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Research Article

Salinity stress amelioration and morpho-physiological growth stimulation by silicon priming and biochar supplementation in *Chenopodium quinoa*

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Abstract

The effect of silicon (Si) priming and soil amendment with biochar (BC) was analysed in *Chenopodium quinoa* under normal and salinity stressed conditions. Reduced growth parameters, chlorophyll content, and photosynthesis under salinity stress were significantly ameliorated by Si priming and soil amendment with BC. Applied Si and BC treatment also enhanced the chlorophyll content, stomatal conductance, transpiration rate, net photosynthesis, and maximal photochemical efficiency. In addition to this, Si and/or BC amendments alleviated the oxidative damage by reducing the generation of toxic reactive oxygen species including hydrogen peroxide and superoxide. Moreover, the activities of antioxidant enzymes and the content of ascorbate and glutathione increased significantly in Si and BC treated plants reflecting efficient alleviation of oxidative damage. Besides, the content of compatible osmolytes was increased in Si and/or BC treated seedlings, thereby contributing to improved growth and salinity tolerance by maintaining higher leaf water potential and water use efficiency. Si and BC treatment increased the uptake of key mineral elements. Moreover, salinity induced decline in the nutritional components of seeds of *C. quinoa* were considerably increased under Si and BC treatments.

Keywords: antioxidants; *C. quinoa*; nutritional aspects; osmoregulation; oxidative stress

Introduction

Salinity is considered as one of the devastating stress factors causing significant decline in global food production. Prevailing salinity conditions has shrinked the agricultural productive lands by converting them into unproductive waste lands by inducing negative effects on germination, root growth, restricting access to major mineral ions, altering metabolism, and hampering key tolerance pathways (Soliman *et al*., 2020; Joshi *et al*., 2022). Excess accumulation of sodium (Na) ions, over-production of reactive oxygen species (ROS), structural and functional alteration of the membranes, photo-inhibition, and enzyme inactivation are key consequences of salinity stress (Ahanger *et al*., 2019, 2020; Challabathula *et al*., 2022). In order to reduce the damaging effects of salinity, plants have evolved some key mechanisms which regulate growth and metabolism at physiological, biochemical, and molecular levels (Ahmad *et al*., 2010; Elkelish *et al*., 2019; Noman *et al*., 2021; Fatima *et al*., 2022).

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Silicon (Si) is the second most abundant element in earth's crust and is considered as beneficial but non-essential element for growth and development of plants (Luyckx *et al*., 2017; Ahanger *et al*., 2020). Plants can be Si accumulators or non-accumulators and it has been established that monocots accumulate more Si as compared to dicots (Liang *et al*., 2017). Silicon accumulation strengthens cell wall by improving silicification, suberization and lignification's (He *et al*., 2013). Due to the accumulation of silica in apoplast, formation of amorphous silica barriers takes place providing an important defence against stresses (Guerriero *et al*., 2016; Luyckx *et al*., 2017), therefore, has led to interesting research on Si so far. Improved stress tolerance in plants by Si application has been reported due to significant modification in tolerance mechanism (Ahmad *et al*., 2019; Ahanger *et al*., 2020). Improved salinity tolerance by applying Si mainly results due to the regulated uptake and accumulation of Na and K ions (Zhu and Gong, 2013; Naz *et al*., 2022).

Biochar (BC) is a carbon rich material produced by pyrolysis process and is usually employed for both scientific and commercial purposes as soil amendment to improve product quantity and quality. It mediates carbon sequestration, agricultural soil amendments, waste management and environmental remediation (Barrow, 2012; Adekiya *et al*., 2020) as well as soil physiochemical properties (Jien and Wang, 2013; Adekiya *et al*., 2020). It is well known in decreasing soil acidity and improving fertility, thereby mediating growth and yield improvement as well as stress tolerance (Shetty and Prakash, 2020). Therefore, BC can be an achievable amendment for better agricultural management regarding sustainable food security (Biederman and Harpole, 2013). Soil treatment with BC improves cation exchange and nutrient retention and promotes growth of beneficial soil microbes including plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhiza fungi (AMF) (Changxun *et al*., 2016) as well as stress tolerance (Hashem *et al*., 2019). BC mediated growth improvement has been reported in several crop species (Zhao *et al*., 2016; Changxun *et al*., 2016; Hashem *et al*., 2019; Jabborova *et al*., 2021). BC strengthens tolerance mechanisms leading to improved growth and productivity under stressful conditions (Hashem *et al*., 2019; Shetty and Prakash, 2020; Bornø *et al*., 2022).

Quinoa (*Chenopodium quinoa*) is a dicotyledonous annual plant belonging to Amaranthaceae family. It is primarily grown for edible seeds rich in proteins, vitamin B, dietary fibres, and minerals. Global climate changes and pollution pressures can lead to significant decline in its growth and hence reducing its production. Soil amendment with BC can be a promising strategy for protecting growth and production of quinoa under saline affected conditions. In present study, we hypothesised that Si priming and soil amendment with BC can improve growth, photosynthesis, and nutritive value of quinoa by up-regulating the key salinity tolerance mechanisms.

Materials and Methods

Plant material, priming and biochar treatments

Seeds of *C. quinoa* cultivar ('Giza 1') were obtained from Agricultural Research station. Seeds were sterilized by immersing in 70% ethanol for 10 sec followed by further surface sterilization with 2% (w/v) NaOCl for 5 min. Sterilized seeds were washed five times with distilled water. Here after seeds were soaked in distilled water or 50 mM sodium silicate [$Na_2O(SiO_2)$ was obtain from Sigma-Aldrich] in petri dishes for 24 h. After soaking, seeds were rinsed and blot dried at 25± 2 °C under shade. Primed seeds were sown in earthen pots (20x 15 cm) filled with 1.2 kg soil with and without BC amendment. Eucalyptus wood derived biochar (EW Biochar) was obtained from experimental farm at Faculty of Agriculture. Biochar was grinded, sieved, and stored until further use. Concentration of BC used was 5% and was thoroughly mixed with soil. The physiochemical properties of soil and BC used are given in Table 1. At the time of sowing, pots were irrigated with 300 mL Hoagland solution. After germination, one healthy seedling was maintained and others were thinned. Pots were irrigated with half strength Hoagland solution after every three days. After three weeks of successful growth, pots were divided in two groups and salinity stress was induced by irrigating one group of

pots with modified Hoagland solution containing 300 mM NaCl for two weeks. So, detailed experimental treatments can be summarised as (1) Control, (2) Si (50 mM Si priming), (3) salinity stress (300 mM NaCl), (4) biochar (5% BC), (5) Si + BC, (6) NaCl + Si, (7) NaCl + BC, and (8) NaCl + Si + BC. Pots were arranged completely randomized block design (CRBD) with three replicates for each treatment and were maintained under greenhouse conditions with 60-65% relative humidity and light/dark cycle of 12/12 h. Thirty-five days old seedlings (14 days after salinity treatment) were analysed for different parameters as described below.

