





Article

Evaluation of Salicylic Acid Effects on Growth, Biochemical, Yield, and Anatomical Characteristics of Eggplant (*Solanum melongena* L.) Plants under Salt Stress Conditions

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Abstract: Salt stress is a major issue in agriculture and crop production that influences global food security. Mitigation options to address salt stress through agronomic practices can help manage this issue. Experiments were performed in two summer seasons in an experimental farm to test the impact of three salinity levels (S): 300 (control), 1000, 2000, and 3000 ppm, and two salicylic acid (SA) levels, including 1.0 and 1.50 mM, and their interaction on growth and yield of eggplant (*Solanum melongena* L.) hybrid Suma. The results showed that increasing S levels up to 3000 ppm reduced plant and fruit physical characteristics, as well as leaf and fruit chemical characteristics, especially leaf total chlorophyll, carotenoids, relative water, fruit nitrogen, phosphorus, and potassium contents, which led to a reduction in total yield per plant. However, an insignificant effect was observed in the control level and 1000 ppm saline water in leaf area, fruit length, leaf total chlorophyll content, fruit phosphorus content, and total yield per plant. In contrast, leaf sugars, proline contents, electrolyte leakage, fruit TSS (total soluble solids), and ascorbic acid contents were improved with S levels up to the concentration of 3000 ppm compared to the control. However, tested parameters were significantly higher due to the SA foliar spray of 1.0 mM besides photosynthetic pigments of leaves enhanced by using 1.0 and 1.50 mM. Using 1.0 mM SA concentration alleviated the adverse impact of S on eggplant plants until 1000 ppm saline water, reflecting an increase in eggplant yield. The anatomical structure of eggplant leaves revealed positive variations in mature leaf blades in both the stressed and SA-treated plants. Based on these results, the use of SA at a concentration of 1.0 mM may lessen the negative impacts of salt on the growth of eggplant, which increases the overall yield.

Keywords: eggplant; salinity; salicylic acid; photosynthesis pigments; yield; anatomical structure

1. Introduction

In Egypt, one of the most significant summer crops is eggplant (*Solanum melongena* L.). Its fruits are a good source of vitamins, minerals, protein, and carbohydrates. Increasing

the productivity of eggplant with good quality is a major goal of the growers for domestic and foreign consumption. One of the most crucial factors that may directly impact plant development and productivity is the irrigation water source. With the rising water shortage, one of the suggested solutions for the irrigation of eggplant is the use of drainage water. In many regions of the world, salinity (S) poses a significant environmental challenge to the cultivation of crops. At every stage of the plant's life cycle, it has a negative impact. This results in numerous particular structural alterations that disrupt the water balance in plants. Moreover, salt stress affects plants in multiple ways: toxicity, a decrease in water potential, ion imbalance, and disruptions in ion homeostasis [1]. This modified state results in growth decline and lower plant productivity. As a result, the metabolic deficiency that is present inhibits the growth and development of plants [2].

In order to increase plant development, flowering, fruit set, and overall output of vegetable crop plants under saline conditions, there is currently a lot of attention being paid to the idea of utilizing natural and safe substitutes. As a result, different strategies were applied to alleviate the deleterious impacts of S on plants, such as foliar applications of growth regulators [3]. Salicylic acid (SA) is an endogenous growth regulator that induces plant tolerance against various abiotic stresses such as S [4], and it plays a vital role in the regulation of some physiological processes in plants [5]. So, many abiotic stresses can be mitigated by SA, which functions as a signaling mechanism [6]. Several studies show that SA possesses a high level of biostimulation potential for salt tolerance via promoting the regulation of the ascorbate glutathione pathway, enhancing the tolerance of plants to ion toxicity, as well as inhibiting abrupt growth decline [7–9]. Moreover, SA improves turgor in stressed plant cells and activates antioxidant enzymes while increasing the levels of osmoprotectants [10,11].

Photoprotectants are highly recommended for enhancing growth and increasing the productivity of many plant species under abiotic stresses. Among them, salicylic acid (SA) plays an important role in defending plants against both biotic and abiotic stress conditions [12]. Exogenously applied SA has been shown to influence a wide range of plant processes, including seed germination, stomatal closure, ion uptake, cell membrane permeability, and photosynthetic rate, including pigment content, growth rate, and fruit yield [13,14]. It also enhances the activities of antioxidant enzymes and proline content [15]. The beneficial effects of SA in terms of S have been studied in several crops, such as peppers (*Capsicum annuum*) [16], strawberries (*Fragaria × ananassa*) [17], and common beans (*Phaseolus vulgaris*) [18].

SA has been shown to improve plant tolerance to major abiotic stresses such as metal [19], salinity [20], osmotic [21], drought [22], chilling damage [23], and heat stress [24]. Exogenously sourced SA applied to stressed plants, either through seed soaking, adding to the nutrient solution, irrigating, or spraying, was reported to induce major abiotic stress tolerance mechanisms [25,26]. SA influences plant functions in a dose-dependent manner, where inducing or inhibiting plant functions can be achieved with low and high SA concentrations, respectively. Recent molecular studies have established that SA can regulate many aspects in plants at the gene level and thereby can improve plant abiotic stress tolerance.

Many researchers have demonstrated that SA can alleviate salt stress in fruits and vegetables such as bell peppers [9,27], pumpkins [28], and tomatoes [29]. However, this effect varies depending on the species, application methods, SA concentration, and growing environments. Ribeiro et al. [30] evaluated watermelon characteristics in relation to water S and SA exogenous treatment and observed that the SA did not alleviate the detrimental impact of salinity but rather that it positively enhanced the plant morphophysiology up to the concentration of 0.85 mM. In light of the aforementioned discussions, the SA foliar spray produced thicker leaflets in bean plants due to the increment in the lamina's thickness at midvein. The increased thickness of lamina might be due to the thickness increase in palisade layers, spongy tissues, as well as the vascular bundle [31]. In a report by [32], the SA application enhanced the stem diameter, vessel number per midvein bundle, leaflet

lamina thickness, along with the average diameter of the xylem vessels in faba bean plants subject to drought stress. In addition, they reported that the SA foliar spray minimized the adverse drought stress and enhanced the plant tolerance to drought in faba beans. Exogenous SA treatment improved the thickness of palisade and spongy tissues, the thickness of the midrib and leaflet lamina, along with increments in the midvein bundle size [33]. The current study was conducted to examine the impact of foliar applications of SA on the growth, chemical composition of leaves and fruits, fruit yield, quality, and anatomical structure of eggplant under S stress. So, the purpose of this research was to improve eggplant ability to tolerate salt stress and lessen salinity's detrimental effects.

2. Materials and Methods

This study was conducted at the experimental farm in Al-Azhar University, Cairo, Egypt, in two summer seasons (2019 and 2020) to study the impact of various levels of saline drainage water for irrigation. Also, the effects of foliar spray of SA and its interaction with saline water levels on growth parameters and yield characteristics of eggplant (*Solanum melongena* L.) were studied. The saline water was sourced from Qarun Lake, which is located at El-Fayoum Governorate, where 28,000 ppm was detected during collection. Then, saline water was diluted to three levels of S concentrations (1000, 2000, and 3000 ppm). The control was maintained at 300 ppm saline water for irrigation. Table 1 presents the results of the chemical analysis of the three levels of diluted water.

Table 1. Analysis of the chemical contents in the saline water expressed as meq/L.

Level	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺
300 ppm (Control)	-	2.8	1.3	0.1	1.6	1.3	1.1	0.2
1000 ppm	-	2.8	9.6	0.2	2.1	3.1	7.1	0.3
2000 ppm	-	2.8	21.5	0.5	2.5	5.9	15.9	0.5
3000 ppm	-	2.8	32.1	0.8	3.1	9.3	22.6	0.7

Imported eggplant seeds, hybrid Suma (sourced from Syngenta, Giza, Egypt), characterized by white and long-shaped fruits, were sown in the nursery using foam trays on 1 March in both seasons. After 40 days from sowing, each seedling was transplanted in a 40 cm pot containing 15 kg of soil of sandy clay loam type. Table 2 shows the physical and chemical properties of the potted soil. After 10 days of transplantation, saline water irrigation started. The experiment was conducted in the open field using potted plants. The weather conditions, including air temperature and relative humidity during the growing season, are presented in Table 3. Each pot received 2.5 L of water to keep the soil consistently moist, and plants were watered with saline solution twice a week. Plants were exposed to two concentrations of SA spray (1.0 and 1.5 mM), as well as the control treatment after 20 days of transplantation, and continued at 20-day intervals during the growing season at three times: 20, 40, and 60 days after transplanting. Plants were sprayed with SA by the aid of the sprayer, so that they were completely wet. The pots were set up as a randomized complete blocks design with three replications. The experiment had 12 treatments, as presented in Table 4. Each treatment contained three pots as replicates. Agronomic practices such as fertilization and disease and pest control were performed following the standard recommendations of the Egyptian Ministry of Agriculture and Land Reclamation.

