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SALT STRESS ALLEVIATION IN *FRAGARIA x ANANASSA* PLANTS BY FOLIAR APPLICATION OF ASCORBIC ACID

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Abstract

Foliar application of ascorbic acid (AsA) was investigated to evaluate its potential for alleviating the salinity stress on strawberry plants. Plants grown in the plastic pots were irrigated with various concentrations of NaCl (0, 50, 100 and 200 mM) to induce salinity stress. Simultaneously, foliar spray of ascorbic acid (0, 0.5 and 1 mM) was applied on plants at regular intervals of 7 days over a period of 30 days. The results revealed that most vegetative and reproductive growth attributes were significantly reduced under salt stress, particularly at 200 mM NaCl. Foliar application of ascorbic acid partially mitigated the adverse effects of salt. All the vegetative growth parameters such as stem diameter, leaf length, leaf width and number of leaves were increased significantly by applying 1 mM ascorbic acid treatment. However, reproductive growth attributes except the number of flowers did not show significant improvement following AsA application under salt stress condition. The plants' protein contents were decreased under NaCl treatment and were not significantly increased with the ascorbic acid application. Similarly, antioxidant enzyme activities including superoxide dismutase (SOD) and peroxidases (POD) were increased under salt stress but did not show much enhancement in response to AsA application. This suggests that their activity primarily reflected stress responsive rather than yield protective role. The outcomes of this investigation indicated that applied AsA provided only partial stress mitigation. Therefore, high AsA concentrations (>1 mM) should be investigated in future studies to determine optimal dose for improving both vegetative and reproductive growth under saline conditions.

Key words: Ascorbic acid; Foliar application; *Fragaria* x *ananassa*; Salt stress

Introduction

Strawberry (*Fragaria x ananassa* (Duchesne ex Weston) Duchesne ex Rozier) an important commercial fruit plant, is cultivated all over the world on a large scale for its nutritious fruits (Crizel *et al*., 2020). Strawberry is a short-day plant and produces a single main crop per year (Asrey *et al*., 2004; Singh *et al*., 2021). It is a significant source of natural antioxidants, minerals, vitamins, flavonoids, phenolics and anthocyanins. These bioactive compounds are helpful for heart health and control of diabetes (Wang & Lin, 2000). Strawberry has high oxygen radical absorbance capacity against superoxide, hydroxyl radicals, peroxyl radicals, singlet oxygen and hydrogen peroxide (Wang & Jiao, 2000).

Among all the abiotic stresses, salinity is the major problem that reduces the plant growth and yield at the global level (Kamran *et al*., 2020). A soil is called saline when it contains approximately 40 mM or high NaCl (Van-Zelm *et al*., 2020). The total area of irrigated land worldwide is 583.1 million hectares (Turdaliev *et al*., 2022), about 25% of which is affected by salinity (Kholliyev & Teshaeva, 2021). Soluble salt (NaCl) in large amounts causes the osmotic stress and sodium acts as an exchangeable ion which causes ion toxicity (Tavakkoli *et al*., 2011; Munns *et al*., 2020). Sodium ions accumulate in the plant leaves and affect cell metabolism, different stages of development, seed germination, vegetative growth, seedlings growth, flowering and fruiting of the plants (Munns *et al*., 2002; Javed *et al*., 2007; Ahsan & Sajid, 2026). Photosynthesis is also greatly affected by salinity as it reduces the functioning and efficiency of both photosystems (PSI and PSII), damages the ultrastructure of chloroplasts, and reduces the stomatal conductance as a result of osmotic stress (Gao *et al*., 2016; Pan *et al*., 2020). Under saline conditions, sodium and chloride ions accumulate and inhibit the uptake of potassium and calcium, resulting in an ionic imbalance. It is also worth mentioning that these physiological and biochemical changes are growth stage-specific and related to yield attributes (Isayenkov & Maathuis, 2019; Khalil *et al*., 2025). Salinity also increases the reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), singlet oxygen (O_2) in different plant causing protein and DNA damage, membrane deterioration, lipid peroxidation and disruption of various macromolecules (Oukarroum *et al*., 2015; Lu *et al*., 2016; El-Ghazali, 2020; Rasool *et al*., 2025). There are several strategies to cope with the salt stress problem in crops like screening and developing of salt tolerant varieties, but such processes are usually time-consuming (Wu *et al*., 2015; Khan *et al*., 2016). Under these circumstances, the exogenous application of osmo-protectants, minerals, vitamins, and plant growth regulators has emerged as a practical and rapid alternative for enhancing stress tolerance. This approach has been successfully investigated in several

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important commercial crops including rice (Jiang *et al*., 2010), wheat (Anosheh *et al*., 2012), corn (Fahad & Bano, 2012), barley (Fayez & Bazaid, 2014), sunflower (Noreen *et al*., 2019), and potato (Gull *et al*., 2022).

Ascorbic acid (AsA) is an important water-soluble vitamin which is important in cell signaling process during plant stress to combat the oxidative stress and production of ROS under stressful conditions (Foyer & Noctor, 2011; Abdelgawad *et al*., 2020; Ahmad *et al*., 2024). Foliar application of ascorbic acid allows its rapid absorption and direct utilization in different plant cells, which may enhance plant tolerance to abiotic stresses (Billah *et al*., 2017). The application of ascorbic acid to mitigate different abiotic stresses is reported in barley (Agami, 2014), maize (Hussein and Alva, 2014; Billah *et al*., 2017), rice (Alhasnawi *et al*., 2016), sweet pepper (Barzegar *et al*., 2018), and wheat (Zhou *et al*., 2022). Ascorbic acid functions as an enzyme cofactor, and participates in photosynthesis, photoprotection, synthesis of ethylene, gibberellins, anthocyanins, and hydroxyproline (Smirnoff & Wheeler, 2000; Bybordi, 2012). It is also reported to play its important role in production of various hormones in plants (Fahad *et al*., 2016), cell wall expansion in many perennial halophytes (Hameed *et al*., 2015), photosynthetic pigments in tomato (Alayafi, 2020), ion uptake in okra (Wang, 2019) and regulation of antioxidant system in tomato (Hussein and Alva, 2014; Siddiqui *et al*., 2019). Previous studies have reported the beneficial effects of ascorbic acid application on strawberry plants under stress (Ergin *et al*., 2014; Ahmad *et al*., 2024). Although the stress mitigating role of AsA is well documented in other crop, studies focusing on foliar AsA mediated salinity tolerance in strawberry are limited. Therefore, the present study was conducted to evaluate the effect of foliar application of different levels of ascorbic acid on the vegetative growth, reproductive attributes, total soluble proteins content and antioxidant enzymes activities like superoxide dismutase and peroxidase of strawberry plants grown under salt stress.

