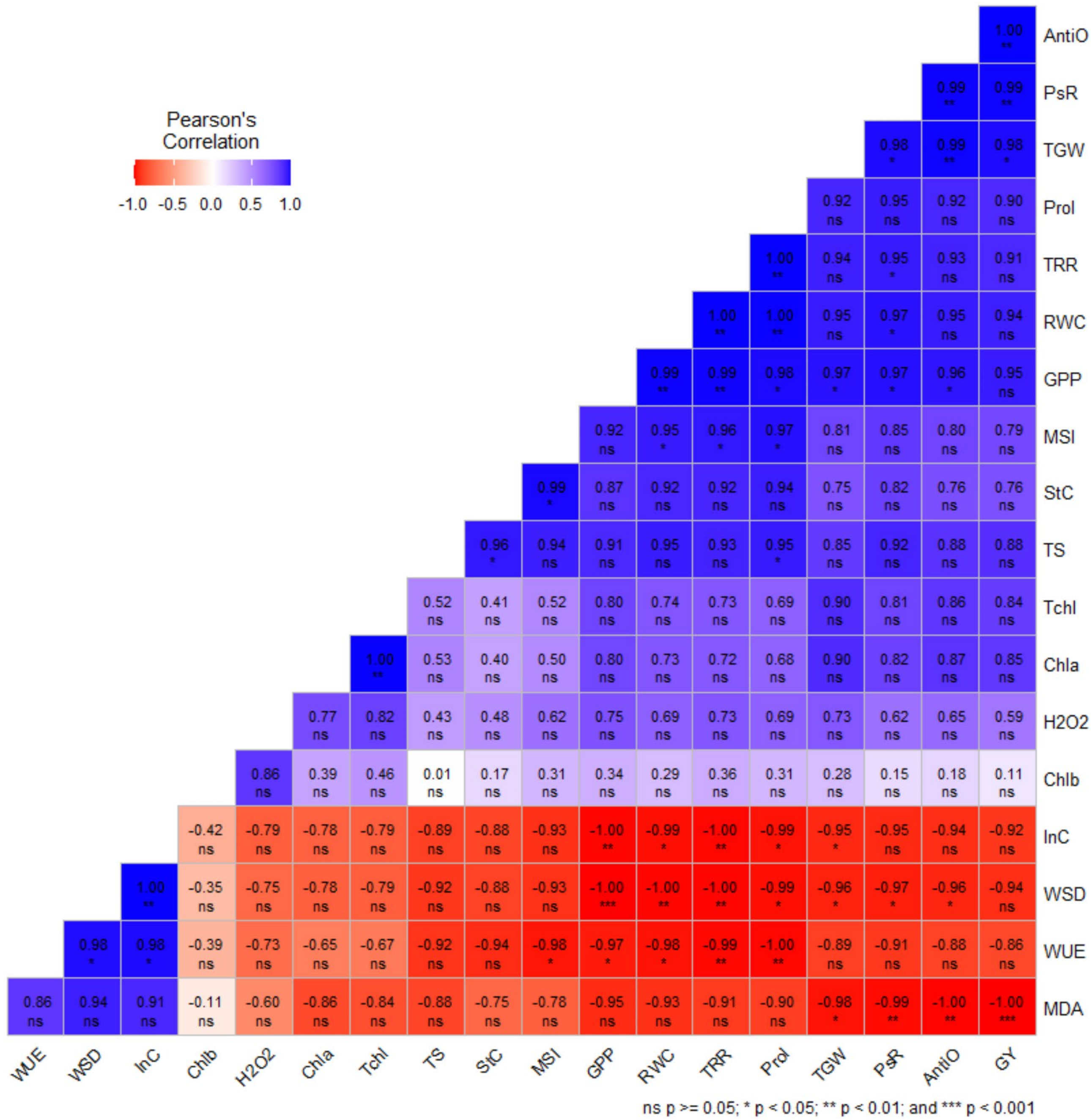

Fig. 5. Pearson's correlation coefficients among various traits were assessed under both drought and control conditions in buckwheat. Correlated marked with ns = non- significant at $P > 0.05$; ∗ = significant at $P \leq 0.05$; ∗ ∗ = highly significant at $P \leq 0.01$; ∗ ∗ ∗ = very highly significant at $P \leq 0.001$. The evaluated traits include relative water content (RWC), photosynthetic rate (PsR), stomatal conductance (StC), transpiration rate (TRR), chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (Tchl), membrane stability index (MSI), proline (Prol), antioxidant (AntiO), hydrogen peroxide (H2O2), malondialdehyde (MDA), water saturation deficit (WSD), water use efficiency (WUE), and intercellular $CO_{2,}$ (InC), total sugar (TS), grain per plant (GPP), thousand-grain weight (TGW) and grain yield (GY).

Principal component analysis

The interrelationship among the evaluated treatments across three different varieties and traits was assessed using principal component analysis (PCA) (Fig. 6). Here, PCA1/Dim1 accounts for 83.7% of the total variance, while PCA2/Dim2 accounts for 9.7%. Treatments combined with various varieties are represented in red, whereas the blue vectors signify the original variables and their influence on the principal components. The vectors' length and direction indicate the magnitude and direction of the variables' impact on the principal components.