Table 2. The physical properties and chemical contents in the potted soil.

Soil Physical Characteristics				Soil Chemical Contents							
Sand	Clay	Silt	Texture	pH	Ca ⁺⁺	Cation (meq/L)			Anion (meq/L)		
66.1	23.25	10.7	Sand	7.61		Mg ⁺⁺	Na ⁺	K ⁺	HCO ₃	Cl ⁻	SO ₄ ⁻
			Clay		0.56	CO ₃ ⁻	0.88	0.17	0.51	1.02	0.46
			Loam			0.00					

Table 3. The average monthly air temperature and relative humidity at the experimental location during the two seasons of 2019 and 2020.

Months	Air Temperature (°C)		Relative Humidity (%)	
	2019	2020	2019	2020
Seasons				
March	21.1	18.1	48.00	51.1
April	27.4	21.1	38.6	50.00
May	29.5	25.5	50.2	42.8
June	30.2	27.8	53.4	44.4
July	31.2	29.2	54.4	55.9

Table 4. Treatment combinations of saline drainage water and salicylic acid (SA) levels.

Treatments	Description
T1	Control 300 ppm
T2	S 1000 ppm
T3	S 2000 ppm
T4	S 3000 ppm
T5	SA (1.0 mM)
T6	SA (1.5 mM)
T7	S 1000 ppm + SA (1.0 mM)
T8	S 2000 ppm + SA (1.0 mM)
T9	S 3000 ppm + SA (1.0 mM)
T10	S 1000 ppm + SA (1.5 mM)
T11	S 2000 ppm + SA (1.5 mM)
T12	S 3000 ppm + SA (1.5 mM)

2.1. Determination Procedures

2.1.1. Plant Physical and Chemical Characteristics

From each experimental treatment, the plants were chosen at random after 80 days from transplanting, and their physical and chemical characteristics were measured. The physical records were plant height (cm), number of leaves, and leaf area (cm²). Using the recent full expanded fourth leaf from the plant's top, leaf area was calculated as a relationship between unit area and leaf fresh weigh according to Koller [34]. Leaf chemical analysis for total chlorophylls and carotenoids (mg/100 g fresh weight) was measured using spectrophotometry in the acetone leaf extract [35]; proline content (mg/g fresh weight) was extracted in 3% (*w/v*) aqueous sulfosalicylic acid, then identified using the acid ninhydrin reagent and reading at 520 nm [36]; total sugar content (g/100 g dry weight) present in the dried leaves was determined by using the phenol–sulfuric acid method following Smith et al. [37]; electrolyte leakage (%) was determined in 0.3 g fresh leaf, which was cut from the

middle portion of leaf samples and placed in test tubes containing 15 mL of distilled water for 2 h at 25 °C. By using an electrical conductivity meter, the initial electrical conductivity of the solution (EC1) was measured. After that, the samples were placed into an autoclave at 120 °C for 20 min., then the final electrical conductivity (EC2) was determined. The formula $L = EC1/EC2 \times 100$ was followed to calculate electrolyte leakage (EL) [38]; also, relative water content (%) was calculated according to Korkmaz et al. [39]. Briefly, from plant samples in each replicate, discs (1 cm in diameter) were taken from the center of fully formed leaves and weighed (FW). Discs were floated on distilled water for five hours in the dark. After using paper towels to dry any excess surface water, leaf discs' turgid weights (TW) were determined. Following a 48-h drying period at 75 °C, the dry weights (DW) of the discs were calculated. The formula $RWC = (FW - DW) / (TW - DW) \times 100$ was used to calculate the relative water content.

2.1.2. Fruit Physical and Chemical Characteristics

Fruits from each plot were picked at the appropriate mature stage, counted, and weighed after each picking, and the following data of physical parameters was recorded: fresh weight (g), diameter (cm), length (cm), and total yield per plant. In addition, fruit chemical constituents such as total soluble solids (TSS) were determined as a percentage with the digital hand-held "Prochet" refractometer by the method published by AOAC [40]; ascorbic acid (mg/100g fresh weight) was determined by using 2,6-dichlorophenolindophenol dye and oxalic acid as reported by Rao and Deshpande [41]. Additionally, nitrogen, phosphorus, and potassium (%) contents were tested in the mixture of dried fruits according to the AOAC methods [40]. Nitrogen content was determined by using the micro-Kjeldahl method, while phosphorus content was determined colorimetrically by the hydroquinone and sodium sulfite method. Potassium content was determined using a flame photometer (PFP7- Jenway, Staffordshire, UK).

2.2. Statistical Analysis

Data from the two seasons were subjected to statistical analysis using CoStat software (Ver. 6.4, CoHort Software, USA). The analysis of variance and the means of treatments were compared using least significant difference (LSD) at the 5% level according to Snedecor and Cochran [42].

2.3. Anatomical Characteristics of Eggplant Leaf

Tested material including the lamina of the developed leaf on the fourth internode of the main stem was taken throughout the second growing season of 2020. Anatomical characteristics of eggplant leaves included upper and lower epidermis thickness (μm), palisade and spongy tissue thickness (μm), and midrib zone thickness (μm). Each value refers to five sections and five readings for each. The microtechnique was performed after the procedure described by Nassar and El-Sahar [43] and El-Taher et al. [44].

3. Results

3.1. Plant Physical Parameters

All of the vegetative growth parameters of eggplant were negatively impacted by the increasing irrigation with saline water up to 3000 ppm, as shown in Figure 1. The plant height and the number of leaves values were elevated in the control plants (300 ppm), while the values of leaf area did not significantly differ by using 300 and 1000 ppm. The concentration of 1.0 mM SA increased plant height, leaf number, and leaf area more than at 1.5 mM concentration. Also, it was found that both S levels and SA application interfere concurrently with the growth of eggplant plants. Thereafter, the plants treated with saline water of 1000 ppm and sprayed with 1.0 mM of acid showed the highest improvements in plant physical parameters, while the least values were obtained for 3000 ppm saline water combined with 1.5 mM SA.

