RESEARCH ARTICLE

Compost and biochar application mitigates salt stress on barley: monitoring of agronomic, physiological, biochemical and molecular parameters

Emna Ghouili · Yordan Muhovski · Richard Hogue · Rim Nefissi Ouertani · Souhir Abdelkrim · Zhengguo Li · Jianghua Cai · Fatma Souissi · Salwa Harzalli Jebara · Moez Jebara · Ghassen Abid

Abstract

Background and aims   Salinity is a global issue that adversely affects crop yield and soil fertility. This study aimed to evaluate the impact of compost and biochar on mitigating salt stress in barley.

Methods   Barley (*Hordeum vulgare* L.) seeds were grown under controlled conditions with additions of amendments separately or combined. When plants were 45 days old, salt stress was applied for 16 days and then the different parameters were studied.

Results   The use of amendments enhanced plant growth under salinity stress, increasing RWC and chlorophyll levels while reducing electrolyte leakage. In general, the addition of compost or biochar separately increased the concentrations of osmoprotectants and the enzymatic activities of SOD and CAT, while significantly decreasing the concentration of H_2O_2. Conversely, the combined addition of compost and biochar significantly reduced the concentrations of osmoprotectants, the enzymatic activities of CAT and APX as well as the levels of H_2O_2. At the molecular level, the compost and biochar significantly upregulate stress-responsive genes (such as *HvDREB*, *HvHSP*, *HvP5CS* and *HvPIP* genes), while their combination moderates gene expression, suggesting a balanced and effective regulation of stress response pathways.

Conclusion   Adding compost or biochar alone offers optimal protection against saltinity. In contrast, the combination of amendments appears to mitigate the effects of salinity, thereby reducing the need for the plant to develop a strong stress response and express

E. Ghouili · S. Abdelkrim · F. Souissi · S. H. Jebara · M. Jebara · G. Abid (✉)
Laboratory of Legumes and Sustainable Agrosystems, Centre of Biotechnology of Borj-Cedria, (L2AD, CBBC), Hammam-Lif, Tunisia 2050, PB 901
e-mail: gha_abid@yahoo.fr

Y. Muhovski
Biological Engineering Unit, Department of Life Sciences, Walloon Agricultural Research Center, BP 234, 5030 Chaussée de Charleroi, Gembloux, Belgium

R. Hogue
Microbial Ecology Laboratory, Research and Development Institute for the Agri-Environment (IRDA), Einstein Street 2700, Québec City, QC G1P 3W8, Canada

R. N. Ouertani
Laboratory of Plant Molecular Physiology, Centre of Biotechnology of Borj Cedria (LPMP, CBBC), BP 901, 2050 Hammam-Lif, Tunisia

Z. Li · J. Cai
Key Laboratory of Plant Hormones and Molecular Breeding of Chongqing, School of Life Sciences, Chongqing University, Chongqing 401331, China

Z. Li · J. Cai
Center of Plant Functional Genomics, Institute of Advanced Interdisciplinary Studies, Chongqing University, Chongqing 401331, China

high levels of stress response genes to combat oxidative stress. These results underscore the complementary roles of compost and biochar in enhancing plant resilience in arid and semi-arid environments.

Introduction

Climate change significantly impacts environmental systems, particularly agriculture, where it reduces crop yields due to alterations in temperature, precipitation patterns, and an increase in extreme weather events (Antonopoulou 2022; Baldi 2017). Elevated global temperatures enhance water evaporation from soil and plant surfaces, resulting in salt accumulation and increased soil salinity (Shaygan & Baumgartl 2022; Yao et al. 2020). This accumulation induces osmotic stress, which restricts plant water uptake and disrupts metabolic processes (Shaygan & Baumgartl 2022). Furthermore, modified precipitation patterns lead to extended drought periods, reducing water availability and limiting salt leaching, thereby intensifying soil salinity (Arif et al. 2020; Chhabra 1996). Salt stress is thus a significant abiotic factor, intensified by climate change, and is known to compromise plant growth and productivity (Corwin 2021; Eswar et al. 2021; Mukhopadhyay et al. 2021). Globally, salt-affected soils cover approximately one billion hectares (Kumawat et al. 2022), posing a serious threat to food security and sustainable agriculture, particularly in arid and semi-arid regions (Mehdi et al. 2018; Omar et al. 2023). Salt stress limits seed germination in plants such as *Arabidopsis thaliana*, broccoli (*Brassica oleracea* L.), and cauliflower (*Brassica oleracea* L.) (Nasri et al. 2016; Wu et al. 2019) and negatively impacts plant growth at various development stages, reducing both crop yield and quality (Arif et al. 2020; Nawaz et al. 2023; Nefissi Ouertani et al. 2022a). Furthermore, the large accumulation of Na^+ and Cl^- ions inhibits the uptake of K^+ and $Ca2^+$, creating an ionic imbalance (Arif et al. 2020). Physiologically, salt stress affects ion homeostasis leading to osmotic and ionic stress resulting in water and nutrient imbalance (Arif et al. 2020; Nawaz et al. 2023). Indeed, it causes morphological, physiological, and biochemical changes, such as reduced shoot and root length and dry weight, photosynthesis, stomatal closure, and increased accumulation of reactive oxygen species (ROS) causing damage to cell membranes, proteins, and DNA (Arif et al. 2020; Nawaz et al. 2023; Nefissi Ouertani et al. 2022a).

Plants respond to salinity stress through a sophisticated interplay of physiological responses, metabolic pathways, molecular networks, and integrated responses (Gupta & Huang 2014; Pandita 2023). Physiologically, plants perform osmotic adjustments by accumulating osmolytes such as proline and sugars to maintain cell turgor and protect cellular integrity, which also regulates water uptake and transport by modifying root architecture and aquaporins expression (Parihar et al. 2015; Zhao et al. 2021). To maintain ion balance, plants employ specific transporters to exclude Na^+ and retain K^+, with excess Na^+ sequestered into vacuoles to prevent cytotoxicity (Parihar et al. 2015). Growth reduction and stomatal closure minimise water loss and conserve energy (Arif et al. 2020; Nawaz et al. 2023). Metabolically, plants enhance their antioxidant defence mechanisms to scavenge ROS, utilising enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (POD), as well as non-enzymatic antioxidants such as ascorbate and glutathione (Mbarki et al. 2018). Photosynthetic and nitrogen metabolism adjustments optimise energy use (Rasool et al. 2022). At the molecular level, signal transduction pathways involving calcium signalling and hormonal regulation activate stress-responsive gene expression, mediated by various transcription factors such as *DREB*, *MYB* and *NAC*, while epigenetic modifications such as DNA methylation and histone modifications further fine-tune gene expression (Arif et al. 2020; Gupta & Huang 2014; Jin et al. 2018; Nefissi Ouertani et al. 2022a). Proteomic adjustments through post-translational modifications and targeted protein degradation help to maintain cellular functions (Dzinyela et al. 2023). These molecular networks ensure a coordinated response to salinity stress by orchestrating a range of biochemical and physiological adjustments, thereby enhancing plant resilience and adaptation. These processes are integrated through cross-talk between signalling pathways, feedback mechanisms for fine-tuning responses and developmental plasticity, allowing plants to survive and adapt to challenging stress environments (Pandita 2023).

The sustainable management of salinity is crucial to ensure farmers' prosperity in concerned areas. Reclamation methods commonly include salt leaching, adding amendments, revegetation with halophytes, and salt scraping (Shaygan & Baumgartl 2022). Rusan (2023) explained that integrating soil and crop nutrient management, including 4R nutrient stewardship (Right Source, Right Rate, Right Time, and Right Place), is essential. Organic amendments, such as compost and biochar, improve the structure of the soil and facilitate salt leaching (Ali et al. 2017; Rusan 2023). The use of compost and biochar has been shown to enhance the physical and chemical properties of sandy soils (Kavvadias et al. 2024). Compost improves soil physical properties and can partially replace mineral fertilisers, leading to increased crop yields under normal conditions (Ghouili et al. 2024a; Mehdi et al. 2018). Compost also enhances soil fertility and crop production under saline conditions (Meena et al. 2019). Our previous studies demonstrated that applying date palm (*Phoenix dactylifera* L.) waste compost increased the expression of stress-related proteins in barley (*Hordeum vulgare* L.) leaves and roots (Ghouili et al. 2022a; 2023a). These results suggest that compost can improve stress adaptation. On the other hand, biochar application has been shown to increase plant growth, photosynthetic activity, nutrient uptake, and yield under salt stress (Ali et al. 2017; Helaoui et al. 2023; Huang et al. 2022; Murtaza et al. 2024). Indeed, biochar can mitigate the negative effects of salt stress on plant physiology by reducing salt accumulation and improving adaptability to saline soils (Ali et al. 2017). Biochar also decreases Na^+ uptake while increasing K^+ uptake, thereby enhancing salt tolerance and improving plant water status (Akhtar et al. 2015).

The present study aimed to investigate the impact of salt stress on barley (*Hordeum vulgare L.*) plants and elucidate the role of biochar and date palm waste compost as soil amendments in alleviating the adverse effects of salt stress. This research seeks to provide practical solutions for enhancing barley productivity in saline-affected areas. To the best of our knowledge, this is the first comprehensive and multifaceted investigation exploring the synergistic effects of biochar and compost under saline conditions.

Materials and methods

Plant material and growth conditions

This experiment was conducted in a glasshouse at the Experimental Station of the Biotechnology Center of Borj Cedria (Tunisia) under controlled conditions (temperature of 23 ± 2 °C, relative humidity 65%−70%, light 270 µmol of photons $m^{-2} \cdot s^{-1}$ photosynthetic active radiations and a 14/10 h day/night photoperiod). Barley seedlings were grown in plastic pots with a capacity of 5 L. The experiment was conducted in a completely randomised design with ten replications.

Eight treatments were applied namely **CK**: control (only soil); **B**: soil+Biochar (6% w/v = 300 g/pot); **C**: soil+Compost (1.9% w/v = 96 g/pot); **CB**: soil+Compost (96 g/pot)+Biochar (300 g/pot); **S**: Salt stress (200 mM); **SB**: Salt+Biochar (300 g/pot); **SC**: Salt+Compost (96 g/pot); **SCB**: Salt+Compost (96 g/pot)+Biochar (300 g/pot).

Ten pots were employed in every treatment. The main specifics of the experimental soil and the physicochemical characteristics of date palm waste compost used in this study were reported by Ghouili et al., (2024b). Briefly, the aerobic compost was made from date palm waste and sheep manure and prepared following the Turned Windrow method at the composting station of NGmOASOC (Association for Saving Oasis of Chenini, Gabes, Tunisia). The biochar provided by the Biofire Society (Tunisia), is derived from the pyrolysis of pine (*Pinus halepensis* L.) wood in an oxygen-poor environment for 10 h at 450 °C. It is characterised by an organic matter content of 81.2%, pH of 7.63 and EC of 1.3 dS.cm^{-1} as reported by (Rajhi et al. 2024).

In this study, we used the Sahli barley cultivar, the most commonly grown variety in Tunisia's organic farming systems. Sahli is a local spring six-row cultivar, provided by the Technical Centre of Organic Agriculture (TCOA) in Tunisia. Ten seeds were sown in each pot at a depth of 0.5 to 1 cm. The seeds were irrigated twice weekly with tap water for 45 days to ensure ordinary growth. Then, the seedlings were progressively exposed to salt stress. Salt stress was gradually applied for 16 days with four equal durations; plants were irrigated with 50 mM NaCl solution in the first duration (4 days), and then the salt concentration was increased by 50 mM in each of

the next durations until it reached a concentration of 200 mM NaCl. At the tillering stage, various growth and physiological parameters were recorded. For further biochemical and molecular analyses, leaves were collected randomly for each treatment from different plants, snap-frozen in liquid nitrogen, and then stored at −80 °C.

Evaluation of agronomic parameters: shoot and root lengths and dry weights

Growth traits were recorded to evaluate the impact of compost and biochar application on plant performance. Ten plants were sampled from each treatment at the tillering stage, and the shoot (SL) and root (RL) lengths were measured. Shoot length (SL) and root length (RL) were measured as the distance from the crown to the leaf tip and from the crown to the root tip, respectively, both in centimetres. The shoot (SDW) and root (RDW) dry weights were measured after being oven-dried at 65 °C for 72 h.

Evaluation of physiological parameters: relative water content (RWC), chlorophyll pigments (Chl), and electrolyte leakage (EL) content

The RWC was determined according to Barrs and Weatherleyt (1962) method. The fresh mass (FW) of the leaves was determined immediately after sampling, and the turgid mass (TW) was determined after soaking the leaves in distilled water for 24 h. The dry mass (DW) was then determined after drying the saturated leaves at 70 °C for 72 h. The RWC was calculated using the following equation: RWC = [(FW − DW)/(TW − DW)] × 100%.

