

The Role of Proline Acid in Enhancing the Yield Characteristics of Hot Pepper (*Capsicum annuum* L.) under Salt Stress Conditions.

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Abstract

This experiment was conducted in the greenhouse of the College of Education for Women, University of Tikrit, from 22 October 2025 to 12 May 2026, to evaluate the response of hot pepper (*Capsicum annuum* L.) plants to foliar application of proline in terms of growth, yield, and selected chemical and anatomical traits under salt stress conditions. The trial was carried out in plastic pots using a two-factor factorial arrangement.: the first factor involved three salt concentrations (0, 3, 5 g/L), and the second factor involved the addition of proline acid at three concentrations (0, 100, 150 ml/L). The results indicated a clear response of hot pepper plants (*Capsicum annuum* L.) to proline application and salinity levels. Foliar spraying with proline at a concentration of 150 mg L⁻¹ had a significant positive effect on most vegetative growth parameters. This treatment recorded the highest mean values for plant height (45.0 cm), number of branches (17.0 branches plant⁻¹), number of leaves (68.0 leaves plant⁻¹), leaf area (409.2 cm²) and total chlorophyll content (1943 µg g⁻¹ fresh weight). With regard to yield-related traits, The results indicated a clear response of hot pepper plants (*Capsicum annuum* L.) to proline application and salinity levels. Foliar spraying with proline at a concentration of 150 mg L⁻¹ had a significant positive effect on most vegetative growth parameters. This treatment recorded the highest mean values for plant height (45.0 cm), number of branches (17.0 branches plant⁻¹), number of leaves (68.0 leaves plant⁻¹), leaf area (409.2 cm²) and total chlorophyll content (1943 µg g⁻¹ fresh weight). With regard to yield-related traits.

Key Words: Proline Acid ,*Capsicum annuum* L. , Salt Stress

Introduction

Hot pepper (*Capsicum annuum* L.), a member of the Solanaceae family, is considered the third most important solanaceous crop after potato and tomato in terms of global economic importance. The species is native to tropical regions of Central and South America.(1). Hot pepper contains alkaloids, specifically Capsicum, and its fully ripe fruits are red in color and rich in Vitamin C compared to green ones, and it is cultivated in tropical and subtropical regions (2). Proline is a proteinogenic amino acid essential for protein biosynthesis. Its exogenous application enhances plant water uptake and mitigates the adverse effects of abiotic stresses, such as salinity and drought. Additionally, it plays a crucial role in

stabilizing protein structures and preserving cell membrane integrity against oxidative damage.(3). It has a physiological effect on the plant under salt stress by increasing chlorophyll content, improving the ratio of sodium and calcium ions, and conversely improving anatomical characteristics through the regulation of cells and vascular bundles and maintaining tissue integrity (3). Salt stress negatively affects plants and their development by hindering the absorption of essential nutrients needed for growth, affecting cell shape and appearance. When plants grow in a saline environment, it is observed that leaves are smaller in size due to the effect of salts on their growth, and cell walls become thicker and less flexible due to salt accumulation. The plant is

physiologically affected by reduced growth

Aim of the Study

Proline is a proteinogenic amino acid essential for protein biosynthesis. Its exogenous application enhances plant water uptake and mitigates the adverse effects of abiotic stresses, such as salinity and drought.

3. Materials and Methods

3-1 Location of the Experiment

The experiment was conducted in the glasshouse belonging to the scientific departments of the College of Education for Girls / University of Tikrit, during the agricultural season 2025-2026, from October 22, 2025, to May 12, 2026. The

3-2 Preparation of Used Seeds

Seeds of pepper (*Capsicum annuum* L.) of the Turkish variety (LEZER) were obtained from local markets in Tikrit Governorate. This variety is intended for protected

3-3 Soil Preparation and Planting Method

The experiment was carried out using plastic pots for cultivation, with a height of 35 cm and a diameter of 25 cm, each with a capacity of 12 kg of soil. The pots were filled with a mixture of soil and peat moss in a 2:1 ratio, where the soil was taken from the bank of the Tigris River. The number of experimental treatments was 9, with a total of 27 experimental units for the complete experiment with three replications. Irrigation and other agricultural operations were carried out according to the plant's needs. It

rate and inhibited cell division (4; 5).

Additionally, it plays a crucial role in stabilizing protein structures and preserving cell membrane integrity against oxidative damage.

study aimed to investigate the effect of proline at concentrations (0, 100, 150 mg/L) and salt stress at concentrations (0, 3, 5 g/L) on the growth, yield, and some anatomical characteristics of pepper plants, according to the following steps:

cultivation, is highly productive, and is resistant to various environmental conditions.

is noted that the number of plants per experimental unit was 5 plants, with 2 plants placed per planting hole after mixing with urea and superphosphate fertilizers according to the recommended rates. After ensuring the health of the plants, thinning was carried out so that each pot contained three plants. The chemical and physical properties of the soil were analyzed in the Soil Laboratory of the College of Agriculture / University of Tikrit, as shown in Table (3-1):

Table (3-1) Physical and Chemical Properties of the Field Soil Before Planting

(Traits)	(Unit of Measurement)	(Value)
Trait	Unit of Measurement	Value
Phosphorus	mg. kg ⁻¹	4.2
Nitrogen	mg. kg ⁻¹	9
Potassium	mg. kg ⁻¹	129
pH	-	7.9
EC	mS/cm	2.23
Sand	%	76
Silt	%	12
Clay	%	8

3-4 Experimental Design

The experiment was conducted as a factorial experiment arranged in a Randomized Complete Block Design (RCBD) with two factors. The first factor was proline (B) at three levels, including the control (0, 100 and 150 mg L⁻¹), and the second factor was salinity stress (NaCl) at three levels, including the control (0, 3 and 5 g L⁻¹). The

