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Full Length Article

Reversal of Salt-Induced Oxidative Damage to Buckwheat by Medium Supplementation of Some Nitrogenous and Non-Nitrogenous Stress Relieving Chemicals

Bushra Mehreen[1], Abdul Wahid[1*], Muhammad Sajid Aqeel Ahmad[1] and Shahzad Maqsood Ahmad Basra[2]
[1]*Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan*
[2]*Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan*
[*]For correspondence: drawahid@uaf.edu.pk; ORCID: 0000-0002-6107-4340

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Abstract

Food scarcity is a global issue due to various biotic and abiotic stresses, and salinity stress is a major one. Human activities are adding various salts to agricultural soils, which deteriorate plant growth, development and economic yield. The adverse effects of salinity can be lessened by using various salts in lower amounts. This pot experiment was conducted to improve salt tolerance in buckwheat (*Fagopyrum esculentum* Moench) by using selected levels of some nitrogenous [potassium nitrate (KNO_3; 15 mM), glycine betaine (GB; 160 mM), calcium nitrate ($Ca(NO_3)_2$; 0.8 mM) and thiourea (TU; 0.4 mM)] and non-nitrogenous [calcium chloride ($CaCl_2$; 16 mM), potassium chloride (KCl; 6 mM), ascorbic acid (AsA; 2 mM), salicylic acid (SA; 0.4 mM)] compounds hereinafter called as stress relieving compounds (SRCs). Eight kg of loam soil was filled and ten seeds were sown in each pot. Fifteen days after germination, half of the pots were salt stressed with 100 mM NaCl solution in three installments while the remaining half were grown without salt stress. Just after that, selected levels of SRCs were medium supplemented. After 15 days of treatment application, the plants were harvested for estimation of various growth, oxidants [hydrogen peroxide (H_2O_2) and malondialdehyde (MDA)], antioxidants (vitamins and phenolics) and mineral nutrient contents. Results showed that all parameters were reduced under salinity stress except shoot and root Na^+, H2O2, MDA, phenolics, flavonoids and anthocyanins due to salt stress. Application of SRCs alleviated the adverse effects of salt and enhanced the values of attributes by preventing the entry of Na^+ into the root and its aerial transport. Among SRCs, for most of the parameters, $CaCl_2$ followed by KCl was more effective, especially under salinity stress. In crux, chlorides of Ca and K were more effective than the other SRCs applied in this study in improving salinity tolerance in buckwheat. The selected levels of SRCs are recommended for growing buckwheat and fetching satisfactory yield in marginally saline soils in future.

Keywords: Buckwheat; Calcium; Medium supplementation; Osmoprotectants; Salinity

Introduction

Salinity is one of the major stresses that causes severe damage to food quality and quantity globally (Ma *et al.* 2019). This is due to accumulation of various salts of calcium, magnesium, and sodium in soil where accretion of NaCl is more hazardous because of its easy ionization into Na^+ and Cl^- (Pérez-Labrada *et al.* 2019) that hinders growth and development of plants which ultimately reduces their productivity (Ma *et al.* 2019). It is estimated that saline stress caused by anthropogenic activities have damaged 70% of the area of land, still this area is increasing frequently, and due to overpopulation adding fuel in enhancing food scarcity issues (Hossain 2019; Yousaf *et al.* 2022). Salinity in soil causes oxidative damage due to the accretion of hazardous ions in plant tissues, resulting in ionic imbalance and water unavailability. These changes adversely influence normal metabolic activities of cells and cause oxidation of crucial structures such as membranes of chloroplast resulting in deterioration of photosynthetic pigments and decline in photosynthesis which ultimately reduce normal plant growth (Zhang *et al.* 2023; Zhou *et al.* 2024).

According to researchers, eradication of salinity is problematic but use of salt infected areas for cultivation is possible by application of some stress relieving chemicals (SRCs) in different crops (Srivastava and Sharma 2022). Some of the most intensely investigated chemicals chlorides and nitrates of potassium (Chen *et al.* 2021) and calcium (Nessim and Kasim 2019; Nizam *et al.* 2019), as well as some organic metabolites including ascorbic-acid (AsA)

