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Salicylic Acid Alleviates Moderate Salinity-Induced Stress in *Petroselinum crispum* (Mill.), within Reduced Effectiveness under Prolonged High Salinity Conditions

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Abstract

This study elucidates the role of salicylic acid (SA) in modulating salinity stress responses in parsley through a controlled RCBD experiment at Taiz University. Plants exposed to incremental NaCl concentrations (0–200 mM) either alone or in combination with three SA concentrations (0, 0.1, and 0.5 mM) exhibited dose-dependent growth inhibition. With 200 mM NaCl, reduced shoot length by 42% and dry matter by 35%, while concurrently suppressing protein content (28%) and catalase activity (40%) but elevating proline accumulation (69%). SA application (0.1 mM) significantly alleviated moderate stress (100 mM NaCl), restoring physiological parameters and enhancing antioxidant defenses, notably increasing catalase activity by 30–35% and reducing proline by 25–30%. Intriguingly, SA's efficacy was temporally constrained, with optimal protection at 15 days that diminished under prolonged exposure. Biochemical profiling revealed a stress-threshold response: ascorbate peroxidase activity remained stable at $\leq$ 150 mM NaCl but increased 20–25% at 50–100 mM NaCl with 0.5 mM SA. Critically, high SA (0.5 mM) synergized with extreme salinity (200 mM NaCl) to induce complete mortality, demonstrating a concentration-dependent phytotoxic shift. These findings establish 0.1 mM SA and 100 mM NaCl as critical thresholds for stress mitigation.

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Antioxidant, Osmatic stress, Parsley, Salicylic acid, Salinity

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1. INTRODUCTION

Parsley (*Petroselinum crispum* Mill..), a member of the Apiaceae family, is widely cultivated in tropical, subtropical, and temperate regions for its diverse uses as a vegetable, medicinal herb, and spice [26, 53, 4]. It is a rich source of bioactive compounds, including ascorbic acid, carotenoids, flavonoids, apiole, terpenoids, coumarins, phenylpropanoids, phthalides, tocopherols, and furanocoumarins. The leaves are particularly abundant in vitamins A, C, and K, as well as β-carotene, lutein, zeaxanthin, folate, choline, niacin, and pantothenic acid. Furthermore, the roots serve as an excellent source of essential minerals [53].

Salinity, a significant environmental stressor, poses a major threat to plant growth and agricultural productivity. The problem is further exacerbated by factors such as soil degradation, poor drainage, inappropriate irrigation practices, and excessive fertilizer use [49].

Additionally, urban expansion and the growing competition for water resources between industrial and agricultural sectors further restrict the availability of irrigation water [81], compounding the challenges faced by farmers in saline-affected regions.

Over 800 million hectares of agricultural land worldwide are severely affected by salinity, making it one of

the most critical challenges to global food security. Salinity is a major abiotic stress that significantly constrains crop production, particularly in arid and semi-arid regions [86]. Approximately 23% of the world's cultivated land is classified as saline, while 37% is sodic [47].

Yemen features a diverse range of climates, including semi-humid, semi-arid, and arid tropical regions [13]. Within the country, approximately 37,100 hectares of non-desert agricultural land are affected by salinity. Additionally, 12 million hectares are impacted by erosion, and 3.8 million hectares exhibit varying degrees of salinity, with 3–5% of this land at risk of desertification [35, 84]. Notably, the highland regions remain unaffected by salinity [15].

Salt stress impacts plant growth through three primary mechanisms: (i) reduced water uptake due to osmotic stress, which lowers soil water potential and restricts water absorption by plant tissues; (ii) disruption of ion homeostasis, leading to nutrient imbalances; and (iii) ion toxicity, particularly from the accumulation of sodium (Na^+) in leaves, which impairs the plant's ability to absorb water and essential nutrients [86, 58, 31, 79].

Salinity profoundly affects plant growth by altering morphological, physiological, biochemical, molecular, and anatomical traits [31, 12, 5, 46], as well as influencing the production of secondary metabolites [75].

Elevated salt concentrations negatively impact the germination, growth, development, and productivity of vegetable crops such as chili pepper [60, 56] and *Cucurbita* genotypes [41].

Parsley exhibits moderate sensitivity to salinity, with optimal growth observed at electrical conductivity (EC) levels below 4.5 dS m^{-1}. However, elevated salinity levels can negatively impact its growth parameters, such as plant height, fresh weight, shoot and root length, leaf number, and overall biomass [30, 45].

Salt-stressed plants often exhibit reduced fruit yield and fewer umbels [21]. Furthermore, salinity diminishes carbohydrate content by reducing photosynthetic activity, inducing hyperosmotic stress, and causing nutritional imbalances, as observed in fennel [85]. While salt stress affects all stages of plant growth, seed germination and seedling development are particularly sensitive in most species [29].

Tolerance to salt stress varies significantly among plant species and cultivars [60], primarily due to differences in osmotic adaptation mechanisms such as ion partitioning and the synthesis of compatible solutes like proline [34]. Additionally, the regulation of reactive oxygen species (ROS) and cell growth rates [38] plays a critical role in enabling plants to cope with saline environments. However, these adaptive mechanisms often come at the cost of reduced overall yield, producing smaller plants with altered morphological and physiological characteristics [69].

Interestingly, salinity can have varying effects on different plant species. For instance, it increases the dry matter percentage in eggplant [83] but reduces it in *Schizonepeta tenuifolia* [87] and parsley [30]. In parsley, high salinity levels (e.g., 240 mM NaCl) have been shown to reduce petiole elongation without significantly altering leaf area, color, electrolyte leakage, or petiole firmness [24].