Materials and Methods

Procurement of plant material: Strawberry (*Fragaria x ananassa*) offshoots (about 2.5 cm) from three months old vigorously growing plants were procured from Chaudhry Bashir Ahmed Nursery located in Model Town Lahore, Pakistan (31.4821 °N, 74.3220 °E).

Preparation of potting mix and the planting of strawberry offshoots: Potting mix for transplantation of strawberry offshoots was prepared by mixing garden soil and organic matter obtained from the Botanical Garden, University of the Punjab, Lahore. The soil was first sieved to remove straws, stones and all the particles having the diameter more than 5 mm. It was then mixed with organic matter in 1:1 ratio and this potting mix was taken in plastic pots of 20 cm diameter. Healthy offshoots of strawberry were planted into these pots at the depth of 1 cm with one offshoot per pot. These pots were kept under controlled conditions of greenhouse (26/16°C day/night temperature, 16/8 h. photoperiod and 60% relative humidity).

Experimental design and salt/ ascorbic acid treatment: This experiment was started in the last week of January 2024 at the Botanical Garden, University of the Punjab, Lahore (Pakistan). The experiment was conducted in a complete randomized block design (CRBD) under controlled environmental conditions. Each treatment consisted of three replicates with one pot containing a single plant, resulting in three independent plants per treatment. All the plants were irrigated as required and weeding was performed manually. Pots were covered with polythene sheet during rainfall and later placed under open field conditions of natural light and temperature. Salt stress was imposed using sodium chloride (NaCl) solutions at concentrations of 0, 50, 100, 200 mM through root drenching method after plant establishment (four weeks after planting). Simultaneously, foliar application of ascorbic acid (0, 0.5, 1mM) was also applied to these salt treated plants. The selected concentration of ascorbic acid was based on previously reported effective range in other crops. In total, twelve treatments (T_0-T_{11}) combination were evaluated (Table 1). Salt and ascorbic acid treatments were applied at an interval of 7 days for 30 days.

Sampling and data collection for Morphological attributes: Plants were harvested at random from each treatment for recording the data of different attributes. Data were collected for vegetative growth parameters such as shoot length, shoot diameter, leaf length, and leaf width at 30th day of salt and ascorbic acid treatment. A measuring tape was used to record various growth parameters. For reproductive growth parameters, the data were collected for number of flowers and number of fruits, fruit size, fresh and dry weight of fruits at 60th day of experiment at the time of final harvest.

Data collection for Biochemical attributes: Total soluble proteins and activities of antioxidant enzymes like SOD and POD were also estimated in the leaves collected at the time of final harvest at 60th day of experiment by the following method.

Extraction of Protein: For protein and antioxidant assay, fresh plant material (leaves) was ground in liquid nitrogen with 0.1 g of polyvinyl-pyrrolidone (PVP) into a fine powder using a pestle and mortar. Then 2.0 ml of 0.1 M phosphate buffer, pH 7.2 was added to the ground tissue containing 0.5% (*v/v*) Triton X-100 and the homogenate was centrifuged (Sorval RB-5 refrigerated super-speed centrifuge) for 30 min at 14,000 rpm at 4°C. Finally, the supernatant was stored at 0°C for estimation of protein and antioxidant enzyme activities.

Total soluble protein content was analyzed using the supernatant by Racusen & Johnstone (1961) method with certain modifications. Biuret reagent (2.0 ml) was added in two test tubes separately. Supernatant was added in experimental sample and the control sample consisted of 0.2 ml of distilled water. The optical density was measured at 545 nm using a spectrophotometer (UV 4000, 21029 Hamburg, Germany). Total protein contents were estimated using standard curve prepared by bovine serum albumin. The following formula was used for the estimation of protein contents.

$$\text{Protein contents (mg/g)} = \frac{\text{CV x TE}}{\text{EU x Wt x 1000}}$$

CV = Curve value
TE = Total extract
EU = Extract used
Wt = Fresh weight of plant material

Estimation of peroxidase and superoxide dismutase: For peroxidase (E.C 1.11.1.7) activity, Guaiacol-H_2O_2' method of Luck (1974) was performed with certain modification. According to this 3.0 ml 0.1 M phosphate buffer (pH 7.2), 0.05 ml of 20 mM guaiacol solution (2-methoxyphenol), 0.1 ml crude enzyme extract and 0.03 ml of 12.3 mM H_2O_2 solution was taken in a test tube. From this mixture 1 mL was added in a cuvette and peroxidase activity was measured by time required to increase the absorbance by a value of 0.1 (e.g., 0.4-0.5) at 240 nm and expressed as U/ml of enzyme.