Table (3-2) Experimental Treatments

Code	Treatment
B0S0	Without treatment (distilled water only)
B0S1	Irrigation with saline water at a concentration of 3 g/L
B0S2	Irrigation with saline water at a concentration of 5 g/L
B1S0	Spraying with proline acid at a concentration of 100 mg/L
B1S1	Spraying with proline acid at a concentration of 100 mg/L + Irrigation with saline water at a concentration of 3 g/L
B1S2	Spraying with proline acid at a concentration of 100 mg/L + Irrigation with saline water at a concentration of 5 g/L
B2S0	Spraying with proline acid at a concentration of 150 mg/L
B2S1	Spraying with proline acid at a concentration of 150 mg/L + Irrigation with saline water at a concentration of 3 g/L
B2S2	Spraying with proline acid at a concentration of 150 mg/L + Irrigation with saline water at a concentration of 5 g/L

Salt stress is denoted by the symbol (S) and proline acid by the symbol (B)

3-5-2 The Second Factor

Addition of three levels of proline acid (B):

1. B0: Spraying with distilled water only.
2. B1: Spraying with proline acid at 100 mg in one liter of distilled water.
3. B2: Spraying with proline acid at 150 mg in one liter of distilled water.

combination of the two factors resulted in nine treatments, each replicated three times, for a total of 27 experimental units, with three plants per unit. Data on growth, yield, biochemical and anatomical traits were recorded from all three plants in each experimental unit(49).

3-5 Study Factors

The study was conducted as a bifactorial experiment. The first factor involved soil application of sodium chloride at three concentrations, designated as S0 control receiving only distilled water, S1 at 3 grams per liter and S2 at 5 grams per liter. Each concentration was prepared by dissolving the required amount of NaCl in one liter of distilled water and applied to the soil in two equal doses, the first on 10 December 2025 and the second fifteen days later using the same levels. The second factor consisted of foliar application of proline at three levels, coded as B0 control sprayed with distilled water only, B1 at 100 milligrams per liter and B2 at 150 milligrams per liter. Proline was obtained from a certified agricultural supplier in Baghdad Governorate and solutions were prepared by dissolving 100 milligrams and 150 milligrams in one liter of distilled water for B1 and B2 respectively. Foliar spraying was performed in the early morning to maximize absorption, with the first application on 20 December 2025 and the second application fifteen days thereafter at identical concentrations.

Proline acid was procured from one of the offices in Baghdad Governorate. It was prepared by weighing 100 mg of proline and dissolving it in one liter of distilled water for treatment B1, and 150 mg dissolved in one liter of distilled water for treatment B2. The

spraying was carried out in the early morning. The first dose was applied on (20/12/2025), and the second dose was

3-6 Studied Traits of the Experiment

Three similar plants from each experimental unit were selected, and their average was recorded at the end of the growing season.

Vegetative Growth Traits

Plant Height (cm. plant⁻¹)

Measured at the end of the growing season from the point where the stem meets the soil

Number of Branches (branch. plant⁻¹)

The number of main fruiting branches of the selected plants from the experimental unit

Number of Leaves (leaf. plant⁻¹)

This trait was measured by counting the number of leaves on the plant at the end of the growing season.

Total Leaf Area per Plant (cm². plant⁻¹)

The average leaf area of three randomly selected leaves from each plant was measured (Saieed, 1997). The plant leaf was photocopied using an A4 scanner/copier. The full A4 copy paper was weighed, then the shapes of the copied plant leaves were cut out from the A4 paper and weighed using a sensitive balance. Since the

Estimation of Leaf Dry Matter Content (%)

Leaves from the vegetative growth were taken, weighed, placed in paper bags, and dried in an electric oven at a temperature of 65-70 °C until a constant weight was achieved. The reading was recorded, and the

Total Chlorophyll Content in Leaves (µg/mg)

Leaves were washed under running water to remove soil and dust from the leaf surface. One gram of fresh leaf tissue was weighed and ground in a clean mortar with 50 ml of 96% ethanol. The extract was centrifuged at 1000 rpm, then filtered through two layers of gauze, and centrifuged again at 2500 rpm for 10 minutes. The absorbance was measured using a Spectrophotometer to determine the Optical Density (OD) at: Chlorophyll a at 666 nm and Chlorophyll b

added 15 days after the first treatment, using the same concentrations

to the growing tip of the main branch using a measuring tape.

was recorded at the end of the growing season.

dimensions of the A4 paper are known (length 29.7 cm and width 21.1 cm), the actual leaf area was calculated according to the following equation:

Leaf area of the plant leaf = (Weight of the cut-out leaf shape × Area of A4 paper) / Weight of the A4 paper

percentage of dry matter was calculated according to the following equation.

Dry matter percentage of vegetative growth (leaves) = (Dry weight / Fresh weight) × 100

at 653 nm. Absorbance density was calculated by applying the equations mentioned by (Dere et al., 1998):

Chlorophyll a = $(12.7 \times A_{663}) - (2.69 \times A_{645}) \times V / (1000 \times W)$

Chlorophyll b = $(22.9 \times A_{645}) - (4.68 \times A_{663}) \times V / (1000 \times W)$

Total Chlorophyll = $20.2 \times (A_{645}) + 8.02 \times (A_{663}) \times V / (1000 \times W)$

Where:

- A663: Optical density at wavelength 663 nm
- A645: Optical density at wavelength 645 nm
- V: Volume of extract (50 ml)
- W: Weight of plant tissue (1 g)

Statistical Analysis

4. Results and Discussion

Vegetative Growth Traits

Plant Height (cm. plant⁻¹)

It is evident from Table (1) that there are significant differences among treatment means for plant height. S1 recorded the highest mean of 42.67 cm, which was significantly different from the mean of S2 (38.00 cm). Both concentrations significantly outperformed the S0 treatment, which recorded the lowest plant height value of 35.67 cm. Regarding the effect of proline acid, the B2 treatment gave the highest mean

The data were subjected to statistical analysis following a Randomized Complete Block Design (RCBD). Means were separated using Duncan's Multiple Range Test at . All statistical analyses were performed using SAS and Microsoft Excel software

plant height of 42.00 cm, compared to the lowest height of 35.67 cm recorded for treatment B1. From the two-way interaction between salt and proline concentrations, proline significantly affected total plant height. The B1S1 treatment outperformed others, giving the highest plant height of 45.0 cm, compared to the lowest length of 31.0 cm recorded for treatments B1S0 and B1S2.