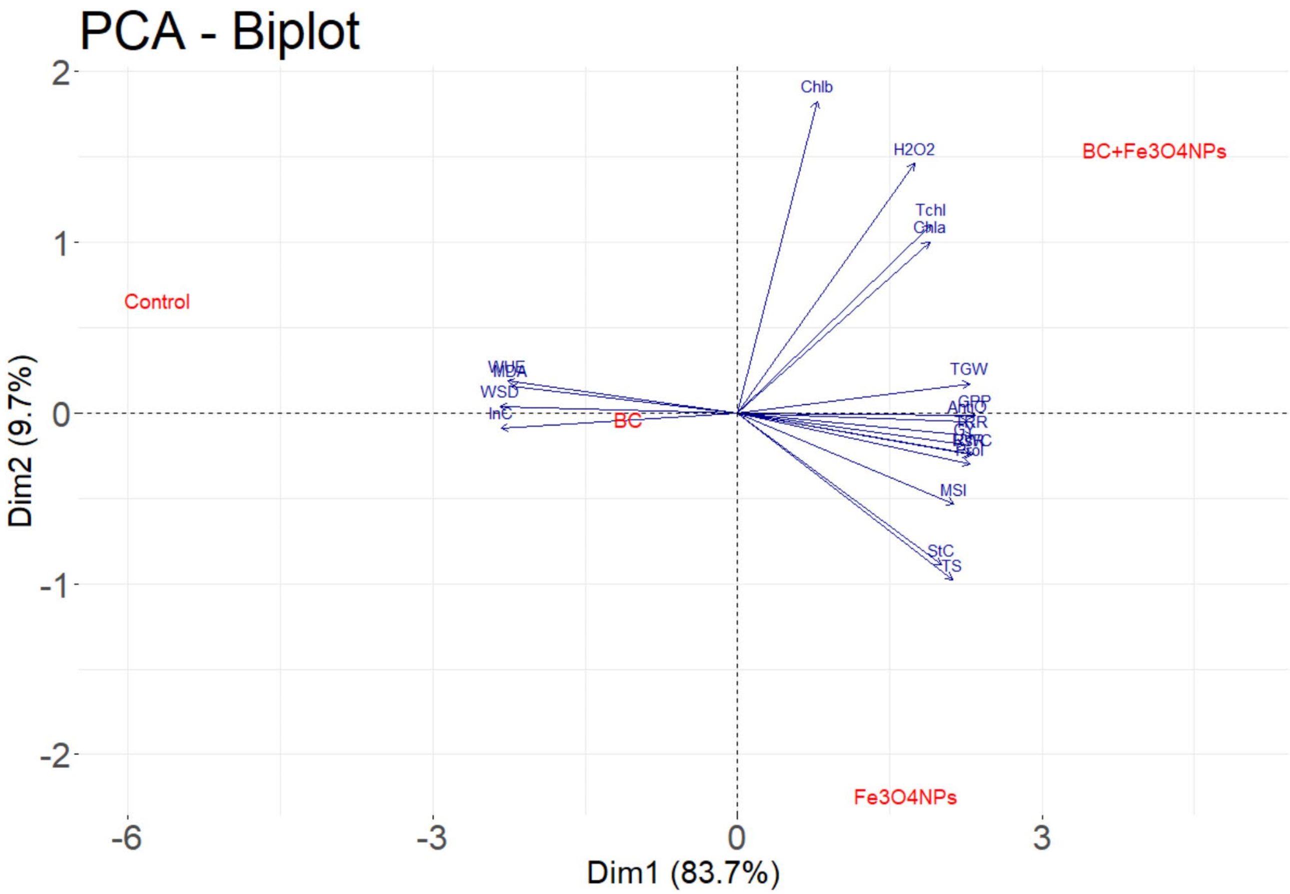

Fig. 6. PCA biplot for projection of the two principal components showing the performance of treatments for the different physiological, biochemical, and yield traits of common buckwheat.

The treatments include - control, rice husk biochar, 400 ppm Fe_3O_4 nanoparticle solution, and rice husk biochar + 400 ppm Fe_3O_4 nanoparticle solution.

A strong positive relationship was observed among traits such as relative water content (RWC), photosynthetic rate (PsR), stomatal conductance (StC), transpiration rate (TRR), chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (Tchl), membrane stability index (MSI), proline (Prol), antioxidant (AntiO), hydrogen peroxide (H_2O_2), total sugar (TS), grain per plant (GPP), thousand-grain weight (TGW) and grain yield (GY). Conversely, there was a negative association found between yield-contributing features and intercellular CO_2 (InC), malondialdehyde (MDA), water saturation deficit (WSD), and water use efficiency (WUE). There is a positive correlation between these four characteristics.

Notably, the treatment "BC + Fe_3O_4 NPs" is positioned on the positive side of both Dim1 (83.7%) and Dim2 (9.7%), which are the two principal components explaining the most variance in the data. That means the treatment BC + Fe_3O_4 NPs likely has a significant positive effect on traits like chlorophyll content and hydrogen peroxide concentration, suggesting enhanced photosynthetic activity or stress mitigation resulting in increased yield compared to other treatments, such as "Control" and "BC" alone, which are positioned more negatively in Dim1 and "Fe_3O_4 NPs" positioned more negatively in Dim2.

Discussion

One of the main abiotic stressors on plant growth, yield, and biomass production is drought, which also limits the physiological, biochemical, and molecular traits of the plants[58–62]. Drought stress significantly reduced the RWC and increased the WSD and WUE in our current study (Table 2). Mesophyll cell turgidity and low leaf water potential, thicker leaf tissue, or decreased soil moisture could all be contributing factors to this obstruction of water transfer from the roots to the shoots[63,64]. Biochar alters the soil's hydrological properties and increases the soil's capacity to hold water. Consequently, the physiological and biochemical properties of plants can be enhanced[65,66]. This suggests that making water available in soil aids in the completion of a number of physiological processes that take place inside plant cells. These could be the reasons why, in our experiment, buckwheat plants grown in BC-treated pots showed comparatively better physiological features than plants that were not treated. Sattar et al.[67] observed BC significantly increased the RWC of maize cultivated in drought-affected areas. Furthermore, the application of BC alone increases the cultivated soil's capacity to hold water, which has a favorable impact on boosting water availability and the effectiveness of water usage features[68,69]. One possible explanation for the favorable benefits of BC application in this study is the preservation of appropriate soil moisture surrounding the root zone, which can protect plant systems from drought stress[70]. Probably, BC can successfully contribute to lowering wilting points while raising soil moisture constants because of its advantages in the buckwheat root zone[71]. Therefore, the effectiveness of water consumption is probably going to be significantly impacted by adding BC as a soil amendment to the cultivated soil. Dola et al.[72] reported that Fe_3O_4 NPs improved the relative water content and reduced the water saturation deficit in soybean leaves under drought stress. Taran et al.[73] reported that Cu and Zn NPs induced an increase in RWC by 8–10% in the leaves of seedlings of two different wheat varieties exposed to drought stress. According to Deepa et al.[74], compared to the regular relative water content (RWC) of plants (64%), the application of Fe and Cu NPs can maintain a higher relative water content (RWC) level of 71% in plants. For the transportation of nutrients and water content into the required region of the plant body, nano-fertilizers can perform reliably[74]. Compared to applying biochar and NPs alone, our work has shown that applying biochar and Fe_3O_4 NPs together was the most efficient way to increase the relative water content, decrease the water saturation deficit, and improve the water usage efficiency in buckwheat leaves under drought stress.

The impact of drought stress on buckwheat resulted in a decrease in photosynthesis rate, transpiration rate, and stomatal conductance, and increased intercellular CO_2 (Table 3; Fig. 1). However, the application of Fe_3O_4 NPs and rice husk biochar either alone or in combination, led to an expansion in photosynthesis rate, transpiration rate, stomatal conductance, and intercellular CO_2under both well-watered and drought-stress conditions. This increase in gas exchange parameters was attributed to the ability of nanoparticles to conserve water during drought, as suggested by Zhu and Haijun[75]. Previously, it has been reported that BC amendment buffers the effects of drought stress on carbon assimilation and photosynthesis, which is linked with boosted chlorophyll synthesis and a less pronounced reduction in stomata conductance[76,77]. Additionally, biochar was found to maintain higher rates of photosynthesis, intracellular CO_2 concentration, transpiration rate, and stomatal conductance in maize plants in comparison with those grown in untreated soil, as reported by Haider et al.[25].

