RESEARCH

Enhancing rice resilience to drought by applying biochar–compost mixture in low-fertile sandy soil

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Abstract

Background Climate change alters modern drought episode patterns by making them longer, more frequent and more severe, in particular in arid and semi-arid agroecosystems. Amending soil properties and enhancing its fertility is a needed sustainable strategy for mitigating drought's damaging effects on crop production and food security. Here, we planned to investigate the potential benefits of biochar–compost mixture (B×C) as a biochar-based fertilizer (BCF) in enhancing the drought tolerance of rice plants cultivated in low-fertile sandy soil.

Results Under drought stress, rice plants cultivated in unamended soil (no B×C) exhibited severely wilted, rolled and discolored shoots. Furthermore, the shoot dry biomass reduction ratio was 73.3% compared to 44.2 and 27.6% for plants treated with 5 and 15% B×C, respectively. Root anatomical and architectural traits were significantly less impaired in B×C plants and reflected better performance under drought compared to no B×C plants. During the induced drought episode, soil moisture content was enhanced by 2.5-fold through adding B×C, compared to unamended soil, thereby reducing the negative impact of drought stress. Moreover, the less drought-stressed rice plants (B×C-treated) rapidly recovered after rewatering and displayed the unwinding of previously rolled leaves and reproduced panicles. On the other hand, no B×C plants failed to recover and eventually perished completely. The expression profiles of several drought responsive genes suggest that leaves of more stressed rice plants (no B×C) significantly accumulated more cytosolic free calcium (OsCML3) and apoplastic H_2O_2 (OsOXO4) which eventually may trigger fast and prolonged stomatal closure (OsSRO1c). In addition, more drought-stressed plants (no B×C) may over-produce the reactive oxygen species (ROS) superoxide anion molecules (OsRbohB), the negative situation that has been further complicated by a possible reduction in the activity of the antioxidative enzyme SOD (OsSOD), and thus more lipid peroxidation (3.5-fold increase MDA) in drought-stressed (no B×C) plant shoots compared to B×C plants.

Conclusion It is suggested that soil amendment B×C (biochar–compost mixture) could promote drought stress tolerance in rice plants by retaining more soil moisture content, thereby mitigating the negative effects of drought stress, such as the over-production of ROS in leaves, and thus eventually facilitating recovery after rewatering.

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1 Background

Climate change is defined as the shift in climate patterns mainly caused by greenhouse gas emissions produced from natural systems and human activities [1]. Climate change is expected to directly impose additional burdens on global agricultural productivity by elevating temperature and intensifying drought episodes over many agroecosystems. In arid and semi-arid areas, such as northern Africa and the Mediterranean regions, climate change is anticipated to exacerbate drought episodes by making them, more frequent, longer, and more severe [2–4]. Global climate models predict that these areas will become drier by the end of the current century [5, 6].

Egypt, as a semi-arid area, is one of the most vulnerable regions to agricultural drought and its negative consequences [7, 8]. Even without climate change, Egypt's water scarcity is already pronounced due to accelerated population growth which has necessitated an increase in food production (https://www.worlddata.info/africa/egypt/populationgrowth.php). Land suitable for agriculture in Egypt (only 3.5 million hectares, including recently reclaimed land) is rather limited when compared to country total area (nearly 100 million hectares), in addition to a high annual population growth rate (2%). Egypt relies primarily on the Nile for irrigation water (95% of Egypt's water demand) whereby 85% of Nile water is consumed by the agricultural sector [9]. Therefore, managing water resources by maximizing water use efficiency per unit of cultivated land area is critically needed to avoid uncertain catastrophic scenarios affecting local agricultural production and, by extension, national food security.

Amending soil is a major sustainable adaptive investment strategy for enhancing crop productivity under undesirable edaphic environmental conditions such as drought or salinity [10, 11]. Soil amendments can be classified into two distinct categories: (i) organic amendments (essential source for carbon and nitrogen) including farm crop residues, compost, biochar, and biochar–compost mixture, and (ii) inorganic amendments such as gypsum, langbeinite, and zeolite [12]. Biochar is a solid carbonaceous material, synthesized by the thermal decomposition (300–700 ℃) of biomass in the absence or partial presence of oxygen, a process referred to as pyrolysis [13, 14]. Biochar can be produced from crop or forest residues, wood processing waste, algae, sewage sludge, and manure. The primary physical and chemical properties of biochar (pH, elemental composition, particle size, surface area, and pore size distribution) are strongly influenced by both pyrolysis conditions and feedstock type; the two factors that ultimately determine its potential beneficial applications [15].

Biochar can improve soil quality and fertility, enhance crop productivity, and mitigate climate change by sequestering carbon [3]. Soil properties such as pH, bulk density (BD), water-holding capacity (WHC), and cation exchange capacity (CEC) may be relatively enhanced by optimized biochar addition, providing that soil type and mixture are efficiently taken into account [7]. Biochar has been repeatedly reported as an effective management tool for mitigating drought stress impact on crop productivity [16]. Although biochar has been used as a soil amendment, its incorporation into soil should be managed with utmost caution to avoid adverse changes in soil biological, physical, and chemical properties [17, 18]. Moreover, biochar entails significant cost burdens, in particular with large quantities for extended cultivated areas [7]. Therefore, the careful assessment of the interaction between biochar and the employed agroecosystem is essential for achieving superior results.

Egypt is the largest rice producer in the near east and north Africa region with a cultivated area of 554,205 ha and a production yield of 3,263,969 tons of milled rice in 2020 [19]. Although rice agriculture is simple with assured results, its cultivation in Egypt has reached a point where limited fresh water resources pose a significant impact on local production potential. The semi-aquatic crop is extremely susceptible to drought and thus inevitably needs a significant amount of irrigation water, approximately 2–3 times higher than is required for producing other cereals, such as maize or wheat [20]. To address these challenges, Egypt is considering cultivating rice, in newly reclaimed land, where fresh water scarcity and soil salinity are the most significant obstacles encountered. Therefore, we hypothesize that amending such type of low-fertile sandy soil is a sustainable investment for expanding national agricultural production and thereby contributing to food security stabilization. Rice was intentionally selected in this study due to its significance as a strategic crop and also as a model plant for monocots [21].

Although biochar is a well-acknowledged soil amendment, it is apparently not a fertilizer; therefore, charging biochar with compost is thought to be beneficial for both soil properties and nutrition, and hence crop

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performance [22]. The main aim of the proposed plan is to investigate the potential role of biochar–compost mixture (B×C) as a soil amendment in mitigating the negative effects of drought stress on rice seedlings cultivated in infertile sandy soil (representing the common type of soil that exists in newly reclaimed land in Egypt, desert and semi-desert areas). Using quantitative measurement of several phenological, physiological, and molecular parameters, the response of rice seedlings to administered treatments was monitored. We hypothesize that biochar–compost mixture (B×C) may aid in enhancing rice cultivation (and potentially other cereals such as wheat) on newly reclaimed low-fertile sandy land (desert and semi-desert) under a water-saving irrigation regime.

2 Materials

2.1 Experimental layout, plant materials, and drought treatment

Pot experiment was conducted at the greenhouse facilities of the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt. The used soil mixture was sandy in texture (75% medium-sized commercial grade sand, 15% peat moss, and 10% pearlite) with a low salinity (EC ranged from 1.8 to 2.3 ds m^{-1}) and slight alkalinity (pH ≈7.5). Temperature inside the greenhouse unit was maintained at 27 ℃ and light was adjusted to approximately 30,000 to 35,000 Lux during daytime, ∼ 16 h. Seeds of Egyptian rice cultivar Sakha 101 were provided by the Rice Research and Training Center (RRTC), Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Egypt. The seeds (un-hulled) were surface-disinfected using 50% commercial bleaching agent (2.5% NaOCl) for 30 min, then washed 5 times thoroughly with water, and then kept at 4℃ for two days to achieve a homogenous germination rate. The seeds were then incubated at room temperature for extra two days.

2.2 Soil potting mixture and biochar–compost mixture (B×C) addition

The evenly germinated seeds were further sowed in soil boxes for two months and transplanted into pots filled with sandy low-fertile soil ($\approx$ 5000 cm^3) supplemented with the following rates of biochar–compost mixture (B×C): 0 (no B×C), 5, and 15% B×C (v/v). The investigated concentrations of B×C in this work (5, and 15%, v/v) were prepared according to [23] where compost to biochar ratio was 50% for each mixture. The transplanted rice seedlings were irrigated with maximum soil capacity (approximately 1000 ml per day of tap water) for an extra week to recover and adapt after transplantation. For

inducing drought stress, irrigation was completely suspended for one month, whereas control rice plants were irrigated daily with nearly 1000 ml of tap water. The control or well-watered plants are referred to as WW, while drought-stressed plants are referred to as DS.