The activity of superoxide dismutase (E.C 1.15.1.1) was measured following the method of Maral *et al*., (1977). SOD activity was analyzed by measuring the capability to inhibit photochemical reduction of nitroblue tetrazolium (NBT). For this purpose, reaction mixture containing 2.0 ml of 1.0 mM sodium cyanide (NaCN), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA and 2.0 µM riboflavin was taken in two test tubes. Enzyme extract or supernatant 5.0 µl was added in one tube and distilled water in same amount was added in other tube considered as a control. Both the test tubes were placed approximately 30 cm under the bank of two 30-W fluorescent tubes for 25 min. After the required time, the absorbance (560 nm) of both samples was compared. Superoxide dismutase activity was calculated by the percentage inhibition of NBT. The SOD activity was calculated based on the fact that one unit of SOD caused 50 % inhibition and expressed as U/mg of Protein.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control sample} - \text{Absorbance of experimental sample}}{\text{Absorbance of experimental sample}} \times 100$$

Statistical analysis: One way ANOVA was performed on the data using SPSS version 23.0.0 to determine the mean values of replicates for all the treatments at $p \leq 0.05$. Duncan's multiple range tests were also performed for the comparison of the mean values to assess any significant differences. Pearson correlation between any two parameters was calculated through OriginPro 23.0.0 and Principal component analysis was performed by using Minitab 17.

Results

Vegetative growth parameters: The results revealed that the shoot length of strawberry plants without any treatment of salt and AsA, (T_0) was 9.21 cm was decreased to 8.81 cm at 200 mM salt concentration (T_3). This effect was mitigated on applying AsA and shoot length was increased up to 10.1 cm on applying 1 mM AsA (T_{11}) with a significant difference with all the other treatments except T_8 in which there was no salt applied. Foliar application of AsA (1 mM) did not affect the stem diameter significantly which was reduced to 0.41 cm at 200 mM salt application (T_3) from 0.46 cm at control (T_0) and increased just up to 0.42 cm at T_{11} upon applying 1 mM AsA. Leaf length on 30th day of the experiment was reduced from 6.20 cm at control (T_0) to 5.80 cm upon applying 200 mM of salt (T_3) which was increased up to 6.12 cm upon applying 1mM AsA (T_{11}) although it had no significant difference with all other treatments. Leaf width which was reduced to 5.92 cm at T_3 from 6.11 cm at T_0 also did not increase significantly at T_{11} being 5.90 cm. A reduction in number of leaves was also observed from 8.50 at T_0 to 7.99 at T_3 which was not increased significantly (7.82) at T_{11} (Table 1).

Reproductive growth parameters: The number of flowers was also observed to decrease from 1.32 at T_0 to 0.99 at 200 mM salt treatment (T_3) with a significant difference which was increased up to 1.32 at T_{11} on applying 1 mM AsA. The number of fruits was reduced up to 6.51 at T_3 with a significant difference from 7.52 at T_0 which was further decreased to 4.30 on applying 1 mM AsA (T_{11}) at the time of final harvest. Reduction in fruit size was also recorded from 2.70 at T_0 to 2.21 cm at T_3 which was further decreased up to 1.52 cm at T_{11} with a significant difference with T_0 and T_3. Fruit fresh weight was decreased from 12.08 g at T_0 to 9.18 g at T_3 with a significant difference which was further decreased up to 8.55 g T_{11} even after applying 1 mM AsA. Fruit dry weight was also reduced from 9.15 g to 7.59 g with increasing salt concentration up to 200 mM (T3) and was further decreased up to 6.99 g at 1mM AsA application in case of T_{11} with a non-significant difference (Table 2).

Total soluble protein, superoxide dismutase and peroxidase activity: Total soluble protein contents of strawberry plants exhibited a decrease from 4.25 at T_0 to 3.65 mg/g at T_3 which was further decreased up to 2.48 mg/g at T_{11} with a significant difference with T_0 and T_{11}. The activity of superoxide dismutase was increased from 109.44 at T_0 to 138.9 U/mg of protein on applying 200 mM salt, with a significant difference which exhibited a non-significant an increase up to 140.55 U/mg of protein at T_{11}. Peroxidase activity also showed a direct relationship with salinity, increased significantly from 0.15 to 0.50 U/ml of enzyme as salinity increased from 0 to 200 mM. This value was further increased significantly up to 0.72 U/ml of enzyme on applying 1 mM AsA in case of T_{11} (Table 3). The correlation matrix was obtained by Pearson correlation analysis exhibited a significant correlation between the parameters having the correlation coefficient above 0.7 whereas the parameters with correlation coefficient from 0.5 - 0.7 were moderately correlated with each other (Fig. 1). The principal component analysis further revealed the trend of response amongst different parameters studied during this experiment. The components 1 (83.6%) and 2 (9.3%) explained a total of 92.9% variance in the data. Figure 2A showed that all the parameters except SOD and POD activity responded in the same manner. The distance amongst the lines representing the parameters indicated that how closely similar was their response with each other. In addition, the treatments in the same groups had a non-significant difference in their effect on strawberry plants while those located in different groups had significant difference in their effect (Fig. 2B). Figure 3(A, B, C, and D) represents the effect of different treatments on reproductive growth parameters of strawberry plants in current experiment.

Table 1. Effect of different levels of AsA and NaCl on growth parameters of strawberry plants.