Table (1) Effect of Salt Stress and Proline Addition on Plant Height (cm. plant⁻¹):

Salt Stress	B0	B1	B2	S Mean
S0	33d	31d	43ab	35.67c
S1	42bc	45a	41c	42.67a
S2	41c	31d	42bc	38.00b
Mean B	38.67b	35.67c	42.00a	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results showed that the proline treatment at a concentration of 150 mg/L (B2) outperformed the other treatments, giving the highest mean plant height of 42.00 cm, while treatment B1 recorded the lowest mean of 35.67 cm. This superiority is attributed to the role of proline in improving osmotic balance within cells, maintaining the integrity of cell membranes, and increasing photosynthetic efficiency, which positively reflects on cell division and elongation, and consequently increases plant height (6). Recent studies have indicated that exogenous proline application improved vegetative growth and plant height of pepper plants under various stress conditions by enhancing physiological processes and

reducing oxidative damage (7). Regarding salt stress, treatment S1 (3 g NaCl/L) achieved the highest mean plant height of 42.67 cm, while the mean decreased at the higher salinity level (S2) to 38.00 cm. This may be attributed to the moderate salinity level stimulating some physiological adaptation mechanisms in the plant, whereas the high salt concentration increased osmotic stress and disrupted water and nutrient uptake, thus limiting growth. This result is consistent with studies on pepper indicating that increased salinity negatively affects vegetative growth due to reduced photosynthetic efficiency and disturbed metabolic processes (8). During salt stress, physiological and biochemical processes are

affected, severely impacting crop growth and development (9). Researchers have identified proline as one of the metabolites contributing to plant stress tolerance mechanisms. Exogenous proline can alleviate damage caused by abiotic stress and improve growth (10). The results of a study by (11) showed that Siete pepper plants were highly sensitive to water deficit, and at the same time, proline pretreatment significantly improved their tolerance, highlighting its effectiveness in mitigating the negative effects of stress. The interaction between the two factors also showed that treatments B1S1 and B2S0 gave the highest plant height, indicating the ability of proline to mitigate the harmful effects of salt stress and maintain vegetative growth. This is

2 Total Number of Branches (branch. plant⁻¹)

The results in Table (2) indicate significant differences among treatment means for the number of branches trait. Salt treatment at concentration S1 recorded the highest mean of 14.33 branches per plant, which did not significantly differ from the mean of treatment S0 (14.00 branches per plant), and both significantly outperformed treatment S2, which recorded the lowest number of branches at 12.00 branches per plant. Regarding proline acid, treatment B2 gave the highest mean number of branches at

Table (2): Effect of Salt Stress and Proline Addition on Total Number of Branches (branch. plant⁻¹):

Salt Stress	B0	B1	B2	S Mean
S0	12ef	13de	17a	14.00a
S1	13de	16ab	14cd	14.33a
S2	11fg	10g	15bc	12.00b
Mean B	12.00b	13.00b	15.33a	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results in Table (2) showed that spraying with proline led to a significant increase in the total number of branches, as treatment B2 (150 mg/L) outperformed and recorded the highest mean of 15.33 branches

attributed to proline's role as a cell protectant by regulating osmotic potential, reducing the accumulation of reactive oxygen species, and improving water use efficiency. Many studies have shown that treatments that enhance proline accumulation or its exogenous application contributed to improving the growth of pepper plants exposed to salinity compared to untreated plants (12). Some studies have also shown that treatment with appropriate concentrations of exogenous proline can effectively reduce the harmful effects of abiotic stress, while high doses negatively affect plant growth by significantly reducing total soluble protein in the root or causing toxic effects (13).

15.33 branches per plant, compared to the lowest number of branches at 12.00 branches per plant for the control treatment B0. The same table shows the two-way interaction between salt and proline concentrations; proline significantly affected total plant height, with treatment B2S0 giving the highest number of branches per plant at 17.0 branches, compared to the lowest number of branches at 10.0 branches for treatment B1S2.

plant⁻¹ compared to treatments B0 and B1. This improvement is attributed to the physiological role of proline in maintaining the osmotic balance of plant cells, protecting membranes and proteins from stress-induced

damage, as well as its contribution to improving photosynthetic efficiency and increasing the energy production necessary for the formation of lateral shoots and new branches (14). (15) explained that exogenous proline treatment enhances photosynthetic processes and reduces oxidative stress, which positively reflects on the vegetative growth of plants under stress. A study conducted on pepper also showed that proline addition improved many morphological traits of plants and increased growth efficiency compared to untreated plants (16). Regarding salt stress, the results showed a decrease in the number of branches at the high salinity level S2, with a mean of 12.00 branches plant⁻¹ compared to S0 and S1. This is explained by the fact that increased salt concentrations in the root zone lead to a decrease in soil water potential and difficulty in absorbing water and nutrients, in addition to the toxic effect of accumulated sodium and chloride ions within plant tissues, which inhibits cell division and elongation and reduces the formation of lateral branches (17). Many recent studies have confirmed that pepper is a relatively salt-sensitive crop, and that increasing NaCl concentrations leads to a clear decline in vegetative growth, number of branches, and shoot fresh weight due to disrupted physiological processes and reduced photosynthetic efficiency (18). It is noted from the salt stress means that treatment S1 gave the highest mean number