The rate of photosynthesis in plants is closely linked to the chlorophyll content, which can be negatively affected by drought stress-induced oxidative damage, leading to a disruption in the photosynthetic apparatus and a decrease in chlorophyll content[78]. In our experiment, the buckwheat plants' chlorophyll levels dramatically increased as a result of the application of nano-iron. Our result was supported by Mohammadi et al.[79], who suggested that the application of 750 ppm Fe_3O_4 nanoparticles as foliar spray increased the total chlorophyll content (0.55 mg g^{-1}) of the peppermint plants. In sunflower leaves, the total chlorophyll content increased positively after the application of biocompatible magnetic nanofluid (MNF)[80]. After the exogenous application of nano-TiO_2, the content of chlorophyll, carotenoids, and anthocyanin in barley also increased significantly[81]. More specifically, chlorophyll structure, reception of sunlight, production of pigment, and RUBISCO activity all improved noticeably after the application of nano-TiO_2, which further enhanced the amount of photosynthesis in the plant. Also, according to Tarafdar et al.[82], the chlorophyll content of pearl millet crops and savory plants can be increased with the foliar application of Zn nano-fertilizer. BC application mitigated the adverse effect of drought stress by improving WUE, stomata conductance, and chlorophyll synthesis in cowpea, okra, and tomato plants[83]. In another study, Lyu et al.[28] noted that BC application improves electron transport and enzymatic activities, by reducing the damaging effects of drought stress on the photosynthetic apparatus. In essence, BC promotes the synthesis of photosynthetic pigments and shields the photosynthetic machinery from oxidative damage brought on by dryness. But in both well-watered and drought-stressed settings, the application of Fe_3O_4 NPs and biochar was found to improve chlorophylls; in our investigation, this impact was more noticeable when the nanoparticles and biochar were applied together (Table 4).

Drought stress decreased the membrane stability and increased the reactive oxygen species (ROS) like MDA content in buckwheat plants. Reduction in chlorophyll concentration in buckwheat leaves may be attributed to the detrimental effects of osmotic stress on the chloroplast layers, which leads to an increase in membrane permeability[84,85]. In this study, the decreased chlorophyll could be due to damage to the thylakoid membranes, as a result of the destructive effect of reactive oxygen species (ROS) on chloroplasts[86]. The generation of reactive oxygen species (ROS) was significantly increased due to water deficiencies[87] which was observed in our study. Drought induces osmotic stress, thereby eliciting cellular stress responses and fostering the generation of reactive oxygen species (ROS) disrupting regular cellular activities. However, the application of biochar and NPs alone and in combination increased the membrane stability and decreased the MDA content in our study (Table 5). Our results was supported by Hafez et al.[88], who observed that the BC addition enhanced drought stress tolerance in barley plants by decreasing lipid peroxidation by improving membrane stability, RWC, and water pressure. In another study, *Medicago ciliaris* plants grown under drought stress treated with BC showed a significant reduction in MDA concentration. Lashari et al.[89] noted that the application of manure and BC application appreciably improved the membrane stability by decreasing MDA concentration. The application of NPs (ZnO) significantly decreased the MDA accumulation and maintained membrane stability which reduced the loss of essential osmolytes[90,91]. Water deficiency substantially reduced membrane stability, efficiency of PS-II, and chlorophyll contents[44]. However, the exogenous application of NPs (ZnO) maintained membrane stability and cell water status under drought stress, thereby improving efficiency of PS-II and metabolic processes[92].

Proline accumulation improves plant–water status, stabilizes proteins, DNA, enzymes, and cellular membranes, and scavenges the ROS, protecting plants from drought-induced oxidative damage[93]. In our investigation, we found that applying Fe_3O_4 NPs and biochar enhances the accumulation of proline in buckwheat leaves. Mohammadi et al.[94] corroborated our findings, suggesting that TiO_2NPs application stimulates the P5CS1 gene, which codes for d1-pyrroline-5-carboxylate synthetase, a proline synthesis-related enzyme. NPs up-regulate the synthesis of proline and sugars that maintain cellular membranes, proteins, and enzymes under drought stress, thereby improving plant performance under water-scarce conditions[95,96]. Similarly, NPs (TiO_2)

also appreciably improved the accumulation of proline, total sugars, total proteins, glycine betaine, and phenolic compounds, and improve plant growth under drought stress[97,98]. Khan et al.[99] noted that BC application increased the total soluble proteins (TSP), free amino acids (FAA) and proline synthesis which countered the toxic effects of drought stress that result was supported of our findings. In another study BC countered the toxic effects of drought stress by increasing osmolytes synthesis, maintaining hormonal balance and antioxidant activity[100]. Therefore, BC maintains the osmolytes and hormones accumulation which protect plants from the damaging impacts of drought and improve plant performance under drought stress.

We also discovered that drought stress caused significant plant damage as seen by elevated MDA, H_2O_2, and reduced total antioxidant (IC50) activities (Table 5; Fig. 3, and Fig. 4). Drought stress modifies the composition of cell membranes, leading to changes in membrane permeability, integrity, and stability[101,102]. Biochar amendments improve antioxidants by improving plant metabolic functions, and cell growth, reducing ROS production, and better soil-plant and water relations[103,104]. BC also improves antioxidant activities and increase in ascorbate peroxidase (APX) and SOD activity has been observed with BC application[105]. Additionally, BC treatment mitigates the harmful effects of drought stress on plants by boosting antioxidant activities[106]. Zhang et al.[107] found that biochar increased plant stress tolerance by decreasing oxidative stress and increasing antioxidant enzyme activity. Dusenge et al.[108] opined that biochar enhances plant development during drought stress by lowering MDA levels and enhancing total antioxidant activities. We discovered that using BC minimized the deleterious effects of drought on buckwheat, as evidenced by lower H_2O_2, and MDA levels. Since these amendments enhanced plant metabolic processes, cell development, decreased ROS formation, and improved soil, plant, and water relations, they enhanced antioxidant activities[109,110]. Moreover, researcher observed that the collective treatments of BC with NPs give rise to maximal antioxidant production[111], confirming the efficacy of our treatments against drought stress. Our results were in harmony with the results of Cui et al.[64] and Ye et al.[112], suggesting that exogenous melatonin application boosted stress tolerance by improving the plant's total antioxidant activities.

Drought stress caused a reduction in yield metrics of buckwheat in our study. Fe_3O_4 NPs and rice husk biochar positively impact buckwheat yield and yield components (Table 6). The results of Haider et al.[26] corroborate our findings, which showed that the application of BC enhanced wheat yield in both drought-prone and controlled environments. Iqbal et al.[113] reported that water-stressed conditions decreased plant metabolic activity, which reduced the number of grains/plant and 1000-grain weight. Zaheer et al.[114] stated that using BC enhanced grain output by improving source-sink relationships and nutritional availability in wheat under drought conditions. Fe_3O_4NPs, on the other hand, improved the yield and yield contributing factors during drought conditions, according to our findings. The direct absorption by leaves of nano-Fe_3O_4through foliar spray resulted in a considerable increase in grain yield, as reported by Sabet and Mortazaeinezhad[115]. According to Sheykhbaglou et al.[116], the Fe_3O_4nanoparticle dramatically increased harvest index (3.86%), biological yield (22.91%), and grain production (37.43%) under drought stress. It was observed that under drought, ZnO NPs increased soybean yield[117]. Dola et al.[72] also reported that Fe3O4 NPs increased the soybean yield under drought environments. Many studies revealed that the combined treatment of nanoparticles and BC enhances the yield of plants under water deficit conditions[118–120].

