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Biochar and Selenium Nanoparticles Induce Water Transporter Genes for Sustaining Carbon Assimilation and Grain Production in Salt-Stressed Wheat

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Abstract

In a controlled environment experiment, we studied how physiological changes in leaves during the vegetative phase regulate final grain yield of wheat crops in salt-affected soils. We also hypothesized that amendments such as biochar (SB) and selenium-chitosan nanoparticles (Se-NPs) can protect wheat plants from salt injury. 20-day-old wheat plants were submitted to 4-week salt stress (3000 ppm NaCl). Soybean straw biochar was mixed with soil media at planting and Se-NPs (30 ppm) was sprayed 5 days after the first salt stress treatment. At the end of 4-week Se-NPs treatment, one set of plants was harvested for studying leaf level physiological changes. The salt-stressed plants accumulated significantly high leaf Na^+ (~ 13-fold increase), which trigged oxidative and osmotic damage. This salt-induced cellular injury was evident from significantly high levels of lipid membrane peroxidation and inhibited photosynthesis. Our study suggested that leaf physiological impairment in wheat plants was translated into poor biomass production and grain yield loss at crop maturity. Compared with control, salt-stressed plants produced 43% lesser biomass during vegetative phase, and 62% lesser grain yield at maturity. Amendments such as SB and Se-NPs protected the plants from salt-induced cellular injury by restricting Na^+ transport toward leaf tissues. Plants treated with NaCl + SB + Se-NPs accumulated 50% less Na^+ concentrations in leaves compared with NaCl-treated plants. Our study also suggested that SB and Se-NPs can restore ionic homeostasis and carbon assimilation in salt-stressed wheat by upregulating key transporter genes in leaves.

Keywords Oxidative stress · Osmolytes · Ion homeostasis · Water relations · Grain yield

Abbreviations

SB	Biochar
Se-NPs	Selenium-chitosan nanoparticles
EL	Electrolyte leakage
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde

Introduction

Feeding the ever-growing world population in an unceasingly changing climate is one of the most significant challenges for crop producers worldwide. To meet human food consumption, an estimated 119% increase in the productivity of major food crops is needed by 2050 (Berners-Lee et al 2018). Crop productivity in many parts of the world is already challenged by the depleting natural resources. This issue has further been complicated by extreme environmental factors such as salinity and drought, which are the key growth and yield limiting factors for many food crops (Munns and Tester 2008). For instance, approximately 60 million hectares of global land area are affected by soil salinity, accounting for 20% of the total irrigated area (Machado and Serralheiro 2017). Salt levels in irrigated lands predominantly increase due to inappropriate irrigation practices, leading to sodium chloride (NaCl) build-up (Munns et al. 2019). Plants growing on the salt-affected soils take up excessive Na^+ ions, which impair their normal metabolic functioning. For instance, oxidative stress caused by cellular Na^+ ion build-up alters water and nutrient uptake, carbon assimilation, and biomass production (Gurmani et al. 2013; Soliman et al. 2020a). Some plant

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species have developed sophisticated mechanisms of mitigating salinity stress, i.e., through anatomical modifications and physiological and metabolic adaptations (Munns and Tester 2008). Nevertheless, an effective defense against oxidative stress is being played by the modulation of antioxidant enzymes in plants (Soliman et al. 2020b). In addition, several methods have been proposed to increase salt tolerance in plants; however, lesser attention has been paid to developing environment-friendly techniques (Ghoname et al. 2010).

One such environment-friendly approach for improving crop performance under stressed environments is addition of biochar to the affected soils. Biochar (SB), a solid and rich form of carbon, is produced by heating organic waste from large biomass through pyrolysis. In agricultural systems, SB is often used as a soil refinement for improving fertility, e.g., soil pH, electrical conductivity (EC), and water holding capacity (Sun et al. 2020). Addition of organic carbon and enhanced mineral composition contribute to superior crop performance under stressed environments (Hanna et al. 2021). Positive effects of SB supplementation leaf physiological processes such as photosynthesis and carbon assimilation have also been reported on salt-stressed crops (Iqbal et al. 2021). High adsorption potential of biochar has been linked to mitigating adverse salt effects by minimizing Na^+ uptake, hence reducing electrolyte leakages even under high salt concentrations (Akhtar et al. 2015).

Selenium (Se) is an essential trace element necessary for normal plant functioning (Feng and Wei 2012). Interestingly, Se has been found effective in counteracting the harmful effects of environmental stresses such as heavy metals, salinity, and pathogens on plants (Schiavon et al. 2017). At low concentrations, Se acts as an anti-oxidative agent and promotes plant growth but it may suppress growth when applied in large quantities (Feng and Wei 2012; Cittrarasu et al. 2021). Appropriate Se concentration can help plants maintaining membrane structure and fluidity and protect cellular organs from damage (Diao et al. 2014). Salts such as sodium selenite (Na_2Se) and potassium selenate (KSe) have been used for promoting crop growth and stress tolerance; however, high Se concentrations could accelerate ROS generation and oxidative damage (Hugouvieux et al. 2009). It has been found that elevated levels of Se can reduce leaf area, biomass production by disrupting cellular structure in plants (Molnár et al. 2018). Nanoparticles offer an attractive solution for managing agricultural production systems due to their tiny size and surface characteristics (Pramanik et al. 2020). Slow-release of nutrients from nanoparticles could promote plant growth by optimizing nutrient supplies and supporting antioxidant defense system (Hamouda et al. 2019). More recently, there has been an increased interest in nanoparticles such as selenium nanoparticles (Se-NPs), which can improve plant performance under stressed environments by stimulating their growth recovery (Mosallam et al. 2018; Farooq et al. 2022).

The negative impact of salinity on plant growth is simultaneously characterized by ionic toxicity, osmotic, and oxidative stress (Tanveer and Shabala 2018). Maintaining cellular osmotic potential is a significant challenge for plants to sustain growth under salt-stressed environments. In living organisms, including plants, aquaporins (AQPs) are the most common transmembrane transporters of water and small ionic substrates such as glycerol, urea, CO_2, NH_3, metalloids, and reactive oxygen species (ROS) (Groszmann et al. 2017). Based on amino acid sequences, four different members of plant AQPs family have been defined (Johanson and Gustavsson 2002). These include plasma membrane intrinsic proteins (PIPs); tonoplast intrinsic proteins (TIPs) (Johansson et al. 2000); nodulin 26-like intrinsic proteins (NIPs) (Roberts and Routray 2017); and small essential intrinsic protein (SIPs) (Ahmed et al. 2020). Twenty-four PIP and 11 TIP genes were recently identified in wheat (Forrest and Bhave 2008; Maurel et al. 2015); however, no NIP genes have been reported in wheat to date.

Wheat (*Triticum aestivum* L.) is an important food crop cultivated in arid and semiarid regions. Despite undeniable status in the human food supply chain, wheat production has been facing a stiff hindrance due to environmental stresses. For instance, soil salinity alone can cause up to 60% loss in wheat grain yield in many parts of the world (Wani et al. 2018). Thus, improving wheat performance under saline environments is crucial for sustaining wheat grain supplies. In this study, we explored the impact of long-term salinity on growth and biomass production in wheat, intending to unravel the biochemical and genetic pathways affected by salt stress. We also investigated the potential of using amendments such as soil SB and Se-NPs to regulate salt tolerance in wheat plants. Relationship between post-stress leaf physiology and grain yield at maturity is also explored to understand how these amendments protect wheat plants from salt-induced cellular injury.

Materials and Methods

Experimental Setup

One week before the start of experiment, plastic pots (40 × 40 cm) were filled with 2 kg sterilized sandy, loamy soil. For biochar amendment (SB), half of these pots contained 5% (W/V) soybean straw. Healthy and uniform grains of a salt-tolerant wheat (*Triticum aestivum* L.) cultivar Sakha 93 (Mahmoud et al. 2019) were obtained from Agriculture Research Centre, Giza, Egypt. The grains were surface sterilized with 0.01% $HgCl_2$ solution and then thoroughly washed with distilled water. Four grains per pot were

established, and the pots were organized to achieve a target density of 100 plants m^{-2}. All pots were kept in a greenhouse under natural day/night conditions [65 ± 2% relative humidity, 23 °C/17 °C (± 3 °C) average day/night temperature and 680 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR). The pots were watered using 100 mL Hoagland nutrient solution on alternate days. Eight treatments with three independent replicates were arranged in a complete randomized block design.

Characterization of Soil and Biochar

The soil and soybean straw SB used in this experiment were oven-dried at 50 °C for 24 h, then homogenized in a grinder and sieved through 2-mm openings and mixed. The soil characteristics such as soil pH and texture were presented in Table 1. Electrical conductivity (EC) and pH were measured in deionized water at 1:5 soil/water suspensions. The basic properties of biochar used in this study are shown in Table 2. Soil organic matter was measured according to (Sikora and Moore 2014). Soil organic carbons and SB samples were measured as described by Nelson protocol (Nelson and Sommers 1996). Total nitrogen in soil and SB were measured according to the sulfuric-salicylic acid mixture method by (Brush et al. 1982). The extractable potassium was measured in soil and SB by the flame atomic absorption spectrophotometer (Thermo Fisher). Total P in soil and SB were performed as described in Olsen and Sommers (1982) method (Table 3).

The Selenium Nanoparticles and SS Biochar Treatments

The selenium nanoparticles (Se-NPs) were obtained from (Sigma-Aldrich, Germany) in physical dispersion form with average particle size ranging from 80 to 100 nm and concentration 0.15 wt% of selenium in water. Soybean straw biochar (SB) was obtained from the experimental farm at the Faculty of Agriculture, Alexandria University. Briefly, the biochar was prepared from soybean straw material. The plant material was thoroughly dried under the sun and converted into biochar under pyrolysis temperature of 400 °C in an oven for 2 h (Joseph 2015). Then, the biochar was ground, sieved, and stored for further use.

