



Influence of Salicylic Acid Concentrations and Spraying Times on Growth and Physiology of Eggplant

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ABSTRACT

Salicylic acid (SA) is a key signaling molecule that regulates plant growth, flowering, and responses to abiotic and biotic stresses. This study evaluated the effects of different SA concentrations (0, 0.5, and 1.0 mM) and application frequencies (once and twice) on the growth, pigment composition, and yield of eggplant (*Solanum melongena* L.) under field conditions during the 2023–2024 growing season. Eggplant seeds were sown in a cocopeat and perlite growing medium at a ratio of 3:1 and the seedlings were watered daily in greenhouse. Foliar spraying was done in two stages before transplanting (four-leaf stage) in greenhouse and before flowering in farm. After the first foliar spray, the eggplant seeds were transferred to the field and the plants were irrigated every three days throughout the growth period. A factorial experiment arranged in a completely randomized design revealed significant SA effects on most measured traits, with 0.5 mM producing the most favorable outcomes. Compared with the control, this treatment increased relative water content by 11%, reduced electrolyte leakage by 30–35%, and enhanced SPAD values, total chlorophyll, and carotenoid contents by up to 120%. Plant height, fruit number, and total yield rose by 38.1%, 85.7%, and 88.6%, respectively. Two applications further improved growth and yield compared to a single spray, indicating that repeated applications amplify the physiological benefits of SA. In contrast, 1.0 mM SA provided no additional improvement, suggesting a threshold beyond which inhibitory effects are not observed. The observed enhancements were associated with improved water balance, enhanced photosynthetic efficiency, and increased membrane integrity. Overall, applying 0.5 mM SA twice during both the vegetative and reproductive stages proved to be an optimal, low-cost, and environmentally safe strategy for enhancing eggplant growth and productivity. Future studies should elucidate SA's molecular mechanisms and interactions with other biostimulants and stresses to optimize its use across crops and production systems.

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1. Introduction

Vegetables play a crucial role in global food and nutritional security, providing essential vitamins and minerals at affordable costs (Gruda et al., 2024, Gruda et al., 2025a, Gruda et al., 2025b). Their production not only ensures dietary quality for consumers but also creates income opportunities, supports rural employment, and contributes to poverty reduction (Schreinemachers et al., 2018, Al Salmi et al., 2020). Among them, eggplant (*Solanum melongena* L.) is an economically significant crop cultivated in both open fields and greenhouses to enhance farmers' livelihoods (Al-Salmi and Nadaf, 2025). It thrives in warm climates, mainly in tropical and subtropical regions, and is valued for its nutritional benefits due to its low caloric content and richness in vitamins, minerals, and bioactive compounds (Taher et al., 2017, Ienciu et al., 2022). According to FAO (2024), global eggplant production in 2022 reached 59.31 million metric tons, representing a 1% increase from the previous year, with China (38.3 million metric tons) and India (12.8 million metric tons) being the leading producers, followed by Egypt and Turkey (Solberg et al., 2022).

Salicylic acid (SA) is a naturally occurring phenolic compound present throughout the plant kingdom, though its basal concentration varies widely among species (Janda et al., 2020). It functions as a growth regulator and signaling molecule involved in modulating plant development and stress responses under diverse environmental conditions (Jahan et al., 2019, Dasgan et al., 2024, İkiz et al., 2024, İkiz et al., 2025). As a photoprotectant, SA has been widely applied to enhance crop productivity (Mady et al., 2023). Exogenous SA influences numerous physiological and biochemical processes, including seed germination, membrane stability, photosynthesis, proline accumulation, and biomass production (Muthulakshmi and Lingakumar, 2017, Tohma and Esitken, 2011, Faghih et al., 2019, Misra and Saxena, 2009, Wang et al., 2022). Positive effects have been reported across several crops, including pepper (*Capsicum annuum*), strawberry (*Fragaria × ananassa*), common bean (*Phaseolus vulgaris*), pumpkin, and tomato (Karami Chame et al., 2016, Roshdy et al., 2021, Bin-Jumah et al., 2021, Rafique et al., 2011, Mimouni et al., 2016).

It exerts dose-dependent effects, where lower concentrations stimulate beneficial responses and higher levels may inhibit plant functions. Beyond growth promotion, SA modulates root system architecture (Khan et al., 2014a), photosynthetic efficiency (Hussain et al., 2021), redox homeostasis (Singh and Gautam, 2013), and flowering (Wang and Li, 2008). SA is a versatile regulator with multifaceted roles in the growth and metabolism processes in plants. Researchers found that the effect of foliar application of salicylic acid increased the Plant height, leaf number, leaf area, number of main stems, and foliage fresh weight per plant, chlorophyll a, chlorophyll b, and carotenoids compared to untreated potato plants, except for electrolyte leakage (Metwaly and El-Shatoury, 2017). Additionally, in *Salvia officinalis* L., plants treated with SA at 0.5 mM showed increases in growth of 44%, 56%, and 79% for the stem, root parts, and chlorophyll content, respectively, compared to the control (Es-Sbihi et al., 2020). An increase in plant fresh matter and chlorophyll b was observed in white and red Swiss chard under foliar application of SA, but chlorophyll a was decreased (Piñero et al., 2025). In Poinsettia (*Euphorbia pulcherrima* Willd.), the high levels of SA had no significant influence on the plant height, leaf number, or biomass of stems, leaves, and roots (Esposito et al., 2025). Salicylic acid (SA) application enhanced vegetative growth in basil and marjoram by increasing plant height, the number of branches, spikes, and leaves per plant, as well as leaf area and both fresh and dry biomass (Gharib and Abed, 2006). Furthermore, the application of 0.5 mM salicylic acid (SA) enhanced the dry weights of roots, shoots, and nodules, as well as the number of flowers and pods in chickpea (*Cicer arietinum*) (Kaur et al., 2022). Similar stimulatory effects of SA have been reported in wheat and mung bean, where it significantly improved photosynthetic activity and overall plant growth (Khan et al., 2013, Khan et al., 2014b). Additionally, Salicylic acid (SA) is among the most extensively studied elicitors (Abdolmaleki et al., 2015). SA is a phenolic compound, synthesized from t-cinnamic acid, which is widely present in plants. SA is a multifunctional molecule that plays roles as both an elicitor and a growth promoter in plants. As an elicitor, it is involved in activating natural defenses in plants (Chaudhary et al., 2015). A study on tomato plants investigated the effects of various salicylic acid (SA) concentrations applied as foliar sprays four times at 10-day intervals, starting two weeks after planting. The findings revealed that treatment with 0.5 mM SA significantly enhanced several parameters, including fruit quality, plant growth, leaf chlorophyll levels, early yield, and overall productivity (Yildirim et al., 2008). Additionally, researchers found that the foliar application of salicylic acid significantly promoted nearly all growth metrics compared to the control in Pea (*Pisum sativum* L.) (Anwar et al., 2025). Researchers have stated that Low concentrations of SA improve the yield of cucumbers, while high concentrations decrease it (Preciado-Rangel et al., 2019). In a study with the Spraying of 1 mM SA (1 mM) was done once (SA1), twice (SA2), three times (SA3), or four times (SA4) during the vegetation period with 7 d intervals in strawberry results showed that spraying more frequently in strawberries increased the early yield and total yield of and content of photosynthetic pigments (Karlidag et al., 2009).

Although numerous studies have documented the positive effects of SA in crops such as tomato, cucumber, pepper, and strawberry, evidence in eggplant remains comparatively limited and fragmented. Most available studies have focused on stress conditions or single-stage applications, with less attention given to repeated foliar treatments under normal field environments. Consequently, the interactive effects of SA concentration and application frequency on eggplant physiology and yield formation are not yet fully understood. Understanding

these relationships is essential for developing precise, cost-effective, and sustainable management practices. Therefore, the present study was designed to evaluate how different concentrations and spraying frequencies of SA influence morpho-physiological traits, photosynthetic pigments, water relations, and fruit productivity in eggplant. By integrating agronomic, physiological, and multivariate analyses, this research aims to establish evidence-based recommendations for optimizing SA use in commercial eggplant cultivation.