As described by Ghouili et al. (2022b), chlorophyll pigments were extracted from fresh leaves with cold acetone (80% v/v). After centrifugation at 12,000 rpm for 15 min, the absorbance of extracts was read spectrophotometrically, and total chlorophyll (Chl tot), Chl a and Chl b were calculated.

For electrolytes leakage, which reflects the permeability of cellular membranes, the Abid et al. (2020a) protocol was applied. The initial (Ci) and the final conductivity (Cf) obtained from each sample were transformed into a percentage using the formula: EL (%) = (Ci/Cf) × 100%.

Evaluation of biochemical parameters: hydrogen peroxide (H_2O_2), malondialdehyde (MDA), proline, soluble sugar content and antioxidant activity

H_2O_2 level in fresh tissues was evaluated as reported by Velikova et al. (2000). Briefly, fresh leaf tissue was homogenised in trichloroacetic acid (TCA) and then centrifuged. Potassium phosphate buffer and KI were added to the supernatant and then the absorbance was read at 390 nm.

To estimate lipid peroxidation levels using MDA content in plant tissues, the Dhindsa et al. (1981) protocol was used. For the essay, fresh leaf samples were homogenised in trichloroacetic acid, centrifuged, and then mixed with thiobarbituric acid before heating at 100 °C for 30 min and cooling to room temperature. The absorbance was recorded at 532 nm and 600 nm.

Proline was extracted from dry leaf by ninhydrine reaction according to Bates et al. (1973), with minor modifications as described by Abid et al. (2020b). Dry leaf samples were homogenised in sulfosalicylic acid solution, centrifuged, mixed with glacial acetic acid and ninhydrin reagent, incubated at 100 °C for 1 h, cooled on ice, and then extracted with toluene, separating the upper phase. Then, proline content was assessed spectrophotometrically at 520 nm using the L-proline standard.

The total soluble sugar content was quantified following the phenol sulfuric acid method reported by Abid et al. (2020a). About 100 mg of dry leaf was homogenized in 5 ml of 80% ethanol, filtered, and mixed with phenol and sulfuric acid before incubation for 1 h and measuring absorbance at 490 nm.

Total soluble proteins were extracted as detailed by Ghouili et al. (2021). Briefly, 1 g of frozen powdered leaf and root tissues were mixed in 1 ml of an ice-cold potassium phosphate buffer (50 mM, pH 7.8) and then the mixture was centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was used to estimate antioxidant enzyme activities and the total protein concentrations were quantified according to Bradford (1976).

Catalase (CAT) activity was measured using the method described by Cakmak and Marschner (1992) by following the decomposition of H_2O_2 at 240 nm. The CAT activity was expressed in mmol H_2O_2 mg^{-1} protein min^{-1}.

Superoxide dismutase (SOD) activity was evaluated through its capacity to inhibit nitro-blue

tetrazolium (NBT) photoreduction at 560 nm in a mixture according to the method by Del Longo et al. (1993). As mentioned by Ghouili et al. (2021), one unit (U) of SOD inhibited 50% of NBT photoreduction. The SOD activity was expressed in Units SOD mg^{-1} protein.

Ascorbate peroxidase (APX) activity was assayed by monitoring the breakdown of ascorbate with enzyme extract at 290 nm, as reported by Nakano & Asada (1981). At 25 °C, the amount of enzyme required for the oxidation of 1 μmol of ascorbate per min corresponding to one unit of APX activity was defined. APX activity was expressed in mmol H_2O_2 mg^{-1} protein min^{-1}.

Guaiacol peroxidase (GPOX) activity was quantified spectrophotometrically by monitoring guaiacol oxidation according to the protocol reported by Polle et al. (1994). The GPOX activity was expressed in mmol guaiacol mg^{-1} protein min^{-1}.

Total RNA extraction, cDNA synthesis, and analysis of gene expression by qRT-PCR

The barley leaves stored at −80 °C were used for total RNA extraction following the protocol detailed by Chang et al., (1993) and treated with RNase-free DNase I (Biomatik) to remove any residual genomic DNA. RNA was then quantified by Nanodrop spectrophotometer. First-strand cDNA was synthesised by First Strand cDNA Synthesis Kit (Biomtik) following the manufacturer's instructions. Genes of interest were quantitatively amplified in a 7300 Real-Time PCR Detection System (Applied Biosystems, Foster City, USA) using specific primers (Table 1) and using the Maxima SYBR Green/ROX qPCR Master Mix (Biomatik) based on the protocol detailed beforehand by Abid et al. (2020b). The Primer3 Input (version 0.4.0) software (Rozen & Skaletsky 2000) (http://frodo.wi. mit.edu/primer3/) was used to design the specific primers and the internal actin gene control

Table 1 List of gene-specific primers used for real-time RT-PCR analysis

Gene name	GenBank accession ID	Primer pair sequence (5´→3´)	Product size (bp)
HvP5CS	BK007070.1	*HvP5CSF*: GAGACAAGTCCCGTGTTGGT *HvP5CSR*: CCCACGGAGAACCTTAACAA	134
HvHSP	L32165.1	*HvHSPF*: CAAGATCAAGGATGGGGAGA *HvHSPR*: GTACGCAGGAACAGTGACGA	136
HvMYB	X70877.1	*HvMYBF*: ATCTAAAGCGATGGGGAGGT *HvMYBR*: CCTTGATGTAGGCGGTGAGT	101
HvHKT1	DQ912169.1	*HvHKT1F*: TATGGAGCAAGGGTTCAAGG *HvHKT1R*: ACGCTCAGGTAGACCAGCAT	135
HvWRKY	AY541586.1	*HvWRKYF*: CAAGCTTCTCTCCCCCTCTT *HvWRKYR*: TGTTCACTTCCTTCCGGTTC	81
AP2/ERF	AK364030.1	*AP2/ERFF*: GATGAGCATGGGATCGAACT *AP2/ERFR*: TCGATGGATTTCCTCTGGAC	141
HvNAC	DQ869678.1	*HvNACF*: CCGAGGTGGACCTCTACAAG *HvNACR*: CAAGCACGATCGACGAGATA	95
HvDREB	DQ012941.1	*HvDREBF*: TTGAGTCTGCAGGGACTGTG *HvDREBR*: CCTTCCCTTTTGGTTGGAAT	111
HvbHLH	AK363663.1	*HvbHLHF*: TCATCTCCAACCACCTCACA *HvbHLHR*: CAGAGGCCCTTGTTCTGAAG	137
HvPIP	AB286964.1	*HvPIPF*: CTGCTCGTTGTTGTGCAAAT *HvPIPR*: TTTCTACCGCGCGATAACTT	124
HvCAT	U20777.1	*HvCATF*:TGGACGGATGGTACTGAACA *HvCATR*: AGTGGATTCCAGGGACAGTG	95
HvSOD	KU179438.1	*HvSODF*: CCCTCCCCAAGTCAGTCATA *HvSODR*: CACAGCTACAGCCTTCACCA	134
HvAPX	AJ006358.1	*HvAPXF*: CCAAGGGTTCTGACCACCTA *HvAPXR*: AGCCAGACCTCTCCTTGTGA	120

used to normalise the data using the $2^{-\Delta Ct}$ method (Schmittgen & Livak 2008). Finally, the R-package (http://www.r-project.org/) was used to generate the heat maps in order to compare the expression profiling in different treatments.

Statistical analysis

For all experimental data, the significance of the difference between mean values was determined at ($p < 0.05$) using a one-way analysis of variance (ANOVA). Treatment means were separated using Duncan's multiple range test at the 5% level of significance using the SPSS 19.0 program. Moreover, a principal component analysis (PCA), correlation test and a hierarchical ascending clustering (HAC) were performed using XLSTAT.

Results

The effect of compost and biochar on plant growth and physiological parameters under salt conditions

The effects of different treatments on plant growth and physiological parameters are presented in Table 2. The amendment treatments (B, C, and CB) significantly increased SL and SDW compared to the

CK treatment. Notably, B plants showed the highest increase in SL, while CB plants had the greatest increase in SDW. The growth parameters of S plants have decreased significantly due to salt stress. The SL and SDW of CK plants in comparison to S plants ranged from 39 to 32 cm and from 363.67 mg to 213 mg, respectively. However, amendments have a significant impact on the negative effects of salt stress. The SL (16%, 33%, and 29%) and SDW (145%, 102%, and 120%) reflected significant increases in SB, SC, and SCB plants compared with S plants, respectively. The negative impact of salt stress on SDW was reduced by the amendment treatments.

Similarly, the amendment treatments (B, C, and CB) increased RL and RDW compared to the CK treatment, except for RL under the CB treatment. Salt stress negatively affected root growth. Overall, amendments increased RL (105%, 132% and −5%) and RDW (55%, 89% and 136%) reflected significant increases in SB, SC, and SCB plants respectively compared with S plants. Overall, SB and SC treatments increased RL by 105% and 132% respectively however SCB decreased RL by 5%. Our results reflected significant increases of RDW in SB, SC, and SCB plants by 55%, 89% and 136% respectively compared with S plants.

The RWC increased from 76.80% in CK plants to 96.87%, 93.48%, and 95.86% in plants B, C, and CB

Table 2 Effect of different treatments on shoot length (SL), root length (RL), shoot (SDW) and root dry weight (RDW), leaf relative water (RWC), electrolytes leakage (EL) level, chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (Chl tot) content at the tillering stage

Parameters	CK	B	C	CB	S	SB	SC	SCB
SL (cm)	39.00 ± 0.67^{de}	54.16 ± 0.56^{a}	49.00 ± 0.67^{b}	41.03 ± 2.64^{cd}	32.00 ± 0.67^{f}	37.33 ± 1.11^{e}	42.67 ± 0.56^{c}	41.50 ± 1.33^{cd}
RL (cm)	20.33 ± 1.53^{d}	24.50 ± 1.32^{c}	22.83 ± 1.04^{c}	12.03 ± 0.25^{f}	15.5 ± 0.50^{e}	31.83 ± 1.04^{b}	36.00 ± 2.00^{a}	14.73 ± 0.93^{e}
SDW (mg plant^{-1})	363.67 ± 11.59^{f}	443.33 ± 4.04^{e}	545.00 ± 8.54^{b}	628.00 ± 13.11^{a}	213.00 ± 6.00^{g}	523.00 ± 1.00^{c}	430.33 ± 2.52^{e}	470.33 ± 4.93^{d}
RDW (mg plant^{-1})	40.83 ± 1.61^{e}	46.57 ± 1.50^{d}	66.00 ± 2.00^{a}	59.67 ± 1.53^{b}	22.40 ± 1.35^{f}	34.73 ± 1.42^{f}	42.40 ± 1.57^{e}	53.00 ± 2.00^{c}
EL (%)	$8.47\% \pm 0.64^{e}$	$5.19\% \pm 0.58^{f}$	$12.33\% \pm 0.79^{d}$	$8.85\% \pm 1.33^{e}$	$23.83\% \pm 0.45^{a}$	$16.70\% \pm 0.99^{b}$	$14.35\% \pm 2.50^{c}$	$9.10\% \pm 1.12^{e}$
RWC (%)	$76.80\% \pm 0.82^{e}$	$96.87\% \pm 0.52^{a}$	$93.48\% \pm 0.30^{c}$	$96\% \pm 0.7^{ab}$	$74\% \pm 1.43^{e}$	$76\% \pm 1.29^{e}$	$80\% \pm 2.84^{d}$	$94\% \pm 0.91^{bc}$
Chl a (mg g^{-1} FW)	38.65 ± 0.51^{c}	37.68 ± 2.42^{cd}	50.31 ± 8.53^{b}	57.08 ± 2.04^{a}	17.49 ± 1.56^{f}	25.12 ± 0.37^{e}	32.03 ± 0.01^{d}	24.44 ± 0.80^{e}
Chl b (mg g^{-1} FW)	16.20 ± 0.19^{c}	16.14 ± 0.84^{c}	21.67 ± 0.23^{b}	24.24 ± 0.81^{a}	7.37 ± 0.63^{f}	12.30 ± 0.81^{d}	13.05 ± 0.71^{d}	9.80 ± 0.32^{e}
Chl tot (mg g^{-1} FW)	$54.83 \pm 0.6c$	$53.81 \pm 3.26c$	$78.18 \pm 2.46b$	$81.30 \pm 1.26a$	$24.86 \pm 0.94g$	$37.41 \pm 0.69e$	$45.08 \pm 0.70d$	$34.24 \pm 0.82f$

With *CK*: control (only soil); *B*: soil + Biochar; *C*: soil + Compost; *CB*: soil + Compost + Biochar; *S*: Salt stress; *SB*: Salt + Biochar; *SC*: Salt + Compost; *SCB*: Salt + Compost + Biochar. Different letters indicate significant difference at $P < 0.05$ based on multiple comparisons (Duncan test) in ANOVA

respectively (Table 2). Salt stress slightly reduced the RWC without being significantly different compared to the CK plants. The addition of amendment improved the RWC compared to the S plants. This improvement is significantly different in the SC and SCB plants (the RWC is equal to 79% and 93% respectively). The impact of salt stress on RWC was eliminated in SC and SCB plants.