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(Hasanuzzaman *et al.* 2023), salicylic-acid (SA) (El-Beltagi *et al.* 2023), glycinebetaine (GB) (Rady *et al*. 2018) and thiourea (TU) (Patade *et al*. 2020). K^+ is crucial for normal plant growth, balances ionic contents by inducing efflux of toxic ions like Na^+ and Cl^- and influx of essential ions, protects tissue from peroxidation, enhances photosynthesis and other metabolic activities need for normal growth and development of plants (Taha *et al.* 2020). Ca^{2+} is known as signaling molecules, balances ionic uptake, activates antioxidants to reduce oxidative damage caused by salinity and improves cellular metabolism which enhances plant growth (Abeed *et al.* 2023). AsA is one of the important antioxidants and is involved in scavenging harmful oxidants, activating other antioxidants, increasing photosynthesis, and improving ionic balance (Azeem *et al.* 2023). SA belongs to phenolic compounds, involved in the activation of antioxidants, reduces oxidative stress, increases gas exchange, improves accumulation of secondary metabolites, enhances influx of essential ions and efflux of toxic ions (Azeem *et al.* 2023). TU is a synthetic bioregulator, reduces peroxidation of cellular structures by modulating redox reactions (Nazir and Wahid 2023). GB application enhances water absorption, and douses ROS by activating antioxidants, and improves photosynthesis and plant growth (Dawood *et al.* 2021).

Buckwheat is considered as one of the important crops that have a high nutritional profile due to the presence of higher levels of flavonoids such as rutin and other secondary metabolites, having medicinal properties such as anti-tumor and anti-inflammatory etc. (Kim *et al.* 2020) and its flour is gluten free (Giménez-Bastida *et al.* 2015). Buckwheat is tolerant to various environmental stresses due to production of high phenolic contents, but this crop is sensitive to salinity. High salt contents influence plant growth by deteriorating quality and quantity of metabolic compounds produces by buckwheat plants (Ma *et al.* 2019). The already explored information is not enough to explain adverse effects of high salt contents on buckwheat plants and their mitigation strategies. Furthermore, the data are entirely scanty that compares the influence of externally applied nitrogenous and non-nitrogenous organic and inorganic compounds in lessening the salinity stress induced oxidative damage on buckwheat. It is predicted that these compounds have a tendency to improve salinity tolerance by dousing the ROS. The purpose of this experiment was to determine the comparative efficacy of some selected nitrogen containing and non-nitrogen containing chemicals in improving the oxidative damage tolerance of common buckwheat under salinity by supplementation of SRCs. It is postulated that the medium supplementation of SRCs may enhance common buckwheat growth by alleviating saline stress.

Materials and Methods

Experimental details

For this study, a completely randomized pot experiment was conducted on common buckwheat in (late-October to mid-November) year 2020 in old botanical garden. Buckwheat germplasm was taken from Department of Agronomy, University of Agriculture, Faisalabad. Ten seeds were soon in each pot containing 8 kg loam soil. The height of the pot was 15 inches while diameter was 10 inches. In total there were 162 pots. The design of the experiment was completely randomized (CRD) factorial with three replications per treatment. After germination, the plants were thinned to three in each pot. After 15 days of growth, half of the potted plants were treated with salt stress (100 mM) by applying saline solution in three equal installments whereas the remaining half were growing as control (no salt stress) alongside the salt treated plants. Shortly after last installment of saline solution, medium application of the non-nitrogenous stress relieving compounds (SRCs) including calcium chloride ($CaCl_2$; 16 mM), potassium chloride (KCl; 6 mM), ascorbic acid (AsA; 2 mM), salicylic acid (SA; 0.4 mM), and nitrogenous SRCs i.e., potassium nitrate (KNO; 15 mM), glycinebetaine (GB; 160 mM), calcium nitrate ($CaNO_3$; 0.8 mM) and TU; 0.4 mM) were applied to control and salinity stressed plants. These levels of SRCs were selected for showing maximum buckwheat shoot and root dry weight and leaf area per plant in a screening experiment. After treatment application, the plants were grown for 15 more days, and then harvested.

Estimation of growth parameters

After 15 days of treatment application, the plants were uprooted, washed to remove dust particles and their leaves were counted. The leaf width and length were measured to estimate leaf area (Carleton and Foote 1965). The roots were gently removed from the soil and washed in running water, briefly blot dried, and their fresh weight was taken. A portion of the freshly harvested shoot and root material was frozen in a freezer at -40ºC for further analyses. A portion of the materials measured for fresh weight was dried in an oven at 70°C for a week to determine dry weight.

Measurement of oxidants

To determine the extent of oxidative damage due to salinity stress, the concentration of H_2O_2 and malondialdehyde (MDA) were measured in shoot and root. For H_2O_2 determination, 0.1 g sample was homogenized with 1 mL TCA (0.1%) and centrifuged (12000 rpm) for 15 min. Took 0.5 mL supernatant, mixed with 0.5 mL buffer (phosphate; pH=7), added 1 mL of 1 M potassium iodide and vortexed. Readings were taken on 390 nm and for estimation of final quantities standard curve was used (Velikova *et al.* 2000). For the estimation of MDA, 1 mL supernatant from the above was mixed with 1 mL of 0.5% of thiobarbutaric acid, heated in water bath for 50 min at 95°C. Then chilled in ice and observations were noted on 532 and 600 nm (Heath and Packer 1968).