2. Material and Methods

2.1. Experimental Design and Location

The effects of different concentrations of salicylic acid (SA) (Sigma-Aldrich, United States) 0 mM (control), 0.5 mM, and 1.0 mM and two application times (once and twice) on the growth and physiological responses of eggplant were evaluated using a factorial experiment arranged in a completely randomized design with four replicates. The first foliar application of salicylic acid was conducted before transplanting, at the 2- to 4-leaf stage in the greenhouse. In contrast, the second application was performed during the pre-flowering stage of eggplant under field conditions. The experiment was conducted in the research farm of the Faculty of Agriculture at Shahid Bahonar University of Kerman during the 2023-2024 growing season.

2.2. Experimental setup

Eggplant seeds (Arettusa RZ F1 variety), sourced from Rijk Zwaan Co. to Sepahan Rooyesh Isfahan, Iran. The seeds germinated 7–10 days after sowing under greenhouse conditions. This period was observed in the cocopeat–perlite (3:1) growing medium with daily irrigation, which provided favorable moisture and aeration for uniform emergence. Foliar spraying was done in two stages before transplanting (four-leaf stage) in greenhouse and before flowering in farm. After the first foliar spray, the eggplant seeds were transferred to the field and the plants were irrigated every three days throughout the growth period. Fertility management was carried out in two stages: first at planting and then 45 days later, using a combination of rotted animal manure, urea, potassium sulfate, and phosphoric acid. Weeding was performed as needed to prevent competition with the crop.

2.3. Plant Water Relations

This section focused on leaf relative water content (RWC). Several 6-mm diameter leaf discs were punched from fresh leaves, and their fresh weight was measured immediately to calculate RWC. The discs were floated in distilled water in Petri dishes for 6 hours to allow them to reach full turgidity. After soaking, the discs were blotted dry and weighed again to determine their turgid weight. Finally, the discs were oven-dried at 70 °C for 48 hours to determine their dry weight. RWC was calculated using the following equation (Trueba et al., 2019):

$$\text{Relative Water Content (\%)} = 100 \times ((\text{Fresh Mass} - \text{Dry Mass}) / (\text{Turgid Mass} - \text{Dry Mass})).$$

2.4. Ion Leakage

Electrolyte leakage was assessed by incubating leaf samples in distilled water at 40°C (EC₁) and 100°C (EC₂) (Dionisio-Sese and Tobita, 1998). The leakage percentage was calculated as:

$$EL = \left(\frac{EC_2}{EC_1} \right) \times 100$$

2.5. Greenness index (SPAD)

To assess chlorophyll content (SPAD index), four leaves from the 3rd and 6th nodes of each plant were selected and measured using a SPAD-502 Chlorophyll Meter (Konica Minolta, Japan).

2.6. Photosynthetic Pigments

To quantify chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, 0.25 g of fresh leaf tissue was finely ground in a porcelain mortar with 10 mL of 80% acetone until a homogeneous slurry was obtained. The resulting mixture was transferred into 20 mL Falcon tubes and centrifuged at 3500 rpm for 10 minutes. The absorbance of the supernatant was measured at 480, 510, 645, 652, and 663 nm using a T80 UV/VIS spectrophotometer (PG Instruments Ltd, UK). The concentrations of pigments were calculated using the following equations (Li et al., 2020)

$$\text{Chlorophyll a (mg g}^{-1}\text{FM)} = [(12.7 \times (A_{663}) - (2.69 \times (A_{645}))] \times (V/1000 \times W)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{FM)} = [(22.9 \times (A_{645}) - (4.68 \times (A_{663}))] \times (V/1000 \times W)$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{FM)} = ((A_{652} \times 1000)/34.5) \times (V/1000 \times W)$$

$$\text{Carotenoids (mg g}^{-1}\text{FM)} = [(7.6 \times (A_{480}) - (1.49 \times A_{510}))] \times (V/1000 \times W)$$

Where: A = absorbance at the specified wavelength, V = volume of acetone used (10 mL), W = fresh sample mass (0.25 g).

2.7. Vegetative Parameters

The vegetative characteristics assessed in this study were plant height, stem diameter, days to flowering, number of fruits, fruit length and width, and fruit yield. Plant height was measured using a ruler, while stem diameter and fruit length and width were determined using a digital caliper. The number of fruits and their yields were continuously counted and weighed from the beginning until the time of harvesting the plants. Ultimately, the yield was determined in relation to the cultivated area.

2.8. Statistical Analysis

The experiment followed a completely randomized design (CRD) with a three-factor arrangement and three replications. Data collected were subjected to statistical analysis using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). A two-way analysis of variance (ANOVA) was conducted to evaluate the effects of different treatments and their interactions. Duncan's Multiple Range Test (DMRT) was applied as a post-hoc analysis to compare the mean values across treatments. Statistical significance was accepted at the level of $p \leq 0.05$.

3. Results

3.1. Electrolyte Leakage

Electrolyte leakage showed a significant response to SA concentrations but not to AR or their interaction (Table 1). Compared to the control (SA0), SA1 (0.5 mM) decreased leakage by 35% to 39%, and SA2 (1 mM) by 30% to 42%. Application frequency had no significant effect, with AR1 at 47.6% and AR2 at 46.3%. The non-significant interaction indicates that SA reductions were consistent across frequencies (Figure 1A).

Table 1. The effect of varying salicylic acid concentrations and spraying intervals on the physiological traits of Eggplant

Source of variation	df	Mean Square						
		Electrolyte leakage	Relative water content	SPAD	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid
Salicylic acid (SA)	2	774**	134**	518**	6.5ns	0.24ns	13.5**	5.18**
Application Repetition (AR)	1	8ns	0.88ns	162ns	2ns	8.82**	0.5ns	0.80**
SA*A	2	2ns	0.72ns	156ns	0.5ns	3.34*	6.5*	0.23**
Experimental error	12	2.08	1.56	7.34	1.87	0.81	1.22	0.17
Coefficient variance (CV)	-	4.42	1.97	14.79	11.9	11.59	5.44	5.01

**, * and ns indicate significance at the 1% and 5% levels, respectively, and non-significance at the 0.05 level according to Duncan's range test. The same letters in each column indicate no significant difference according to the Duncan Test.

3.2. Relative Water Content

Relative water content responded significantly to SA concentrations but not to AR or their interaction (Table 1). Relative to the control (SA0) at 73.6%, SA1 (0.5 mM) increased content by 11.5% to 82.1%, and SA2 (1 mM) by 10.7% to 81.5%. Spraying frequency showed no significant difference, with AR1 at 79.3% and AR2 at 78.8%. With a non-significant interaction, SA effects were uniform regardless of AR (Figure 1B).

3.3. SPAD and chlorophyll content

The chlorophyll content index (SPAD) exhibited a significant response to SA concentrations, but not to AR or their interaction (Table 1). Compared to the control (SA0), SA1 (0.5 mM) increased SPAD by 42.9% to 60, while SA2 (1 mM) increased it by 11.9% to 47. Application frequency had no significant impact, with AR1 at 46.6 and AR2 at 52.6. The non-significant interaction suggests that SA influences were independent of spraying frequency (Figure 2).