The chlorophyll content (chl tot, chl a and chl b) increased significantly in the C and CB plants, by 42% and 48% for chl tot respectively. Salt stress resulted in a 55% decrease in each of chl a, chl b, and chl tot (Table 2). The chlorophyll content was highest in SC plants, followed by SB plants and then SCB plants, compared with the S plants. The SC plants reflected a significant increase in chl a (77%), chl b (83%), and chl tot (81%) compared with the S plants. Compared with the same control, SB and SCB plants showed a significant increase in chl a (43% and 39%), chl b (66% and 32%), and chl tot (50% and 37%) respectively.

The EL level increased significantly in B plants, decreased in C plants, and was not significantly different in CB plants compared to CK plants (Table 2). Salt stress drastically increased EL content from 8.47% in CK plants to 23.83% in S plants. Applying amendments reduced the negative effect of salt stress on RWC, chlorophyll content and EL level. Indeed, the EL level significantly decreased to 16.70%, 14.35% and 9.10% in SB, SC, and SCB plants respectively. Statistical analysis showed that the difference in EL level between CK plants and SCB plants was not significant.

The effect of compost and biochar on hydrogen peroxide, malondialdehyde, proline and soluble sugars content under salt conditions

The content of H_2O_2, a reactive oxygen species, decreased consecutively in B, C, and CB plants in response to the addition of amendments (Fig. 1.A) compared to the control, with a higher decrease
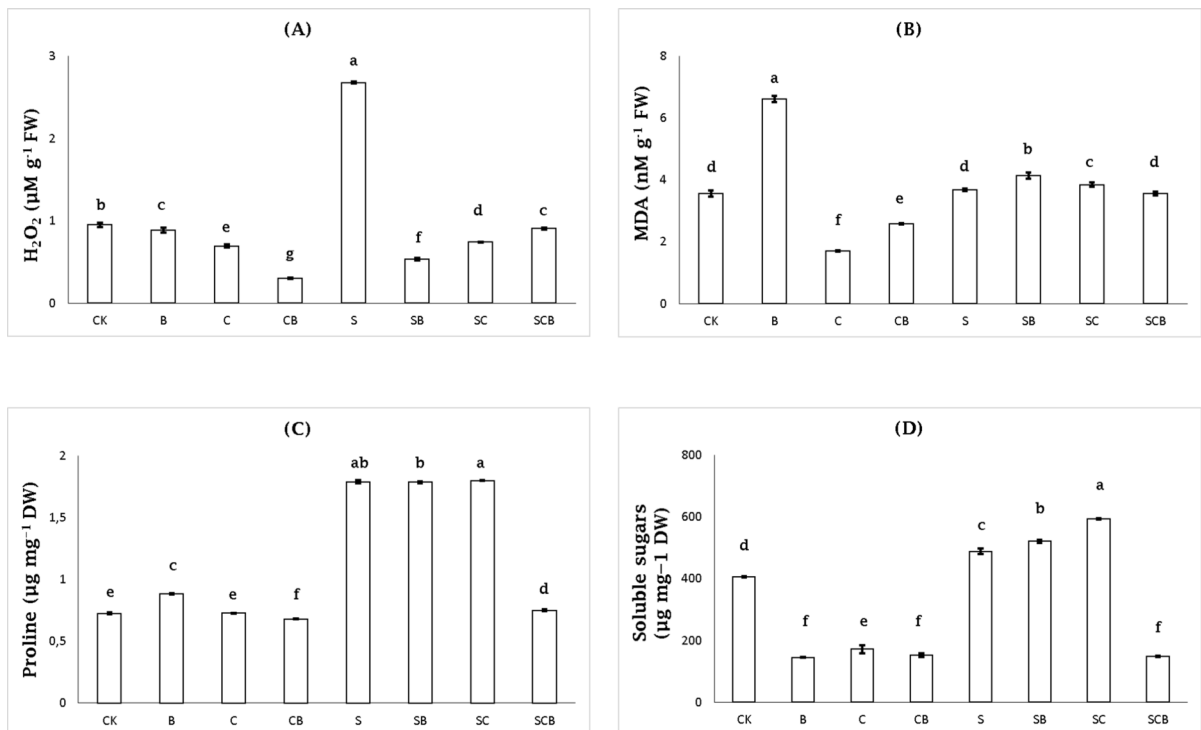

Fig. 1 Effect of different treatments on H_2O_2 (**A**), MDA (**B**), proline (**C**), and soluble sugars (**D**) under salt conditions. With CK: control (only soil); B: soil+Biochar; C: soil+Compost; CB: soil+Compost+Biochar; S: Salt stress; SB: Salt+Biochar; SC: Salt+Compost; SCB: Salt+Compost+Biochar. Different letters indicate significant difference at $P < 0.05$ based on multiple comparisons (Duncan test) in ANOVA

being observed in CB plants. Consistently, salt stress elevated the H_2O_2 concentration by 181%. H_2O_2 accumulation decreased by 80% to 72% and 66% in B, C, and BC plants, respectively, compared with the values recorded for S plants.

As an indicator of lipid peroxidation, MDA content almost doubled (86%) in B plants, but decreased however in C and CB plants (52% and 27% respectively) compared with CK plants (Fig. 1.B). Salt stress has no significant effect on MDA content. MDA content was increased in both SB and SC plants by 12% and 4% and decreased by 3% in SCB plants compared to S plants. Statistical analysis showed no significant effect between CK and SCB plants.

Proline increased by 21% in B plants, did not significantly change in C plants, and decreased by 6% in CB plants compared to CK plants (Fig. 1C). Salt stress significantly increased the proline content, and only SCB treatment significantly and remarkably attenuated this increase by 58% compared to S plants.

Soluble sugars significantly decreased in B, C, and CB plants. On the other hand, it increased by 20% under stress conditions (Fig. 1D). In SB and SC plants, the sugar content increased by 6% and 21% respectively, compared to S plants. Under the same stress conditions, this content decreased by 70% in SCB plants.

The effect of compost and biochar on antioxidant activity under salt conditions

The enzyme activities of the barley plant are presented in Fig. 2. For SOD activities (Fig. 2A), our result showed a significant and progressive decrease in B, C, and CB plans respectively, and values vary between 11.91 ± 0.08, and 5.99 ± 0.13 Units mg^{-1} of protein against a control value of 18.93 ± 0.08 Units mg^{-1} of protein. In the presence of saline conditions, SOD activity decreased by 53% compared to the control CK plants. Amendments supply increased SOD units compared to S plants by 16%, 15%, and
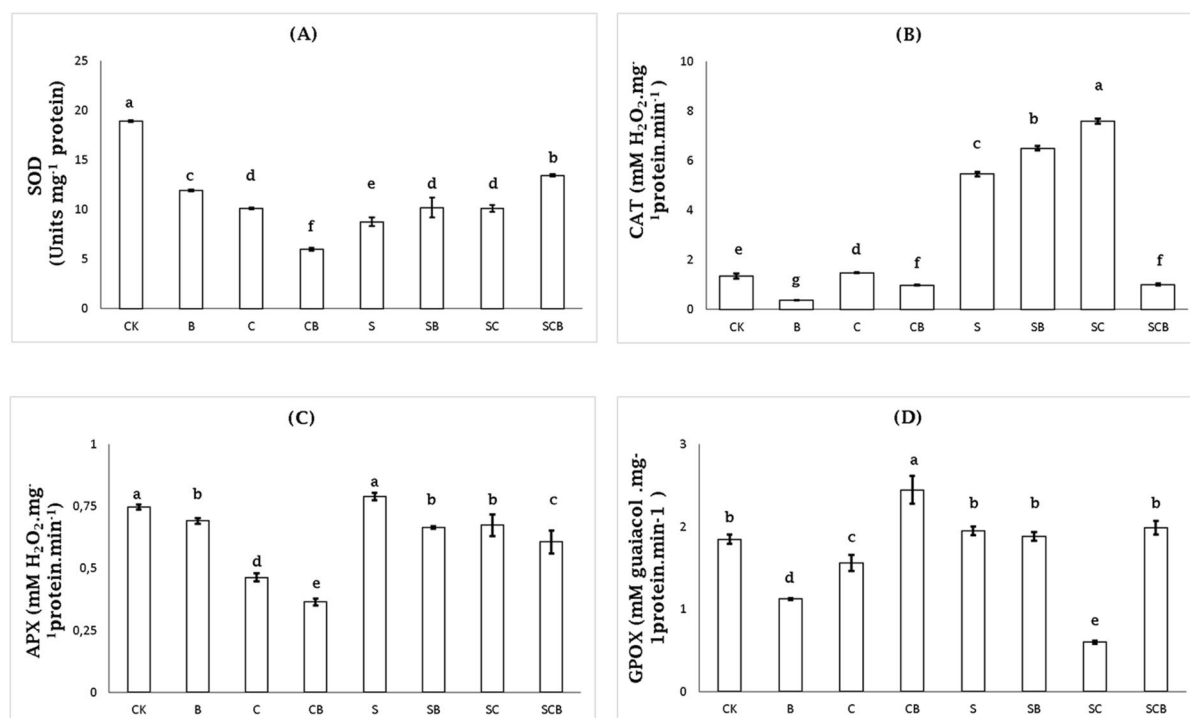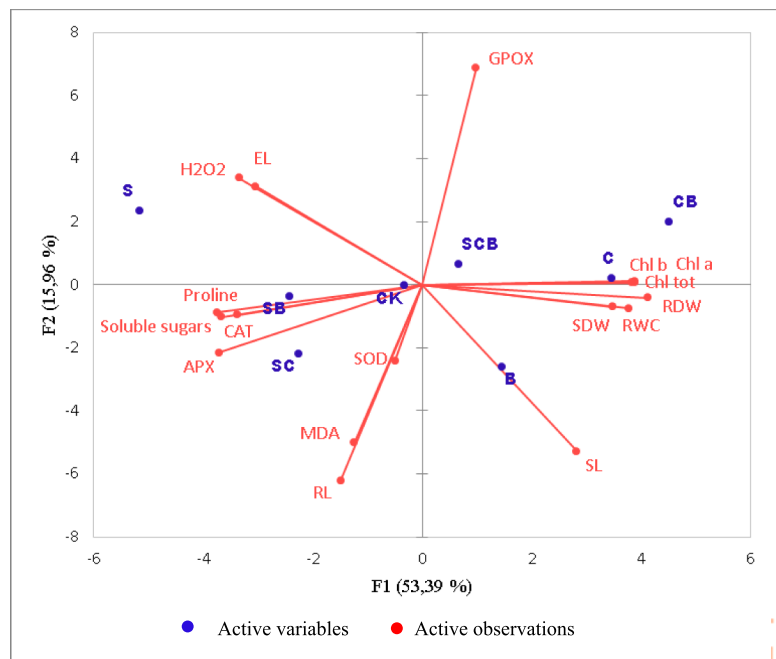

Fig. 2 Effect of different treatments on the activities of SOD (A), CAT (B), APX (C) and GPOX (D) under salt conditions. With CK: control (only soil); B: soil+Biochar; C: soil+Compost; CB: soil+Compost+Biochar; S: Salt stress; SB: Salt+Biochar; SC: Salt+Compost; SCB: Salt+Compost+Biochar. Different letters indicate significant difference at $P < 0.05$ based on multiple comparisons (Duncan test) in ANOVA

53% in SB, SC, and SCB plants, respectively. In B and CB plants, CAT activity decreased by 72% and 27%, respectively and increased by 6% in C plants compared to CK plants (Fig. 2B). Salt stress sharply increased CAT activity by 305% compared to normal conditions. As shown in Fig. 2A, the interaction between salinity and treatment with amendments was significant. Indeed, SB and SC plants showed a significant increase in CAT activity by 19% and 39%, respectively, however SCB plants showed a significant drop in CAT activity by 81% compared to the S plants. As shown in Fig. 1C, APX activity decreased progressively and significantly in B, C, and CB plants compared to CK plants. However, the highest APX activity was observed in the S plants with a value of 0.788 ± 0.014 mM. mg^{-1} protein. min^{-1}. There was no significant difference compared to the control treatment. Compared to CK plants, SB, SC, and SCB plants showed a significant decrease in APX activity by 15%, 14%, and 23%, respectively. GPOX activities in maize plants are presented in Fig. 2.D. The APX activity was lower in B and C plants by 39% and 15% respectively but higher in CB plants by 32% compared to CK plants. Unlike the control condition, no significant change was noticed between S, SB, and SCB plants. However, GPOX activity decreased in SC plants by 70% compared to CK plants.

