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Amino acids application alleviated salinity stress in spinach (*Spinacia oleracea* L.) by improving oxidative defense, osmolyte accumulation, and nutrient balance

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Abstract: Salinity is affecting more than 6 million hectares of cultivated area in Pakistan. The use of amino acids offers a pragmatic solution for minimizing the adverse effects of salinity on *Spinacia oleracea* L. (spinach). The present study evaluated the possible potential of amino acids in enhancing the salinity tolerance in spinach and identified the probable underlying mechanisms. The experiment comprised of two factors viz. amino acids with total of seven treatments; control, salinity (sal) 100 mM, Methionine + sal, Phenylalanine + sal, Proline + sal, Tyrosine + sal, Combined amino acids + sal, and two spinach cultivars (Desi Palak and VRI-2019). Salinity stress decreased the morpho-physiological attributes of both spinach cultivars, nevertheless, the application of combined amino acids effectively improved the tolerance against salinity stress. Compared with control, all applications of amino acids increased the root and shoot length, fresh and dry weight, number of leaves, and plant yield per plant of both spinach cultivars. However, the maximum was noted by the application of combined amino acids. Combined amino acids triggered the activities of antioxidants (catalase, superoxide dismutase, peroxidase), increased the contents of free proline, phenolics, flavonoids, chlorophyll a, b, carotenoids, calcium, and potassium in root and shoot while decreasing the reactive oxygen species (hydrogen peroxide) and sodium contents in both spinach cultivars under saline conditions. Overall, the VRI-2019 performed better than Desi Palak. The vigorous growth along with higher salinity tolerance because of amino acid treatments was linked to better chlorophyll contents, higher accumulation of osmolytes, maintenance of ionic balance, and an improved antioxidant defense system in *Spinacia oleracea*.

Key words: Osmoregulations, antioxidants, growth and yield, salinity stress

1. Introduction

Among abiotic stresses, soil salinity is the most devasting as it reduces the cultivated area, crop yield, and quality in the world (Shrivastava and Kumar, 2015, Hafeez et al., 2021). About 19.5% of the total world irrigated area is salt-affected, and it is expected to increase by 50% by 2050 due to crops irrigated with saline water (Arun et al., 2016; FAO, 2018), due to this the potential yield decrease is 20% (Rauf et al., 2010). Salinity stress comprises of ionic and osmotic stress that retard the growth, physiological, and biochemical mechanisms which ultimately decrease the yield of crops (Arif et al., 2020, Saddiq et al., 2021a). The osmotic stress can be strong by a higher update of sodium ions in plant tissues (Santander et al., 2017) causing rapid closure of stomata (Hedrich and Shabala 2018), which declines the photosynthetic efficiency and chlorophyll content (Zahra et al., 2022), inhibition of photosystems (PSI and PSII) (Saddiq et al., 2019), and interrupts the sense of balance among antioxidants and reactive oxygen species (ROS) (Huang et al., 2021), ions uptake (Iqbal et al., 2019), as well as the crop growth and yield (Saddiq et al., 2021b). However, sustained sugar production, photosynthetic, and antioxidants activities possibly will provide energy and defense to guarantee successful acclimation under saline conditions (Muhammad et al., 2021, Munir et al., 2021).

Spinach (*Spinacia oleracea* L.) is an annual leafy vegetable which considered as the rich source of ascorbic acid, beta-carotene, calcium (Ca), iron (Fe), potassium (K), phosphorus (P) and bioactive compounds such as 20-hydroxyecdysone, glucuronic acid, and polyphenols (Massa et al., 2018, Bokov et al., 2020). The consumption of a specific quantity of vegetables will help in reducing the

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risk of many diseases (Altemimi et al., 2015). Furthermore, *Spinacia oleracea* demand is also increasing within the food process industry worldwide (Nguyen et al., 2019).

Since the requirements of an increasing world population, it is needed to improve the production of the crops which will have the ability to endure salinization, so exogenous application of amino acids has been revealed to lessen the salinity-induced yield losses in crops (Sabagh et al., 2017, Hussain et al., 2018, Marium et al., 2021). Amino acids are the chief part of proteins that deploys many physio-chemical modulations to provide tolerance against abiotic stresses (Bakhoum et al., 2019).

The cucumber growth, biomass, and nutrient uptake are improved with the exogenous application of methionine and phenylalanine in saline soils (Marium et al., 2021). Tyrosine foliar treatment increased the plant height, root shoot ratio, and yield of sugar beet under normal conditions (El-Sherbeny and Teixeira da Silva, 2013). The mustard plant growth, efficiency of PSII, photosynthetic and antioxidant activities, and grain yield was improved with foliar sprayer of proline under salt stress (Wani et al., 2019). In the present study, we evaluated the role of sole or combined applied amino acids in modulating photosynthetic activity, antioxidant defense system, and yield of two *Spinacia oleracea* varieties under saline conditions.

2. Material and methods

2.1. Experimental site and plant material

A pot experiment was conducted in the warehouse of Department of Botany (31.4177° N, 73.1196° E), Government College Women University Faisalabad (GCWUF). Sandy loam soil was used in the experiment having properties as nitrogen 0.81%, exchangeable K 112 ppm, phosphorus 118 ppm, pH 8.3, electric conductivity 0.45 dSm^{-1}, and organic matter 0.82%. *Spinacia oleracea* seeds of different varieties mainly V1 (Desi Palak) and V2 (VRI-2019) were obtained from Ayub Agriculture Research Center, Faisalabad.

2.2. Foliar application of amino acids and NaCl stress treatments

Plants at vegetative stage (3 weeks old) were irrigated with a solution of 100 mM salt level for next 15 to 20 days until significant stress signs started to appear, while control plant were irrigated with normal water. When salinity stress sign started to appear, foliar treatments with amino acid level (250 mg L^{-1}) were carried out two times per week interval by using a hand sprayer. Data for growth and morphological parameters were recorded after 20 days of foliar treatment. *Spinacia oleracea* was exposed to 7 different treatments, i.e. T1 = Control, T2 = 100mM NaCl stress + without amino acids, T3 = 100mM NaCl stress + 250mg/L methionine, T4 = 100mM NaCl stress + 250mg/L phenylalanine, T5 = 100mM NaCl stress + 250mg/L proline, T6 = 100mM NaCl stress + 250mg/L tyrosine, T7 = 100mM NaCl stress + 250mg/L methionine + 250mg/L phenylalanine + 250mg/L proline + 250mg/L tyrosine.