Table 1. Physico-chemical properties of Eucalyptus wood Biochar (EW Biochar) and sandy loam soil used

Component	EW Biochar	Sandy loam soil
EC, dS m^{-1}	1.5	0.9
PH	8.35	7.1
Total N (mg g^{-1} DW)	15.6	265
Total P (mg g^{-1} DW)	1.05	1.25
Total K (mg g^{-1} DW)	2.7	117
Organic matter (mg g^{-1} DW)	125	26.7
Organic carbon (mg g^{-1} DW)	78.6	17.2

Growth parameters

Plant height was measured using a manual scale. Fresh weight of shoot and root was measured immediately after uprooting the plants. However dry weight of shoot and root was recorded after oven-drying the samples at 80 °C for 48 h.

Determination of relative water content, leaf water potential, and membrane stability index

Relative water content was determined by following the method of Smart and Bingham (1974) and following formula was used for calculation (equation I).

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100 \quad (I)$$

For measurement of leaf water potential, mature leaves were selected to measure leaf water potential using a psychrometer between 09:00 and 11:00 am. For each treatment, 10 measurements were recorded.

Membrane stability index (MSI) was determined by following Sairam (1994). Fresh leaf samples (0.1 g) were cut into small discs in test tubes containing 10 ml distilled water and boiled at 25 °C for the measurement of electrical conductivity (C1). Thereafter, same tubes were boiled at 120 °C for 20 min and subsequently electrical conductivity (C2) was measured. MSI was calculated using the following formula – equation II:

$$MSI\ (\%) = \{1-(C1/C2)\} \times 100 \quad (II)$$

Photosynthetic pigments and gas exchange parameters

Chlorophyll content of leaves was determined spectrophotometrically according to method of Arnon (1949). Briefly, 0.2 g fresh leaf samples extracted in acetone (80%), followed by centrifugation for 10 min at 12000 g. The absorbance of supernatant was recorded at 663 and 645 nm using UV/VIS spectrophotometer (Genway, Japan). The net photosynthetic rate (Pn), stomatal conductance (gs), and transpiration rate (E) of fully expanded leaves was measured between 09.00 and 11.00 AM using a portable infrared gas analyzer system (TPS-2, USA). Water use efficiency (WUE) was calculated as the ratio of Pn and E by following Zhang *et al*. (2016) method. Maximum quantum efficiency of PSII photochemistry (Fv/Fm) was measured using Modulated Chlorophyll Fluorometer (PAM 2500; Walz, Germany) after dark adapted the leaves for 30 min.

Determination of oxidative stress parameters

Hydrogen peroxide (H_2O_2) levels were measured according to method described by Najafi Kakavand *et al*. (2019). Briefly, 100 mg fresh leaf samples were extracted in trichloroacetic acid followed by centrifugation

at 12,000 g for 15 min. The supernatant (0.5 mL) was added to phosphate buffer (0.5 mL, pH 7.0) and potassium iodide (1 mM). Absorbance was recorded at 390 nm and calculations were done from the standard curve of H_2O_2 as a standard. Content of superoxide anion was measured according to the method described by Elstner and Heupel (1976).

Malondialdehyde (MDA content was measured according to the method described by Heath and Packer (1968). Fresh 0.5 leaf samples were homogenized in trichloroacetic acid (TCA) and homogenate was centrifuged at 10,000 g for 10 min. One mL supernatant was added to 2 mL mixture of thiobarbituric acid (TBA, 0.5%) in 20% TCA. The mixture was boiled for 30 min and then cooled rapidly. After centrifugation at 10,000 g for 5 min, content of MDA was determined from the difference in non-specific absorption at 600 and 532 nm using UV/VIS spectrophotometer (Genway, Japan).

Electrolyte leakage (EL) was measured by boiling fresh leaf discs in 10 ml deionized water and electrical conductivity (EC1) was measured. Thereafter, tubes were heated at 55 °C for 30 min and again electrical conductivity (EC2) was measured. Then boiling the tissue for 10 min at 100 °C, electrical conductivity (EC3) was recorded (Sullivan 1979). Calculation was done using following formula (equation III):

Electrolyte leakage (%) = {EC2- EC1) /EC3} ×100 (III)

Estimation of osmolytes

Content of total soluble protein was estimated following Bradford (1976) using Folin phenol reagent and absorbance was recorded at 700nm using bovine serum albumin as standard. Total soluble sugars were estimated according to the modified method of Irigo-yen *et al*. (1992) using an anthrone reagent and the absorbance was recorded at 625 nm using glucose as a standard. The Method of Moore and Stein (1948) was used for estimation of free amino acids. Glycine betaine (GB) was estimated according to Grieve and Grattan (1983). For this, 0.5 g leaf powder was homogenized in 10 ml distilled water and was incubated for 24 h at 25 °C. The homogenate was filtered and the filtrate was mixed with 2 N sulphuric acid in the ratio of 1:1 (v/v). Thereafter, 0.2 ml cold potassium tri-iodide reagent was added to each tube and kept at 4 °C for 16 h, followed by centrifugation at 14,000 g for 15 min at 0 °C. Absorbance of the supernatant was read at 365 nm and standard curve of GB was used for calculation.

Assay of antioxidant enzymes

For extraction of antioxidant enzymes, fresh 1.0 g leaves were macerated in chilled 50 mM phosphate buffer (pH 7.0), supplemented with 1% polyvinyl pyrolidine and 1 mM EDTA using prechilled pestle and mortar. After centrifuging the homogenate at 15,000 g for 20 min at 4 °C, the supernatant was collected and used to determine activity of different antioxidant enzymes.

For determination of superoxide dismutase (SOD, EC 1.15.1.1) activity, Bayer and Fridovich's (1987) method and photochemical reduction of NBT was recorded at 560 nm after 15 min of incubation under light. Assay mixture contained sodium phosphate buffer (50 mM, pH 7.5), 100 µL EDTA, L-methionine, 75 µM NBT, riboflavin and 100 µL enzyme extract in a final volume of 1.5 mL. The activity was expressed as EU mg^{-1} protein. Activity of catalase (CAT, EC1.11.1.6) was measured following Luck, (1974) and change in absorbance was monitored at 240 nm for 2 min. For calculation, an extinction coefficient of 39.4 mM^{-1} cm^{-1} was used. Activity of ascorbate peroxidase (APX, EC 1.11.1.11) was determined by monitoring change in absorption at 290 nm for 3 min in 1mL reaction mixture containing potassium phosphate buffer (pH 7.0), 0.5 mM AsA, H_2O_2 and enzyme extract. An extinction coefficient of 2.8 mM^{-1} cm^{-1} was used for calculation (Nakano and Asada, 1981). Glutathione peroxidase (GPX, EC 1.11.1.9) activity was determined using UV/visible spectrophotometer (Jenway, Japan) as described by Hossain *et al*. (2010).