Principal component analysis

The parameters obtained were subjected to principal component analysis (Fig. 3). Results showed that the first axis explained 53.39% of the variability. This axis consisted primarily of shoot and root weight, EL, chlorophyll (a, b, and tot), CAT, APX, H$_2$O$_2$, proline, and soluble sugars. The second axis varied by 15.96%, including shoot and root lengths, GPOX, and MDA. The data clearly separated the salt stress treatment from the other treatments. There was also a clear separation between the combined application and the sole application of biochar and compost under salt stress.

Correlation analysis of different parameters

Estimates of correlation between different parameters are presented in Table 3. Based on the correlation analysis, it is observed that (i) the RDW had a significant positive correlation of 0.797 with the SDW; (ii) EL had a significant negative correlation of −0.721 with SL, (iii) RWC had a significant positive correlation of 0.827 with the RDW, and a negative correlation of −0.710 with EL; (iv) Chl a had a significant positive correlation of 0.803 with RDW; (v) Chl b had a significant positive correlation of 0.738, 0.781,

Fig. 3 Principal component analysis (PCA). With CK: control (only soil); B: soil + Biochar; C: soil + Compost; CB: soil + Compost + Biochar; S: Salt stress; SB: Salt + Biochar; SC: Salt + Compost; SCB: Salt + Compost + Biochar

Table 3 Pearson's correlation coefficient (r) for measured parameters under different treatments

Variables	SL	RL	SDW	RDW	EL	RWC	Chl a	Chl b	Chl tot	CAT	SOD	APX	GPOX	H2O2	MDA	Pro	Soluble sugars
SL	1																
RL	0,205	1															
SDW	0,444	0,036	1														
RDW	0,646	−0,204	**0,797**	1													
EL	**−0,721**	0,124	−0,530	−0,664	1												
RWC	**0,748**	−0,371	0,630	**0,827**	**−0,710**	1											
Chl a	0,514	−0,196	0,703	**0,803**	−0,588	0,604	1										
Chl b	0,503	−0,155	**0,738**	**0,781**	−0,557	0,577	**0,993**	1									
Chl tot	0,528	−0,171	**0,709**	**0,818**	−0,548	0,599	**0,994**	**0,993**	1								
CAT	−0,520	0,653	−0,314	−0,629	**0,760**	**−0,754**	−0,552	−0,520	−0,539	1							
SOD	0,056	0,063	−0,336	−0,117	−0,374	−0,225	−0,192	−0,219	−0,202	−0,278	1						
APX	−0,323	0,304	**−0,870**	**−0,857**	0,367	−0,695	**−0,801**	**−0,804**	**−0,809**	0,410	0,518	1					
GPOX	−0,484	**−0,783**	0,182	0,075	0,062	0,082	0,136	0,161	0,127	−0,374	−0,126	−0,349	1				
H₂O₂	−0,499	−0,278	**−0,902**	−0,700	0,682	−0,474	−0,636	−0,660	−0,626	0,254	−0,003	0,634	0,057	1			
MDA	0,315	0,295	−0,292	−0,425	−0,239	0,010	−0,381	−0,371	−0,421	0,010	0,215	0,571	−0,418	0,112	1		
Proline	−0,508	0,580	−0,445	**−0,763**	**0,802**	**−0,739**	−0,674	−0,636	−0,661	**0,960**	−0,304	0,535	−0,347	0,419	0,200	1	
Soluble sugars	−0,606	0,608	−0,498	**−0,755**	0,679	**−0,930**	−0,555	−0,532	−0,555	**0,913**	0,054	0,613	−0,337	0,320	0,058	**0,868**	1

Values in bold are different from 0 at significance level alpha=0.05

and 0.993 with SDW, RDW, and Chl a, respectively; (iv) Chl tot had a significant positive correlation of 0.709, 0.818, 0.994, and 0.993 with SDW, RDW, Chl a, and Chl b, respectively; (vii) CAT had a significant positive correlation of 0.760 with EL, and a negative correlation of −0.754 with RWC; (viii) APX had a significant negative correlation of −0.870, −0.857, −0.801, −0.804, and −0.809 with SDW, RDW, Chl a, Chl b, and Chl tot, respectively; (ix) GPOX had a significant negative correlation of −0.783 with RL; (x) H2O2 had a significant negative correlation of −0.902 with SDW; (xi) proline had a significant correlation of 0.960 with CAT and 0.802 with EL, while negative correlation of −0.763 RDW and −0.739 with RWC; and (xii) soluble sugars had a significant positive correlation of 0.913 with CAT and 0.868 with proline, while it had a significant negative correlation of −0.755 with RDW and −0.930 RWC.

Hierarchical Ascending Clustering (HAC): insights into treatment groupings

To determine the effect of compost and biochar alone or combined on reducing the salt stress effects, a hierarchical ascending clustering (HAC) was carried out for the salt treatments (S, SC, SB, and SCB) only (Fig. 4). The dendrogram shows the hierarchical clustering of items S, SB, SC, and SCB, illustrating how these items are grouped based on their dissimilarities, with the height of the lines indicating the level of dissimilarity at which clusters are formed. The dendrogram presents two classes. The first class consists solely of saline stress (S). The second class includes salt treatments with the addition of amendments and is arranged in two sub-classes. The first sub-class contains the saline stress treatment with biochar application (SB), while the second sub-class contains the saline stress treatment with compost application (SC) and the saline stress treatment with combined biochar and compost application (SCB).

The effect of compost and biochar on gene expression under salt conditions

An in silico analysis was performed to identify genes involved in barley's stress response metabolism. Table 1 highlights 13 genes in barley that share significant similarities with those of other plants, including Arabidopsis. Specific primers were assigned to each
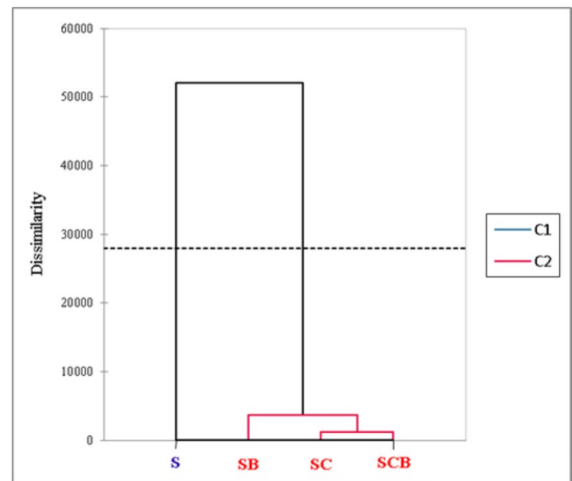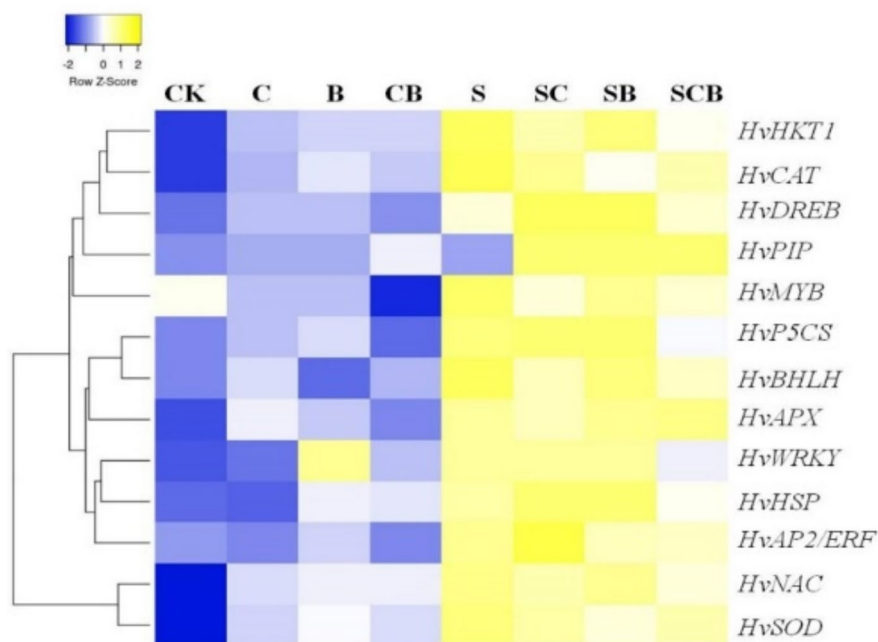
Fig. 4 Hierarchical ascending clustering (HAC). With CK: control (only soil); B: soil + Biochar; C: soil + Compost; CB: soil + Compost + Biochar; S: Salt stress; SB: Salt + Biochar; SC: Salt + Compost; SCB: Salt + Compost + Biochar

of the 13 selected genes (*HvP5CS, HvHSP, HvMYB, HvHKT1, HvWRKY, AP2/ERF, HvNAC, HvDREB, HvBHLH, HvPIP, HvCAT, HvSOD* and *HvAPX*). Quantitative real-time PCR was used to investigate the expression patterns of these transcripts to elucidate their potential roles in stress response to CK, B, CB, S, SB, SC, and SCB treatments. Log2 fold signal intensities were used to visualise the gene expression as a heat map. The heatmap (Fig. 5) illustrated the expression levels of genes involved in the plant stress response. The CK, C, B, and CB treatments represent gene expression in normal conditions, where varying shades of blue indicate baseline expression levels. In contrast, the S, SC, SB, and SCB treatments represent expression under salt stress, with shades of yellow indicating changes in gene activity. The shift from blue to yellow suggests that these genes are differentially expressed in response to salt stress, with brighter yellow signifying greater upregulation as part of the plant's adaptive response. This visualization demonstrates that these genes are particularly responsive to salt stress. The heatmap classified these genes into 6 clusters. Cluster 1 contains 2 members (*HvHKT1* and *HvCAT*, 15.38%) of the 13 genes being studied. The 2 genes in this cluster showed limited expression under CK, low expression under C, B, and CB, high expression under S and SC, and high expression under SB and SCB for *HvHKT1* and *HvCAT*

Fig. 5 Heat map representation of the effects of different treatments on the gene expression in the barley leaves. Blue and yellow indicate lower and higher expression values, respectively. The intensity of the colors is proportional to the absolute value of $\log^2$ of the fold difference in expression. With CK: control (only soil); B: soil + Biochar; C: soil + Compost; CB: soil + Compost + Biochar; S: Salt stress; SB: Salt + Biochar; SC: Salt + Compost; SCB: Salt + Compost + Biochar

respectively. Cluster 2 contains 2 members (*HvDREB* and *HvPIP*, 15.38%). The genes in this cluster tend to show no change or low expression difference under CK, C, B, and CB but also salt stress (S). In contrast, bioamendment application combined with salt stress (SC, SB, SCB) induced particularly high expression of the *HvDREB* and *HvPIP* genes. Cluster 3 contains only 1 member (*HvMYB*, 7.69%). The *HvMYB* gene revealed low expression under C, B, and SB, moderate expression under CK, SC, and SCB, and high expression under S and SB. Cluster 4 contains 3 members (HvP5CS, *HvBHLH* and *HvAPX*, 23.07%). These genes are weakly to moderately expressed under CK, C, B, and CB conditions, whereas they are generally highly expressed under S, SC, SB, and SCB conditions. Cluster 5 contains 3 members (*HvWRKY, HvHSP*, and *AP2/ERF*, 23.07%). These genes are poorly expressed under CK and C, low to moderately expressed under B and CB, and usually highly expressed under salt stress with or without bioamendment applications (S, SC, SB, and SCB). Cluster 6 contains 2 members (*HvNAC* and *HvSOD*, 15.38%). The genes have a too-low expression in CK, relatively constant and low in C, B, and CB, and constant and slightly high in S, SC, SB, and SCB. Overall, both SC and SB treatments appear to result in mostly yellow tones, indicating that many genes are upregulated in

the presence of either compost or biochar under salt stress. This could imply that both amendments help plants activate stress response genes under salt conditions. However, under SCB treatment, while the expression of some genes remains high (yellow), the majority of genes show reduced or minimal expression (white or light shades). This suggests that the combination of both amendments may result in a balanced response, with some genes not requiring further upregulation.