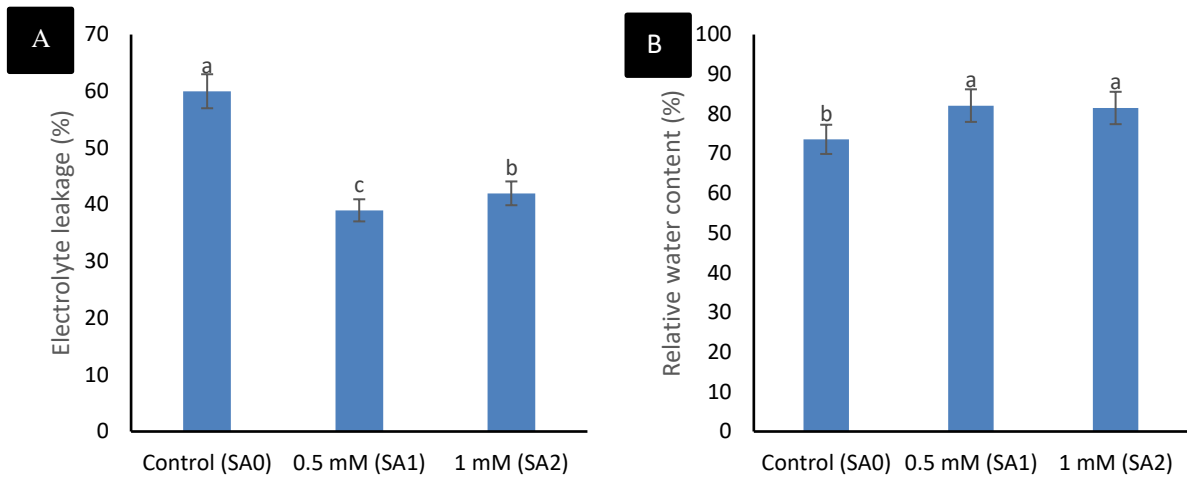


Figure 1. The effect of salicylic acid concentration on electrolyte leakage (A) and relative water content (B)

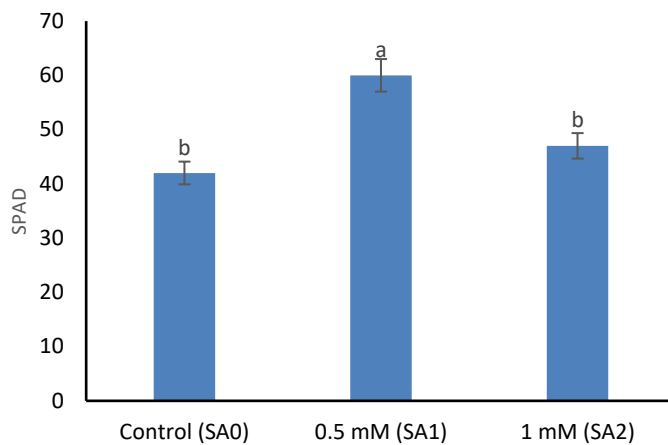


Figure 2. The effect of salicylic acid concentration on SPAD

Chlorophyll a showed no significant response to SA concentrations, AR, or their interaction (Table 1). Chlorophyll b responded significantly to AR and the SA \times AR interaction but not to SA alone (Table 1). Overall SA means were 6.85 mg/g in SA0, 7 mg/g in SA1, and 7.25 mg/g in SA2, showing no main SA effect. However, AR2 decreased levels by 18.1% compared to AR1, from 7.73 mg/g to 6.33 mg/g. For interactions, compared to SA0 at 7 mg/g, SA1 \times AR1 increased by 14.3% to 8 mg/g, SA1 \times AR2 decreased by 14.3% to 6 mg/g, SA2 \times AR1 increased by 21.4% to 8.5 mg/g, and SA2 \times AR2 decreased by 14.3% to 6 mg/g (Figure 3).

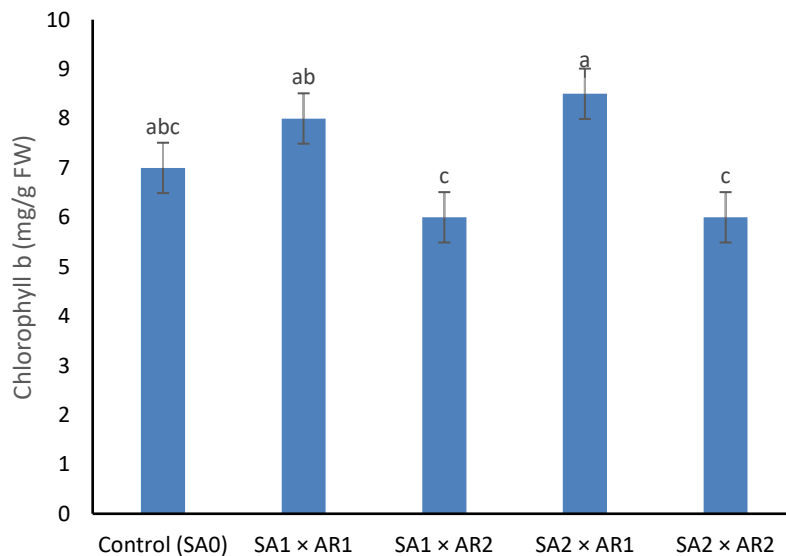


Figure 3. The effect of varying salicylic acid concentrations and spraying intervals on chlorophyll b

Total chlorophyll showed significant responses to SA concentrations and the SA \times AR interaction but not to AR alone (Table 1). Compared to the control (SA0) at 21 mg/g, SA1 (0.5 mM) increased total chlorophyll by 7.1% to 22.5 mg/g, and SA2 (1 mM) by 14.3% to 24 mg/g. Application frequency had no main effect, with AR1 at 22.66 mg/g and AR2 at 22.33 mg/g. In interactions, SA1 \times AR1 increased by 9.5% over SA0 (to 23 mg/g), SA1 \times AR2 by 4.8% (to 22 mg/g), SA2 \times AR1 by 19% (to 25 mg/g), and SA2 \times AR2 by 9.5% (to 23 mg/g), using SA0 at 22 mg/g as the baseline (Figure 4).

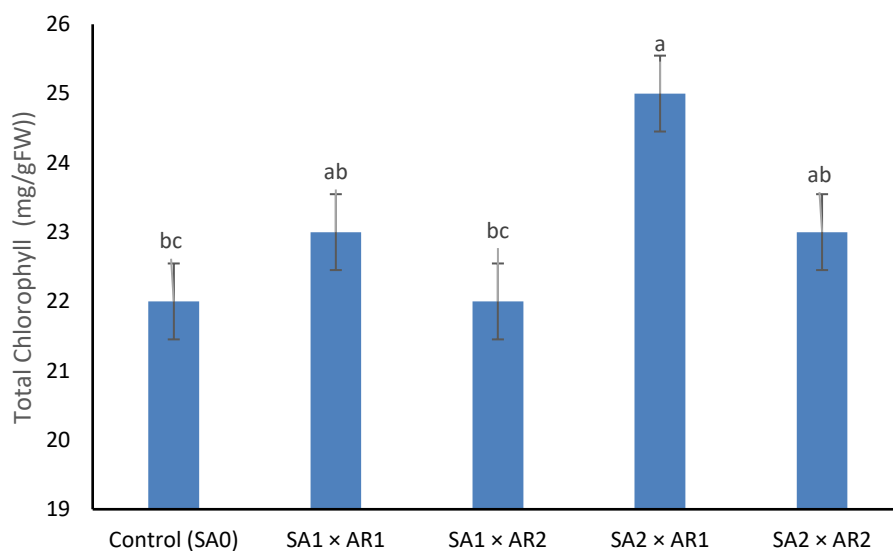


Figure 4. The effect of varying salicylic acid concentrations and spraying intervals on total chlorophyll

3.4. Carotenoid

Carotenoid content exhibited significant responses to SA concentrations, AR, and their interaction (Table 1). Relative to the control (SA0) at 2.6 mg/g, SA1 (0.5 mM) increased carotenoids by 71.2% to 4.45 mg/g, while SA2 (1 mM) increased them by 41.5% to 3.68 mg/g. Twice spraying (AR2) raised levels by 12.5% over AR1, from 3.36 mg/g to 3.78 mg/g. For interactions, SA1 \times AR1 increased by 36% over SA0 (to 3.4 mg/g), SA1 \times AR2 by 120% (to 5.5 mg/g), SA2 \times AR1 by 60% (to 4 mg/g), and SA2 \times AR2 by 34.4% (to 3.36 mg/g), with SA0 at 2.5 mg/g as the reference (Figure 5).