Estimation of non-enzymatic antioxidants

Content of ascorbic acid (AsA) was determined according to Jagota and Dani (1982). Leaf samples (0.2 g) were extracted in 2 ml of 5% TCA and homogenate was centrifuged at 10,000 g for 15 min. Then, 0.5 ml of

the extract was diluted to 2.0 ml using double distilled water followed by addition of 0.2 ml of diluted Folin-Ciocalteu reagent and the absorbance was measured after 10 min at 760 nm. Reduced and oxidized glutathione (GSH and GSSG, respectively) were estimated using the protocol described by Yu *et al*. (2003). After this, 0.4 ml aliquot was neutralized using 0.6 ml of 500 mM K phosphate buffer (pH 7.0). Finally, GSH was calculated by the changes in absorption rate at 412 nm wavelength for NTB (2-nitro-5-thiobenzoic acid) generated by the reduction of DTNB (5, 5′- dithio-bis (2-nitrobenzoic acid)) and GSSG was quantified by eliminating GSH using a derivatizing agent (2-vinylpyridine).

Nutritional value of seeds

After crop harvest, seeds were collected and analysed for nutritional components. For estimating total carbohydrates in *C. quinoa* seeds, anthrone method was followed and calibration curve of glucose was used for calculation (Osborne, 1986). Total dietary fiber was determined by neutral detergent fiber method (Goering and Van Soest, 1970). Crude protein was estimated by the Kjeldahl method and a conversion factor of 6.25 was multiplied to nitrogen. Total lipid was extracted from the dried *C. quinoa* seeds with petroleum ether (60-80 °C) in a Soxhlet apparatus for about 20h. The residual solvent was evaporated in a pre-weighted beaker and increase in weight of beaker gave total lipid (AOAC, 2000). Content of α-tocopherol content was analysed according to Linow and Pohl (1970). Total ash was determined by incineration of a representative 0.5 g sample in an oven at 450 °C for 48 h.

Mineral analysis

Phosphate content was determined by vanadomolybdo phosphoric acid colorimetric method. Potassium (K^+) ion concentration was estimated by flame photometer (Fisher scientific, USA). Estimation of magnesium (Mg^{2+}), calcium (Ca^{2+}), and sodium (Na^+) ions was determined by atomic absorption spectrophotometer using Systronics Type 130 flame photometer (Rowell, 1994) and Fe was extracted from the samples with DTPA and aqua regia digestion and quantified by flame atomic absorption spectrometry (AAS) using 6300- flame atomic absorption spectrometer (Shimadzu, Japan).

Statistical analysis

Data presented is mean (±SE) of three replicates. Least significant difference was calculated at $P<0.05$ using ANOVA.

Results

Silicon priming and biochar amendment increased C. quinoa growth parameters

Table 2 shows the effect of salinity stress, soil BC amendment and Si application on the plant growth parameters including plant height, leaf area, fresh and dry weight of root and shoot. Relative to control, growth parameters exhibited a significant improvement due to BC treatment and Si priming with maximal enhancement in plants treated with BC + Si. Under normal conditions, BC + Si treated seedlings height increased by 38.53%, shoot fresh weight by 78.47%, root fresh weight by 48.28%, shoot dry weight by 36.95%, root dry weight by 17.01%, and leaf area by 34.54%, over control. Treatment of salinity resulted in decline of 31.66, 40.41, 38.17, 24.68, 39.27, and 30.74% in plant height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, and leaf area, respectively. Priming of Si or BC amendment significantly ameliorated the decline in growth parameters and their combined treatment maximally mitigated the salinity mediated decline (Table 2).

Table 2. Effect of salinity stress (300 mM NaCl) on the plant height, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and leaf area in *Chenopodium quinoa* with and without Si (50 mM) priming and biochar (BC, 5%) amendment. Data presented is mean of three replicates and different alphabets denote significant difference at P<0.05.

Treatment	Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Leaf Area (cm^2)
Control	34.33 ±2.57d	21.60 ±1.21d	11.08 ±0.85c	13.61 ±0.67c	11.05 ±0.91c	17.40 ±1.15d
Si	40.60 ±3.21c	28.45 ±1.39c	14.20 ±1.07b	15.07 ±0.96b	11.98 ±0.94b	20.71 ±1.21c
BC	43.43 ±3.07b	34.98 ±2.31b	15.45 ±1.09ab	15.96 ±0.94b	12.41 ±0.99ab	22.51 ±1.86b
BC + Si	47.56 ±3.57a	38.55 ±2.67a	16.43 ±1.11a	18.64 ±1.11a	12.93 ±1.01a	23.41 ±1.93a
NaCl	23.46 ±1.52g	12.87 ±0.98g	6.85 ±0.30f	10.25 ±0.44f	6.71 ±0.52e	12.05 ±0.81f
NaCl + Si	26.80 ±1.54f	15.64 ±1.02f	7.36 ±0.78f	11.86 ±0.51e	7.31 ±0.63e	12.81 ±0.97f
NaCl + BC	29.23 ±2.27e	16.41 ±1.04f	8.41 ±0.79de	12.31 ±0.87d	9.683 ±0.66d	14.90 ±1.08e
NaCl + BC + Si	32.53 ±2.42d	18.71 ±1.17e	9.54 ±0.81d	12.58 ±0.88d	10.48 ±0.72c	16.81 ±1.09d

Silicon and biochar improved gas exchange parameters

Soil amendment with BC and priming with Si resulted in significant enhancement in leaf chlorophyll, stomatal and non-stomatal photosynthetic parameters (Figure 1). Relative to control, total chlorophyll, photosynthesis, stomatal conductance, transpiration, and maximal photochemical efficiency reduced by 29.68%, 26.34%, 32.67%, 33.01%, and 21.53%, respectively due to salinity stress. Maximal increase of 40.85% for total chlorophyll, 35.55% for photosynthesis, 14.88% for stomatal conductance, 5.48% for transpiration, and 33.84% for maximal photochemical efficiency was observed in plants treated with BC + Si. Salinity mediated decline was significantly mitigated by BC and Si treatment individually and maximal amelioration was observed due to their combined application. Water use efficiency (WUE) and leaf water potential (LWP) were also increased due to the application of BC and Si, otherwise declined under salinity stress over the control plants (Figure 1).

Impact on osmolytes

Silicon and biochar increased osmolytes

Content of soluble sugars, protein, free amino acids, and GB increased due to the application of BC and Si, exhibiting a maximal enhancement of 69.81%, 40.36%, 27.71%, and 52.68%, respectively, by BC + Si treatment (Figure 2). Salinity stress resulted in an increase of 90.04% for soluble sugars, 30.42% for protein, and 51.21% for GB, while free amino acids declined by 38.56% over the control. In NaCl + BC + Si treated seedlings, content of soluble sugars, protein, and GB increased by 159.43%, 64.15%, and 139.02%, respectively, over the control plants (Figure 2). Salinity also reduced RWC by 30.91%, and an increase of 2.11% for BC, 0.851% for Si and 6.16% for BC + Si was observed over the control. Relative to NaCl treated plants, decline in RWC was maximally ameliorated by 36.66% in NaCl + BC + Si treated plants (Figure 2).