Vitamins

In non-nitrogenous compounds AsA and total soluble phenolics were determined. For AsA, a 0.24 g sample was homogenized with 6% TCA. One mL of the extract was mixed with acidified dinitrophenyl hydrazine. Added 1 drop of TU (10%), boiled (10 min), chilled and added 5 mL of 80% H_2SO_4, and took the absorbance at 530 nm and final values were calculated from the standard curve (Mukherjee and Choudhuri 1983). To measure the concentrations of niacin with the method of Okwu and Josiah (2006), 0.1 g sample was mixed with 1 mL H_2SO_4 (1 N), shaken for 20 min, added a drop of ammonia solution, and filtered. Took 1 mL filtrate, mixed with 0.5 mL KCN (10%) and H_2SO_4 (0.02N), vortexed and absorbance was noted on 470 nm. For final calculation of niacin standard curve was used. To measure riboflavin, 0.1 g of fat free sample homogenized with 2 mL ethanol (50%). Took 1 mL extract and mixed with 1 mL $KMnO_4$ (5%), added 1 mL 30% H_2O_2, and heated for 30 min. A 0.2 mL solution of sodium sulfate (40%) was added to the mixture and diluted with water. Waited for 5 min, separated the colorless layer and readings were noted at 510 nm using 50% ethanol as blank (Okwu and Josiah 2006).

Soluble phenolics

To measure total soluble phenolics, 0.1 g sample was crushed with 1 mL acetone (80%). Heated at 50°C, centrifuged (15 min), took 100 µL aliquot and diluted with 1 mL water. Added Folin phenol reagent (0.5 mL), shaken, mixed with 20% $NaHCO_3$ (2.5 mL) and vortexed. After 20 min readings were taken at 750 nm (Julkunen-Tiitto 1985). The flavonoids were measured with the method of Zhishen *et al.* (1999), while anthocyanins were estimated with the protocols of Stark and Wray (1989).

Analysis of elements

To digest the dried leaf and root samples, 0.5 g material was added in a flask with nitric acid (5 mL) and few drops of perchloric acid. Heated at 150–200°C until mixture got cleared; filtered and diluted with water. This extract was used to estimate Na^+, K^+ and Ca^{2+} by using flame photometer (PEP7-Jenway, UK). To estimate phosphate-P, 1 mL extract was mixed with 2 mL HNO_3, diluted with water (8 mL), added Barton's reagent (1 mL), mixed and diluted, waited for 10 min. The absorbance of the colored complex was taken at 420 nm by using a spectrophotometer (Yoshida *et al.* 1976). To measure sulfate-S, 10 of mL extract mixed with 1 mL HCl (6N) and arabica-gum solution (0.5 %), added $BaCl_3$ (0.5 g), shake until solution got cleared, measurements were taken at 340 nm to estimate sulfate-S (Tendon 1993). To estimate nitrate-N, 3 mL of sample taken from the extract (extracted using a mixture of conc. H_2SO_4 and 35% H_2O_2) was added to 7 mL of 0.1% chromotropic acid (CTA; prepared by dissolving CTA in 0.1 N H_2SO_2) and vortexed. After 20 min readings were taken at 430 nm (Kowalenko and Lowe 1973).

Statistical analysis

"Statistix 8.1" was used for applying two-way ANOVA. For comparison of treatments, mean values of treatments were used to run LSD test. Software R studio was used to draw chord diagram and correlations matrix heatmap to draw the relationships among the different parameters.

Results

Growth attributes

SRCs markedly enhanced growth attributes of buckwheat plants under saline stress. Shoot and root dry weights showed significant reduction under salinity when no SRCs were applied. However, $CaCl_2$ exhibited the maximum value of this parameter under no stress and saline stress followed by KCl. Likewise, leaf area and leaf number were also enhanced under salinity when SRCs were applied. The supplementation of $CaCl_2$ followed by KCl revealed the maximum value of these characteristics in no stress. However, the minimum value of these parameters was recorded under salinity stress with no SRCs application. Overall, TU followed by GB were the least effective among all SRCs under normal as well as saline conditions for all growth attributes (Fig. 1).

Oxidants

Although salinity stress greatly increased the concentrations of H_2O_2 and MDA in both shoot and root of buckwheat, a significant ($P < 0.01$) reduction in these oxidants was observed by SRCs supplementation under salinity stress. A similar trend of changes was recorded in the plant parts, root indicated more concentration of these compounds than shoot. Among SRCs, $CaCl_2$ was highly effective and showed a minimum value of these parameters under followed by KNO_3 while thiourea was the least effective in reducing their amounts in both shoot and root (Fig. 2).

