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Role of *L*-phenylalanine in enhancing salt tolerance in carrot (*Daucus carota* L.) through morpho-physiological, antioxidant, and ionic modification**

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A b s t r a c t. Salt stress is one of the major abiotic stresses that affects the growth and development of the carrot. To evaluate its function in carrot salt tolerance, a pot experiment was conducted with three salinity levels (0, 60, and 120 mM NaCl) and three *L*-phenylalanine concentrations (0, 100, and 200 ppm). Salt stress significantly reduced the shoot fresh weight by 28% and the root dry weight by 50%, respectively. Under salinity (120 mM), photosynthetic pigments such as total chlorophyll content decreased by 35% and carotenoids by 28%, while H_2O_2 and MDA increased by 35.5 and 27%. Additionally, Na^+ accumulation increased and K^+ and Ca^{2+} accumulation declined. The foliar application of *L*-phenylalanine (200 ppm) increased the shoot fresh weight by 38% and the root dry weight by 92%, total chlorophyll and carotenoids increased by 54 and 39%, respectively. Upon the foliar application of *L*-phenylalanine, SOD increased by 54%, CAT by 43%, and POD by 36% under salt stress. Non-enzymatic antioxidants, such as leaf ascorbic acid content increased by 32%, flavonoids by 39%, and anthocyanins by 50%. K^+ and Ca^{2+} accumulation increased after the application of *L*-phenylalanine. Overall, *L*-phenylalanine improved morpho-physiological performance, suggesting its potential as a biostimulant for carrot production under saline conditions.

K e y w o r d s: carrot (*Daucus carota* L.), salinity stress, *L*-phenylalanine, antioxidant defense, secondary metabolites, osmolytes

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1. INTRODUCTION

One of the significant cool-season root vegetables cultivated and benefited from worldwide is the carrot (*Daucus carota* L.) (Kalia *et al.,* 2023). Carrots are considered to be crucial to the livelihoods of millions of people worldwide. They are grown around the world, but particularly in temperate climates (Djoufack *et al.,* 2024). Carrot grain characteristics are influenced differently by moisture content (Gaadhe *et al.,* 2022). It is widely recognized for its nutritional content and is rich in antioxidants, particularly carotenoids, which have been demonstrated to have anticancer effects. With 50% carotene content, carrot pomace has been identified as a useful byproduct that may be used to make a variety of utilitarian items and enhance culinary products like bread, cakes, and biscuits (Ikram *et al.,* 2024).

Carrot yields range from 30 to 100 tons per hectare worldwide. Vitamins A, C, and B, as well as proteins, minerals, and fiber, are abundant in carrots. Vitamin C helps repair wounds and regulate allergies. In the meantime, vitamin A is essential for immune system function, bone formation, eyesight, and reproduction (Gelaye *et al.,* 2024). Carrots can be a valuable produce for combating food insecurity. Currently, large amounts of carrots are canned or frozen, either as standalone products or in combination with other vegetables (Eze *et al.,* 2024).

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Salinity stress is harmful to the health of carrot (*Daucus carota* L.) plants, as it causes osmotic stress, ionic toxicity, oxidative damage, and metabolic disruption. High sodium content in the root zone decreases the potential of soil water to take in water, decreasing water uptake and inducing osmotic stress, which decreases cell turgor, inhibits cell expansion, and reduces root and shoot development (Kiełkowska *et al.,* 2019). At the same time, Na^+ and Cl^- ion excess disrupts ionic homeostasis and competes with important nutrients like K^+ and Ca^{2+}, leading to nutrient imbalance and disrupted enzymatic functions (Ramzan *et al.,* 2025; Kamińska *et al.,* 2022). Salinity causes a decrease in the content of chlorophyll and photosynthetic efficiency, as well as closing stomata and destroying the ultrastructure of chloroplasts, thereby limiting CO_2 uptake and energy generation (Kamińska *et al.,* 2022). Besides, salinity stress increases the production of reactive oxygen species (ROS), leading to lipid peroxidation, protein oxidation, and destabilization of the membrane; despite inducing antioxidant defense systems and accumulating osmoprotectants such as proline to counteract oxidative stress in carrots, continued or extreme salinity overloads these systems, and eventually halts carrot growth, root quality, and plant vigor (Simpson *et al.,* 2018).

Salt stress can significantly impair plant cell walls and interfere with regular plant growth and development, which lowers yield and productivity (Ramzan *et al.,* 2026ab). Excessive salt buildup in soil, largely driven by human activities such as overuse of fertilizers, poor land management, and unsuitable irrigation practices, is a major cause of this condition (Balasubramaniam *et al.,* 2023). Salt stress causes oxidative and osmotic stress, ion toxicity, nutritional and hormonal abnormalities and a rise in plant disease vulnerability (Hasanuzzaman *et al.,* 2022). By altering the metabolic processes of plants, it causes an electrolyte imbalance and has a significant impact on their growth and production globally (Ramzan *et al.,* 2025; Kesawat *et al.,* 2023). To combat abiotic challenges, plants can use a variety of defense strategies such as application of different plant growth regulators (Muzafar *et al*., 2026; Khursheed *et al.,* 2024; Haider *et al.,* 2026ab; Ramzan *et al.,* 2025).

An essential aromatic amino acid, *L*-phenylalanine (Phe), serves as a primary and secondary metabolite involved in various biological processes (Ramzan *et al.,* 2023; Almas *et al*., 2021). *L*-phenylalanine is a potential and inexpensive biostimulant that can increase plant resistance to abiotic stress (Ramzan *et al.,* 2023). Additionally, according to Adams *et al.* (2019), it acts as a precursor for a number of phenolic chemicals, including flavonoids, lignin, benzenoids, condensed tannins, and phenyl propanoid volatiles, all of which support the plant's defense against adverse environmental circumstances such as salinity. Studies on the antioxidant activities and growth of soybean (Teixeira *et al.,* 2019), common sage (Samani *et al.,* 2019), and tomato (Almas *et al.,* 2021), have shown favorable outcomes from the foliar application of *L*-phenylalanine.

It has been demonstrated that foliar supplementation of aromatic amino acids, such as Phe, increases the accumulation of total phenolics and flavonoids, improving the antioxidant capacity of plants under stress (Abd El-Mageed *et al.,* 2023). Adding nutrients from bioregulators, which control numerous physiological and biological processes at the metabolism and whole plant levels, can strengthen plant's defenses against environmental stress (Hayat *et al*., 2024; Maqbool *et al.,* 2024). Low production and productivity are still problems that can be resolved through the biosynthesis of molecules produced from *L*-phenylalanine (Qiu *et al.,* 2024). To combat salt stress, the extract of *L*-phenylalanine is an easy-to-use, reasonably priced, locally produced, and environmentally acceptable substitute for growth regulators. It was proposed that using *L*-phenylalanine topically might increase carrot tolerance to salinity stress. Despite these innovations, the role of *L*-phenylalanine in regulating salinity responses of carrot was not studied earlier, and this is a critical knowledge gap. This study hypothesized that foliar application of *L*-phenylalanine could alleviate the detrimental impacts of salinity stress in carrots. The objective of this study was to assess the impact of *L*-phenylalanine on morpho-physiological aspects of carrot under salinity stress and to investigate the activity of antioxidants, osmolytes, and mineral ions in carrot under salinity stress.

2. MATERIAL AND METHODS

The experimental work was executed at the Old Botanical Garden, University of Agriculture Faisalabad, Pakistan, to evaluate the effects of *L*-phenylalanine (Phe) on the morphological and physiological traits of carrot (*Daucus carota* L.). The experiment was conducted in 36 pots and one variety of *D. carota* L. T29, and its seeds were acquired from Ayub Agriculture Research Institute, Faisalabad. Each pot was loaded with 7 kg of sand and 15 seeds sown in each pot. After establishment of seedlings for two weeks, thinning was done to retain 6 plants per pot. The experiment was laid out in a Completely Randomized Design (CRD) with four replications per treatment. Full-strength Hoagland's solution was applied to all pots, and salinity stress was imposed after 4 weeks of sowing at three levels (0 mM, 60 mM, and 120 mM). *L*-phenylalanine at 100 and 200 ppm was separately mixed with distilled water, and the solution was administered as foliar sprays at the vegetative stage of carrot 45 DAS. One liter of distilled water was used to dissolve 100 and 200 mg L^{-1} of *L*-phenylalanine to create 100 and 200 ppm *L*-phenylalanine solutions, respectively. The solutions were applied as foliar sprays until the leaves were uniformly wet in the early morning after a few drops of Tween-20 were added as a surfactant. To provide the carrot crop with sufficient nutrients, Hoagland's

solution was used weekly until harvesting. After two weeks of foliar application with *L*-phenylalanine, plants were harvested to examine the morphological, photosynthetic, and biochemical attributes of carrot.

2.1. Determination of morphological parameters

After harvesting plant samples, shoots and roots were separated to record the fresh weight of roots with the use of an electronic weighing balance. A digital electronic balance (Model; OHAUS Corporation, USA) was used to measure the shoot fresh weight of the plant samples. A measuring tape was used to record the length of plant shoots and roots. After harvesting, the plant samples were air dried for three days. After drying the plants, the samples were kept in an oven at 65°C for two weeks. Subsequently, the dry weight of the roots and shoots was measured using a weight balance machine.

2.2. Photosynthetic pigments

Photosynthetic pigments, including chlorophyll *a*, chlorophyll *b*, and carotenoids, were quantified according the procedure outlined by Arnon (1949). For each replicate, approximately 0.1 g of leaf tissue was collected, finely chopped, and placed into labeled plastic containers. Each sample was treated with 5 mL of 80% acetone. The containers were placed in darkness at room temperature overnight to allow pigment extraction. Following incubation, a spectrophotometer was used to measure absorbance at wavelengths of 663, 645, and 480 nm. The concentrations of chlorophyll and carotenoids were then calculated using specific equations derived from the absorbance readings.

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{ FW)} = (12.7 \times A663) - (2.69 \times A645),$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{ FW)} = (22.9 \times A645) - (4.68 \times A663),$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{ FW)} = (20.2 \times A645) + (8.02 \times A663),$$

$$\text{Carotenoids (mg g}^{-1}\text{ FW)} = (A480 + 0.114 \times A663 - 0.638 \times A645) \times V / (1000 \times W),$$

where: V is the volume of the extract (mL), W is the weight of the fresh leaf (g), A is the absorbance at the respective wavelengths.