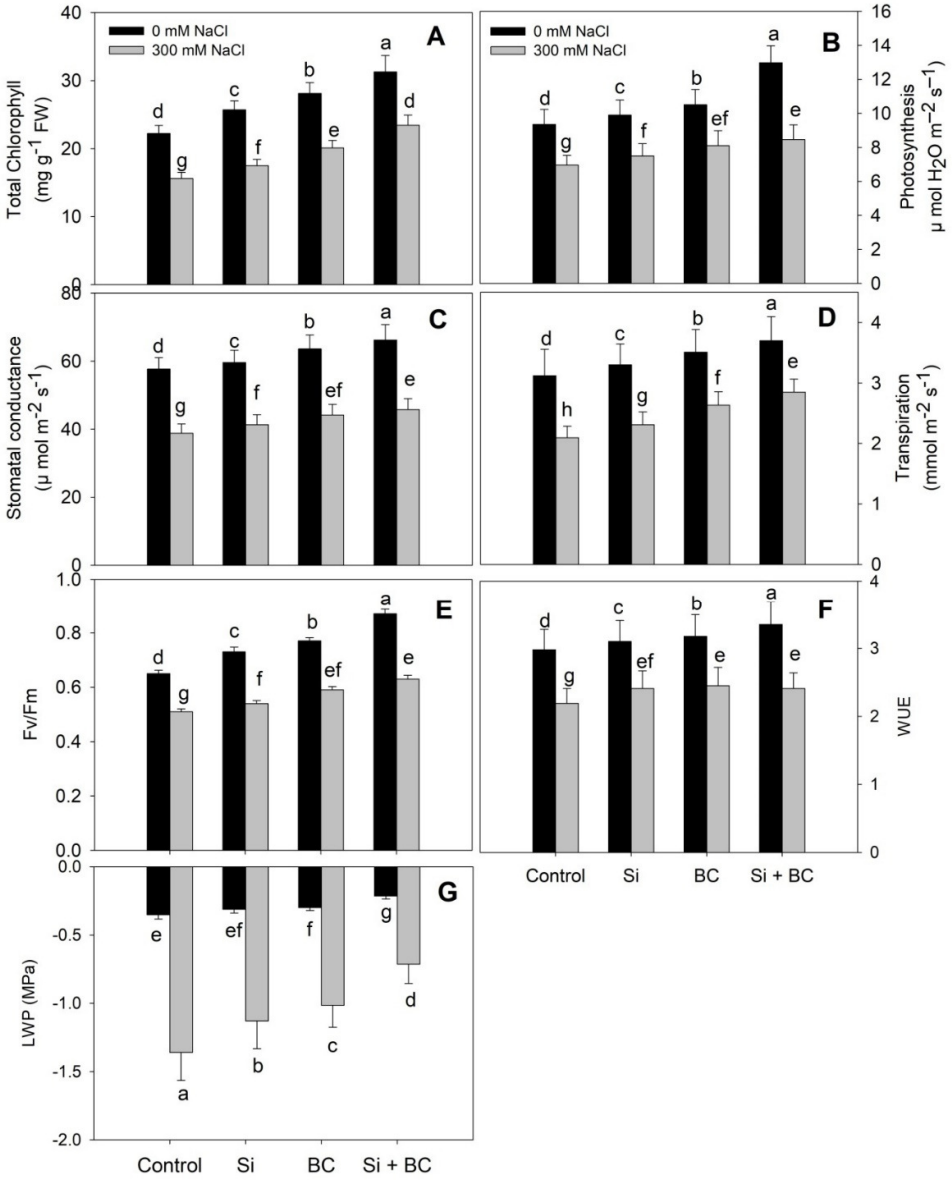

Figure 1. Effect of salinity stress (300 mM NaCl) on the (A) total chlorophyll, (B) photosynthesis, (C) stomatal conductance, (D) transpiration, (E) maximal photochemical efficiency, (F) water use efficiency and (G) leaf water potential in *Chenopodium quinoa* with and without Si (50 mM) priming and biochar (BC, 5%) amendment. Data presented is mean of three replicates and different alphabets denote significant difference at $P<0.05$

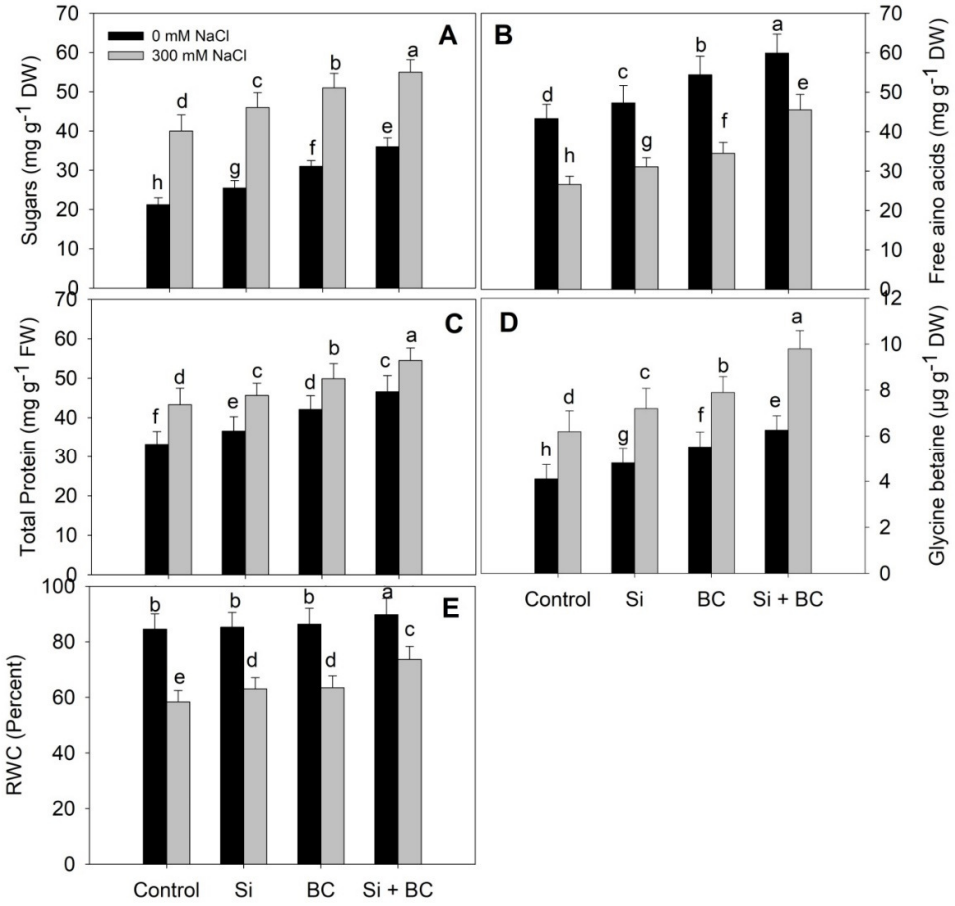

Figure 2. Effect of salinity stress (300 mM NaCl) on the content of (A) sugars, (B) free amino acids, (C) total protein, (D) glycine betaine and (E) relative water content of *Chenopodium quinoa* with and without Si (50 mM) priming and biochar (BC, 5%) amendment. Data presented is mean of three replicates and different alphabets denote significant difference at $P<0.05$