Antioxidants

Vitamins and phenolic compounds were measured for their roles in nullifying oxidative damage under stressful conditions. Medium supply of SRCs, with some differences, significantly ($P<0.01$) increased the amounts of ascorbic acid, niacin and riboflavin in the buckwheat shoot and root tissues both under control and salinity stress conditions, although the effectiveness of the SRCs was greater under salt stress and root tissue was more positively affected. Among the SRCs, $CaCl_2$ KCl, SA and AsA were more effective in enhancing shoot and root vitamin (antioxidant)

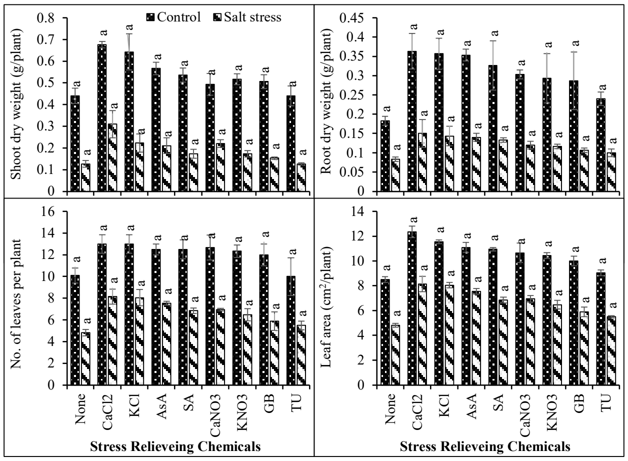

Fig. 1: Effect of nitrogenous and non-nitrogenous SRCs on growth characteristics of buckwheat under salt stress. In this and subsequent figures, the alphabets on bars show significant ($P < 0.05$) differences between the treatments means

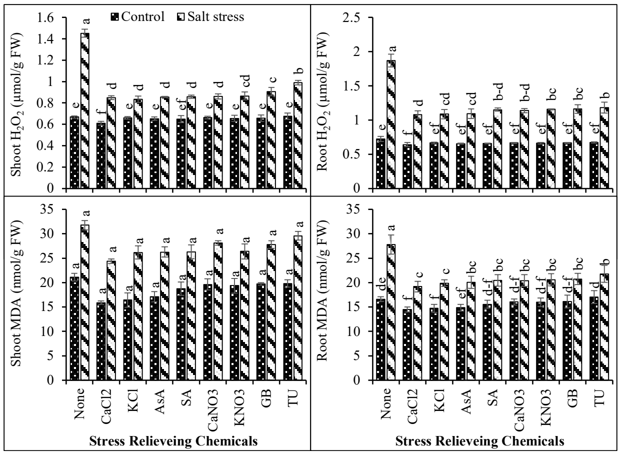

Fig. 2: Changes in the concentrations of H_2O_2 and MDA as indicators of oxidative stress with the exogenous application of nitrogenous and non-nitrogenous SRCs in the shoot and root of buckwheat under salt stress

contents under salinity stress (Fig. 3).

Applied salinity conditions significantly increased the soluble phenolic content, while their accumulation was greater in the shoot tissue. All the SRCs were greatly effective in reducing the soluble phenolics contents but $CaCl_2$ was relatively more effective while thiourea and glycinebetaine were the least effective. These effects were more obvious in the root than shoot when expressed based on control (Fig. 4). The flavonoids and anthocyanins contents were higher in shoot than root under either condition. When calculated over control, applied salinity stress reduced the content of both these metabolites while $CaCl_2$, KCl, AsA and SA were more promising and GB and thiourea were on a bottom edge. Nonetheless such as effect was more clearly seen in root than shoot tissue (Fig. 4).