Ongoing research efforts are focused on developing agricultural technologies to mitigate the adverse effects of salinity on crop production, including the use of growth regulators [17].

Plant growth regulators (PGRs) are widely used to influence and regulate plant growth and development. These substances, which include both natural plant hormones and synthetic compounds, can promote, inhibit, or modify various physiological processes in plants, depending on their type, concentration, and application method [16, 11, 59].

Salicylic acid (SA), a plant growth regulator, has been demonstrated to enhance salt stress tolerance by improving physiological traits and alleviating the detrimental effects of salinity. This is achieved through increased antioxidants.

Enzyme activity and elevated soluble sugar content in plants [1, 7].

Furthermore, SA plays a regulatory role in essential plant processes, including seed germination, growth, and development [50], while also influencing ion absorption, stomatal conductance, photosynthetic activity, and transpiration rates [12, 39].

Despite the widespread use of parsley in Yemeni households, the salt tolerance of local parsley genotypes remains poorly understood. Furthermore, there is a significant gap in research specifically addressing the response of these plants to salinity, highlighting the importance of this study in addressing this critical knowledge gap.

2. MATERIALS AND METHODS

2.1. Location of Study

This experiment was conducted in two distinct phases: sowing and treatment application. Growing plants and treatments were applied at Taiz University during the spring season. All chemical analyses were carried out in the pharmaceutical laboratory at Al-Saeed University, ensuring precise and reliable measurements of the relevant parameters.

2.2. Experimental Design and Treatments

The pot experiment was meticulously designed to achieve the study's objectives and was conducted using a factorial Randomized Complete Block Design (RCBD)

with three replicates.

The experimental design incorporated three key factors: Five levels of NaCl salinity: 0, 50, 100, 150, and 200 mM. Three concentrations of Salicylic Acid (SA): 0, 0.1, and 0.5 mM. Two harvest time points: 15 and 30 days after the initiation of treatments (salinity and SA).

2.3. Experimental layout

The experimental pots were filled with a growth medium consisting of a 2:1 volume-to-volume ratio (v/v) of soil: sand mixture. Parsley seeds were sown and initially irrigated with tap water to ensure uniform germination. Fifteen days post-emergence (two-leaf stage), plants were subjected to salinity stress through irrigation with NaCl solutions (0, 50, 100, 150, and 200 mM) either alone or in combination with salicylic acid (0, 0.1, and 0.5 mM) in a completely randomized design with three replicates per treatment.

2.4. Soil and water characterize

Electrical Conductivity (EC) and pH were measured for soil and sand samples separately and in combination. Additionally, the water used in the experiment (normal water as control and salinity solution with or without SA) was characterized (Table 1).

2.5. Parameters of the study

To evaluate treatment effects, a subset of pots was harvested 15 days after initiating salinity stress, while the remaining pots were maintained for an additional 15 days (30 days total after treatment initiation). At each harvest interval (15 and 30 days), the following parameters were measured:

2.5.1. Morphology Parameters by Using Tomato Analyzer

The method outlined by Arraf and Al-Madhagi [18] was employed for plant analysis. After weighing the fresh plants, they were carefully spread on a labeled square black sheet to ensure clear visibility. Photographs of the plants were captured using a mobile digital camera (Figure 1a), and the images, saved in JPEG format, were transferred to a laptop for further processing.

Digital images were processed using Adobe Photoshop to enhance contrast by standardizing background illumination. All images were saved at 200 dpi resolution and scaled using a calibration ruler for dimensional accuracy (Figure 2b). Morphometric parameters including plant height, shoot length, and root length (cm) were quantified using Tomato Analyzer 3.0 software developed by the Department of Horticulture and Crop Science, Ohio State University (available at; http://oardc.osu.edu/vanderknaap/tomato_analyzer.htm) (Figure 1c).

2.5.2. Dry matter contact

The shoots and roots of ten plants from each replicate were carefully harvested and immediately weighed using a sensitive balance to determine their fresh weight. The plant samples were then dried under shade conditions at room temperature until they reached a constant weight. The dry matter content was calculated using the following equation:

$$\text{DM\%} = \frac{\text{dry weight}}{\text{fresh weight}} \times 100 \quad [32] \tag{1}$$

2.5.3. Biochemical parameters:

Estimation of proteins (mg/g FW):

The method used to determine shoot protein content was adapted from Lowry et al. [57]. The procedure involved preparing an extraction buffer by combining 8.33mL of dipotassium phosphate (K_2HPO_4) (8 mL + 330 μL) and 1.67 mL of monopotassium phosphate (KH_2PO_4) (1 mL + 670 μL), which was then diluted to a final volume of 200 mL with distilled water.

The Coomassie Blue dye solution was prepared by dissolving 0.1 g of Coomassie Brilliant Blue dye in a mixture of 50 mL of 95% ethanol and 100 mL of phosphoric acid, followed by dilution to 1 L with distilled water, settling, and filtration. For sample preparation, 0.1 g of frozen plant tissue was homogenized with 1 mL of the prepared buffer solution and centrifuged at 6000 rpm for 10 minutes to obtain the supernatant. Protein quantification was performed by mixing 1000 μL of the Coomassie Blue dye solution with 800 μL of the buffer solution and 200 μL of the supernatant. The absorbance of the mixture was then measured at 575 nm using a JENWAY 6305 spectrophotometer.

2.5.4. Estimation of Proline (mg/g DW):

The proline content in the shoot was measured using the protocol established by Bates *et al.* [23]. The reaction solution was prepared under dark conditions by dissolving 1 g of ninhydrin in 60 mL of glacial acetic acid, followed by the addition of 20 mL of 70% ethanol and 20 mL of distilled water. The solution was kept cool until use. Proline was extracted from shoot tissue by homogenizing 0.1 g of dry weight in a mortar and pestle with 2 mL of 70% ethanol.