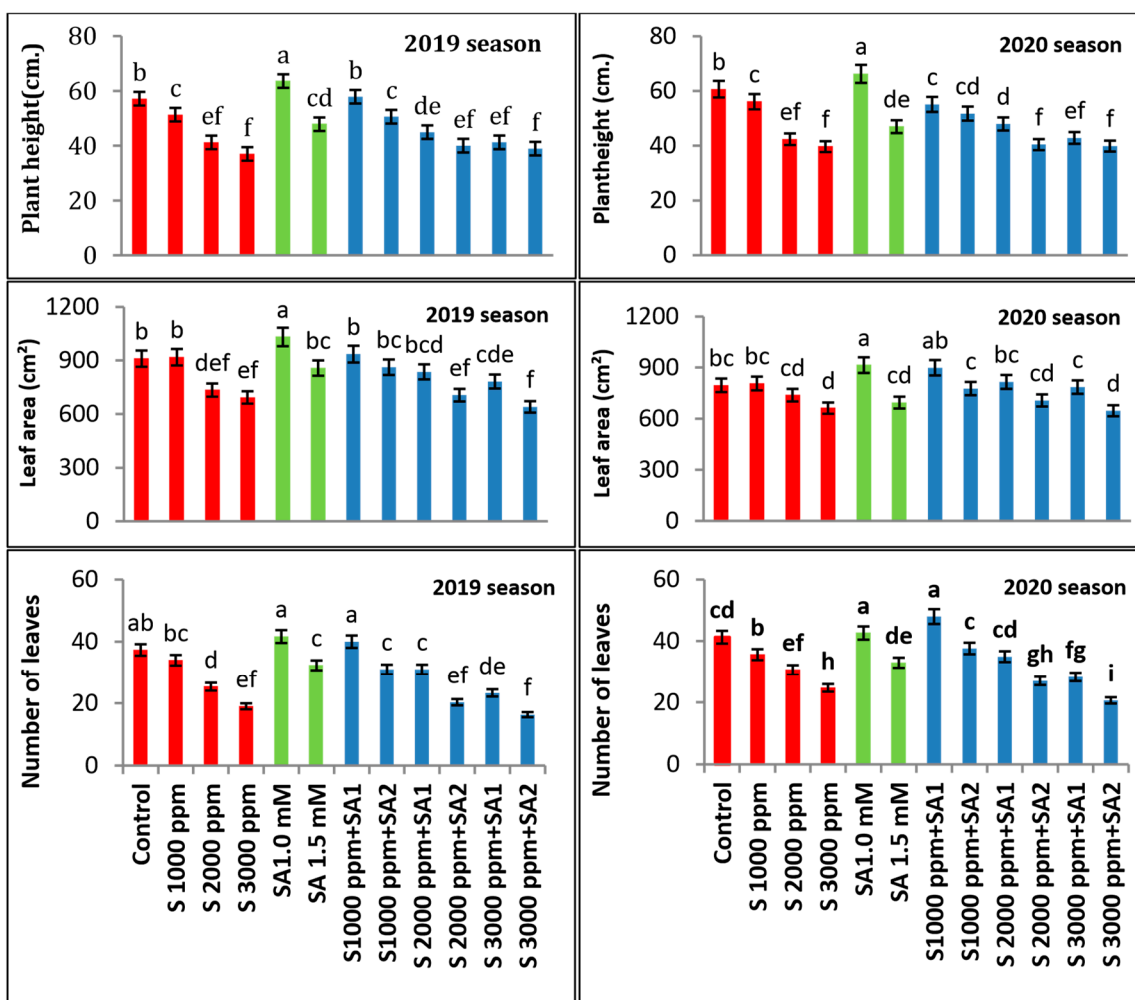


Figure 1. Plant height, leaf area, and number of leaves of eggplants as affected by irrigation with saline water levels, salicylic acid concentrations, and their interaction during the two seasons of 2019 and 2020. Significant differences between treatments are labelled by different alphabets from a to i to indicate significant levels ($p < 0.05$), i.e., a treatment with the letter “a” is significantly different from “b”, and “b” is significantly different from “c”, and so forth. However, if two treatments have a shared letter, there is no significant difference. Red: S level; Green: SA level; Blue: S and SA levels.

3.2. Plant Chemical Constituents

Regarding photosynthesis pigments, an insignificant influence was observed in the control plants and the level of 1000 ppm saline water in total chlorophyll content; in addition, the highest values in carotenoid content resulted from the control at 300 ppm (Figure 2), while higher levels at 2000 ppm and 3000 ppm led to a proportional significant decrease in both contents. Foliar spray of SA at the tested concentrations increased the values of total chlorophylls and carotenoids significantly to the highest level by SA at 1.5 mM. Thus, the data indicated that the applied SA up to 1.5 mM completely enhanced total chlorophyll and carotenoid content in the stressed plant, especially the irrigated plants with 1000 ppm S level.

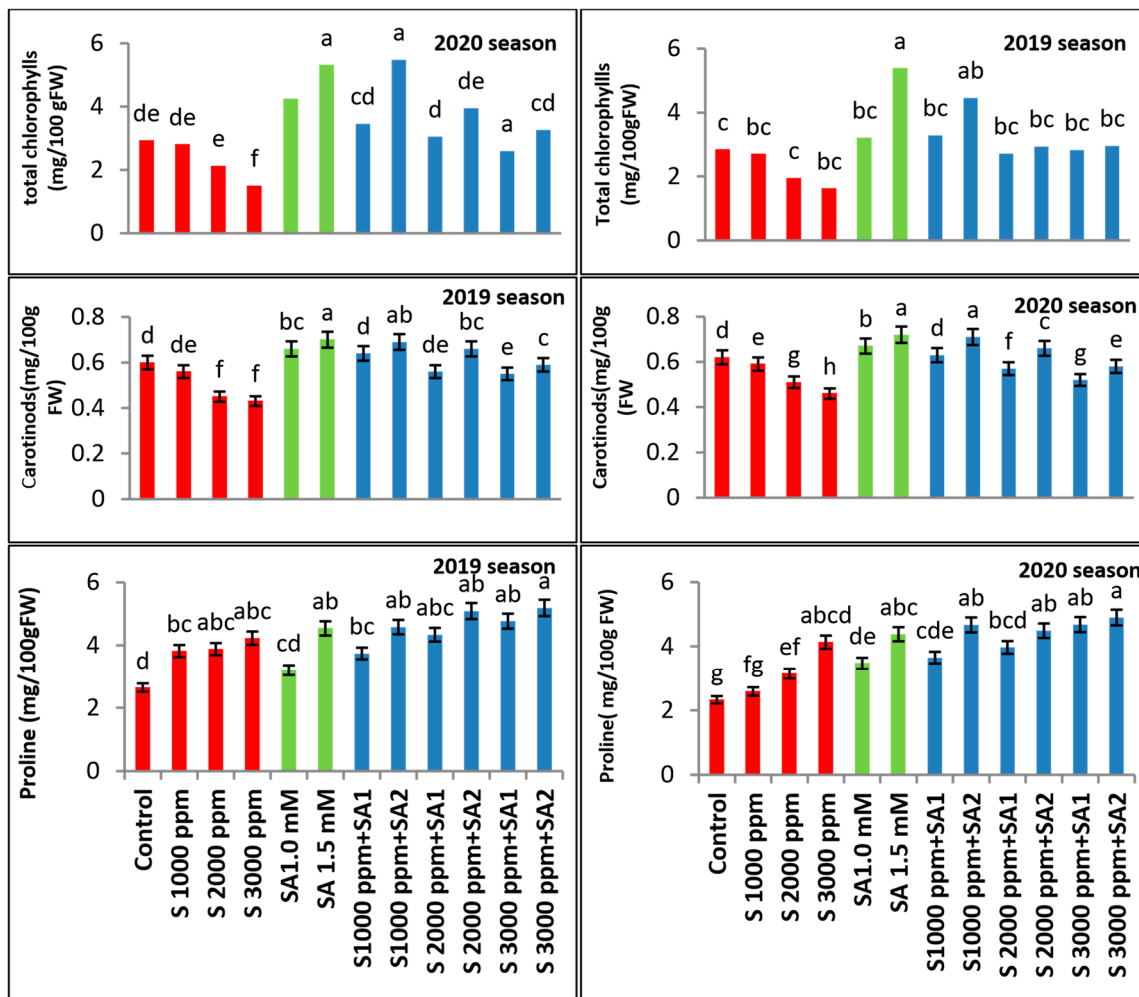


Figure 2. Total chlorophylls, carotenoid, and proline contents of eggplant leaves as affected by irrigation with saline water levels, salicylic acid concentrations, and their interaction during the two seasons of 2019 and 2020. Significant differences between treatments are labelled by different alphabets from a to h to indicate significant levels ($p < 0.05$), i.e., a treatment with the letter “a” is significantly different from “b”, and “b” is significantly different from “c”, and so forth. However, if two treatments have a shared letter, there is no significant difference. Red: S level; Green: SA level; Blue: S and SA levels.

Concentrations of proline and total sugars as osmolytes in leaves were presented in Figures 2 and 3. S levels up to 3000 ppm recorded a significant increase in proline and total sugars above control at 300 ppm. Also, spraying SA at 1.5 mM concentration induced the accumulation of leaf proline and total sugar content. Hence, the most effective treatment that enhanced the proline concentration subject to all S stress conditions was SA at 1.5 mM, while SA at 1.00 and 1.5 mM recorded the high value for total sugar content under stress conditions at 3000 ppm.