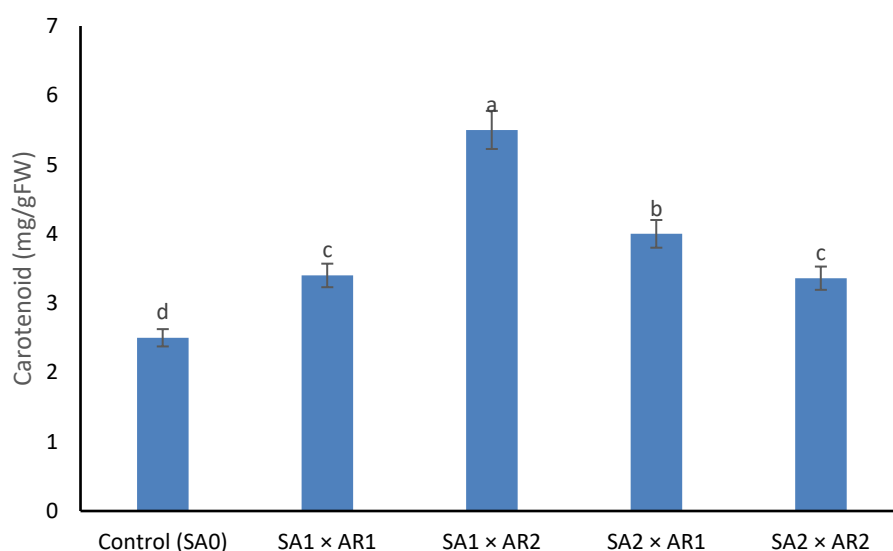


Figure 5. The effect of varying salicylic acid concentrations and spraying intervals on carotenoid

3.5. Plant Height

Plant height showed significant responses to salicylic acid (SA) concentrations, application repetition (AR), and their interaction (Table 2). Compared to the control (SA0) with a height of 77.5 cm, SA1 (0.5 mM) increased height by 30.3% to 101 cm, while SA2 (1 mM) increased it by 16.1% to 90 cm. For application frequency, twice

spraying (AR2) resulted in a 6.6% increase over once spraying (AR1), from 86.6 cm to 92.3 cm. In interactions, SA1 × AR1 increased height by 22.6% over SA0 (to 95 cm), SA1 × AR2 by 38.1% (to 107 cm), SA2 × AR1 by 13.5% (to 88 cm), and SA2 × AR2 by 18.7% (to 92 cm), with SA0 at 78 cm serving as the baseline (Figure 6).

Table 2. The effect of varying salicylic acid concentrations and spraying intervals on growth traits of Eggplant

Source of variation	df	Mean Square						
		Plant Height	Stem Diameter	Days to Flowering	Number of Fruits	Fruit Length	Fruit Width	Fruit Yield
Salicylic acid (SA)	2	829.5**	30.5**	171.5**	32.5**	1178**	105.5*	10.46**
Application Repetition (AR)	1	144.5**	4.5ns	4.5ns	72**	12.5ns	16.05ns	1.62**
SA*A	2	48.5**	4.5ns	10.5ns	19.5**	6.5ns	1.05ns	0.42**
Experimental error	12	2.38	1.82	1.87	1.22	7.79	3.92	0.17
Coefficient variance (CV)	-	2.65	10.63	3.12	4.22	5.15	6.75	3.51

**, * and ns indicate significance at the 1% and 5% levels, respectively, and non-significance at the 0.05 level according to Duncan's range test. The same letters in each column indicate no significant difference according to the Duncan Test.

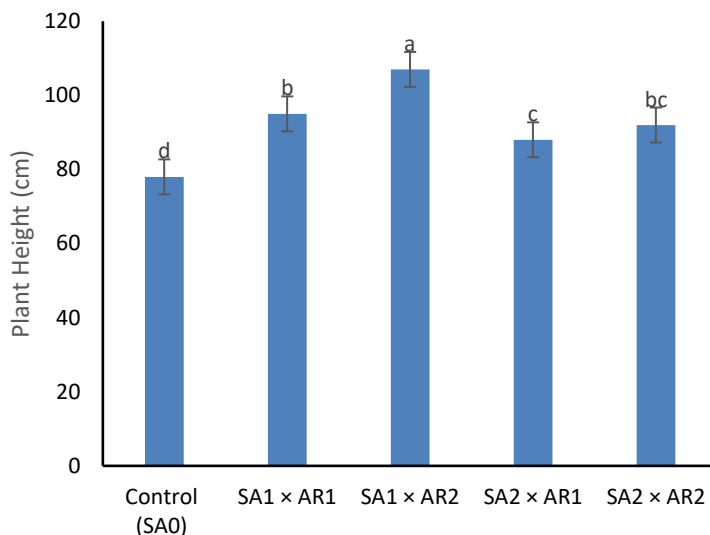


Figure 6. The effect of varying salicylic acid concentrations and spraying intervals on plant height

3.6. Stem Diameter

Stem diameter exhibited a significant response to SA concentrations but not to AR or their interaction (Table 2). Relative to the control (SA0) at 15 mm, SA1 (0.5 mM) increased the diameter by 30% to 19.5 mm, whereas SA2 (1 mM) increased it by 13.3% to 17 mm. Application frequency showed no significant difference, with AR1 at 16.6 mm and AR2 at 17.6 mm. Since the interaction was non-significant, the combined effects were not distinctly separable beyond the main SA effects (Figure 7A).

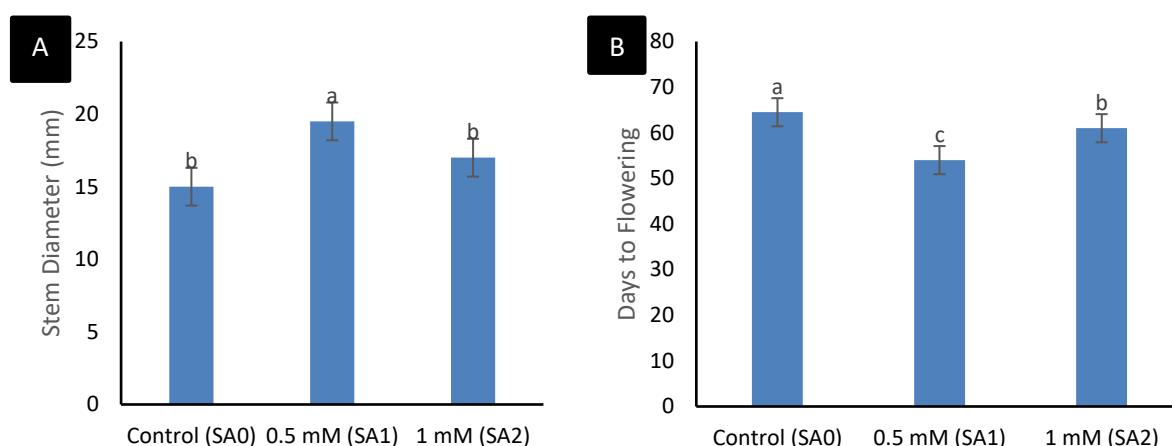


Figure 7. The effect of salicylic acid concentration on stem diameter (A) and days to flowering (B)

3.7. Days to Flowering

Days to flowering responded significantly to SA concentrations but not to AR or their interaction (Table 2). Compared to the control (SA0) at 64.5 days, SA1 (0.5 mM) decreased days by 16.3% to 54 days, and SA2 (1 mM) decreased them by 5.4% to 61 days. Application frequency had no significant impact, with AR1 at 59.3 days and AR2 at 60.3 days. The non-significant interaction indicates that SA effects were consistent across spraying frequencies (Figure 7B).

3.8. Number of Fruits

The number of fruits showed significant responses to SA concentrations, AR, and their interaction (Table 2). Against the control (SA0) at 21 fruits, SA1 (0.5 mM) increased the number by 69% to 35.5, and SA2 (1 mM) by 45.2% to 30.5. Twice spraying (AR2) increased fruits by 14.8% over AR1, from 27 to 31. For interactions, SA1 × AR1 increased fruits by 52.4% over SA0 (to 32), SA1 × AR2 by 85.7% (to 39), SA2 × AR1 by 33.3% (to 28), and SA2 × AR2 by 57.1% (to 33), using SA0 at 21 as the baseline (Figure 8A).