2.3. Hydrogen peroxide (H_2O_2)

The hydrogen peroxide concentration was assessed in accordance with a modified protocol based on Velikova *et al.* (2000). A 0.5% trichloroacetic acid (TCA) solution (5 mL) was prepared, and approximately 0.25 g of leaf tissue was finely ground using a chilled mortar and pestle. The resulting homogenate was then centrifuged at 12 000 rpm for 12 min to separate the supernatant. To 500 µL of the obtained supernatant, equal volumes (500 µL each) of phosphate buffer and potassium iodide (KI) solution were added. The mixture was then vortexed thoroughly to ensure proper mixing. The absorbance readings of the prepared solution were taken at 390 nm using a spectrophotometer (Model, WE 721 WeLab instrument Limited).

2.4. Malondialdehyde (MDA)

Malondialdehyde (MDA) content was determined following the method described by Cakmak and Horst (1991), with slight modifications for precision. A quantity of 0.25 g of leaf tissue was homogenized in 3 mL of 0.5% trichloroacetic acid (TCA). The homogenate was then transferred to Eppendorf tubes and centrifuged at 12 000 rpm for 12 min to isolate the supernatant. One milliliter of the clear supernatant was mixed with 1 mL of 0.5% thiobarbituric acid (TBA) in labeled test tubes. To prevent oxidation, the tubes were covered with aluminum foil and incubated in a water bath at 95°C for 15 min. Immediately after incubation, the tubes were rapidly cooled in an ice bath for 15 min to halt the reaction. The optical density of the samples was determined at 532 nm using a spectrophotometer, and nonspecific turbidity was corrected by measuring absorbance at 600 nm. (Model: WE 721 WeLab Instruments Ltd.).

2.5. Determination of antioxidants

2.5.1. Superoxide dismutase

The role of superoxide dismutase was evaluated based on the protocol described by Spitz and Oberley (2001), using a quartz cuvette for accurate spectrophotometric measurements. Reagents were introduced in a defined order; initially, 400 µL of distilled water was added. Subsequently, 250 µL of potassium phosphate buffer, 100 µL of *L*-methionine, 100 µL of Triton X, 50 µL of nitro blue tetrazolium (NBT), and 50 µL of the leaf extract were added. Lastly, 50 µL of riboflavin was mixed to complete the reaction mixture. The cuvettes were illuminated with a steady light source for 15-20 min to induce the photochemical reaction. A blank was prepared and measured after 15 min to ensure accuracy. Absorbance for all samples was then recorded at a wavelength of 560 nm using a spectrophotometer (Model, WE 721 WeLab instrument Limited).

2.5.2. Peroxidase

Peroxidase activity was assessed based on the method of Chance and Maehly (1955), with slight modifications. The reaction mixture was prepared in a cuvette by sequentially combining 750 µL of phosphate buffer, 100 µL of guaiacol, 50 µL of leaf extract, and 100 µL of hydrogen peroxide (H_2O_2). The change in absorbance, indicating enzyme activity, was monitored at 470 nm using

a spectrophotometer (Model: WE 721 We Lab Instruments Ltd.). Readings were recorded at regular time intervals of 0, 30, 60, and 90 s to assess the rate of the reaction.

2.5.3. Catalase

Catalase activity was tested using the Chance and Maehly (1955) approach. The cuvette was loaded with 1,000 µL of carrot leaf extract homogenized in 1.9 mL of chilled phosphate buffer and 1 mL of hydrogen peroxide (H_2O_2) in order to measure the absorbance at 240 nm using a spectrophotometer (Model, WE 721 WeLab instrument Limited) at 0, 30, 60, and 90%.

2.6. Determination of non-enzymatic antioxidants

Fresh leaf material (100 mg) was mashed and stored from each sample using the ascorbic acid content method outlined by Mukherjee and Chaudhuri (1983). For this, 5 mL of TCA at 6% were used. The sample was centrifuged at 12 000 rpm for 12 min, and the resulting supernatant was collected and stored for further analysis. These test tubes were filled with one milliliter of the supernatant, 2 mL of 25% DNPH, and one drop of thiourea. The test tubes were incubated in a water bath at 50°C for 20 min and subsequently placed in an ice bath to stop the reaction. Next, each test tube received 2.5 ml of 80% H_2SO_4. A spectrophotometer (Model: WE 721WeLab instrument Limited) was used for measuring absorbance at 530 nm.

The Starck and Wray (1989) method was used to estimate the quantity of anthocyanin. Two milliliters of acidified methanol and 0.1 g of freshly crushed leaf material were added to each test tube containing a sample. After incubation at 90°C for 60 min, the absorbance of each sample was determined at 535 nm using a spectrophotometer.

Flavonoid levels in the samples were assessed using the Marinova *et al.* (2005) method. 100 mg of fresh leaf material was steeped in 5 mL of an 80% acetone solution for 10 to 20 h. Each test tube received 1 mL of the leaf extract and 4 mL of distilled water on the next day. The test tubes were then allowed to stand for five minutes. Subsequently, 600 µL of 55% sodium nitro oxide ($NaNO_2$) and several liters of 10% $AICl_3$ were added. After approximately one minute, the mixture in the test tubes was adjusted with 24 mL of distilled water and 2 µL of 1M sodium hydroxide (NaOH). Absorbance was measured using a spectrophotometer (Model: WE 721 WeLab instrument Limited) at 510 nm.

2.7. Determination of total soluble protein content

According to the method of Bradford (1976), 0.25 g of fresh leaf tissue was homogenized in 5 mL of phosphate buffer. The resulting homogenate was transferred into labeled Eppendorf tubes and centrifuged at 12 000 rpm for 12 min. After centrifugation, the supernatant was carefully collected into fresh Eppendorf tubes, and the pellet was discarded. After only five milliliters of this supernatant were extracted, five milliliters of Bradford reagent was dispensed into all test tubes. The materials are vortexed and their absorbance was confirmed using a spectrophotometer (Model, WE 721 Welab instrument Limited) adjusted to 595 nm.

2.8. Determination of total soluble sugars

Total soluble sugars were quantified using the method proposed by Yoshida *et al.* (1976). Each test tube received 10 mL of distilled water and 100 µL of the leaf samples separately. The incubation of the test tubes was carried out in a water bath set at 90°C for one hour. After removing the samples, 50 mL of distilled water were added to the test tubes to dilute the samples. Five milliliters of Anthrone reagent was added to 1.5 mL of the diluted samples in new test tubes. Next, the samples were immersed in a water bath at 90°C for 20 min. After 20 min, the samples were allowed to settle to ambient temperature for a long time. Lastly a spectrophotometer (WeLab instrument Limited, model WE 721) was used to measure absorbance at 620 nm.

2.9. Determination of ions

The Allen *et al.* (1986) method was used to measure ions. For this, the digestion flasks only received 0.1 g of the chopped, oven-dried plant samples. Additionally, two milliliters of pure H_2SO_4 were added to the flasks, which were then kept in the dark for ten to twenty hours. Ten hours later, one milliliter of H_2O_2 was added to the digesting flasks, which were placed on a hot plate. Until the mixture became transparent or colorless, the hot plate was left on. After removing the samples from the hot plate, each sample was diluted with 50 mL of distilled water. The solutions were filtered using the Whatman filter paper contained in plastic bottles. The concentrations of Na^+, Ca^{2+}, and K^+ in the shoot and root tissues were determined using a flame photometer (Model, Sherwood 410).

2.10. Statistical analysis

The experiment followed a completely randomized design (CRD) with four replications. Data were analyzed using CO-STAT software to determine the significance of treatment effects, and analysis of variance (ANOVA) was applied to assess the results. Statistix 8.1 software was used for lettering, and Tukey's test was used to compare the means. R-studio (v4.3.3), a statistical tool, was utilized for creating the heatmap with a dendrogram, and the Pearson correlation coefficient, Originpro (V2024), was used to perform PCA analysis. The graphical abstract diagram was made by using Biorender, and Microsoft Excel (Version, 2016) was used to make the graphs.

3. RESULTS

3.1. Morphological parameters

The statistical analysis revealed that salinity stress, *L*-phenylalanine (Phe) application, and their interaction significantly influenced the response on the morpho-physiological parameters of carrot plants. Shoot fresh weight (SFW) was reduced by 12%, root fresh weight (RFW) by 20%, shoot dry weight (SDW) by 13%, root dry weight (RDW) by 24%, shoot length (SL) by 11%, and root length (RL) by 7% under 60 mM salinity and further declined: SFW by 28% and RFW by 43%, SDW by 36% and RDW by 50%, SL by 33% and RL by 20%, respectively, under 120 mM salinity, compared to the control. Under 60 mM salinity stress, the application of 100 ppm Phe enhanced the shoot fresh weight by 8%, root fresh weight by 27%, shoot dry weight by 32%, root dry weight by 33%, shoot length by 17%, and root length by 8%, while the application of 200 ppm Phe improved SFW by 38%, RFW by 46%, SDW by 51%, RDW by 45%, SL by 23%, and RL by 20%, relative to 0 ppm *L*-phenylalanine. At 120 mM salinity, the respective enhancements were 14% in shoot fresh weight, 35% in root fresh weight, 55% in shoot dry weight, 67% in root dry weight, 16% in shoot length, and 17% in root length under 100 ppm Phe, while the variant with 200 ppm Phe increased SFW by 28%, RFW by 68%, SDW by 64%, RDW by 92%, SL by 34%, and RL by 31% in comparison with 0 ppm *L*-phenylalanine. The overall results showed that the salinity stress decreased the morphological parameters of carrot, while the foliar application with Phe showed improvement in all morphological parameters, and the maximum enhancement was observed at 200 ppm Phe (Fig. 1).