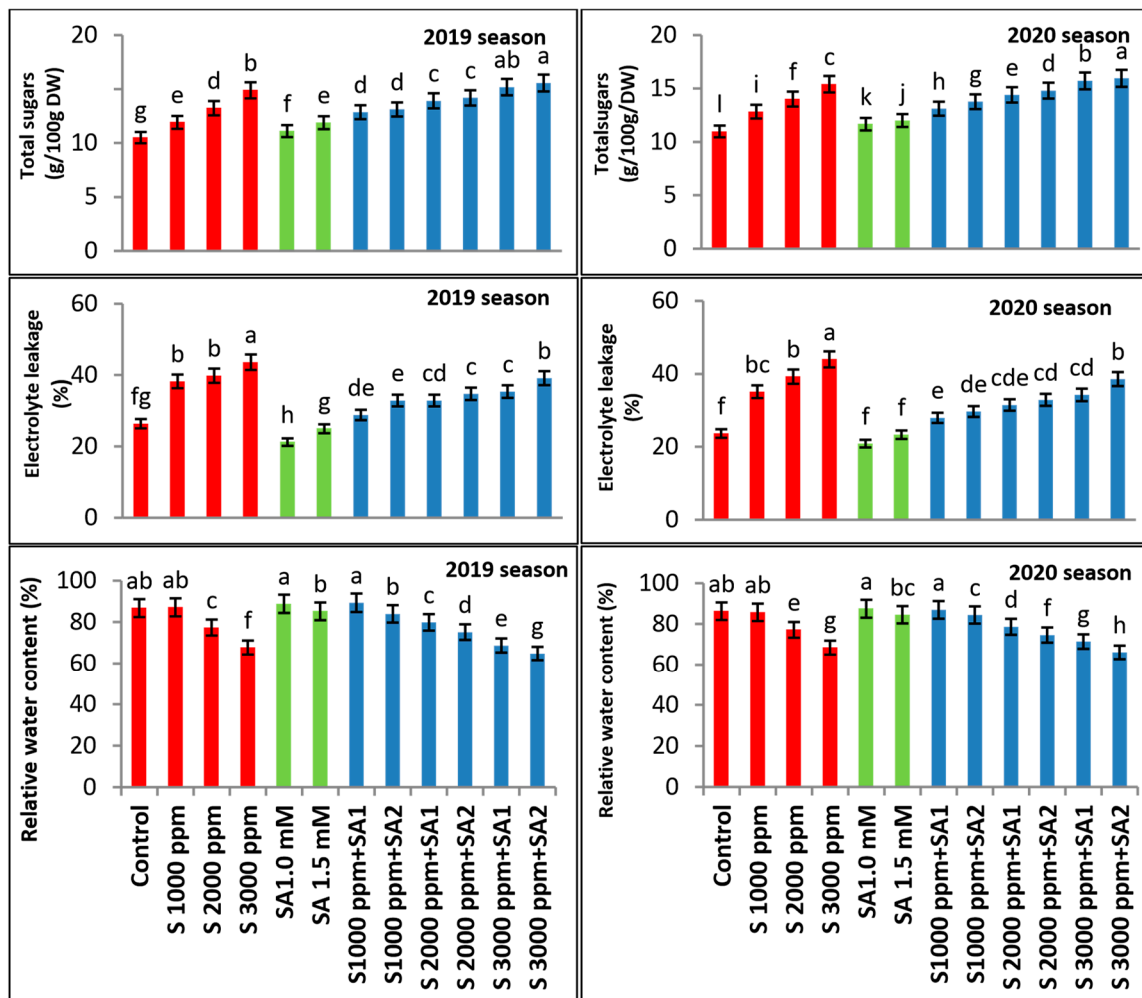


Figure 3. Total sugar content, electrolyte leakage, and water relative content of eggplant leaves as affected by irrigation with saline water levels, salicylic acid concentrations, and their interaction during the two seasons of 2019 and 2020. Significant differences between treatments are labelled by different alphabets from a to l to indicate significant levels ($p < 0.05$), i.e., a treatment with the letter “a” is significantly different from “b”, and “b” is significantly different from “c”, and so forth. However, if two treatments have a shared letter, there is no significant difference. Red: S level; Green: SA level; Blue: S and SA levels.

The electrolyte leakage in eggplant leaves had higher values at the S level of 3000 ppm (Figure 3). This finding indicates that S stress impaired membrane permeability. The results also revealed that 1.5 and 1.0 mM of SA treatments obtained the highest and lowest levels of electrolyte leakage, respectively. Generally, the data discovered that an increase in acid decreased the proportion of electrolyte leakage even at the maximum salt concentration of 3000 ppm. Hence, applications of SA partly reduced the adverse S effect on cell membrane degradation. However, the electrolyte leakage in control plants at 1000 ppm treated with SA revealed a significant decrease compared to stressed plants with SA.

Figure 3 shows significant decreases in relative water content with every increase in S concentrations up to 3000 ppm when compared to both 300 and 1000 ppm saline water. Conversely, the plants sprayed with SA at 1.0 mM concentration resulted in the highest significant rise compared to all other studied concentrations. In addition, the increase in leaf relative water content is higher at the 1000 ppm S level with SA spray of 1.0 mM.

3.3. Fruit Physical Characteristics

Figure 4 shows that there is a decrease in fruit fresh weight, length, and diameter with the increase in saline water up to the level of 3000 ppm, but insignificant changes were noted for fruit length between the control plant and 1000 ppm. Also, the 1.0 mM concentration was the most obvious increase in the tested fruit physical characteristics than the 1.5 mM concentration. Plants treated with 1000 ppm saline water and SA sprayed at a 1.0 mM concentration resulted in the highest values of physical fruit characteristics, while it was the lowest in plants treated with 3000 ppm saline water combined with 1.5 mM SA.

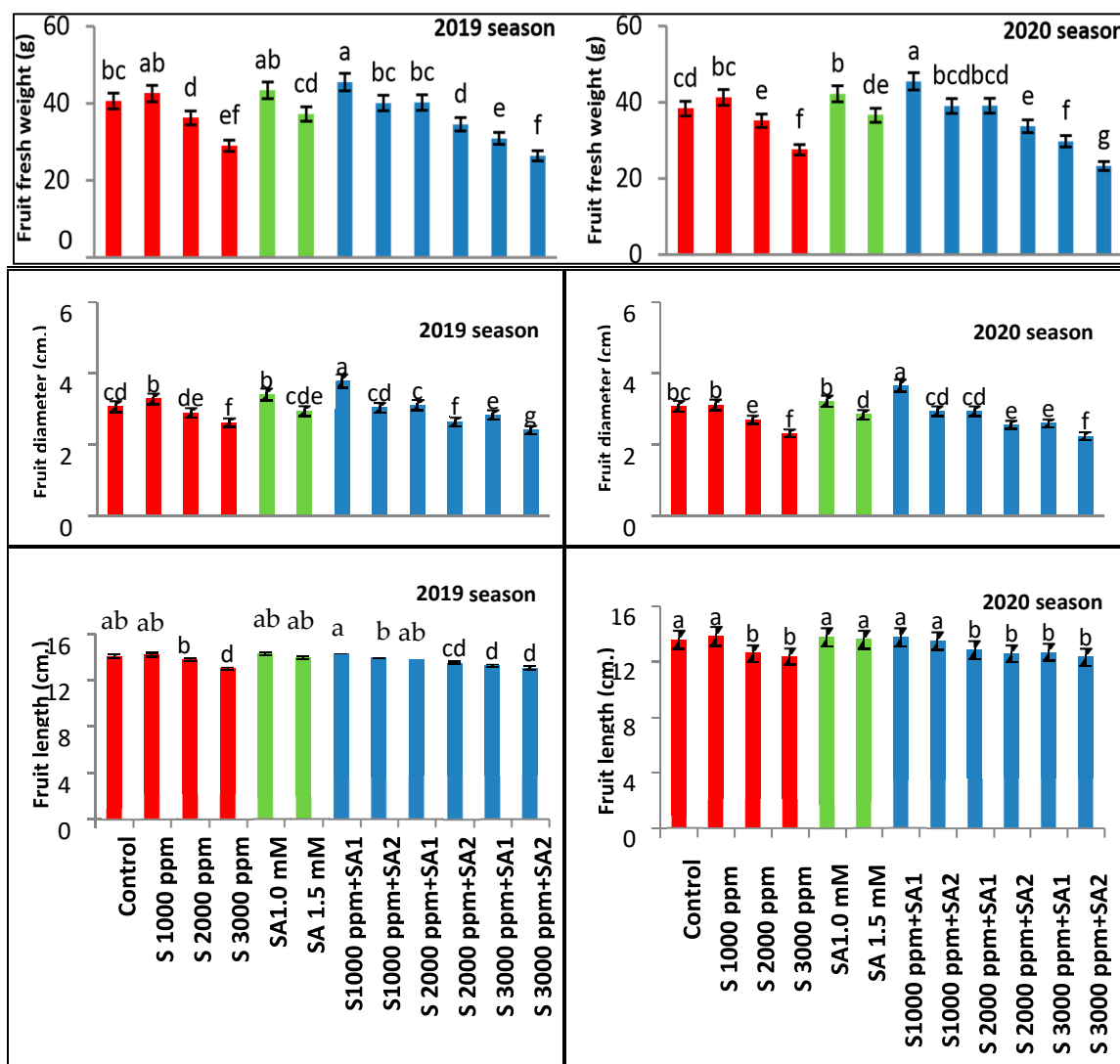


Figure 4. Fruit fresh weight, diameter, and length of eggplant as affected by irrigation with saline water levels, salicylic acid concentrations, and their interaction during the two seasons of 2019 and 2020. Significant differences between treatments are labelled by different alphabets from a to g to indicate significant levels ($p < 0.05$), i.e., a treatment with the letter “a” is significantly different from “b”, and “b” is significantly different from “c”, and so forth. However, if two treatments have a shared letter, there is no significant difference. Red: S level; Green: SA level; Blue: S and SA levels.