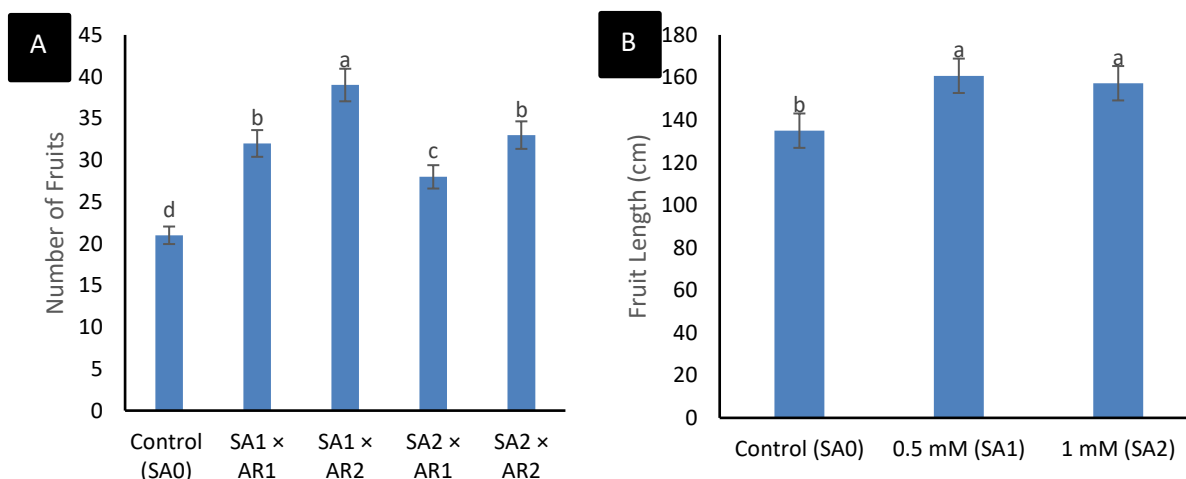


Figure 8. The effect of varying salicylic acid concentrations and spraying intervals on number of fruits (A) and salicylic Acid concentrations on fruit length (B)

3.9. Fruit Length

Fruit length was significantly affected by SA concentrations but not by AR or their interaction (Table 2). Compared to the control (SA0) at 135 mm, SA1 (0.5 mM) increased length by 19.1% to 160.8 mm, and SA2 (1 mM) by 16.5% to 157.3 mm. Application frequency showed no significant difference, with AR1 at 150.2 mm and AR2 at 151.8 mm. The non-significant interaction suggests uniform SA effects regardless of spraying frequency (Figure 8B).

3.10. Fruit Width

Fruit width responded significantly to SA concentrations but not to AR or their interaction (Table 2). Relative to the control (SA0) at 55.5 mm, SA1 (0.5 mM) increased the width by 13.5% to 63 mm, while SA2 (1 mM)

increased it by 0.9% to 56 mm. Application frequency had no significant effect, with AR1 at 57.2 mm and AR2 at 59.1 mm. With a non-significant interaction, SA influences were independent of AR (Figure 9A).

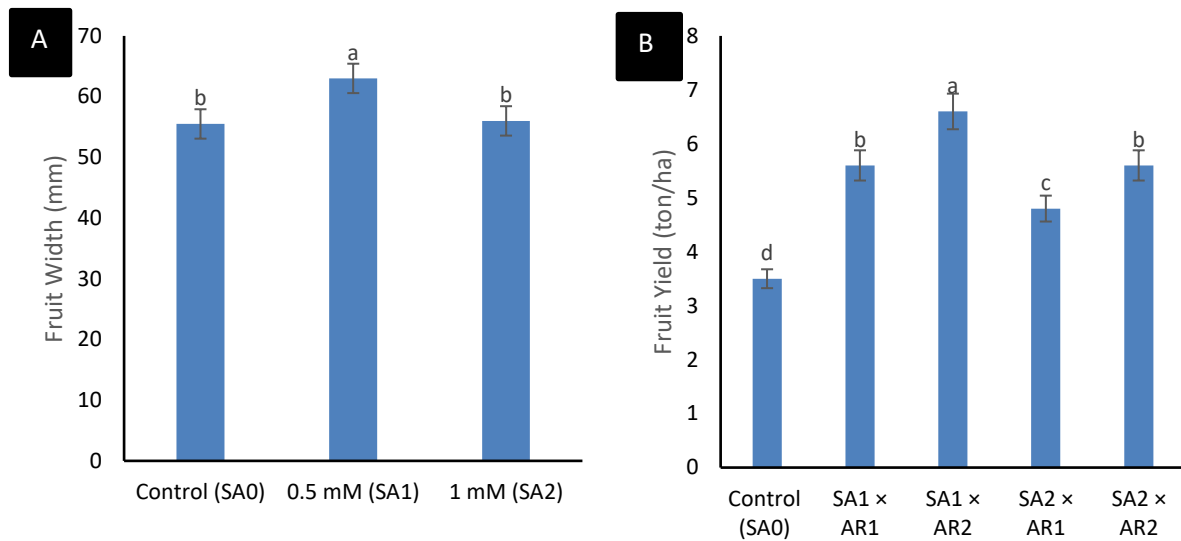


Figure 9. The Effect of salicylic acid concentrations on fruit width (A) and the effect of varying salicylic acid concentrations and spraying intervals on fruit yield (B)

3.11. Fruit Yield

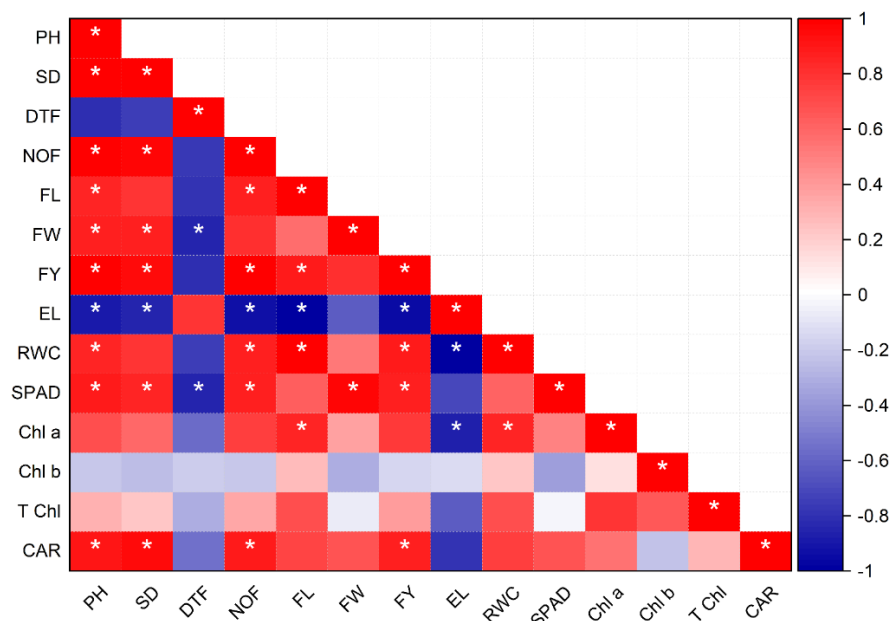
Fruit yield exhibited significant responses to SA concentrations, AR, and their interaction (Table 2). Compared to the control (SA0) at 3.5 kg, SA1 (0.5 mM) increased yield by 74.3% to 6.1 kg, and SA2 (1 mM) by 48.6% to 5.2 kg. Twice spraying (AR2) boosted yield by 13.7% over AR1, from 4.6 kg to 5.23 kg. In interactions, SA1 × AR1 increased yield by 60% over SA0 (to 5.6 kg), SA1 × AR2 by 88.6% (to 6.6 kg), SA2 × AR1 by 37.1% (to 4.8 kg), and SA2 × AR2 by 60% (to 5.6 kg), with SA0 at 3.5 kg as the reference (Figure 9B).

3.12. Correlation Analysis

The correlation matrix provided more profound insights into the relationships among traits. Strong positive correlations were observed between vegetative and reproductive growth parameters such as plant height, stem diameter, number of fruits, fruit length, fruit width, and fruit yield, suggesting that enhanced vegetative growth contributed to improved yield. Physiological attributes, including chlorophyll pigments (Chl a, Chl b, total chlorophyll), carotenoids, SPAD values, and RWC, also showed strong interrelationships, reflecting their coordinated role in photosynthesis and water balance. Fruit yield exhibited robust correlations with the number of fruits, fruit size, SPAD, and RWC, confirming that improved physiological status directly supports productivity. On the other hand, electrolyte leakage showed significant negative correlations with nearly all growth and physiological traits, indicating that membrane instability reduced performance. Similarly, DTF displayed negative correlations with growth and yield, suggesting that delayed flowering is associated with weaker plant productivity (Figure 10).