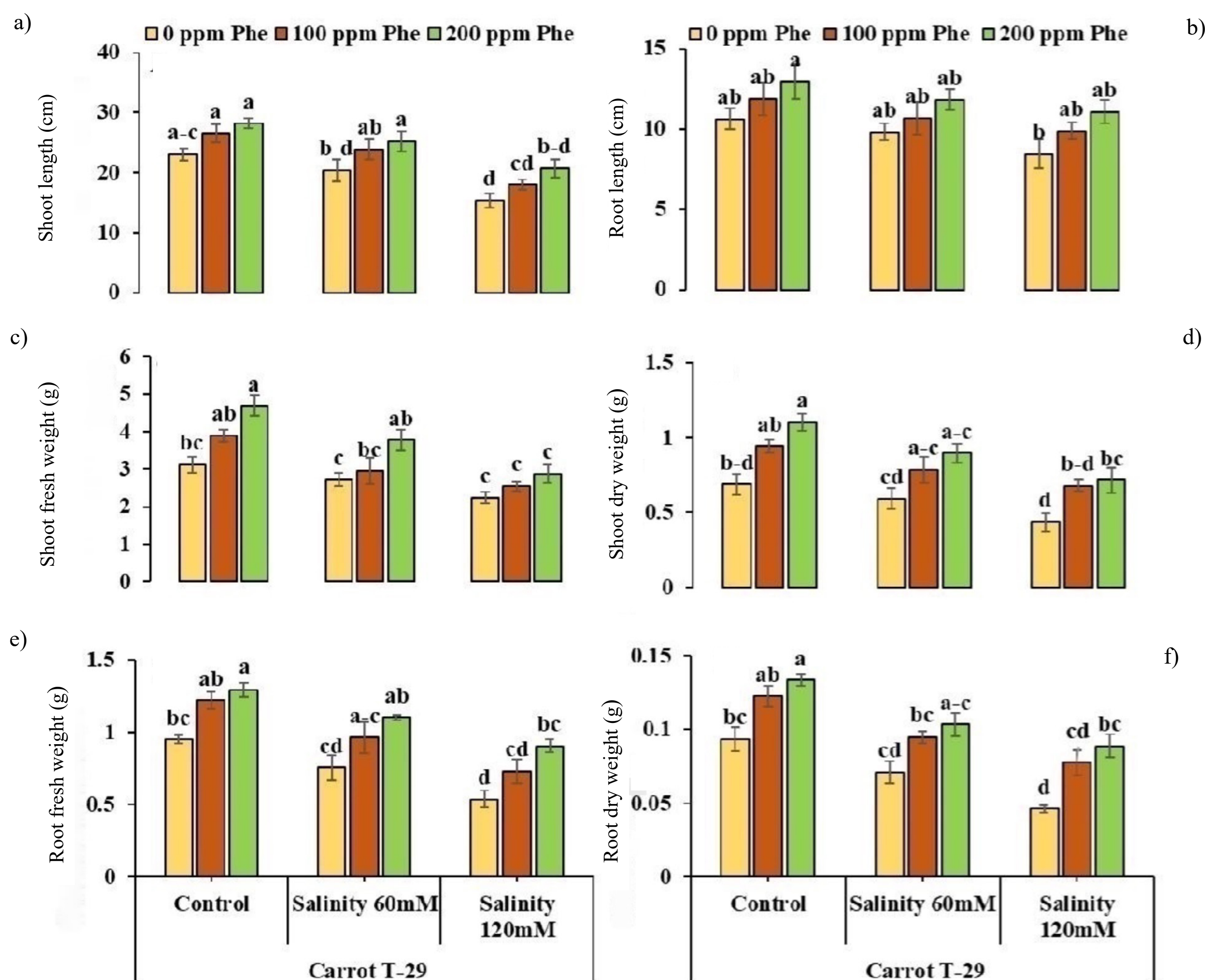

Fig. 1. Effect of *L*-phenylalanine on: a) shoot length (cm), b) root length (cm), c) shoot fresh weight (g), d) shoot dry weight (g), e) root fresh weight (g), f) and root dry weight (g) in carrot (T29) under salinity stress. Variety (V1) = T-29, S0 = control, S1 = 60 mM, S2 = 120 mM, *L*-phenylalanine = control (0 ppm), *L*-phenylalanine (100 ppm) and *L*-phenylalanine (200 ppm). Error bars sharing the same lettering for a parameter indicate non-significant differences at a significance level of $p \leq 0.05$.

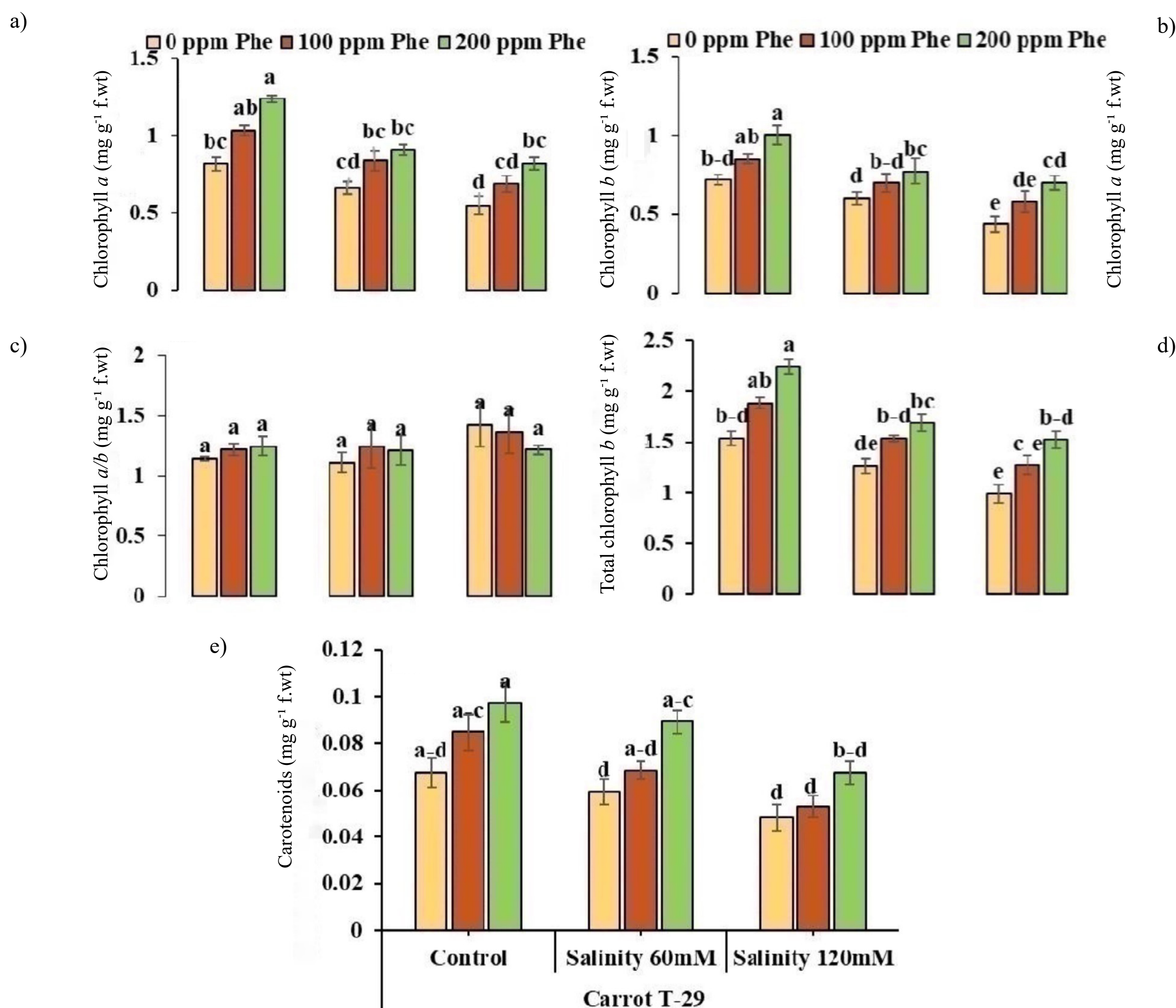

Fig. 2. Effect of *L*-phenylalanine on: a) chlorophyll *a* (mg g^{-1} f.wt), b) chlorophyll *b* (mg g^{-1} f.wt), c) chlorophyll *a/b,* d) total chlorophyll (mg g^{-1} f.wt) and e) carotenoids (mg g^{-1} f.wt) ratio in carrot (T29) under salinity stress. Variety (V1) = T-29, S0 = control, S1 = 60 mM, S2 = 120 mM, *L*-phenylalanine = control (0 ppm), *L*-phenylalanine (100 ppm) and *L*-phenylalanine (200 ppm). Error bars sharing the same lettering for a parameter indicate non-significant differences at a significance level of $p \leq 0.05$.

3.2. Photosynthetic pigments

The photosynthetic pigments in the carrot plants were markedly influenced by salinity stress and the *L*-phenylalanine application. Chlorophyll *a* was suppressed by 19%, chlorophyll *b* by 16%, the chlorophyll *ab* ratio by 2.7%, total chlorophyll by 17%, and carotenoids by 12% under 60 mM salinity and further declined: Chl. *a* by 32%, Chl. *b* by 38%, total Chl. by 35%, and carotenoids by 28%, with a 9% increase in the Chl. *ab* ratio under 120 mM salinity, compared to the control. At 60 mM salinity, the application of 100 ppm Phe increased these pigments as Chl. *a* by 26%, Chl. *b* by 16%, Chl. *ab* ratio by 12%, total Chl. by 21%, and carotenoids 15%, while with 200 ppm Phe enhanced Chl. *a* by 37%*,* Chl. *b* by 28%*,* Chl. *ab* ratio by 9%, total Chl. by 33%, and carotenoids by 50% relative to 0 ppm *L*-phenylalanine. At 120 mM salinity, the respective improvements were 25% in Chl. *a,* 32% in Chl. *b,* 28% in total Chl., and 9% in carotenoids, with a 4.2% decrease in the Chl. *ab* ratio at 100 ppm Phe. The treatment with 200 ppm resulted in an increase by 49% in Chl. *a,* 59% in Chl. *b,* 58% in total Chl., and 39% in carotenoids and a 14% decrease in the Chl. *ab* ratio, compared to 0 ppm *L*-phenylalanine. The overall results showed that salinity stress decreased the photosynthetic pigments in carrot while the foliar application of Phe caused improvements in all chlorophyll pigments and carotenoids, and the maximum enhancement was observed at 200 ppm Phe (Fig. 2).