3.4. Fruit Chemical Constituents

The results of Figure 5 exhibited that the fruit TSS and ascorbic acid contents increased with every increase in saline water levels despite that the significance level was not achieved for T.S.S content. In dealing with the effect of SA, it was found that the difference did not attain the significance level for T.S.S content, but SA at 1.0 mM concentration accumulated

more ascorbic acid in fruit than the 1.5 mM level. Regarding treatment interactions, applying the SA spray at 1.0 mM concentration recorded the highest increases in TSS and ascorbic acid contents, which received the 3000 ppm saline water.

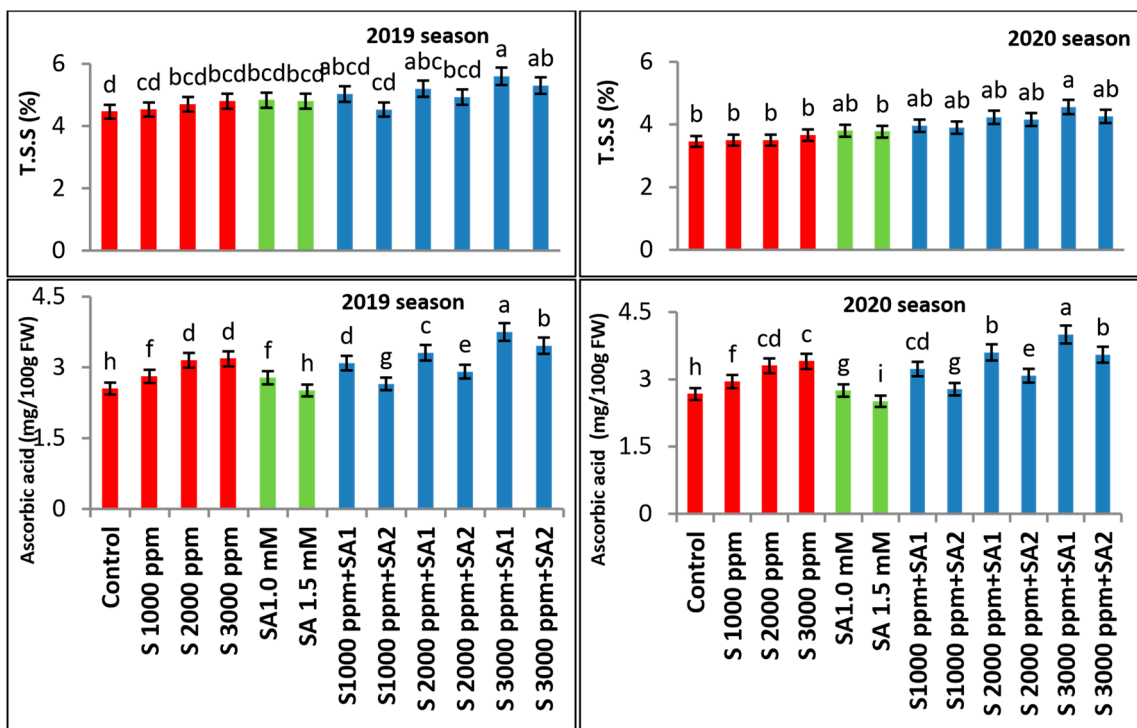


Figure 5. Contents of TSS and ascorbic acid in eggplant fruits as affected by irrigation with saline water levels, salicylic acid concentrations, and their interaction during the two seasons of 2019 and 2020. Significant differences between treatments are labelled by different alphabets from a to i to indicate significant levels ($p < 0.05$), i.e., a treatment with the letter “a” is significantly different from “b”, and “b” is significantly different from “c”, and so forth. However, if two treatments have a shared letter, there is no significant difference. Red: S level; Green: SA level; Blue: S and SA levels.

Data in Figure 6 showed that irrigation with a control level of 300 ppm resulted in high values in fruit N and K contents, but an insignificant increase in P content was recorded between levels of 300 and 1000 ppm. The SA concentration of 1.0 mM increased the contents of N, P, and K more compared to the 1.5 mM concentration. The interaction showed that the SA concentration at 1.0 mM produced the maximum effect in increasing N, P, and K contents under the S level of 1000 ppm.

The data of Figure 7 show that yield per plant did not significantly differ by using the control level and 1000 ppm, then a significant decrease occurred with the high levels. On the other hand, yield per plant did not change with the use of the concentrations of SA in the first season, but the 1.0 mM concentration was more effective compared with 1.5 mM SA in the second season. Also, the maximum yield per plant was harvested from those exposed to the level of 1000 ppm level, combined with a foliar spray of SA at 1.0 mM concentration, while the lowest yield was obtained from plants treated with a 3000 ppm S level and SA foliar spray of 1.5 mM.

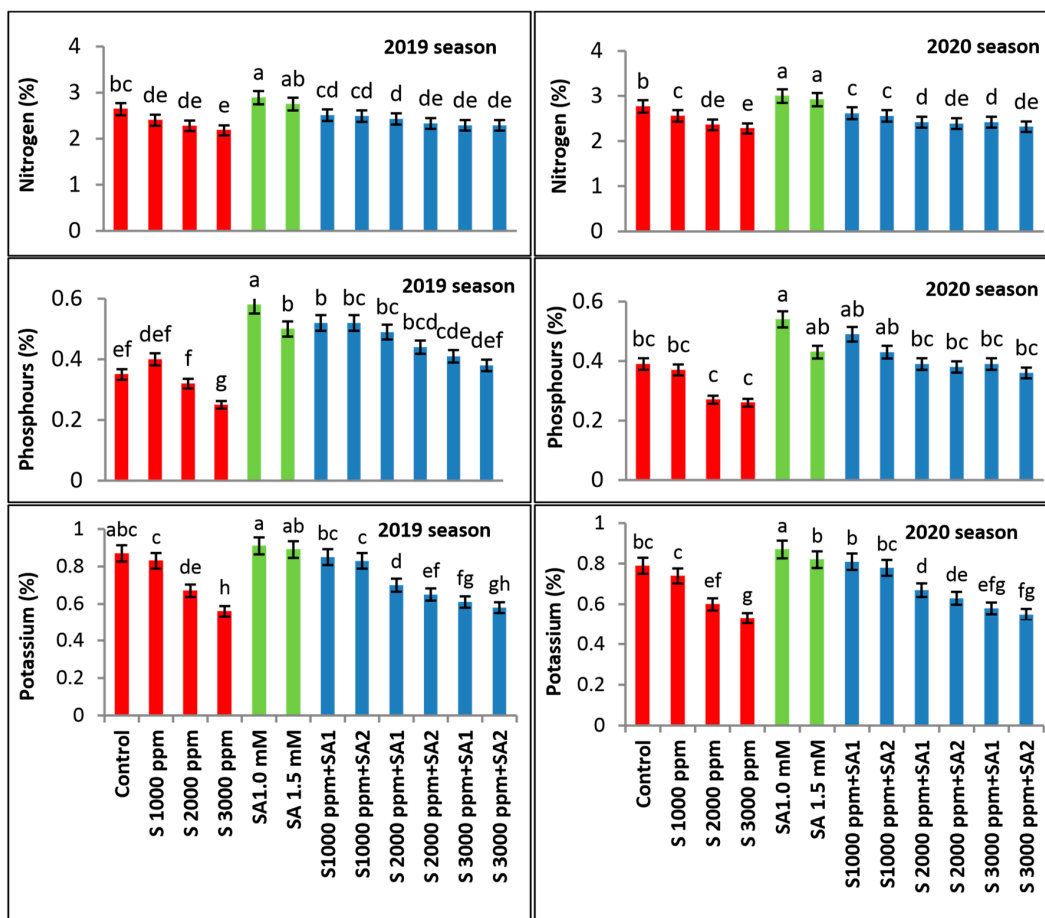


Figure 6. NPK contents of eggplant fruits as affected by irrigation with saline water levels, salicylic acid concentrations, and their interaction during the two seasons of 2019 and 2020. Significant differences between treatments are labelled by different alphabets from a to h to indicate significant levels ($p < 0.05$), i.e., a treatment with the letter “a” is significantly different from “b”, and “b” is significantly different from “c”, and so forth. However, if two treatments have a shared letter, there is no significant difference. Red: S level; Green: SA level; Blue: S and SA levels.