The homogenate was centrifuged at 6000 rpm for 10 minutes to obtain the supernatant. The supernatant was then mixed with the reaction solution in a 1:2 volume ratio (supernatant to reaction solution) and incubated for 20 minutes at 95℃ in a water bath. After incubation, the mixture was cooled in an ice bath. Absorbance was measured at a wavelength of 520 nm using a JENWAY 6305 spectrophotometer. Proline content was calculated using an optimized calibration curve developed with

Table 1. Soil and water characteristics used in the experiment.

NO.	Simple	EC (µs/cm)	pH	TDS (PPM)
1	Soil	0.2955	8.2	151.75
2	Sand	0.319	8.3	162
3	Soil: Sand (2:1 V: V)	0.3195	8.3	160.75
4	normal Water	0.5585	8.4	281.25
5	NaCl 50	1.37	8.5	682.25
6	NaCl 100	3.27	8.3	1046.4
7	NaCl 150	16.6	8.3	5601.6
8	NaCl 200	29.9	8.3	5972.8
9	SA 0.1	0.55625	8.4	178080
10	NaCl 50+SA 0.1	1.2675	8.3	406560
11	NaCl100+SA 0.1	2.1575	8.3	694.4
12	NaCl 150+SA 0.1	3.2	8.4	1011.2
13	NaCl 200+SA 0.1	4.03	8.4	1286.4
14	SA 0.5	0.5335	8.5	171680
15	NaCl 50 +SA 0.5	0.001265	8.1	403200
16	NaCl 100 + SA 0.5	0.0021825	8.1	1.09
17	NaCl 150+ SA 0.5	0.00314	8.2	1.6225
18	NaCl 200 +SA 0.5	0.00395	8.2	1.965

The number is the average of four samples. NaCl in mM and SA (Salicylic Acid) in mM

varying concentrations of proline standard, based on dry weight.

2.5.5. Estimation of antioxidant enzymes:

The Velikova *et al.* [14] method was employed to assess enzyme activation in the shoot. The preparation of solutions involved dissolving 34.836 grams of K_2HPO_4 in 200 mL of distilled water for the K_2HPO_4 solution, and 27.218 grams of KH_2PO_4 in 200 mL of distilled water for the KH_2PO_4 solution. For the enzyme extraction solution, 0.184 mL of mono potassium phosphate KH_2PO_4 (184 mµ) and 1.8 mL of dipotassium phosphate K_2HPO_4 (1 mL + 800 mμ) were combined and adjusted to a final volume of 20 mL with distilled water.

The extraction process involved homogenizing 0.1 g of frozen tissues with 1 mL of the previously prepared extraction solution, followed by centrifugation at 6000 rpm for 10 minutes. The resultant extract was kept on ice until further measurements.

A buffer solution was prepared by mixing 1.230 mL of dipotassium phosphate K_2HPO_4 (1 mL + 230 mμ) with 0.77 mL of mono potassium phosphate KH_2PO_4 (770 mμ) and adding 40 mL of distilled water. Additionally, 0.834 mL of CAT H_2O_2 was prepared and adjusted to a final volume of 10 mL.

For the APX H_2O_2 solution, 1.70 mL of H_2O_2 was brought up to a volume of 10 mL.

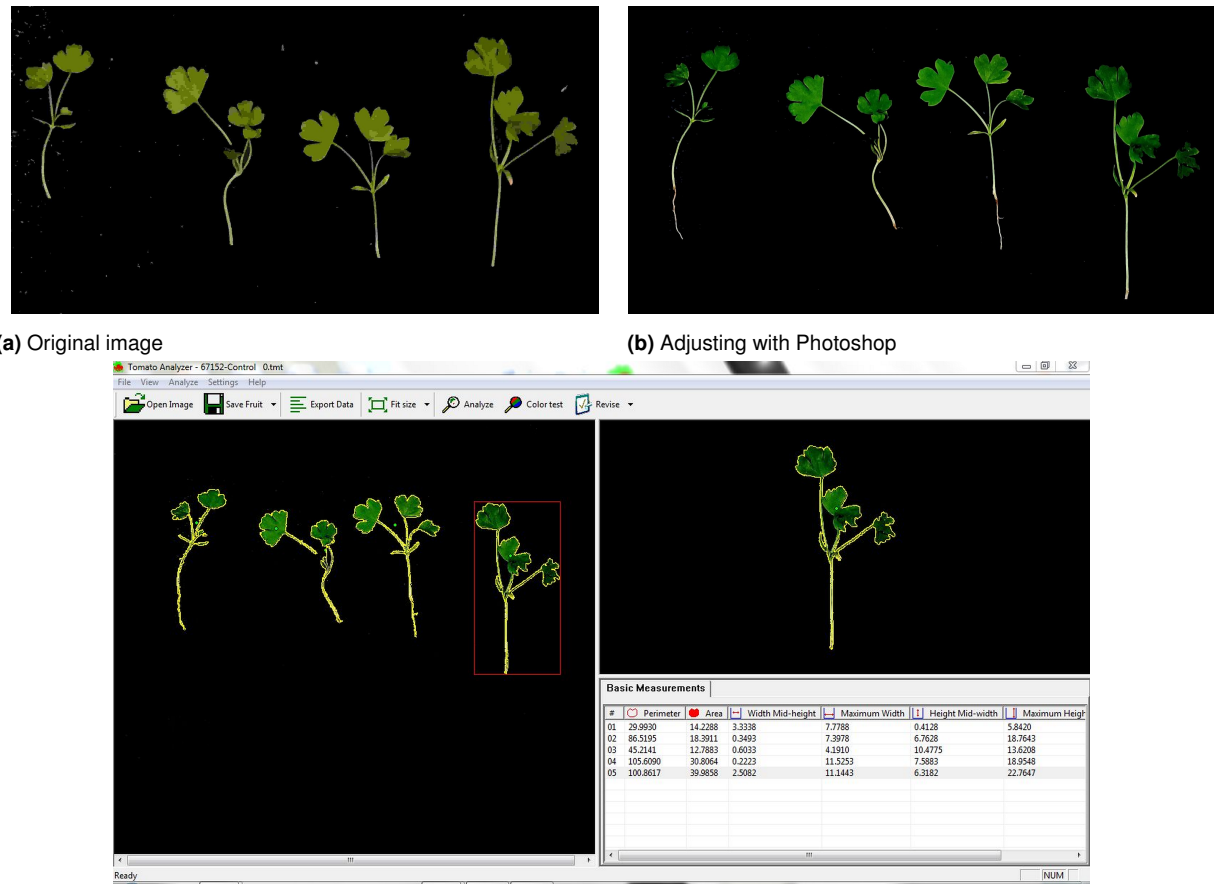
The ascorbate solution was created by dissolving 0.0158 grams of ascorbate in 20 mL of purified water. Setup of Measurement Tubes:

Catalase (CAT) activity was evaluated following Aebi [2] by mixing 2.7 mL of the prepared buffer solution with 0.05 mL of the supernatant. The absorbance was measured at 240 nm using a JENWAY 6305 Spectrophotometer at a rate of 0-30 sens -1 min.

APX activation was determined according to Asada [19] by combining 1.8 mL of the prepared buffer solution with 0.1 mL of H_2O_2 solution, 1 mL of ascorbate solution, and 0.1 mL of the supernatant.

Absorbance was measured at 290 nm using a JENWAY 6305 Spectrophotometer at a rate of 0-30 s within one minute.

The enzyme activity in nanomoles per gram of pro-



(a) Original image

(b) Adjusting with Photoshop

(c) analyzed by Tomato analysis software

Figure 1. Images analyzed by Tomato Analyzer.

tein (nmol/g protein) was calculated using the specified formula:

$$\text{Enzyme Activity}\left(\frac{\text{nmol/min}}{\text{g protein}}\right) = \frac{\text{Change inOD}}{\text{Time (minutes)}} \times \frac{1}{\text{Extinction Coefficient}} \times \frac{\text{Total Reaction Volume}}{\text{Volume of Enzyme Extract}} \times \frac{\text{Total Volume of Enzyme}}{\text{Fresh Weight}} \times \frac{\text{Total Protein (mg/mL)}}{100} \quad (2)$$

with the extinction coefficient for peroxidases being 26.6 $mM^{-1}cm^{-1}$ and for catalase being 39.4 mM^{-1} cm^{-1}.

2.6. Statistical Analysis

The data were evaluated using GenStat 12.1 software as a factorial experiment (RCBD), taking into account the concentration of salt and salicylic acid, and measuring period as factors. The significance of individual factor means was determined using the least significant difference (LSD) at a significant level of less than 0.05. Interactions were evaluated using Duncan's multiple range test (DMRT) method. Microsoft Excel was also used to estimate regression equations for linear effects.

3. RESULTS

3.1. Effect of NaCl, SA, and time on shoot length (cm):

The results generally indicated that the independent factors (SA and NaCl) significantly affected the shoot length (cm) at a probability level of ($P < 0.001$), except for the effect of time, which was not statistically significant (Table 2).

The shoot length of parsley exhibited a significant reduction with increasing salinity levels. In the control treatment, the shoot length measured 12 cm, whereas at the highest salinity concentration of 200 mM NaCl, it decreased to 3.37 cm (Figure 2a). The decline in shoot length followed a progressive pattern, with an approximate reduction of 10% for every incremental increase of 50 mM NaCl above the control, up to 100 mM. Beyond 100 mM, the reduction became more pronounced, reaching approximately 26% (Figure 2a).

This trend was further supported by the linear regression equation (y = -2.084x + 14.85, R^2 = 0.928), which indicates that shoot length decreased by an average of 2.084 cm for every 50 mM increase in NaCl concentration (Figure 2a). Notably, the time factor did not exhibit a significant effect on plant height (Table 2).

However, the application of salicylic acid (SA) also had a significant impact on shoot height, resulting in a

reduction compared to the control. Specifically, the shoot length decreased by approximately 15.5% and 19.2% in treatments with 0.1 mM and 0.5 mM SA, respectively (Figure 3a).

The interaction effects of the second and third factors were statistically significant (Table 2). The results of the three-way interaction (N × SA × T) revealed that shoot length was significantly influenced by the combined effects of salinity, SA application, and measurement time (Table 3).

During the first measurement period (15 days), the application of 0.1 mM SA did not significantly alleviate the adverse effects of salinity on shoot length. For instance, at 200 mM NaCl and 0.1 mM SA, the shoot length was 8.25 cm, which was comparable to the control treatment (0 mM NaCl and 0 SA) at 15 days (7.59 cm) (Table 3).