2.3. Growth and yield indicators

Twenty days after foliar treatments, four plants were collected from each treatment, shoot and root length were taken with a meter rod, an electric balance was used to determine plant fresh weight of shoot and root. Plant root and shoot were oven dried at 65 °C for 72 h and their respective dry weights were measured.

2.4. Chlorophyll a, b and carotenoids

By using the procedure of Arnon (Arnon, 1949), chlorophyll (a and b) contents were estimated, and carotenoids content was measured by using the method of Davies (Davies, 1976). Fresh leaf material (0.5g) of plant was chopped and acetone solution (80%) mixed with ethanol 1:1 were used. After grinding samples in acetone, the suspension was filtered to remove turbidity. The absorbance of samples was measured at three different wavelengths (645nm, 663nm, and 480nm) using spectrophotometer.

2.5. Total free proline, flavonoids, and total phenolic contents

Total free proline content was measured by following Bates et al. (Bates et al., 1973) method. A 0.5 g fresh leaf tissue sample was homogenized with 10 mL $C_7H_6O_6S$ and filtered. Then 2 mL of filtrate was mixed with 2 mL acid ninhydrin solution, 2 mL glacial acetic acid and 4.0 mL of toluene a test tube, the absorbance was taken at 520 nm.

By using Zhishen et al. (Zhishen et al., 1999) method, the flavonoids contents were determined. Test tubes were filled with 0.5 mL of plants leaf sample extract 80% acetone (80+20).. Then 2 mL of distilled water and aluminium chloride 2.5mL were mixed in each sample after 10 min, 5% $NaNO_3$ (4 mL) was added then after 3min, 1 M NaOH (7.5 mL) was added then put it at room temperature for 5min absorbance at 510 nm using a spectrophotometer.

Method defined by Ainsworth and Gillespie (Ainsworth and Gillespie, 2007) was followed for measuring total phenolics content. A 0.2 g leaf sample was homogenized with 0.8 mL methanol and centrifuged. A (100 mL) supernatant was mixed with (100 mL) F-C reagent and 800 mL of (700 mM) Na_2CO_3, the absorbance was taken at 765 nm.

2.6. Antioxidant assays and H_2O_2 content

By following the procedure of Giannopolitis and Ries (Giannopolitis and Ries, 1977) activities of SOD were recorded. The reaction solution was prepared by mixing proposed quantity of nitroblue tetrazolium test, methionine, riboflavin, ethylenediaminetetraacetic acid and potassium phosphate buffer (pH 7.8) with 20–50

µl enzyme extract. The test tubes containing the reaction solution were irradiated under a light of fluorescent lamp for 15 min. The absorbance was recorded at 560 nm with a spectrophotometer.

For the estimation of CAT and POD, method of Chance and Maehly (Chance and Maehly, 1955) was followed. Leaf sample (0.5 g) was homogenized with (50 mM) potassium phosphate buffer. For the estimation of POD activity assay solution was prepared by mixing 50 mM potassium phosphate buffer, 40 mM H_2O_2, 20 mM guaiacol, and 0.1 mL enzyme extract. Absorbance was taken at 470 nm was recorded after every 20 s. For measurement of CAT activity assay solution was prepared by mixing 50 mM phosphate buffer, 5.9 mM H_2O_2, and (0.1 mL) enzyme extract. A decrease in absorbance was recorded at 240 nm after every 20 s spectrophotometrically.

Hydrogen peroxide was estimated by following Alexieva et al. (Alexieva et al. 2001) method. The reaction mixture having 0.5 mL $C_2HCl_3O_2$, 100 mM K phosphate buffer (0.5 mL) and reagent (2 mL) 1 M KI. With the help of spectrophotometer, absorbance was recorded at 390 nm.

2.7. Statistical analysis

The experiment was laid out in a completely randomized design with factorial arrangements with three replications. The data attained from the experiment was analyzed through analysis of variance (ANOVA) using Statistics 8.1 to spot the significance, and data and means were compared using LSD at 5%. Graphs were prepared using origin Pro. 9.1 (Origin Lab Corporation, 2014)

3. Results

3.1. Influence of foliar applied treatments on growth and yield indicators of Spinacia oleracea varieties

Data revealed that growth and yield indicators were significantly ($p \leq 0.05$) influenced by the treatments of amino acids. Variations also recorded within the varieties (Figures 1,2,3). Salinity stress significantly ($p \leq 0.05$) lessen all growth and yield attributes, i.e. root length (RL), root fresh weight (RFW), root dry weight (RDW), shoot length (SL), shoot fresh weight (SFW), shoot dry weight (SDW), number of leaves per plant (NLP) and yield per plant (YP) (Figures 1,2,3). SL from foliarly applied treatments were in the order Comb+S > Phe+S Met+S > Pro+S > Tyr+S with respective increases of 94.6%, 73.6%, 72.5%, 64.4%, and 60.9% relative to the saline treatment. SFW from foliarly applied treatments were in the order Comb+S >> Phe+SMet+S > Pro+S > Tyr+S with respective increases of 122.2%, 92.2%, 91.4%, 79.6%, and 72.6% relative to the saline treatment. SDW from foliarly applied treatments were in the order Comb+S > Met+S > Phe+S > Pro+S > Tyr+S with respective increases of 98.4%, 73.3%, 72.6%, 58.9%, and 57.8% relative to the saline treatment. RL from foliarly applied treatments were in the order Comb+S > Phe+S > Met+S > Tyr+S > Pro+S with respective increases of 100.6%, 94.2%, 67.9%, 65.3%, and 64.1% relative to the saline treatment. RFW from foliarly applied treatments were in the order Comb+S > Phe+S Met+S > Pro+S > Tyr+S with respective increases of 157.7%, 130.9%, 97.1%, 83.4%, and 74.4% relative to the saline treatment. RDW from foliarly applied treatments were in the order Comb+S > Met+S > Phe+S > Tyr+S > Pro+S with respective increases of 149.6%, 123.6%, 95.6%, 77.7%, and 72.1% relative to the saline treatment. NLP from foliarly applied treatments were in the order Comb+S > Phe+S > Met+S > Pro+S > Tyr+S with respective increases of 60.9%, 48.7%, 46.3%, 36.5%, and 34.1% relative to the saline treatment. PY from foliarly applied treatments were in the order Comb+S > Phe+S > Met+S > Tyr+S > Pro+S with respective increases of 58.8%, 42.9%, 40.1%, 34.9%, and 32.8% relative to the saline treatment. SFW, SDW, RL, RFW, RDW, NLP, and YP under saline (100 mM NaCl) stress were decreased as compared to control (Figures 1,2,3). Overall the performance of VRI-2019 was better desi palak irrespective of treatment differences.