In this study, rice plants were subjected to six different treatments: (i) fresh tap water irrigation of unamended (no B×C) soil, (ii) fresh tap water irrigation of soil amended with 5% B×C (v/v), (iii) fresh tap water irrigation of soil amended with 15% B×C (v/v), (iv) suspended irrigation of unamended (no B×C) soil, (v) suspended irrigation of soil amended with 5% B×C, and (vi) suspended irrigation of soil amended with 15% B×C. The biochar–compost mixture was homogeneously mixed with the soil to ensure a uniform distribution around the cultivated rice root systems.

2.3 Shoot growth measurement

At harvest, following a one-month episode of drought stress, shoots of all treated plants were disjunct from the root system using sharp and clean garden scissors and then dried in an oven at 80 ℃ for three days, and then weighed to determine dry biomass. The percentage reduction of shoot dry biomass in drought-stressed (DS) rice plants compared with well-watered (WW) plants was calculated according to the following equation: (WW–DS/WW)*100.

2.4 Leaf relative water content (LRWC)

Leaf RWC (relative water content) of all treated rice plants was estimated according to [24]. Essentially, a 6-cm-long leaf blade end (third leaf) was seized from all rice plants and immediately weighed to record fresh weight (FW), then immersed in distilled water for two days and then weighed to determine turgid weight (TW). The leaves were then dried in an oven at 80 °C for two days and weighed again to determine the dry weight (DW). For each treatment, LRWC was calculated according to the following equation: LRWC (%) = [(FW–DW) / (TW–DW)] * 100.

2.5 Determination of lipid peroxidation in shoots

Lipid peroxidation of shoots was estimated in terms of MDA (Malondialdehyde) using the thiobarbituric acid (TBA) method as described by [25]. Briefly, 500 mg of rice shoots were thoroughly homogenized using mortar and pestle in 1 ml of 0.1% TCA (trichloroacetic acid, w/v). The homogenate was then centrifuged for 20 min at 10,000 g, then 800 µl of the supernatant was added to 1 ml of 0.5% TBA dissolved in 20% TCA. The mixture was incubated in a boiling water bath for 60 min using thick-wall glass test tubes and then transferred to an ice bath for 10 min to stop the reaction. The mixtures were

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individually transferred into regular plastic microcentrifuge tubes and then centrifuged for 15 min at 12,000 g. The absorbance of the supernatant of each sample was spectrophotometrically determined at 532 nm and 600 nm as specific and non-specific values, respectively. The value of the non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 $mM^{-1}cm^{-1}$.

2.6 Root anatomical and architectural measurements
The root systems of treated rice plants were extracted from the soil in the greenhouse using a high-pressure water stream, and then delicately washed in the laboratory several times using tap water to remove any possible traces of soil granules, and then stored at 4 ℃ in 70% ethanol for subsequent root traits investigations. For studying anatomical traits, root segments from the basal position were hand-sectioned using sharp mid-thickness blades, and stained with 1.5% Toluidin Blue O for 5 min then washed 2–3 times with distilled water [26]. The transverse sections were examined and imaged using the stereoscope SZ61 (Olympus, Japan) supplied with a high-resolution digital camera DP23 (Olympus, Japan). Using the software ImageJ, the cross section area and stele area were measured. For architectural traits: nodal root number and total root length were also determined. The ethanol- preserved root samples were rinsed briefly with distilled water and then dried in a regular oven at 80 ℃ for two days to record root dry biomass. Root biomass reduction ratio was calculated for each sample as follows: (WW–DS/WW) * 100.

2.7 Determination of soil moisture content
50 g of soil samples that were subjected to drought stress (no B×C or amended with B×C) were retrieved from middle depth of each pot, then soil samples were oven-dried at 65 ℃ for 4 days until reaching a constant weight. Soil moisture content (%) was calculated according to the following equation: [(weight of fresh soil sample − weight of oven-dried soil) / weight of oven-dried soil] *100 [27].

2.8 Total RNA isolation and cDNA synthesis
Total RNA was isolated from rice shoots, and genomic DNA was in-column digested using Direct-zol™ RNA MiniPrep (Zymo Research, USA) according to the manufacturer manual. The quantity and purity of RNA samples were confirmed to be highly acceptable by using NanoDrop® spectrophotometer (Thermo Scientific, USA). Additionally, RNA was visualized on agarose gel (1.3%, w/v) stained with ethidium bromide (1 mg/ml final concentration) to examine integrity and the absence of genomic DNA contamination. cDNA was synthesized from 400 ng of total RNA using Cosmo cDNA synthesis kit (Willowfort, England) according to the supplied protocol from the manufacturing company. The synthesized cDNA was diluted 1:5 with nucleases-free water and used for subsequent gene expression analysis.

2.9 Quantifying gene expression profile
The molecular quantification of several stress marker genes was carried out by the quantification of mRNA levels relative to a reference gene. This was achieved by quantifying the PCR amplicons integrated density (IntDen) using the Gel express method developed by Hazman [28]. The sequences of forward and reverse primers are provided in the Additional file 1: Table S1. For setting up PCR reactions, a 25 µl mixture for each reaction was prepared as follows: 12.5 µl of amaR PCR master mix (GeneDireX, Taiwan), 5 µl of diluted cDNA (1:5), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM), and nuclease-free water was added up to 25 µl. The thermal cycling protocol used with T100™ Thermal Cycler (Bio-Rad, USA) was as follows: initial denaturation step 95 ℃ for 3 min, 30 cycles of denaturation step at 95 ℃ for 30 s, annealing step at 58 ℃ for 1 min, extension step at 72 ℃ for 1 min. The final extension step was done at 72 ℃ for 5 min. The reaction was stopped by incubating the tubes at 4 ℃ for 30 min. The PCR product was electrophoresed and then visualized on a 1.5% agarose gel stained with ethidium bromide (1mg/ml final concentration). The agarose gel was photographed by the gel documentation system (Bio-Rad, USA).

2.10 Experimental design and statistical analysis
The experiment plots were arranged in a complete randomized design (CRD). SPSS (IBM Statistics, USA) software was used for statistical tests including mean separations by Duncan's multiple range test (DMRT), with a significance level of $P \leq 0.05$. All analyzed data represented three independent biological replications.

3 Results
3.1 Morphological response of rice plants under drought stress
Drought stress was induced by suspending irrigation (for four weeks) applied to rice plants (9 weeks old) cultivated in low-fertile sandy soil supplemented with two different rates of biochar–compost mixture (B×C): 5 and 15% (v/v) in addition to an unamended soil treatment containing no B×C. Both B×C treatments (5 and 15%, v/v) appeared to mitigate the negative effects of drought stress compared to the non-amended drought-stressed plants (no B×C) with respect to exogenous wilting and necrosis symptoms (Fig. 1a). Figure 1b demonstrates that the leaf blades of rice plants grown in soil lacking B×C
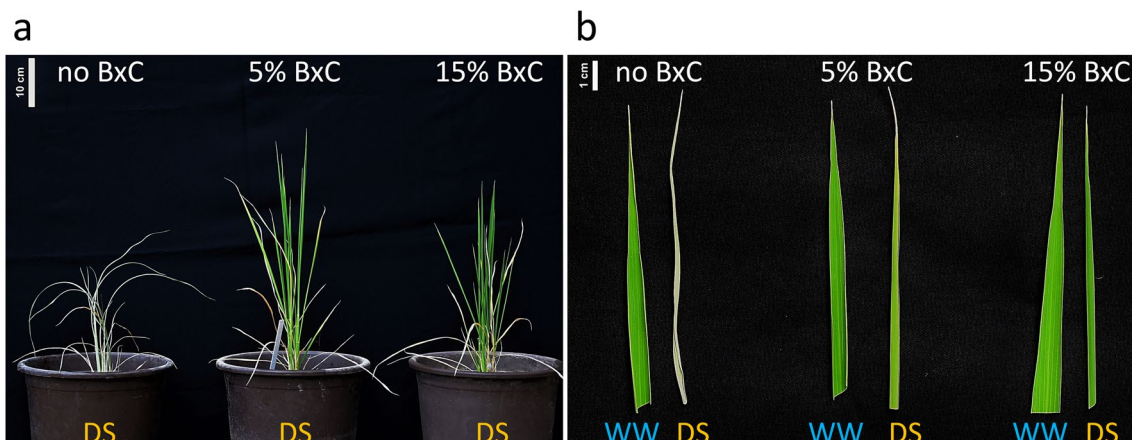
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Fig. 1 Phenotyping of Egyptian rice cultivar Sakha 101 (13 weeks old) in response to drought stress (four weeks of suspended irrigation), and cultivated in soil amended with biochar–compost mixture (B×C): 0, 5 and 15% (v/v). **a** Rice plant shoots (13 weeks old) subjected to a drought stress episode of four-weeks suspended irrigation, **b** The third leaf of 13-week old rice plants cultivated in low-fertile sandy soil amended with 0, 5 and 15% B×C under well watering (WW) and drought stress (DS) conditions

amendment were obviously shrunken, dehydrated, and wilted, in contrast to plants grown in soil containing 5 and 15% B×C, where shoots were less wilted. Generally, applying a drought stress regime by suspending irrigation for one month had a negative effect on the growth shape of Sakha 101 rice shoots; however, the damage was more apparent in the rice plants grown with no B×C compared to rice plants cultivated in B×C-amended soil.