3.3. Antioxidant enzymes and oxidative stress

The statistical analysis revealed that the levels of oxidative stress indicators (H_2O_2 and MDA) as well as the activities of antioxidant enzymes in the carrot plants were significantly modulated by the salinity stress and *L*-phenylalanine treatment. H_2O_2 was increased by 1.2% and

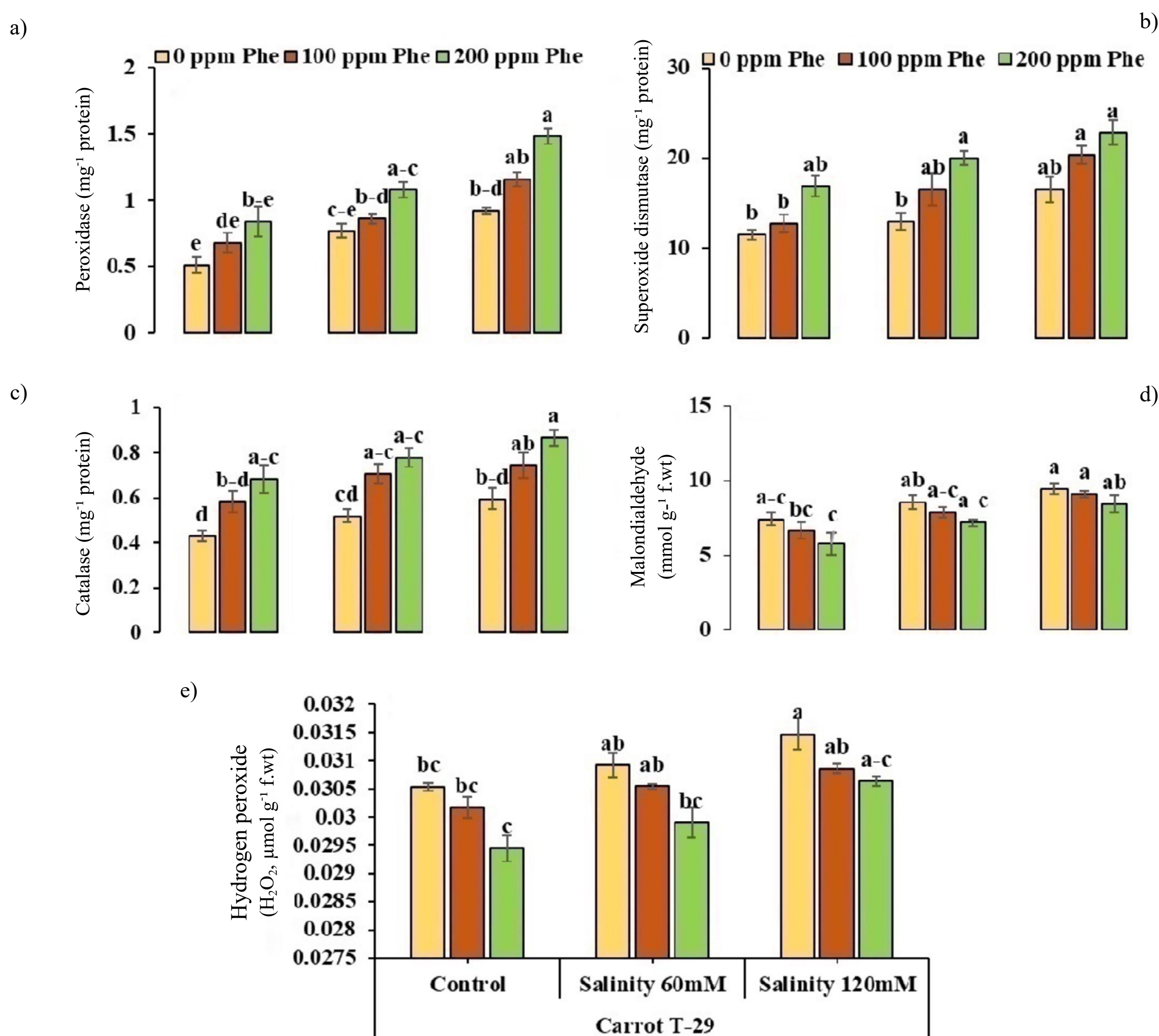

Fig. 3. Effect of *L*-phenylalanine on: a) peroxidase (POD, mg^{-1} protein), b) superoxide dismutase (SOD, mg^{-1} protein), c) catalase (CAT, mg^{-1} protein), d) malondialdehyde (MDA, mmol g^{-1} f.wt), and e) hydrogen peroxide (H_2O_2, µmol g^{-1} f.wt) in carrot (T29) under salinity stress. Variety (V1) = T-29, S0 = control, S1 = 60 mM, S2 = 120 mM, *L*-phenylalanine = control (0 ppm), *L*-phenylalanine (100 ppm) and *L*-phenylalanine (200 ppm). Error bars sharing the same lettering for a parameter indicate non-significant differences at a significance level of $p \leq 0.05$.

MDA by 15% under 60 mM salinity and further increased by 3.05% in H_2O_2 and 27% in MDA respectively under 120 mM salinity, compared to the control. At 60 mM salinity, the application of 100 ppm Phe reduced H_2O_2 and MDA by 1.2 and 8.3%, respectively, while 200 ppm Phe further decreased these parameters by 3.3% and 16%, relative to 0 ppm Phe. At 120 mM salinity, H_2O_2 declined by 1.9% and MDA by 3.6% at 100 ppm Phe and by 2.6 and 10% at 200 ppm, compared to 0 ppm Phe, respectively. Similarly, the levels of antioxidant enzymes were increased: peroxidase by 49%, superoxide dismutase by 13%, and catalase by 20% under 60 mM salinity and further increased by 79% in POD, 44% in SOD, and 38% in CAT, respectively, under 120 mM salinity, compared to the control. At 60 mM salinity, the application of 100 ppm Phe increased these enzymes as follows: peroxidase by 12%, superoxide dismutase by 27%, and catalase by 36%, respectively, while 200 ppm Phe increased POD by 40%, SOD by 54%, and CAT by 50%, relative to 0 ppm Phe. At 120 mM salinity, the respective enhancements were 25% in peroxidase, 23% in superoxide dismutase, and 25% in catalase with 100 ppm Phe, while the treatments with 200 ppm Phe increased their levels by 60% in POD, 38% in SOD, and 46% in CAT, compared to 0 ppm *L*-phenylalanine. The overall results showed that the salinity stress increased the H_2O_2 and MDA content as well as enzymatic antioxidants in carrot, while the foliar application of Phe caused maximum enhancement in enzymatic antioxidants but decreased the H_2O_2 and MDA content (Fig. 3).

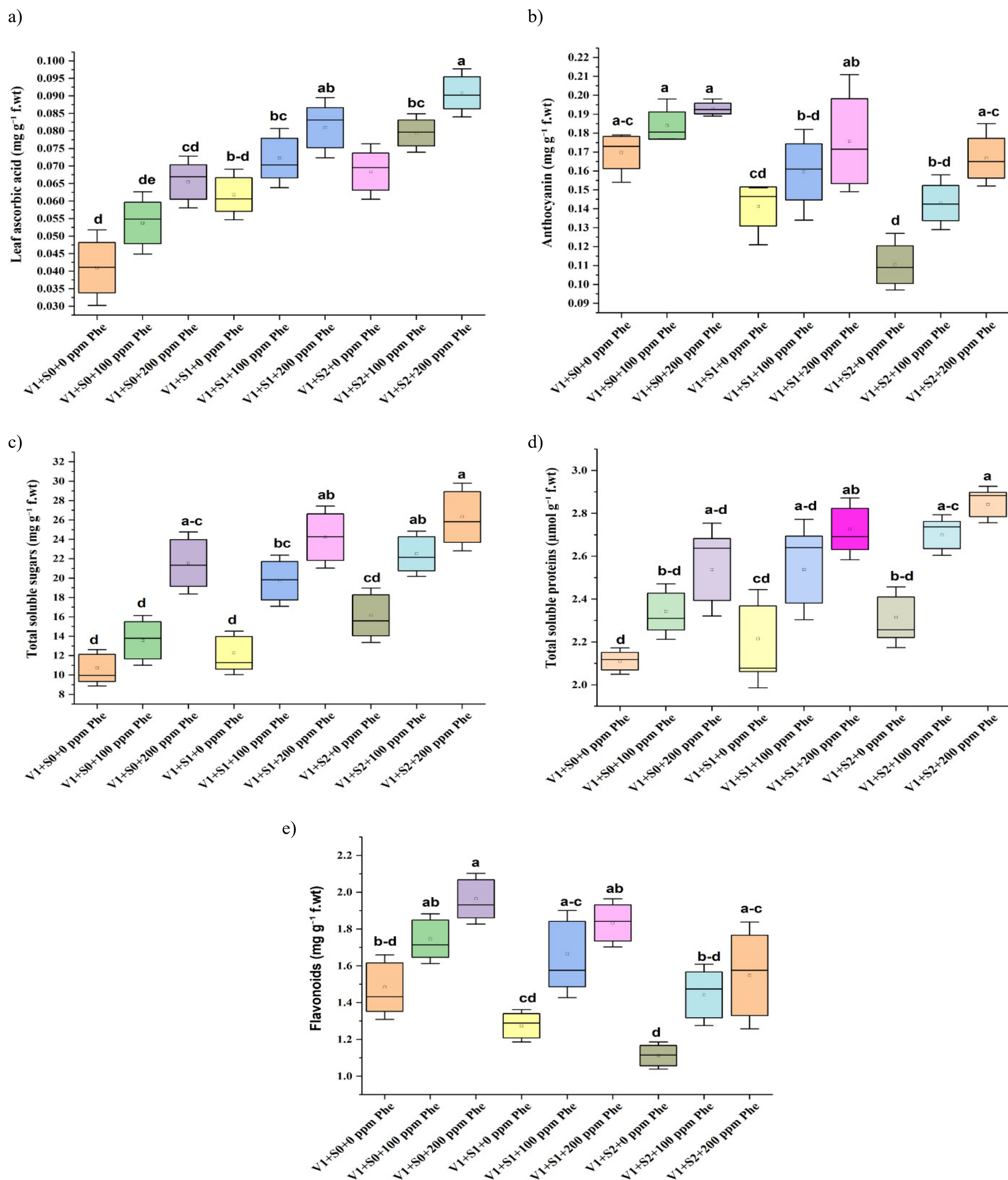

Fig. 4. Effects of *L*-phenylalanine on: a) leaf ascorbic acid (mg g^{-1} f.wt), b) anthocyanin (mg g^{-1} f.wt), c) total soluble sugars (TSS, mg g^{-1} f.wt), d) total soluble proteins (TSP, µmol g^{-1} f.wt) and e) flavonoids (mg g^{-1} f.wt) in carrot (T29) under salinity stress. Variety (V1) = T-29, S0 = control, S1 = 60 mM, S2 = 120 mM, *L*-phenylalanine = control (0 ppm), *L*-phenylalanine (100 ppm) and *L*-phenylalanine (200 ppm). Error bars sharing the same lettering for a parameter indicate non-significant differences at a significance level of $p \leq 0.05$.

3.4. Non-enzymatic antioxidant and osmolytes

The statistical analysis revealed antioxidant and biochemical parameters that were significantly influenced by the treatments. Anthocyanins decreased by 16%, flavonoids by 14%, and ascorbic acid increased by 50%, TSS by 4.9%, and TSP by 11% under 60 mM salinity. A further decline was recorded in anthocyanins by 34% and flavonoids 25%, but the levels of leaf ascorbic acid was enhanced by 66%, TSS by 50%, and TSP by 9.7% under 120 mM salinity, compared to the control. At 60 mM salinity, the application of 100 ppm Phe increased the anthocyanin content by 12%, flavonoids by 30%, ascorbic acid by 16%, TSS by 60%, and TSP by 14%, while the 200 ppm Phe treatment further enhanced anthocyanins by 24%, flavonoids by 43%, ascorbic acid by 30%, TSS by 97%, and TSP by 23%, relative to 0 ppm Phe. At 120 mM salinity, the enhancements were 29% in anthocyanins, 29% in flavonoids, 16% in ascorbic acid, 39% in TSS, and 16% in TSP at 100 ppm Phe, while the 200 ppm Phe treatment resulted in an increase by 50% in anthocyanins, 39% in flavonoids, 32% in ascorbic acid, 62% in TSS, and 22% in TSP, compared to 0 ppm *L*-phenylalanine. The overall results showed that the salinity stress increased the non-enzymatic antioxidants and organic osmolytes in carrot, and the foliar application of Phe caused maximum enhancements in non-enzymatic antioxidants and organic osmolytes, with the maximum enhancement observed at 200 ppm Phe (Fig. 4).