3.2. Influence of foliar applied treatments on reactive oxygen species and antioxidants of Spinacia oleracea varieties

The antioxidants and ROS activities significantly ($p \leq 0.05$) increased under salinity medium compared to nonsaline medium (Figures 3,4). H_2O_2 from foliarly applied treatments were in the decreaseing order; Comb+S < Phe+S < Met+S < Pro+S = Tyr+S (–23.6%, –18.6%, –15.5%, 9.9%, and 9.9% relative to the saline treatment (Figure 3a). SOD from foliarly applied treatments were in the order Comb+S > Phe+S > Met+S > Pro+S > Tyr+S with respective increases of 21.1%, 18.1%, 12.1%, 8.4%, and 5.9% relative to the saline treatment. POD from foliarly applied treatments were in the order Comb+S > Met+S > Phe+S > Pro+S > Tyr+S with respective increases of 32.9%, 22.3%, 21.5%, 13.8%, and 9.4% relative to the saline treatment. CAT from foliarly applied treatments were in the order Comb+S > Phe+S > Pro+S > Tyr+S > Met+S with respective increases of 34.6%, 21.5%, 12.8%, 10.1%, and 8.7%, relative to the saline treatment. Moreover, the antioxidant activities were enhanced under salinity medium compared to nonsaline medium (Figure 4).

3.3. Influence of foliar applied treatments on photosynthetic pigments of Spinacia oleracea varieties

Under saline medium caused a drastic influence on the photosynthetic pigments (chl-*a* and chl-*b*) as compared to control conditions. Chl-*a* from foliarly applied treatments were in the order Comb+S > Phe+S > Met+S > Pro+S > Tyr+S with respective increases of 25.1%, 22.3%, 19.8%, 14.4%, and 12.8%, relative to the saline treatment. Chl-*b* from foliarly applied treatments were

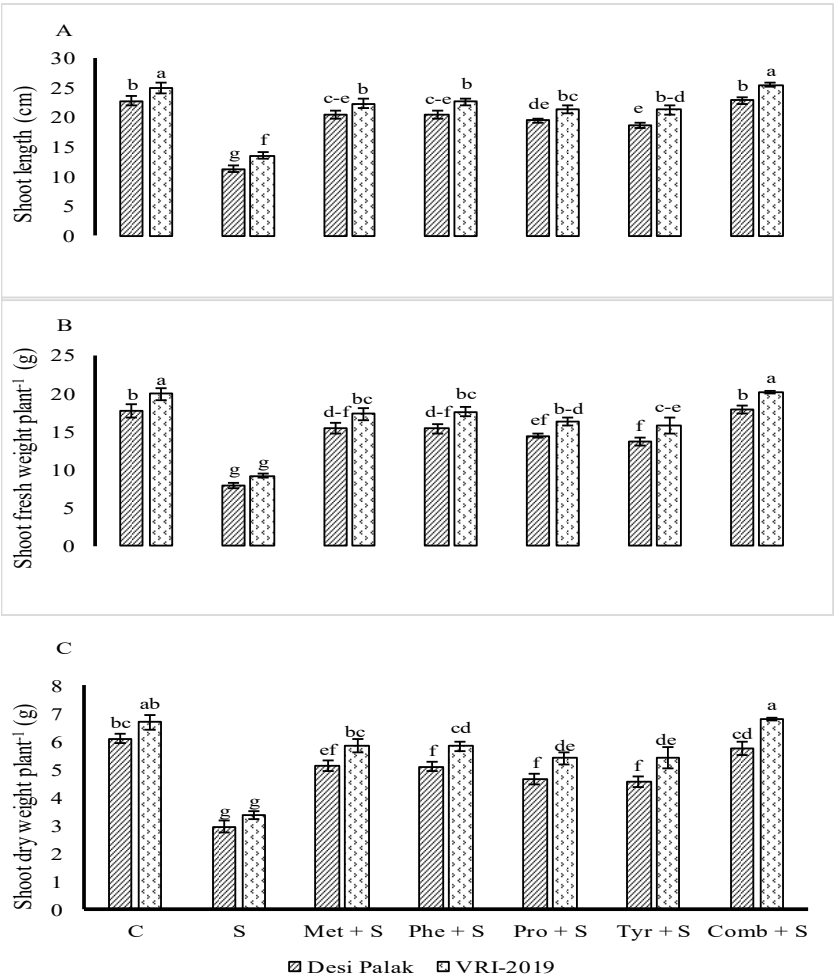

Figure 1. Influence of amino acids on A) shoot length B) shoot fresh weight C) shoot dry weight of spinach cultivated under saline conditions. Error bars denote the standard error of three replications. Bars with the same letters do not differ significantly at $p \leq 0.05$. Whereas, C = Control, S = Salinity, Met+S = Methionine+Salinity, Phe+S = Phenylalanine+Salinity, Pro+S = Proline+Salinity, Tyr+S = Tyrosine+Salinity, Comb+S = Combined amino acids+Salinity.

in the order Comb+S > Phe+S > Met+S > Pro+S > Tyr+S with respective increases of 41.1%, 35.3%, 29.1%, 19.9%, and 13.6%, relative to the saline treatment (Figures 5a,5b). The carotenoid contents from foliarly applied treatments were in the order Comb+S > Phe+S > Tyr+S > Pro+S > Met+S with respective increases of 62.8%, 56.6%, 47.1%, 43.6%, and 26.6%, relative to the saline treatment (Figure 5c). Overall the performance of VRI-2019 was better desi palak irrespective of treatment differences (Figure 5).