3.2 Shoot biomass reduction ratio, leaf RWC, and lipid peroxidation levels

The induced drought stress significantly reduced dry biomass of rice shoots in the presence and absence of B×C soil amendment (Fig. 2a). However, the reduction ratio was significantly higher in rice plants cultivated in soil without B×C (73.3%) compared to soil amended with B×C ratios of 5 and 15% (44.2 and 27.6%, respectively) as shown in Fig. 2b. Under drought stress, the reduction ratios in shoot biomass were comparable in both B×C blending ratios, of 5 and 15%. Figure 2c illustrates the determined levels of leaf relative water content (Leaf RWC) under control and drought stress conditions in the presence and absence of B×C in sandy low-fertile soil. It is observed that drought stress significantly reduced leaf RWC in rice plants grown in no B×C soil compared with control well-watered conditions by a reduction ratio of nearly 41%, whereas the reduction ratios in cases of 5 and 15% B×C were only 13.7 and 6.9%, respectively. The leaf RWC levels were not significantly different under drought stress in plants cultivated in both 5 and 15% B×C-amended soil (Fig. 2c). On the basis of lipid peroxidation, the extent of oxidative damage is determined in Fig. 2d. Expectedly, the shoots of drought-stressed rice

plants in no B×C soil accumulated 3.56-fold more MDA-TBA complex (68.8 nmol/gFw) than the leaves of well-watered plants (19.3 nmol/gFw). MDA-TBA complex levels showed no significant difference in B×C-treated plants in well-watered and drought stress conditions.

3.3 Root anatomical and architectural traits

Figure 3a, b demonstrates that the mean root cross section area of B×C-treated plants was reduced by 23% as a result of the induced drought stress compared to well-watered conditions. In contrast, no B×C rice plants demonstrated a more significant reduction in root cross section area by 64.5% under drought stress relative to well-watered conditions. Similarly, the stele area of all rice plants decreased in response to drought (Fig. 3c). With no B×C plants, the reduction ratio was 57%, whereas it was around 26% with B×C-treated rice plants. For root architectural traits, the entire root systems of rice plants were extracted, delicately washed, and presented in Fig. 4a, where it appears that B×C addition substantially alleviated the negative effects of drought stress on the root system. All drought-stressed plants exhibited a significant reduction in total root length, but the reduction ratio in the absence of B×C was greater than in the presence of B×C (mean value), 55.3% and 37.2%, respectively, as shown in Fig. 4c. Similarly, nodal root number diminished by 70% in the absence of B×C compared with 42.5% in B×C-treated plants, relative to control conditions (Fig. 4c). Root dry biomass was significantly reduced in drought-stressed plants compared with well-watered plants, yet B×C plants showed a lower reduction ratio (72%) compared with plants grown in unamended
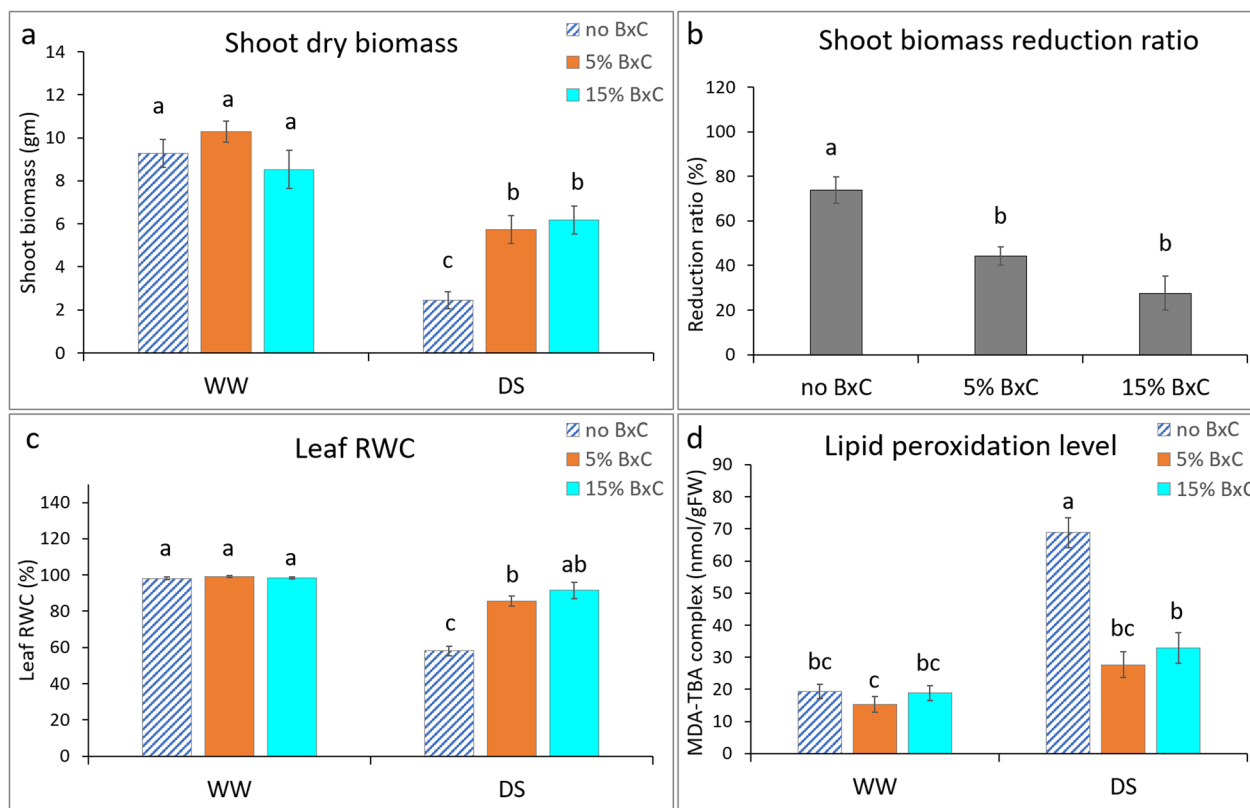
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Fig. 2 Effect of amending low-fertile sandy soil with biochar–compost mixture on growth and physiological parameters of rice plants in response to drought stress. **a** Determination of shoot dry biomass of rice plants, **b** represents calculated shoot biomass reduction ratios, **c** calculated leaf relative water content (Leaf RWC), and **d** for estimated amounts of MDA-TBA complex as a marker for lipid peroxidation due to oxidative stress. Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$)

sandy soil (88%) (Fig. 4d). Root/shoot dry biomass ratio of all drought-stressed plants decreased significantly compared with well-watered plants, yet with comparable values in no B×C and B×C soil treatments (Fig. 4e).

3.4 Elevated soil moisture content ratio after short-term drought episode in B×C-amended soil

The percentage of soil moisture was calculated according to the following equation: soil moisture content % = [(weight of fresh soil sample − weight of oven-dried soil) / weight of oven-dried soil]* 100. Figure 5 demonstrates that the moisture content of B×C-amended soil was substantially greater than that of unamended soil by a factor of 2.4. Furthermore, the percentage of soil moisture in the 15% B×C-amended soil was lower (although not significant) than the 5% B×C-amended soil.