Discussion

Compost and biochar prove plant growth and physiological parameters under salt conditions

Salt stress significantly affects plant growth, particularly by reducing the length and dry mass of barley shoots and roots, chlorophyll pigments (chl a, b, and tot), RWC and increasing EL. Our results align with previous research showing that salt stress reduces growth traits in barley (Nefissi Ouertani et al. 2021; 2022b) and faba bean (Vicia faba L.) (Nasrallah et al. 2022), as well as chlorophyll content in various plants (Nefissi Ouertani et al. 2022a; Parihar et al. 2015). Salt stress also decreases water availability to plants

and affects cell turgidity (Parihar et al. 2015). Additionally, similar to our findings, EL increased in lettuce (Hniličková et al. 2019) and faba bean (Ghouili et al. 2021) under salt and osmotic stress, respectively. Parihar et al. (2015) explained that salt ions (Na^+ and Cl^-) can interfere with the absorption of essential nutrients, leading to nutritional deficiencies that hamper plant growth. They added that the excess accumulation of these ions in chloroplasts disrupts chlorophyll production, causing chlorotic toxicity. Additionally, salt stress alters the ionic and osmotic balance of the cells, damaging cell membranes and resulting in electrolyte leakage (Demidchik et al. 2014).

In our study, the application of biochar and compost, whether alone or in combination under salt stress (SB, SC, and SCB), significantly improved growth traits (except RL under SCB), chlorophyll content, and relative water content (RWC) compared to the salt-only treatment. Furthermore, the amendments (SB, SC, and SCB) reduced EL under salt stress. Similar to our results, several studies have reported the beneficial effects of compost and biochar application on growth and physiological parameters in various plants, both under normal (Ghouili et al. 2022b; 2024b) and stressed conditions (Nadeem et al. 2017; Rahayu et al. 2019; Rekaby et al. 2020; Zulfiqar et al. 2022). The combined application of compost and biochar (SCB) given the highest RWC and the lower EL. Ud Din et al. (2023) found that compost and biochar, either alone or combined, improved relative water content (RWC) in tomatoes (*Solanum lycopersicum L.*) under salt stress. Nadeem et al. (2017) reported that the synergistic use of biochar, compost, and plant growth-promoting rhizobacteria (PGPR) significantly increased chlorophyll content and reduced EL in cucumber under water deficit. Sandy soils in arid and semi-arid regions are characterised by their low organic carbon content and low water-holding capacity (Le Guyader et al. 2024). Using organic amendments can greatly improve soil characteristics and plant growth even under stress by providing them with a continuous source of moisture and nutrients. On the one hand, compost enriches soil with organic matter, enhancing its structure, aeration, and water-retention capacity (Scotti et al. 2015). It promotes root growth and nutrient uptake (Ghouili et al. 2022b; 2024b; Janati et al., 2022) and gradually releases essential nutrients, improving plant nutrition

even in saline conditions (Ait-El-Mokhtar et al. 2022; Savy et al. 2022). This explains the improved values observed especially for shoot (SL) and root (RL) length as well as chlorophyll contentment under SC treatment compared to salt treatment. Compost also supplies nutrients to beneficial microorganisms, improving plant health and increasing nutrient availability (Ghouili et al. 2023a; 2022b). On the other hand, biochar is characterised by its high cation exchange capacity, which enables it to sequester Na + and Cl- ions, thereby reducing their toxicity to plants (Murtaza et al. 2024). Biochar is also carcaterised by its porous structure that improves porosity, structure and water-holding capacity of the soil (Chi et al. 2024). This water can then be released slowly, providing a continuous source of plant moisture. This is particularly beneficial in sandy soils, such as the soil used in this study. The improvement of soil porosity and structure facilitate also root growth and water infiltration, which reduces plant stress (Zulfiqar et al. 2022). These benefits of compost and biochar explain the improvement in plant growth and physiological responses observed under salt stress when compost is applied, compared to stress plants without compost. The combined use of compost and biochar can have synergistic effects, allowing the benefits of both amendments to be fully realised. Together, they can reduce the negative impacts of salt stress on barley plants (Sharma et al. 2021). Compost enriches the soil with essential nutrients and organic matter, while biochar enhances nutrient retention and stabilizes soil structure. This combination creates an optimal environment for root growth, nutrient uptake, and water use efficiency, leading to improved plant growth, chlorophyll production, and to reduced EL. Furthermore, biochar and compost work synergistically to improve nutrient availability and water retention in the soil, which is particularly reflected in SL, RDW, RWC and EL of the SCB plants.

The effect of compost and biochar on oxidative stress markers and metabolite accumulation under salt stress

The results show that salt stress induces elevated H_2O_2 production, proline and soluble sugars accumulation, which is a typical response to oxidative stress caused by saline conditions (Nasrallah et al. 2022). Our results align with other research on

barley (Nefissi Ouertani et al. 2021) and faba bean (Nasrallah et al. 2022). However, MDA levels has not changed compared with the control, suggesting that plants can tolerate this level of salt stress. Biochar (SB) and compost (SC) reduced H_2O_2 levels, with biochar being particularly effective, in contrast they slightly increase MDA compared with salinity conditions. These overall effects suggests that these amendments are effective in improving the plant's ability to handle oxidative stress caused by salt, but they do not entirely eliminate the negative impacts of salinity. Biochar reduces the toxicity of salt to plants by adsorbing excess sodium and chlorine ions from saline soil (Chi et al. 2024), while compost enriches the soil with organic matter and nutrients, improving soil structure and water retention capacity. These improvements reduce the impact of oxydative stress as reflected by H_2O_2 levels. The slight increase in MDA could reflect a minor trade-off in metabolic pathways, where the plants balance between ROS scavenging and membrane stability. Compared to previous studies, MDA content increased in faba bean plants when subjected to salt treatment (Nasrallah et al. 2022) and barley (Nefissi Ouertani et al. 2021) and also in date palm trees even in presence of compost (Ait-El-mokhtar et al. 2022). This indicates that while the amendments are beneficial, they do not entirely eliminate the stress but help the plants cope more effectively with the salinity-induced oxidative damage.

Compared to S, compost increases proline and soluble sugars synthesis under salt stress, whereas biochar tends to reduce proline and increase soluble sugars synthesis. Previous studies reported that compost application (Ait-El-mokhtar et al. 2022) and combining biochar and Pseudomonas sp. (Abideen et al. 2022) improved proline content under salt conditions. In contrast, Abdel-Ati & Eisa, (2015) found that proline content decreased in barley plants amended by compost under saline conditions. The differing effects of proline between compost and biochar suggest that while both amendments help the plant cope with salt stress, they might do so through slightly different mechanisms. Compost enhances the plant's ability to accumulate proline as an osmotic regulation. Indeed, plants accumulate compatible solutes such as proline in order to maintain cell turgor pressure and stabilise cellular structures to counteract stress (Szabados & Savouré, 2010). Biochar seems to reduce the need

for proline by improving overall soil conditions and reducing stress on the plant. The increase in soluble sugars under both SB and SC treatments is a positive response to reduce the negative effects of salt stress, as these sugars help balance osmotic pressure, protect cellular structures, and support metabolic activities under stress. Previous studies recorded high levels of soluble sugars in faba bean plants under osmotic stress (Ghouili et al. 2021) and also in the presence of compost under salt conditions in date palms and tomato plants (Ait-El-mokhtar et al. 2022; Savy et al. 2022). Indeed, soluble sugars, such as glucose, fructose, and sucrose, help maintain the osmotic balance of plant cells (Ghouili et al. 2021). In summary, compost and biochar both help plants cope with salt stress by improving soil conditions, but they affect osmotic regulators like proline and soluble sugars differently.

The SCB treatment decreased H_2O_2 levels, maintains MDA content similar to that of the control, and reduced the synthesis of proline and soluble sugars compared to S treatment. Our result suggests that a synergetic effect of compost and biochar treatments on salt stress response. This combination effectively reduces oxidative stress, as indicated by the decrease in H_2O_2 levels and the maintenance of MDA content at control levels. It could modify the plants' perception of salt stress or their ability to cope with the stress thereby reducing the need to accumulate high levels of proline and soluble sugars, typically synthesised in response to stress. This suggests that the amendments create a more favourable growing environment by improving soil conditions, thus alleviating the plant's need to rely heavily on these stress metabolites. In essence, the combination of compost and biochar helps the plant handle salt stress more efficiently, reducing the severity of oxidative damage and limiting the accumulation of stress-related metabolites.

Role of compost and biochar in modulating antioxidant activity under salt stress

SOD, CAT, GPOX, and APX enzymes play a crucial role in defending plants against oxidative stress caused by abiotic conditions, such as salinity. Our analyses revealed that salt stress significantly increased the activity of CAT and APX, decreased SOD activity, and had no effect on GPOX activity compared to the control. This indicates that plants

are capable to reduce the oxidative stress induced by salt. On the light of our results, salt stress increased the activity of these antioxidants on barley (Nefissi Ouertani et al. 2021; 2022a; 2022b) and faba bean (Abid et al. 2020a; Nasrallah et al. 2022). Overall, SB and SC treatments increased SOD and CAT activities, while decreasing APX and GPOX activities compared to the salt-only treatment. The increase in SOD and CAT activities indicates that biochar and compost enhance the plant's ROS scavenging capabilities, likely improving its overall salt tolerance and oxidative stress management. By scavenging ROS, antioxidant enzymes protect membrane lipids, proteins, and DNA from oxidative damage, which is crucial for maintaining cellular integrity and plant survival (Hanana et al. 2011; Nasrallah et al. 2022). At the same time, the reduction in APX and GPOX activities suggests that the plant's defense system is becoming more efficient and balanced, as it can manage ROS effectively with fewer resources. SB and SC treatments seem to support the plant's ability to handle oxidative stress in a more coordinated manner, ensuring that the plant's antioxidant defences are tuned to the appropriate levels for coping with salt stress.

SCB treatment increased SOD activity, decreased CAT and APX activities and has not affect GPOX activity compared to salt treatment. SOD plays a critical role in the first line of defense against oxidative stress by converting superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), which is then further detoxified by other enzymes like CAT and APX. The increase in SOD activity under the SCB treatment suggests that the combination of compost and biochar enhances the plant's ability to manage superoxide radicals generated by salt stress. This could be due to improvements in soil conditions provided by biochar and compost, such as better nutrient availability (Ghouili et al. 2022b; 2023b), moisture retention, and soil aeration (Ud Din et al. 2023), which help the plant grow more robustly and improve its defense responses. The decrease in CAT and APX activities and the lack of effect on GPOX activity could be an indication that the plant, due to the improved soil conditions from biochar and compost, is better managing oxidative stress at an earlier stage in the ROS cascade. Since SOD activity is increased, more superoxide radicals are being converted into H_2O_2, but the reduced activity of CAT and APX indicates that less hydrogen peroxide is being generated overall or that

other mechanisms are compensating for the need for H_2O_2 detoxification. Overall, these findings imply that SCB treatment enhances the plant's ability to manage salt-induced oxidative stress by improving early-stage antioxidant defense (via SOD), but the need for later-stage H_2O_2 detoxification (via CAT and APX) is reduced, possibly due to improved overall plant health and stress resilience.

Role of compost and biochar in regulating gene expression in response to salt stress

In this work, we studied the expression of certain genes involved in different metabolic responses to salt stress, as shown by the heat map. These genes demonstrate a coordinated and differential response to stress conditions.

Genes involved in ion homeostasis

Four genes are involved in maintaining ionic balance and osmosis in cells namely *HvHKT1* transporter and *HvMYB, HvNAC*, and *HvAP2/ERF* transcription factors. The *HvHKT1* transporter is primarily involved in the transport of Na+ and K+ ions (Horie et al. 2009). Under salt stress, *HvHKT1* expression has been upregulated to reduce sodium toxicity and manage competition between Na+ and K+. By promoting selectivity for K+ at the root level, *HvHKT1* enables the plant to maintain adequate levels of K+ despite the high presence of Na+ (Munns & Tester 2008). Then, *HvHKT1* transports ions from the roots to the leaves and limits excessive Na+ accumulation in aerial plant parts by sequestering Na+ in cell vacuoles (Hauser & Horie 2010; Ud Din et al. 2023). Based on our results, SB particularly and SC upregulated *HvHKT1* expression. Biochar or compost application can modulate the expression of *HvHKT1*, by improving the soil's physical and chemical properties, thereby reducing the uptake of toxic ions and enhancing nutrient availability (Bagues et al. 2024; Ghouili et al. 2022b). In tomato, the combined application of compost and biochar significantly enhanced plant growth and yield under salinity stress by reducing Na^+ levels in plant tissues and improving overall nutrient uptake (Ud Din et al. 2023).

In S plants exposed to salt stress, transcription factor *HvMYB* expression increased. These outcomes resonate with the work of He et al. (2019), who found

that in *D. candidum* genome, nine genes of *DoMYB* were up-regulated under salinity stress. This upregulation is an adaptive response aimed at activating genes involved in stress defence mechanisms, such as antioxidants, heat shock proteins and ionic transport systems that help maintain ionic balance and osmosis in cells (Cao et al. 2020). Amendments application modulated the expression of *HvMYB*, this effect is generally less pronounced than that observed under salinity stress. This may be due to the improvement of soil fertility, water and nutrient availability following compost and biochar input (Ghouili et al. 2023a; Sharma et al. 2021). The combined use of amendments can have a synergistic effect on the expression of stress-related genes, including *HvMYB* and *HvHKT1*, further improving salt stress tolerance by improving nutrient availability and soil physicochemical properties.