3.4. Influence of foliar applied treatments on free proline, phenolics and flavonoids of Spinacia oleracea varieties

Under saline medium a significant ($p \leq 0.05$) improvement in the synthesis free proline, phenolics and flavonoids as compared to control conditions (Figure 6). Proline from foliarly applied treatments were in the order Comb+S > Phe+S > Met+S > Pro+S > Tyr+S with respective increases of 16.7%, 11.9%, 9.9%, 2.1%, and 1.8%, relative to the saline treatment (Figure 6a). Phenolics from foliarly applied treatments were in the order Comb+S > Met+S > Phe+S > Pro+S > Tyr+S with respective increases of 13.7%, 10.5%, 9.0%, 7.1%, and 4.7%, relative to the saline treatment (Figure 6b). Flavoniods from foliarly applied treatments were in the order Comb+S > Met+S > Phe+S > Pro+S > Tyr+S with respective increases of 42.7%,

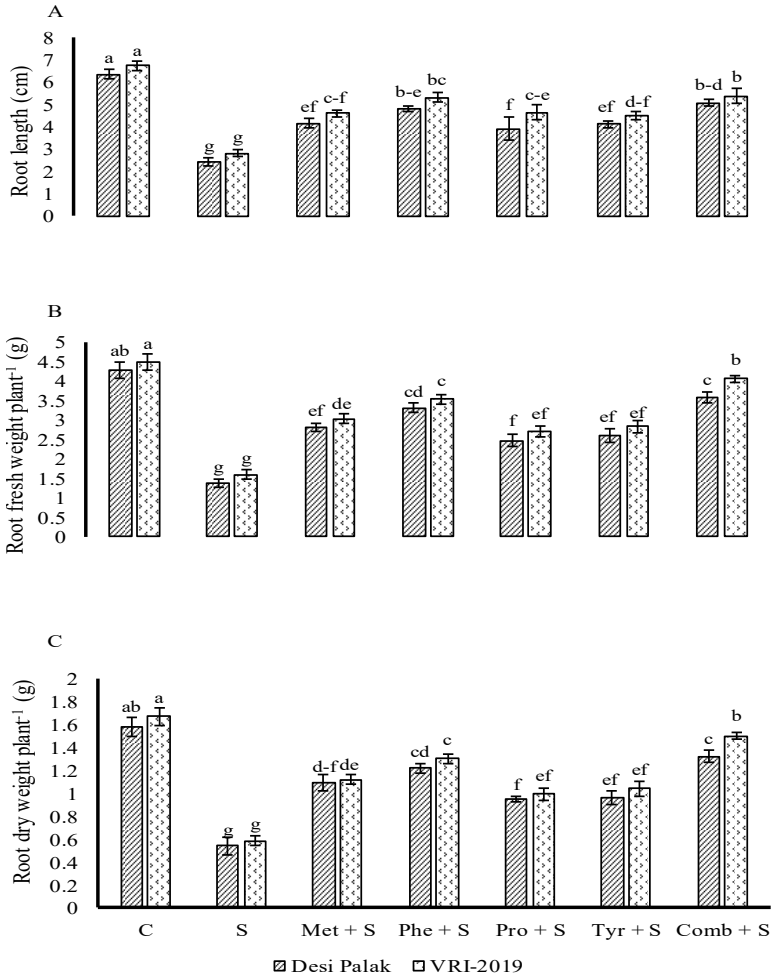

Figure 2. Influence of amino acids on A) root length B) root fresh weight C) root dry weight of spinach cultivated under saline conditions. Error bars denote the standard error of three replications. Bars with the same letters do not differ significantly at $p \leq 0.05$. Whereas, C = Control, S = Salinity, Met+S = Methionine+Salinity, Phe+S = Phenylalanine+Salinity, Pro+S = Proline+Salinity, Tyr+S = Tyrosine+Salinity, Comb+S = Combined amino acids+Salinity.

35.3%, 33.2%, 26.7%, and 18.3% , relative to the saline treatment. However, the varietal difference among them was nonsignificant (Figure 6c).

3.5. Influence of foliar applied treatments on ions of Spinacia oleracea varieties

The root and shoot Ca^{+2} and K^{+} ions were decreased due at 100 mM saline medium as compared to control (Figures 7a and 7b, Figures 8a and 8b). Root Ca^{+2} from foliarly applied treatments were in the order Comb+S > Phe+S > Pro+S > Met+S = Tyr+S with respective increases of 59.0%, 52.9%, 48.1%, 42.7%, and 42.7% , relative to the saline treatment. Shoot K^{+} from foliarly applied treatments were in the order Comb+S > Phe+S > Met+S > Tyr+S > Pro+S with respective increases of 146.1%, 121.1%, 115.2%, 95.1%, and 92.9% relative to the saline treatment. Root Na^{+} from foliarly applied treatments were in the decreasing order; Comb+S < Met+S = Phe+S = Tyr+S < Pro+S (–23.3%, –15.9%, –15.9%, 15.9%, and –11.3%) relative to the saline treatment (Figure 7c).