Application of silicon and biochar reduced oxidative stress parameters under salinity stress

Treatment of BC or Si or BC + Si resulted in significant reduction in the content of H_2O_2 and O_2^- over control and also resulted in significant decline in their concentration under salinity conditions (Figure 3). Maximal reduction of 42.84% and 46.76% in H_2O_2 and $O_2^{\bullet-}$ was observed due to BC + Si treatment over the control plants resulting in decline of 28.61% in lipid peroxidation and 28.04% in electrolyte leakage. Salinity stress increased H_2O_2, $O_2^{\bullet-}$, lipid peroxidation and electrolyte leakage by 184.46%, 71.28%, 94.50%, and 250.57%, respectively, over the control plants (Figure 3). Application of BC and Si priming individually as well as combinedly resulted in decline of H_2O_2, $O_2^{\bullet-}$, lipid peroxidation, and electrolyte leakage maximally by 45.93%, 39.76%, 35.96%, and 22.12% under BC + Si treated plants over the NaCl stressed counterparts (Figure 3).

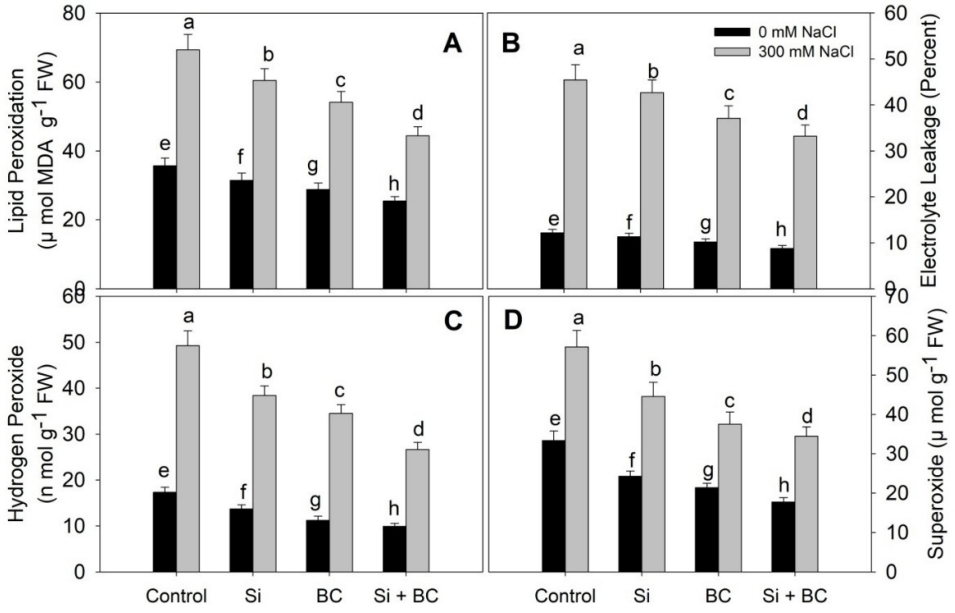

Figure 3. Effect of salinity stress (300 mM NaCl) on the (A) lipid peroxidation (B) electrolyte leakage, (C) hydrogen peroxide and (D) superoxide in *Chenopodium quinoa* with and without Si (50 mM) priming and biochar (BC, 5%) amendment. Data presented is mean of three replicates and different alphabets denote significant difference at $P<0.05$

Impact on antioxidant enzymes

Silicon and biochar boosted antioxidant activities under salt stress

Activity of SOD, CAT, APX, and GR increased by 47.87%, 79.29%, 33.59%, and 47.71%, respectively, due to NaCl treatment over the control plants. Treatment of BC or Si or BC + Si showed significant improvement in the activity of all antioxidant enzymes assayed. Under normal conditions, relative to control, activity of SOD (23.63%), CAT (26.32%), APX (15.74%), and GR (21.56%) was increased maximally by BC + Si treated plants. In NaCl treated plants, treatment of BC and Si individually increased the activity of enzymes with maximal enhancement of 36.17% for SOD, 17.53% for CAT, 14.91% for APX and 15.92% for GR in NaCl + BC + Si treated plants (Figure 4). In addition to this, contents of AsA, GSH, and GSSG also increased by 8.70%, 21.63%, and 29.90%, respectively, due to NaCl treatment over the control. In plants treated with NaCl + BC + Si, increase of 46.03%, 48.16%, and 36.50% for AsA, GSH, and GSSG was observed over the control plants (Figure 5).

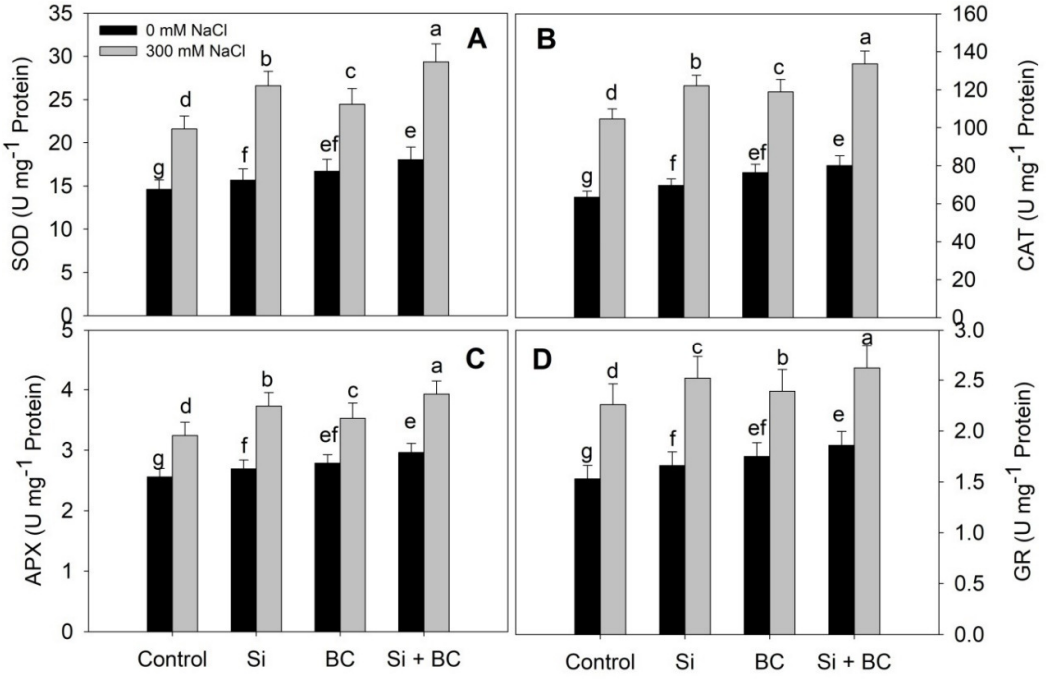

Figure 4. Effect of salinity stress (300 mM NaCl) on the activity of (A) superoxide dismutase, (B) catalase, (C) ascorbate peroxidase and (D) glutathione reductase in *Chenopodium quinoa* with and without Si (50 mM) priming and biochar (BC, 5%) amendment. Data presented is mean of three replicates and different alphabets denote significant difference at $P<0.05$.