To investigate the interacting relationships between the indices and their principal components for acclimating the plants under drought stress, Pearson's correlation analysis and PCA biplot were performed. Traits including relative water content (RWC), photosynthetic rate (PsR), stomatal conductance (StC), transpiration rate (TRR), total chlorophyll (Tchl), proline (Prol), antioxidant (AntiO), hydrogen peroxide (H_2O_2), total sugar (TS), grain per plant (GPP), thousand-grain weight (TGW), and grain yield (GY) were found to have a strong positive relationship in our study. Conversely, there was a negative association found between yield-contributing features and intercellular CO_2 (InC), malondialdehyde (MDA), water saturation deficit (WSD), and water usage efficiency (WUE). There is a positive correlation between these four characteristics. (Fig. 5). A heatmap is a visual aid that helps clarify intricate relationships between several indices gathered from different treatments. On the other hand, the relative contributions of the treatments on the measured parameters might be investigated using PCA biplot analysis (Fig. 6). The objective of this study was to assess the effects of Fe_3O_4 NPs and rice husk biochar (BC) on plants to improve their tolerance to drought stress. The results of PCA and PCA multiple scoring analysis made it evident that 400 ppm Fe_3O_4 NPs and BC could effectively mitigate oxidative damaging effects by enhancing gas exchange characters, antioxidants, and chlorophylls, as well as by reducing ROS and regulating RWC and osmotic adjustment.

Our research indicates that when BC and Fe_3O_4 NPs were applied together, as opposed to when they were applied separately, there was a notable improvement in the physiological, biochemical attributes, yield component, and grain quantity. This may be because buckwheat plants grown in irrigation deficit conditions benefit from the addition of BC as a soil amendment and Fe_3O_4 NPs as an exogenous application, which can improve various nutrient acquisition and water enabling in the roots zone. Based on this, under irrigation deficiency treatments, the combined action of BC as a soil amendment and Fe_3O_4 NPs as exogenous applications can positively improve water relations and promote crop yield output.

Conclusions

The physio-biochemical process, grain yield, and drought stress tolerance of common buckwheat were all increased by the application of rice husk biochar and Fe_3O_4 NPs. Although drought stress modifies buckwheat plants' physiology, treated buckwheat plants exhibited improved physiological and biochemical traits when exposed to 400 ppm of Fe_3O_4 NPs and rice husk biochar. In addition, buckwheat plants treated with Fe_3O_4 NPs and rice husk biochar showed a synergistic impact that improved plant physiology and, eventually, increased buckwheat yield. The most effective method for reducing the effects of drought stress on buckwheat was a soil amendment using rice husk biochar combined with 400 ppm of Fe_3O_4 NPs. Thus, utilizing nanoparticles in combination with biochar may be a suitable substitute to lessen the adverse effects of drought on buckwheat. Future studies should investigate the molecular mechanisms that underlie the advantageous and complementary effects of biochar and Fe_3O_4 NPs in the cultivation of buckwheat under water-limited conditions.

Data availability
Data will be supplied by the 1st corresponding author upon the request.