3.5. Ions

The statistical analysis demonstrated that the ionic parameters decreased as follows: shoot Ca^{2+} by 9%, root Ca^{2+} by 8%, shoot K^+ by 12%, and root K^+ by 14%, while shoot Na^+ and root Na^+ increased by 13 and 14% under 60 mM salinity. They further declined by 31% in shoot Ca^{2+}, 37% in root Ca^{2+}, 33% in shoot K^+, and 38% by root K^+, while an increase was observed in shoot Na^+ by 31% and root Na^+ by 30%, respectively, under 120 mM salinity, compared to the control. At 60 mM salinity, the application of 100 ppm Phe caused an increase of 20% in shoot Ca^{2+}, 18% in root Ca^{2+}, 26% in shoot K^+, and 15% in root K^+ and decreased shoot Na^+ by 9% and root Na^+ by 4%, respectively. The treatment with 200 ppm Phe led to a decrease of 30% in shoot Ca^{2+}, 59% in root Ca^{2+}, 41% in shoot K^+, 30% in root K^+, 16% in shoot Na^+, and 9% in root Na^+, relative to 0 ppm *L*-phenylalanine. At 120 mM salinity, the improvements reached 53% in shoot Ca^{2+}, 73% in root Ca^{2+}, 30% in shoot K^+, and 21% in root K^+. The values declined by 5.2% in shoot Na^+ and 1.4% in root Na^+ under 100 ppm Phe, while under 200 ppm Phe, they increased by 66% in shoot Ca^{2+}, 88% in root Ca^{2+}, 46% in shoot K^+, and 47% in root K^+ and declined by 8% in shoot Na^+ and 11% in root Na^+, compared to 0 ppm *L*-phenylalanine. The overall results showed that the salinity stress increased the root and shoot Na^+ ions in carrot but decreased the root and shoot K^+ and shoot Ca^{2+}. However, the foliar application of Phe caused reduction in the root and shoot Na^+ ions but increased the root and shoot K^+ and shoot Ca^{2+}; the maximum enhancement was observed at 200 ppm Phe (Table 1).

3.6. Heatmap analysis

A two-way heatmap accompanied by a dendrogram was generated to examine the effects of *L*-Phe on carrot under salinity stress (Fig. 5). Based on the similarity of observations at different points during the treatment, the observations were grouped, and the relationships between the groups were visualized as colored squares. The lavender

Table 1. Mean square values for the effects of *L*-phenylalanine and salinity stress on ion accumulation in shoot and root of carrot (*Daucus carota* L.)

Varieties	Treatment	Shoot Ca^{2+}	Root Ca^{2+}	Shoot K^+	Root K^+	Shoot Na^+	Root Na^+
Carrot (T29)	S0T0	5.5±0.28 bc	6±0.40 bc	9.75±0.85bc	15.5±1.32 ac	18.25±1.2 b-d	26.5±0.64 b-d
	S0T1	7±0.40 ab	7.5±0.64 ab	12.5±0.64ab	17.25±0.75 ab	16.25±1.25 cd	22.25±1.03 cd
	S0T2	7.75±0.62 a	9.5±0.64 a	13.25±0.85a	19.25±0.85 a	13.75±0.85 d	20.5±1.55 d
	S1T0	5±0.40 bc	5.5±0.28 bc	8.5±0.64cd	13.25±1.3 b-d	20.75±1.25 a-c	30.25±2.01 ab
	S1T1	6±0.40 ab	6.5±0.64 b	10.7±0.8 a-c	15.25±0.8 a-c	18.75±0.85 a-d	29±1.29 a-c
	S1T2	6.5±0.64 ab	8.75±0.47 a	12±0.91ab	17.25±0.62 ab	17.25±1.25 b-d	27.25±1.79 a-c
	S2T0	3.75±0.47 e	3.75±0.62 c	6.5±0.64 d	9.5±0.64 d	24±0.91 a	34.5±1.55 a
	S2T1	5.7±0.47 a-c	6.5±0.64 b	8.5±0.64 cd	11.5±1.19 cd	22.75±0.85 ab	34±0.91 a
	S2T2	6.25±0.47 ab	7.75±0.75ab	9.5±0.64 bc	14±0.81 bc	22±1.47 a-c	30.5±1.19 a-c

Values represent means ± standard error of three replicates of carrot variety (T29). Treatments sharing the same lettering within a column indicate nonsignificant differences at $p \leq 0.05$. S0 = 0 mM NaCl, S1 = 60 mM NaCl, S2 = 120 mM NaCl; T0 = 0 ppm *L*-phenylalanine, T1 = 100 ppm *L*-phenylalanine, T2 = 200 ppm *L*-phenylalanine.

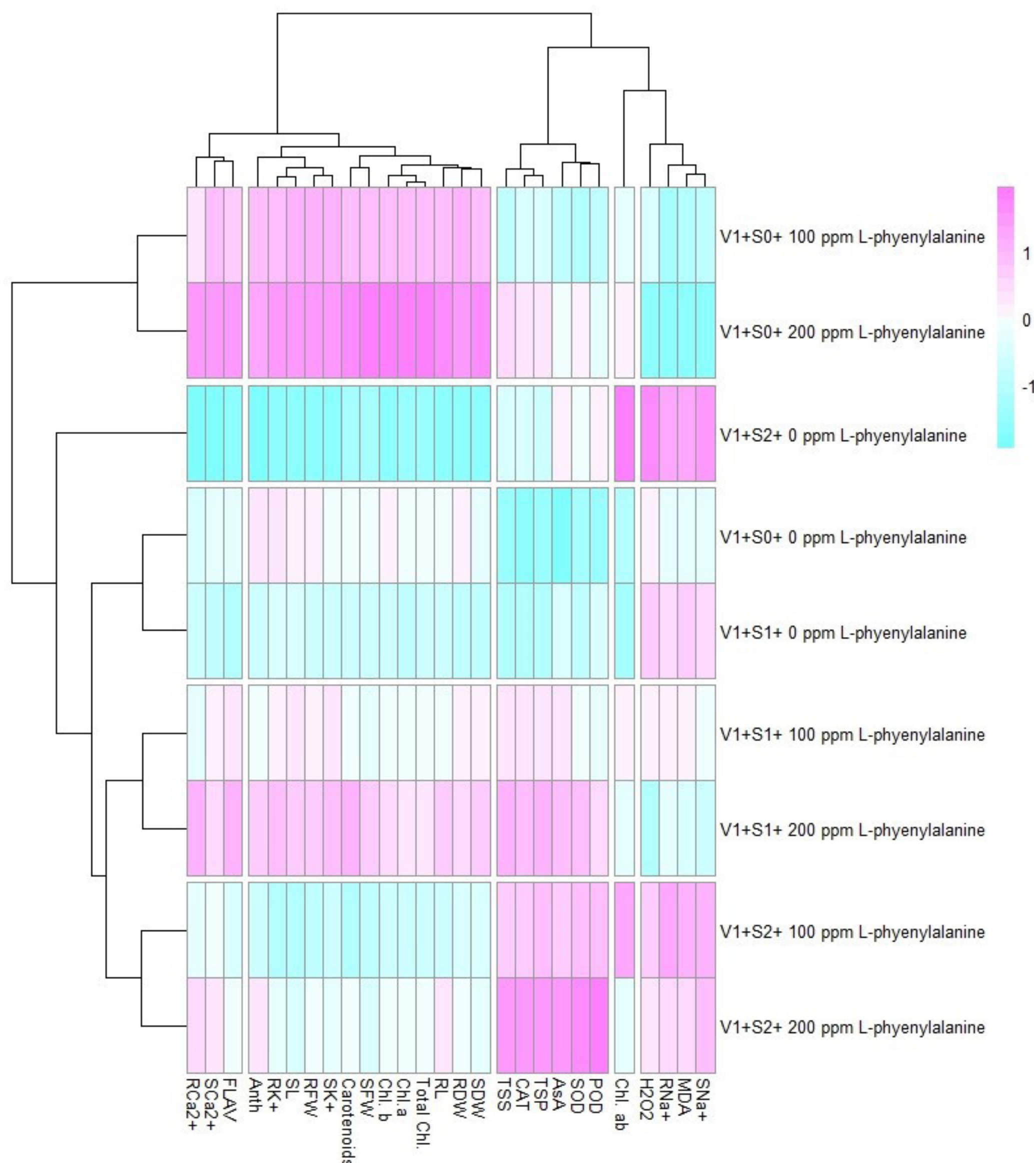

Fig. 5. Heatmap analysis with dendrogram between various morpho-biochemical, physiological parameters, osmolytes and ionic attributes of carrot exposed to salinity stress and Phe treatment. Variety: T-29 S0 = control, S1 = 60 mM, S2 = 120 mM, *L*-phenylalanine = control and *L*-phenylalanine (100 and 200 ppm). SL – shoot length, RL – root length, SFW – shoot fresh weight, RFW – root fresh weight, SDW – shoot dry weight, RDW – root dry weight, Chl. a – chlorophyll *a*, Chl. b – chlorophyll *b*, total Chl. – total chlorophyll, carotenoids, SOD – superoxide dismutase, POD – peroxidase, CAT – catalase, MDA – malondialdehyde, H_2O_2 – hydrogen peroxide, Flav. – flavonoids, AsA. – ascorbic acid, Anth. – anthocyanin, TSP – total soluble protein, TSS – total soluble sugar, RNa^+ – root sodium, SNa^+ – shoot sodium, RK^+ – root potassium, SK^+ – shoot potassium, RCa^{2+} – root calcium, SCa^{2+} – shoot calcium.

color denoted a strong positive association, and the blue color demonstrated a strong negative correlation for various observations, which were impacted by Phe under salinity stress (Fig. 5). The Heatmap was clustered into five groups. In the first group, H_2O_2, MDA, and root and shoot Na^+ was clustered. These parameters are strongly positively correlated with S2 (120 mM NaCl) and no treatment in carrot and showed a negative connection under S0 (no stress) and Phe (200 ppm), respectively. This group indicated that the use of Phe (200 ppm) decreased the contents of H_2O_2 and MDA and mitigated the adverse impacts of salinity stress. The 2nd group included the Chl. *a*/*b* ratio; this parameter is strongly positively correlated with S2 (120 mM NaCl) and no treatment in carrot and showed a negative connection under S0, and S1 (no stress and 60 mM NaCl), and no treatment, respectively.