3 Number of Leaves (leaf. plant⁻¹)

It is noted from the results presented in Table (3) that there are significant differences among treatment means for the number of leaves trait. Salt treatment at concentration S0 recorded the highest mean of 56.67 leaves, which did not significantly differ from the mean of treatment S1 (56.33 leaves), and both concentrations significantly outperformed treatment S2,

of branches (14.33 branches plant⁻¹), slightly outperforming the control treatment S0. This can be explained by the fact that moderate stress levels may stimulate some defense and adaptation mechanisms in the plant, temporarily increasing the efficiency of some physiological processes before the negative effects appear at higher salinity levels (19). A study by (20) indicated that the response to salinity varies depending on stress intensity and plant variety, as low or moderate salinity levels may show limited stimulatory effects on some growth traits before the effect turns negative with increased salt concentrations. Regarding the interaction between the two factors, treatment B2S0 showed superiority by giving the highest number of branches at 17 branches plant⁻¹. These results indicate that proline contributed to reducing salt-induced damage and maintaining the vital activity of the vegetative growth, leading to continued branch formation even under stress conditions. This is due to proline's ability to act as an osmotic regulator and antioxidant, as well as its role in protecting enzymes and cell membranes from damage caused by free radicals generated under salt stress conditions (7). A study by (21) found that proline treatment improved the growth of pepper and other horticultural crops under stress conditions by enhancing antioxidant defense efficiency and improving the plant's water status.

which recorded the lowest number of leaves at 51.67 leaves. Regarding the effect of proline acid, treatment B2 gave the highest mean number of leaves at 58.67 leaves, compared to the lowest number of leaves at 51.67 leaves for the control treatment B0. The same table shows the two-way interaction between salt and proline concentrations on leaf number; treatment

B2S0 outperformed and gave 68.0 leaves, compared to the lowest number of leaves at

37.0 leaves for treatment B0S0.

Table (3): Effect of Salt Stress and Proline Addition on Number of Leaves (leaf. plant⁻¹):

Salt Stress	B0	B1	B2	S Mean
S0	37g	65b	68a	56.67a
S1	62c	53e	54e	56.33a
S2	56d	45f	54e	51.67b
Mean B	51.67c	54.33b	58.67a	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results in Table (3) showed that spraying with proline led to a significant increase in the number of leaves, as treatment B2 recorded the highest mean of 58.67 leaves plant⁻¹ compared to treatments B1 and B0. This improvement is attributed to the important role of proline in maintaining cellular osmotic balance and increasing the plant's ability to retain water within tissues, as well as its role in protecting cell membranes and chloroplasts from oxidative damage. Proline also works to improve photosynthetic efficiency and sustain meristematic tissue division activity, which is reflected in increased formation of new leaves and continued vegetative growth (22). A recent study specialized in pepper plants showed that proline is one of the most important protective compounds that contribute to enhancing growth and maintaining the efficiency of physiological processes under difficult environmental conditions (23). This result is consistent with the findings of (6), who found that proline treatment improved the morphological and physiological traits of pepper plants and helped maintain vegetative growth compared to untreated plants, as a result of improved water status and reduced stress-induced damage. Regarding salt stress, treatments S0 and S1 recorded close means for the number of leaves at 56.67 and 56.33 leaves plant⁻¹, respectively, while the number decreased at the higher salinity level S2 to 51.67 leaves

plant⁻¹. This decrease is explained by the fact that increased salt concentrations reduce the water potential around the roots and hinder water and nutrient uptake, in addition to the accumulation of sodium and chloride ions within plant cells, which causes disruption of metabolic processes and reduced rates of cell division and elongation, thus decreasing the formation of new leaves. Salinity also leads to reduced photosynthetic efficiency due to damage to chloroplasts and decreased leaf water content (24). Many studies on pepper have indicated that salt stress is one of the most important limiting factors for vegetative growth and causes a clear decrease in leaf number, leaf area, and plant fresh weight (25). It is noted that the moderate salinity level (S1) did not significantly differ from the control treatment (S0), which may indicate the ability of pepper plants to adapt to moderate salinity levels through activating physiological defense mechanisms and increasing the production of protective compounds such as proline, soluble sugars, and antioxidant compounds. Studies have shown that salt damage symptoms in pepper increase gradually with rising stress levels, while the response is limited at moderate levels due to the effectiveness of the plant's internal adaptation mechanisms (26). Regarding the interaction between salt stress and proline, the results showed that treatment B2S0 outperformed, giving the highest number of leaves at 68 leaves

plant⁻¹, while treatment B0S0 recorded the lowest number of leaves at 37 leaves plant⁻¹. These results indicate that proline was the most influential factor in increasing leaf number compared to the effect of salinity itself, as it helped provide suitable physiological conditions for vegetative growth and maintained leaf activity and continuous formation. The continued superiority of proline-treated plants under

4 Total Leaf Area per Plant (cm². plant⁻¹)

The results in Table (4) indicate significant differences among treatment means for the total leaf area trait. Salt treatment at concentration S1 recorded the highest mean of 376.5 cm². plant⁻¹, which differed from the control treatment S0 mean of 328.1 cm². plant⁻¹, and both concentrations significantly outperformed treatment S2, which recorded the lowest leaf area value of 299.4 cm². plant⁻¹. Regarding the effect of proline acid, treatment B2 gave the highest

Table (4): Effect of Salt Stress and Proline Addition on Total Leaf Area per Plant (cm². leaf⁻¹):

Salt Stress	B0	B1	B2	S Mean
S0	205.42h	409.24b	369.78e	328.1b
S1	302.49g	425.64a	401.27c	376.5a
S2	318.75f	196.96h	382.53d	299.4c
Mean B	275.6c	343.9b	384.5a	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results in Table (4) showed a significant effect of proline in increasing the total leaf area of pepper plants, as treatment B2 recorded the highest mean of 384.5 cm² leaf⁻¹ compared to treatments B1 and B0. This improvement is attributed to the role of proline in maintaining cellular turgor pressure and increasing the efficiency of water and nutrient uptake, as well as its contribution to protecting membranes and proteins from oxidative stress-induced damage. These effects are reflected in increased cell elongation and leaf expansion, leading to an increase in total leaf area (11).

salinity conditions also confirms its role in mitigating the negative effects of salt stress by protecting cell membranes, reducing free radical accumulation, and maintaining leaf water content (27). (15) confirmed that exogenous proline enhances photosynthesis, improves antioxidant efficiency, and limits cellular damage caused by salinity, which directly reflects on leaf growth and development.

mean leaf area of 384.5 cm². plant⁻¹, compared to the control treatment B0, which recorded the lowest leaf area value of 275.6 cm². plant⁻¹. The same table shows the two-way interaction between salt and proline concentrations on plant leaf area; treatment B1S1 outperformed and gave the highest leaf area of 425.64 cm². plant⁻¹, compared to the lowest value recorded for treatment B1S2 at 196.96 cm². plant⁻¹.