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References
1. Hunt, H. V., Shang, X. & Jones, M. K. Buckwheat: a crop from outside the Major Chinese Domestication Centres? A review of the Archaeobotanical, Palynological and genetic evidence. *Veg. Hist. Archaeobotany*. **27**, 493–506 (2018).
2. Kumari, A. & Chaudhary, H. K. Nutraceutical crop buckwheat: a concealed wealth in the lap of Himalayas. *Crit. Rev. Biotechnol.* **40**, 539–554 (2020).
3. Koval, D. et al. Buckwheat secondary metabolites: potential antifungal agents. *J. Agric. Food Chem.* **68**, 11631–11643 (2020).
4. Kreft, I. et al. Breeding buckwheat for Nutritional Quality. *Breed. Sci.* **70**, 67–73 (2020).
5. Jacquemart, A. L., Cawoy, V., Kinet, J. M., Ledent, J. F. & Quinet, M. Is buckwheat (Fagopyrum esculentum Moench) still a Valuable Crop today? *Eur. J. Plant. Sci. Biotechnol.* **6**, 1–10 (2012).
6. Ahmad, M. et al. Impact of buckwheat stover-and pine nee-dle-derived biochars on Pb and As mobility, microbial community, and carbon stability in a contaminatedagricultural soil. *J. Environ. Manage.* **166**, 131–139. https://doi.org/10.1016/j.jenvman.2015.10.006 (2015).
7. Alvarez-Jubete, L., Arendt, E. K. & Gallagher, E. Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trends Food Sci. Technol.* **21**, 106–113 (2010).
8. Zhu, F. Chemical Composition and Health effects of Tartary Buckwheat. *Food Chem.* **203**, 231–245 (2016).
9. Martínez-Villaluenga, C., Peñas, E. & Hernández-Ledesma, B. Pseudocereal grains: Nutritional Value, Health benefits and current applications for the development of Gluten-Free Foods. *Food Chem. Toxicol.* **137**, 111178 (2020).
10. Huda, M. N. et al. Treasure from Garden: Bioactive compounds of Buckwheat. *Food Chem.* **335**, 127653 (2021).
11. Aubert, L., Decamps, C., Jacquemin, G. & Quinet, M. Comparison of Plant Morphology, Yield and Nutritional Quality of *Fagopyrum esculentum* and *Fagopyrum tataricum* Grown under Field Conditions in Belgium. *Plants*. **10**, 258 (2021).
12. Wan, Y. Water Deficit and Recovery-Induced changes in Growth, photosynthetic characteristics, antioxidant enzymes and yield of two Tartary buckwheat genotypes. *IJAB* **25**, 483–491 (2021).
13. Aubert, L., Konrádová, D., Barris, S. & Quinet, M. Different Drought Resistance mechanisms between two buckwheat species Fagopyrum esculentum and Fagopyrum tataricum. *Physiol. Plant.* **172**, 577–586 (2020).
14. Rajakumar, R. & Sankar, J. Biochar for sustainable agriculture – a review. *Int. J. Appl. Pure Sci. Agric.* **2**, 173–184 (2016).
15. Oliveira, F. R. et al. Environmental application of biochar: current status and perspectives. *Bioresour Technol.* **246**, 110–122. https://doi.org/10.1016/j.biortech.2017.08.122 (2017).
16. Ahmed, F., Arthur, E., Plauborg, F. & Andersen, M. N. Biochar effects on maize physiology and water capacity of sandy subsoil. *Mech. Agric. Conserv. Resour.* **62**, 8–13 (2016).
17. Lehmann, J. & Joseph, S. (eds.) *Biochar for Environmental Management: Science, Technology and Implementation* 2nd ed. (Routledge, 2015). https://doi.org/10.4324/9780203762264.
18. Shaon, M. J. I., Mannan, M. A., Khaliq, Q. A. & Rahman, M. M. Growth and yield response of maize to rice husk biochar. *Aust. J. Crop Sci.* **12** (12), 1813–1819 (2018).
19. Ali, S. et al. Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environ. Sci. Pollut Res.* **24**, 12700–12712. https://doi.org/10.1007/s11356-017-8904-x (2017).
20. Cornelissen, G., Martinsen, V., Shitumbanuma, V. & Agronomy, V. A. Undefined. Biochar effect on maize yield and soil characteristics in five conservation farming sites in Zambia. *mdpi com.* **3**, 256–274. https://doi.org/10.3390/agronomy302025627 (2013).
21. Paneque, M., Jose, M., Franco-Navarro, J. D., Colmenero-Flores, J. M. & Knicker, H. Effect of biochar amend-ment on morphology, productivity and water relations of sunflower plants under non-irrigation condi-tions. *Catena* **147**, 280–28728 (2016).
22. Bamminger, C. et al. Short-term response of soil microor-ganisms to biochar addition in a temperate agroecosystem under soil warming. *Agric. Ecosyst. Environ.* **233**, 308–31729 (2016).
23. Manolikaki, I. & Diamadopoulos, E. Positive effects of Biochar and Biochar-Compost on Maize Growth and Nutrient availability in two agricultural soils. *Commun. Soil. Sci. Plant. Anal.* **50**, 512–526 (2019).
24. Akhtar, S. S., Andersen, M. N. & Liu, F. Residual effects of biochar on improving growth, physiology and yield of wheat under salt stress. *Agric. Water Manag.* **158**, 61–6831 (2015).
25. Haider, G. et al. Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations. *Plant. Soil.* **395**, 141–157. https://doi.org/10.1007/s11104-014-2294-3 (2015).
26. Haider, I. et al. Potential effects of biocharapplication on mitigating the drought stress implications on wheat (Triticum aestivum L.) under variousgrowth stages. *J. Saudi Chem. Soc.* **24**, 974–981. https://doi.org/10.1016/j.jscs.2020.10.00530 (2020).
27. Kammann, C. & Graber, E. R. Biochar effects on plant ecophysiology. *Biochar Environ. Manag.* 423–452. https://doi.org/10.4324/9780203762264-2133 (2019).
28. Lyu, S., Du, G., Liu, Z., Zhao, L. & Lyu, D. Effects of biochar on photosystem function and activities of protectiveenzymes in Pyrus ussuriensis Maxim. Under drought stress. *Acta Physiol. Plant.* **38**, 1–10. https://doi.org/10.1007/s11738-016-2236-134 (2016).
29. Flexas, J., Bota, J., Loreto, F., Cornic, G. & Sharkey, T. D. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biol.* **6**, 1–11 (2004).
30. Wang, Y., Wei, Y. & Sun, J. Biochar Application promotes Growth parameters of Buckwheat and reduces theGrowth Difference. *Commun. Soil. Sci. Plant. Anal.* **47**, 1493–1502. https://doi.org/10.1080/00103624.2016.119498835 (2016).
31. Manolikaki, I. & Diamadopoulos, E. Positive effects of Biochar and Biochar-Compost on Maize Growth andNutrient availability in two agricultural soils. *Commun. Soil. Sci. Plant. Anal.* **50**, 512–526. https://doi.org/10.1080/00103624.2019.1566468 (2019).
32. Raza, M. A. S. et al. Integrating Biochar,Rhizobacteria and Silicon for Strenuous Productivity of Drought stressed wheat. *Commun. Soil. Sci. Plant. Anal.* **52**, 338–352. https://doi.org/10.1080/00103624.2020.1853149 (2021).
33. Alabdallah, N. M. & Hasan, M. M. Plant-based green synthesis of silver nanoparticles and its effective role in abiotic stress tolerance in crop plants. *Saudi J. Biol. Sci.* **28**, 5631–5639. https://doi.org/10.1016/j.sjbs.2021.05.081 (2021).
34. Hafeez, A., Razzaq, A., Mahmood, T. & Jhanzab, H. M. Potential of copper nanoparticles to increase growth and yield of wheat. *J. Nanosci. Adv. Technol.* **1**, 6–11 (2015).
35. Hashem, A. H., Abdelaziz, A. M., Askar, A. A., Fouda, H. M., Khalil, A. M., Abd-Elsalam,K. A., et al. Bacillus megaterium-mediated synthesis of selenium nanoparticles and their antifungal activity against *Rhizoctonia solani* in faba bean plants. *J. Fungi* 7, 195. https://doi.org/10.3390/jof7030195 .