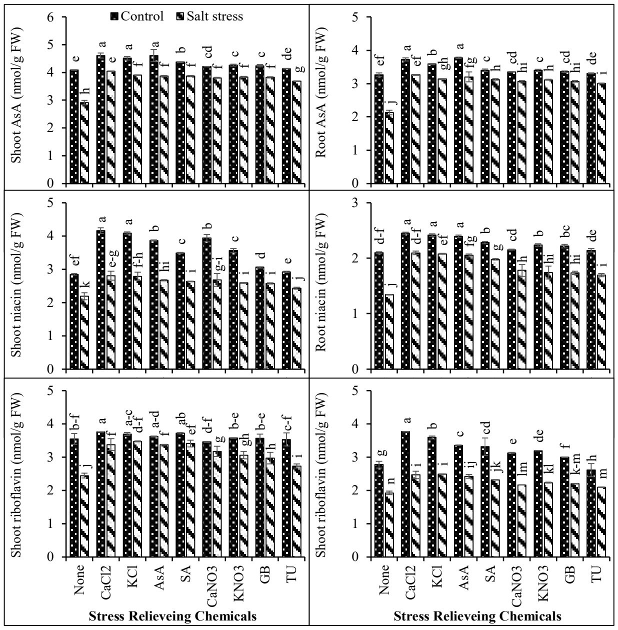

Fig. 3: Changes in the vitamin concentrations with the exogenous application of nitrogenous and non-nitrogenous SRCs in the shoot and root of buckwheat under salt stress

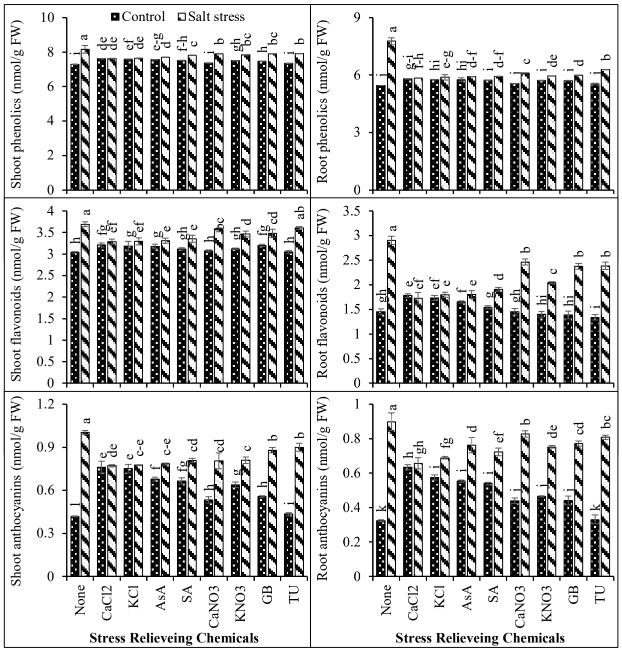

Fig. 4: Changes in the concentration of some secondary metabolites with the exogenous application of nitrogenous and non-nitrogenous SRCs in the shoot and root of buckwheat under salt stress

Ionic contents

Salinity caused a severe reduction in elemental contents of buckwheat plants while applied SRCs significantly ($P < 0.05$) enhanced their concentration by alleviating adverse effects of high salt contents except Na^+ which exhibited opposing results. The elevation in shoot and root Na^+ contents were recorded under saline conditions when no SRCs were applied. Supplementation of SRCs reduced the accumulation of Na^+ in shoot than in root (Fig. 5a). Conflicting to these results, the decline in shoot and root K^+ and Ca^{2+} contents were estimated under salt stress without SRCs application. The medium supplementation of SRCs increased the content of both these ions under control and salt stress conditions. However, the effectiveness of the SRCs was more clearly noticed in root than in shoot when expressed over control (Fig. 5a).

In case of shoot and root nitrate-N contents, KNO_3 exhibited maximum increase in this parameter under no stress and saline stress. High salt contents showed the lowest value of this ionic content without SRCs supplementation. Among the SRCs, $Ca(NO_3)_2$, followed by KNO_3 and GB were the most promising in enhancing nitrate-N content in shoot and root under control condition, while $Ca(NO_3)_2$, KNO_3, GB and $CaCl_2$ were quite effective under salinity stress (Fig. 5b). Applied salinity also adversely influenced the shoot and root phosphate-P contents, whilst all the applied SRCs were almost equally effective in improving phosphate-P in both shoot and root. Nonetheless, $CaCl_2$, KCl and AsA among the SRCs were relatively more promising in improving this nutrient in shoot and root tissues (Fig. 5b). In case of shoot sulfate-S contents, all the SRCs enhanced this parameter in shoot and root tissue under control condition. Under salinity stress, the medium supplementation of SRCs almost equally improved shoot sulfate-S content, while root sulfate-S, $CaCl_2$, KCl, AsA and thiourea were more effective (Fig. 5b).

Chord diagram and correlation matrix

The chord diagram showed balanced and evenly distributed color intensities depicting stable plant growth, nutrient contents, and secondary metabolite production, while under salinity stress concentrated interactions were observed suggesting a disturbed or weaker relationship for plant growth and nutrient uptake, however, the interaction of antioxidants and secondary metabolites exhibit stronger interactions, marked by thicker and more densely colored arcs. TU and CaNO3 showed stronger interaction for ion uptake and growth parameters, while SA and AsA treatments showed thicker arcs of antioxidants and secondary metabolite production (Fig. 6).