Treatments	AsA + NaCl	Leaf length (cm)	Leaf width (cm)	Shoot length	Stem diameter	Number of leaves
T_0	0 + 0	6.20 ± 0.24^{ab}	6.11 ± 0.71^{b}	9.21 ± 1.06^{b}	0.46 ± 0.05^{b}	8.50 ± 0.70^{de}
T_1	0 + 50	6.31 ± 0.53^{ab}	6.16 ± 0.28^{a}	9.91 ± 1.06^{b}	0.49 ± 0.05^{b}	9.90 ± 1.14^{e}
T_2	0 + 100	6.70 ± 0.07^{ac}	6.21 ± 0.14^{a}	11.00 ± 0.42^{d}	0.55 ± 0.02^{c}	10.71 ± 0.63^{ef}
T_3	0 + 200	5.80 ± 0.42^{a}	5.92 ± 0.27^{a}	8.81 ± 0.21^{a}	0.41 ± 0.09^{b}	7.99 ± 0.01^{b}
T_4	0.5 + 0	6.02 ± 1.00^{a}	6.03 ± 0.90^{bc}	9.02 ± 1.61^{a}	0.46 ± 0.05^{b}	8.70 ± 1.12^{de}
T_5	0.5 + 50	6.42 ± 0.41^{ab}	6.14 ± 0.57^{b}	10.70 ± 0.28^{c}	0.50 ± 0.03^{c}	9.41 ± 1.12^{e}
T_6	0.5 + 100	5.51 ± 0.49^{a}	5.81 ± 0.49^{a}	8.79 ± 0.77^{a}	0.37 ± 0.04^{a}	7.43 ± 0.81^{a}
T_7	0.5 + 200	5.70 ± 0.35^{a}	5.90 ± 0.22^{ab}	8.75 ± 0.01^{a}	0.42 ± 0.06^{b}	8.20 ± 1.41^{c}
T_8	1 + 0	6.22 ± 1.01^{ab}	6.08 ± 0.45^{b}	10.40 ± 0.56^{c}	0.48 ± 0.03^{b}	9.10 ± 0.70^{e}
T_9	1 + 50	5.24 ± 0.28^{a}	5.57 ± 0.62^{a}	8.63 ± 0.91^{a}	0.35 ± 0.02^{a}	6.59 ± 1.12^{a}
T_{10}	1 + 100	5.55 ± 0.43^{a}	5.68 ± 0.85^{ab}	8.81 ± 0.92^{a}	0.38 ± 1.01^{a}	7.70 ± 1.12^{cd}
T_{11}	1 + 200	6.12 ± 0.22^{ab}	5.89 ± 0.47^{ab}	10.10 ± 1.05^{c}	0.42 ± 0.04^{b}	7.82 ± 0.12^{d}

Data represented is the mean value ± S.E

Different alphabets on mean values represent the significant differences among treatment according to DMRT ($p \leq 0.05$)

Table 2. Effect of different levels of AsA and NaCl on the number of fruits, fruit size, fruit fresh weight and fruit dry weight in strawberry plants.

Treatments	Number of flowers	Number of fruits	Fruit size (cm)	Fruit fresh weight (g)	Fruit dry weight (g)
T_0	1.22 ± 0.05^{b}	7.51 ± 0.70^{d}	2.70 ± 0.44^{b}	12.08 ± 0.54^{c}	9.15 ± 1.12^{bc}
T_1	1.38 ± 0.07^{b}	7.90 ± 1.41^{d}	2.83 ± 0.12^{b}	14.81± 0.31^{cd}	11.04 ± 0.85^{cd}
T_2	1.70 ± 0.04^{bc}	8.30 ± 1.11^{e}	3.12 ± 0.22^{d}	17.45 ± 0.33^{e}	14.58 ± 0.66^{d}
T_3	0.99 ± 0.07^{a}	6.52 ± 1.15^{c}	2.21 ± 0.36^{b}	9.18 ± 0.65^{c}	7.59 ± 0.39^{b}
T_4	1.25 ± 0.07^{b}	6.91 ± 0.35^{c}	2.79 ± 0.22^{bc}	11.14 ± 0.78^{bc}	9.23 ± 0.98^{bc}
T_5	1.47 ± 0.07^{bc}	7.4 ± 0.01^{d}	2.91 ± 0.18^{c}	13.55 ± 0.45^{c}	10.01 ± 0.51^{c}
T_6	0.63 ± 0.06^{a}	5.40 ± 0.52^{b}	1.98 ± 0.09^{b}	7.19 ± 0.69^{a}	5.07 ± 0.93^{a}
T_7	1.10 ± 0.04^{ab}	5.88 ± 0.70^{b}	2.65 ± 0.31^{bc}	9.49 ± 0.88^{bc}	7.42 ± 1.13^{b}
T_8	1.45 ± 0.03^{b}	6.23 ± 1.01^{c}	2.80 ± 0.20^{bc}	11.87 ± 0.86^{c}	8.83 ± 0.86^{bc}
T_9	0.32 ± 0.05^{a}	3.00 ± 0.87^{a}	1.82 ± 0.33^{a}	6.21 ± 0.55^{a}	4.31 ± 0.47^{a}
T_{10}	1.08 ± 0.08^{a}	4.01 ± 1.05^{ab}	1.44 ± 0.36^{a}	7.75 ± 0.23^{ab}	5.55 ± 0.38^{a}
T_{11}	1.32 ± 0.07^{b}	4.30 ± 1.01^{ab}	1.52 ± 0.28^{a}	8.55 ± 0.28^{b}	6.99 ± 0.29^{ab}

Data represented is the mean value ± S.E

Different alphabets on mean values represent the significant differences among treatment according to DMRT ($p \leq 0.05$)

Table 3. Effect of different levels of AsA and NaCl on total soluble proteins, superoxide dismutase activity and peroxidase activity in strawberry plants.

Treatments	Total soluble proteins (mg/g)	Superoxidase dismutase activity (U/mg of protein)	Peroxidase activity (U/ml of enzyme)
T_0	4.25 ± 0.43^{d}	109.44 ±1.57^{a}	0.15 ± 0.03^{a}
T_1	4.47 ± 0.22^{d}	117.31 ± 0.98^{ab}	0.22 ± 0.01^{a}
T_2	5.01 ± 0.75^{e}	129.75 ± 1.34^{ab}	0.25 ± 0.064^{ab}
T_3	3.65 ± 1.23^{bc}	138.89 ± 1.75^{bc}	0.50 ± 0.02^{c}
T_4	3.81 ± 0.88^{bc}	119.93 ± 1.63^{b}	0.38 ± 0.03^{ab}
T_5	4.01 ± 0.43^{c}	121.23 ± 1.22^{b}	0.43 ± 0.03^{ab}
T_6	3.42 ± 0.12^{b}	146.31 ± 1.41^{c}	0.64 ± 0.02^{c}
T_7	3.60 ± 0.53^{b}	124.48 ± 1.56^{b}	0.41 ± 0.04^{ab}
T_8	3.75 ± 0.65^{b}	130.67 ± 1.49^{b}	0.53 ± 0.01^{bc}
T_9	2.48 ± 0.38^{a}	151.96 ± 1.38^{cd}	0.87 ± 0.33^{d}
T_{10}	2.76 ± 0.26^{a}	138.32 ± 1.61^{bc}	0.68 ± 0.02^{cd}
T_{11}	2.90 ± 0.54^{a}	140.55 ± 1.59^{bc}	0.72 ± 0.54^{cd}