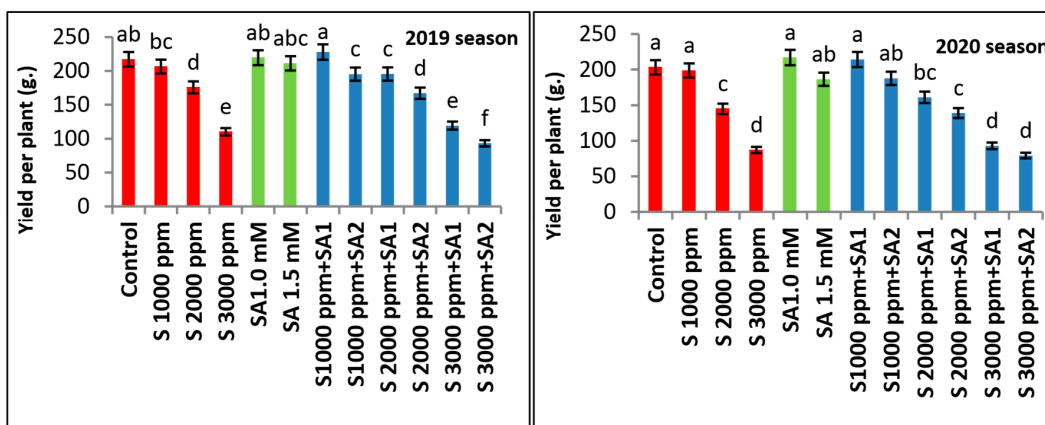


Figure 7. Yield per plant of eggplant fruits as affected by irrigation with saline water levels, salicylic acid concentrations, and their interaction during the two seasons of 2019 and 2020. Significant differences between treatments are labelled by different alphabets from a to f to indicate significant levels ($p < 0.05$), i.e., a treatment with the letter “a” is significantly different from “b”, and “b” is significantly different from “c”, and so forth. However, if two treatments have a shared letter, there is no significant difference. Red: S level; Green: SA level; Blue: S and SA levels.

4. Anatomical Studies

Microscopic measures of certain anatomical characters in transverse sections treated with different levels of saline water, SA, and their interaction in eggplant (*Solanum melongena* L.) plant as compared to the control.

Data in Figure 8 show the effect of salt stress at levels 1000, 2000, and 3000 ppm on the leaf anatomical structure as compared to untreated plants in the control was as follows: the thickness of upper epidermis, lower epidermis, palisade tissue, spongy tissue, and midrib zone were slightly decreased (Figure 9B) compared to the control (Figure 9A). Also, the aforementioned characteristics decreased by -16.6% , -12.5% , -27.8% , -9.9% , and 10.0% , respectively, under the intermediate level of S 2000 ppm compared with the control, while the highest level of S at 3000 ppm led to a sharp decrease in the studied traits by -33.3% , -25.0% , -36.1% , -20.5% , and -21.8% , respectively (Figure 9C), as compared to the control (Figure 9A). In addition, parenchymatous cells under S treatments appeared smaller in size with less intercellular spaces between parenchymatous cells of mesophyll tissue. Moreover, upper and lower epidermal cells were reduced in size and rolled in shape.

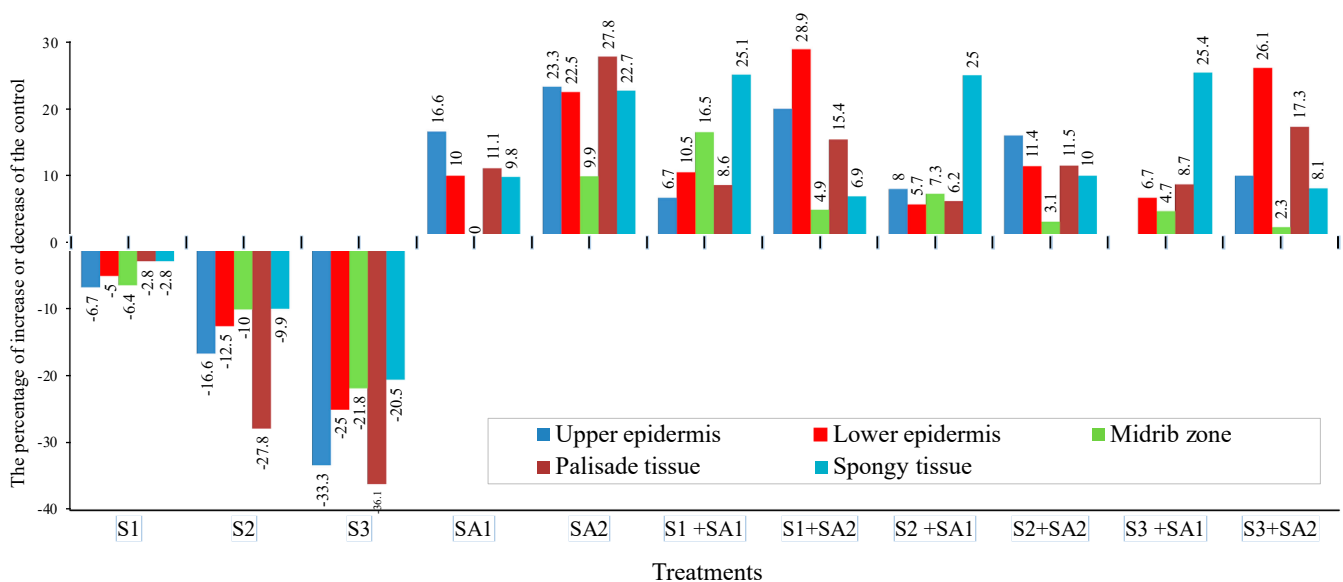


Figure 8. Effect of salinity (S), salicylic acid (SA) concentrations, and the interaction between them on anatomical structure in eggplant leaves.

4.1. SA Effect on Anatomical Structure in Leaves

The presented data revealed that foliar spray of SA at 1 mM concentration increased leaf blade thickness more than the control; such an increase appeared to mainly correspond with the increment that occurred in the thickness of the upper epidermis, lower epidermis, palisade tissue, and spongy tissue by $+16.6\%$, $+10.0\%$, $+11.1\%$, and $+9.8\%$, respectively, more than the control, noting a constant value of midrib zone thickness at 1 mM concentration of SA (Figure 9D) as compared to the control (Figure 9A). Also, the aforementioned characteristics clearly increased by 23.3% , 22.5% , 27.8% , 22.7% , and 9.9% , respectively, over the control at 1.5 Mm concentration of SA (Figure 9E) as compared to the control (Figure 9A). Foliar spray of SA resulted in a clear arrangement of parenchymatous cells in mesophyll tissue as compared to the control, parenchymatous cells of mesophyll tissue are slightly packed with less intercellular spaces between mesophyll tissue parenchymatous cells, and the shape of parenchymatous cells of mesophyll tissue tended to be more or less rounded in shape.