The heatmap correlation matrix depicted a strong linear correlation of $CaCl_2$, SA, KCl, and AsA on all the studied indicators except for shoot and root H_2O_2, MDA, and NA, while an opposite relationship was observed for control and TU applications under control conditions (Fig. 7). Moreover, $CaNO_3$, KNO_3 and GB showed a negative relationship with RNiacin, SAsA, RFlavo, Rantho, SAntho, SRiFlav, SDW, SP, Sflavo, RS, RRiFlav, Sphenol, and Rphenol, while the positive linear relationship was seen for other parameters. However, salinity-exposed plants without priming treatment showed a strong negative relationship with LA, RDW, Rniacin, Sniacin, RRiFlav, SRiFlav, NOL, RK, SK, SS, SS, SP, RS, RP, RAsA, SasAm RCa, SCa, and

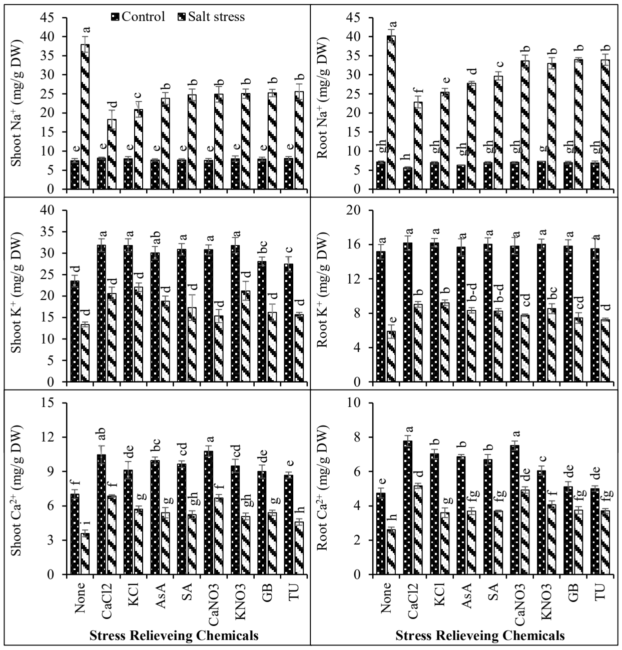

Fig. 5a: Changes in the concentration of Na^+, K^+ and Ca^{2+} with the exogenous application of nitrogenous and non-nitrogenous SRCs in the shoot and root of buckwheat under salt stress

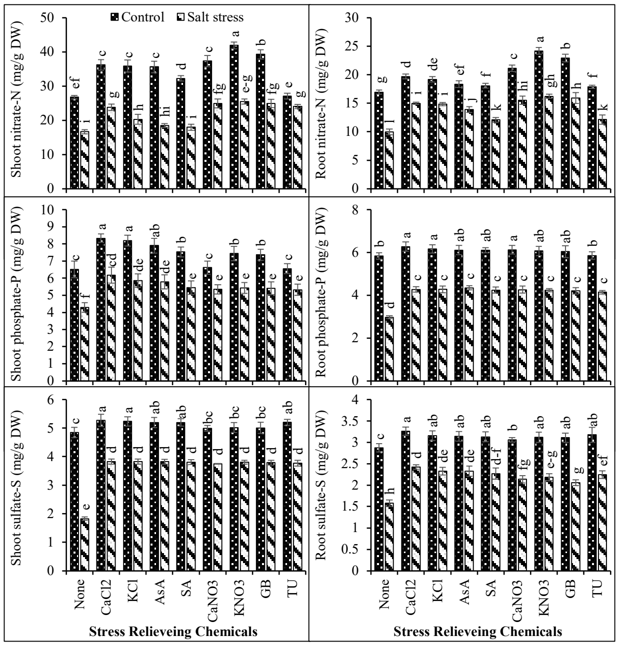

Fig. 5b: Changes in the concentration of nitrate-N, phosphate-P and sulfate-S with the exogenous application of nitrogenous and non-nitrogenous SRCs in the shoot and root of buckwheat under salt stress