3.5 Biochar–compost mixture (B×C) facilitates the recovery of drought-stressed plants after rewatering

As previously indicated, at the late vegetative stage, an induced drought stress episode affected rice plants cultivated in B×C-amended soil less severely than in unamended soil, where the latter were significantly stunted (Fig. 1a, b). Furthermore, we examined whether rewatering drought-stressed B×C-treated rice plants would restore normal growth and facilitate development to the reproductive stage. After one week of daily base rewatering, previously drought-stressed B×C plants with partially wilted leaves (Fig. 6a) were able to eventually re-extend and reproduce panicles (Fig. 6b). In response to drought, the number of panicles and panicle length values were markedly reduced compared to well-watered plants (data not shown). In contrast, no B×C drought-stressed rice plants were not able to recover and ultimately perished (Fig. 6b).
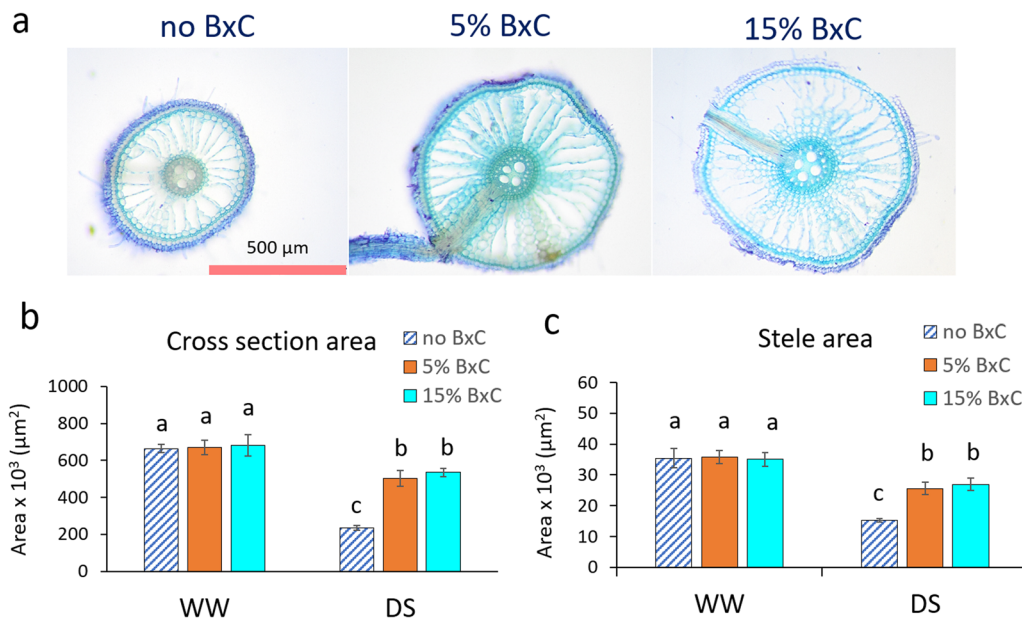
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Fig. 3 Effect of drought stress on root anatomical traits in the presence and absence of soil amendment biochar–compost mixture (B×C). **a** Toluidine Blue O staining (1.5%) of hand-made cross sections of fresh basal nodal root segments (5 cm from root base) of cultivar Sakha 101 under drought stress in 0, 5, and 15% B×C. **b** Effect of B×C addition on cross section area of basal nodal root segment in well-watered and drought-stressed rice plants. **c** Effect of B×C addition on stele area of basal nodal root segment in well-watered and drought-stressed rice plants. Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$)
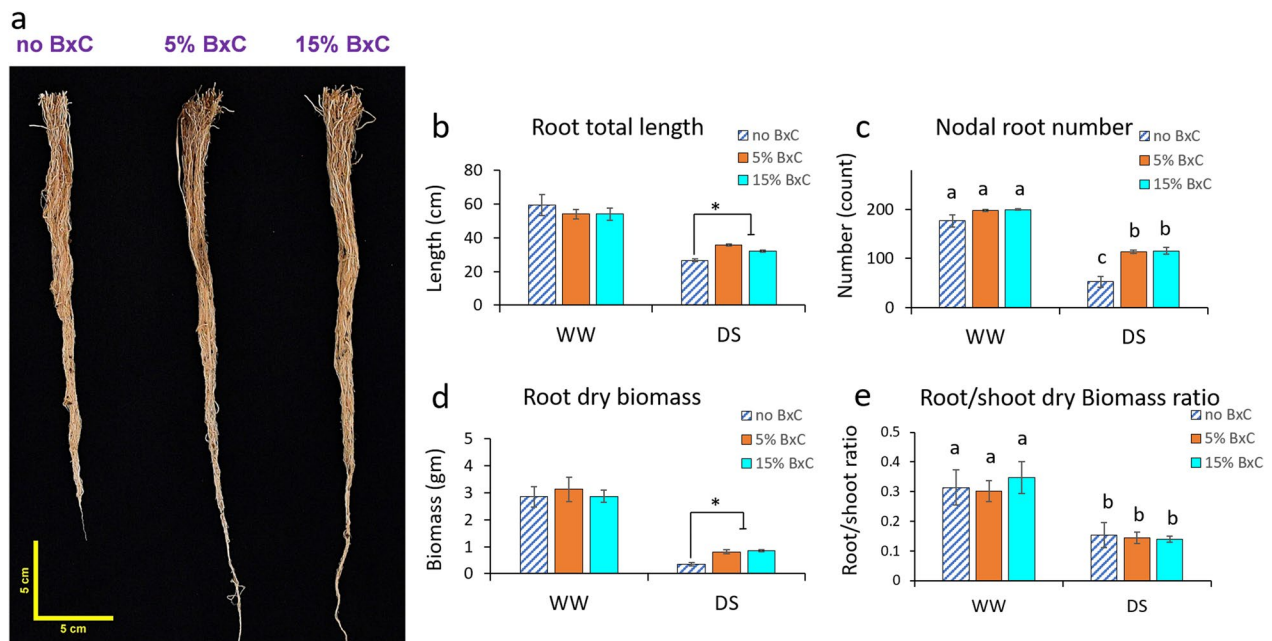

Fig. 4 Effect of adding B×C to low-fertile sandy soil on shaping rice root architectural traits under drought stress. **a** Exogenious shape of extracted whole root system of drought-stressed rice plants (13 weeks old), adding B×C with 5 or 15% could enable developing a better root system compared with no B×C plants. **b** The response of root total length, **c** nodal root number, **d** root dry biomass, and **e** root/shoot dry biomass ratio. Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$)
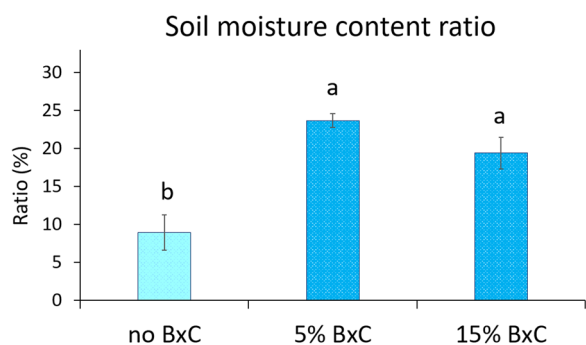
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Soil moisture content ratio

Fig. 5 Soil moisture content of low-fertile sandy soil amended with 5 and 15% B×C compared to no B×C soil. Full irrigation was applied and then drought stress was induced by suspending water for two weeks, then samples were taken from the middle soil strata of the pot (10 cm from top soil). Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$)

3.6 Relative gene expression profiling of several stress marker genes

We quantified the expression of twelve (12) stress marker genes in an effort to elucidate potential molecular responses of rice plants under less drought stress (cultivated in B×C soil) versus greater drought stress (cultivated in soil without B×C). Agarose gel images, representing quantified PCR amplicons, are presented in Figs. 7a, 8a, 9a. Figure 7 presents the expression profile of OsSOD, OsCML3, OsSRO1c, and OsJAR1. The expression of OsSOD (encodes the antioxidant enzyme superoxide dismutase) was elevated in leaves of all drought-stressed plants relative to well-watered

conditions, however at a higher level in B×C plants versus unamended plants under drought stress (Fig. 7b). On the other hand, the expressions of both OsCML3 (encodes calmodulin like protein) and OsSRO1c (encodes similar to radical-induced cell death one protein) were significantly elevated in no B×C plant shoots (Fig. 7c, d). The relative abundance in mRNA of OsCML3 and OsSRO1c in no B×C plant shoots were a 289.7- and 127.5-fold increase compared to mean values of B×C-treated plants under drought stress, respectively. The expression of OsJAR1 gene (encodes enzyme Jasmonate Resistant 1) was significantly diminished by a reduction ratio of 93.22% in no B×C under drought compared to unamended well-watered plants, and by 38.9% in 5% B×C stressed plants compared to well-watered 5% B×C-amended plants (Fig. 7e). It is worth noting that under drought stress, amended plants with 5 and 15% B×C showed a higher OsJAR1 mRNA level relative to no B×C plants with a 16.3- and 8.4-fold increase, respectively.