(28) indicated that proline treatment contributes to improving vegetative growth of plants by enhancing photosynthesis and maintaining the integrity of cellular systems under various stress conditions. This result is consistent with the findings of (29), who explained that proline is one of the most important protective compounds that help plants maintain vegetative growth by regulating osmotic balance and reducing oxidative damage, which is reflected in increased leaf area and photosynthetic efficiency. The researchers also noted that proline-treated plants often show better

vegetative growth compared to untreated plants when exposed to environmental stresses. Regarding salt stress, treatment S1 recorded the highest mean leaf area of $376.5 \text{ cm}^2 \text{ leaf}^{-1}$, while the mean decreased to $299.4 \text{ cm}^2 \text{ leaf}^{-1}$ at the higher salinity level S2. This decrease is attributed to the fact that increased salinity reduces water uptake due to decreased soil water potential, in addition to the toxic effects of accumulated sodium and chloride ions within plant cells. These conditions lead to reduced cell division and elongation and decreased leaf growth, which is reflected in the total leaf area of the plant (30). (31) explained that salinity leads to reduced leaf area in many horticultural crops due to its direct effect on plant water relations and photosynthetic efficiency. It is noted that the moderate salinity level (S1) gave a larger leaf area than the control treatment (S0), which may be due to the moderate stress stimulating physiological adaptation mechanisms within the plant, such as increased accumulation of osmotic regulatory compounds and

activation of antioxidant systems, which helped plants continue growing and maintain leaf area. Some studies have indicated that moderate salinity levels may not cause direct damage to relatively tolerant plants, but may sometimes activate some defense pathways associated with growth and adaptation (12). Regarding the interaction between salt stress and proline, the results showed that treatment B1S1 outperformed, achieving the highest leaf area of $425.64 \text{ cm}^2 \text{ leaf}$. These results indicate that proline helped reduce the negative effects of salt stress and maintain leaf growth and expansion even in saline environments. This is attributed to proline's ability to stabilize cell membranes, reduce chloroplast damage, and maintain photosynthetic efficiency, allowing continued production of the carbohydrates necessary for leaf growth. showed that increased proline accumulation is associated with improved plant tolerance to salinity and maintenance of important morphological traits, including leaf area

5 Dry Matter Percentage in Leaves (%)

It is concluded from Table (5) that there are significant differences among treatment means for the dry matter trait. The control treatment S0 recorded the highest mean of 40.00%, which significantly differed from treatment S1 mean of 36.67%, and both concentrations significantly outperformed treatment S2, which recorded the lowest dry matter percentage of 34.67%. Regarding the effect of proline acid, the control treatment

B0 gave the highest dry matter percentage of 43.33%, compared to treatment B1, which recorded the lowest dry matter percentage of 33.33%. The results of the two-way interaction between salt and proline concentrations also showed a significant effect, with treatments B1S2 and B0S1 giving the highest dry matter percentage of 67.00%, while B2S2 recorded the lowest dry matter value of 14.00%.

Table (5): Effect of Salt Stress and Proline Addition on Dry Matter Percentage in Leaves (%):

Salt Stress	B0	B1	B2	S Mean
S0	40b	15e	65a	40.00a
S1	67a	18d	25c	36.67b
S2	23c	67a	14e	34.67c
Mean B	43.33a	33.33b	34.67b	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results in Table (5) showed a significant effect of proline on the dry matter percentage in leaves, as treatment B0 recorded the highest mean of 43.33% compared to treatments B2 and B1, which recorded 34.67% and 33.33%, respectively. This can be explained by the fact that proline addition improved the water balance within plant cells and increased the leaves' ability to retain water, leading to a decrease in dry matter percentage despite the improvement in the plant's physiological status. Proline is considered one of the most important compounds regulating osmotic pressure and helps maintain cell viability and continued metabolic activity under stress conditions, which is often reflected in increased water content of plant tissues (32). Regarding salt stress, treatment S0 recorded the highest mean dry matter of 40.00%, while the mean decreased to 36.67% and 34.67% at salinity levels S1 and S2, respectively. This is attributed to the fact that increased salt concentrations disrupt the physiological processes associated with dry matter formation due to reduced water and nutrient uptake and decreased photosynthetic efficiency, in addition to the consumption of part of the photosynthetic products in stress adaptation processes rather than directing them toward growth and biomass accumulation. Studies on pepper have shown that salt stress leads to reduced dry matter accumulation due to its direct effect on vegetative growth and the

6 Total Chlorophyll Content in Leaves (µg/mg)

The results in Table (6) show significant differences among treatment means for the total chlorophyll trait in leaves. The control treatment S0 recorded the highest mean of 1571 µg/mg, which significantly differed from treatment S1 at 1393 µg/mg, and both significantly outperformed treatment S2,

efficiency of organic matter production (Haghighi et al., 2025). It is noted that the decrease in dry matter was gradual with increasing salinity levels, indicating that pepper plants were able to adapt relatively to the moderate salinity level before the negative effects became more pronounced at the higher level. This may be attributed to the activation of multiple defense mechanisms, including increased production of protective compounds, regulation of enzymatic activity, and maintenance of ionic balance within cells. These responses help the plant reduce stress-induced damage during the early stages of salinity exposure (5). Regarding the interaction between salt stress and proline, the results showed that treatments B0S1 and B1S2 outperformed, recording the highest dry matter percentages at 67%, while treatment B2S2 recorded the lowest percentage at 14%. These results indicate that dry matter response was more related to the interaction between salinity level and proline than to a single factor. In some cases, stress may lead to increased accumulation of soluble solids, carbohydrates, and some secondary metabolites within leaves, raising the dry matter percentage, while in other cases, improved leaf water content may lower this percentage. Therefore, dry matter evaluation should be linked with other growth traits such as leaf area and leaf number to form a more accurate picture of plant response to different treatments (33).

which recorded the lowest chlorophyll value of 1227 µg/mg. Regarding the effect of proline acid, treatment B2 gave the highest mean chlorophyll content of 1564 µg/mg, compared to treatment B1, which recorded the lowest chlorophyll value of 1087 µg/mg. The results in the same table showed that the

two-way interaction between salt and proline concentrations had a significant effect, with treatment B2S2 outperforming

and giving the highest mean of 1943 $\mu\text{g}/\text{mg}$, while treatment B1S2 recorded the lowest chlorophyll content of 852 $\mu\text{g}/\text{mg}$.