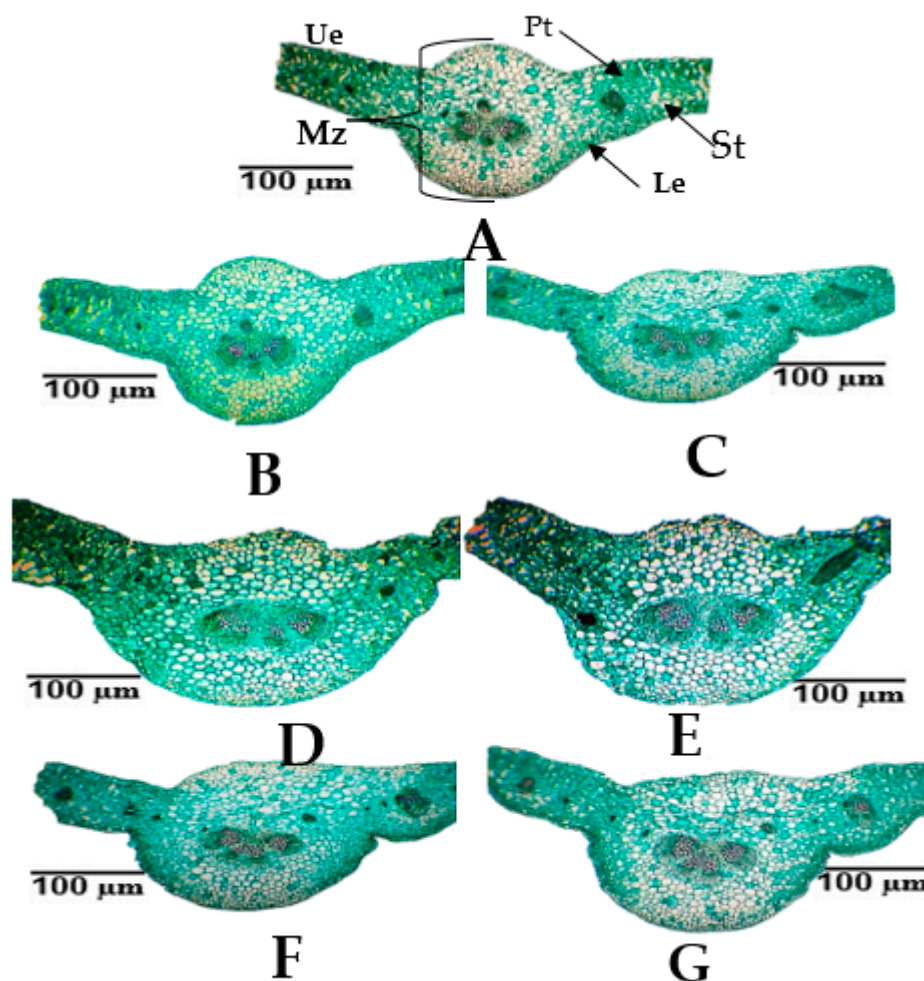


Figure 9. Microphotographs of vertical sections through eggplant leaf blade *Solanum elongena* L. Suma hybrid cultivar as follows: (A) control, (B,C) plant treated with salinity at level 1000 ppm and salinity at level 3000 ppm, (D,E) plant treated with salicylic acid at concentrations (1 and 1.5) mM, and (F,G) plant treated with salicylic acid at concentrations (1 and 1.5) mM under salinity at level 3000 ppm. Abbreviations: Ue: upper epidermis; Mz: midrib zone; Le: lower epidermis; Pt: palisade tissue; and St: spongy tissue.

4.2. The Interaction between S Levels and SA Concentrations on the Anatomical Structure in Leaves of the Studied Eggplant

Data in Figure 8 make it clear that the interaction between a low S level (1000 ppm) and SA foliar spray at concentrations of 1 mM led to improvement in the studied layers, especially the lower epidermal layer and spongy tissue, by +10.5% and +6.9%; also, SA foliar spray at concentrations of 1.5 mM led to a clear increment in most of the studied layers, especially the lower epidermal layer and spongy tissue by +28.9% and +25.1%, respectively, more than plants irrigated with 1000 ppm S level.

The medium S at 2000 ppm level and SA 1 mM and 1.5 mM concentrations noted improvement in most investigated layers, especially only spongy tissue (+10.0% and +25.0%, respectively) more than plants irrigated with a S level of 2000 ppm and the untreated plants.

The highest S level at 3000 ppm and SA 1 mM concentration noted a slight improvement in most investigated layers, but the lower epidermal layer and spongy tissue were clearly increased (+6.7%, +8.1%, respectively), while the treated plants with 1.5 mM SA concentration under S level at 3000 ppm clearly increased (+26.1% and +25.4%, respectively) more than plants that were irrigated with S level at 3000 ppm alone as in Figure 9(C,F,G). In

general, the combined influence of the interaction between S concentration and SA level showed improvement in the studied layers when compared to the control.

5. Discussion

All types of abiotic stresses substantially impact the ability of vegetable crops to produce yield since most of them are sensitive to or only moderately tolerant of abiotic stresses. Salinity stress is recognized as the most harmful stress among all abiotic stresses and greatly lowers the growth and productivity of a number of crops. Both osmotic stress and ion toxicity, which result in secondary oxidative stress, are adversely impacted by S stress, which also severely affects plant growth. According to different research, eggplant has some tolerance to S as it is moderately sensitive to S stress [45–47], which indicates that it can tolerate moderate abiotic stress to some extent. One objective of this research is to evaluate the potential of SA foliar spray in abiotic stress tolerance.

In the present study, all of the vegetative growth parameters of eggplant were negatively impacted by the increasing irrigation with saline water up to 3000 ppm, but leaf number and leaf area did not significantly differ by using the control level and 1000 ppm except during the second season for leaf number. Similarly, the fruit fresh weight, length, and diameter increased in plants irrigated with 1000 ppm S level, but a significant level was not reached in fruit length. After that, a significant decrease was noted in 2000 and 3000 ppm saline levels. According to Kaydan and Okut [48], the inhibitory effect of S on the growth of eggplant might be a result of reduced water uptake and impaired metabolic processes that led to decreased meristematic activity and enlarged cells and increased respiration rate brought on by increased energy needs. Here, it is easy to say that salt stress can slow growth in one of two ways: by destroying growth cells so that they are unable to execute their duties or by reducing their access to vital metabolites [49]. Moreover, higher salt concentrations in irrigation water might induce direct ionic toxicity resulting from accumulating both Na and Cl ions in the cytoplasm, which impacts leaf chlorophyll production [50]. In addition, the reduced light use efficiency from partial stomatal closure and a simultaneous decrease in CO₂ fixation decreased the rate of photosynthesis [51] and the reduction in vegetative growth [50].

In terms of the chemical changes that took place, it is clear that an insignificant effect was observed in the levels of 300 and 1000 ppm saline water in total chlorophyll content; in addition, the highest values in carotenoid content resulted from the control level, while the higher levels of 2000 and 3000 ppm led to a significant proportional decrease in both contents. This decrease might be attributed to the improved activity of chlorophyllase, a chlorophyll-degrading enzyme.

SA might protect the chloroplast pigments from the toxicity probably induced by salinity [52]. The application of SA promotes a pre-adaptive reaction to salt stress, resulting in the encouragement of defensive responses to photosynthetic pigments, therefore preserving membrane straightness in plants, which reinforces the growth of the plant [53]. Hence, SA is a key antioxidant that increases the activity of the enzyme's catalase, peroxidase, and superoxide dismutase to destroy reactive oxygen species, thus enhancing the synthesis of chlorophyll [54,55]. Many researchers also observed a significant improvement in chlorophyll content with the application of SA in other plants [56–58].

In our study, proline and total sugars were found to be the most effective osmolytes in eggplant leaves subject to S up to 3000 ppm as compared to the control. Furthermore, the electrolyte leakage was higher in the leaves with a level of 3000 ppm. These observations showed that eggplants treated with saline water relied largely on total sugars and proline through the osmoregulation process. At the same time, electrolyte leakage shows changes in cell membrane structure under S stress, and it is possible to use it to evaluate the structural and functional damage of cell membranes under stress. Hence, higher values of electrolyte leakage in a saline environment are caused by the phytotoxicity induced by excessive amounts of Na⁺ and Cl⁻ ions in the leaf tissue, and this accumulation causes membrane composition changes, which in turn leads to rupture [59].

Also, it was found that there are significant decreases in relative water content following higher S concentrations up to 3000 ppm. This may be explained by the role of the relative water content of leaves, which is an important parameter determining plant water status. Moreover, the decreased relative water content can result from low water availability in saline conditions resulting from excessive amounts of salts in the rhizosphere [60]. In addition, it also might be a result of an inefficient root system that inhibits the ability of roots for water retrieval after water losses due to the decrease in absorbing surfaces [61].

Regarding the fruit ascorbic acid content, the results indicated that there were increases in these contents with each increase in the level of irrigation saline water. This effect might be connected to the antioxidative role played by ascorbic acid in protecting plants from harmful S effects [62].