The transcripts of OsRbohB, (encodes ROS producing enzyme NADPH oxidase), were greatly induced in response to drought stress, however, the level was significantly higher in B×C plants compared with no B×C plants by a mean value increase of 1.8-fold (Fig. 8b). OsPXA (Peroxidase) gene was over-expressed in no B×C plants under drought compared to well-watered conditions, while both drought-stressed B×C-treated plants displayed comparable transcript levels relative to well-watered B×C-treated plants (Fig. 8c). Similar to the profile of OsCML3 and OsSRO1c, the expression of OsOXO4 (encodes the H_2O_2 producing enzyme oxalic acid oxidase 4) was strongly upregulated in no
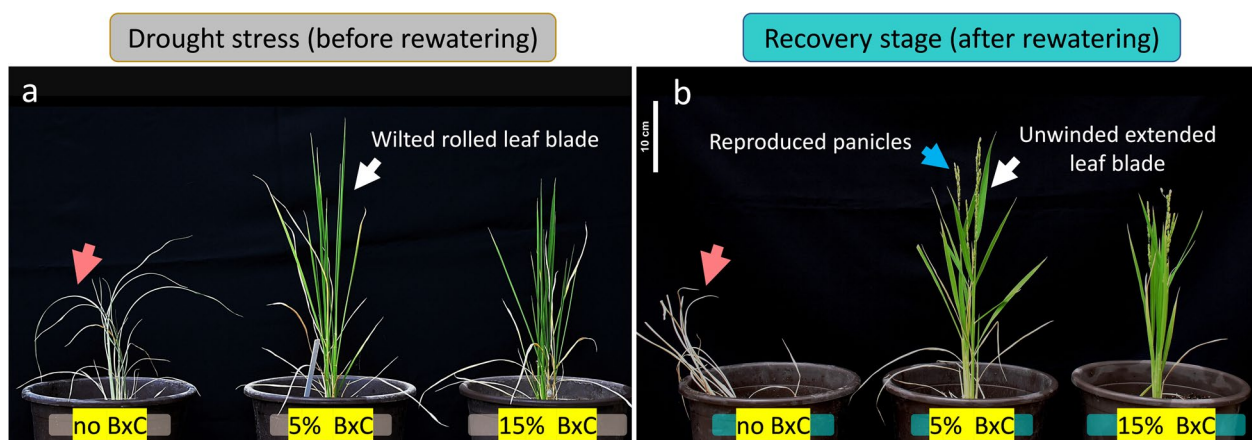

Fig. 6 The effect of B×C amendment on the recovery of Egyptian rice japonica cultivar Sakha 101 post a drought stress episode of one month. **a** Phenotype of rice plants under drought stress grown in no B×C and B×C-amended soil (*presented in* Fig. 1a). **b** Phenotype of the same drought-stressed rice plants in "**a**" after irrigation with fresh tap water for two weeks (daily base, full soil irrigation). Red arrows point at the severely stunted rice plants after exposure to drought stress episode of one month in "**a**" and the completely deceased plants after recovery in "**b**". White arrows point at the wilted rice leaf blade under drought in "**a**" and in "**b**" where the leaf blades recovered after rewatering, and the blue arrow in "**b**" points at the panicles reproduced from recovered B×C plants
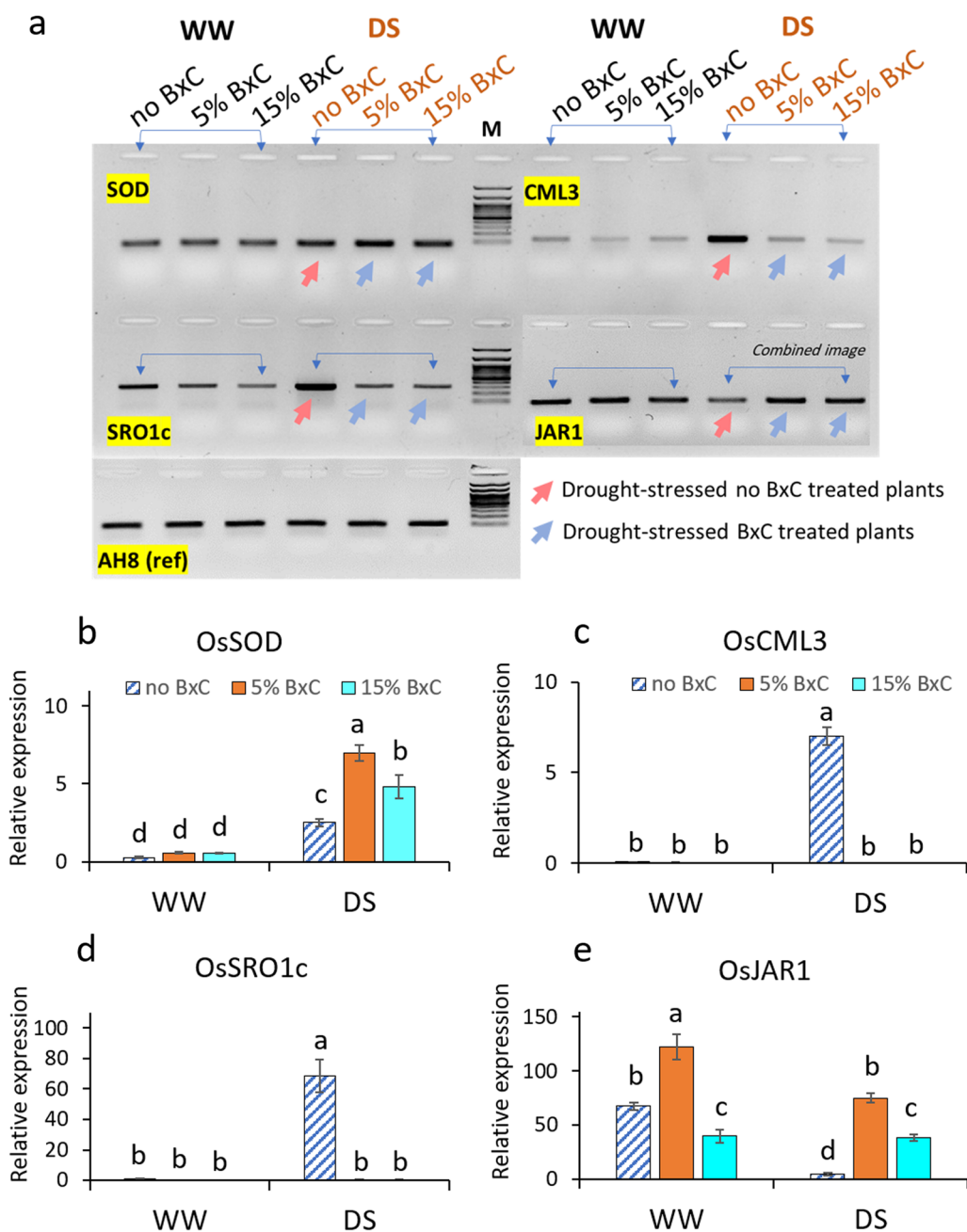
Fig. 7 The relative expression profile of selected stress marker genes in response to drought stress in shoots of Egyptian rice variety Sakha 101 plants under the effect of amending soil with biochar–compost mixture (B×C) with rates of 0, 5 and 15% (v/v). **a** Agarose gel electrophoresis (inverted color image) showing the migration of PCR product of each studied target gene and the reference gene, **M** 100 bp DNA ladder. Quantified gene relative expression of **b** OsSOD (encodes enzyme Superoxide dismutase), **c** OsCML3 (encodes Calmodulin like protein), **d** OsSRO1c (encodes Similar to radical-induced cell death one protein), and **e** OsJAR1 (encodes enzyme Jasmonate resistant 1). Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$)