The 3rd group included SOD, CAT, POD, TSP, TSS, and AsA, which were strongly positively correlated in carrot under salinity S2 (120 mM NaCl) and Phe (200 ppm), but negatively correlated at S0 (no stress) and no treat-

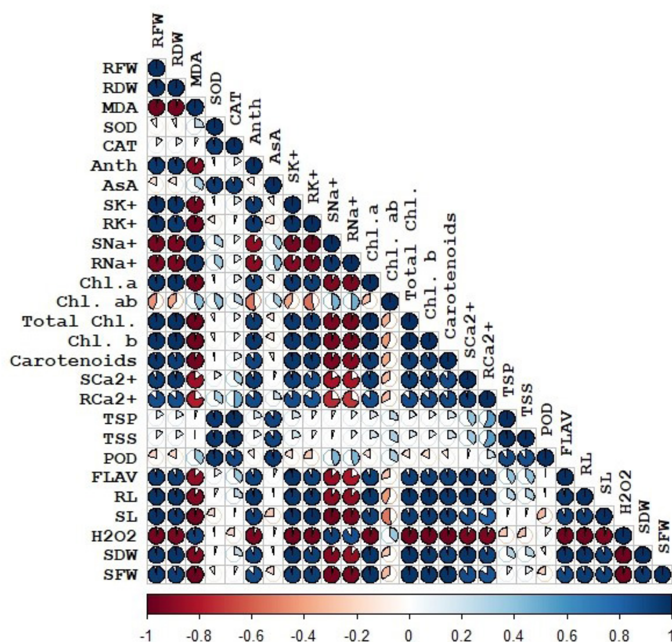

Fig. 6. Correlation matrix between morpho-physiological, biochemical parameters, ionic attributes and organic osmolytes of carrot under salinity stress and Phe treatment. SL – shoot length, RL – root length, SFW – shoot fresh weight, RFW – root fresh weight, SDW – shoot dry weight, RDW – root dry weight, Chl. a – chlorophyll *a*, Chl. b – chlorophyll *b*, total Chl. – total chlorophyll, carotenoids, SOD – superoxide dismutase, POD – peroxidase, CAT – catalase, MDA – malondialdehyde, H_2O_2 – hydrogen peroxide, Flav. – flavonoids, AsA. – ascorbic acid, Anth. – anthocyanin, TSP – total soluble protein, TSS – total soluble sugar, RNa^+ – root sodium, SNa^+ – shoot sodium, RK^+ – root potassium, SK^+ – shoot potassium, RCa^{2+} – root calcium, SCa^{2+} – shoot calcium.

ment (control conditions). These observations showed that under salinity stress, osmolytes and enzymatic antioxidants in carrot were improved by the application of Phe. In the 4th group, shoot and root fresh and dry weight, total Chl., carotenoids, root length, shoot length, root K^+, shoot K^+, and anthocyanins were grouped into the same cluster. These measures show strong positive correlations with S0 (no stress) and Phe (200 ppm), while negative correlations were found under S2 (120 mM NaCl) and no treatment. This group showed that the application of Phe (200 ppm) improved the morphological attributes, photosynthetic pigments, and ionic content in carrot. The 5th group comprised shoot and root Ca^{2+} and flavonoids. These attributes were strongly positively correlated at S0 (no stress) and Phe (200 ppm), while negative correlations were observed under S2 (120 mM NaCl) and no treatment. These observations demonstrated that the foliar application of Phe increased the growth parameters, photosynthetic pigments, and antioxidant activities in carrot under salinity stress (Fig. 5).

3.7. Correlation matrix

The correlation matrix demonstrates both high positive and strong negative relationships between the different attributes of carrot under salinity stress (Fig. 6). The results of the correlation analysis demonstrated that the growth parameters, such as shoot and root length, fresh weight, dry weight, spikelet number, no. of spikes, leaves, and grains were positively correlated with anthocyanins, Chl. *a*, Chl. *b*, total chlorophyll, carotenoids, flavonoids, and root and shoot K^+, Ca^{2+} respectively, and negatively correlated with H_2O_2, MDA, and shoot and root Na^+ ions (Fig. 6). There was a positive correlation between the CAT, POD, and SOD with TSP, TSS, and AsA content. Furthermore, the correlation was strongly positive among morphological traits and photosynthetic pigments (Fig. 6).

3.8. PCA analysis

The PCA analysis revealed that PCA 1 and PCA 2 accounted for 94.7% of the accumulated variations, with 71.05 and 23.65%, respectively (Fig. 7). However, the morphological, photosynthetic, antioxidant, and ionic contents differed significantly under the different Phe treatments (100 ppm and 200 ppm) and salinity (S0 = control, S1 = 60 mM NaCl, and 120 mM NaCl). A very strong connection was noted among different photosynthetic and morphological parameters, flavonoids, and root and shoot Ca^{2+} and K^+ levels in the carrot. In turn, AsA, TSS, and TSP indicated close relationships with antioxidants (SOD, POD, and CAT). Root and shoot Na^+ were closely associated in the same treatment as well as MDA, Chl. *a*/*b*, and H_2O_2, respectively. In our research, the Phe implementation showed to be beneficial in alleviating salinity stress through enhancing morphological characteristics, such as

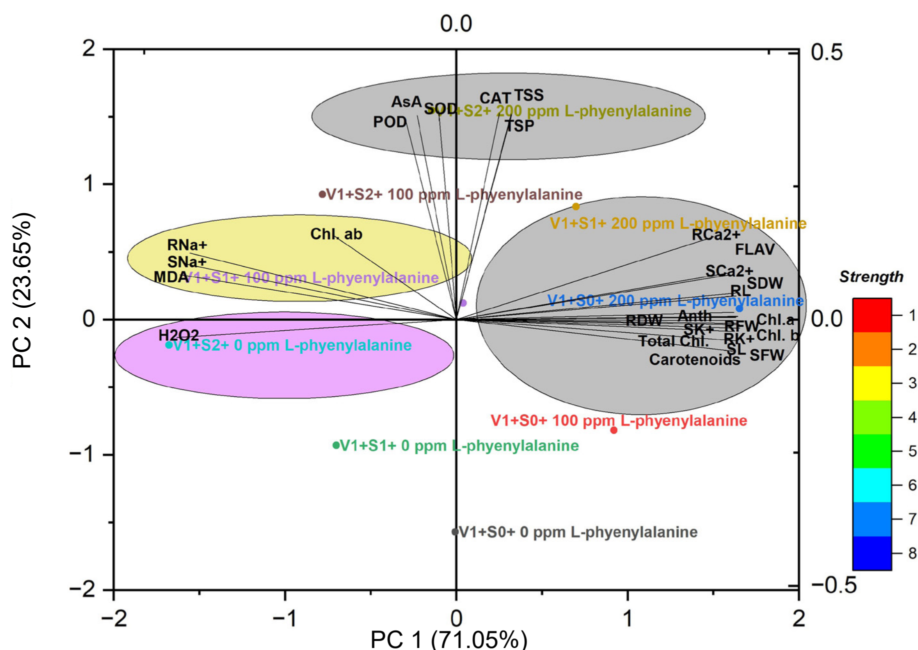

Fig. 7. Principal component analysis between morpho-physiological, biochemical parameters, ionic attributes and organic osmolytes of carrot under salinity stress and Phe treatment. Variety: T-29 S0 = control, S1 = 60 mM, S2 = 120 mM, *L*-phenylalanine = control and *L*-phenylalanine (100 and 200 ppm).

shoot length, photosynthetic pigments, and ionic contents while decreasing the negative SNa^+, RNa^+, MDA, and H_2O_2 in the carrot plants (Fig. 7).

4. DISCUSSION

Salt stress imposed a pronounced reduction in carrot (*Daucus carota* L.) growth, with significant decreases in morphological parameters, including shoot length, root length, and fresh and dry weight under 60 and 120 mM NaCl. The reduction was especially marked at the higher salinity level, reflecting the combined effects of osmotic stress and ionic toxicity on morphogenesis. Similar results were reported in radish, where salinity stress markedly reduced root and shoot length (Inayat *et al.,* 2024). Likewise, in tomato, pea, and black gram, salinity decreased root elongation and shoot biomass due to impaired water uptake and hormonal imbalance (Almas *et al.,* 2021; Fareed *et al.,* 2024; Ahmad *et al.,* 2024). A comparable trend was also observed in *Juglans microcarpa* seedlings, where salt stress caused leaf yellowing, curling, and biomass reduction due to disrupted photosynthesis (Ji *et al.,* 2022). These reports confirm that salt stress universally hampers plant morpho-physiological growth through osmotic imbalance and nutrient deficiency. The foliar application of *L*-phenylalanine improved morphological growth of carrot plants under salt stress, as evident from the enhancement in shoot and root length as well as fresh and dry biomass, compared to untreated stressed plants. The growth-promoting effect of phenylalanine may be attributed to its role as a precursor of phenylpropanoid metabolism, which enhances structural integrity and stress resilience. Similar results were obtained in mustard, where phenylalanine supplementation improved vegetative growth under saline conditions by stimulating secondary metabolism (Ramzan *et al.,* 2023). It was reported that exogenous application of amino acids, including phenylalanine, enhanced biomass accumulation under salt stress. Likewise, in soybeans, foliar amino acid treatment improved plant height and biomass production through enhanced nitrogen metabolism (Sadak *et al.,* 2020). These findings indicate that phenylalanine mitigates the negative impact of salinity on plant growth by stimulating metabolic adjustments and promoting biomass accumulation.

In this study, photosynthetic pigments, including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, exhibited a notable decline in carrots under salinity. This reduction can be attributed to oxidative damage to chloroplast membranes and the inhibition of chlorophyll biosynthesis under Na^+ toxicity. Similar declines were observed in wheat (Ibrahimova *et al.,* 2021), where chlorophyll content was reduced by salinity stress. In carrot, Saad *et al.* (2021) reported downregulation of carotenoid biosynthetic genes under salinity, leading to pigment loss. These consistent findings highlight that pigment reduction is a general mechanism of salt-induced photosynthetic impairment across species. Photosynthetic pigments, such as chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, were significantly improved in phenylalanine-treated carrots compared to untreated stressed plants. Phenylalanine application likely prevented oxidative

degradation of chlorophyll and supported pigment biosynthesis. In pepper (Sheteiwy *et al.,* 2021), phenylalanine application enhanced chlorophyll and carotenoid content, improving photosynthetic efficiency under stress. These reports suggest that phenylalanine sustains pigment stability by promoting chloroplast protection and photosynthetic activity under salinity stress.