Table 1 Physio-chemical properties of soil

Character	Sandy loam
pH	6.43
EC (dS m^{-1})	0.6
Total P (mg g^{-1})	304
Total N (mg g^{-1})	0.63
Total K (mg g^{-1})	168
Organic matter (mg g^{-1})	29.7
Organic carbon (mg g^{-1})	17.3
Water holding capacity (%)	47.8
Soil texture	Sand (%)
	Silt (%)
	Clay (%)

Table 2 Basic properties of soybean straw biochar

Components	Soybean straw biochar	
	Raw material	Biochar
pH	6.45	8.63
Total N (mg g^{-1})	17.9	25.1
Total P (mg g^{-1})	3.62	5.8
Total K (mg g^{-1})	5.26	6.24
Organic matter (mg g^{-1})	187	236
Organic carbon	108	135

Table 3 Primers used in quantitative real-time PCR analysis

Transcript	Primers sequence
NHX1	
F	5′-CTCAAGGGTGACTACCAAGCA-3′
R	5′- CCAATGCATCCATCCCGAC-3′
SOS1	
F	5′-GTTGTCGGTGAGGTCGGAGGG-3′
R	5´-CATCTTCTCCTACCGCCCTGC-3´
CAX1	
F	5′-GCAACAGGAGGAGGAGTTTT-3′
R	5′-AACCCACCCACAAGAAGAAT-3′
HKT1	
F	5′-CTGTCGCTCTTCTGCGCCAT-3′
R	5′-TTATACTATCCTCCATGCCT-3′
ATPase	
F	5′-GCCAACCTTGTATGCGGGTTA-3′
R	5′-GTTGGCCATGTTGCTTGTGC-3′
TIP1;1	
F	5′-CGCTTGCTTTTGGTGTTGGA-3´
R	5′-TGGAGAAGCGGAGGAGGAAG-3′
PIP1	
F	5′-CTACATGATTGCGCAGTGCC-3′
R	5′-GCCGAACTGAACTGTCGAGA-3′
NIP	
F	5′-GCATTACGTCCATCTTCGCA-3′
R	5′-CCTCGAAGCGGATGTAGGTG-3′
β-Actin	
F	5′-GTGCCCATTTACGAAGGATA-3′
R	5′-GAAGACTCCATGCCGATCAT-3′

Salinity Stress and Se-NPs Treatment

Twenty days after germination, one set of plants was submitted to salinity treatment by irrigating the pots with 3000 ppm saline water (NaCl) for 4 weeks. Five days after the start of salinity treatment, the plants (25-day-old) were sprayed with 30 ppm Se-NPs once a week. Se-NPs were dissolved in 2 mL of ethanol and then desired concentration (30 ppm) was prepared by adding double-distilled water. Twenty mL of Se-NPs solution per pot was sprayed on leaves each time, at the same time control plants were also sprayed with water (20 mL pot^{-1}). Experimental treatments included: (a) control, (b) salinity stressed, (c) Se-NPs, (d) soil amended with biochar (SB), (e) salinity + Se-NPs, (f) salinity + SB, (g) SB + Se-NPs and (h) salinity + SB + Se-NPs. After the fourth Se-NPs spray, one set of 55-day-old plants was harvested for further analysis, while the remaining plants were allowed to growth under optimum conditions till maturity.

Plant Growth Parameters and Yield Parameters

Plant height was measured with a scale at harvest, and then the plants were separated into stem and leaves. Green leaf area was measured following Quarrie (Quarrie and Jones 1977).

$$\textit{Leaf area} = \text{Leaf length} \times \text{leaf width} \times 0.75$$

Shoot and root dry weight was measured after oven-drying the samples at 70 °C for 24 h. Mature plants of each treatment were harvested for grain yield components such as number of tillers, spike length, number of spikelet per spike plants.

Measurement of Photosynthesis, PSII Activity, Water Use Efficiency and Leaf Water Potential

Leaf chlorophyll contents were determined by Arnon method (Arnon 1949) and the absorbance of reagent solution containing leaf sample was recorded at 663 and 645 nm with UV/VIS spectrophotometer (Genway, Japan). Chlorophyll fluorescence (*Fv/Fm*) and photosynthetic rate (*Pn*) were measured using a portable gas-exchange system equipped with fluorimeter and infrared gas analyzer (IRGA) system (TPS-2, USA). The measurements were taken under 22 °C temperature and 50–70% relative humidity. Mature leaves having total light exposure (maximum leaf area) were selected to measure leaf water potential using a psychrometer. Each value was an average of at least10 independent measurements. Water use efficiency (WUE) was calculated from the ratio of *Pn* and *Tr* following Zhang (2016) protocol. Measurements were made on fully expanded leaves with a fluorometer after plant adaptation in the dark for 30 min.

Estimation of Stress-Induced Biomarkers

Malondialdehyde Contents

Leaf MDA contents were measured using the thiobarbituric acid (TBA) method according to Heath and Packer (1968) and Erofeeva (2014) with slight modifications. For calculation, a molar extension coefficient of 155 mmol L^{-1} cm^{-1} was used. Absorbance of the mixture was taken at 532 nm and MDA contents were expressed as nmol g^{-1} FW.

Hydrogen Peroxide Contents

Reactive oxygen species such as hydrogen peroxide level was determined from the stressed and non-stressed samples according to Velikova et al (2000). In brief, leaf samples were homogenized in 2 mL 0.1% trichloroacetic acid (TCA) solution. After centrifugation of homogenates at 12,000×*g* for 15 min, 0.5 mL of the supernatant was added into the reaction mixture containing 0.5 mL of 10 mM K phosphate buffer (pH 7.0) and 1 mL of 1 M KI. The absorbance of the mixture was determined at 390 nm.

Electrolytes Leakage

Electrolytes leakage (EL) was measured in 0.2 g leaf segments. Samples were immersed in a test tube containing 4 mL of demineralized water for 30 min, rinsed 3 times to eliminate surface electrolytes (Blum and Ebercon 1981), and then placed on a dry filter paper under dark (Fan and Blake 1994). After 2 h incubation, the segments were placed into another set of tubes containing 15 mL of demineralized water. The conductivity of the solution was determined using a conductivity meter.

Determination of Osmolytes

Glycine betaine contents were measured by homogenizing (0.5 g) leaf samples with 10 mL milli-Q water and incubated for 24 h at 25 °C (Grieve and Grattan 1983). The homogenate was filtered and mixed with 2 N sulfuric acid in a 1:1 (v:v) ratio. 0.5 mL of the mixture was incubated on ice for 1 h and then 0.2 mL cold KI reagent was added into the solution 4 °C. The next day, mixture was centrifuged at 14,000×*g* for 15 min. C. Absorbance of the supernatant was read at 365 nm. A standard curve was prepared and glycine betaine contents were expressed as µmol g^{-1} FW.

Total water soluble carbohydrates (WSC) were measured by Anthrone method (Yemm and Willis 1954). The

absorption of samples was taken at 625 nm using a PD-303 model spectrophotometer. WSC contents were determined from a standard glucose curve and expressed as mg g^{-1} DW.

Proline concentration from fresh leaf samples was measured as described by Bates et al (1973). Phenolic contents (mg 100 g^{-1} DW) were determined with Folin–Ciocalteu reagent (Cumplido-Nájera et al. 2019). Total flavonoids in the extracts were determined by a colorimetric method described by Chang et al (2002).

Total Antioxidant and Oxidant Capacity

The percent ratio of total antioxidant capacity (TAC) and total oxidant capacity (TOC) was assessed following Erel (2004) method.

Antioxidant Enzymes Assay

Fresh (0.1 g) wheat leaf samples were used for enzyme assays from all treatments. Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972). Glutathione reductase (GR) activity was determined by Carlberg and Mannervik (1975) method. Monodehydroascorbate reductase (MDAR) activity was determined by Hossain et al (1984) protocol. Catalase (CAT) activity was determined by recording consumption of H_2O_2 for 30–90 s of the reaction, according to Aebi (1984). Ascorbate peroxidase (APX) activity was measured by Asada (1992) method.

Estimation of Element Contents

Dried leaf samples were used for measuring elemental concentrations in wheat tissues. P were measured using vanadomolybdate indication method, and total N was measured by McGill and Figueiredo (1993). Na and K ion concentrations were estimated by a flame photometer (Fisher scientific, USA). Estimation of Ca and Mg were performed according to Padhye (1957).

Ion transporter Genes and AQPs Expression Analysis

Gene expression analysis was performed following the MIQE guidelines (Bustin et al, 2009). Total mRNA was isolated from leaf samples (0.25 g) of all the treatments including control plants, using a Spectrum™ Plant Total RNA Kit (Sigma-Aldrich, USA). The extracted RNA was treated with DNase I (Sigma-Aldrich, USA) for the removal of any gDNA contamination and purified RNA concentration was determined using a SPECTROstar Nano (BMG LABTECH, Ortenberg, Germany) spectrophotometer at 260/280 nm with 1% agarose gel**.** From total RNA samples, cDNA was constructed using a GoScript™ Reverse Transcriptase assay kit (Promega, Germany). A reaction mixture (20 mL) containing 10.8 µL RNase free water, 2.5 µL 10 × buffer with MgCl2, (10 mM), 3 µL RNA (30 ng), 2.5 µL deoxynucleotide triphosphates (dNTPs), 1 µL oligo (dT) primer (10 pmol μL^{-1}), and 0.2 µL reverse transcriptase enzyme was used (Promega, Walldorf, Germany). PCR was performed using a SureCycler 8800 (Agilent, Santa Clara, CA, USA) programmed at 42 °C for 2 h, and then at 70 °C for 5 min, and the product was stored at − 80 °C. qPCR reactions were performed to quantify mRNA level using a Rotor-Gene-6000-system (Qiagen, Valencia, CA, United States), real-time PCR through Sybr Green GoTaq® qPCR Master Mix (2X) (Promega, Germany). A reaction mixture was prepared, comprising 2 µL of template, 10 µL of GoTaq® qPCR Master Mix (2X) (Promega, Germany), 2 µL of reverse primer, 2 µL of forward primer, and DD water was added for a total volume of 20 µL. PCR assays were performed under the following conditions: 95 °C for 2 min, 95 °C for 15 s and 60 °C for 60 s followed by 40 cycles. The wheat β-actin gene (housekeeping gene) was used as an internal control for all qRT-PCR expression analysis. The reaction was performed in three biological replicates. The expression level of gene of interest (targeted AQPs gene) was normalized to that of β-Actin (internal control) by subtracting β-Actin gene CT from target gene CT. The relative gene expression was determined using $2^{-\Delta\Delta Ct}$ method (Togawa et al. 2008). For each sample, triplicate biological and technical replications were done. Primer sequences used in this analysis are given in Table 2.