Data represented is the mean value ± S.E

Different alphabets on mean values represent the significant differences among treatment according to DMRT ($p \leq 0.05$)

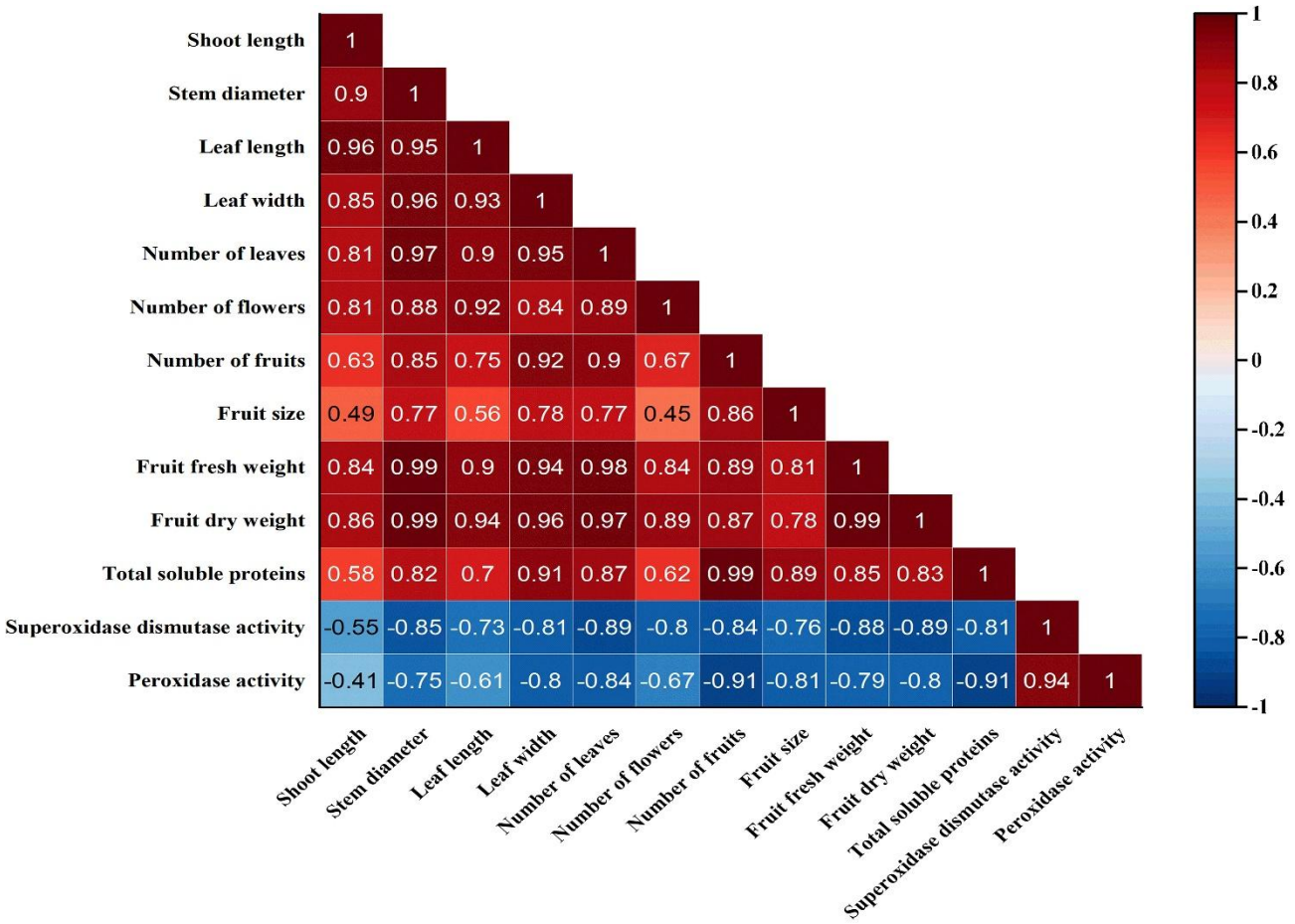

Fig. 1. Pearson correlation between different parameters of strawberry plants.