Table (6): Effect of Salt Stress and Proline Addition on Total Chlorophyll Content ($\mu\text{g}/\text{mg}$):

Salt Stress	B0	B1	B2	S Mean
B0	1853b	1523c	1336e	1571a
B1	1880b	887f	1413d	1393b
B2	887f	852g	1943a	1227c
Mean B	1540b	1087c	1564a	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results in Table (6) showed a significant effect of salt stress, proline addition, and their interaction on the total chlorophyll content in pepper plant leaves. Treatment B2 recorded the highest mean chlorophyll content of 1564 $\mu\text{g}\cdot\text{mg}^{-1}$ compared to treatments B0 and B1, which recorded 1540 and 1087 $\mu\text{g}\cdot\text{mg}^{-1}$, respectively. This superiority is attributed to the protective role of proline in maintaining chloroplast integrity and reducing oxidative damage caused by stress, as well as its contribution to stabilizing cell membranes and protecting photosynthetic pigments from degradation. Proline also works to enhance the activity of antioxidant systems and reduce the accumulation of reactive oxygen species, which are among the most important causes of chlorophyll deterioration under environmental stress conditions, leading to the maintenance of photosynthetic efficiency and continued production of the energy necessary for plant growth and development (16). A study by de ,showed that exogenous proline treatment led to increased chlorophyll content in pepper plants exposed to salt and drought stress, as a result of reducing chloroplast damage and improving the physiological stability of plant cells. This result is also consistent with the findings of (34), who found that proline addition improved photosynthetic efficiency and increased chlorophyll concentrations in stressed plants, by maintaining the integrity

of the photosynthetic apparatus and reducing oxidative damage caused by harsh environmental conditions. These results indicate that proline not only acts as an osmotic regulator but also plays an important role in protecting the vital systems associated with energy production within the plant cell. Regarding salt stress, the first level recorded the highest mean chlorophyll content of 1571 $\mu\text{g}\cdot\text{mg}^{-1}$, while the mean decreased to 1393 and 1227 $\mu\text{g}\cdot\text{mg}^{-1}$ at the subsequent levels. This decline reflects the negative effect of salinity on the formation and stability of photosynthetic pigments, as increased salt concentrations cause disruption in the absorption of essential nutrients for chlorophyll formation, particularly magnesium and nitrogen, in addition to increased production of free radicals that cause oxidation of membranes and chloroplasts (26). Salinity also leads to inhibition of the activity of enzymes responsible for chlorophyll synthesis and accelerates its degradation, which is reflected in reduced photosynthetic efficiency and decreased vegetative growth of the plant. Many studies have shown that decreased chlorophyll content is one of the most common physiological indicators when pepper plants are exposed to salt stress (24). It is noted that the decrease in chlorophyll content was gradual with increasing salinity stress levels, indicating that pepper plants were able to maintain part of their metabolic

activity at moderate salinity levels before the harmful effects became more pronounced at higher levels. This may be attributed to the activation of some physiological defense mechanisms that help protect photosynthetic pigments and reduce oxidative damage during the early stages of stress exposure. However, the continued increase in salt concentration eventually exceeds the plant's resistance capacity, and the negative effects become clearly apparent on chlorophyll content and photosynthetic efficiency (35). (8) explained that maintaining chlorophyll content is one of the most important indicators associated with pepper tolerance to abiotic stresses, and its decline is directly linked to decreased plant physiological performance. Regarding the interaction between salt stress and proline, the results showed that treatment B2S2 outperformed, recording the highest chlorophyll content of $1943 \mu\text{g.mg}^{-1}$, while treatments B2S0 and

Yield Traits

7 Average Number of Fruits (fruit. plant⁻¹)

The results in Table (7) indicate significant differences among treatment means for the number of fruits trait. Salt treatment at concentration S2 recorded the highest mean of 3.557 fruits. plant⁻¹, which significantly differed from the control treatment S0 at 3.037 fruits. plant⁻¹, and both concentrations significantly outperformed treatment S1, which recorded the lowest number of fruits at 2.963 fruits. plant⁻¹. Regarding the effect of proline acid,

B2S1 recorded the lowest values of 887 and $852 \mu\text{g.mg}^{-1}$, respectively. These results indicate that the effect of proline on maintaining chlorophyll depends greatly on the prevailing stress level, as proline appeared to be more effective under severe saline conditions where it provided additional protection to chloroplasts and helped sustain the photosynthetic process. The large increase in chlorophyll content in some interaction treatments may also reflect stimulation of defense mechanisms responsible for maintaining photosynthetic pigments and reducing their degradation rate (36). This result is consistent with what researchers have indicated, that chlorophyll response to proline is often more pronounced when plants are exposed to stress compared to normal conditions, due to the increased need for protective compounds to maintain the integrity of the photosynthetic system.

treatment B2 gave the highest mean number of fruits at 3.927 fruits. plant⁻¹, while the control treatment B0 recorded the lowest mean of 2.557 fruits. plant⁻¹. The same table shows that the two-way interaction between salt and proline concentrations had a significant effect on fruit number, with treatment B2S2 outperforming and giving the highest number of fruits at 5.00 fruits. plant⁻¹, while treatment B0S0 gave the lowest fruit number of 2.11 fruits. plant⁻¹.