Data Analysis

Data were presented as mean ± CI (95% confidence interval) of three independent replicates. Data were analyzed in R (R Core Team (2018). One-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test to determine significant differences (at $P \leq 0.05$) among treatment means. Association between leaf physiological traits and grain yield components of wheat was calculated using a heat map.

Results

Plant Growth and Biomass Production

Long-term (4 weeks) salinity significantly inhibited growth and biomass production of wheat plants without any immediate recovery. For instance, salt-stressed plants (harvested 11 days after the end of salt treatment) experienced 56%, 53%, 45%, and 40% reduction in leaf area, plant height, root, and shoot dry biomass, respectively, compared with control (Fig. 1). In the absence of salt stress, Se-NPs and SB treatments had no significant effect on plant growth except

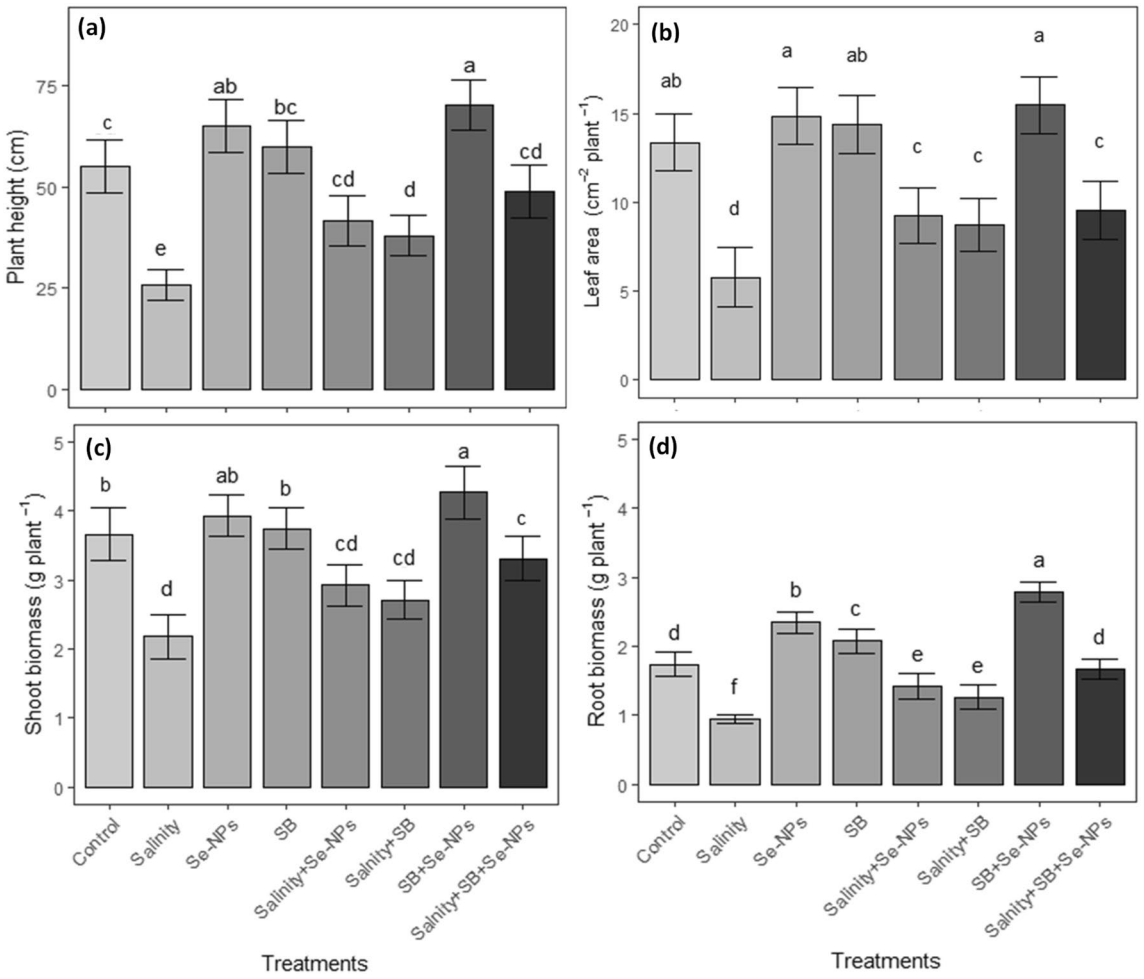

Fig. 1 Changes in **a** height, **b** leaf area, **c** shoot biomass and **d** root biomass in wheat plants treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected 55 days after sowing and were presented as mean ± CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

root biomass, which was significantly increased. In contrast, SB and/or Se-NPs significantly improved the growth of salt-stressed plants. On average, Se-NPs more effectively promoted growth recovery of salt-stressed wheat plants than SB in this study, with maximum a biomass production was achieved under their combined application. For example, salt-stressed plants treated with SB, Se-NPs, and biochar + Se-NPs produced 25%, 33%, and 44% more root dry biomass, respectively, compared with salt treatments alone (Fig. 1).

Grain Yield Components

Excessive salt concentration in the growth media during vegetative phase significantly inhibited grain yield formation at maturity, causing 61%, 47%, 25% reduction in total grain yield per pot, the number of fertile tillers, and the number of spikelet per spike, respectively (Fig. 2). However, spike length remained significantly unaffected (Fig. 2b). A relatively more significant reduction in grain yield than the number of fertile tillers and spikelet suggested that salt stress negatively affected both grain number and weight in wheat. Individual or combined application of amendments such as Se-NPs and SB significantly improved grain yield components, i.e., number of tillers and spikes both under salt-stressed or control conditions. Individual application of Se-NPs and SB had no significant effect on wheat grain yield but BC + Se-NPs-treated plants produced 32% and 41% higher grain yield than their respective untreated control (non-stressed) and salt-stressed plants, respectively (Fig. 2b).

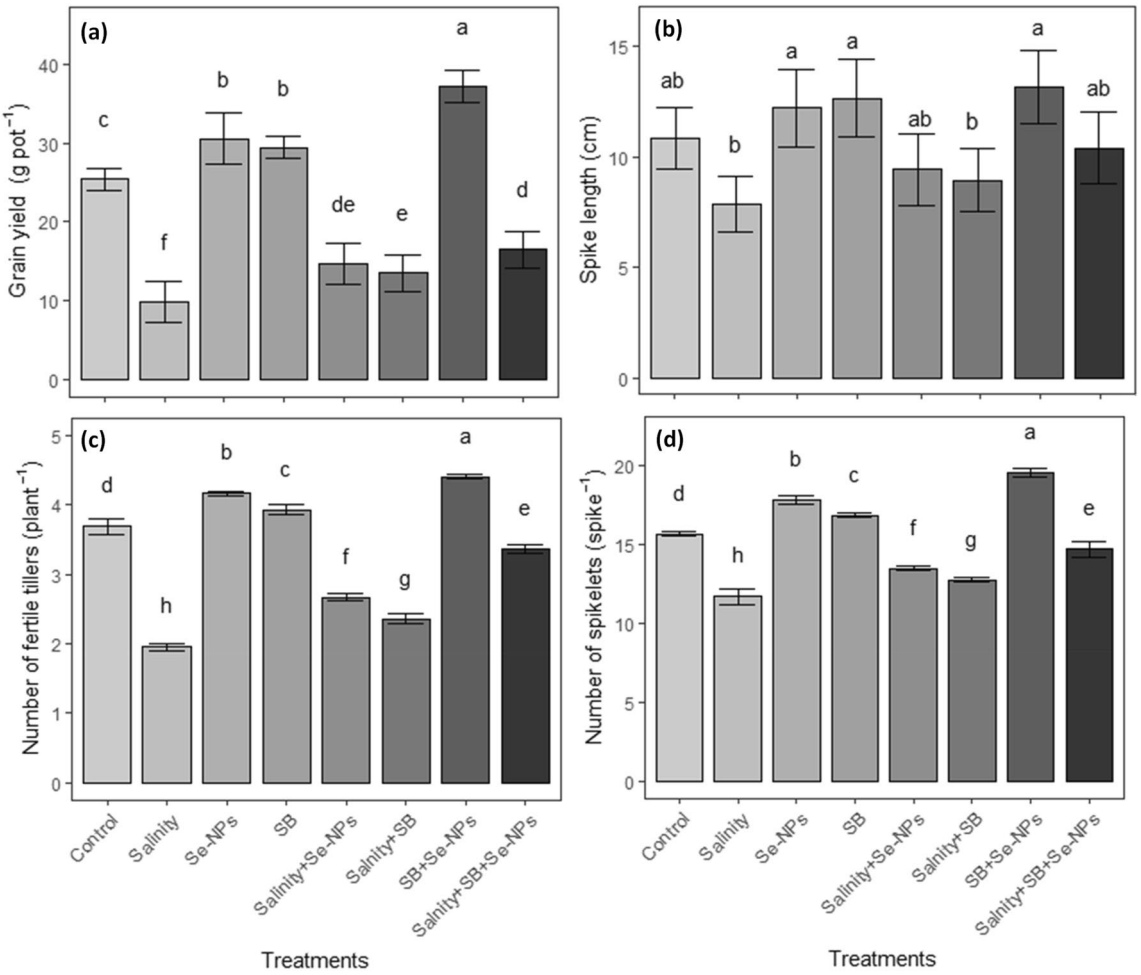

Fig. 2 Changes in **a** grain yield, **b** spike length, **c** number of fertile tillers and **d** number of spikelets per spike in wheat plants treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected at crop maturity and were presented as mean ± CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

Leaf Water Relations

Leaf greenness and other physiological traits of wheat plants were significantly affected by the 4 weeks of growth under salt-affected environments. For instance, compared with control, the salt-stressed plants experienced 39%, 48%, 20% and 31% reduction in chlorophyll, P_n, WUE and PSII efficiency, respectively (Fig. 3). In contrast, LWP of was significantly increased under salt stress. Amendments such as Se-NPs, biochar and Se-NPs + biochar variably affected these traits under different treatments with relatively more significant under salt-stressed than unstressed environments. Individual or combined SB and Se-NPs treatments significantly reduced LWP of salt-stressed plants but this reduction in control (non-stressed) plants was significant under Se-NPs + SB treatment only (Fig. 3a). In contrast, Se-NPs and/or SB significantly increased WUE of wheat plants under non-stressed environments only (Fig. 3b). Se-NPs + SB treatments significantly increased leaf P_n (25% and 17%) chlorophyll (38% and 28%) and PSII efficiency (18% and 12%) in salt-stressed and non-stressed plants (Fig. 3c, d, e).