However, at lower salt concentrations (50 mM NaCl), the use of either 0.1 mM or 0.5 mM SA led to a modest increase in shoot length, with values of 8.10 cm and 8.36 cm, respectively, at 15 days. In the control treatment (0 mM NaCl and 0 SA), the average shoot length reached its maximum value of 16.71 cm at the second measurement time (30 days), indicating optimal growth conditions in the absence of salinity stress. Conversely, the lowest values (0 cm) were observed in plants treated with 0.1 mM SA × 200 mM NaCl × 30 days, 0.5 mM SA × 200 mM NaCl × 15 or 30 days, and 0.5 mM SA × 150 mM NaCl × 30 days. These zero values were attributed to plant mortality that occurred before the measurement period, highlighting the lethal effects of prolonged high salinity under elevated SA levels (Table 3).

Notably, under moderate salinity (100 mM NaCl), shoot length showed variability with SA application and time. For example, at 100 mM NaCl and 0 SA, shoot length increased from 10.64 cm at 15 days to 8.39 cm at 30 days, suggesting that prolonged exposure to moderate salinity may inhibit growth. However, with 0.1 mM SA, shoot length remained relatively stable at 10.09 cm at 15 days and 9.80 cm at 30 days, indicating that SA application can mitigate the adverse effects of moderate salinity over time. At 0.5 mM SA, shoot length decreased to 8.13 cm at 15 days but increased to 11.22 cm at 30 days, further emphasizing the dynamic role of SA in modulating growth under stress (Table 3).

3.2. Effect of NaCl, SA, and time on root length (cm):

Sodium chloride (NaCl) exerted a statistically significant influence on root length, as demonstrated by an *F*-probability value of 0.012 (Table 2 and Figure 2b). The highest NaCl concentration (200 mM) resulted in a pronounced reduction in root length compared to both the control and lower salinity levels, with a decrease of approximately 47.3% relative to the control plants.

Similarly, salicylic acid (SA) also had a significant effect on root length, as indicated by an *F*-probability value of 0.016 (Table 2 and Figure 3b).

Across all tested concentrations, the application of SA led to a significant reduction in root length when compared to the control. Specifically, plants treated with SA exhibited an average decrease in root length of approximately 27% relative to untreated plants (Figure 3b).

In contrast, the time factor did not show a statistically significant effect on root length, as evidenced by an *F*-probability value of 0.201 (Table 2).

The interaction effects among the studied factors (NaCl, SA, and time) were analyzed and found to be statistically significant, with all interactions yielding *F*-probability values below 0.05 (Table 2). The three-way interaction (NaCl × SA × Time) in Table 4, revealed that the application of salicylic acid (SA) at the higher concentration (0.5 mM) partially alleviated the adverse effects of NaCl on parsley by promoting root elongation. Notably, plants treated with 0.5 mM SA × 150 mM NaCl × 15 days achieved the maximum root length of 7.016 cm, demonstrating that SA can enhance root growth under moderate salinity stress during shorter exposure periods. However, under prolonged exposure (30 days) to the same treatment (150 mM NaCl and 0.5 mM SA), root length dropped to 0 cm, indicating plant mortality and the inability of SA to counteract the cumulative effects of prolonged salinity stress.

Conversely, the lowest root length values, including zero values resulting from plant mortality before measurement, were observed in the following treatments: 0.1 mM SA × 200 mM NaCl × 30 days, 0.5 mM SA × 200 mM NaCl × 15 or 30 days, and 0.5 mM SA × 150 mM NaCl × 30 days (Table 4).

These results highlight the lethal effects of prolonged high salinity, even with SA application, and underscore the critical threshold beyond which SA cannot sustain root growth (Table 4).

Under moderate salinity (100 mM NaCl), root length showed variability with SA application and time, for instance, at 100 mM NaCl and 0 SA, root length increased from 5.49 cm at 15 days to 5.64 cm at 30 days, suggesting that moderate salinity alone does not severely inhibit root growth. However, with 0.1 mM SA, root length decreased to 4.33 cm at 15 days and further dropped to 1.68 cm at 30 days, indicating that SA may initially mitigate stress but becomes less effective over time (Table 4).

At 0.5 mM SA, root length remained relatively stable at 3.70 cm at 15 days and increased to 5.01 cm at 30 days, demonstrating that higher SA levels can sustain root growth under moderate salinity over time (Table 4).

In the absence of salinity stress (0 mM NaCl), root length was significantly enhanced by SA application. For example, at 0 mM NaCl and 0.5 mM SA, root length increased from 5.95 cm at 15 days to 6.36 cm at 30 days, highlighting the growth-promoting effects of SA under

Table 2. Probability values *(F-values)* for the effects of NaCl (N), Salicylic Acid (SA), and Measurement Time (T) on the morphological and biochemical characteristics of parsley

Source of variation	Shoot length cm	Root length cm	Shoot DM%	Root DM%	Protein mg/g FW	Proline mg/g DW	Peroxides nmol/min/mg of protein	Catalase nmol/min/mg of protein
NaCl (N)	<.001	0.012	<.001	<.001	<.001	<.001	<.001	<.001
Salicylic acid (SA)	<.008	0.016	<.001	<.001	<.001	<.001	0.529	0.002
***Time (T)**	0.118	0.201	<.001	0.137	<.001	0.674	0.067	0.015
15	**8.2 [a]**	**4.07[a]**	**13.32 [a]**	**13.19**	**18.12 [a]**	**8.91[a]**	**183.3[a]**	**17 [a]**
30	**8.99 [a]**	**3.56[a]**	**10.59 [b]**	**14.17**	**14.41 [b]**	**8.83[a]**	**141.55[a]**	**12.72 [b]**
N ×SA	0.021	0.001	<.001	<.001	<.001	<.001	0.03	<.001
N×T	<.001	0.003	<.001	<.001	<.001	<.001	<.001	<.001
SA × T	<.01	0.012	0.001	<.001	<.001	<.001	0.023	0.219
N×SA×T	0.001	0.019	<.001	<.001	<.001	<.001	0.287	0.003

*Means of the time (days), the values with different Latin letters in the same column are significantly different at 0.05

Table 3. Mean shoot length (cm) of the parsley plants under NaCl × SA × Time interactions.