The *HvNAC* transcription factor plays a part in the regulation of numerous genes involved in the biosynthesis of osmolytes, the synthesis of protective proteins and antioxidant molecules. These osmolytes help cells cope with the damage caused by salt (Chen et al. 2019; Nefissi Ouertani et al. 2022a). Plants overexpressing *HvNAC* show improved growth and survival under high salinity conditions compared to wild-type plants (Xue et al. 2011). This is due to the activation of adaptive mechanisms such as regulating ion balance, reducing sodium accumulation in cells, and increasing antioxidant capacity. In this study, *HvNAC* was enhanced under salinity, slightly upregulated when amendment was applied (SB, SC), and was reduced in combined treatment SCB. While the specific studies on NAC gene expression under these amendments are limited, it is understood that improved soil conditions and nutrient availability can influence the expression of stress-responsive genes such as *NAC*s.

HvAP2/ERF has been slightly upregulated under salinity. Ma et al. (2024) reported that in rice the *OsERF19* expression was inhibited by salt stress. However, overexpressing the same gene in line increased the tolerance of plants to salt stress and upregulated Late embryogenesis abundant protein gene (*OsLEA3*), Vacuolar Na+/H+antiporter gene (*OsNHX1*), Low-affinity Na+transporter (*OsHKT6*), and Overly tolerant to salt 1 (*OsOTS1*) genes under salt stress. Indeed, *HvAP2/ERF* factors regulate the expression of key genes involved in the salt stress

response. In the presence of salt stress, these factors can activate metabolic pathways that increase the production of osmoprotective compounds and enhance ROS detoxification mechanisms (Licausi et al. 2013; Ma et al. 2024; Mizoi et al. 2012). Our results showed that although the expression of this factor increased slightly under SB and SCB, it increased more under SC. Amendments, especially compost, could modulate the expression of *HvAP2/ERF*-regulated genes by influencing hormonal signalling pathways and ROS levels.

Genes involved in the production of osmoprotectants

Three genes are involved in the mechanisms of osmoprotectant production namely *HvP5CS HvDREB*, and *HvWRKY*. The *HvP5CS* gene encodes the enzyme P5CS, which catalyses an essential step in proline biosynthesis (Szabados & Savouré, 2010). In our investigation, the overexpression of *HvP5CS* in S, SC, and SB is reflected by the elevation of proline levels under the same treatments. Similar to our results, P5CS increased under salt stress in barley plants (Nefissi Ouertani et al. 2022a). Other studies demonstrate that transgenic plants over-expressing this enzyme show better growth and reduced cell damage in saline conditions (Szabados & Savouré, 2010). These results may be strongly linked to an increased tolerance of salt stress in barley. In addition, a proteomic analysis carried out in barley plants grown in the presence of compost found that the relative abundance of the P5SC protein increased in leaf and root tissue compared to the control (Ghouili et al. 2022a; 2023a). Our results showed that *HvP5CS* gene expression was stable in SCB. When the conditions are not severely stressful, the combined application of amendments in saline conditions can indirectly decrease the expression of *HvP5CS*by improving the physicochemical and nutritional characteristics of the soil (Hazman et al. 2023). These amendments can therefore potentially increase or stabilise *HvP5CS* expression, thereby facilitating better proline accumulation under salt stress.

HvWRKY is a transcription factor involved in various physiological processes, especially in responses to biotic and abiotic stress. Under salinity, they can activate or repress the expression of genes involved in osmoprotectants biosynthesis to help the plant adapt to unfavourable conditions (Isah 2019; Karam et al.

2022; Li et al. 2021; Nakashima et al. 2009). WRKYs can also interact with other transcription factors and regulators, such as bZIP, which are known to regulate the stress response and osmolyte synthesis (Rushton et al. 2010). Increased under SC, and SB, the expression of *HvWRKY* can be modulated by these amendments, indicating an activation of defence mechanisms to improve stress tolerance. Similar to the *HvP5CS* gene, *HvWRKY* gene expression was stable in SCB. The over-expression of WRKY genes in various plant species enhances their resistance to saline conditions by promoting their capacity to maintain a lower Na+/K+ ratio (Li et al. 2021; Nefissi Ouertani et al. 2022a). Compost and biochar are known to improve soil health and plant resilience, potentially leading to more stable gene expression profiles under stress conditions (Chi et al. 2024; Sharma et al. 2021).

The *HvDREB* transcription factor is involved in the regulation of responses to abiotic stresses. It can activate or repress the expression of genes involved in adaptation to stress (Agarwal et al. 2006). The transgenic plants overexpressing DREB1A/CBF3 exhibited an accumulation of osmoprotectants, including proline and various sugars, under control and drought conditions (Rushton et al. 2010). Our findings indicate that *HvDREB* expression is increased in response to the provision of amendments. This observed adaptation may occur as a result of improvements in the microenvironment of the root.

Genes involved in antioxidant enzymes

Antioxidant enzymes work in orchestrated synergy to protect plant tissue from oxidative damage that can be generated by ROS. In *H. vulgare*, genes encoding enzymes such as *HvSOD*, *HvCAT*, and *HvAPX* play an essential role in protecting cells against ROS generated in response to salt stress (Nefissi Ouertani et al. 2022b). Several studies reported that salt stress increases antioxidant gene expressions (Nefissi Ouertani et al. 2021; 2022a; 2022b). In our research, *HvSOD* is upregulated in response to salt stress to better manage the increased ROS levels produced by osmotic and ionic stress caused by salinity. Its slight overexpression in the presence of amendments (SB, SC, and SCB) suggests that *HvSOD* is not strongly regulated by these amendments, perhaps as a result of sufficient antioxidant mechanisms provided by

other genes or soil organic matter. *HvCAT* expression increased under S. This increase was less pronounced in SC and SCB. The presence of soil amendments may attenuate the salt effect, hence the decrease in *HvCAT* expression compared with S plants. In support of this, the abundance of CAT protein was significantly lower under compost treatment (Ghouili et al. 2022a). *HvCAT* expression remained relatively stable under SB where biochar may have mixed effects on plant response to stress (Bagues et al. 2024). *HvAPX* expression increased slightly in S and SC but increased more in SB and SCB. The high expression of *HvAPX* suggests an active response in order to manage oxidative stress, potentially increased by salt stress but also microbial interactions or decomposition of organic matter.

Transport proteins

The *HvPIP* gene was down-regulated in S plants and over-expressed in SC, SB, and SCB plants. This variation is reflected in the RWC. Indeed, proteins of the aquaporins (PIPs) family form channels in the cell membrane to facilitate the transport of water and, in some cases, small solutes across the membrane (Ghouili et al. 2023a; Horie et al. 2011). Under saline conditions, maintaining a water balance is crucial for plant survival. The upregulation of *HvPIPs* under amendments in salt conditions helps regulate water uptake and transport. So, plant cells can maintain turgor pressure and avoid dehydration. In barley roots, compost treatment increased the abundance of proteins involved in the transport of water and other small neutral molecules across membranes, including PIP (Ghouili et al. 2023a).

Regulation of genes involved in salt tolerance

The bHLH transcription factors regulates various genes involved in salt tolerance (Wang et al. 2023). The *HvbHLH* has been upregulated in S, SC, SB, and SCB plants. Quan and colleagues (2023) report several ways in which bHLH TFs influence plant stress reactions. They can confer drought tolerance, some can improve plant growth under nutrient deprivation, and others, when overexpressed, can improve salt stress in plants (Quan et al. 2023). Compost and biochar can enhance this response under salt conditions by modifying soil conditions and providing nutrients

and microbial support, which can also influence the expression of *HVbHLH* genes and enhance plant resilience.

Gene involved in protein protection

HSPs function as molecular chaperones, playing a vital role in the stabilisation of proteins under both normal and stressful conditions (Timperio et al. 2008). In the present study, *HvHSP* expression was slightly upregulated under salinity, upregulated under SB and SC treatment, and stable under SCB treatment. The *OsHSP* 17.9 overexpression in rice plants increases antioxidant activity under heat and salt stress (Wang et al. 2023). The increased expression of *HvHSP* under salt stress is an adaptive response by plants to protect and stabilise their proteins and cellular structures. Several studies reported that HSPs reduce intracellular levels of reactive ROS and maintain membrane integrity by facilitating protein folding, degradation, complex assembly, and translocation (Do et al. 2023; Ghouili et al. 2022a; Yer et al. 2018). Compost application induces the accumulation of HSP in barley leaves (Ghouili et al. 2022a), and also heat shock protein (HSP 70) and small heat shock protein (sHSP) in barley roots (Ghouili et al. 2023a). The combination of compost and biochar can improve soil conditions, reducing the impact of salt stress and helping plants to tolerate such conditions.

The different molecular mechanisms of salt stress tolerance induced by the separate or combined application of biochar and compost

Our results show that biochar and compost influence the expression of defence genes differently in response to salt stress, each modulating specific tolerance mechanisms that can be exploited depending on the specific needs of the plant and the environmental conditions.

In fact, biochar mainly promotes ion regulation (*HvHT1*) and the stabilisation of cell structures (*HvP5CS, HvDREB, HvBHLH* and *HvHSP*), probably reducing the need for certain antioxidant enzymes (*HvSOD* and *HvCAT*) by compensating for stress by other mechanisms. In this way, biochar appears to trigger proactive defence mechanisms, helping the plant to manage salt stress more effectively,

thereby reducing the need for additional antioxidant responses.

Compost activates genes related to transcription factors (*HvDREB* and *HvAP2/ERF*), proline accumulation (*HvP5CS*) and water regulation (*HvPIP*), but decreases the expression of genes related to ion regulation (*HvHT1*), targeted transcription factors (*HvMYB* and *HvBHLH*), and antioxidant enzymes (*HvAPX, HvSOD* and *HvCAT*), suggesting a different modulation of the stress response. Compost seems to offer a more favourable environment, where the plant experiences less oxidative stress, reducing the need to trigger its antioxidant mechanisms intensively.

The combined application of compost and biochar under salt stress has a particular effect on the expression of plant genes. It stimulates the expression of *HvPIP* and *HvAPX*, but reduces that of many other stress response genes compared with S-stressed plants. The increase in *HvPIP* indicates that the plant benefits from better water management, a crucial aspect under salt stress conditions. This helps the plant to avoid dehydration by facilitating the circulation of water through the cell membranes, an effect that is amplified by the presence of the two amendments. The increase in *HvAPX* indicates that the biochar-compost combination helps to activate a specific antioxidant defence, probably to eliminate moderate levels of free radicals without requiring activation of the full range of antioxidant enzymes. The expression of many other stress response genes was reduced. The combined input of compost and biochar seems to create a sufficiently stable environment for the plant to perceive less salt stress. This reduces the need to stimulate genes associated with responses to severe stress, such as those involved in ion management (*HvHT1*), protection against oxidative stress (*HvHSP, HvSOD,* and *HvCAT*), and the activation of intense response signals via transcription factors (such as *HvDREB, HvWRKY,* and *HvNAC*). Furthermore, compost and biochar combined can provide complementary benefits: compost improves soil nutrition and structure, while biochar stabilises water and ion levels. This combination reduces the need to activate defence genes intensively. The plant activates only certain specific genes (*HvPIP* and *HvAPX*) to respond moderately to stress, which is probably sufficient given the benefits provided by the amendments. Thus, in the absence of high stress levels, the plant can devote more of its energy resources to growth and

metabolism, rather than mobilising energy-intensive defence mechanisms.

Conclusion

The application of date palm waste compost and biochar, either individually or combined, effectively mitigates the negative effects of salt stress on barley plants. These treatments enhance plant growth, RWC, and chlorophyll levels while reducing oxidative stress markers such as EL and H_2O_2. Compost and biochar alone increase antioxidant activity, soluble sugar content, and maintain proline levels, while their combined use stabilizes MDA levels and balances antioxidant activities. Gene expression analysis shows that both amendments significantly upregulate stress-related genes, with the combination treatment providing a moderated effect that may selectively activate specific responses. This synergy reduces ROS production, limiting the need for intense antioxidant mechanisms and reallocating plant resources more efficiently. The combined application of compost and biochar proves more advantageous by improving soil moisture, nutrient availability, and stress tolerance mechanisms. This strategy holds potential for enhancing crop resilience in arid and semi-arid regions, though field studies are recommended to confirm these findings. The results underscore the complementary effects of compost and biochar in reducing stress perception and optimizing plant resource use.