In our experiment, salt stress also triggered oxidative stress in carrots, as indicated by elevated hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents. This demonstrates enhanced lipid peroxidation and ROS accumulation. Similar oxidative damage was reported in mustard (Ramzan *et al*., 2023), where NaCl increased MDA and ROS levels, impairing membrane stability. Likewise, in basil (Sirousmehr *et al.,* 2020), salinity-induced ROS accumulation disrupted cell metabolism. In canola (Ramzan *et al*., 2025), excessive oxidative stress markers were observed under salt conditions. These findings confirm that oxidative damage is a conserved response in plants in saline environments. Phenylalanine also reduced oxidative stress markers in carrot, where hydrogen peroxide and malondialdehyde contents were significantly lowered, while an increase was observed in the activities of antioxidant enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). This indicates that phenylalanine strengthened the antioxidant defense system, minimizing ROS-mediated damage. A similar protective role was observed in rice, where phenylalanine application reduced lipid peroxidation and enhanced antioxidant enzyme activity under salinity (Ramzan *et al*., 2025). In mustard, amino acid supplementation boosted antioxidant capacity, reducing ROS accumulation under abiotic stress (Ramzan *et al*., 2023). Moreover, in basil, Sirousmehr *et al. (*2020) reported that exogenous amino acids enhanced antioxidant enzyme activities, thereby lowering oxidative stress levels. These results highlight the role of phenylalanine in maintaining redox homeostasis under adverse conditions.

In the current study on carrot plants, salt stress led to a significant decline in anthocyanins and flavonoids, while the levels of ascorbic acid, total soluble proteins (TSP), and total soluble sugars (TSS) were elevated, suggesting a metabolic allocation to sustain osmotic adjustment. The foliar application of phenylalanine reversed the negative effects on secondary metabolites, leading to increased anthocyanin and flavonoid accumulation, while further enhancing the levels of ascorbic acid, proteins, and sugars. Similar findings were observed in *Amaranthus tricolor*, where salinity reduced flavonoids and anthocyanins but pretreatment with exogenous regulators restored their accumulation (Sarker and Oba, 2018). In Ginkgo cultures, phenylalanine-derived compounds, including ascorbic acid, were reported to increase under stress, indicating its role in maintaining redox balance (Suminar *et al.,* 2025). In safflower, soluble sugars were found to accumulate under salinity, supporting osmotic regulation (Jamshidi *et al.,* 2023). These findings indicate that phenylalanine plays a dual role by activating the phenylpropanoid pathway for secondary metabolites and by supporting osmolyte accumulation, thereby improving salt stress tolerance in carrot.

Ionic imbalance was evident in the carrot, where Na^+ accumulation increased while K^+ and Ca^{2+} levels decreased in both roots and shoots. This is a typical hallmark of salt stress, leading to nutrient deficiency and reduced enzyme activity. Similar ionic disturbances were reported in peanut, where NaCl stress reduced K^+ and Ca^{2+} uptake (Ramzan *et al*., 2023). In lettuce (Smolen *et al.,* 2020), it was observed that salinity disrupted ion homeostasis, reducing essential nutrient absorption. Likewise, in jasmine (Shahmoradi and Naderi 2018), it was reported that high Na^+ disrupted K^+/Na^+ balance, impairing stomatal conductance. These results support the idea that ionic imbalance under salinity is a universal physiological constraint in plants. With respect to ion regulation, phenylalanine significantly reduced Na^+ accumulation while enhancing K^+ and Ca^{2+} contents in carrot tissues, thereby improving ionic balance and nutrient uptake. The regulation of the Na^+/K^+ ratio is crucial for maintaining cellular metabolism and osmotic adjustment under salt stress. Similar findings were reported in canola, where amino acid treatment improved K^+/Na^+ homeostasis under saline irrigation (Ramzan *et al*., 2026a). In lettuce (Smoleń *et al.,* 2020), it was observed that amino acid applications improved Ca^{2+} and K^+ uptake, reducing salt-induced ionic toxicity. These results indicate that phenylalanine supports ion homeostasis and nutrient regulation, thereby alleviating salinity-induced ionic stress.

5. CONCLUSIONS

The morpho-physiological and biochemical performance of carrot plants was impaired by salinity stress, which inhibited growth and interfered with the absorption of nutrients. However, by stabilizing photosynthetic pigments and reducing the negative effects of salt, the *L*-phenylalanine exogenous application as a foliar spray increased plant growth and development. In addition to enhancing the accumulation of secondary metabolites and non-enzymatic antioxidants, *L*-phenylalanine increased the ability of SOD, CAT, and POD to scavenge hydrogen peroxide and malondialdehyde. Additionally, by increasing the content of K^+ and Ca^{2+} in roots and shoots and decreasing the intake of Na^+, *L*-phenylalanine controlled ionic equilibrium. Consequently, these implications suggest that *L*-phenylalanine would make a good low-cost foliar supplement to improve salt tolerance in crops planted in salty soils, especially salt soils. Future studies ought to be based on field experiments in multi-locations, dose-effect, long-term yield and quality outcomes and clarification of the underlying molecular pathways to confirm and expand the role of *L*-phenylalanine-mediated salt stress mitigation mechanisms.

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7. REFERENCES

Abd El-Mageed T.A., Ihab R., Rady M.M., Belal H.E.E., Mostafa F.A., Galal T.M., *et al*., 2023. A novel nutrient- and antioxidant-based formulation can sustain tomato production under full watering and drought stress in saline soil. Plants 12, 3407. https://doi.org/10.3390/plants12193407

Adams, Z.P., Ehlting, J., Edwards, R., 2019. The regulatory role of shikimate in plant phenylalanine metabolism. J. Theor. Biol. 462, 158-170. https://doi.org/10.1016/j.jtbi.2018.11.005

Ahmad, W., Waraich, E.A., Haider, A., Mahmood, N., Ramzan, T., Alamri, S., Akhtar, M.S., 2024. Silicon-mediated improvement in drought and salinity stress tolerance of black gram (*Vigna mungo* L.) by modulating growth, physiological, biochemical and root attributes. ACS Omega 9, 37231-37242. https://doi.org/10.1021/acsomega.4c04727

Alhudhaibi, A.M., Ibrahim, M.A., Abd-Elaziz, S.M., Farag, H.R., Elsayed, S.M., Ibrahim, H.A., *et al*., 2024. Enhancing salt stress tolerance in wheat (*Triticum aestivum*) seedlings: insights from trehalose and mannitol. BMC Plant Biol. 24(1), 472. https://doi.org/10.1186/s12870-024-04964-2

Allen, S.E., Grimshaw, H.M., Rowland, A.P., 1986. Methods in plant ecology. In: Moore P.D., Chapman S.B. (eds) Chemical Analysis. Blackwell Scientific Publications, Oxford, London, 285-344.

Almas, H.I., Anwar, S., Kausar, A., Farhat, F., Munawar, M., Khalizadieh, R., 2021. Exogenous application of methionine and phenylalanine confers salinity tolerance in tomato by concerted regulation of metabolites and antioxidants. J. Soil Sci. Plant Nutr. 21, 3051-3064. https://doi.org/10.1007/s42729-021-00588-9

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris.* Plant Physiol. 24, 1-15. https://doi.org/10.1104/pp.24.1.1

Balasubramaniam, T., Shen, G., Esmaeili, N., Zhang, H., 2023. Plants' response mechanisms to salinity stress. Plants 12, 2253. https://doi.org/10.3390/plants12122253

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Cakmak, I., Horst, J.H., 1991. Effects of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine* max). Physiol. Plant.83,463-468.https://doi.org/10.1111/j.1399-3054.1991.tb00121.x

Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidases. Methods Enzymol. 2, 764-775. https://doi.org/10.1016/S0076-6879(55)02300-8

Djoufack, M.M.T., Kouam, E.B., Foko, E.M.K., Anoumaa, M., Meli, G.R.L., Kaktcham, P.M., *et al.*, 2024. Determinants and constraints of carrot (*Daucus carota* L.) production and marketing in Cameroon. PLoS One 19:e0296418. https://doi.org/10.1371/journal.pone.0296418

Eze, F.O., Mukosha, C.E., Anozie, C., Moudry, J., Ali, S., Ghorbani, M., *et al*., 2024. Response of carrots (*Daucus carota*) on the growth, yield, and nutritional composition to varying poultry manure rates. Agric. Res. 13, 841-850. https://doi.org/10.1007/s40003-024-00723-9

Fareed, S., Haider, A., Ramzan, T., Ahmad, M., Younis, A., Zulfiqar, U., *et al*., 2024. Investigating the growth promotion potential of biochar on pea (*Pisum sativum*) plants under saline conditions. Sci. Rep. 14, 10870. https://doi.org/10.1038/s41598-024-59891-x

Gaadhe, S.K., Tiwari, V.K., 2022. Carrot harvesting methods: a review. Int. J. Plant Soil Sci. 34, 7-16. https://doi.org/10.9734/ijpss/2022/v34i1030988

Gelaye, Y., Getahun, S., 2024. Potential influence of nitrogen fertilizer rates on yield and yield components of carrot (*Daucus carota* L.) in Ethiopia: systematic review. Open Agric. 9, 20220335. https://doi.org/10.1515/opag-2022-0335

Haider, A., Waraich, E.A., Hussain, S. and Shahbaz, M., 2026. Characterization of morpho-physiological, biochemical, ionic, and root responses of camelina sativa genotypes under drought stress by corn-cob-based biodegradable super-absorbent polymer. New Zealand J. Crop Hortic. Sci. 54(1), p.e70054. https://doi.org/10.1002/nzc2.70054

Haider, A., Ahmad M.S.A., Riffat, A., Ramzan, T., Rani, W., 2026b. Biochar-mediated amelioration of nickel-induced stress in spinach (*Spinacia oleracea* L.) plants: a phytoremediation approach. Pak. J. Bot. 58(2), 379-393. http://dx.doi.org/10.30848/PJB2026-2(1)

Hasanuzzaman, M., Fujita, M., 2022. Plant responses and tolerance to salt stress: physiological and molecular interventions. Int. J. Mol. Sci. 23(9), 4810. https://doi.org/10.3390/ijms23094810

Hayat, U., din, K.U., Haider, A., Ramzan, T., Shahzad, B.A., Ahmad, M., Elshikh, M.S., 2024. Salicylic acid-induced antioxidant defense system alleviates cadmium toxicity in wheat. J. Soil Sci. Plant Nutr. 24, 3068-3086. https://doi.org/10.1007/s42729-024-01732-x

Ibrahimova, U., Zivcak, M., Gasparovic, K., Rastogi, A., Allakhverdiev, S.I., Yang, X., *et al*., 2021. Electron and proton transport in wheat exposed to salt stress: Is the increase of the thylakoid membrane proton conductivity responsible

for decreasing the photosynthetic activity in sensitive genotypes? Photosynth. Res. 150, 195-211. https://doi.org/10.1007/s11120-021-00853-z

Ikram, A., Rasheed, A., Ahmad Khan, A., Khan, R., Ahmad M., Bashir, R., *et al*., 2024. Exploring the health benefits and utility of carrots and carrot pomace: a systematic review. Int. J. Food Prop. 27, 180-193. https://doi.org/10.1080/10942912.2023.2290132

Inayat, H., Mehmood, H., Danish, S., Alharbi, S.A., Ansari, M.J., Datta, R., 2024. Impact of cobalt and proline foliar application for alleviation of salinity stress in radish. BMC Plant Biol. 24, 287. https://doi.org/10.1186/s12870-024-04998-6

Jahan, M.A.H.S., Hossain, M.S., Karim, A.J.M.S., 2020. Growth and physiological responses of carrot (*Daucus carota* L.) to salinity stress. Progressive Agriculture 31, 55-63.