3.13. PCA Biplot Analysis

The Principal Component Analysis (PCA) biplot illustrates the relationships among salicylic acid (SA) treatments and the evaluated physiological and growth traits of eggplant. The first two principal components (PC1 and PC2) explain 66.78% and 21.09% of the total variance, respectively. Treatments with 0.5 mM SA (both single and double applications) are grouped on the positive side of PC1, showing strong associations with most growth and physiological variables such as relative water content (RWC), chlorophyll a (Chl a), fruit length (FL), fruit width (FW), number of fruits (NOF), fruit yield (FY), SPAD, and plant height (PH). These correlations indicate that moderate SA concentration (0.5 mM) markedly enhanced plant vigor, photosynthetic pigments, and yield parameters. In contrast, the control (SA0) treatment is located on the negative side of PC1, correlating with higher electrolyte leakage (EL) and delayed flowering (DTF), which reflect stress and reduced physiological efficiency. Treatments with 1 mM SA showed intermediate behavior, where a single application correlated more with chlorophyll b (Chl b) and total chlorophyll (T Chl), while repeated application moderately improved water status and pigment content (Figure 11).



* p<=0.05

Figure 10. Correlation analysis of salicylic acid concentrations and spraying times on growth and physiology of Eggplant. The variables are: Plant Height (PH), Stem Diameter (SD), Days to Flowering (DTF), Number of Fruits (NOF), Fruit Length (FL), Fruit Width (FW), Fruit Yield (FY), Electrolyte leakage (EL), Relative water content (RWC), SPAD, Chlorophyll a (Chl a), Chlorophyll b (Chl b), Total Chlorophyll (T Chl) and Carotenoid (Car).

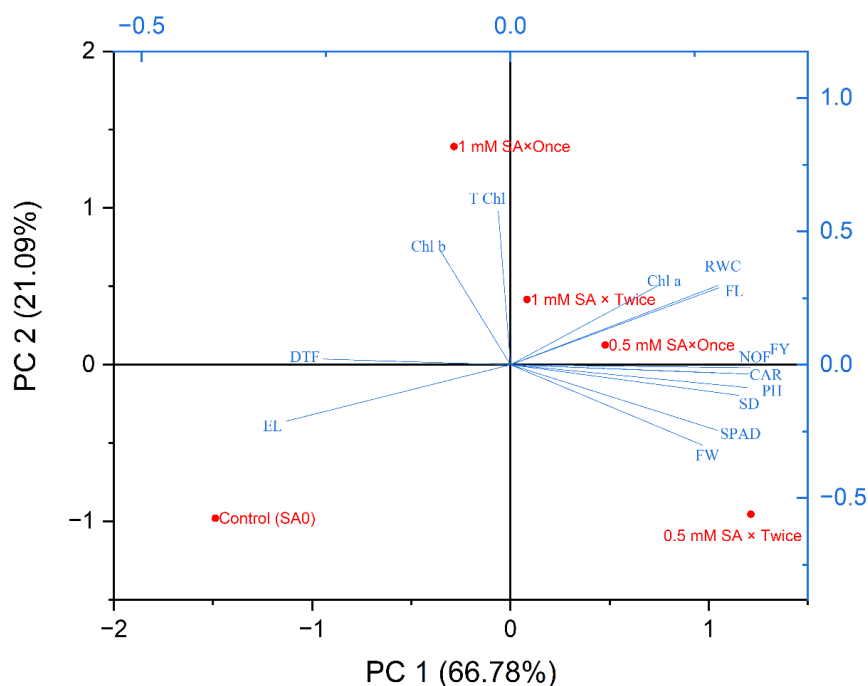


Figure 11. PCA Biplot analysis of salicylic acid concentrations on growth and physiology of Eggplant. The variables are: Plant Height (PH), Stem Diameter (SD), Days to Flowering (DTF), Number of Fruits (NOF), Fruit Length (FL), Fruit Width (FW), Fruit Yield (FY), Electrolyte leakage (EL), Relative water content (RWC), SPAD, Chlorophyll a (Chl a), Chlorophyll b (Chl b), Total Chlorophyll (T Chl) and Carotenoid (Car).

3.14. Heatmap and Cluster Analysis

The hierarchical clustering heatmap supports the PCA interpretation, clearly distinguishing treatment effects based on the intensity and frequency of SA application. The 0.5 mM SA treatments, particularly when applied twice, are clustered together and exhibit strong positive responses (green coloration) for most parameters related to photosynthetic activity (Chl a, RWC, SPAD) and productivity (NOF, FL, FY, PH). The 1 mM SA treatments form a separate cluster, showing moderate enhancement in chlorophyll pigments and growth traits but with some reduction in overall performance compared to 0.5 mM SA. The control group (SA0) is clearly separated, displaying high EL and DTF values (red coloration), which indicate lower membrane stability and delayed flowering. Overall, the heatmap confirms that 0.5 mM SA applied twice optimally improved physiological performance, photosynthetic pigment concentration, and yield-related traits in eggplant compared with other treatments (Figure 12).

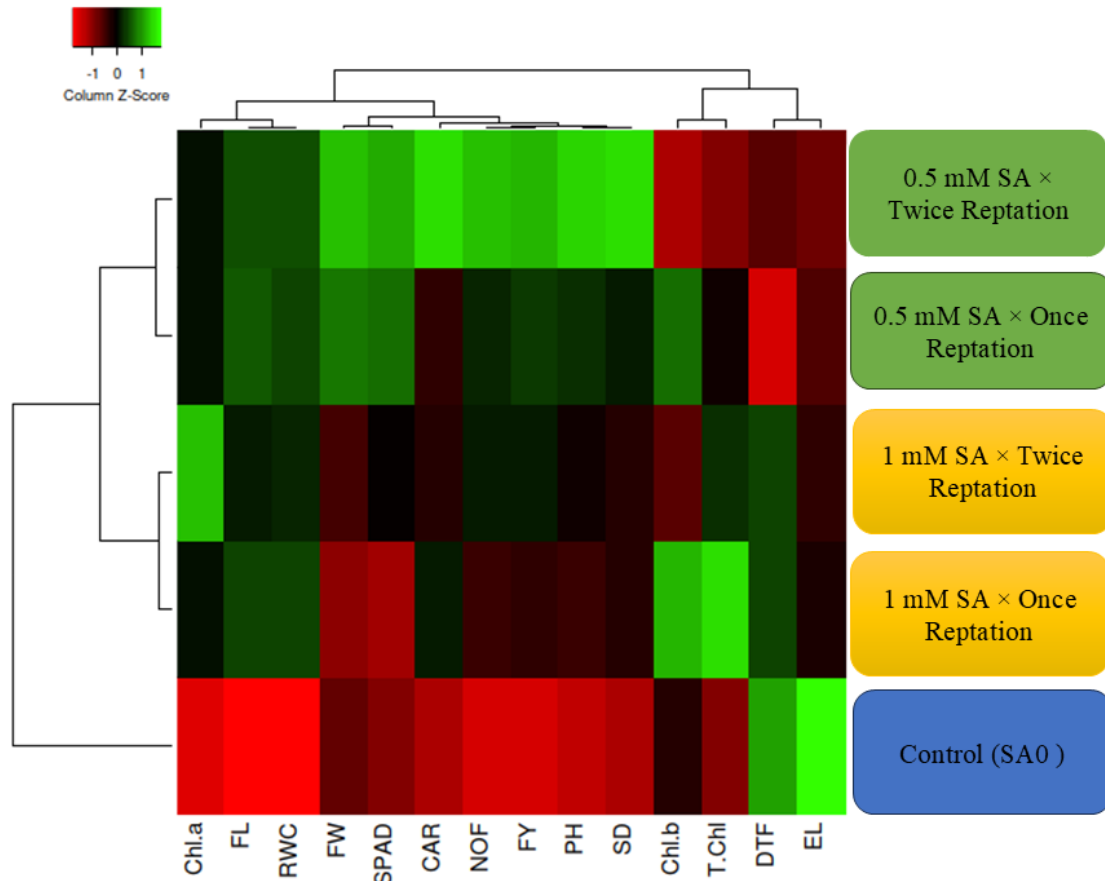


Figure 12. Heatmap and Cluster Analysis of salicylic acid concentrations on growth and physiology of Eggplant. The variables are: Plant Height (PH), Stem Diameter (SD), Days to Flowering (DTF), Number of Fruits (NOF), Fruit Length (FL), Fruit Width (FW), Fruit Yield (FY), Electrolyte leakage (EL), Relative water content (RWC), SPAD, Chlorophyll a (Chl a), Chlorophyll b (Chl b), Total Chlorophyll (T Chl) and Carotenoid (Car).