B×C plants under drought (147-fold) compared to well-watered plants, while drought-stressed B×C treated plants showed comparable levels with well-watered conditions (Fig. 8d). OsAPX (encodes the antioxidative enzyme Ascorbate peroxidase) transcripts were significantly downregulated under drought stress relative to well-watered conditions, whereas the reduction ratio in no B×C plants was 99.8% and approximately 69% for mean values of B×C-treated plants (Fig. 8e). It is worth noting that the addition of B×C to soil resulted in a 74.7%
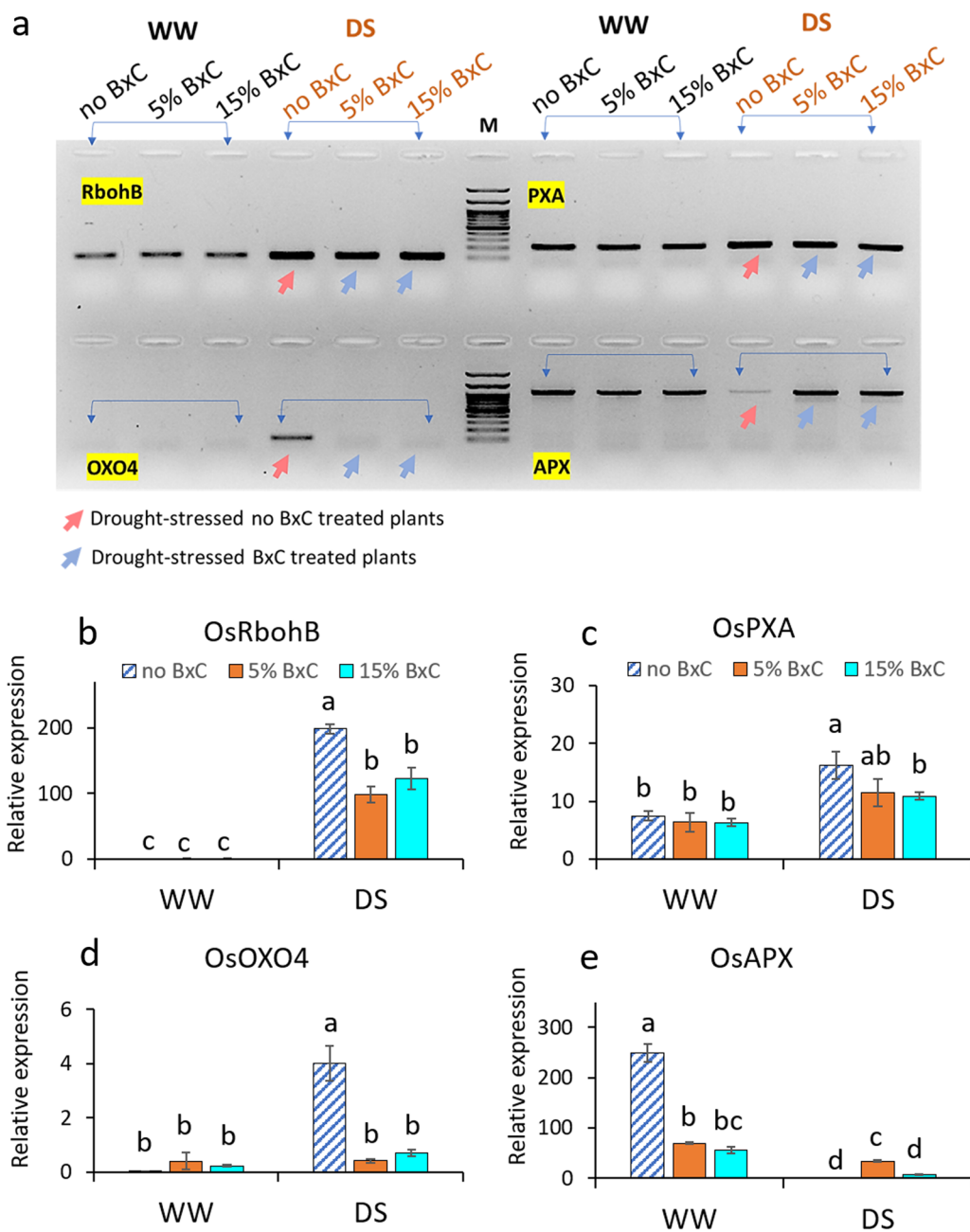
Fig. 8 The relative expression profile of selected stress marker genes in response to drought stress in shoots of Egyptian rice variety Sakha 101 plants under the effect of amending soil with biochar–compost mixture (B×C) with rates of 0, 5 and 15% (v/v). **a** Agarose gel electrophoresis (inverted color image) showing the migration of PCR product of each studied target gene and the reference gene, **M** 100 bp DNA ladder. Quantified gene relative expression of **b** OsRbohB (encodes enzyme NADPH oxidase), **c** OsPXA (encodes enzyme peroxidase), **d** OsOXO4 (encodes enzyme oxalate oxidase), and **e** OsAPX (encodes enzyme Ascorbate peroxidase). Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$)

reduction in the level of OsAPX transcripts under well-watered conditions.

OsWR1 (encodes for wax synthesis regulator protein 1) was highly expressed in both B×C plants under drought relative to well-watered conditions, while no B×C plants showed comparable levels (Fig. 9b). We further investigated the response of two genes related to nitrogen assimilation in plants, OsNR (nitrate reductase) and OsNOS1 (nitric oxide synthase 1). Figure 9c shows that OsNR was strongly downregulated in
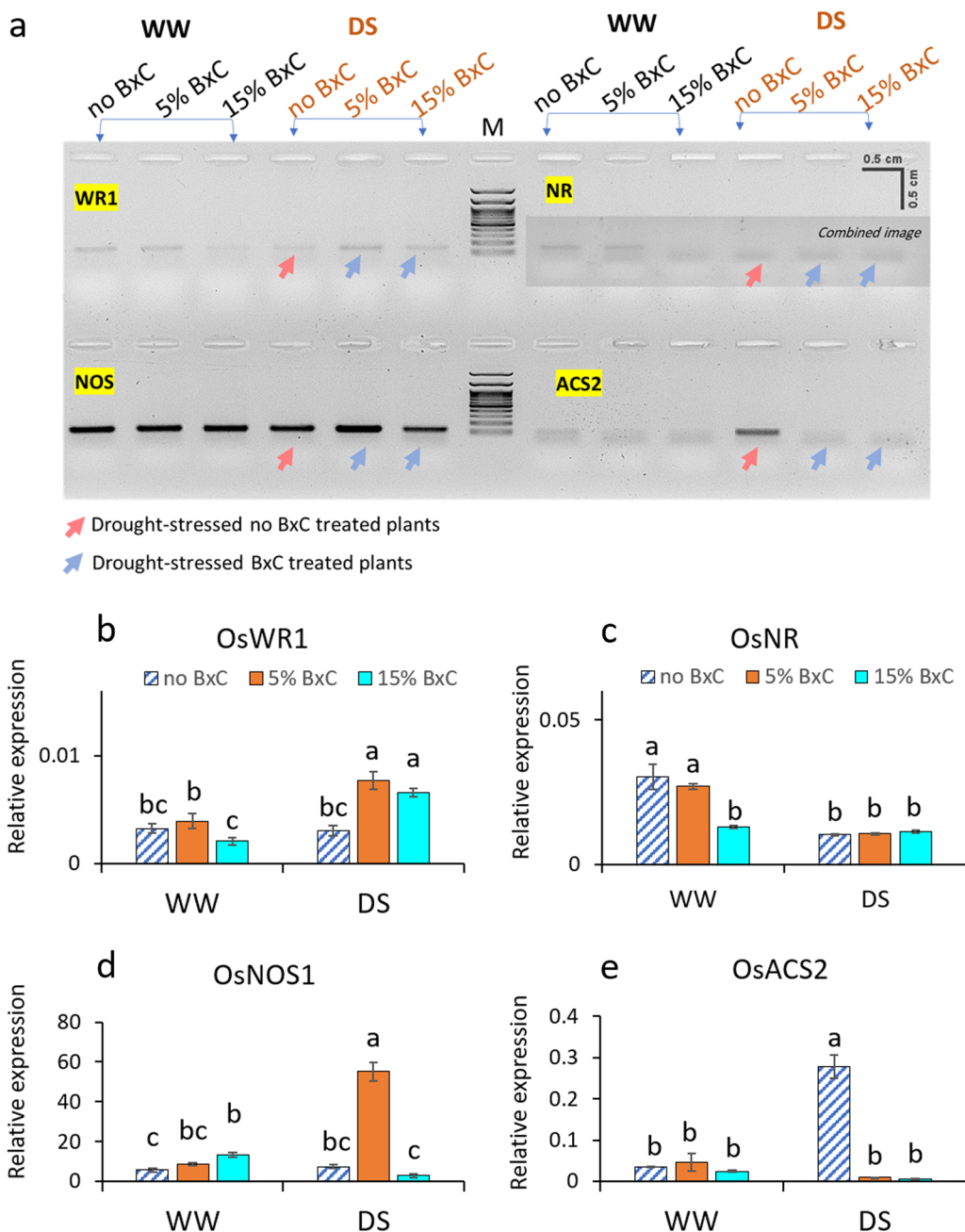
Fig. 9 The relative expression profile of selected stress marker genes in response to drought stress in shoots of Egyptian rice variety Sakha 101 plants under the effect of amending soil with biochar–compost mixture (B×C) with rates of 0, 5 and 15% (v/v). **a** Agarose gel electrophoresis (inverted color image) showing the migration of PCR product of each studied target gene and the reference gene, **M** 100 bp DNA ladder. Quantified gene relative expression of **b** OsWR1 (encodes Wax regulator protein 1), **c** OsNR (encodes enzyme Nitrate reductase), **d** OsNOS1 (encodes Nitric oxide synthase 1), and **e** OsACS2 (encodes enzyme 1-aminocyclopropane-1-carboxylic acid synthase). Values represent the means of three replications ± SE. Means with the same letters are not significantly different according to Duncan's multiple range test (DMRT) ($p \leq 0.05$)

response to drought in case of 0 and 5% B×C plants by a reduction ratio of 66.6 and 60%, respectively. Under well-watered conditions, the expression of OsNR gene in 15% B×C-treated plants was downregulated by a reduction ratio of 56.6% and 51.8% compared to unamended (no B×C) and amended plants with 5% B×C, respectively. On the other hand, OsNOS1 transcripts were elevated under drought stress only in the case of 5% B×C-treated plants by nearly a six fold increase compared to well-watered 5% B×C plants

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(Fig. 9d). The expression of OsACS2 (encodes ethylene biosynthesis key enzyme) was strongly elevated in no B×C plants by an 8 and 36-fold increase under drought compared to well-watered no B×C conditions and drought-stressed B×C plants, respectively (Fig. 9e).