Jamshidi Jam, B., Shekari, F., Andalibi, B., Fotovat, R., Jafarian, V., Dolatabadian, A., 2023. The effects of salicylic acid and silicon on safflower seed yield, oil content, and fatty acids composition under salinity stress. Silicon 15, 4081-4094. https://doi.org/10.1007/s12633-023-02308-7

Ji, X., Tang, J., Zhang, J., 2022. Effects of salt stress on the morphology, growth and physiological parameters of *Juglans microcarpa* L. seedlings. Plants 11, 2381. https://doi.org/10.3390/plants11182381

Kalia, P., Singh, B.K., Patel, V., Selvakumar, R., 2023. Carrot: breeding and genomics. Veg. Sci. 50, 221-230. https://doi.org/10.61180/vegsci.2023.v50.spl.08

Kamińska, I., Lukasiewicz, A., Klimek-Chodacka, M., Długosz-Grochowska, O., Rutkowska, J., Szymonik, K., *et al*., 2022. Antioxidative and osmoprotecting mechanisms in carrot plants tolerant to soil salinity. Scientific Reports 12(1), 7266. https://doi.org/10.1038/s41598-022-10835-3

Kesawat, M.S., Satheesh, N., Kherawat, B.S., Kumar, A., Kim, H.U., Chung, SM., *et al*., 2023. Regulation of reactive oxygen species during salt stress in plants and their crosstalk with other signaling molecules: current perspectives and future directions. Plants 12, 864. https://doi.org/10.3390/plants12040864

Khursheed, M.H.U.R., Shahbaz, M., Ramzan, T., Haider, A., Maqsood, M.F., Khan, A., *et al*., 2024. Enhancing wheat tolerance to cadmium stress through moringa leaf extract foliar application. Scientifica 2919557. https://doi.org/10.1155/2024/2919557

Kiełkowska, A., Grzebelus, E., Lis-Krzyścin, A., Maćkowska, K., 2019. Application of the salt stress to the protoplast cultures of the carrot (*Daucus carota* L.) and evaluation of the response of regenerants to soil salinity. Plant Cell Tiss. Organ Cult. 137, 79-395. https://doi.org/10.1007/s11240-019-01578-7

Maqbool, S., Ramzan, T., Haider, A., Waraich, E.A., Fatima, A., Siddiqui, M.H., *et al*., 2025. Impact of thiourea on wheat's morpho-physiological and ionic attributes (*Triticum aestivum* L.) under lead stress: reducing the translocation of lead from soil to roots, shoots, and grains. ACS Omega 10, 3054-3066. https://doi.org/10.1021/acsomega.4c09941

Marinova, D., Ribarova, F., Atanassova, M., 2005. Total phenolic and total flavonoids in Bulgarian fruits and vegetables. J. Univ. Chem. Technol. Metall. 40, 255-260.

Mukherjee, S., Choudari, M., 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol. Plant. 58, 166-170. https://doi.org/10.1111/j.1399-3054.1983.tb04162.x

Munns, R., Tester, M., 2020. Mechanisms of salinity tolerance. Ann. Review Plant Biol. 71, 59-81.

Muzafar, A., Shahbaz, M., Ramzan, T., Haider A, Fatima M., 2026. Assessing the efficacy of exogenous application of glycine betaine in canola (*Brassica napus* L.) under nickel stress. J. Soil Sci. Plant Nutr. 26, 1-20. https://doi.org/10.1007/s42729-025-02912-z

Qiu, M., Jiang, J., Jiang, W., Zhang, W., Jiang, Y., Xin, F., *et al.*, 2024. The biosynthesis of *L*-phenylalanine-derived compounds by engineered microbes. Biotechnol. Adv. 77, 108448. https://doi.org/10.1016/j.biotechadv.2024.108448

Ramzan, T., Shahbaz, M., Ahmad F., Waraich, E.A., 2025. Changes in the morpho-physiological, and biochemical attributes of canola (*Brassica napus* L.) varieties caused by seed priming with melatonin and ascorbic acid under salt stress. Pakistan J. Agric. Sci. 62, 605-622. https://doi.org/10.21162/PAKJAS/25.650

Ramzan, T., Shahbaz, M., Maqsood, M.F., Zulfiqar, U., Saman, R.U., Lili, N., *et al*., 2023. Phenylalanine supply alleviates the drought stress in mustard (*Brassica campestris*) by modulating plant growth, photosynthesis, and antioxidant defense system. Plant Physiol. Biochem. 201, 107828. https://doi.org/10.1016/j.plaphy.2023.107828

Ramzan, T., Shahbaz, M., Ahmad F., Waraich E.A., 2026a. Modulation in growth, yield, water relations, and mineral nutrients of canola (*Brassica napus* L.) by foliar application of melatonin and ascorbic acid under salinity stress. New Zea J. Crop Hortic. Sci. 54(1), e70003. https://doi.org/10.1002/nzc2.70003

Ramzan, T., Shahbaz, M., Ahmad F., Waraich E.A., 2026b. Foliar Application of melatonin and ascorbic acid mitigates salinity stress by improving the morpho-physiological, and antioxidant defense system in canola (*Brassica napus* L.). J. Soil Sci Plant Nutr. https://doi.org/10.1007/s42729-025-02862-6

Saad, K.R., Kumar, G., Mudliar, S.N., Giridhar, P., Shetty, N.P., 2021. Salt stress-induced anthocyanin biosynthesis genes and MATE transporter involved in anthocyanin accumulation in *Daucus carota* cell culture. ACS Omega 6, 24502-24514. https://doi.org/10.1021/acsomega.1c02941

Sadak, M.S., Abdalla, A.M., Abd, Elhamid, E.M., Ezzo, M.I., 2020. Role of melatonin in improving growth, yield quantity and quality of *Moringa oleifera* L. plant under drought stress. Bull. Natl. Res. Cent. 44, 18. https://doi.org/10.1186/s42269-020-0275-7

Samani, M.R., Pirbalouti, A.G., Moattar, F., Golparvar, A.R., 2019. *L*-phenylalanine and bio-fertilizers interaction effects on growth, yield and chemical compositions and content of essential oil from the sage (*Salvia officinalis* L.) leaves. Ind. Crops Prod. 137, 1-8. https://doi.org/10.1016/j.indcrop.2019.05.019

Sarker, U., Oba, S., 2018. Augmentation of pigments, vitamins, phenolic acids, flavonoids, and antioxidant activity in selected *Amaranthus tricolor* under salinity stress. Sci. Rep. 8, 12349. https://doi.org/10.1038/s41598-018-30897-6

Shahmoradi, H., Naderi, D., 2018. Improving effects of salicylic acid on morphological, physiological and biochemical

responses of salt-imposed winter jasmine. Int. J. Hortic. Sci. Technol. 5, 219-230. https://doi.org/10.22059/ijhst.2018.259507.246

Sheteiwy, M.S., Xiong, Y., Abd, Elgawad, H., Macovei, A., Brestic, M., Skalicky, M., *et al*., 2021. Phenylalanine improves pigment and osmotic adjustment in pepper under stress. Plant Physiol. Biochem. 161, 6168. https://doi.org/10.1016/j.plaphy.2021.01.030

Simpson, K., Fuentes P., Quiroz-Iturra, L.F., Flores-Ortiz, C., Contreras R., Handford M., *et al.,* 2018. Unravelling the induction of phytoene synthase 2 expression by salt stress and abscisic acid in *Daucus carota*. J. Exp. Bot. 69, 4113-4126. https://doi.org/10.1093/jxb/ery207

Sirousmehr, H., Moaveni, P., Farahvash, F., Nejad, T.S., 2020. Amino acids reduce oxidative stress in basil under salinity. J. Plant Interact. 15, 101-111.

Smoleń, S., Kowalska, I., Skoczylas, L., Tabaszewska, M., Pitala, J., Mrożek, J., *et al.*, 2022. Effectiveness of enriching lettuce with iodine using 5-iodosalicylic and 3,5-diiodosalicylic acids and the chemical composition of plants depending on the type of soil in a pot experiment. Food Chem. 382, 132347. https://doi.org/10.1016/j.foodchem.2022.132347

Spitz, D.R., Oberly, L.W., 2001. Measurement of MnSOD and CuZnSOD activity in mammalian tissue homogenates. Curr. Protoc. Toxicol. 8, 751-758.

Spoljarić, D., Agić, D., Lisjak, M., Špoljarić Maronić, D., 2022. Physiological, biochemical, and antioxidative responses of carrot (*Daucus carota* L.) genotypes to salinity stress. Scientific Reports, 12, 10835.

Starck D., Wray V., 1989. Anthocyanins. In: Harborne J.B. (ed) Methods in Plant Biochemistry, Vol. 1. Academic Press, London, 325-356. https://doi.org/10.1016/B978-0-12-461011-8.50015-9

Stefanov, M.A., Rashkov, G.D., Yotsova, E.K., Borisova, P.B., Dobrikova, A.G., Apostolova, E.L., 2021. Different sensitivity levels of the photosynthetic apparatus in *Zea mays* L. and *Sorghum bicolor* L. under salt stress. Plants, 10(7), 1469. https://doi.org/10.3390/plants10071469

Teixeira, W.F., Fagan, E.B., Soares, L.H., Reichardt, K., Silva, L.G., Dourado-Neto, D., 2019. Dry mass increment, foliar nutrientes and soybean yield as affected by amino acid application. J. Agric. Sci. 11, 230-242. https://doi.org/10.5539/jas.v11n18p230

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

Yoshida, S., Forno, D.A., Cock, J.H., Gomez, K.A., 1976. Laboratory Manual for Physiological Studies of Rice. Int. Rice Res. Inst., Los Baños, Philippines, p. 99.

Zelm, E. van, Zhang, Y., Testerink, C., 2020. Salt tolerance mechanisms of plants. Annual Rev. Plant Biol. 71, 403-433. https://doi.org/10.1146/annurev-arplant-050718-100005