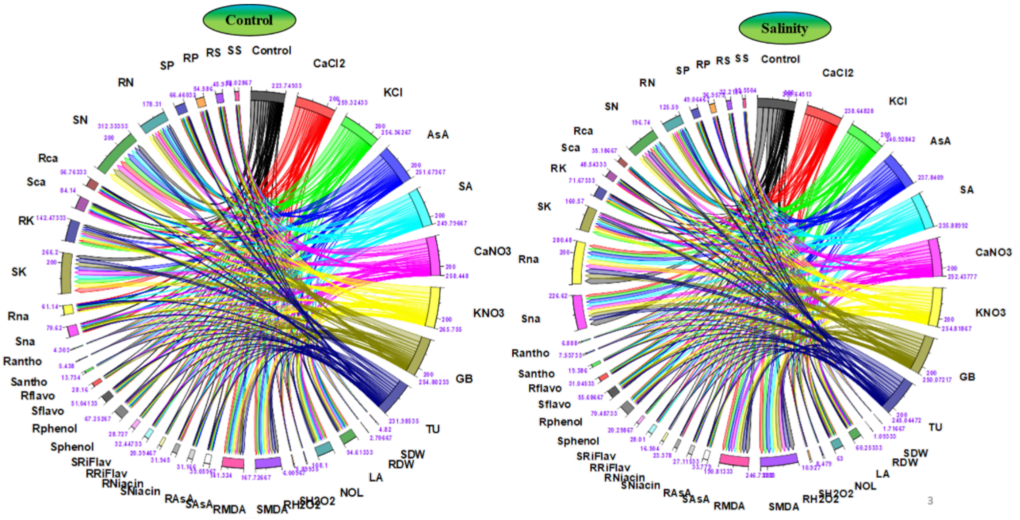

Fig. 6: Chord diagram of different growth parameters and physiological attributes of buckwheat under control and salt stress conditions

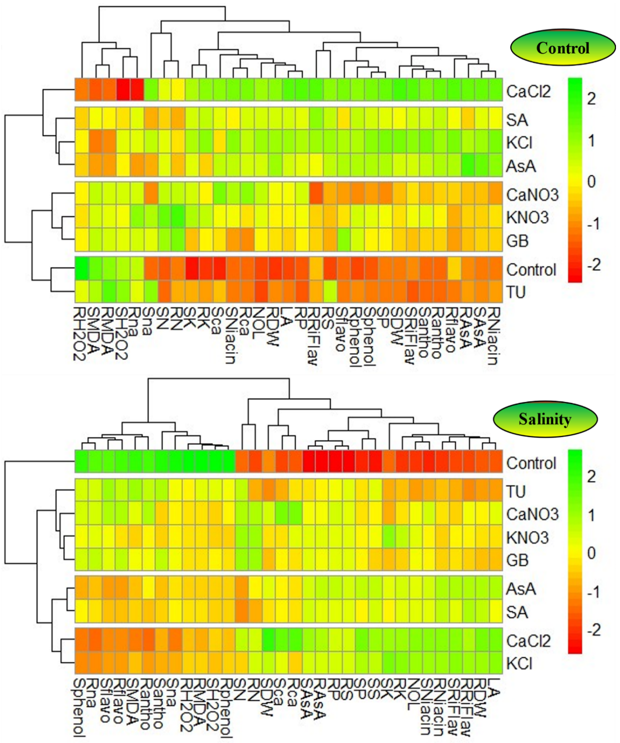

Fig. 7: Heatmap matrix of different growth parameters and physiochemical attributes of buckwheat under salinity and control conditions

SDW, while a significant positive correlation was observed for SNa, RNa, SMDA, RMDA, Santho, Rantho, Rflavo, Sflavo, Rphenol, and Sphenol. However, the opposite trend of correlation was recorded for $CaCl_2$, KCl, AsA, and SA priming treatments. Furthermore, TU, $CaNO_3$, GB, and KNO_3 showed non-significant contributions for almost all the studies indicators (Fig. 7).

Discussion

Accumulation of high salt contents such as NaCl adversely influences normal growth of plants by deteriorating their metabolic processes. The decrease in production of photosynthates reduces plant biomass (Abeed *et al.* 2023). In current study, decline in plant biomass, leaf number and leaf area were recorded under saline stress (Fig. 1), which is due to oxidative stress (Fig. 2). The accumulation of these perilous ions generates oxidative stress, which damages cellular structure by peroxidation of biomolecules, causing decline in plant metabolic functions which culminates in reduced biomass yield. However, reversal of salinity stress effects was noted with the medium supplementation of SRCs, resulting in improved biomass yields (Fig. 1).