36. Ahmed, T. et al. Nanoparticle-based amelioration of drought stress and cadmium toxicity in rice *via* triggering the stress responsive genetic mechanisms and nutrient acquisition. *Ecotoxicol. Environ. Saf.* **209**, 111829. https://doi.org/10.1016/j.ecoenv.2020.111829 (2021).
37. Adrees, M. et al. Simultaneous mitigation of cadmium and drought stress in wheat by soil application of iron nanoparticles. *Chemosphere* **238**, 124681. https://doi.org/10.1016/j.chemosphere.2019.124681 (2020).
38. Maity, A. et al. Influence of metal nanoparticles (NPS) on germination and yield of oat (Avena sativa) and berseem (*Trifolium alexandrinum*). *Pro Nat. Acad. Sci. India Sect. B Biol. Sci.* **88**, 595–607. https://doi.org/10.1007/s40011-016-0796-x (2018).
39. Khan, I., Saeed, K. & Khan, I. Nanoparticles: Properties, applications and toxicities. Arab. *J. Chemi*. **12**, 908–931. https://doi.org/10.1016/j.arabjc.2017.05.011 (2019).
40. Abdelkhalik, A. et al. Effect of deficit irrigation on the productive response of drip-irrigated onion (*Allium cepa* l.) in mediterranean conditions. *Hortic. J.* **88**, 488–498. https://doi.org/10.2503/hortj.UTD-081 (2019).
41. Ahmad, J. et al. Drought and salinity induced changes in ecophysiology and proteomic profile of *parthenium hysterophorus*. *PloS One*. **12**, e0185118. https://doi.org/10.1371/journal.pone.0185118 (2017).
42. Djanaguiraman, M., Nair, R., Giraldo, J. P. & Prasad, P. V. V. Cerium oxide nanoparticles decrease drought-induced oxidative damage in sorghum leading to higher photosynthesis and grain yield. *ACS Omega*. **3**, 14406–14416. https://doi.org/10.1021/acsomega.8b01894 (2018).
43. Zahedi, S. M., Hosseini, M. S., Meybodi, N. D. H. & Da Silva, J. Foliar application of selenium and nano-selenium affects pomegranate (*Punica granatum* Cv. Malase saveh) fruit yield and quality. *South. Afri J. Bot.* **124**, 350–358. https://doi.org/10.1016/j.sajb.2019.05.019 (2019).
44. Van Nguyen, D., Nguyen, H. M., Le N. T., Nguyen, K. H., Nguyen, H. T., Le, H. M.,et al. Copper nanoparticle application enhances plant growth and grain yield in maize under drought stress conditions. *J. Plant Growth Reg*. **41**, 364–375. https://doi.org/10.1007/s00344-021-10301-w .
45. Islam, S. J. M., Mannan, M. A., Khaliq, Q. A. & Rahman, M. M. Growth andyield response of maize to rice husk biochar. *Australian J. CropScience*. **12** (12), 1813–1819. https://doi.org/10.21475/ajcs.18.12.12.p944 (2018).
46. Sigma, A. Certificate of Analysis. Iron (II, III) oxide-nanopowder, 50–100 nm particle size (SEM), 97% trace metal basis. (2016). 3050 Spruce Street, Saint Louis, MO 63103, USA.
47. Sandhya, M. A., Johnson, R. C., Carver, B. F. & Harun, W. S. W. Ultrasonication an intensifying tool for preparation of stable nanofluids and study the time influence on distinct properties of graphene nanofluids – a systematic overview. *Ultrason. Sonochem*. **73**, 105479 (2021).
48. Mannan, M. A. et al. Response of buckwheat to salinity: III. Water status and accumulation of mineral ions. *Trop. Agric. Dev.* **57 91**, 41–48 (2013).
49. Yuping, L., Hongbin, L., Yuanyuan, L. & Suiqi, Z. Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in drought-resistant wheat. *Crop J.* **5**, 231–239. https://doi.org/10.1016/j.cj.2017.01.001 (2017).
50. Mannan, M. A. et al. Biostimulant red seaweed (*Gracilaria tenuistipitata* var. Liui) extracts spray improves yield and drought tolerance in buckwheat. *PeerJ*. Jun **23**; 11, e15588. https://doi.org/10.7717/peerj.15588. (2023).
51. Rady, M. M. Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. *Sci. Hort.* **129** (2), 232–237. https://doi.org/10.1016/j.scienta.2011.03.035 (2011).
52. Rao, K. R. & Sresty, T. V. S. Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Sci.* **157** (1), 113–128. https://doi.org/10.1016/s0168-9452(00)00273-9 (2000).
53. Bates, L. S., Waldren, R. P. & Teare, I. D. Rapid determination of free proline for water-stress studies. *Plant. Soil.* **39** (1), 205–207. https://doi.org/10.1007/bf00018060 (1973).
54. Somogyi, H. Notes on sugar determination. *J. Biol. Chem.* **195**, 19–23 (1952).
55. Velikova, V., Yordanov, I. & Edreva, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants. *Protective role Exogenous Polyamines Plant. Sci.* **151**, 59–66 (2000).
56. Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S. & ChowwanapooNPsohn, S. Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. *Food Chem.* **103** (3), 839–846. https://doi.org/10.1016/j.foodchem.2006.09.034 (2007).
57. Gomez, K. A. & Gomez, A. A. Statistical procedures for agricultural research (2nd edition). International Rice Research Institute. John Willey and Sons, Inc. Singapore. pp. 139–240. (1984).
58. Alam, S. A. et al. Effects of Competition, Drought stress and Photosynthetic Productivity on the Radial Growth of White Spruce in Western Canada. *Front. Plant. Sci.* **8**, 1915 (2017).
59. Caser, M. et al. Drought stress adaptation modulates plant secondary metabolite production in Salvia Dolomitica Codd. *Ind. Crop Prod.* **129**, 85–96 (2019).
60. Saudy, H. S., El-Bially, M. E., Hashem, F. A., Shahin, M. G. & El-Gabry, Y. A. The changes in yield response factor, Water Use Efficiency, and physiology of sunflower owing to ascorbic and citric acids application under mild deficit irrigation. *Gesunde Pflanz*. https://doi.org/10.1007/s10343-022-00736-w (2022).
61. Ojuederie, O. B., Olanrewaju, O. S. & Babalola, O. O. Plant Growth promoting Rhizobacterial Mitigation of Drought stress in crop plants: implications for sustainable agriculture. *Agronomy* **9**, 11–712. https://doi.org/10.3390/agronomy9110712 (2019).
62. El-Metwally, I. M. & Saudy, H. S. Interactional impacts of Drought and Weed stresses on Nutritional Status of seeds and Water Use Efficiency of Peanut plants grown in arid conditions. *Gesunde Pflanz*. **73**, 407–416 (2021).
63. Rahimi, Y., Taleei, A. & Ranjbar, M. Changes in the expression of key genes involved in the biosynthesis of menthol and menthofuran in Mentha Piperita L. under drought stress. *Acta Physiol. Plant.* **39**, s11738–s11017 (2017).
64. Cui, G. et al. Beneficial effects of melatonin in overcoming drought stress in wheat seedlings. *Plant. Physiol. Biochem.* **118**, 138–149 (2017).
65. Seleiman, M. F. & Hafez, E. M. Optimizing Inputs Management for Sustainable Agricultural Development. In *Mitigating Environmental Stresses for Agricultural Sustainability in Egypt* (eds Awaad, H., Abu-hashim, M. & Negm, A.) 487–507 (Springer, Cham, Switzerland, 2021).
66. Seleiman, M. F. et al. Effects of ZnO nanoparticles and Biochar of Rice Straw and cow manure on characteristics of contaminated soil and sunflower Productivity, Oil Quality, and Heavy metals Uptake. *Agronomy* **10**, 790 (2020).
67. Sattar, A. et al. Interactive effect of biochar and silicon on improving morpho-physiological and biochemical attributes of maize by reducing drought hazards. *J. Soil. Sci. Plant. Nutr.* **20**, 1819–1826 (2020).
68. Pandit, N. R. et al. Nutrient effect of various composting methods with and without biochar on soil fertility and maize growth. *Arch. Agron. Soil. Sci.* **66**, 250–265 (2019).
69. Yu, O. Y., Harperm, M., Hoepfl, M. & Domermuth, D. Characterization of biochar and its effects on the water holding capacity of loamy sand soil: comparison of hemlock biochar and switchblade grass biochar characteristics. *Environ. Prog Sustain. Energy*. **36**, 1474–1479 (2017).
70. Roy, R. et al. Additions of Optimum Water, spent Mushroom Compost and Wood Biochar to improve the growth performance of *Althaea rosea* in Drought-Prone coal-mined spoils. *J. Environ. Manag.* **295**, 113076. https://doi.org/10.1016/j.jenvman.2021.113076 (2021).
71. Chintala, R., Mollinedo, J., Schumacher, T. E., Malo, D. D. & Julson, J. L. Effect of biochar on chemical properties of acidic soil. *Arch. Agron. Soil. Sci.* **60**, 393–404 (2014).