Osmolytes in Leaf Tissues

Salt stress and amendments significantly affected concentrations of various osmolytes in wheat leaves (Fig. 4). For instance, compared with control, the salt-stressed plants accumulated significantly lower proline (44%) and glycine betaine (34%) contents in leaves, but there was a significant increase in WSC, phenolic, and flavonoids contents. On average, salt-stressed plants accumulated 73%, 103%, and 136% more phenolic, flavonoids, and WSC contents in leaf

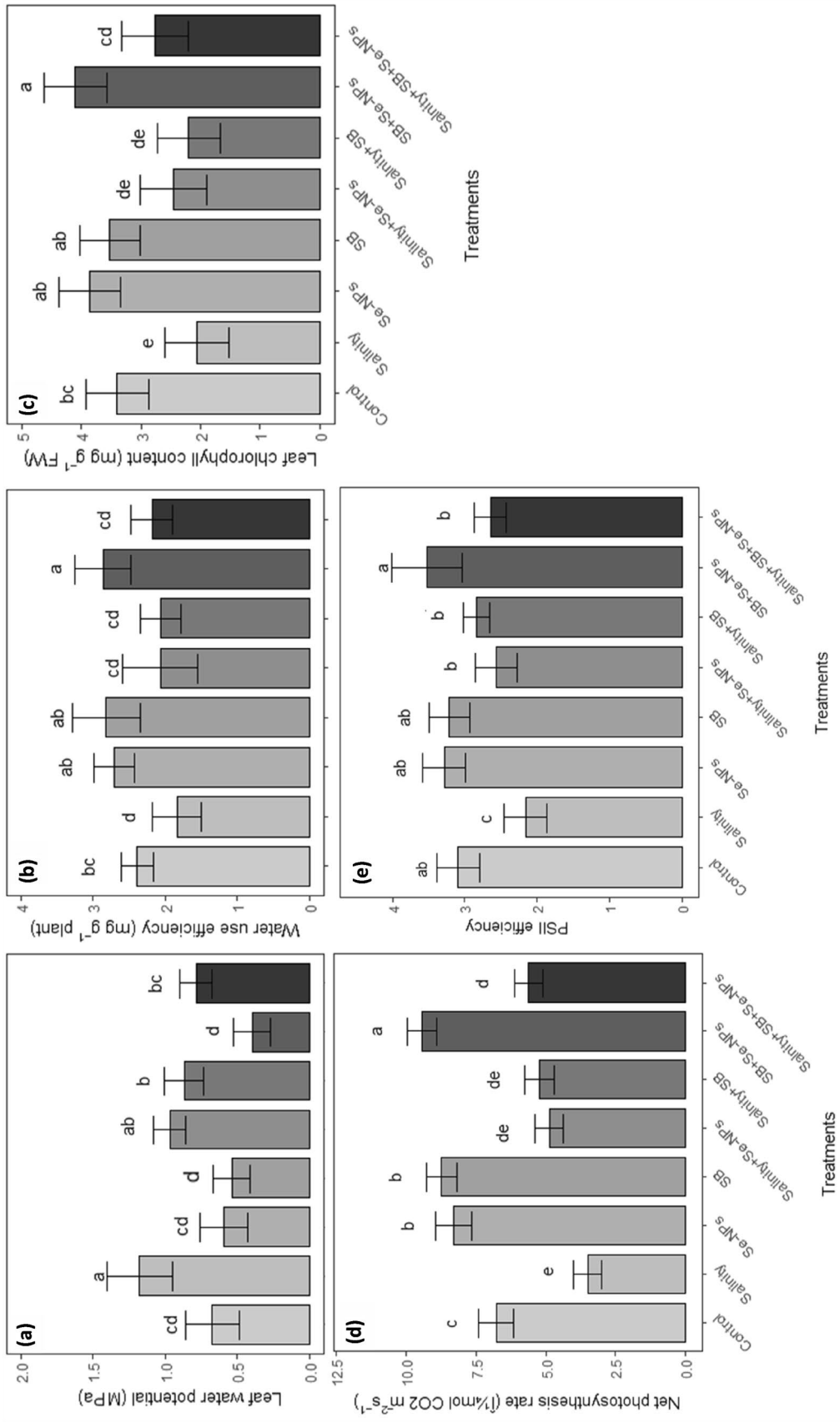

Fig. 3 Changes in **a** leaf water potential, **b** water use efficiency, **c** chlorophyll, **d** net photosynthesis and **e** PSII efficiency in wheat plants treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected 55 days after sowing and were presented as mean ± CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

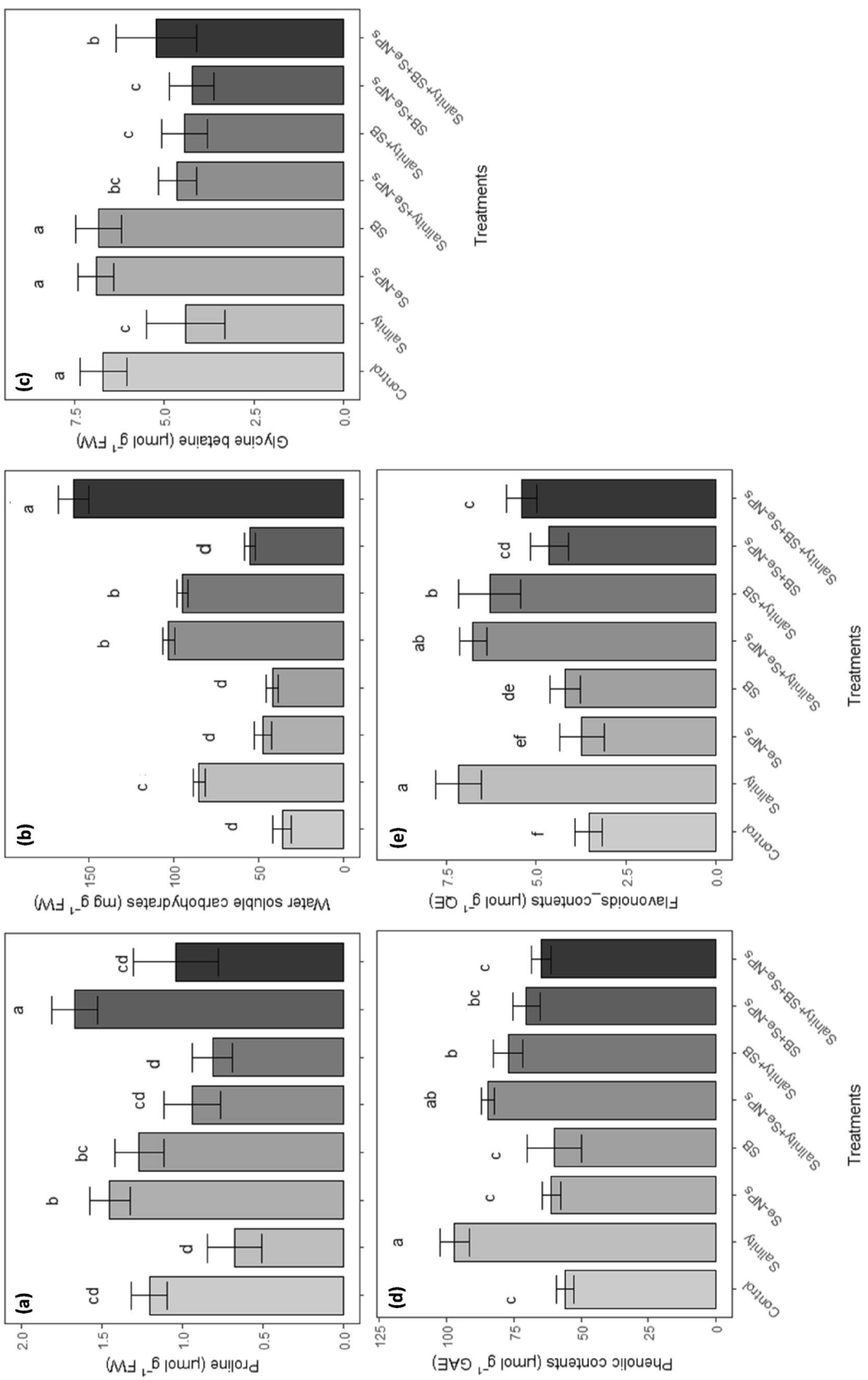

Fig. 4 Changes in **a** proline, **b** water soluble carbohydrates, **c** glycine betaine, **d** phenolics and **e** flavonoids in wheat plants treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected 55 days after sowing and were presented as mean ± CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

tissues, respectively, compared with control (Fig. 4b d, e). Se-NPs + SB, restored osmolytes production in plants with a significant impact under salt-stressed environments. Proline contents in wheat leaves were significantly increased under Se-NPs applied as individually or in combination with SB both under salt-stressed and non-stressed environments (Fig. 4a). A significant increase in WSC contents was recorded in wheat leaves in response to Se-NPs + SB treatment, with treated leaves contained 47% and 224% greater WSC contents that their respective salt-stressed and non-stressed leaves, respectively (Fig. 4b). No significant change in glycine betaine was observed in response to any amendment treatments under non-stressed environments but Se-NPs + SB significantly increased glycine betaine contents in salt-stressed plants (Fig. 4c). Leaf phenolic and flavonoid contents were significantly reduced in response to Se-NPs + SB treatments both under salt-stressed and non-stressed environments (Fig. 4d, e).

Reactive Oxygen Species and Malondialdehyde

Elevated salt levels in the growth media significantly induced oxidative damage to wheat leaves. For instance, the plants exposed to 4-week salinity had 44%, 37%, and 50% higher leaf MDA, H_2O_2 contents, and EL (%), respectively, compared with control (Fig. 5). Amendments such as Se-NPs and SB significantly reduced oxidative stress, particularly in salt-stressed plants. For example, leaf MDA contents of salt-stressed plants were significantly reduced by SB as alone or with Se-NPs, although Se-NPs alone had no significant effect on leaf MDA under any environments (Fig. 5a). Leaf H_2O_2 and EL in the salt-stressed plants were significantly reduced by the individual or combined applications of Se-NPs and SB (Fig. 5b, c).