SA (mM)	Time (Days)	NaCl mM				
		0	**50**	**100**	**150**	**200**
0	**15**	7.594^{efgh}	6.144^{fgh}	10.643^{cdef}	9.025^{defg}	7.75^{efgh}
	30	16.705^{a}	15.001^{abc}	8.385^{efgh}	11.735^{bcde}	4.267^{hi}
0.1	**15**	8.518^{efgh}	8.103^{efgh}	10.089^{def}	6.251^{fgh}	8.252^{efgh}
	30	10.51^{def}	15.367^{ab}	9.8^{defg}	5.233^{gh}	0^{i}
0.5	**15**	13.527^{abcd}	8.362^{efgh}	8.133^{efgh}	10.648^{cdef}	0^{i}
	30	15.189^{ab}	11.467^{bcde}	11.221^{bcde}	0^{i}	0^{i}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test, $p \leq 0.05$.

non-stress conditions (Table 4).

3.3. Effect of NaCl, SA, and time on percentage of Shoot Dry matter (SDM%):

Overall, the shoot dry matter percentage (SDM%) of parsley was significantly influenced by sodium chloride (NaCl) at concentrations exceeding 100 mM. The SDM% exhibited a notable decline at the highest NaCl concentration (200 mM), reaching 9.5%, which was significantly lower compared to both the control and other treatments. Notably, this treatment resulted in a zero value at the 30-day measurement interval due to complete plant mortality. In contrast, no significant differences in SDM% were observed among treatments with NaCl concentrations ranging from 0 to 100 mM (Figure 2c).

Salicylic acid (SA) also had a significant effect on SDM%, as indicated by an *F*-probability value of <0.001 (Table 2). The application of SA led to a marked reduction in SDM% compared to the control. However, no significant differences were observed between the two SA concentrations (0.1 mM and 0.5 mM) tested in this study (Figure 3c).

Additionally, the time factor significantly influenced SDM%, with the highest percentage recorded at 15 days compared to the lower values observed at the 30-day measurement interval (Table 2).

All interaction effects among the studied factors were statistically significant, with probability values for all interactions being <0.001 (Table 2).

Analysis of the full three-way interaction (NaCl × SA × Time) is shown in Table 5. The data demonstrate that the shoot DM% of parsley plants is influenced by