4. Discussion

4.1. Membrane Stability and Water Relations

Salicylic acids significantly reduced electrolyte leakage by 30–35% and increased relative water content (RWC) by approximately 11%. These improvements indicate stronger membrane stability and better osmotic adjustment. Such results are consistent with reports in cowpea (Dutra et al., 2017) and tomato (Kowalska and Smoleń, 2013), where SA treatment enhanced antioxidant enzyme activities, reducing lipid peroxidation and improving RWC. The non-significant frequency effect suggests that SA's membrane-protective role is concentration-dependent, and additional sprays provide limited further advantage under non-stressed conditions.

4.2. Pigment Composition and Photosynthetic Efficiency

The SPAD index increased significantly, reflecting higher chlorophyll accumulation, particularly under 0.5 mM SA. Total chlorophyll and carotenoid contents also increased, with carotenoids showing the strongest response (up to +120% under 0.5 mM twice-spray). Similar trends were observed by Esposito et al. (Esposito et al., 2025), who reported an increase in chlorophyll content and leaf darkening following the application of SA. Souri et al. (Kowalska and Smoleň, 2013) also confirmed that SA enhances pigment biosynthesis and nutrient uptake, thereby boosting photosynthetic capacity. Carotenoids function as photoprotective pigments that dissipate excess light energy and reduce oxidative stress. Their strong response under repeated spraying suggests that sustained SA exposure supports long-term photoprotection (Li et al., 2022). Conversely, chlorophyll a showed no significant change, while chlorophyll b exhibited minor fluctuations, possibly reflecting a rebalancing of pigments. Such selective pigment responses were also noted in cucumber and basil under SA treatment (Yildirim et al., 2008, Gharib, 2007). Our findings are consistent with those of El-Tayeb (El-Tayeb, 2005) and Yildirim et al. (Yildirim et al., 2006), who demonstrated that foliar application of salicylic acid (SA) enhances leaf chlorophyll content in plants grown under both stress and non-stress conditions. This improvement can be attributed to SA's ability to promote the uptake of essential nutrients and to stimulate the activity of the photosynthetic machinery and stomatal function, thereby increasing carbon dioxide assimilation and overall photosynthetic efficiency. The well-documented effects of salicylic acid (SA) on stomatal regulation, chlorophyll accumulation, transpiration, and respiratory activity have led to the hypothesis that SA may also play a physiological role in modulating photosynthetic processes (Popova et al., 1997). In a detailed investigation, Pancheva et al. (Pancheva et al., 1996) reported that prolonged exposure of barley seedlings to SA (for seven days) resulted in reduced photosynthetic rates and lower activity of ribulose-1,5-bisphosphate (RuBP) carboxylase, accompanied by increases in both the CO₂ compensation point and stomatal resistance. In contrast, short-term SA treatments (ranging from a few minutes to two hours) produced no significant changes in photosynthetic performance or biochemical capacity compared with untreated control plants. SA at 1 mM can degrade or reduce the synthesis of chlorophyll (a and b) and carotenoids by inducing oxidative damage and inhibiting enzyme activities. In barley, 0.1–1 mM SA reduced chlorophyll content, linked to decreased RuBP carboxylase activity and protein levels, causing lower CO₂ fixation and net photosynthetic rate in a concentration-dependent manner (declines observed 6–24 hours post-treatment) (Pancheva et al., 1996). In tomato, foliar application of one mM SA lowered chlorophyll content under normal conditions, diverting energy from pigment maintenance to defense (Aires et al., 2022, Moustakas et al., 2022). Mechanisms involve the overproduction of ROS, which oxidizes pigments, and reduced Rubisco content/activation, impairing light-harvesting efficiency (Moustakas et al., 2022).

4.3. Effects of Salicylic Acid on Growth and Morphology

Salicylic acid (SA) is a key signaling molecule that regulates plant growth, development, and stress responses through interactions with hormones and modulation of reactive oxygen species (ROS) metabolism (Li et al., 2022). In the present study, plant height increased significantly with SA application, particularly at 0.5 mM, where twice spraying enhanced the response by 38.1% over the control. This improvement can be attributed to enhanced cell division and elongation mediated by SA-induced hormonal crosstalk and improved nutrient mobilization (Anwar et al., 2025). Similar results were reported in tomato, where foliar SA applications increased stem diameter, leaf area, and total yield under both stress and non-stress conditions, with a concentration of 0.5 mM being the most effective (Kowalska and Smoleň, 2013, AHMED et al., 2024). Yildirim et al. (Yildirim et al., 2008) also found that 1.0 mM SA improved plant height by 15–20% in eggplants under non-saline conditions, which supports the findings of the current study. However, higher concentrations (1 mM or above) may cause hormonal imbalance or oxidative stress, resulting in reduced benefits—a phenomenon often described as a hormetic response (Li et al., 2022). Repeated spraying further improved growth, suggesting that maintaining SA activity during key developmental phases enhances the continuity of signaling. Youssef et al. (Youssef et al., 2017) found that frequent foliar SA sprays, applied every 10 days, increased growth, leaf area, and tissue thickness in strawberry, reinforcing the idea that sustained application amplifies SA effects. The stem diameter increased significantly with SA concentration, particularly at 0.5 mM, whereas spray frequency had no effect. This indicates that once SA activates structural pathways such as xylem expansion and cell wall thickening, further applications add limited benefits. Similar structural improvements have been observed in tomato under salinity, where foliar SA increased epidermal and mesophyll thickness (Souri and Tohidloo, 2019). These anatomical changes likely enhance stem rigidity and vascular capacity, thereby improving resource transport and overall growth stability.

A notable finding from studies on salicylic acid (SA) application in habanero pepper (*Capsicum chinense*) was the pronounced increase in plant vigor observed in treated seedlings compared with untreated controls. Foliar application of 1 μM SA to young shoots significantly enhanced overall growth and increased the fresh and dry weights of roots, stems, leaves, and fruits at harvest, demonstrating SA's broad promotive influence on both vegetative and reproductive development in this species (Tucuch-Haas et al., 2017). SA at 1 mM can inhibit vegetative growth by antagonizing key hormones, such as auxin (IAA) and gibberellin (GA), leading to reduced cell division, expansion, and overall biomass (Souri and Tohidloo, 2019). This results in stunted stature, smaller leaves/roots, and delayed development. For example, in barley seedlings, SA concentrations between 0.1 and 1 mM inhibited leaf and root growth in a dose-dependent manner, with reductions attributed to suppressed cyclin

gene expression and altered auxin transport via hyperphosphorylation of PIN proteins (Li et al., 2022). In Arabidopsis, SA concentrations above 0.05 mM (up to 1 mM) deplete auxin levels, repressing root elongation and meristem activity through the ROS-mediated downregulation of PLT1/2 and WOX5 genes. Mechanisms include NPR1-dependent signaling, which represses growth-promoting pathways, and the activation of the unfolded protein response (UPR), which antagonizes organ expansion (Zhu et al., 2020). In rice, root application of SA inhibits growth, likely due to similar hormone crosstalk (Zhu et al., 2020).