Antioxidant Enzymes

Antioxidant enzyme activities of wheat leaves responded variably to different treatments used in this study. For instance, salt stress significantly upregulated most of the tested enzymes, i.e., SOD, APX, GR, and TOC but it significantly deceased CAT and TAC activity (Fig. 6). Amendments also variably affected the antioxidant enzymes under different environments. SB and Se-NPs treatments significantly increased SOD activity of salt-stressed and non-stressed plants, with a maximum increased achieved under Se-NPs applications alone (Fig. 6a). Similarly, Se-NPs, was relatively more effective in upregulating CAT and TAC enzymes in leaves than SB under saline or non-stressed environment (Fig. 6b, f). GR and APX responded significantly more strongly to SB + Se-NPs than their individual applications (Fig. 6c, d). TOC activity was significantly reduced by individual and combined SB and Se-NPs treatments both under salt-stressed and non-stressed environments (Fig. 6e).

Nutrient Concentrations in Wheat Leaves

Salt stress significantly inhibited the accumulation of different macro- and micro-nutrients in wheat leaves, except Na, which showed a significantly greater accumulation in salt-stressed plants. On average, salt-stressed plants contained 35%, 61%, 59%, 12%, and 11% lower N, P, K, Ca, and Mg in leaf tissues than control plants (Fig. 7). The combined application of Se-NPs and biochar significantly promoted the uptake of these ions both under salt-stressed and unstressed environments. Further, individual effect of these amendments was also significant on macronutrients uptake particularly under non-stressed environments. Individual or combined application of Se-NPs and biochar significantly reduced Na accumulation in salt-stressed wheat leaves.

Changes in Expression of Key Genes

Soil salinity significantly induced the expression of various ion transporter genes, i.e., plasma membrane H^+-ATPase (*ATPase*) Ca^{++} protein exchanger (*CAX1*), sodium/proton exchanger (*NHX1*), and salt overly sensitive (*SOS1*) in wheat leaves (Fig. 8). In the absence of salt stress, amendments had no significant effect on expression of these genes when applied alone, but there was a significant increase in the expression of ion transporter genes when Se-NPs were applied to SB-treated plants. All the tested ion transporter genes in salt-stressed leaves were further induced by SB + Se-NPs treatments, although the effect was variable when SB or Se-NPs were applied individually. For example, Se-NPs upregulated *ATPase* and *SOS1*, while SB induced expression of *CAX1* in salt-stressed leaves. In contrast, *NHX1* expression in salt-stressed leaves was significantly downregulated by individual application of SB or Se-NPs (Fig. 8c).

Salt stress also significantly induced water transporter proteins, i.e., nodulin intrinsic protein (*NIP*), plasma membrane intrinsic protein (*PIP1*), and tonoplast intrinsic protein (*TIP1*) in leaves. Amendments, i.e., SB and/or Se-NPs significantly induced expression of these genes in control leaves, but their effect on salt-stressed plants was significant when applied in combination (Fig. 9). Induction of ionic and water transporter genes in response to SB + Se-NPs application played a crucial role in ion homeostasis and ROS regulation in salt-stressed wheat plants (Fig. 10).

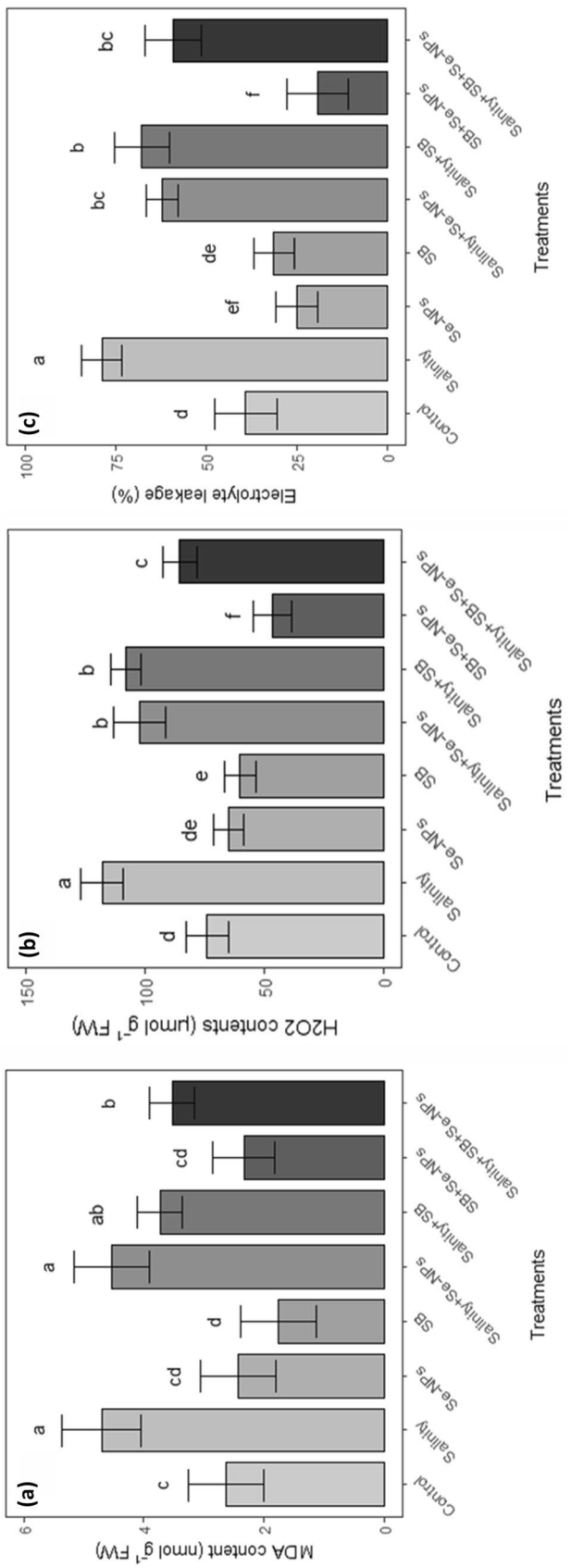

Fig. 5 Changes in **a** lipid peroxidation, **b** hydrogen peroxide and **c** electrolyte leakage in wheat plants treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected 55 days after sowing and were presented as mean ± CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

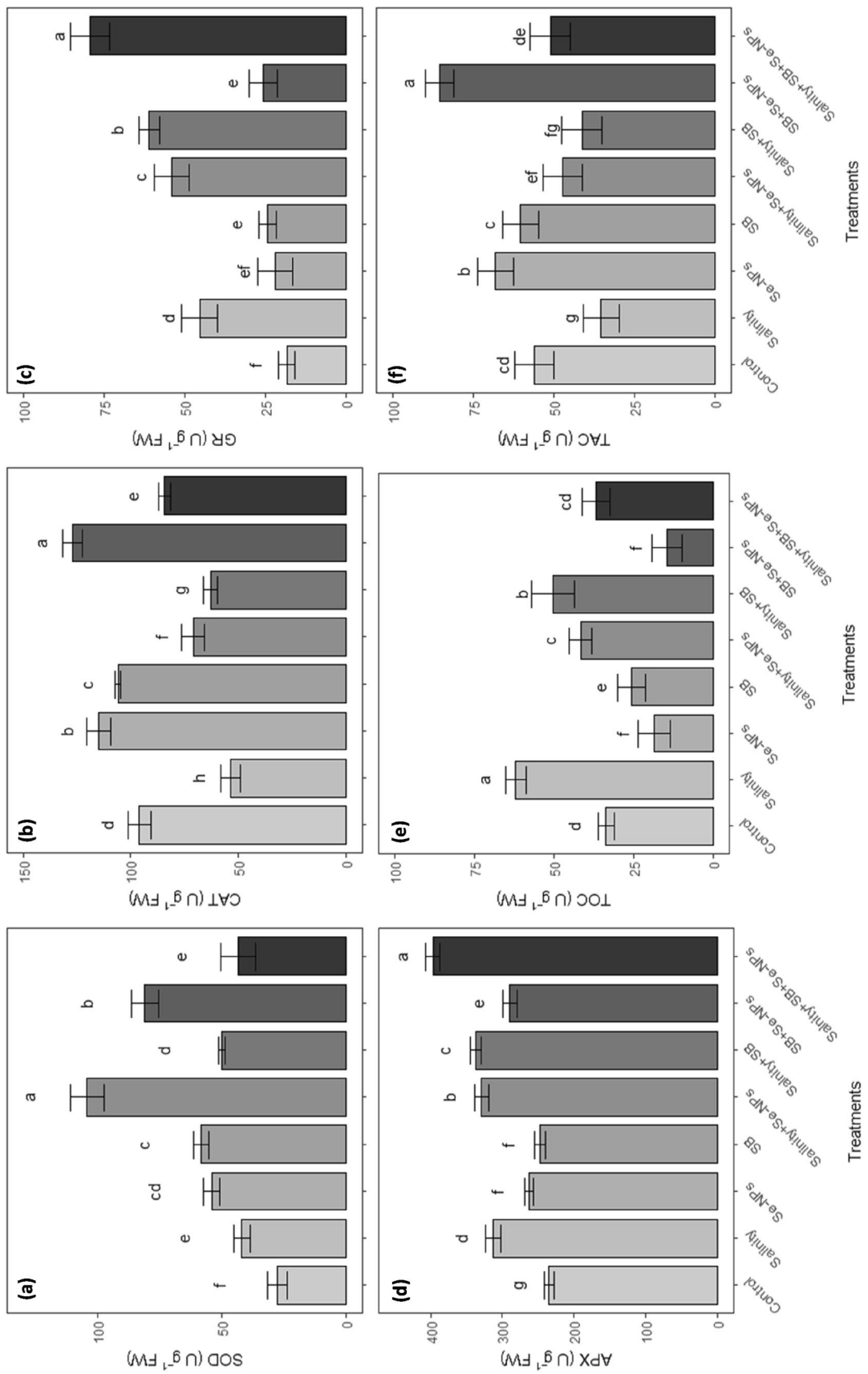

Fig. 6 Changes in activity of **a** SOD, **b** CAT, **c** GR, **d** APX, **e** total oxidant capacity and **f** total antioxidant capacity in wheat plants treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected 55 days after sowing and were presented as mean ± CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