4 Discussion

Biochar has been widely used to improve poor soil quality and thus acclimating crops to edaphic stresses such as drought. This is particularly relevant for sandy loam soil, which is commonly found in newly reclaimed land in Egypt, and is characterized by a low level of fertility and high porosity [11]. Nevertheless, if biochar is applied exclusively, it can have limitations due its nutrient deficiency; therefore, deploying biochar-based fertilizers (BCFs) is a sustainable necessity for amending soil and enhancing its fertility [29]. In this study, we intended to examine the effect of biochar–compost mixture (B×C) as a biochar-based fertilizer on enhancing drought stress tolerance in rice; a strategic universal crop that is notoriously susceptible to drought, and considered as a well-acknowledged model plant for monocots [21].

Rice (*Oryza sativa* L.) is a semi-aquatic plant with a fibrous root system and the lowest water use efficiency in comparison to other cereal crops such as maize, wheat and barley [26]. Therefore, soil properties are crucial for rice performance in agroecosystems with limited water input [30]. In this study, we applied a one-month drought stress episode in pots, under greenhouse-controlled conditions, to severely challenge rice seedlings of Sakha 101 as a drought-sensitive rice variety [31]. The drought-sensitive cultivar Sakha 101 was selected for this study to establish a realistic parameter to evaluate whether the applied soil amending agent (biochar–compost mixture, B×C) effectively enhanced plant drought tolerance.

As expected, the induced drought stress episode strongly damaged rice shoots in the form of wilted and rolled leaf blades, whereas the B×C-treated rice plants were more effective in enhancing drought stress tolerance, i.e., alleviating visible severe drought stress damage (Fig. 1a, b). The presented phenotypical results correlate with a lower ratio of shoot biomass reduction and a higher leaf relative water content in rice plants treated with B×C and subjected to drought (Fig. 2a, b and c). Sadegh-Zadeh et al. [32] reported that applying a biochar–compost mixture to calcareous sandy soil could enhance rice plant growth and grain yield. Similarly, a biochar–compost mixture promoted shoot dry biomass of sweet pepper under a dry irrigation strategy, thereby enhancing plant growth [33]. The level of lipid peroxidation, which represents the oxidative damage level, was substantially elevated in no B×C rice leaves exposed to drought stress (Fig. 2d). This is consistent with Yildirim et al. [34] who reported that amending soil by biochar could reduce lipid peroxidation level in cabbage seedlings under drought stress. Reactive oxygen species (ROS) are generated in large amounts within plants subjected to drought as a result of imbalanced gas exchange through partially closed stomata. ROS target several highly-critical molecules such as DNA, proteins and lipids of cell membranes via a process termed lipid peroxidation [35]. Lipid peroxidation (in terms of MDA accumulation, see methodology) is a stable marker for osmotic stress (or drought) in rice compared to other abiotic forms of stress such as salinity and alkalinity [36].

Roots are the primary target to drought stress where soil moisture rapidly declined, in particular at the top soil strata where roots are connected to the shoot system. Since roots and shoots are interdependent, the healthy shape of shoots reflects on roots. In this study, we examined and evaluated several root anatomical and architectural traits under well-watered and drought-stressed conditions. Under drought stress, root cross section area and stele area of B×C-treated plants were larger than those cultivated in no B×C soil (Fig. 3b, dc). In a previous study conducted on several Egyptian rice varieties, Hazman and Brown [26] reported that traits such as cross section and/or stele area are associated with shoot dry biomass and root length. In the current study, B×C-treated plants exhibited the highest value of root length compared to unamended soil plants (no B×C) under drought stress (Fig. 4a, b). There is a significant reduction in nodal root number and root dry biomass in no B×C compared to B×C-amended soil (both 5 and 15% rates), under drought stress (Fig. 4c, d). Interestingly, several reports explained such reduction as an adaptive response to save resources and metabolic costs (e.g., root respiration) under stress rather than as mere damage symptoms [37, 38].

Root/shoot biomass ratios were statistically comparable in drought-stressed plants cultivated in B×C-amended or unamended soil (Fig. 4e). Root/shoot ratio is a well-acknowledged trait for monitoring plant response to drought stress in terms of estimating relative biomass allocation between roots and shoots [39]. Although shoot and root dry biomass values were enhanced with the addition of B×C to soil (Figs. 2a and 4d), root/shoot ratios were statistically comparable across all B×C treatments. This could possibly be attributed to fact that B×C addition could evenly promote both shoot and root growth. The situation could be further explained by the elevated moisture ratio in B×C-amended soil with a mean 2.5-fold increase in comparison to soil without B×C amendment (Fig. 5). It has been demonstrated that biochar can

induce soil physical properties by several aspects including enhancing water-holding capacity (WHC) or soil moisture, therefore, making water more abundant in the rhizosphere for a much longer period during drought episodes, in particular in coarse or sandy soil [7, 11]. The elevated soil moisture content in B×C-treated soil accelerated the recovery of stressed plants following rewatering. B×C-treated plants recovered rapidly, with the unwinding of previously rolled leaf blades and the reproduction of panicles. On the other hand, in the absence of B×C amendment, drought-stressed plants perished even after two weeks of daily-based rewatering (Fig. 6a, b).

The molecular tools for stress perception, signaling and adaptation to applied stress are further investigated through the quantification of several stress marker genes (Figs. 7, 8, 9). The most notable observation is the strongly upregulated transcripts of OsCML3 (calcium sensing), OsSRO1c (stomatal closure), OsOXO4 (apoplast hydrogen peroxide production), and OsACS2 (Ethylene biosynthesis) exclusively in the shoots of no B×C plants under drought (Figs. 7c, d, 8d, 9e). Calcium is a second messenger possessing extracellular stimuli with a robust intracellular response, yet the exact role of calcium in plant response to drought is still not fully understood [40]. It is suggested that calcium ions are trans-allocated from roots to shoots for better interpretation in drought stress signature, probably through cell influx via the mechanosensitive calcium channel OSCA1 (reduced hyperosmolality-induced $[Ca^{2+}]$ Increase 1) which is gated by hyperosmotic stress [41]. Ca^{++} entry into cells can trigger apoplast H_2O_2 production by the activation of membrane-located NADPH oxidases so that the primary calcium influx is followed (probably with some delay) by a transient oxidative burst [42]. Indeed, in this work, the level of OsRbohB transcripts was significantly higher in no B×C rice shoots compared with B×C drought-stressed rice plants (Fig. 8b). Furthermore, it is suggested that H_2O_2 was produced in large amounts by the enzyme oxalate oxidase as represented by the upregulated OsOXO4 gene expression under no B×C treatment but not in B×C-treated plants which seemed to be less stressed (Fig. 8d) [43]. The over-production of extracellular H_2O_2 is thought to be driven by the elevated intracellular level of calcium ions, however, H_2O_2 would further elevate cytosolic free calcium that eventually can promote stomatal closure [44, 45]. This assumption is in line with our findings; OsSRO1c gene (related to stomatal closure) was strongly upregulated in no B×C drought-stressed plants compared to B×C-treated plants (Fig. 7d). The over-expression of OsACS2 is thought to be associated with arrested growth of the more drought-stressed plants (no B×C). Ethylene (C_2H_2) as a gaseous stress hormone could be over-produced to inhibit growth in order

to manage the balance between drought tolerance mechanisms and growth arrest, a fine-tuned trade-off strategy that is recently reported [46].