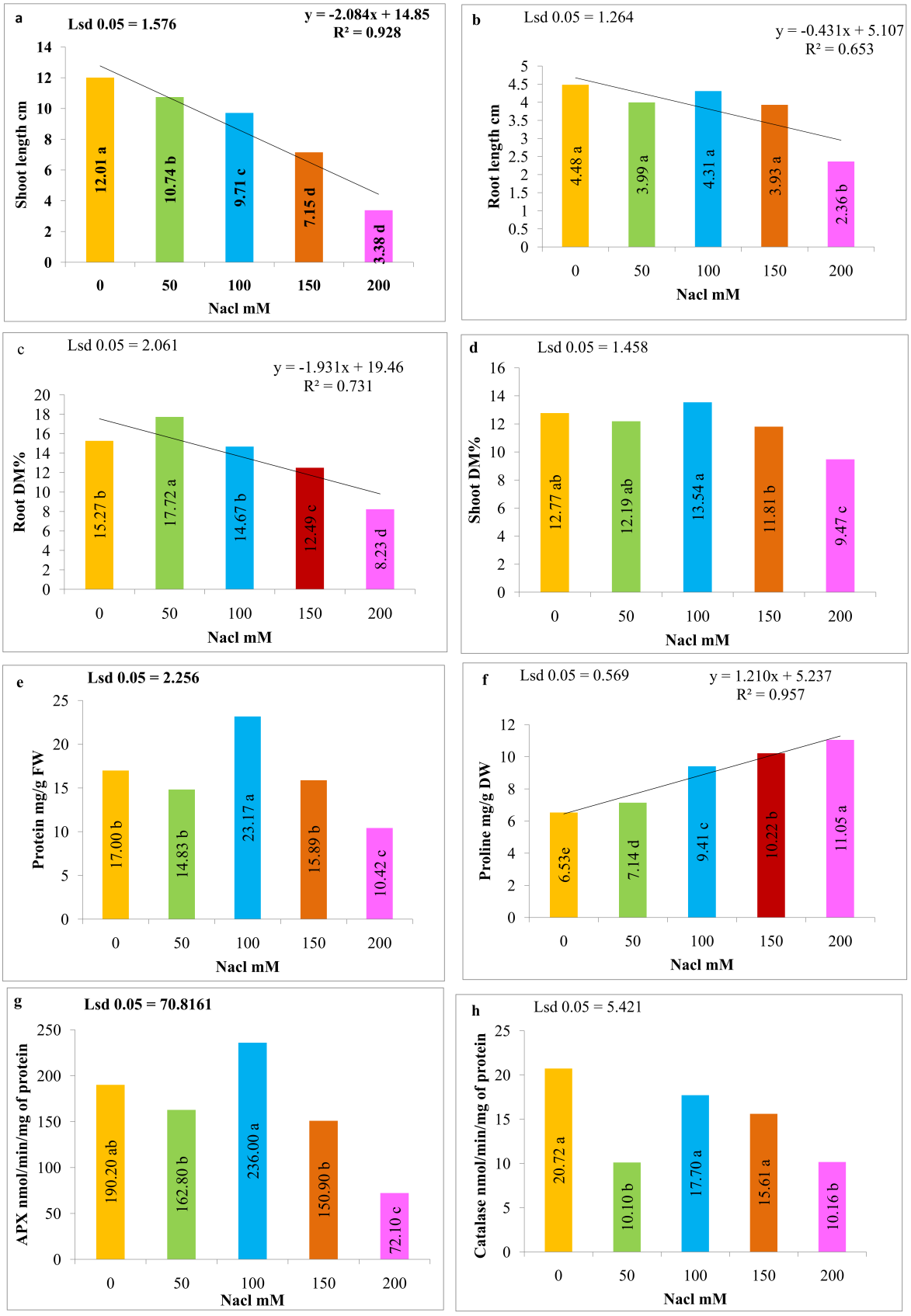


Figure 2. Effect of salt on the morphological and biochemical characteristics of parsley. Bars sharing the same lowercase letter within each parameter are not significantly different according to the L.S.D test $p \leq 0.05$.

the interaction of salicylic acid (SA) concentration, time, and NaCl stress levels. Under control conditions (0 mM NaCl), the shoot DM% ranged from 10.17 to 14.48, with the highest value observed at 15 days with 0.5 mM SA

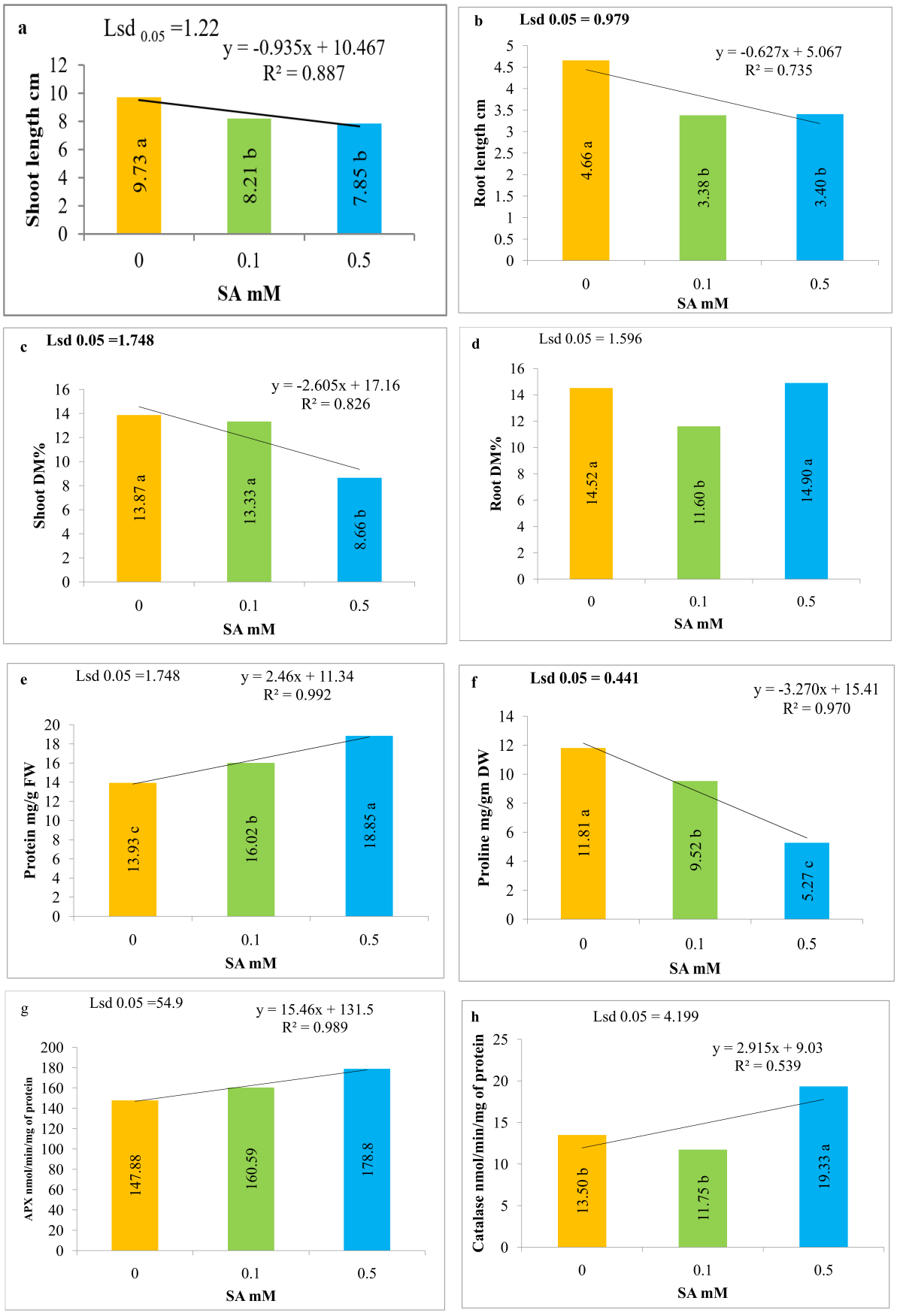


Figure 3. Effect of SA on the morphological and biochemical characteristics of parsley. Bars sharing the same lowercase letter within each parameter are not significantly different according to the LSD test, $p \leq 0.05$.

Table 4. Means root length (cm) of the parsley plants under NaCl × SA × Time interactions.