72. Dola, D. B. et al. Nano-iron oxide accelerates growth, yield, and quality of *Glycine max* seed in water deficits. *Front. Plant. Sci.* **13**, 992535. https://doi.org/10.3389/fpls.2022.992535 (2022).
73. Taran, N. et al. Effect of zinc and copper nanoparticles on drought resistance of wheat seedlings. *Nanoscale Res. Lett.* **12**, 60. https://doi.org/10.1186/s11671-017-1839-9 (2017).
74. Deepa, M., Sudhakar, P. & Nagamadhuri, K. V. First evidence on phloem transport of nanoscale calcium oxide in groundnut using solution culture technique. *Appl. Nanosci.* **5**, 545–551. https://doi.org/10.1007/s13204-014-0348-8 (2015).
75. Zhu, Y. & Haijun, G. Beneficial effects of silicon on salt and drought tolerance in plants. *Agron. Sustain. Dev.* **34**, 455–472 (2014).
76. Zainul, A., Koyro, H. W., Huchzermeyer, B., Gul, B. & Khan, M. A. Impact of a biochar or a compost-biochar mixture on water relation, nutrient uptake and photosynthesis of phragmites karka. *Pedosphere* **160**, 1–22 (2017).
77. Wang, S. et al. Photosynthesis, chlorophyll fluorescence, and yield of peanut in response to biochar application. *Front. Plant. Sci.* **12**, 1000. https://doi.org/10.3389/fpls.2021.650432 (2021).
78. Meher, S. P., Ashok, R. K. & Manohar, R. D. Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. *Saudi J. Biol. Sci.* **25** (2), 285–289. https://doi.org/10.1016/j.sjbs.2017.04.008 (2018).
79. Mohammadi, M. et al. Influence of nano-iron oxide and zinc sulfate on physiological characteristics of peppermint. *Commun. Soil. Sci. Plant. Anal.* **49**, 2315–2326 (2018).
80. Pirvulescua, A., Salaa, F. & Boldea, M. Variation of chlorophyll content in sunflower under the influence of magnetic nanofluids, in *Proceedings of the International Conference on Numerical Analysis and Applied Mathematics (ICNAAM-2014)*. https://doi.org/10.1063/1.4912904 (2014).
81. Janmohammadi, M., Amanzadeh, T., Sabaghnia, N. & Dashti, S. Impact of foliar application of nano micronutrient fertilizers and titanium dioxide nanoparticles on the growth and yield components of barley under supplemental irrigation. *Acta Agric. Slov.* **107**, 265–276 (2016).
82. Tarafdar, J. C., Raliya, R., Mahawar, H., Rathore, I. & and Development of zinc nanofertilizer to enhance crop production in pearl millet (*Pennisetum americanum*). *Agric. Res.* **3**, 257–262 (2014).
83. Farooq, M. et al. Integration of seed priming and biochar application improves drought tolerance in cowpea. *J. Plant. Growth Regul.* **40**, 1972–1980. https://doi.org/10.1007/s00344-020-10245-7 (2021).
84. Ma, J. et al. Impact of foliar application of syringic acid on tomato (*Solanum lycopersicum* L.) under heavy metal stress-insights into nutrient uptake, redox homeostasis, oxidative stress, and antioxidant defense. *Front. Plant. Sci.* **13**, 950120 (2022).
85. Al-Elwany, O. A. et al. Impact of folic acid in modulating antioxidant activity, osmoprotectants, anatomical responses, and photosynthetic efficiency of Plectranthus amboinicus under salinity conditions. *Front. Plant. Sci.* **13**, 887091 (2022).
86. Mady, E. et al. Evaluation of salicylic acid effects on growth, biochemical, yield, and Anatomical Characteristics of Eggplant (Solanum melongena L.) plants under salt stress conditions. *Agronomy* **13** (9), 2213 (2023).
87. Abbas, A. et al. NaCl stress, tissue specific na $^+$ and K $^+$ up-take and their effect on growth and physiology of *Helianthus annuus* L. and *Solanum lycopersicum* L. *Sci. Hort*. **326**, 112454 (2024).
88. Hafez, Y. et al. Beneficial effects of biochar and chitosan on antioxidative capacity, osmolytes accumulation, and anatomical characters of water-stressed barley plants. *Agron* **10**, 630. https://doi.org/10.3390/agronomy10050630 (2020).
89. Lashari, M. S., Ye, Y., Ji, H., Li, L., Kibue, G. W., Lu H., et al. Biochar–manure compost in conjunction with pyroligneous solution alleviated salt stress and improved leaf bioactivity of maize in a saline soil from central China: a 2-year field experiment.*J. Sci. Food Agric.* 95, 1321–1327. https://doi.org/10.1002/jsfa.6825 .
90. Sun, L., Song, F., Guo, J., Zhu, X., Liu, S., Liu, F., et al. Nano-ZnO-induced drought tolerance is associated with melatonin synthesis and metabolism in maize. *Int. J. Mol. Sci.* 21, 782. https://doi.org/10.3390/ijms21030782 .
91. El-Zohri, M., Al-Wadaani, N. A. & Bafeel, S. O. Foliar sprayed green zinc oxide nanoparticles mitigate drought-induced oxidative stress in tomato. *Plants* **10**, 2400. https://doi.org/10.3390/plants10112400 (2021).
92. Zhao, L.-S., Li, K., Wang, Q.-M., Song, X.-Y., Su, H.-N., Xie, B.-B., et al. Nitrogen starvation impacts the photosynthetic performance of porphyridium cruentum as revealed by chlorophyll a fluorescence. *Sci. Rep.* **7**, 1–11. https://doi.org/10.1038/s41598-017-08428-6 .
93. Farooq, M., Nawaz, A., Chaudhry, M., Indrasti, R. & Rehman, A. Improving resistance against terminal drought in bread wheat by exogenous application of proline and gamma-aminobutyric acid. *J. Agron. Crop Sci.* **203**, 464–472. https://doi.org/10.1111/jac.12222 (2017).
94. Mohammadi, H., Esmailpour, M. & Gheranpaye, A. Effects of TiO2 nanoparticles and water-deficit stress on morpho-physiological characteristics of dragonhead (*Dracocephalum moldavica* l.) plants. *Acta Agricul Slo*. **107**, 385–396 (2016).
95. Gohari G., Mohammadi A., Akbari A., Panahirad S., Dadpour M. R., Fotopoulos V., et al. Titanium dioxide nanoparticles (TiO_2 NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of *Dracocephalum moldavica*. *Sci. Rep.* 10, 1–14. https://doi.org/10.1038/s41598-020-57794-1 .
96. Zhang, Y., Liu, N., Wang, W., Sun, J. & Zhu, L. Photosynthesis and related metabolic mechanism of promoted rice (*Oryza sativa* l.) growth by TiO2 nanoparticles. *Front. Environ. Sci. Engin*. **14**, 1–12 (2020).
97. Kardavan Ghabel, V. & Karamian, R. The effects of titanium dioxide nanoparticles and spermine on physiological responses of licorice (*Glycyrrhiza glabra* l.) to cold stress. *J. Plant. Physiol. Breed.* **10**, 93–110 (2020).
98. Mustafa, H. et al. Biosynthesis and characterization of titanium dioxide nanoparticles and its effects along with calcium phosphate on physicochemical attributes of wheat under drought stress. *Ecotoxicol. Environ. Saf.* **223**, 112519. https://doi.org/10.1016/j.ecoenv.2021.112519 (2021).
99. Khan, Z. et al. The application of biochar alleviated the adverse effects of drought on the growth, physiology, yield and quality of rapeseed through regulation of soil status and nutrients availability. *Ind. Crops Prod.* **171** https://doi.org/10.1016/j.indcrop.2021.113878 (2021).
100. Mickan, B. S., Abbott, L. K., Stefanova, K. & Solaiman, Z. M. Interactions between biochar and mycorrhizal fungi in a water-stressed agricultural soil. *Mycorrhiza* **26** (6), 565–574. https://doi.org/10.1007/s00572-016-0693-4 (2016).
101. Batool, A., Rashid, A. & Aziz, I. Amending soil with Rhizobium carrying Biochar ameliorates Drought stress on *Phaseolus vulgaris*: Drought stress amelioration in Phaseolus using Biochar. *Proc. Pakistan Acad. Sciences: B Life Environ. Sci.* **60** (3), 515–537 (2023).
102. Mega, R. et al. Tuning water-use efficiency and drought tolerance in wheat using abscisic acid receptors. *Nat. Plants*. **5**, 153–159 (2019).
103. Ghazouani, H., Ibrahimi, K., Amami, R., Helaoui, S., Boughattas, I., Kanzari, S., Sher F. (2023). Integrative effect of activated biochar to reduce water stress impact and enhance antioxidant capacity in crops Sci. *Total Environ*. 905, Article 166950.
104. Zulfiqar, B., Raza, M.A.S., Saleem, M.F., Aslam, M.U., Iqbal, R., Muhammad, F., Khan, I.H. (2022). Biochar enhances wheat crop productivity by mitigating the effects of drought: insights into physiological and antioxidant defense mechanisms. PLoS ONE,17 (4), Article e0267819.
105. Gharred, J. et al. Impact of biochar application at water shortage on biochemical and physiological processes in medicago ciliaris. *Plant* **11**, 2411. https://doi.org/10.3390/plants11182411 (2022).
106. Chaves, M. M., Flexas, J. & Pinheiro, C. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* **103**, 551–560. https://doi.org/10.1093/aob/mcn125 (2009).
107. Zhang, P. et al. Preparation of ball-milled phosphorus-loaded biochar and its highly effective remediation for Cd- and Pb-contaminated alkaline soil. *Sci. Total Environ.* **813**, 152648. https://doi.org/10.1016/j.scitotenv.2021.152648 (2022).