Shoot Ca^{+2} from foliarly applied treatments were in the order Comb+S > Phe+S > Pro+S > Met+S > Tyr+S with respective increases of 52.6%, 46.1%, 40.8%, 36.7%, and 35.1% , relative to the saline treatment. Shoot K^{+} from foliarly applied treatments were in the order Comb+S >

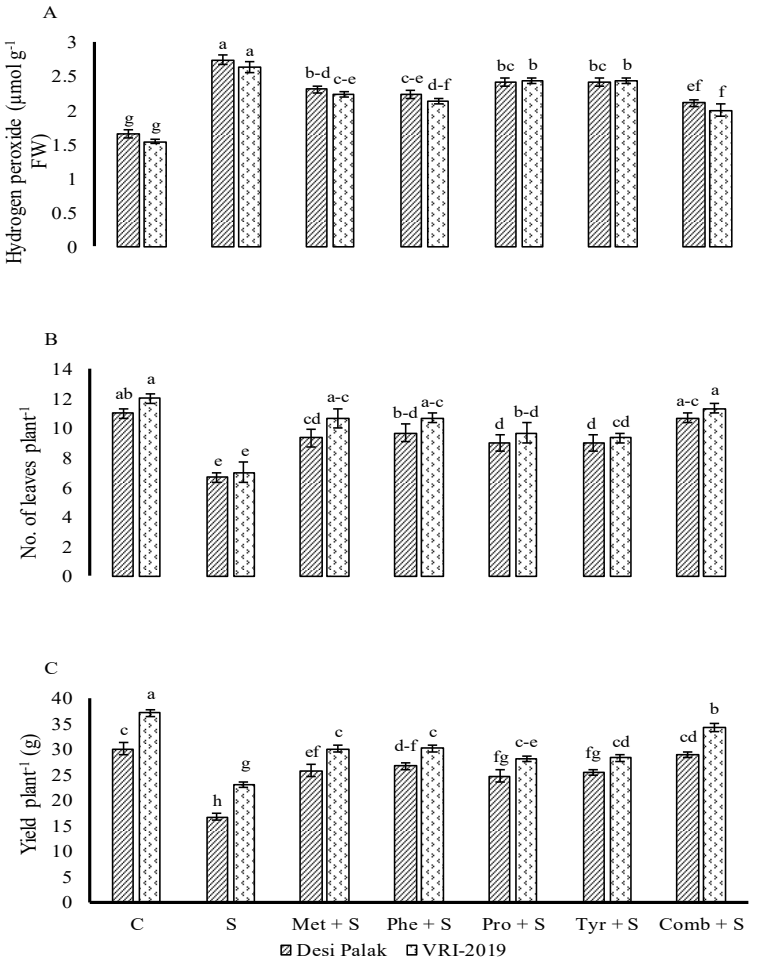

Figure 3. Influence of amino acids on A) hydrogen peroxide B) no. of leaves plant^{-1} C) yield plant^{-1} of spinach cultivated under saline conditions. Error bars denote the standard error of three replications. Bars with the same letters do not differ significantly at $p \leq 0.05$. Whereas, C = Control, S = Salinity, Met+S = Methionine+Salinity, Phe+S = Phenylalanine+Salinity, Pro+S = Proline+Salinity, Tyr+S = Tyrosine+Salinity, Comb+S = Combined amino acids+Salinity.

Met+S > Phe+S > Tyr+S > Pro+S with respective increases of 91.1%, 67.9%, 61.6%, 53.1%, and 52.4% relative to the saline treatment. Shoot Na^+ from foliarly applied treatments were in the decreasing order; Comb+S < Phe+S < Met+S = Tyr+S < Pro+S (–25.1%, –17.8%, –17.5%, 17.5%, and –12.3%) relative to the saline treatment (Figure 8c).

4. Discussion

Abiotic stresses like high and low temperature limits, scarcity of water, saline conditions, and heavy metals are chief elements that limit the crop productivity and sustainability globaly. Among abiotic stresses, soil salinity is themost devasting, as itreduces the cultivated area, crop yield and quality in the world (Shrivastava and Kumar, 2015, Hafeez et al., 2021). Salinity also influences the developmental mechanisms, i.e. disrupting synthesis of proteins and metabolism of lipids, the biochemical mechanisms, causing osmotic, oxidative and ionic stress (Hasanuzzaman et al., 2020, Zahra et al., 2021). In the present trial, it is found that amino acids sole or combined not only improve growth and yield but also protect *Spinacia oleracea* plants from the detrimental effects of salinity. The

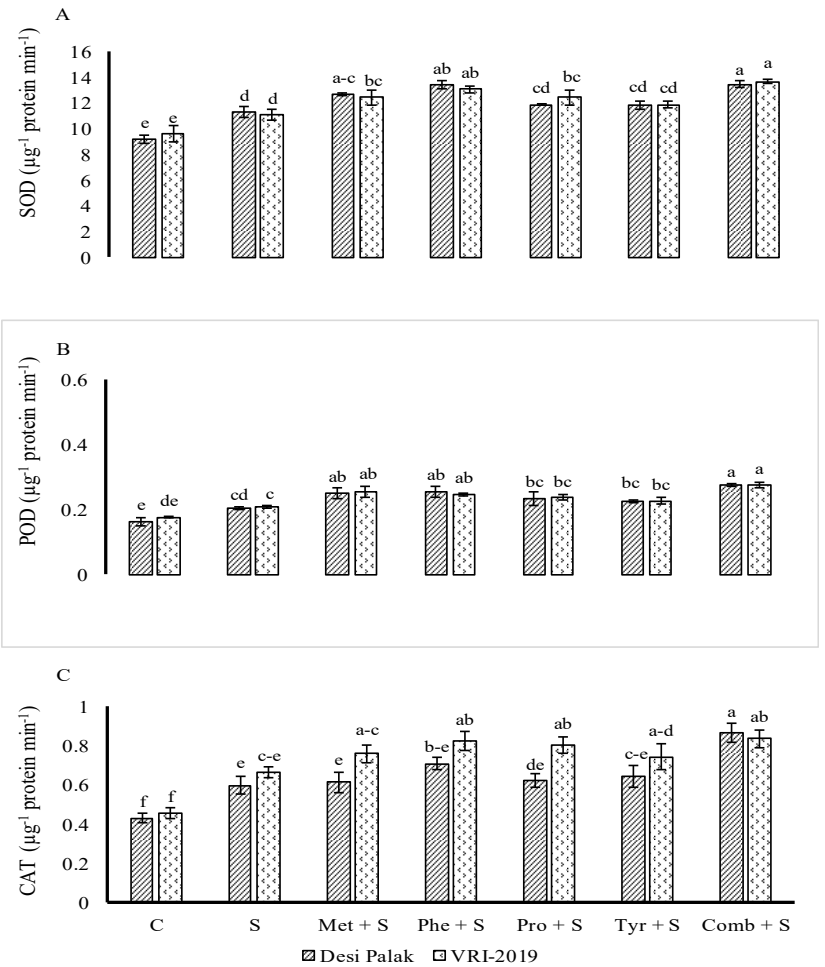

Figure 4. Influence of amino acids on A) SOD B) POD C) CAT of spinach cultivated under saline conditions. Error bars denote the standard error of three replications. Bars with the same letters do not differ significantly at $p \leq 0.05$. Whereas, C = Control, S = Salinity, Met+S = Methionine+Salinity, Phe+S = Phenylalanine+Salinity, Pro+S = Proline+Salinity, Tyr+S = Tyrosine+Salinity, Comb+S = Combined amino acids+Salinity.

combined treatment of amino acids persuading a higher degree of protective mechanism under saline medium.