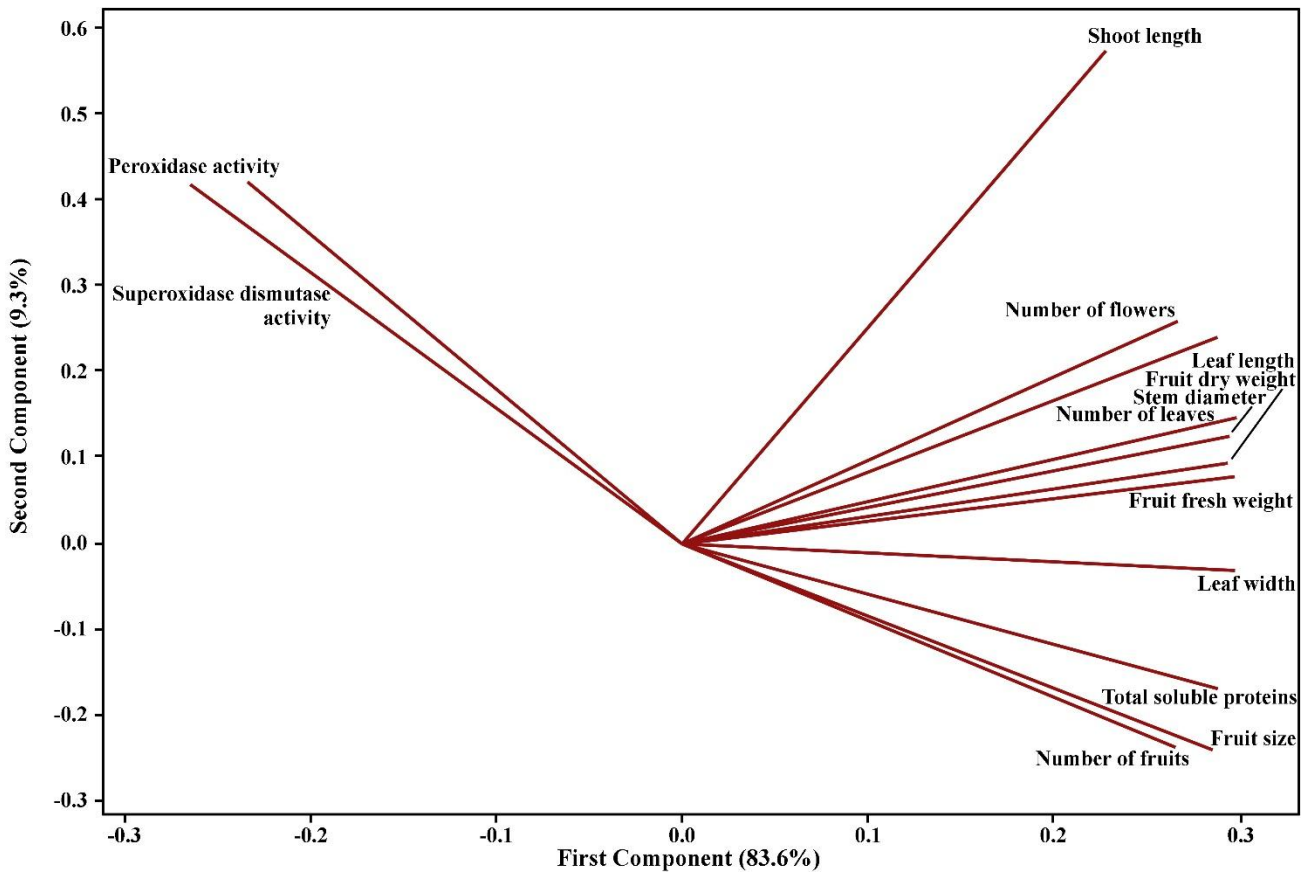

Fig. 2A. Response of different parameters of strawberry plants grown at different treatments of salt and ascorbic acid.

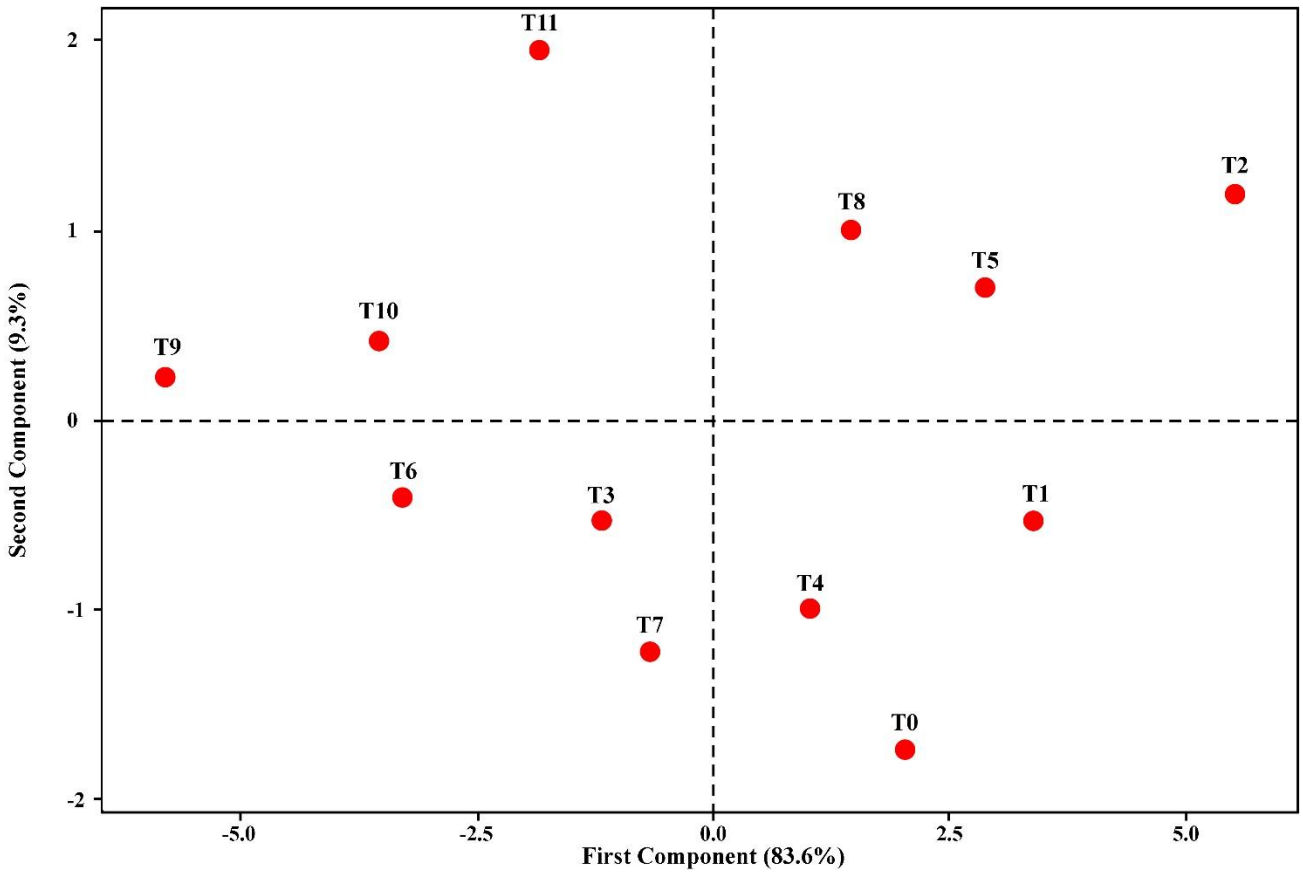

Fig. 2B. Relationship in efficacy of different treatments of salt and ascorbic acid.