4.4. Flowering Response and Fruit Number, Yield

SA significantly accelerated flowering, reducing the number of days to flowering by 16.3% at 0.5 mM. This early transition may result from SA's interaction with flowering hormones such as gibberellins and ethylene, which regulate floral initiation (Li et al., 2022). Comparable findings were reported by Gharib (Gharib, 2007), who observed earlier flowering in solanaceous crops treated with 150 mg L⁻¹ SA. Likewise, Priya et al. (Priya and Singh, 2022) demonstrated that SA enhanced flowering percentage in mungbean (*Vigna radiata* L. Wilczek), even under the control treatment. The absence of a frequency effect implies that a single SA application is sufficient to trigger the developmental shift toward flowering. In addition, Raskin (Raskin, 1992) reported that SA application stimulated flowering in several angiosperm species. The earliest evidence of salicylic acid (SA) exerting a physiological role was its observed ability to induce flowering and bud formation in tobacco cell cultures (Eberhard et al., 1989). The promotive influence of salicylic acid (SA) on flowering was later confirmed in various plant species, leading to the suggestion that SA may act as an endogenous regulator of floral induction (Luo et al., 2022). This regulatory effect is not exclusive to SA alone, as its flowering promotion often occurs synergistically with other plant hormones, such as gibberellins, indicating that SA interacts within a broader hormonal network controlling reproductive development (Popova et al., 1997). Salicylic acid markedly increased fruit number and yield, particularly at 0.5 mM and under twice-spray conditions. This concentration resulted in an 85.7% increase in fruit number and an 88.6% increase in total yield compared to the control. The improvement likely results from enhanced pollination efficiency, resource partitioning, and reduced flower or fruit abortion (Gharib, 2007). Similar enhancements in yield were recorded in tomato and strawberry treated with foliar SA (Mohamed et al., 2018, AHMED et al., 2024).

The non-linear concentration response (where 0.5 mM outperformed 1 mM) suggests an optimal physiological window for SA action, beyond which inhibitory effects may occur (Li et al., 2022). Repeated spraying ensured SA availability during critical reproductive stages, consistent with Youssef et al. (Youssef et al., 2017), who observed that frequent foliar SA applications enhanced fruit yield by sustaining hormonal signaling and improving antioxidant protection. Foliar application of salicylic acid (SA) promoted both early and total fruit yield in strawberry plants. Comparable outcomes were documented by Kling and Meyer (Kling and Meyer, 1983) in beans, Zhou et al. (Zhou et al., 1999) in maize, Yildirim et al. (Yildirim et al., 2006) in cucumber, and Karlidag et al. (Karlidag et al., 2009) in strawberry. The beneficial influence of SA may be linked to its ability to enhance CO₂ fixation, elevate chlorophyll content, and improve photosynthetic efficiency, as well as to facilitate greater mineral absorption in plants treated with SA. Yield reductions result from inhibited reproductive development and altered resource allocation, often accompanied by growth suppression. High SA (0.1–1 mM) reduces pollen tube length, trichome density, and seed production by antagonizing ethylene (ET) and GA, leading to smaller floral organs and lower fruit/seed set (Li et al., 2022). In SA-over-accumulating mutants (e.g., *acd6*, *cpr5*), dwarfism correlates with decreased seed yield, as SA represses GA marker genes and promotes immunity at the expense of reproduction. Additionally, dosage-dependent inhibition occurs by impairing assimilate transport and nutrient uptake, thereby reducing overall plant vigor and productive output (Khan et al., 2015, Li et al., 2022).

4.5 Correlation and multivariate analyses of dosage and time application of SA on morpho-physiological parameters of eggplant

Salicylic acid (SA) is widely recognized as a plant growth regulator that influences physiological, morphological, and biochemical processes even under non-stress conditions. In the present study, the application of SA significantly enhanced vegetative and reproductive growth traits of eggplant, including plant height, stem diameter, fruit number, and fruit yield, while simultaneously improving physiological parameters such as chlorophyll content, carotenoid concentration, SPAD index, and relative water content (RWC). These results confirm that SA acts as a biostimulant capable of optimizing growth and yield potential when applied at appropriate concentrations and intervals (Gruda et al., 2024). Among the tested concentrations, 0.5 mM SA consistently produced superior results across most measured traits. Plants treated with 0.5 mM SA exhibited marked increases in plant height, stem diameter, fruit number, fruit yield, and chlorophyll pigments compared with the untreated control. These findings suggest that moderate SA concentrations stimulate physiological processes associated with cell division, chlorophyll biosynthesis, and the allocation of assimilates. Similar outcomes were reported by Yildirim et al. (Yildirim et al., 2008), who observed that 1.0 mM SA improved growth and chlorophyll content in eggplants grown under non-saline conditions. Likewise, Gharib (Gharib, 2007) found that SA at 150 mg L⁻¹ (~1 mM) enhanced growth and yield parameters in solanaceous crops, supporting the concentration-dependent nature of SA responses. The superior performance at 0.5 mM compared with 1.0 mM SA indicates that excessive

concentrations may lead to metabolic feedback inhibition or mild oxidative effects, resulting in reduced efficiency of physiological pathways. This nonlinear, dose-dependent behavior has been noted by Li et al. (Li et al., 2022), who emphasized that low to moderate SA levels enhance photosynthetic capacity and biomass accumulation, whereas higher doses can disrupt cellular homeostasis. Therefore, the optimal concentration of SA in eggplant cultivation appears to be below 1.0 mM for maximizing physiological and agronomic performance.

Foliar spray frequency also significantly affected several traits, especially those related to cumulative growth and productivity. Twice spraying (AR2) consistently outperformed single spraying (AR1) for plant height, fruit number, fruit yield, and carotenoid accumulation. This result suggests that repeated SA application helps sustain its physiological activity over time, ensuring that growth-regulating effects persist during critical vegetative and reproductive stages. Similar trends have been reported in strawberry, where Youssef et al. (Youssef et al., 2017) observed that more frequent SA applications (every 10 days) led to sustained pigment accumulation and higher fruit yield compared with less frequent spraying. The PCA and heatmap analyses in the current study further confirmed that repeated SA application maintained higher correlations among growth, pigment, and yield traits, while separating treated plants distinctly from controls. Treatments involving twice-spraying clustered plants with higher SPAD, chlorophyll, and yield components indicate that the frequency of SA exposure enhances the coordination between physiological efficiency and reproductive success. Conversely, the control treatments were associated with higher electrolyte leakage and delayed flowering, reflecting a comparatively weaker physiological status. The enhanced chlorophyll and carotenoid levels under SA treatments indicate stimulation of photosynthetic pigment synthesis and improved light-harvesting efficiency, which are key contributors to yield formation. The increased RWC and reduced electrolyte leakage further demonstrate that SA improves water balance and membrane integrity, thereby maintaining optimal cellular function. Although the experiment was conducted under non-stressed conditions, these improvements signify enhanced metabolic vigor rather than stress alleviation, as supported by the control data interpretations of Yildirim et al. (Yildirim et al., 2008) and Souri et al. (Souri and Tohidloo, 2019). Overall, the current findings demonstrate that salicylic acid acts as an effective growth-promoting regulator when used at moderate concentrations and with appropriate application frequency. The positive correlations between vegetative and reproductive parameters, as well as between pigment content and yield, underscore the integrated nature of SA's effects.

Conclusion

This study demonstrates that the foliar application of salicylic acid (SA) significantly enhances the vegetative growth, physiological performance, and reproductive productivity of eggplant, with effects dependent on both the concentration and frequency of application. The optimal treatment (0.5 mM SA applied twice) resulted in significant improvements in plant height, stem diameter, leaf greenness, chlorophyll and carotenoid contents, flower initiation, and total fruit yield compared to untreated plants. These enhancements were associated with improved water retention, reduced membrane injury, and greater pigment accumulation, indicating that SA positively regulates photosynthetic and stress-related pathways even under non-stress conditions. Increasing the SA concentration to 1.0 mM provided no further benefits and occasionally reduced performance, confirming that SA exhibits a concentration-dependent (hormetic) response. Application frequency was particularly important for cumulative traits such as plant height, fruit number, and yield, emphasizing the role of repeated SA exposure in sustaining metabolic activation during critical growth stages. From a practical perspective, foliar application of 0.5 mM SA twice during the growth cycle can be recommended as a low-cost, effective, and environmentally safe approach to enhance eggplant productivity. These findings contribute to a better understanding of SA's multifaceted physiological role and offer a foundation for its integration into sustainable horticultural practices. Future research should further elucidate the molecular and enzymatic mechanisms underlying SA-induced improvements, including its effects on antioxidant defense, hormonal balance, and gene regulation.

Data Availability Statement: The authors declare that data supporting the findings of this study are available in the article. If raw data files are required, they are available from the corresponding author upon reasonable request.

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