The photosynthetic machinery is profoundly sensitive to saline medium as compared to other physiological mechanisms, due to this chlorophyll pigments were decreased under such conditions (Acosta-Motos et al., 2017). The chloroplast's thylakoid membrane is disintegrated due to deterioration of protein and PS-II present in the thylakoid membrane is exceedingly subtle to saline conditions (Mittal et al., 2012, Saddiq et al., 2019). Imposition of salinity triggered a reduction in chlorophyll constituents; though, the foliar application of amino acids caused a significant improvement in chlorophyll contents. Comparable upshots are defined in maize (Zahra et al., 2018, Zahra et al., 2020) and wheat (Bahari et al., 2013). This improvement in chlorophyll constituents is probably accompanying to antioxidant enzymes function because amino acids help to decrease the different ROS (Bahari et al., 2013, Zahra et al., 2018). Our outcomes are also linked that foliarly applied chemicals tangled in the defense mechanism from oxidative stress and reinforced by a scientific paper in which foliar treatment with amino acids mixture enhanced the chlorophyll constituents in faba bean (Sadak and Abdelhamid, 2015).

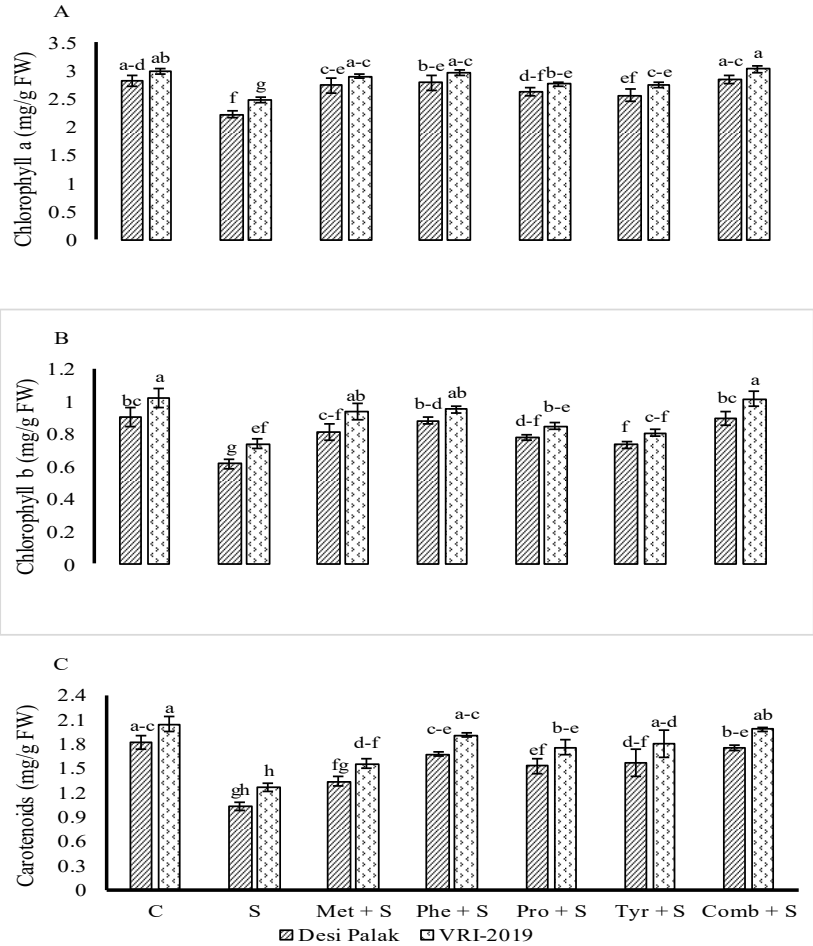

Figure 5. Influence of amino acids on A) chlorophyll a B) chlorophyll b C) caroteniods of spinach cultivated under saline conditions. Error bars denote the standard error of three replications. Bars with the same letters do not differ significantly at $p \leq 0.05$. Whereas, C = Control, S = Salinity, Met+S = Methionine+Salinity, Phe+S = Phenylalanine+Salinity, Pro+S = Proline+Salinity, Tyr+S = Tyrosine+Salinity, Comb+S = Combined amino acids+Salinity.

In the presence of salts, an over-production of ROS (H_2O_2) and the preliminary influence of ROS accumulation in cell is lipid peroxidation that is a sign of oxidative stress (Noreen et al., 2021). Salinity stress increases ROS production and lessens the integrity of membranes, which causes the leakage of nutrients (Ions e.g., Na, K and Ca) from the cell organelles (Tuna et al., 2007). Our outcomes exposed that saline medium higher H_2O_2 that increased lipid peroxidation, while the treatment of amino acids sole or combined resulted in reduced accumulation of H_2O_2. Current fallouts are in agreement with others (Sadak and Abdelhamid, 2015, Zahra et al., 2018, Butt et al., 2020). The salinity causes ROS is radially alleviated by boosted activities of antioxidant enzymes (Muhammad et al., 2021, Munir et al., 2021). Under stressful conditions, the enzymatic antioxidants stand at the front-line to rescue plants. Saline medium greater the activities of antioxidant enzymes that is a shielding machinery in plants (Huang et al., 2021). The antioxidant enzymes are also boosted by foliar treatment with amino acids in a saline medium (Butt et al., 2020, Perveen and Hussain, 2021). The spray of mixture of amino acids boosts the enzymatic antioxidant response under stress that helps the plants to maintain membrane integrity (Sadak and Abdelhamid, 2015).