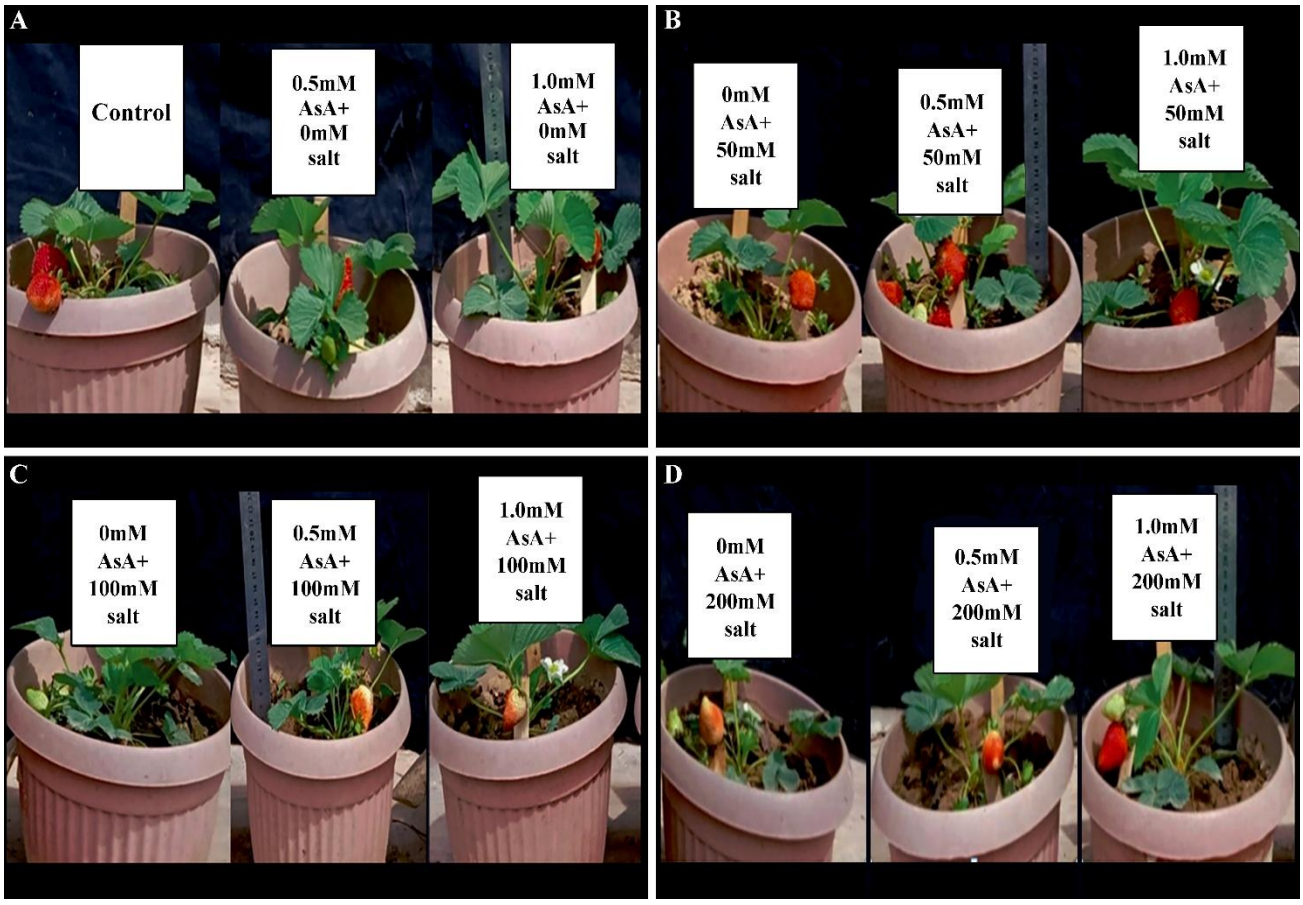

Fig. 3. **A)** Effect of AsA (0, 0.5, and 1.0 mM) without salt stress; **B)** Effect of AsA (0, 0.5, and 1.0 mM) under 50 mM NaCl stress; **C)** Effect of AsA (0, 0.5, and 1.0 mM) under 100 mM NaCl stress; **D)** Effect of AsA (0, 0.5, and 1.0 mM) under 200 mM NaCl stress.

Discussion

Salinity is a drastic abiotic stress that damages plants by causing ionic imbalance, osmotic stress and inhibition of photosynthesis (Dedejani & Ghaderi, 2021). The growth and yield of strawberry plants is particularly sensitive to salt stress and effected severely (Yaghubi *et al*., 2019). Foliar application of some protective compounds has been proposed as an effective strategy to mitigate the deleterious effects of salinity. It allows rapid absorption by aerial tissues and further salt accommodation in root zone unlike soil amendments. Since, ascorbic acid has been reported as an important signal molecule for plant responses against salinity and other abiotic stresses (Hassan *et al*., 2021). Therefore, it was selected for foliar application in the present study. AsA can lead to the synthesis of hydroxyproline-containing proteins (Bilska *et al*., 2019) and can reduce oxidative stress by removing free radicals and by acting as a substrate of APX which is an essential enzyme of the ascorbate–glutathione pathway (Sharma *et al*., 2019). The significant increase in the activity of most antioxidant enzymes, such as SOD, POD, CAT, and APX, are also reported in potato plants grown under salt stress after exogenous application of ascorbic acid (Sajid & Aftab, 2009). Elkelish *et al*., (2020) also reported in tomato plants that ascorbic acid was very important in the improvement of various physiological as well as biochemical activities such as cell division, flowering, pathogen resistance and cell enlargement.