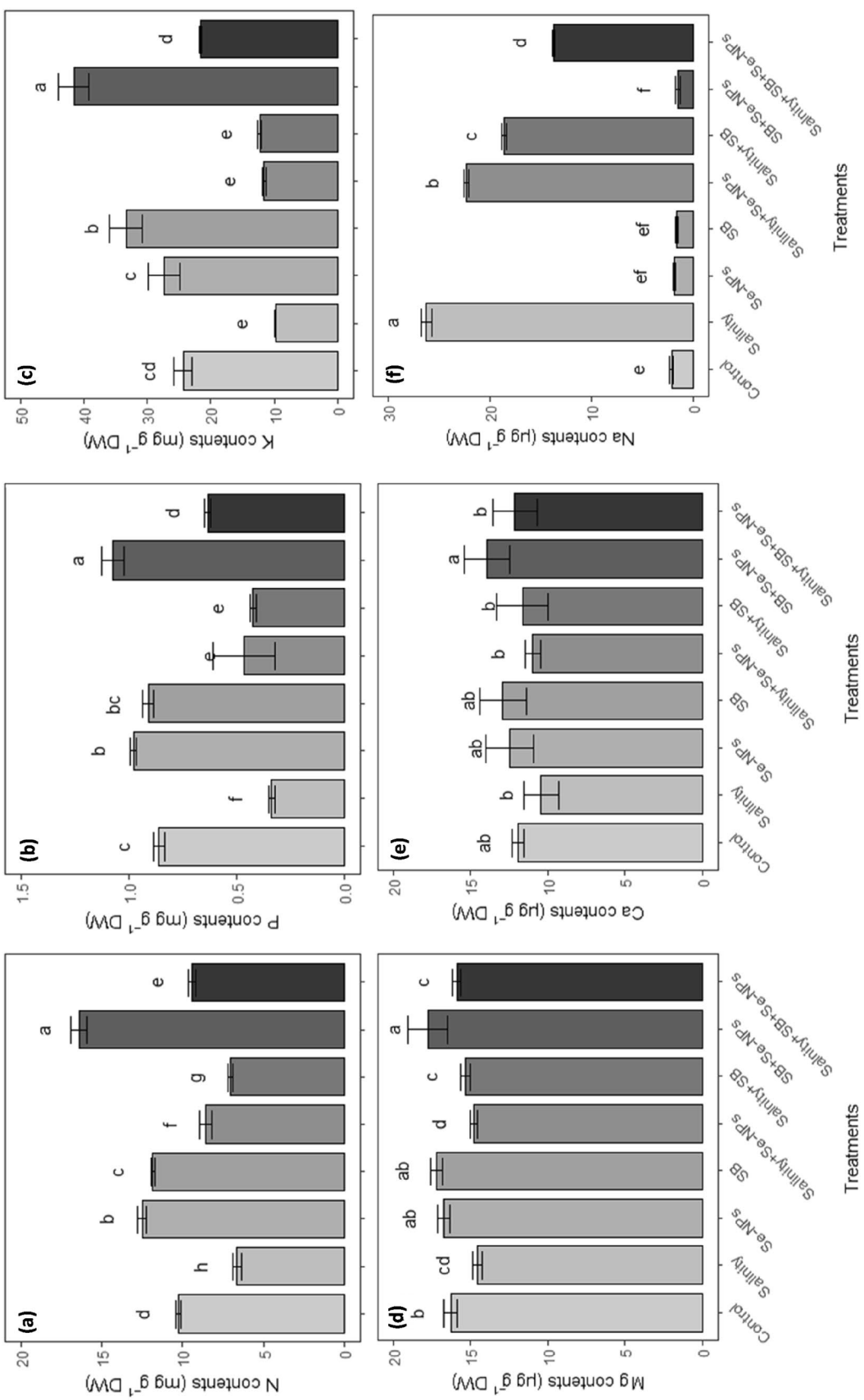

Fig. 7 Changes in content of **a** nitrogen, **b** phosphorous, **c** potassium, **d** magnesium, **e** calcium and **f** sodium in flag leaves of wheat treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected 55 days after sowing and were presented as mean ±CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

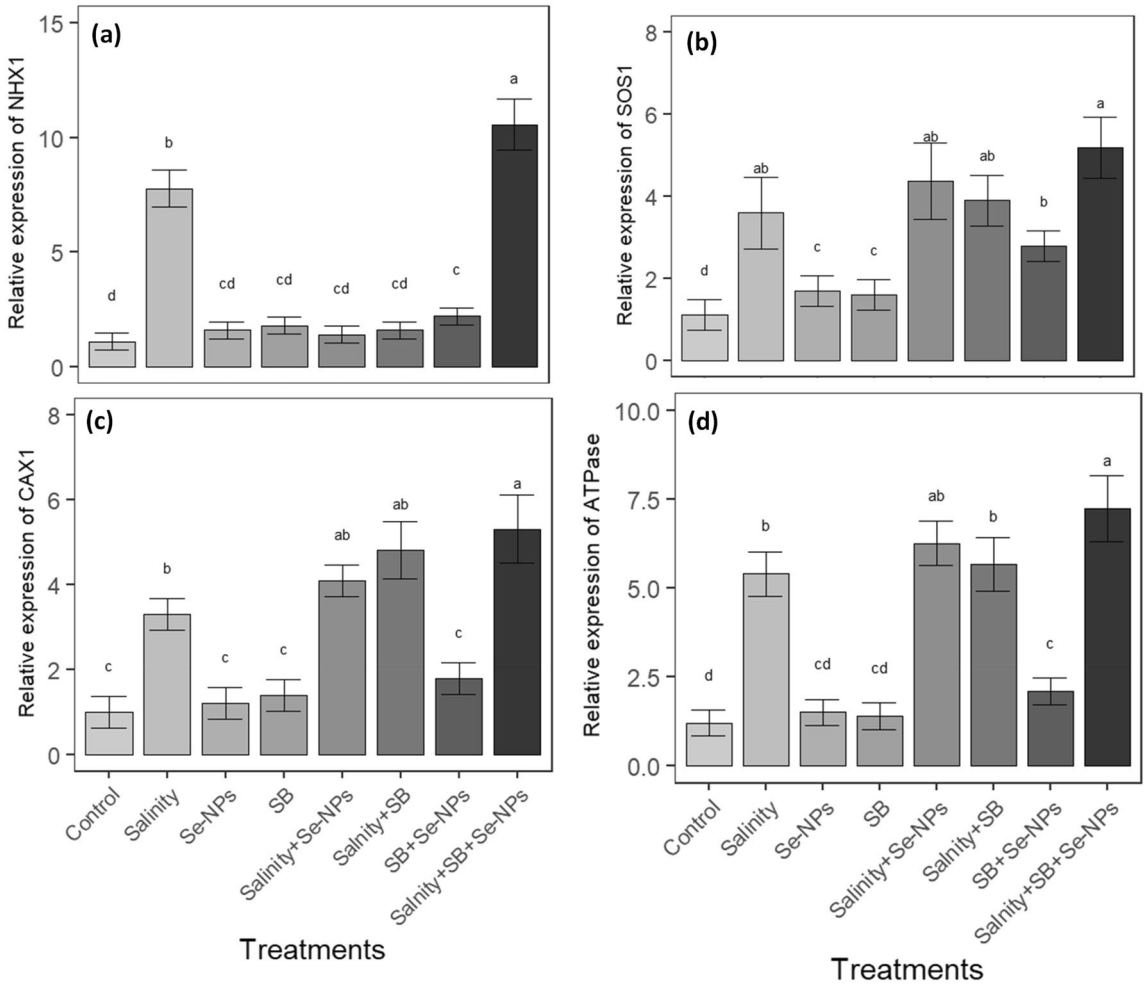

Fig. 8 Changes in expression of **a** sodium/proton exchanger (NHX1); **b** salt overly sensitive 1 (SOS1); **c** Ca^{++} protein exchanger (CAX1); **d** plasma membrane H^{+}-ATPase (ATPase) in wheat treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected 55 days after sowing and were presented as mean ± CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

Correlation Between Key Leaf Physiological Traits and Expression of Transporter Genes

Leaf levels changes in wheat physiological traits under salt and amendment treatments can be categorized into three distinct groups (1) traits regulating biomass accumulation and grain yield formation, (2) changes associated with cellular damage and (3) key transporter genes (Fig. 11). The expression of key ion and water transporter genes in wheat leaves were strongly positively correlated with each other (r^2 values of 0.94 to 0.37) as well as with growth related traits (except NHX1, which had a relatively poor association). Similarly, stress related traits, i.e., leaf proline, ROS and LWP and Na concentration were strongly positively correlated with each other (r^2 values of 0.92 to 0.57) but negatively associated with growth related traits (r^2 values of 0.94 to 0.34).