Plant species have an innate tendency to overcome oxidative damage with the expression of antioxidants, which may be enzymatic and non-enzymatic in nature. Their functions can be enhanced by exogeneous supplementations (Rudenko *et al.* 2023). Among the non-enzymatic antioxidants, phenolics and vitamins are considered as the most important (Rudenko *et al.* 2023; Mehdizadeh *et al.* 2024). In the present study, as a sensitivity of buckwheat to salt stress, there was quite a reduced synthesis of vitamins (ascorbic acid, niacin and riboflavin), which were measured as antioxidants, while medium supplemental SRCs were quite effective (Fig. 3). However, contrary to the vitamins, amount of phenolic metabolites (soluble phenolics, flavonoids and anthocyanins) was increased due to salinity stress and declined due to the SRCs supplementation (Fig. 4). The phenolics are widely reported to act as antioxidants to protect the cytoplasmic membranes due to their peculiar molecular structures (Foss *et al.* 2022). This implied that phenolic accumulation acted as first line of defense against the salt toxicity, while their amounts were normalized with the exogenous application of SRCs (Fig. 4).

The accumulation of perilous Na^+ ions generates oxidative stress, which damages cellular structure due to peroxidation of biomolecules. Excessive Na^+ in soil solution occupies protein channels on the root plasmalemma thus reducing the absorption of essential nutrients into the plant roots, resulting in influx of toxic ions and causing imbalance of ionic contents (Shahid *et al.* 2020). All plants have multiple sets of transporter proteins such as HKTs, NRTs, IRTs, PIPs responsible for the uptake of essential elements. So, higher concentrations of toxic ions in soil can easily occupy these transporters and cause ionic imbalance (Barzana *et al.* 2021). A rise in Na^+ and decline in the tissue levels of K, Ca, N, P and S were noted in buckwheat plants while SRCs were able to reduce Na^+ and enhance essential nutrient content more in root than in shoot (Fig. 5a-b). This implied that the medium supplementation of SRCs improved the activity of transporters on the root plasmalemma to improve the essential nutrient contents (Feng *et al.* 2023).

The application of various SRCs increases plant growth by alleviating salinity damage (Feng *et al.* 2023). Applied Ca^{2+} signals K^+ influx which reduces the uptake of Na^+ because K^+ and Na^+ compete at the root surface (Abeed *et al.* 2023). Ca and K not only enhance the influx of Ca^{2+} (Behtash *et al.* 2023) and K^+ (Moghaddam *et al.* 2023) but also increases the uptake of nitrogen containing ions. Plants use nitrogen in the biosynthesis of photosynthetic pigments, increases accumulation of photosynthates, which improves plant biomass. The high contents of H_2O_2 and MDA results in oxidative damage caused by accretion of Na^+ ions also reduced by efflux of hazardous Na^+ ions. These changes balance the antioxidant-ROS system (Maeda 2019). Nonetheless, externally applied AsA (Kanwal *et al.* 2024), SA (Azeem *et al.* 2023), GB (Dawood *et al.* 2021) and TU (Nazir and Wahid 2023) improved antioxidants activity, reduced oxidative damage, enhanced influx of essential elements to improve buckwheat growth. In the current study too, supplementation of SRCs blocked the entry of Na^+, declined oxidants (H_2O_2 and MDA) generation (Fig. 4), enhanced influx of essential ions especially K, Ca, and N, caused ionic balance (Figs. 5a-b), which normalized metabolic activities to result in increased plant biomass, and foliage development (Fig. 1). All these results were substantiated from the chord diagram (Fig. 6) and correlations matrix heatmap (Fig. 7).

A comparison of SRCs revealed that although medium supplementation of all the compounds was impressive in alleviating the salinity toxicity on buckwheat, $CaCl_2$, KCl, AsA and SA were more effective. Ca and AsA have signaling roles (Feng *et al.* 2023) while K has an osmoregulatory role in plant cells (Kumar *et al.* 2020). However, most of the organic SRCs irrespective of the containing N were less effective in reducing oxidative stress improving growth. Reduction in oxidants production due to specific roles of Ca, K and AsA with enhanced vitamins content due to the medium supplementation of SRCs emerged as a mechanism for salinity tolerance in buckwheat.

Conclusion

The medium supplementation of SRCs was effective in reduced oxidative damage with the enhanced vitamins (antioxidants) synthesis that helped buckwheat to grow successfully under applied salinity stress. Among the SRCs, $CaCl_2$ and KCl followed by AsA and SA were more effective while thiourea was the least effective. Hence, to obtain acceptable buckwheat yield, chlorides of Ca and K at the recommended levels can be used for successful buckwheat cultivation in marginally saline fields.

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Author Contributions

BM conceived idea; performed experiments, collected data and prepared initial draft; AW conceived the idea, interpreted data and finalized draft; MSAA performed statistical analysis, interpreted data and improved initial draft; SMAB arranged seed material, finalized results and improved initial draft.

Conflict of Interest

No conflict of interest by any author.

Data Availability

The data reported in this will be provided on a fair request to the corresponding author.

Ethics Approval

Not applicable.

Funding Source

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