The response of antioxidative enzyme genes was also examined in this work. Modulating ROS levels by antioxidative enzymes is an essential protective strategy for ROS detoxification and modulation of stress signaling [36]. OsSOD (encodes superoxide dismutase) is found to be upregulated in response to drought stress in 0, 5 and 15% B×C-treated plants, although with a greater magnitude in the less drought-stressed plant shoots, i.e., cultivated in 5 and 15% B×C-amended soil (Fig. 7b). SOD is a vital antioxidative enzyme in regulating ROS accumulation in rice in response to different types of abiotic stress, such as salinity and osmotic stress. It is essential in detoxifying superoxide anion molecules, which if highly accumulated could damage cell and mitochondrial membranes [47, 48]. Since drought-stressed rice plants grown in unamended soil (no B×C) could access and thus uptake less water relative to B×C plants, it is expected that drought stress could negatively impair the performance of several antioxidative enzymes within the cell, such as SOD. Additionally, the significant reduction in the expression of OsAPX (encodes Ascorbate peroxidase) under drought could be possibly attributed to drought negative impact on basal metabolism of plants, in particular for sensitive species such as rice [36]. In the same context, Hazman et al. [49] found that salinity stress unexpectedly inhibited the activity of the antioxidant enzyme catalase (CAT) compared to control fresh water conditions. The higher expression of OsAPX in no B×C than B×C plants under well-watered conditions might be an adaptive response to a possible higher level of H_2O_2 in shoots (originally could be produced in roots and translocated to shoots) due to severe nutrient deprivation after long-term growth in low-fertile sandy soil [50]. We suggest that no B×C rice plants under drought stress accumulated higher amounts of ROS (primarily superoxide anion O_2^-) through NADPH oxidase, the action that was accompanied with a lower SOD activity (as proposed by gene expression profile data), thus a higher magnitude of damaging cell membranes in no B×C plants under drought (see high lipid peroxidation in Fig. 2d).

Drought-stressed rice plants cultivated in no B×C soil (unamended soil) severely inhibited the expression of OsWR1 (related to wax biosynthesis). It is suggested that the severe drought damage effect observed in these plants is due to less synthesized wax in the leaves which can promote better tolerance during extended drought episodes by reducing water loss (Fig. 9b, see also Fig. 1) [51]. OsJAR1 (encodes Jasmonate Resistant 1) is essential in producing Jasmonile isoleucine (JA-Ile), the active biological form of the plant hormone jasmonic acid. The

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role of jasmonic acid or its derivative JA-Ile in abiotic stress response is not well understood [52]. Although not entirely verified, JA-Ile is thought to be associated with enhanced drought tolerance in rice, therefore, we assume that the less drought-stressed rice plants (B×C) might accumulate higher amounts of JA-Ile (high OsJAR expression), thus better adaptive response to drought (Fig. 7e) [53, 54].

Nitrogen assimilation-related genes (OsNR and OsNOS1) could highlight the possible differences between 5% B×C and 15% B×C plants in terms of behavior under well-watered and stress conditions (Fig. 9c, d). It is suggested that the higher ratio of applied B×C (15%) to sandy soil may delay the nitrogen uptake from soil due to biochar-induced elevated cation exchange capacity (CEC) [7, 10]. The expression of OsNR (nitrogen assimilation key enzyme nitrate reductase) was significantly inhibited in the shoots of plants cultivated in 15% B×C-amended soil under well-watered conditions compared to no B×C and 5% B×C plants. On the other hand, OsNOS1 (nitric oxide synthase 1) was solely overexpressed in 5% B×C plants in response to drought stress by a 6.11-fold increase compared to well-watered plants, suggesting better nitric oxide (NO) production. NOS enzyme activity is associated with nitrogen availability and uptake (ammonia in particular), and is reported to be elevated in response to osmotic stress, a basic single stressor type of complex syndromes such as drought and salinity. NO contributes to adaptation to drought stress by facilitating stress signaling and adaptive responses such as stomatal closure, alleviation of toxic effects caused by various stressors by modulating oxidative stress, antioxidant defense mechanism, metal transport, and ion homeostasis. [55, 56].

5 Conclusion
Amending low-fertile sandy soil with biochar–compost mixture (B×C) can be beneficial in alleviating the detrimental impacts of drought stress on rice plants. Plants cultivated in B×C-treated soil outperformed untreated plants in terms of shoot and root system growth traits under drought stress. The applied B×C rates (5 and 15%, v/v) successfully enhanced soil ability to retain moisture under drought and thus facilitated a rapid recovery post soil rewatering. Based on gene expression profiles, we hypothesize that the more stressed plants (no B×C) accumulated more ROS with weaker antioxidative enzymatic ability, eventually developing unrecoverable damage symptoms. Furthermore, the excessive use of B×C is assumed to relatively reduce nitrogen uptake under well-watered and drought-stressed conditions, emphasizing the necessity to optimize the efficient application rate of B×C. Collectively, we propose improving sandy
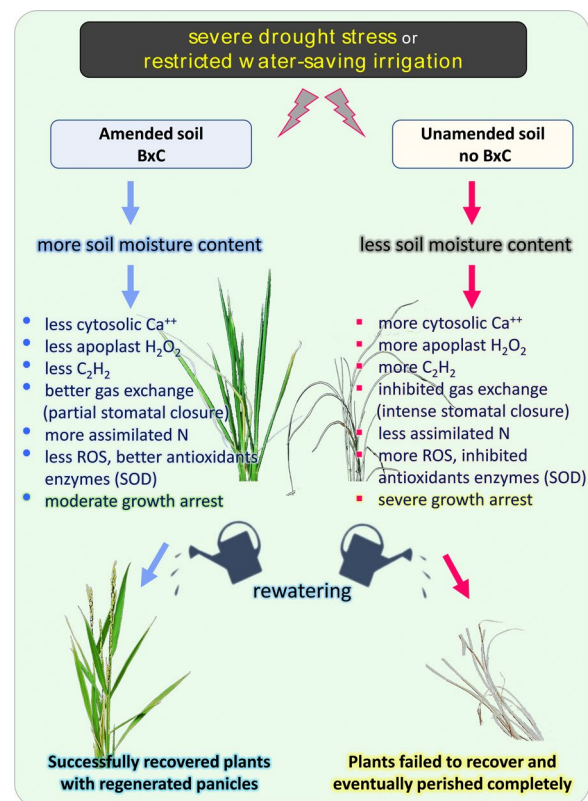

Fig. 10 Schematic model for the molecular basis of rice plant response to severe drought or restricted water-saving irrigation regime after amending soil with a biochar–compost mixture (B×C). The observed phenotype of less-stressed rice plants that are able to recover rapidly after rewatering could be associated with the improved moisture content in B×C-amended soil throughout the induced drought episode. The enhanced rice resilience in B×C-amended soil could be associated with less stress signal perception (Ca^{++} and H$_2$O$_2$), and attributed to better gas exchange quality due to partial stomatal closure, better nitrogen assimilation, and ROS/antioxidants balance under stress

low-fertile soil in newly reclaimed land or in greenhouses by incorporating a biochar–compost mixture amendment, which could facilitate the cultivation of strategic or cash crops using water-saving irrigation regimes. We interpret these findings in a model for revealing rice molecular response to drought stress in the presence and absence of biochar–compost mixture (Fig. 10). We must emphasize that additional research is required to assess the impact of a single high-dose of biochar–compost mixture after several years in the open field or in greenhouses facilities.

Abbreviations
BCFs	Biochar-based fertilizers
B×C	Biochar–compost mixture
cDNA	Complementary deoxyribonucleic acid
CEC	Cation exchange capacity

CRB	Complete randomized design
DS	Drought stress
DW	Dry weight
FW	Fresh weight
gDNA	Genomic deoxyribonucleic acid
LRWC	Leaf relative water content
MDA	Malondialdehyde
NO	Nitric oxide
OsACS2	1-Aminocyclopropane-1-carboxylic acid synthase
OsAH8	Amidohydrolases
OsAPX	Ascorbate peroxidase
OsCML3	Calmodulin-like protein
OsJAR1	Jasmonate resistant 1
OsNOS1	Nitric oxide synthase 1
OsNR	Nitrate reductase
OsOXO4	Oxalic acid oxidase 4
OsPXA	Peroxidase
OsRbohB	NADPH oxidase
OsSOD	Superoxide dismutase
OsSRO1c	Encodes similar to radical-induced cell death one protein
OsWR1	Wax regulator protein 1
PCR	Polymerase chain reaction
Ref	Reference gene
RNA	Ribonucleic acid
ROS	Reactive oxygen species
TBA	Thiobarbituric acid
TW	Turgid weight
WW	Well-watered

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-023-00411-7.

Additional file 1: Supplementary Material 1.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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