Discussion

Soil Salinity Inhibits Biomass Assimilation Processes

Our study suggested that increased salt levels in soils can arrest plant growth and biomass assimilation, leading to a significant reduction in grain yield at maturity. Detrimental effects of salt stress on growth and yield of crops, including wheat, have already been reported (Gurmani et al. 2013; Ahanger et al. 2019). Increased salt levels in rhizosphere can inhibit root development, water and mineral nutrient uptake

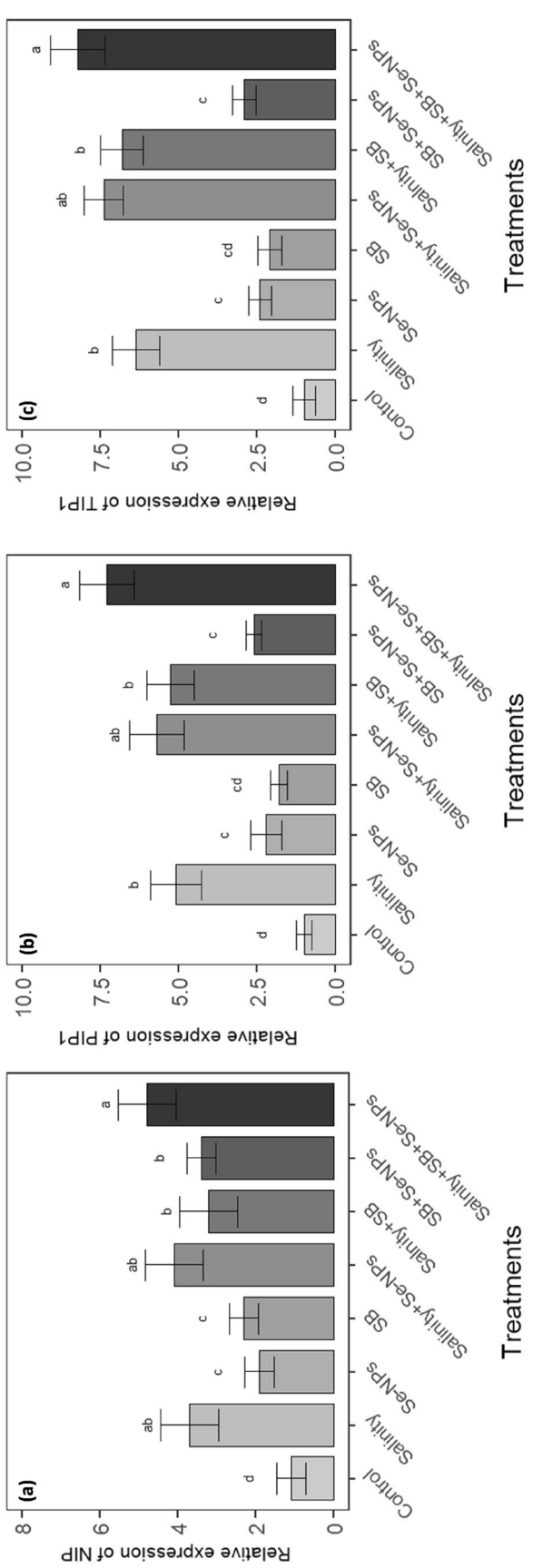

Fig. 9 Changes in expression of **a** nodulin intrinsic protein (NIP), **b** plasma membrane intrinsic protein (PIP1) and **c** tonoplast intrinsic protein (TIP1.1) in wheat treated with salinity, biochar (SB) and selenium nanoparticles (Se-NPs). Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. Starting from 20 days after sowing, every alternate day the plants were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, plants were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. Data were collected 55 days after sowing and were presented as mean ± CI (95% confidence interval) of three independent replicates. Variable means sharing similar letters are not significantly ($P < 0.05$) different from each other

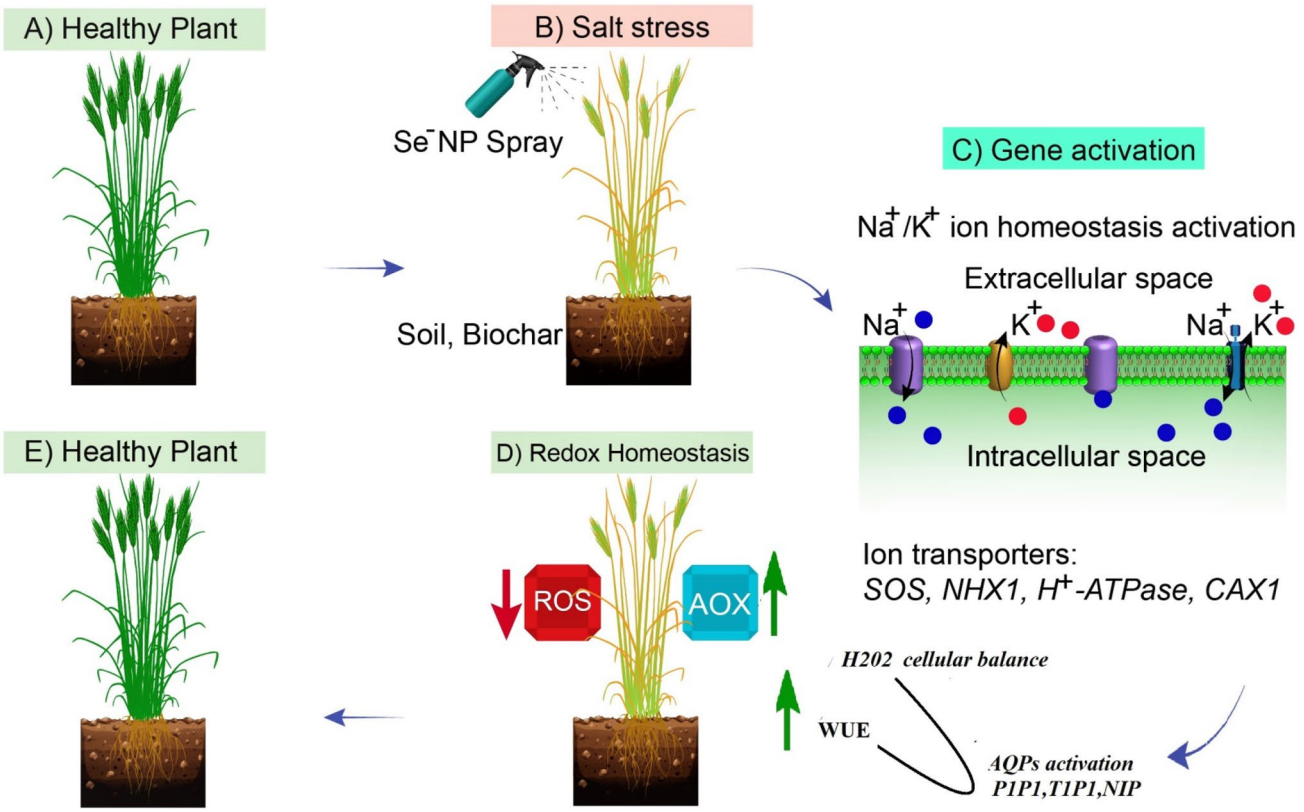

Fig. 10 A schematic diagram of the genetic regulation of salt stress tolerance in wheat through soil amendments (biochar) and Selenium nanoparticle (Se-NPs) application

(Abbas et al. 2017), inducing osmotic stress. Under inhibited water supply, leaves tend to close their stomata, thereby restricting leaf water potential and carbon assimilation, as has been evident in this study (Fig. 3a, d). Under salt-stressed environments, there was a significant increase in Na concentrations in leaf tissues (Fig. 7f), suggesting that wheat plants translocated excessive Na^+ into the aboveground tissues. This induces ionic stress and imbalances cytosolic Na^+/K^+ ratio (Assaha et al. 2017). Further, excessive salt in leaves accelerated ROS generation, damaging membranous cellular organelles as evident from an increased level of MDA and electrolyte leakage in salt-stressed leaves (Fig. 5). This was further supported by the strong positive correlation ($r^2 = 0.58–0.99$) of Na^+ concentration with the key stress indicators such as H_2O_2, MDA and phenolic compounds in leaf tissues (Fig. 11).

Our study suggest that soil salinity induces osmotic, ionic and oxidative stresses in wheat leaves, thereby impairing chloroplast structure (as evidenced by chlorophyll loss) and carbon assimilation process. Although plant species may overcome oxidative stress by activating their antioxidant enzyme system (Sofy 2016), in this study, the antioxidant enzyme capacity of wheat plants appeared to be limited due to an excessive cellular damage (Figs. 4 and 5). This was also confirmed by a limited post-stress recovery in leaf physiological traits (data collected 1 week after the last salt treatment) and reduced grain yield formation at maturity (Fig. 2).

Biochar and Se-NPs Protect Leaf Physiological Traits from Salt Injury by Restricting Na^+ Uptake

Extensive research is underway on the introduction of novel management techniques to improve plant performance on salt-affected soils. In the present study, soil amendment through SB application and foliar supplementation significantly improved the growth and yield of wheat plants both under stressed and unstressed environments. Positive effects of SB (Cui et al. 2021a) and Se (Shekari et al. 2017; Shalaby et al. 2021) on the growth of stressed plants have already been reported. Cellular injury and impaired physiological functioning in salt stress plants are associated with excessive tissue Na^+ concentrations (Theerawitaya et al. 2020). Further, excessive salt in the root zone can inhibit uptake of essential minerals (Fig. 7). In this study, both SB and Se-NPs significantly restricted Na^+ uptake and restored uptake