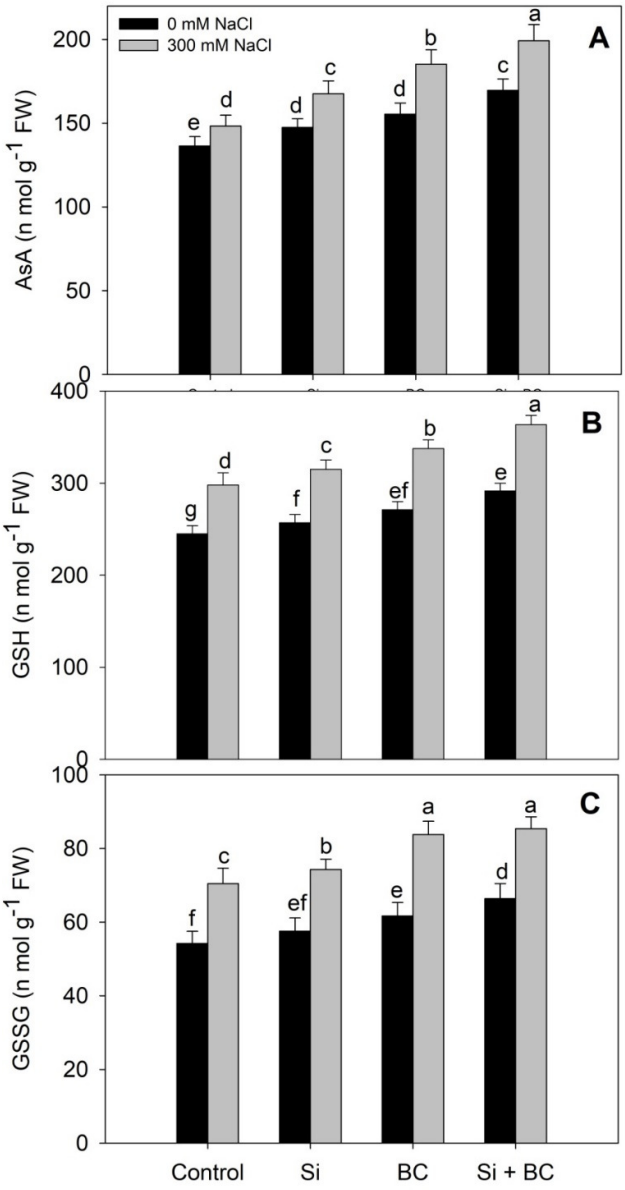

Figure 5. Effect of salinity stress (300 mM NaCl) on the content of (A) ascorbate, (B) reduced glutathione and (C) oxidised glutathione in *Chenopodium quinoa* with and without Si (50 mM) priming and biochar (BC, 5%) amendment. Data presented is mean of three replicates and different alphabets denote significant difference at $P<0.05$

Combined silicon and biochar application restored mineral contents under salt stress

Salinity stress caused significant decline in P (64.97%), K (32.61%), Ca (46.10%), Mg (34.31%), Fe (53.24%), and Na (11.21%) contents over the control. Treatment of BC or Si or BC + Si increased content of P, K, Ca, Mg, and Fe over control with maximal increase observed by combined treatment of Si and BC. Treatment of BC and Si individually or combinedly resulted in amelioration of salinity induced decline in the content of mineral elements with maximal mitigation of 65.51% for P, 40.63% for K, 39.22% for Ca, 22.38% for Mg, 28.50% for Fe, and 11.29% for Na in NaCl + BC + Si treated plants over the NaCl stressed plants (Table 3).

Table 3. Effect of salinity stress (300 mM NaCl) on the content of phosphorous, potassium, calcium, magnesium, iron and sodium in *Chenopodium quinoa* with and without Si (50 mM) priming and biochar (BC, 5%) amendment

Treatments	P (mg g^{-1} DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Mg (mg g^{-1} DW)	Fe (mg g^{-1} DW)	Na (mg g^{-1} DW)
Control	16.56 ±1.17c	32.5 ±2.20d	16.70 ±1.21c	10.20 ±0.66d	14.33 ±0.77d	4.190 ±0.42b
Si	18.20 ±1.33ab	34.6 ±2.61c	18.30 ±1.45a	11.30 ±0.59c	16.10 ±0.81c	3.069 ±0.30c
BC	17.11 ±1.19b	36.2 ±2.83b	17.10 ±1.42ab	12.50 ±0.64b	17.50 ±0.93b	2.523 ±0.31cd
BC + Si	19.01 ±1.68a	39.6 ±3.21a	18.70 ±1.40a	13.60 ±0.73a	21.05 ±1.4a	4.520 ±0.32a
NaCl	5.80 ±0.42f	21.9 ±1.51h	9.00 ±0.78g	6.70 ±0.68g	6.70 ±0.58g	3.720 ±0.21bc
NaCl + Si	7.31 ±0.53e	26.5 ±1.70g	10.20 ±0.83f	7.30 ±0.72f	7.16 ±0.057f	3.870 ±0.33bc
NaCl + BC	7.91 ±0.55e	28.2 ±1.80f	11.40 ±0.89e	7.93 ±0.81f	7.40 ±0.73f	3.966 ±0.39bc
NaCl + BC+Si	9.60 ±0.75d	30.8 ±2.01e	12.53 ±1.02d	8.20 ±0.98e	8.61 ±0.83e	4.140 ±0.46b

Data presented is mean of three replicates and different alphabets denote significant difference at P<0.05.

Silicon and biochar improved nutritional status under salt stress

Salinity stress resulted in significant decline in the nutritional value of seeds by declining total carbohydrates (47.90%), dietary fibres (41.45%), total protein (35.55%), total fat (47.10%), tocopherol (59.67%), total ash (47.11%), and total calories (48.69%) over the control plants. Combined treatment of BC and Si increased total carbohydrates, dietary fibres, total proteins, total fat, tocopherol, total ash and total calories by 23.66%, 11.92%, 20.73%, 14.45%, 14.52%, 8.16%, and 22.18%, respectively, over the control plants. Salinity mediated decline was mitigated by BC amendment as well as Si priming with maximal amelioration by their combined application (Table 4).

Table 4. Effect of salinity stress (300 mM NaCl) on the total carbohydrate, dietary fibre, total protein, total fat, **α**-tocopherol, total ash and total calories in seeds of *Chenopodium quinoa* with and without Si (50 mM) priming and biochar (BC, 5%) amendment.