In present study, increasing NaCl concentration up to 200 mM caused a significant reduction in shoot length, stem diameter, leaf length, leaf width and number of leaves, consistent with the work of Zhang *et al*., (2020) who reported a decrease in leaf length, leaf width and leaf area under different salt concentrations in tomato seedlings. The number of flowers and fruits was significantly decreased under salt stress, similar trend was also reported by Abdelaal *et al*., (2020) in sunflower. This could be attributed to the fact that salt in soil solution decreases the water and nutrients uptake which leads to ion toxicity, water deficiency, osmotic stress and nutrient imbalance which results in damage to photosynthetically active leaves (Zahra *et al*., 2020). Abdel-Farid *et al*., (2020) also stated that the shoot length, root length, and fruit fresh and dry weight were decreased in tomato plants due to the application of different concentrations of salt. The decline in yield related parameters such as fruit size and their fresh and dry weight observed in the present study in salt treated strawberry plants aligned with the findings of Zorb *et al*., (2019) who stated the negative effect of soil salinity on biomass, and yield of different plant species. The results of Azarmi & Sayyari (2020) research work also corresponded with the present research as they reported decrease in fresh weight of fruits in lettuce due to the toxicity caused by sodium and chloride ions. Similar results were obtained by Ali *et al*., (2018) and Tanveer *et al*., (2020) who stated that fruit fresh and dry mass in tomato was reduced, and growth was also inhibited due to salt stress. Salinity induced disruption of phytohormonal balance, particularly reduced auxin, gibberellin activity and impaired partitioning toward reproductive sink, may explain the sensitivity of fruit development compared to vegetative growth (Isayenkov & Maathuis, 2019). This differential sensitivity may account for limited improvement in reproductive parameters despite partial recovery of vegetative growth following AsA application. In the present research, the total soluble protein content was significantly decreased at 200 mM NaCl. Similar reduction has been reported in flax (Dubey *et al*., 2020; Ahsan & Sajid, 2026) and tomato (Arikan *et al*., 2020) under high salinity. This decline may result from inhibited nitrogen assimilation, enhanced proteolysis and oxidative degradation of protein caused by excessive ROS accumulation under severe salt stress. Salinity induced oxidative stress was further evidenced by increased activities of antioxidant enzymes with rising concentrations of salt from 0 to 200 mM. Same trend was observed by Crizel *et al*., (2020) who reported the increase in activities of SOD and POD in strawberry plants with increasing levels of NaCl. The results of Mozanzadeh *et al*., (2021) were also similar with our findings who explained that salinity increased the enzymatic activities of antioxidants such as catalase, peroxidase, and superoxide dismutase. While enhanced antioxidant enzymes activity reflects the activation of defense mechanism, it may also indicate elevated oxidative rather than complete stress alleviation.

The foliar spray of AsA mitigated the effect of salt and enhanced different vegetative growth parameters like shoot length, stem diameter, leaf length, leaf width, and number of leaves when treated with various levels of AsA specifically at 1.0 mM concentration. Our results were similar to Arif *et al*., (2020), who reported an increase in number of leaves with increasing concentration of AsA. A similar trend was also observed in okra plant by Saheed (2020). The outcomes of a study conducted by Fatima *et al*., (2020) were also in accordance with our results who observed significant increase in leaf area, leaf and shoots number in fenugreek plant when treated with AsA. Billah *et al*., (2017) also reported that foliar spray of AsA further increased the production of indigenous AsA in plant cell improving plant resilience and growth under stress. In present study, some reproductive traits such flower number show partial improvement; however, fruit number and fruit biomass did not increase significantly. This suggests that while AsA effectively supports vegetative recovery, its influence on reproductive development under severe salinity (200 mM NaCl) may be limited.

The total soluble protein content was not increased following AsA application in the present study, contrasting with the findings in tomatoes (Dehnadi *et al*., 2020). This discrepancy may be attributed to the severity of salt stress applied; crop specific metabolic responses or insufficient AsA concentration to restore protein synthesis pathways under extreme salt condition. Similarly, the activities of SOD and POD in present experiment increased under salt stress but did not decrease upon AsA application. The work of Noreen *et al*., (2021) was similar to the results in present experiment who also noticed an increase in the activities of SOD and POD in barley plants when they were treated with AsA. The sustained elevation of antioxidant enzymes suggests that AsA enhanced the plant's capacity to cope with oxidative stress rather than completely neutralizing the drastic effect of salt.

Conclusions

The significant and gradual increase in some of the vegetative growth parameters upon applying 1 mM ascorbic acid to the strawberry plants treated with 200 mM salt (NaCl) revealed that this concentration of AsA was effective in alleviating the salt stress on vegetative growth. This effect was however, non-significant on most of the reproductive growth parameters as well as on the total soluble proteins and activities of SOD and POD suggesting that higher than 1 mM concentrations of AsA should be investigated to mitigate the drastic effects of salt stress at 200 mM concentration. Future prospects of present study thus involve investigating higher concentrations to mitigate the drastically damaging effects of salt stress to the plant's physiological and metabolic processes.

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Author's Contribution: SH & MU. performed the experimental work and prepared the preliminary draft, SN&SM analyzed the biochemical parameters and performed the statistical data analysis, SAK & ZAS proofread the draft and supervised the experimental work

Data Availability: All the data to support this study are incorporated in this manuscript and if required further information available upon reasonable request from the corresponding author.

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