References

Abdel-Ati AA, Eisa SS (2015) Response of barley grown under saline condition to some fertilization treatments. Ann Agric Sci 60:413–421. https://doi.org/10.1016/J.AOAS.2015.11.002

Abid G, Ouertani RN, Jebara SH, Boubakri H, Muhovski Y, Ghouili E, Abdelkarim S, Chaieb O, Hidri Y, Kadri S, El Ayed M, Elkahoui S, Barhoumi F, Jebara M (2020a) Alleviation of drought stress in faba bean (*Vicia faba* L.) by exogenous application of β-aminobutyric acid (BABA). Physiol Mol Biol Plants 26:1173–1186. https://doi.org/10.1007/s12298-020-00796-0

Abid G, Ouertani RN, Muhovski Y, Jebara SH, Hidri Y, Ghouili E, Abdelkarim S, Chaieb O, Souissi F, Zribi F, Jebara M (2020b) Variation in antioxidant metabolism of faba bean (*Vicia faba*) under drought stress induced by polyethylene glycol reveals biochemical markers associated with antioxidant defense. Plant Biosyst 0:1–10. https://doi.org/10.1080/11263504.2020.1785964

Abideen Z, Waqif H, Munir N, El-keblawy A, Hasnain M, Radicetti E, Mancinelli R, Nielsen BL, Haider G (2022) Algal-mediated nanoparticles, phycochar, and biofertilizers for mitigating abiotic stresses in plants : a review. Agronomy 12:1–27. https://www.mdpi.com/2073-4395/12/8/1788

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25:1263–1274. https://doi.org/10.1007/s00299-006-0204-8

Ait-El-mokhtar M, Fakhech A, Ben-Laouane R, Anli M, Boutasknit A, Ait-Rahou Y, Wahbi S, Meddich A (2022) Compost as an eco-friendly alternative to mitigate salt-induced effects on growth, nutritional, physiological and biochemical responses of date palm. Int J Recycl Org Waste Agric 11:85–100. https://doi.org/10.30486/ijrowa.2021.1927528.1233

Akhtar SS, Andersen MN, Liu F (2015) Biochar mitigates salinity stress in potato. J Agron Crop Sci 201:368–378. https://doi.org/10.1111/jac.12132

Ali S, Rizwan M, Qayyum MF, Ok YS, Ibrahim M, Riaz M, Arif MS, Hafeez F, Al-Wabel MI, Shahzad AN (2017) 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

Antonopoulou C (2022) Climate change and agriculture. In The academic language of climate change: an introduction for students and non-native speakers. Emerald Publishing Limited, Leeds, pp. 7–12. https://doi.org/10.1108/978-1-80382-911-120221002

Arif Y, Singh P, Siddiqui H, Bajguz A, Hayat S (2020) Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiol Biochem 156:64–77. https://doi.org/10.1016/j.plaphy.2020.08.042

Bagues M, Neji M, Karbout N, Boussora F, Triki T, Guasmi F, Nagaz K (2024) Mitigating salinity stress in barley (*Hordeum vulgare* L.) through biochar and NPK fertilizers: impacts on physio-biochemical behavior and grain yield.

Agronomy 14:317. https://doi.org/10.3390/agronomy14020317

Baldi, M. (2017). Climate change impacts on precipitation and temperature. In Saeid Eslamian, Faezeh A. Eslamian (Eds.), handbook of drought and water scarcity: environmental impacts and analysis of drought and water scarcity. Taylor & Francis, 1st edn. pp 123–145. https://doi.org/10.1201/9781315226781-7

Barrs H, Weatherleyt P (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci 15:413–428. https://doi.org/10.1071/BI9620413

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060/METRICS

Cakmak I, Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol 98:1222. https://doi.org/10.1104/PP.98.4.1222

Cao Y, Li K, Li Y, Zhao X, Wang L (2020) MYB transcription factors as regulators of secondary metabolism in plants. Biology (Basel) 9:1–16. https://doi.org/10.3390/biology9030061

Chang S, Puryear J, JCairney J, (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Report 11:113–116

Chen S, Lin X, Zhang D, Li Q, Zhao X, Chen S (2019) Genome-wide analysis of NAC gene family in Betula pendula. Forests 10:741. https://doi.org/10.3390/f10090741

Chhabra R (1996) Soil Salinity and Water Quality (1st ed.). Routledge, London. https://doi.org/10.1201/9780203739242

Chi W, Nan Q, Liu Y, Dong D, Qin Y, Li S, Wu W (2024) Stress resistance enhancing with biochar application and promotion on crop growth. Biochar 6:43. https://doi.org/10.1007/s42773-024-00336-z

Corwin DL (2021) Climate change impacts on soil salinity in agricultural areas. Eur J Soil Sci 72:842–862. https://doi.org/10.1111/ejss.13010

Del Longo OT, González CA, Pastori GM, Trippi VS (1993) Antioxidant defences under hyperoxygenic and hyperosmotic conditions in leaves of two lines of maize with differential sensitivity to drought. Plant Cell Physiol 34:1023–1028. https://doi.org/10.1093/oxfordjournals.pcp.a078515

Demidchik V, Straltsova D, Medvedev SS, Pozhvanov GA, Sokolik A, Yurin V (2014) Stress-induced electrolyte leakage: The role of K^+-permeable channels and involvement in programmed cell death and metabolic adjustment. J Exp Bot 65:1259–1270. https://doi.org/10.1093/jxb/eru004

Dhindsa RS, Plumb-dhindsa P, Thorpe TA (1981) Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 32:93–101. https://doi.org/10.1093/jxb/32.1.93

Do JM, Kim HJ, Shin SY, Park SI, Kim JJ, Yoon HS (2023) OsHSP 17.9, a small heat shock protein, confers improved productivity and tolerance to high temperature and salinity

in a natural paddy field in transgenic rice plants. Agric 13:931. https://doi.org/10.3390/agriculture13050931

Dzinyela R, Alhassan AR, Suglo P, Movahedi A (2023) Advanced study of functional proteins involved in salt stress regulatory pathways in plants. South African J Bot 159:425–438. https://doi.org/10.1016/j.sajb.2023.06.029

El JM, Akkal-Corfini N, Robin P, Oukarroum A, Sabri A, Thomas Z, Chikhaoui M, Bouaziz A (2022) Compost from date palm residues increases soil nutrient availability and growth of silage corn (*Zea mays* L.) in an arid agroecosystem. J Soil Sci Plant Nutr 22:3727–3739. https://doi.org/10.1007/s42729-022-00922-9

Eswar D, Karuppusamy R, Chellamuthu S (2021) Drivers of soil salinity and their correlation with climate change. Curr Opin Environ Sustain 50:310–318. https://doi.org/10.1016/j.cosust.2020.10.015

Ghouili E, Sassi K, Jebara M, Hidri Y, Ouertani RN, Muhovski Y, Jebara SH, El Ayed M, Abdelkarim S, Chaieb O, Jallouli S, Kalleli F, M'hamdi M, Souissi F, Abid G, (2021) Physiological responses and expression of sugar associated genes in faba bean (*Vicia faba* L.) exposed to osmotic stress. Physiol Mol Biol Plants 27:135–150. https://doi.org/10.1007/s12298-021-00935-1

Ghouili E, Abid G, Jebara M, Ouertani RN, Oliveira AC De, Ayed M El, Muhovski Y (2022a) Proteomic analysis of barley (*Hordeum vulgare* L.) leaves in response to date palm waste compost application. Plants 11:3287. https://doi.org/10.3390/plants11233287

Ghouili E, Hidri Y, Cheikh H, Hamed M, Somenahally A, Xue Q, El I, Znaïdi A, Jebara M, Ne R, Muhovski Y, Riahi J, Abid G, Sassi K (2022b) Date palm waste compost promotes plant growth and nutrient transporter genes expression in barley (*Hordeum vulgare* L.). South African J Bot 149:247–257. https://doi.org/10.1016/j.sajb.2022.06.018

Ghouili E, Abid G, Hogue R, eanne T, D'Astous-Pagé J, Sassi K, Hidri Y, M'Hamed HC, Somenahally A, Xue Q, Jebara M, Nefissi Ouertani R, Riahi J, de Oliveira AC, Muhovski Y (2023b) Date palm waste compost application increases soil microbial community diversity in a cropping barley (*Hordeum vulgare* L.) field. Biology 12:546. https://doi.org/10.3390/biology12040546

Ghouili E, Sassi K, Hidri Y et al (2023b) Effects of date palm waste compost application on root proteome changes of barley (*Hordeum vulgare* L.). Plants 12:1–25. https://doi.org/10.3390/plants12030526

Ghouili E, Abid G, Yahmed J Ben, Jebara M, Muhovski Y, Sassi K (2024a) Date palm waste compost improves the production of organic barley in an oasis environment. In: David W, Hossain ST, Chang J, Feng L (eds) Global organic agricultural innovations. Universitas Bakrie Press, pp 55–59.

Ghouili E, Hidri Y, Cheikh M'hamed H, Somenahally AC, Xue Q, Znaïdi IEA, Jebara M, Nefissi Ouertani R, Muhovski Y, Riahi J, Abid G, Sassi K, (2024b) Assessment of phytotoxicity and efficiency of date palm waste compost on barley seeds germination and seedlings growth. Commun Soil Sci Plant Anal 00:1–14. https://doi.org/10.1080/00103624.2024.2345147

Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular

characterization. Int J Genomics 2014:701596. https://doi.org/10.1155/2014/701596

Hanana M, Hamrouni L, Cagnac O, Blumwald E (2011) Mechanisms and cellular strategies of salinity tolerance (NaCl) in plants. Environ Rev 19:121–140. https://doi.org/10.1139/a11-003

Hauser F, Horie T (2010) A conserved primary salt tolerance mechanism mediated by HKT transporters: A mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress. Plant, Cell Environ 33:552–565. https://doi.org/10.1111/j.1365-3040.2009.02056.x

Hazman M, Fawzy S, Hamdy A, Khaled A, Mahmoud A, Khalid E, Ibrahim HM, Gamal M, Abo Elyazeed N, Saber N, Ehab M, Kabil F (2023) Enhancing rice resilience to drought by applying biochar–compost mixture in low-fertile sandy soil. Beni-Suef Univ J Basic Appl Sci 12:74. https://doi.org/10.1186/s43088-023-00411-7

He C, Teixeira da Silva JA, Wang H, Si C, Zhang M, Zhang X, Li M, Tan J, Duan J (2019) Mining MYB transcription factors from the genomes of orchids (*Phalaenopsis* and *Dendrobium*) and characterization of an orchid R2R3-MYB gene involved in water-soluble polysaccharide biosynthesis. Sci Rep 9:1–19. https://doi.org/10.1038/s41598-019-49812-8

Helaoui S, Boughattas I, Mkhinini M, Ghazouani H, Jabnouni H, El K-B, Marai B, Slimani D, Arfaoui Z, Banni M (2023) Biochar application mitigates salt stress on maize plant: Study of the agronomic parameters, photosynthetic activities and biochemical attributes. Plant Stress 9:100182. https://doi.org/10.1016/j.stress.2023.100182

Hniličková H, Hnilička F, Orsák M, Hejnák V (2019) Effect of salt stress on growth, electrolyte leakage, Na^+ and k^+ content in selected plant species. Plant, Soil Environ 65:90–96. https://doi.org/10.17221/620/2018-PSE

Horie T, Hauser F, Schroeder JI (2009) HKT transporter-mediated salinity resistance mechanisms in Arabidopsis and monocot crop plants. Trends Plant Sci 14:660–668. https://doi.org/10.1016/j.tplants.2009.08.009

Horie T, Kaneko T, Sugimoto G, Sasano S, Panda SK, Shibasaka M, Katsuhara M (2011) Mechanisms of water transport mediated by PIP aquaporins and their regulation via phosphorylation events under salinity stress in barley roots. Plant Cell Physiol 52:663–675. https://doi.org/10.1093/pcp/pcr027

Huang J, Zhu C, Kong Y, Cao X, Zhu L, Zhang Y, Ning Y, Tian W, Zhang H, Yu Y, Zhang J (2022) Biochar application alleviated rice salt stress via modifying soil properties and regulating soil bacterial abundance and community structure. Agronomy 12:409. https://doi.org/10.3390/agronomy12020409

Isah T (2019) Stress and defense responses in plant secondary metabolites production. Biol Res 52:39. https://doi.org/10.1186/s40659-019-0246-3

Jin Y, Xu Z, Zhang H, Wang F, Chen S, Xiong Y, Wei S (2018) Research progress on salt stress of and salt-resistance-related genes in tobacco. Acta Tabacaria Sin 24:116–122. https://doi.org/10.16472/j.chinatobacco.2018.084

Karam A, El-Assal SEDS, Hussein BA, Atia MAM (2022) Transcriptome data mining towards characterization of single nucleotide polymorphisms (SNPs) controlling salinity tolerance in bread wheat. Biotechnol Biotechnol Equip 36:389–400. https://doi.org/10.1080/13102818.2022.2081516