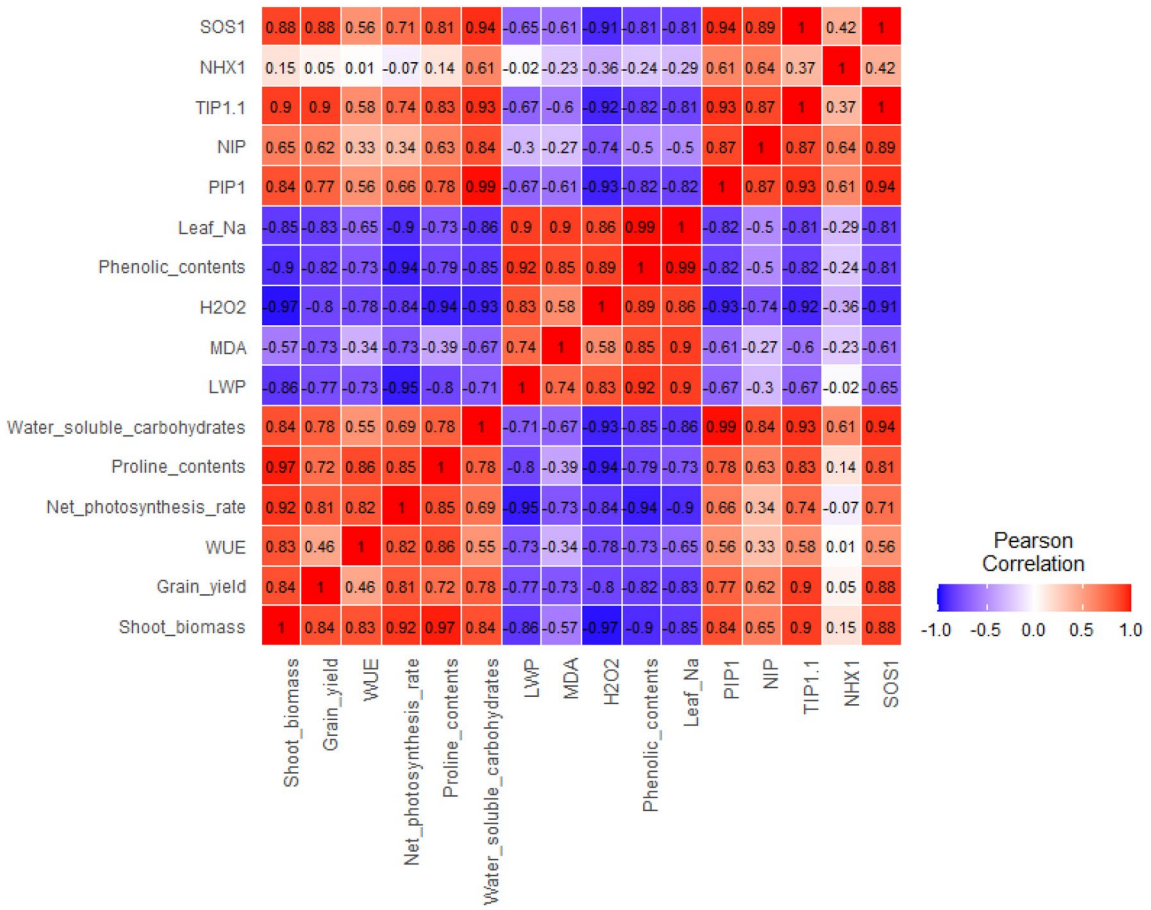

Fig. 11 Heatmap of correlations of grain yield and biomass production in wheat plants treated with salinity, biochar SB amendment, and foliar application of selenium nanoparticles with key leaf physiological traits. Leaf physiology data were collected at 55 days after sowing and grain yield components at crop maturity. Soybean straw biochar (SB) was mixed with soil (5%, W/V) before planting. The plants (20-day-old) were irrigated with saline water (3000 ppm NaCl) for 4 weeks. Five days after the start of salinity treatment, the plants (25-day-old) were sprayed with Se-NPs (30 ppm) or water (control) on a weekly basis for 4 weeks. These changes can be categorized into three distinct groups (1) traits regulating biomass accumulation and grain yield formation, (2) changes associated with cellular damage and (3) key transporter genes. *WUE* water use efficiency, *LWP* leaf water potential, *MDA* leaf malondialdehyde contents, *PIP1* plasma membrane intrinsic protein, *NIP* nodulin intrinsic protein, *NHX1* sodium/proton exchanger, *TIP1.1* tonoplast intrinsic protein, *SOS1* salt overly sensitive gene, *NHX1* sodium/proton exchanger

of essential nutrients in the salt-stressed plants (Fig. 7) and improving their physiological functioning. Increased mineral uptake and assimilation are associated with the growth recovery as these elements regulate plant function, e.g., enzyme activity, chlorophyll synthesis, photosynthesis, and stress tolerance (Fig. 11).

Biochar and Se-NPs Regulate ROS Oxidative Damage in Salt Stress Wheat Leaves

Excessive ROS accumulation in plant cells impairs the structural integrity of key macromolecules, including proteins and lipids, thereby influencing their normal functioning (Mittler 2002). In addition to increasing ion homeostasis in salt-stressed wheat, SB and Se-NPs protected cellular organelles from injury by capturing these excessive ROS. This was achieved through the modification of antioxidant enzyme activities. In salinity stressed plants, an increased lipid peroxidation and subsequent electrolyte leakage have been observed as a result of unregulated ROS, which is directly related to lipoxygenase activity (Ahanger et al. 2019) and can be reversed by amendments such as SB and Se. Hence, in the present study, reduced ROS accumulation and oxidative damage in SB and Se-treated wheat plants is likely associated with increased ROS-scavenging enzymes (Fig. 11).

Biochar-induced recovery of carbon assimilation has been linked with upregulated antioxidant functioning, and PSII stability in drought-stressed *Phragmites karka* (Abideen et al.

2020) and salt-stressed soybean (Mehmood et al. 2020). Similar positive effects of Se have been recorded on photosynthesis and membrane stability of maize (Jiang et al. 2017) and strawberry plants (Refaat et al. 2021). These studies suggested that the anti-oxidative defense mechanism plays a crucial role in protecting major cellular organelles from salinity. For example, critical antioxidant enzymes such SOD mediates dismutation of toxic superoxide. At the same time, H_2O_2 is neutralized by either CAT or through an intriguing pathway called ascorbate–glutathione (AsA-GSH) of which APX, GR, and MDAR are the key components (Alhaithloul et al. 2020). Increased functioning of AsA-GSH cycle prevents H_2O_2 mediated oxidative effects and protects the major functioning of cells by maintaining redox homeostasis, thereby protecting major metabolic pathways, including photosynthesis, enzyme functioning (Alam et al. 2020). In this study, SB and Se-NP modulated various antioxidant enzymes to protect growth and physiological functioning of wheat plants under saline conditions.

Secondary metabolites such as phenols and flavonoids also mediate osmoregulation and ROS scavenging, thereby strengthening the enzymatic antioxidant system to withstand oxidative stress (Austen et al. 2019). The synthesis of secondary metabolites is regulated by various environmental factors, including biotic and abiotic stresses (Ramakrishna and Ravishankar 2013). In this study, SB and Se-NP significantly amplified phenols and flavonoids levels in the salt-stressed wheat with a concomitant increase in osmolytes, including glycine betaine, proline, and carbohydrates. Increased accumulation of phenols and flavonoids strengthened the antioxidant system, thus preventing damaging effects of ROS on cellular organelles. In earlier studies by Hussein et al. (2019) and Zahedi et al. (2019) have also suggested an increased synthesis of secondary metabolites in plants in response to Se-NP application. Similarly, Ciccolini et al. (2017) demonstrated that SB-induced phenols and flavonoids accumulation in lettuce promoted antioxidant efficiency and stress adaptation. Osmolytes also scavenge ROS, maintain cellular osmolarity and eventually maintain water contents, and mediate stress signaling for quick elicitation of stress response to prevent oxidative effects. Recently, Qin et al. (2021) demonstrated that application of acetylcholine, a glycine betaine precursor, can activate signaling pathways and induce salinity stress tolerance in *Nicotiana tobaccumAQ*. In the present study, SB or Se-NP application also resulted in a significant enhancement in the accumulation of compatible osmolytes, contributing to strengthening tolerance mechanisms against salinity (Fig. 10).

Activation of Water Transporter Genes Increases Salt Stress Tolerance in Wheat

Increased carbon assimilation and biomass production in response to SB and Se-NPs in wheat observed in this study could be linked with upregulation of water transporter genes. Aquaporins play an important role in water transport; particularly PIPs not only promote uptake and transport of water to roots and leaves (Saibi 2021; Cui et al. 2021b), but boost CO_2 diffusion, ensuring abundant substrate supply for photosynthesis and carbohydrate assimilation (Groszmann et al. 2017). The findings of this study confirm previous observations, where upregulation of AQP genes was linked with an enhanced water uptake for maintaining cell homeostasis as in *Brassica rapa* (Kayum et al. 2017). Similarly, upregulated AQPs genes can promote CO_2 concentration in mesophyll cells by enhancing stomatal conductance (Groszmann et al. 2017). A strong positive correlation of water transporter genes with plant growth attributes and leaf physiological traits as observed in this study also supported our hypothesis (Fig. 11).

AQPs mediate and regulate rapid transmembrane water flow during growth and developmental processes from the physiological point of view. This has been confirmed through upregulation of wheat ion transporter genes and aquaporin, which are regulate ion homeostasis under salinity conditions (Santander et al. 2021). Moreover, increased Ca^{2++} content in the SB + Se-NPs-treated plants might have induced salt tolerance in wheat plants by modifying cellular signaling pathways (Qin et al. 2019). Consequently, these treatments increased K^+ /Na^+ ratio and Ca^{2+} concentrations primarily via Ca^{2+} -dependent SOS pathway. Ion transport proteins such as *NHX1*, *HKT1* and *SOS1* play a key role in regulating salinity tolerance and elicitation of signaling events (Assaha et al. 2017; Li et al. 2021). For instance, *NHX1, HKT1* mediate cellular Na sequestration (Liu et al. 2018), while *SOS1* forms the salt's key component over a sensitive pathway for mediating ion homeostasis (Ji et al. 2013). In this study, *NHX1, CAX1, SOS1,* and *H + -ATPase* were switched off under non-stressed environments but upregulated under salt and amendment treatments, with a significantly higher expression under their combined (SB + Se-NPs) than individual (SB or Se-NPs) application (Fig. 8). This suggests a synergy between SB and Se-NPs for stabilizing membrane potential for optimal functioning of H^+ATPase, favoring K uptake and protecting wheat plants from salt-induced injury. In terms of aquaporin (AQP) proteins expression, which belongs to a major intrinsic protein superfamily, it has a crucial role in transporting water and some other molecules in a plant cell by phosphorylation. In this experiment, three wheat AQPs, i.e., *P1P1*, *NIP*, and *N1P1* were upregulated in response salinity and were further significantly induced when SB + Se-NPs were applied (Fig. 9). This upregulation of aquaporin (AQP) proteins promoted water status equilibrium in plant cells and adjusted their position concerning ROS accumulation and membrane damage.

Conclusion

This study suggests that elevated salt concentrations in plant tissues impairs leaf functioning and biomass assimilation through oxidative and osmotic stresses. However, SB and Se-NPs applied individually or combined, can protect wheat plants from this salt-induced injury by restricting Na^+ transport and restoring leaf functions. Our findings suggest that upregulation antioxidant system plays a key role in alleviation of negative effects of salinity on wheat physiological functioning. SB and/or Se-NPs can activate this plant defense mechanism by inducing AQPs and ion transporter genes and promoting synthesis of protective metabolites such as phenols and flavonoids and compatible osmolytes. Further, increased mineral uptake and restricted Na accumulation in SB and Se-treated plants also promoted photosynthesis and WUE, thereby contributing to growth and yield enhancement. Further studies are required to unravel the molecular mechanistic and signaling pathways associated with salt stress tolerance in wheat.

Author Contributions MHS, GSHA and UN contributed to the conception and design of the experiments. MHS, TSA, AMA and HSA conducted experiments, collected and analysed plant samples. Data analysis and write up were performed by MHS, AAK, MAF and MY. The first draft of the manuscript was written by MHS and UN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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