Table (7): Effect of Salt Stress and Proline Addition on Average Number of Fruits (fruit. plant⁻¹):

Salt Stress	B0	B1	B2	S Mean
S0	2.11f	2.89d	4.11b	3.037b
S1	2.67e	3.55c	2.67e	2.963c
S2	2.89d	2.78de	5.00a	3.557a
Mean B	2.557c	3.073b	3.927a	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results in Table (7) showed a significant effect of proline addition on the average number of fruits, as treatment B2 recorded the highest mean of 3.927 fruits plant⁻¹ compared to treatments B1 and B0, which recorded 3.073 and 2.557 fruits plant⁻¹, respectively. This improvement is attributed to the role of proline in enhancing photosynthetic efficiency and maintaining the osmotic balance of plant cells, which helps provide larger quantities of carbohydrates necessary for flower and fruit formation. Proline also contributes to protecting reproductive tissues from oxidative damage and maintaining pollen viability and fertilization efficiency, which positively reflects on increased fruit set and the number of formed fruits (37). Recent studies have indicated that improving proline accumulation or its exogenous application contributes to enhancing the reproductive growth efficiency of pepper under various stress conditions (27). This result is also consistent with the findings of (21), who found that improving the physiological status of plants exposed to salinity leads to increased flowering, fruit set, and final yield, as a result of maintaining leaf activity, photosynthetic efficiency, and continued translocation of nutrients toward reproductive organs. Regarding salt stress, level S2 recorded the highest mean number of fruits at 3.557 fruits plant⁻¹, followed by S0 and then S1. This result is unconventional compared to what is common in many studies, but it may indicate that the salinity level used did not reach the point of causing severe damage to the plant, but rather stimulated some physiological adaptation mechanisms that led to the redistribution of photosynthetic products toward reproductive growth instead of vegetative growth (38). Some pepper varieties may also respond to moderate or high stress by increasing investment in seed

and fruit production as a survival and species continuation mechanism. Studies on pepper have recorded significant differences between varieties in their yield response to salinity depending on genetic background and stress level (39). It is noted that level S1 recorded the lowest mean number of fruits compared to the other two levels, which may indicate that this stress level was sufficient to cause disruption in flowering and fruit set processes without stimulating effective adaptive responses like those seen at the higher level. Salinity affects the hormonal balance of the plant and limits nutrient translocation to floral organs, in addition to its effect on pollen viability and pollen tube growth, which may lead to reduced fruit set percentage and fruit number (40). Many physiological studies have indicated that the reproductive stage is more sensitive to salinity than the vegetative stages in many vegetable crops, including pepper (40). Regarding the interaction between salt stress and proline, the results showed that treatment B2S2 outperformed, recording the highest mean number of fruits at 5.00 fruits plant⁻¹, while treatment B0S0 recorded the lowest mean of 2.11 fruits plant⁻¹. These results indicate that proline was more effective at the higher salinity stress level, as it helped maintain the efficiency of biological processes associated with flowering and fruit set. This may be attributed to its role in protecting cell membranes, reducing oxidative damage, and improving the plant's water status, which led to continued flower formation, reduced flower abscission, and increased fruit set percentage. The clear superiority of this treatment is also consistent with the results of previous vegetative traits that showed improvement in leaf number, leaf area, and chlorophyll content, which are traits directly associated with increased plant productivity (41).

Average Yield per Plant (g. plant⁻¹)

The results in Table (8) show significant differences among treatment means for the yield per plant trait. Salt treatment at concentration S2 recorded the highest mean yield per plant of 7.123 g compared to the control treatment S0, which recorded the lowest mean yield per plant of 5.057 g.

Regarding the effect of proline acid, treatment B2 gave the highest mean yield per plant of 7.397 g, while the control

treatment B0 recorded the lowest value of 4.967 g. The results in the same table showed that the two-way interaction between salt and proline concentrations had a significant effect, with treatment B2S2 outperforming and giving the highest yield per plant value of 8.50 g, compared to treatment B0S0, which recorded the lowest yield per plant value of 3.44 g.

Table (8): Effect of Salt Stress and Proline Addition on Average Yield per Plant (g. plant⁻¹):

Salt Stress	B0	B1	B2	S Mean
S0	3.44i	5.00h	6.73e	5.057c
S1	6.07f	7.67b	6.96d	6.900b
S2	5.39g	7.48c	8.50a	7.123a
Mean B	4.967c	6.717b	7.397a	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results in Table (8) showed a significant effect of proline addition on the average yield per plant, as treatment B2 recorded the highest mean of 7.397 g plant⁻¹ compared to treatments B1 and B0, which recorded 6.717 and 4.967 g plant⁻¹, respectively. This improvement is attributed to the physiological role of proline in enhancing plant tolerance to stress by maintaining cellular osmotic balance, protecting membranes and proteins from oxidative damage, as well as improving photosynthetic efficiency and increasing the production of carbohydrate compounds necessary for growth and fruiting. Proline also helps sustain the translocation of photosynthetic products toward fruits, leading to increased yield weight and improved overall plant productivity. Recent studies have indicated that exogenous proline treatment leads to improved yield traits in pepper by enhancing the efficiency of physiological processes associated with growth, fruit set, and fruit development (42).

This result is also consistent with the findings of (43), who showed that improving the plant's ability to cope with salt stress positively reflects on yield components, as maintaining leaf activity and photosynthetic efficiency leads to increased quantities of nutrients directed toward fruits and thus higher final plant production. Regarding salt stress, level S2 recorded the highest mean yield of 7.123 g plant⁻¹, followed by S1, then S0, which recorded 5.057 g plant⁻¹. These results indicate that the salinity level used was not sufficient to cause severe damage to growth and production, but rather may have stimulated some physiological adaptation mechanisms that improved water and nutrient use efficiency and led to increased allocation of manufactured materials toward reproductive organs. The response of pepper to salinity also varies depending on the variety and stress level, and some studies have shown that relatively tolerant varieties can maintain their productivity at certain salinity levels by

activating defense systems and improving the regulation of osmotic balance within cells (44). It is noted that the gradual increase in yield from S0 to S2 is consistent with previous results for fruit number, chlorophyll, and leaf area, indicating a positive relationship between maintaining the plant's metabolic activity and increased production. Higher chlorophyll content and improved leaf area lead to increased light interception efficiency and higher photosynthetic rates, which provide larger quantities of carbohydrates necessary for fruit growth and dry matter accumulation. Studies have confirmed that photosynthetic efficiency is one of the most important factors determining productivity under salt stress conditions (47). Regarding the interaction between salt stress and proline, the results showed that treatment B2S2 outperformed, recording the highest yield of