Moreover, the insignificant increases in TSS content in the eggplant fruits were noticed with the progressive increase in the saline water levels, and these increases reflected that plants regulate the activity of specific metabolic processes under S stress conditions through polysaccharide degradation into simple sugars, thus increasing the TSS that plays a critical role in osmotic regulation in plant cells [63]. Thus, the osmotic adjustment maintains water relations in plants subject to osmotic stress conditions.

Also, irrigation with the control level resulted in a significant increase in N and K contents in the fruits, but the significance level did not achieve P content at levels of 300 and 1000 ppm. Due to competitive interactions between Na^+ and other ions, the uptake of nutrients during S stress was inhibited, which led to nutrient deficiencies and decreased plant growth [47].

Furthermore, total yield significantly improved by using the saline water level of 1.5 dS/m, then a significant reduction occurred with the high levels. These results may be due to the insignificant effect observed in the levels 300 and 1000 ppm in leaf number, leaf area, and total chlorophyll content of leaves and fruit fresh weight resulting in an increase in the total yield.

The obtained results reflected that with the application of 1.0 mM SA concentration combined with the control level of 300 ppm, the values of plant height were increased. At the same time, the plants irrigated with saline water of 300 and 1000 ppm showed a significant increase in leaf number and leaf area when treated with 1.0 mM SA concentration. In addition, the fruit physical characteristics agreed with the findings on leaf number and leaf area. To discuss this trend in the previous results, it could be observed that SA can be considered as a plant endogenous regulator that induces tolerance to S stresses [4]. SA plays a vital role in physiological metabolic processes, such as photosynthesis, nitrogen assimilation, antioxidant defense mechanism, and the plant–water relationship. In contrast, under lower concentrations, these metabolic processes are enhanced, while in high concentrations, they are inhibited, which interferes with plant growth [64]. Hence, these results might be connected to the alleviation effects of SA against harmful S effects by inducing growth hormones like IAA and cytokinins, which in turn reduce the uptake of toxic ions. This also maintains the integrity of the cell membrane, leading to improvements in cell division and plant growth in general [65]. In addition, the increase in CO_2 assimilation and photosynthetic rate increased mineral uptake in stressed plants [66]. So, the enhancement of vegetative growth parameters due to the foliar spray of 1.0 mM SA (Figure 1) reflects increases in fruit traits.

Our results indicated that the applied SA up to 1.5 mM completely enhanced total chlorophylls and carotenoid content in the stressed plant, especially the treated plants, by the saline level of 300 and 1000 ppm. These enhancing effects may result from the SA's beneficial effect in enhancing reactions that protect photosynthetic pigments [67]. Also, the enhancing effects of SA on total chlorophyll content may be attributed to its stimulatory effects on Rubisco activity [68]. The efficiency of exogenous SA application also relies on several factors, including the plant species, stage of development, method of application, and the applied concentration of SA [65].

Foliar applications of SA increased the contents of osmolytes as proline and total sugars in eggplant leaves, especially under S stress conditions. This result might suggest that applying SA leads to amino acid accumulation under S stress by reducing the levels of dissolved proteins [67]. Moreover, the accumulation of proline results in the reduction in the cytoplasmic osmotic potential, which is contributing to the water homeostasis maintenance in cellular compartments [69]. Hence, proline can be considered an important factor that is involved in the SA-induced protective effect in eggplant under S stress conditions. Furthermore, the ability of SA to reduce oxidative, osmotic, and ionic stress, and increase the concentrations of organic solutes for osmoregulation in plants, can be used to explain its effects [70,71].

At the same time, the exogenous application of 1.0 mM SA was the most effective treatment, which resulted in increases in the levels of ascorbic acid and TSS in fruits of plants that were treated with various S levels of irrigation water. This finding confirms the essential SA function in S stress tolerance due to the SA-induced stimulating activity of antioxidant enzymes that protect plants against oxidative damage [72]. In addition, these findings might be explained by the role of SA in improving the permeability of the membrane, which facilitates nutrient absorption and utilization as well as assimilates transport in environmental stress conditions [73,74]. The results revealed that the control and 1.5 mM of SA treatments obtained the highest and lowest levels of electrolyte leakage, respectively, under S stress. This may be a result of reducing the adverse impact of S on the degradation of cell membrane structure by the application of SA. It is concluded that under high salt concentration, increasing the SA level resulted in reducing the damage percentage compared to the absence of SA treatment [75]. According to a study with eggplant, the structure of leaf cell membrane under S stress and SA incurred more deleterious effects at higher salt levels, and the use of SA lessened the harmful effects compared to the control [76]. Leaf relative water content increase is more pronounced when the plants are sprayed with 1.0 mM SA at the S level of 300 and 1000 ppm, and investigators attributed this to the influence of SA in accumulating levels of compatible osmolytes in plants subject to S conditions, and this can maintain the turgor in the leaf cells [27].

Also, these results assured that the levels of SA at 1.0 and 1.5 mM are suggested to have the most effect in elevating N and K contents under irrigation with a control level of 300 ppm; in addition, 1.0 mM SA for the control level and 1000 ppm saline water were the most significant for P content in both seasons. This enhancement in these elements as affected by SA application is attributed to the SA role in inducing electrolyte leakage, along with boosting the functional physiological processes and metabolisms, such as improved chlorophyll pigment accumulation, and stimulating dry weight production due to higher activity in photosynthesis, thus increasing the translocation and accumulation of macro and microelements in plant organs [77].

The ability of SA to reduce ionic, osmotic, and oxidative stresses, increase the levels of organic solutes towards plant osmoregulation, as well as increase the number of stomata can be used to explain its effects [70,71]. This can maintain the turgor in the leaf cells. When plants are subjected to S stress, suitable osmolytes, including proline, accumulate. SA treatments in pepper culture indicated a rise in the relative water content, which is related to the involvement of SA in this process [27].

6. Anatomical Studies

The information about the effect of SA on the anatomical structure, particularly of eggplant leaves, is unavailable, whereas many researchers confirmed the present findings using SA on other field crop plants, for instance, on barley [78], on *Phaseolus vulgaris* L. [31,79], and on *Lupinus termis* L. [33]. The above-mentioned reports suggest that SA treatment has the ability to increase the thickness of both midvein and lamina in leaves, which is in agreement with the results of this study.

Anatomical characteristics were reduced by saline water levels in eggplant. Research by Swathy Lekshmi and Jayadev [80] observed that saline water led to a decrease in the

thickness of many layers, especially the upper epidermal, cortex, hypodermal, and pith areas. In addition, Arnaout et al. [81] reported that drought stress (at 20% or 40%) caused a reduction in the upper and lower epidermis, a decrease in the midvein thickness of the leaflet blade, a lower xylem row number in each midvein bundle, along with a decrease in the vessel diameter of eggplant. In addition, an S concentration of 6000 ppm resulted in a thickness reduction in the lamina as well as midvein of mature basil leaves. Also, the decline in plant growth as affected by S stress might be attributed to reduced cell division and elongation [82].

The exogenous SA treatment at 100 ppm in cowpea plants that were exposed to high levels of salt stress (6000 ppm) resulted in improved anatomical leaf characteristics [83]. They suggested that the foliar spray of SA might mitigate the harmful effect of saline water stress on cowpea growth, including physiological and anatomical features, along with productivity. In addition, SA can help avoid the harmful impact of saline water stress on the anatomical structure.

7. Conclusions

The results suggest that increasing S levels up to 3000 ppm decreases most of the examined characteristics of plants, leaves, and fruit. However, the treated eggplant plants with SA at 1.0 mM concentration can grow well under S stress, especially at 1000 ppm of agricultural drainage saline water, and can tolerate to a great extent the irrigation up to 3000 ppm by increasing plant and fruit physical characteristics, as well as leaf and fruit chemical characters, especially leaf total chlorophyll, carotenoids, proline, total sugars, relative water contents, fruit TSS., ascorbic acid, nitrogen, phosphorus, and potassium contents. In addition, the use of SA lessened the damaging effect of salt on the breakdown of cell membranes by decreasing the electrolyte leakage percentage in leaves. Based on these results, the use of SA at a concentration of 1.0 mM may lessen the negative impacts of salt on the growth of eggplant, resulting in an increase in overall yield per plant. The anatomical structure of eggplant leaves was investigated, and positive variations in the anatomical structure of leaf blades of mature foliage leaves were detected in the stressed and SA-treated plants.

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