Treatments	Total carbohydrate (mg g^{-1} DW)	Dietary fiber (mg g^{-1} DW)	Total Protein (mg g^{-1} DW)	Total Fat (mg g^{-1} DW)	α-tocopherol (mg g^{-1} DW)	Total ash (%)	Total calories (Kcal)
Control	27.26 ±1.77cd	6.43 ±0.52b	4.50 ±0.33c	2.767 ±0.38ab	6.47 ±0.41c	4.166 ±0.33b	165.0 ±5.10c
Si	28.50 ±1.81c	6.90 ±0.58b	5.16 ±0.47ab	2.700 ±0.34b	7.33 ±0.57a	4.280 ±0.32ab	162.0 ±5.21c
BC	31.20 ±2.21b	6.66 ±0.48b	4.76 ±0.36b	2.833±0.35cb	7.16 ±0.51ab	4.200 ±0.31ab	181.0 ±6.61b
BC + Si	33.80 ±2.32a	7.20 ±0.67a	5.43 ±0.52a	3.167 ±0.33a	7.41 ±0.52a	4.506 ±0.41a	201.6 ±8.54a
NaCl	14.20 ±1.01f	3.76 ±0.44d	2.90 ±0.14e	1.467 ±0.121d	2.61 ±0.22f	2.200 ±0.12e	84.6 ±4.57e
NaCl + Si	15.80 ±1.01ef	3.90 ±0.47d	3.15 ±0.33e	1.900 ±0.155c	2.40 ±0.21f	2.330 ±0.18e	84.0 ±4.71e
NaCl + BC	14.13 ±1.01f	4.06 ±0.48c	3.30 ±0.37d	1.853 ±0.136c	3.16 ±0.32e	2.750 ±0.21d	80.0 ±4.41ef
NaCl + BC + Si	16.80 ±1.22e	4.50 ±0.53c	3.66 ±0.35d	2.167 ±0.282c	3.73 ±0.31d	3.110 ±0.22c	93.3 ±5.08d

Data presented is mean of three replicates and different alphabets denote significant difference at P<0.05.

Discussion

In contemporary era, plants are counteracted by various adverse environmental conditions reflecting significant decline in their growth and productivity. Employing novel strategies for minimizing the stress damage has been under extensive experimental trials. In this connection, the potential of BC amendment and Si priming to mitigate the damaging effects of salinity stress in *C. quinoa* was studied. Priming *C. quinoa* with Si or/and growing the seedlings on BC amended soil improved the growth significantly and assuaged the salinity stress induced decline in morphological and growth parameters to considerable extent. Increased growth due to Si (Latef and Tran, 2016) and BC (Bananomi *et al*., 2017) has been reported, however, interactive effect of Si priming and BC have not been reported. Dose and timing of Si treatment also influences the plant growth significantly (Ullah *et al*., 2017). Recently, El-Serafy *et al*. (2021) has demonstrated significant alleviation of salinity induced growth decline in *Lathyrus odoratus* by Si priming. In corroboration to present

study, *Zea mays* plants grown on BC amended soils exhibited significant increase in number of leaves, leaf areas, plant height and biomass under salinity stress (Soothar *et al*., 2021). It has been reported that BC imparts significant impact on the soil properties including N, P, K, pH, soil moisture content, water holding capacity, organic matter content, and bulk density, hence assists in the maintenance of plant growth (Jien and Wang, 2013; Ali *et al*., 2017; Karim *et al*., 2020). In present study, *quinoa* plants grown on BC amended soil exhibited significant enhancement in the uptake of mineral elements. Maintaining significantly higher concentrations of mineral ions affects the functioning of key metabolic pathways by influencing the enzyme functioning, protein synthesis, water balance, photosynthesis and stress tolerance (Ahanger *et al*., 2017, 2019; Soliman *et al*., 2020; Ahmed *et al*., 2022). Application of Si influences the uptake of mineral ions and the growth regulation significantly hereby mitigating the damaging effects of cadmium (Jan *et al*., 2018; Noman *et al*., 2021).

In the present study, both Si priming and BC amendment resulted in improved water potential and relative water content under normal as well as salinity stress conditions. Maintaining increased tissue water content helps to regulate the growth by influencing the cellular division and cell proliferation (Setter and Flannigan, 2001). Salinity adversely affects the tissue potential by inducing the osmotic and ionic stress thereby restricting the uptake of water and essential ions (Soni *et al*., 2021). Si mediated improvement in the leaf water content and K/Na ratio significantly influences the growth and salt tolerance (Latef and Tran, 2016). Salinity mediated decline in water potential and tissue water content can restrict the major plant processes like photosynthesis which was evident in present study also. Reduced water availability together with declined water uptake under salinity stress influences the WUE (Kotagiri and Kolluru, 2017), hence affecting the growth and yield productivity. Here, Si and BC treatment imparted considerable effect on the water uptake, hence improving the WUE. Besides this, salinity mediated significant reduction in growth, chlorophyll synthesis, and photosynthesis, however, Si and BC treatment individually as well as combinedly mitigated the decline. Earlier salinity induced decline in stomatal and non-stomatal photosynthetic parameters has been reported in *Solanum melongena* (Shaheen *et al*., 2013), *Solanum lycopersicum* (Ahanger *et al*., 2019b) and wheat (Soni *et al*., 2021). Salinity declines the synthesis of chlorophyll intermediates, and hence chlorophyll synthesis and photosynthetic functioning (Qin *et al*., 2020). In present study, Si and BC treatment improved Mg uptake which may have also contributed to increased chlorophyll synthesis. Magnesium forms central component of chlorophyll molecules and Si and/or BC treatment mediated increase in uptake of essential mineral elements may have contributed to photosynthetic regulation by improving the functioning of key enzymatic and non-enzymatic components that contribute to redox regulation (Asgher *et al*., 2014; Ahanger and Agarwal, 2017; Elkelish *et al*., 2019; Ahanger *et al*., 2019a; Soliman *et al*., 2020). Salinity stress reduces photosynthesis by inactivating the Rubisco functioning and thereby interfering with the normal metabolism of plants (Fatma *et al*., 2016; Gong *et al*., 2018). Besides, salinity adversely disturbs the chloroplast structure and functioning by changing number, size, and lamellar organization (Hameed *et al*., 2021). Silicon application has been reported to improve photosynthesis in *Pisum sativum* by protecting the oxidative damage (Jan *et al*., 2018). BC treatment has been reported to improve chlorophyll and maximal photochemical activity of maize plants under normal as well as salinity stress conditions (Soother *et al*., 2021). Increased photosynthesis results from the precise regulation of stomatal and non-stomatal components, protection of chloroplast machinery from the oxidative effects by lessening the accumulation of ROS and the maintenance of optimal water content (Ahanger *et al*., 2020; Begum *et al*., 2021; Qin *et al*., 2021). Si and BC mediated photosynthetic regulation can be attributed to up-regulation of antioxidant system, redox and nutrient homeostasis and enzyme functioning. Si up-regulates the expression of key genes like *Psb* and *Pet* isozymes reflecting in increased photosynthesis, PSII activity and electron transport rate in water stressed tomato (Zhang *et al*., 2018). However interactive effects of Si and BC on photosynthesis are not reported earlier.