108. Dusenge, M. E. & Duarte, A. G. D.A. Way Plant carbon metabolism and climate change: elevated CO2 and temperature impacts on photosynthesis, photorespiration and respiration *New Phytol.* **221** (1), 32–49 (2019).
109. Yildirim, E., Ekinci, M. & Turan, M. Impact of biochar in mitigating the negative effect of drought stress on cabbage seedlings. *J. Soil. Sci. Plant. Nutr.* **21**, 2297–2309. https://doi.org/10.1007/s42729-021-00522-z (2021).
110. Mannan, M., Mia, S., Halder, E. & Dijkstra, F. A. Biochar application rate does not improve plant water availability in soybean under drought stress. *Agric. Water Manage.* **253**, 106940. https://doi.org/10.1016/j.agwat.2021.106940 (2021).
111. Zulfiqar, B. et al. Combined application of biochar and silicon nanoparticles enhance soil and wheat productivity under drought: insights into physiological and antioxidant defense mechanisms. *Curr. Plant. Biology*. **40** (100424), 2214–6628. https://doi.org/10.1016/j.cpb.2024.100424 (2024).
112. Ye, J. et al. Melatonin increased maize (Zea mays L.) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage. *Acta Physiol. Plant*. **38**, s11738-015 (2016).
113. Iqbal, R. et al. Foliar applied iron and zinc improves the growth, physiological and yield 704 related traits of wheat under drought. *Int. J. Biosci.* **14**, 376–387 (2019).
114. Zaheer, M. S. et al. Potential effects of biochar application for improving wheat (Triticum aestivum l.) growth and soil biochemical properties under drought stress conditions. *Land. (Basel).* **10**, 1125. https://doi.org/10.3390/land10111125 (2021).
115. Sabet, H. & Mortazaeinezhad, F. Yield, growth and Fe uptake of cumin (*cuminum cyminum* l.) affected by Fe-nano, Fe-chelated and Fe-siderophore fertilization in the calcareous soils. *J. Trace Elem. Med. Biol.* **50**, 60–154 (2018).
116. Sheykhbaglou, R., Sedghi, M., Shishevan, M. T. & Sharifi, R. S. Effects of nano-iron oxide particles on agronomic traits of soybean. *Not Sci. Biol.* **2**, 112–113. https://doi.org/10.15835/nsb224667 (2010).
117. Dola, D. B. & Mannan, M. A. Foliar Application effects of Zinc Oxide nanoparticles on Growth, Yield and Drought Tolerance of soybean. *Bangladesh Agron. J.* **25** (2), 67–76 (2022).
118. Ali, S. et al. Combined use of biochar and zinc oxide nanoparticle foliar spray improved the plant growth and decreased the cadmium accumulation in rice (*Oryza sativa* L.) plant. *Environ. Sci. Pollut. Res.* **26** (622), 11288–11299. https://doi.org/10.1007/s11356-019-04554-y (2019).
119. Gill, S. et al. Effect of silicon nanoparticle-based biochar on wheat growth, antioxidants and nutrients concentration under salinity stress. *Sci. Rep.* **14**, 6380 (2024).
120. Mahmoud, A. W. M. et al. Nanopotassium, nanosilicon, and biochar applications improve potato salt tolerance by modulating photosynthesis, water status, and biochemical constituents. *Sustainability* **14**, 723 (2022).

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Declarations

Competing interests
The authors declare no competing interests.

Statement on 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
We all authors are confirming that our experimental research on plants was comply in accordance with relevant institutional, national, and international guidelines and legislation.

Additional information
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