Accumulation of free proline, phenolics and flavonoids is considered a preliminary shielding response under

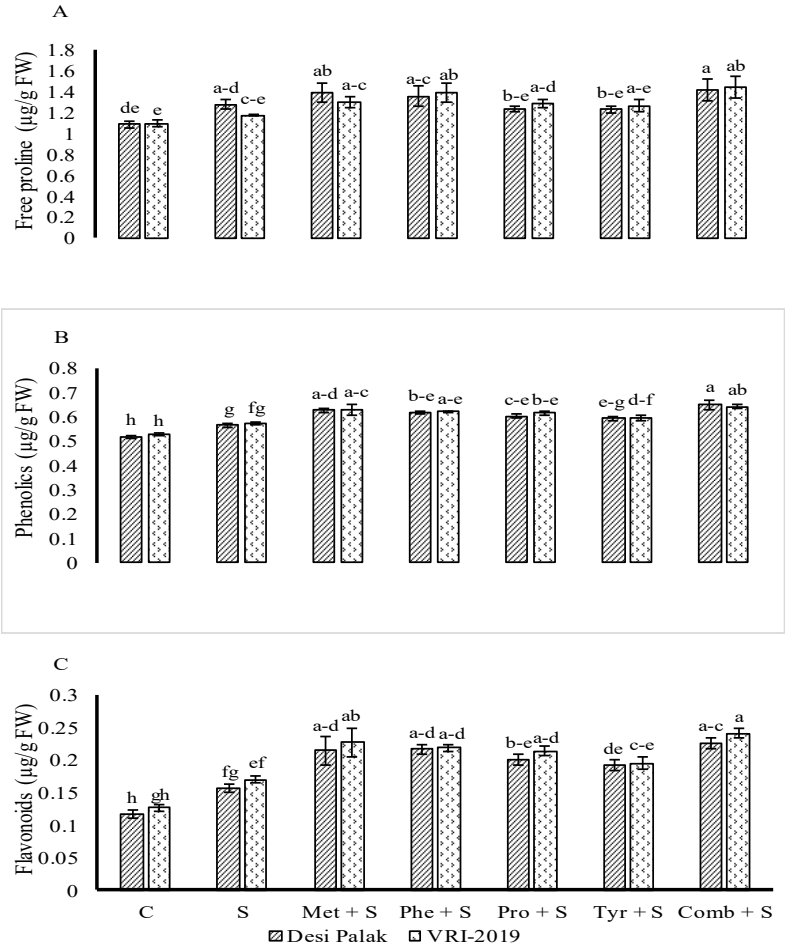

Figure 6. Influence of amino acids on A) free proline B) phenolics C) flavonoids of spinach cultivated under saline conditions. Error bars denote the standard error of three replications. Bars with the same letters do not differ significantly at $p \leq 0.05$. Whereas, C = Control, S = Salinity, Met+S = Methionine+Salinity, Phe+S = Phenylalanine+Salinity, Pro+S = Proline+Salinity, Tyr+S = Tyrosine+Salinity, Comb+S = Combined amino acids+Salinity.

saline environment (Al Hassan et al., 2015). It was detected that free proline, phenolics, and flavonoids contents were improved under saline medium, however, the spray of amino acids sole/combined give rise to in further improvement in free proline, phenolics and flavonoids accumulation. The increase in the amount of free proline, phenolics and flavonoids under saline medium and spraying with amino acids has also been described by prior researchers (Sadak and Abdelhamid, 2015, Zahra et al., 2018, Perveen and Hussain, 2021).

The Na^+ ions were higher in leaf and root organs under saline medium. The ion homeostasis is important mechanism under saline medium (Hasanuzzaman et al., 2020). But Na^+ is profoundly absorbed by roots, with little absorption of H_2O excessive uptake of salts by roots (Munns et al., 2020). The fallouts exposed that foliar spray of amino acids sole/combined lowered the uptake of Na^+ ion from roots. Alike outcomes have been reported by others (Sadak and Abdelhamid, 2015, de Freitas et al., 2018, Zahra et al., 2018, Butt et al., 2020, Perveen and Hussain, 2021), who also documented reduction in the uptake of Na^+ by root after applying amino acids under saline medium.

Under saline medium due to higher uptake of Na^+ ions, the uptake of K^+ ions were affected (Saddiq et al., 2019). Saline environment influences an excessive influx of Na^+ ions in roots and leaves that leads to K^+ ions efflux,