Improved growth and salinity tolerance in Si and BC treated plants was correlated to their effects on ROS production and antioxidant enzyme system. Salinity resulted in accumulation of ROS including H_2O_2

and O_2- reflecting in increased lipid peroxidation and electrolyte leakage. Salinity mediated decline in membrane stability results from increased damage to membrane lipids and proteins, thereby resulting in leakage of essential cellular components (Mansour, 2014). Changes in lipids affect the membrane proteins and functioning of signalling molecules, fluidity, and permeability of membranes (Guo *et al*., 2019). Earlier salinity stress mediated increase in ROS production and lipid peroxidation has been reported in tomato (Ahanger *et al*., 2019b), soybean (Soliman *et al*., 2020), and *A. tricolor* (Sarkar and Oba, 2020). In barley, BC amendment significantly reduced the lipid peroxidation and hence prevented electrolyte leakage (Hafiz *et al*., 2020). Plasma membrane functioning is considered as important indicator of salinity tolerance (Mansour, 2013). Decline in ROS and lipid peroxidation due to application of Si (Jan *et al*., 2018) and BC (Farhangi-Abriz and Torabian, 2017) has been reported earlier. Decline in ROS accumulation was much evident in plants grown with combined treatment of Si and BC. Maintaining optimal concentration of ROS can potentiate the plant responses to stresses by mediating signalling (Huang *et al*., 2019; Castro *et al*., 2021). However, over-accumulation can seriously impede the key structures and their functioning. Reduced oxidative damage and improved membrane stability in Si and BC treated plants can be due to the up-regulation of antioxidant system and the accumulation of osmolytes contributing to quick elimination of ROS. Antioxidant system comprised of enzymatic and non-enzymatic components alleviates the stress mediated growth and metabolic alterations by protecting peroxidation of key macromolecules (Ahanger *et al*., 2017; Soliman *et al*., 2020; Elkelish *et al*., 2020). Upregulation of antioxidant system under stressful conditions contributes significantly to growth, photosynthetic protection and nutrient uptake (Iftikhar *et al*., 2019). Soil amendment with BC has been reported to upregulate the activity of antioxidant enzymes in bean (Farhangi-Abriz and Torabian, 2017), barley (Hafez *et al*., 2020) and faba bean (El-Nahhas *et al*., 2021) reflecting in increased tolerance to stresses. In salt stressed *Glycyrrhiza uralensis*, Zhang *et al*. (2017) has also demonstrated that Si treatment up-regulated the antioxidant system functioning by increasing the activity of CAT and APX, and content of GSH reflecting significant alleviation of salinity mediated oxidative damage. Maintaining higher activities of antioxidant enzymes and the content of non- enzymatic antioxidants can potentially prevent the stress damage on the key cellular structures and their functioning (Ahanger *et al*., 2020). Priming with Si potentiated the BC amendment induced strengthening of antioxidant system reflecting in the significant decline of ROS accumulation and the oxidative damage. Non-enzymatic antioxidants including GSH, AsA and tocopherol contribute to growth and metabolism protection under adverse conditions by (a) scavenging free radicals, (b) maintaining redox homeostasis and (c) protecting the enzyme functioning (Munne-Bosch, 2005; Hasanuzzaman *et al*., 2017; Akram *et al*., 2017; Soliman *et al*., 2020; Ahanger *et al*., 2021).

In addition, the accumulation of osmolytes including sugars, soluble protein, free amino acids and GB due to Si priming and BC amendment was obvious. Significant accumulation of compatible osmolytes contributes to plant growth regulation by maintaining tissue water content thereby preventing the osmotic effects of stresses (Ahanger *et al*., 2014). Supplementation of Si has been reported to improve growth and stress tolerance (Jan *et al*., 2018). Similarly, BC amendment improved growth and RWC in through greater accumulation of sugars in barley thereby helping in withstanding the stress better (Hafez *et al*., 2020). Farhangi-Abriz and Torabian (2017) has also demonstrated significant alleviation of salt stress induced oxidative damage in bean plants through accumulation of soluble sugars, soluble protein, proline, and GB. Silicon mediated increase in compatible osmolyte accumulation in *Acacia gerrardii* has been reported to increase growth and photosynthesis reflecting in reduced oxidative damage (Al-Huqail *et al*., 2019). Si has the potential to alleviate salinity induced ionic and osmotic toxicity in organ specific pattern driven by maintenance of root morphological traits and osmotic potential (Yan *et al*., 2020). Increase in compatible osmolyte accumulation due to Si and BC treatment protected *C. quinoa* seedlings from salinity induced growth and photosynthetic decline through maintenance of osmotic potential and distribution of toxic ions. Sugars have key role in stress signalling, thereby assist in eliciting a quick response for better stress mitigation (Ahmad, 2019). Under

stressful conditions, sugars act as key players of stress perception and signalling as well as regulatory hub for gene-expression for ensuring responses to osmotic adjustment, ROS neutralization, and maintenance of cellular energy status (Saddhe *et al*., 2021). Priming with Si and amendment of soil with BC resulted in significant increase in sugars, thereby contributing to salinity tolerance through maintenance of tissue water and protecting damage to sensitive processes like photosynthesis and structures like membranes.

The salinity stress induced decline in growth was also linked with the considerable decline in the nutritional aspects like carbohydrates, dietary fibres, fats, proteins, tocopherol and total calories. However, it was interesting to observe that plants raised after Si priming or on BC amended soils exhibited significant increase in the nutritional contents in their seeds. This reflects in the beneficial effect of Si and BC in maintaining the nutritional value of seeds of *C. quinoa* which could contribute to its increased medicinal importance. Salinity adversely affects the nutritional quality of seeds by altering the key components including fatty acids, mineral ions, and amino acids (Toderich *et al*., 2020), however, effect varies with the type of genotype. Salinity mediated decline in nutritional components of *C. quinoa* seeds may drastically affect the pharmacological potential of this plants. However, Si and BC treatments prove beneficial in protecting and improving the medicinal aspects. Though the reports discussing the role of Si and BC in maintaining the nutritional aspects of *quinoa* are not available, present study advocates the usage of Si and BC supplementation for better growth and salinity stress tolerance in *quinoa*.

Conclusions

Salinity stress adversely affected the growth and photosynthesis of *C. quinoa* and Si priming andor BC amendment mitigated the decline to a considerable extent. Salinity damaged membranes by increasing the generation of ROS, hence triggering lipid peroxidation. Priming with Si or soil amendment with BC ameliorated the damaging effects of salinity by up-regulating the antioxidant system, osmolyte accumulation, and maintaining the tissue water potential. Moreover, the nutritional aspects of *C. quinoa* seeds were also improved due to Si and BC treatment simultaneously.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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