Average Fruit Length (cm):

The results in Table (9) show significant differences among treatment means for fruit length. Salt treatment at concentration S1 recorded the highest mean of 9.883 cm, which significantly differed from treatment S2 with a mean of 9.203 cm, and both significantly outperformed the control treatment S0, which recorded the lowest mean fruit length of 8.297 cm. Regarding the effect of proline acid, treatment B1 gave

Table (9): Effect of Salt Stress and Proline Addition on Average Fruit Length (cm):

Salt Stress	B0	B1	B2	S Mean
S0	6.67g	9.30c	8.92e	8.297c
S1	9.15d	9.90b	10.60a	9.883a
S2	9.21cd	10.03b	8.37f	9.203b
Mean B	8.343c	9.743a	9.297b	

Means followed by the same letters indicate no significant differences between them according to Duncan's test at a probability level of 0.05.

The results in Table (9) showed a significant effect of proline addition on average fruit length, as treatment B1 recorded the highest

8.50 g plant⁻¹, while treatment B0S0 recorded the lowest yield of 3.44 g plant⁻¹. This result demonstrates that proline was more efficient in improving plant productivity at the higher salinity stress level, as it helped maintain cellular water balance and protect membranes and chloroplasts from oxidative damage, which was reflected in continued fruit formation and increased fruit growth. The clear increase in yield under this treatment is also consistent with the recorded increases in fruit number and chlorophyll content, confirming that proline played a pivotal role in improving the physiological and productive efficiency of the plant under salinity conditions (15). Studies have shown that improving plants' ability to adapt to salt stress leads to increased resource allocation toward reproductive growth and improved final yield (45).

the highest mean fruit length of 9.743 cm, compared to treatment B0, which recorded the lowest mean fruit length of 8.343 cm. The same table shows the two-way interaction between salt and proline concentrations on average fruit length; treatment B2S1 outperformed and gave a fruit length of 10.60 cm, compared to the lowest fruit length of 6.67 cm recorded for treatment B0S0.

mean of 9.743 cm, followed by treatment B2 with a mean of 9.297 cm, while the control treatment B0 recorded the lowest mean of

8.343 cm. This improvement is attributed to the role of proline in maintaining the water status of cells and increasing the efficiency of physiological processes associated with growth, particularly cell division and elongation during fruit development stages. Proline also contributes to improving photosynthetic efficiency and increasing the translocation of photosynthetic products toward developing fruits, which is reflected in increased fruit dimensions and improved quality traits. Studies have indicated that proline treatment leads to improved growth, productivity, and fruit traits in pepper plants by enhancing the plant's ability to tolerate various environmental stresses (41). This result is also consistent with the findings of ,who found that improving osmotic balance and reducing oxidative damage in stressed plants leads to increased fruit growth and improved morphological traits as a result of continued flow of nutrients and organic compounds toward reproductive organs. Regarding salt stress, level S1 recorded the highest mean fruit length of 9.883 cm, followed by S2 with a mean of 9.203 cm, while the control treatment S0 recorded the lowest mean of 8.297 cm. These results indicate that the moderate salinity level may have stimulated some physiological responses that helped improve fruit growth compared to the control treatment. This may be due to increased water use efficiency and stimulation of some defense systems that contributed to maintaining the plant's metabolic activity. The response of pepper to salinity also varies depending on the variety, stress intensity, and growth stage, and some studies have shown that moderate stress levels may lead to improvement in some quality and yield traits of fruits

without negatively affecting their growth (46). It is noted that the higher salinity level S2 did not cause a significant decrease in fruit length compared to level S1, indicating the presence of adaptive capacity in the plants used in the experiment that enabled them to maintain fruit growth despite stress conditions. This may be associated with the activation of defense mechanisms responsible for protecting cell membranes, maintaining ionic balance, and reducing free radical accumulation—factors that help sustain fruit cell division and elongation. Recent studies have shown that the plant's success in maintaining fruit growth under stress is an important indicator of the efficiency of its physiological tolerance mechanisms (27). Regarding the interaction between salt stress and proline, the results showed that treatment B2S1 outperformed, recording the highest mean fruit length of 10.60 cm, while treatment B0S0 recorded the lowest mean of 6.67 cm. This result indicates that proline addition under moderate salinity conditions contributed to enhancing fruit growth to a greater extent than the effect of each factor alone, as proline helped maintain photosynthetic efficiency, improve the plant's water status, and reduce oxidative damage associated with salt stress. The increased fruit length in this treatment may also reflect improved efficiency of nutrient translocation toward fruits and continued cell division and elongation during their development stages. This result is consistent with what researchers have mentioned, that the positive effect of proline is more pronounced when plants are exposed to stress, as it works to alleviate its effects and maintain the plant's productive performance (21).

5. Conclusions and Recommendations

5-1 Conclusions

1. Foliar application of proline significantly improved vegetative growth and yield related traits in pepper plants.
2. Salinity stress markedly suppressed vegetative growth and yield parameters, with the inhibitory effect increasing proportionally with salt concentration.
3. Exogenous proline effectively alleviated salinity induced damage and enhanced the

5-2 Recommendations

1. . Foliar application of proline is recommended as a practical approach to sustain growth and productivity of pepper grown under saline conditions.
2. Future research should evaluate a wider range of proline concentrations, both lower and higher than those tested, across different salinity levels to establish the optimal dose response relationship in pepper.

physiological capacity of plants to tolerate salt stress.

4. The combined application of the highest proline concentration with the lowest salinity level resulted in superior performance for the majority of traits compared to all other treatments.

3. The protective effect of exogenous proline should be investigated in other economically important crops affected by salinity to confirm its broader applicability.
4. Molecular investigations are recommended to elucidate the genetic and regulatory mechanisms underlying proline mediated tolerance to salt stress.

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