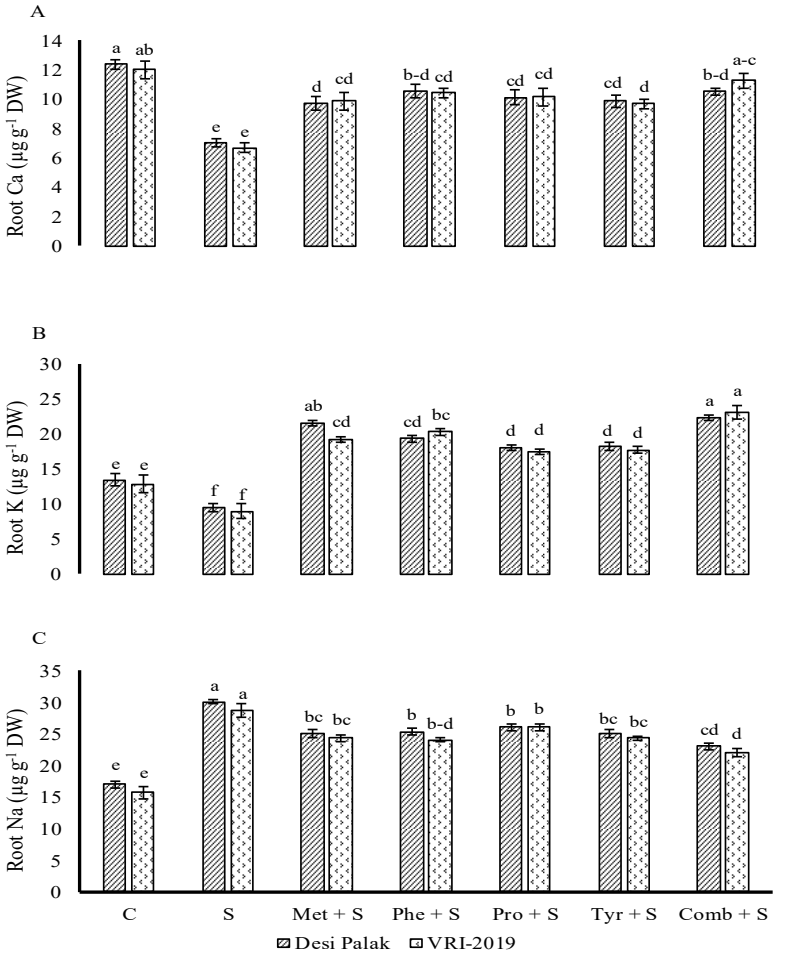

Figure 7. Influence of amino acids on A) Root Ca B) Root K C) Root Na of spinach cultivated under saline conditions. Error bars denote the standard error of three replications. Bars with the same letters do not differ significantly at $p \leq 0.05$. Whereas, C = Control, S = Salinity, Met+S = Methionine+Salinity, Phe+S = Phenylalanine+Salinity, Pro+S = Proline+Salinity, Tyr+S = Tyrosine+Salinity, Comb+S = Combined amino acids+Salinity.

hence causing K^+ ions deficiency in plants (Noreen et al., 2021). In addition, foliar treatments let down Na^+ ions uptake, which concurrently boosted the uptake of K^+ ions in cells of plants. The rise in K^+ ions uptake may be due to enlarged RL (Zahra et al., 2018, Saddiq et al., 2019). The foliar treatment of biomolecules leads to in the improved Ca^{2+} ions in shoots and roots of *Spinacia oleracea* plants under saline medium.

The growth and yield attributes (SL, SFW, SDW, RL, RFW, RDW, NLP, and YP) of *Spinacia oleracea* were significantly reduced under saline medium. The foliar treatment of amino acids sole/combined improved growth and yield attributes under saline medium. Alike fallouts that foliar application of amino acids improves yield attributes have been earlier stated in maize (Zahra et al., 2018, Perveen and Hussain, 2021).

5. Conclusions

The present study demonstrated that amino acid application regulates the synthesis of antioxidants, proline, phenolics, and flavonoids in order to decrease the damaging effect of saline medium by lessening the ROS (H_2O_2). Such alterations in the crops are key mechanisms to better salt tolerance in *Spinacia oleracea*. The improved physiological and biochemical responses under saline medium owing to the application of amino acids led to enhanced growth and yield of *Spinacia oleracea*. Inclusive and wide-scale research is essential to elucidate the definite role of these

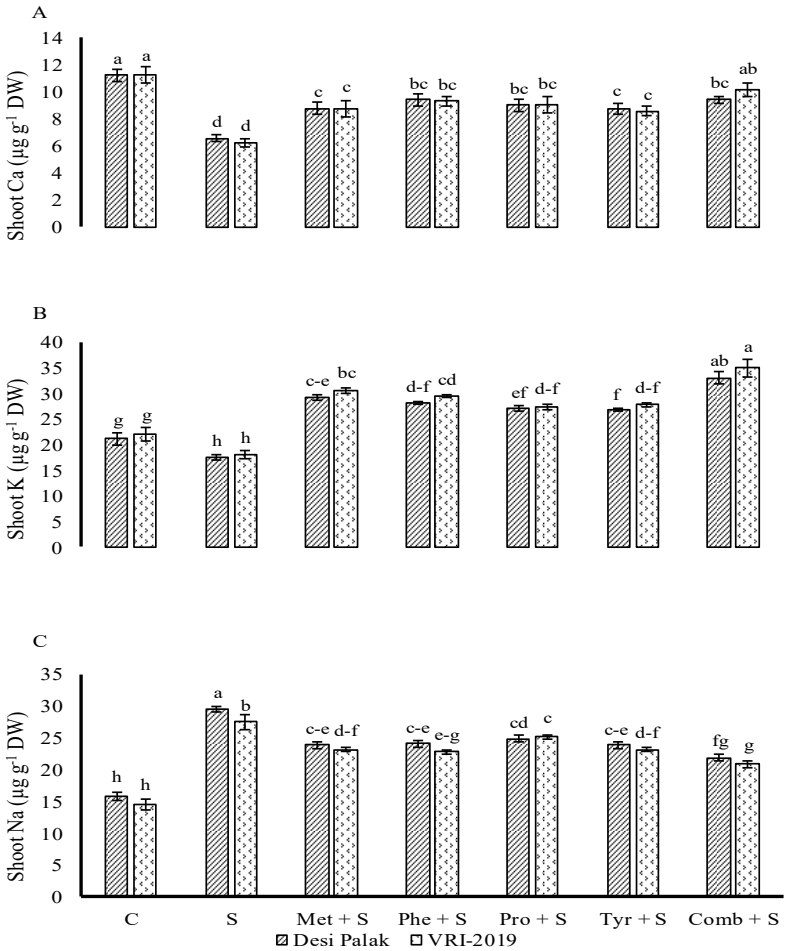

Figure 8. Influence of amino acids on A) Shoot Ca B) Shoot K C) Shoot Na of spinach cultivated under saline conditions. Error bars denote the standard error of three replications. Bars with the same letters do not differ significantly at $p \leq 0.05$. Whereas, C = Control, S = Salinity, Met+S = Methionine+Salinity, Phe+S = Phenylalanine+Salinity, Pro+S = Proline+Salinity, Tyr+S = Tyrosine+Salinity, Comb+S = Combined amino acids+Salinity.

amino acids in plant adaptations to salt stress. This will later add as first step to the development of strategies for designing stress tolerance genotypes

Conflict of interest

Abida Kausar, Maham Saddique worked equally on conceptualization and study design, Iqra performed Lab Analysis, Noreen Akhter and Nazoora Mujahid worked on acquisition of data, Abida Parveen, Qamar uz Zaman and Saddam Hussain did statistical analysis, Abida Kausar, Maham Saddique and Saddam Hussain prepared the manuscript draft, Abida Kausar: Supervision. All Authors approved the final version of the manuscript.

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