Kavvadias V, Organisation-demeter HGA, Le GE, El MM (2024) Using Date Palm Residues to Improve Soil Properties : The Case of Compost and Biochar. Soil Syst 8:69. https://doi.org/10.3390/soilsystems8030069

Kumawat C, Kumar A, Parshad J, Sharma SS, Patra A, Dogra P, Yadav GK, Dadhich SK, Verma R, Kumawat GL (2022) Microbial Diversity and Adaptation under Salt-Affected Soils: A Review. Sustain 14:1–24. https://doi.org/10.3390/su14159280

Le Guyader E, Morvan X, Miconnet V, Marin B, Moussa M, Intrigliolo DS, Delgado-Iniesta MJ, Girods P, Fontana S, Sbih M, Boumaraf B, Tirichine A, Kavvadias V, Gommeaux M (2024) Influence of date palm-based biochar and compost on water retention properties of soils with different sand contents. Forests 15:304. https://doi.org/10.3390/f15020304

Li Y, Li X, Wei J, Cai K, Zhang H, Ge L, Ren Z, Zhao C, Zhao X (2021) Genome-wide identification and analysis of the WRKY gene family and cold stress response in acer truncatum. Genes 12:1–19. https://doi.org/10.3390/genes12121867

Licausi F, Ohme-Takagi M, Perata P (2013) APETALA2/Ethylene Responsive Factor (AP2/ERF) transcription factors: mediators of stress responses and developmental programs. New Phytol 199:639–649. https://doi.org/10.1111/nph.12291

Ma Z, Hu L, Jiang W (2024) Understanding AP2/ERF transcription factor responses and tolerance to various abiotic stresses in plants: A comprehensive review. Int J Mol Sci 25:893. https://doi.org/10.3390/ijms25020893

Mbarki S, Sytar O, Cerda A, Zivcak M, Rastogi A, He X, Zoghlami A, Abdelly C, Brestic M (2018) Strategies to mitigate the salt stress effects on photosynthetic apparatus and productivity of crop plants. In: Kumar, V., Wani, S., Suprasanna, P., Tran, LS. (eds) Salinity Responses and Tolerance in Plants, 1. Springer, Cham, pp 85–136. https://doi.org/10.1007/978-3-319-75671-4_4

Meena MD, Yadav RK, Narjary B, Yadav G, Jat HS, Sheoran P, Meena MK, Antil RS, Meena BL, Singh HV, Singh Meena V, Rai PK, Ghosh A, Moharana PC (2019) Municipal solid waste (MSW): Strategies to improve salt affected soil sustainability: A review. Waste Manag 84:38–53. https://doi.org/10.1016/j.wasman.2018.11.020

Mehdi SM, Sarfraz M, Qureshi MA, Ilyas M, Zaka MA, Qazi MA, Rafa HU (2018) Site-specific phosphorus management with inorganic fertilizer and municipal solid waste compostapplication in salt affected soil. Pakistan J Agric Sci 55:103–110. https://doi.org/10.21162/PAKJAS/18.1660

Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) AP2/ERF family transcription factors in plant abiotic stress responses. Biochim Biophys Acta - Gene Regul Mech 1819:86–96. https://doi.org/10.1016/j.bbagrm.2011.08.004

Mukhopadhyay R, Sarkar B, Jat HS, Sharma PC, Bolan NS (2021) Soil salinity under climate change: Challenges for sustainable agriculture and food security. J Environ

Manage 280:111736. https://doi.org/10.1016/j.jenvman.2020.111736

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Murtaza G, Rizwan M, Usman M, Hyder S, Akram MI, Deeb M, Alkahtani J, Al Munqedhi BM, Hendy AS, Ali MR, Iqbal R, Harsonowati W, Habib ur Rahman M, Rizwan M, (2024) Biochar enhances the growth and physiological characteristics of *Medicago sativa*, *Amaranthus caudatus* and *Zea mays* in saline soils. BMC Plant Biol 24:1–12. https://doi.org/10.1186/s12870-024-04957-1

Nadeem SM, Imran M, Naveed M, Khan MY, Ahmad M, Zahir ZA, Crowley DE (2017) Synergistic use of biochar, compost and plant growth-promoting rhizobacteria for enhancing cucumber growth under water deficit conditions. J Sci Food Agric 97:5139–5145. https://doi.org/10.1002/jsfa.8393

Nakano Y, Asada K (1981) Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. Plant Cell Physiol 22:867–880. https://doi.org/10.1093/OXFORDJOURNALS.PCP.A076232

Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. Plant Physiol 149:88–95. https://doi.org/10.1104/pp.108.129791

Nasrallah AK, Atia MAM, Abd El-Maksoud RM, Kord MA, Fouad AS (2022) Salt Priming as a Smart Approach to Mitigate Salt Stress in Faba Bean (*Vicia faba* L.). Plants 11:1–23. https://doi.org/10.3390/plants11121610

Nasri N, Maatallah S, Kaddour R, Lachâal M (2016) Effect of salinity on *Arabidopsis thaliana* seed germination and acid phosphatase activity. Arch Biol Sci 68:17–23. https://doi.org/10.2298/ABS150620003N

Nawaz MS, Sami SA, Bano M, Khan MRQ, Anwar Z, Ijaz A, Ahmed T (2023) Impact of salt stress on cotton. Int J Agric Biosci 12:98–103. https://doi.org/10.47278/journal.ijab/2023.051

Nefissi Ouertani R, Abid G, Karmous C, Ben Chikha M, Boudaya O, Mahmoudi H, Mejri S, Jansen RK, Ghorbel A (2021) Evaluating the contribution of osmotic and oxidative stress components on barley growth under salt stress. AoB Plants 13:4. https://doi.org/10.1093/aobpla/plab034

Nefissi Ouertani R, Abid G, Ben Chikha M, Boudaya O, Mejri S, Karmous C, Ghorbel A (2022a) Physiological and biochemical analysis of barley (*Hordeum vulgare*) genotypes with contrasting salt tolerance. Acta Physiol Plant 44:51. https://doi.org/10.1007/s11738-022-03388-5

Nefissi Ouertani R, Arasappan D, Ruhlman TA, Ben CM, Abid G, Mejri S, Ghorbel A, Jansen RK (2022b) Effects of salt stress on transcriptional and physiological responses in barley leaves with contrasting salt tolerance. Int J Mol Sci 23:5006. https://doi.org/10.3390/ijms23095006

Omar MM, Shitindi MJ, Massawe BHJ, Fue KG, Meliyo JL, Pedersen O (2023) Salt-affected soils in Tanzanian agricultural lands: Type of soils and extent of the problem. Sustain Environ 9. https://doi.org/10.1080/27658511.2023.2205731

Pandita D (2023) Biochemical, physiological, and molecular mechanisms of plant adaptation to salinity. In Climate-resilient agriculture (Vol. 1). https://doi.org/10.1007/978-3-031-37424-1_40

Parihar P, Singh S, Singh R, Singh VP, Prasad SM (2015) Effect of salinity stress on plants and its tolerance strategies: a review. Environ Sci Pollut Res 22:4056–4075. https://doi.org/10.1007/s11356-014-3739-1

Polle A, Otter T, Seifert F (1994) Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.). Plant Physiol 106:53–60. https://doi.org/10.1104/pp.106.1.53

Quan X, Meng C, Zhang N, Liang X, Li J, Li H, He W (2023) Genome-wide analysis of barley bHLH transcription factors and the functional characterization of HvbHLH56 in low nitrogen tolerance in *Arabidopsis*. Int J Mol Sci 24:9740. https://doi.org/10.3390/ijms24119740

Rahayu, Syamsiyah J, Cahyani VR, Fauziah SK (2019) The effects of biochar and compost on different cultivars of shallots (*Allium ascalonicum* L.) growth and nutrient uptake in sandy soil under saline water. Sains Tanah 16:216–228. https://doi.org/10.20961/STJSSA.V16I2.34209

Rajhi I, Ouertani Nefissi R, Ferchichi N, Khiari B, El-Bassi L, Mhadhbi H (2024) Biochar alleviates single and combined effects of salinity and drought stress in *faba bean* plants. Photosynthetica 62 (2):221–231. https://doi.org/10.32615/ps.2024.019

Rasool A, Shah WH, Mushtaq NU, Saleem S, Hakeem KR, Rehman RU (2022) Salinity-Induced Changes on Different Physiological and Biochemical Features of Plants. In Rehman Hakeem K and Aftab T (ed) Plant Abiotic Stress Physiology 1st edn. Apple Academic Press, New York, pp. 24. https://doi.org/10.1201/9781003180562

Rekaby SA, Awad MYM, Hegab SA, Eissa MA (2020) Effect of some organic amendments on barley plants under saline condition. J Plant Nutr 43:1840–1851. https://doi.org/10.1080/01904167.2020.1750645

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. . In: Krawetz, S., Misener, S. (eds.), Bioinformatics methods and protocols, Methods Mol Biol Humana Press, Totowa, 132:365–386. https://doi.org/10.1385/1-59259-192-2:365

Rusan MJ (2023) Soil and nutrient management under saline conditions. In: Choukr-Allah, R., Ragab, R. (eds) Biosaline Agriculture as a Climate Change Adaptation for Food Security. Springer, Cham, pp 71–85. https://doi.org/10.1007/978-3-031-24279-3_4

Rushton PJ, Somssich IE, Ringler P, Shen QJ (2010) WRKY transcription factors. Trends Plant Sci 15:247–258. https://doi.org/10.1016/j.tplants.2010.02.006

Savy D, Cozzolino V, Vinci G et al (2022) Fertilisation with compost mitigates salt stress in tomato by affecting plant metabolomics and nutritional profiles. Chem Biol Technol Agric 9:1–13. https://doi.org/10.1186/s40538-022-00373-5

Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3:1101–1108. https://doi.org/10.1038/nprot.2008.73

Scotti R, Bonanomi G, Scelza R et al (2015) Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. J Soil Sci Plant Nutr 15:333–352. https://doi.org/10.4067/s0718-95162015005000031

Sharma P, Abrol V, Sharma V et al (2021) Effectiveness of biochar and compost on improving soil hydro-physical properties, crop yield and monetary returns in inceptisol subtropics. Saudi J Biol Sci 28:7539–7549. https://doi.org/10.1016/j.sjbs.2021.09.043

Shaygan M, Baumgartl T (2022) Reclamation of Salt-Affected Land: A Review. Soil Syst 6:61. https://doi.org/10.3390/soilsystems6030061

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15:89–97. https://doi.org/10.1016/j.tplants.2009.11.009

Timperio AM, Egidi MG, Zolla L (2008) Proteomics applied on plant abiotic stresses: Role of heat shock proteins (HSP). J Proteomics 71:391–411. https://doi.org/10.1016/j.jprot.2008.07.005

Ud Din MM, Khan MI, Azam M, Ali MH, Qadri R, Naveed M, Nasir A (2023) Effect of biochar and compost addition on mitigating salinity stress and improving fruit quality of tomato. Agronomy 13:1–13. https://doi.org/10.3390/agronomy13092197

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Sci 151:59–66. https://doi.org/10.1016/S0168-9452(99)00197-1

Wang K, Liu H, Mei Q, Yang J, Ma F, Mao K (2023) Characteristics of bHLH transcription factors and their roles in the abiotic stress responses of horticultural crops. Sci Hortic 310:111710. https://doi.org/10.1016/J.SCIENTA.2022.111710

Wu L, Huo W, Yao D, Li M (2019) Effects of solid matrix priming (SMP) and salt stress on broccoli and cauliflower seed germination and early seedling growth. Sci Hortic 255:161–168. https://doi.org/10.1016/J.SCIENTA.2019.05.007

Xue GP, Way HM, Richardson T, Drenth J, Joyce PA, McIntyre CL (2011) Overexpression of TaNAC69 leads to enhanced transcript levels of stress up-regulated genes and dehydration tolerance in bread wheat. Mol Plant 4:697–712. https://doi.org/10.1093/mp/ssr013

Yao S-T, Lu G-X, Wang J-B, Huang C-X, Wang Z-H, Zhao L-R (2020) Effect of simulated warming on soil conductivity. Arid Zo Res 37:598–606. https://doi.org/10.13866/j.azr.2020.03.07

Yer EN, Baloglu MC, Ayan S (2018) Identification and expression profiling of all Hsp family member genes under salinity stress in different poplar clones. Gene 678:324–336. https://doi.org/10.1016/j.gene.2018.08.049

Zhao S, Zhang Q, Liu M et al (2021) Regulation of plant responses to salt stress. Int J Mol Sci 22:4609. https://doi.org/10.3390/ijms22094609

Zulfiqar F, Moosa A, Nazir MM et al (2022) Biochar: An emerging recipe for designing sustainable horticulture under climate change scenarios. Front Plant Sci 13:1018646. https://doi.org/10.3389/fpls.2022.1018646

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