SA (mM)	Time (Days)	NaCl mM				
		0	50	100	150	200
0	15	1.935 ^{def}	2.656^{bcdef}	5.492^{abcd}	5.501^{abcd}	4.606^{abcde}
	30	4.361^{abcde}	5.846^{abc}	5.642^{abcd}	5.653^{abcd}	4.893^{abcde}
0.1	15	2.493^{cdef}	5.097^{abcde}	4.326^{abcde}	3.817^{abcde}	4.665^{abcde}
	30	5.814^{abc}	4.333^{abcde}	1.678^{ef}	1.567^{ef}	0^{f}
0.5	15	5.946^{abc}	3.829^{abcde}	3.697^{abcde}	7.016^{a}	0^{f}
	30	6.356^{ab}	2.19^{cdef}	5.006^{abcde}	0^{f}	0^{f}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test, $p \leq 0.05$.

(14.48%).

At 50 mM NaCl, the shoot DM% remained relatively stable across SA concentrations and time points, ranging from 10.17 to 13.56 %, with no clear trend. However, at 100 mM NaCl, the shoot DM% showed variability, peaking at 15 days with 0 mM SA (15.4%) and at 30 days with 0.5 mM SA (14.34%) (Table 5).

At 150 mM NaCl, the highest shoot DM% was observed at 30 days with 0 mM SA (15.59%), while other treatments showed moderate values (12.94–14.31%). Notably, at 200 mM NaCl, the shoot DM% exhibited extreme variability (Table 5).

The highest value was recorded at 15 days with 0.1 mM SA (24.45%), which was significantly higher than all other treatments. However, most SA treatments (0.1 and 0.5 mM) at 200 mM NaCl resulted in no shoot dry matter (0), indicating severe stress inhibition. Overall, the data suggest that low to moderate NaCl stress (50–150 mM) can be partially mitigated by SA application, depending on the concentration and time. However, under severe NaCl stress (200 mM), only 0.1 mM SA at 15 days showed a remarkable increase in shoot DM%, while other SA treatments failed to sustain growth. Additionally, longer exposure time (30 days) generally improved shoot DM% under lower NaCl stress but had inconsistent effects under higher stress levels (Table 5).

3.4. .Effect of NaCl, SA, and time on percentage of Root Dry Matter% (RDM%):

In general, the root dry matter percentage (RDM%) of parsley exhibited a significant and linear decline with increasing salinity levels above 50 mM. The highest RDM% (17.73%) was observed in plants treated with 50 mM NaCl. In contrast, roots exposed to 200 mM NaCl accumulated significantly lower root dry matter (8.23%), representing a reduction of approximately 46% compared to the control roots (15.27%) (Figure 22d).

Salicylic acid (SA) also demonstrated a significant effect on RDM%. The application of 0.1 mM SA resulted in a lower RDM% (11.6%), which was significantly reduced compared to both the control and the 0.5 mM SA treatment. This reduction represented a decrease of roughly 20% relative to the control's RDM% (Figure 3d). Conversely, no significant difference in RDM% was observed between plants treated with 0.5 mM SA and the control plants. Additionally, there was no significant variation in RDM% between the two measurement time points (15 and 30 days) (Table 2). However, significant effects were observed in the second- and third-order interactions (Table 2).

Specifically, the three-way interaction (NaCl × SA × Time) in Table 6 revealed that. Under control conditions (0 mM NaCl), root DM% ranged from 8.36 to 33.27, with the maximum value observed at 30 days under 0.5 mM SA (33.27%). This treatment also produced the highest root DM% across all experimental conditions, peaking at 39.36% under 50 mM NaCl at 30 days, highlighting the robust positive influence of 0.5 mM SA on root dry matter accumulation in the absence of severe salt stress.

At 50 mM NaCl, root DM% generally increased with extended exposure time and higher SA concentrations, reaching its peak at 30 days under 0.5 mM SA (39.36%)(Table 6).

Under moderate NaCl stress (100 mM), root DM% exhibited significant variability, with the highest values recorded at 15 days under 0 mM SA (19.67%) and at 30 days under 0.5 mM SA (19.21%). Similarly, at 150 mM NaCl, the maximum root DM% was observed at 15 days under 0.5 mM SA (21.18%), while other treatments showed moderate values ranging from 9.63 to 19.59. In contrast, under severe NaCl stress (200 mM), root DM% was markedly reduced. Most SA treatments (0.1 and 0.5 mM) at 200 mM NaCl resulted in negligible root dry matter accumulation (0), except for 0 mM SA at 15 days (17.33%) and 0.1 mM SA at 15 days (19.21%), which demonstrated relatively higher values (Table 6).

Table 5. Means shoot dry matter (SDM%) of Parsley plants under NaCl × SA × Time interactions.

SA (mM)	Time (Days)	NaCl mM				
		0	50	100	150	200
0	15	12.56^{cdef}	12.58^{cdef}	15.4^{bcd}	14.8^{bcde}	14.93^{bcde}
	30	11.13^{def}	11.92^{cdef}	12.37^{cdef}	15.59^{bc}	17.41^{b}
0.1	15	13.27^{bcdef}	13.55^{bcdef}	14.19^{bcdef}	12.94^{cdef}	24.45^{a}
	30	14.21^{bcdef}	13.56^{bcdef}	13.9^{bcdef}	13.2^{bcdef}	0^{g}
0.5	15	14.48^{bcdef}	11.33^{cdef}	11.01^{ef}	14.31^{bcdef}	0^{g}
	30	10.98^{ef}	10.17^{f}	14.34^{bcdef}	0^{g}	0^{g}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test, $p \leq 0.05$.

Table 6. Means root dry matter (RDM%) of Parsley plants under NaCl × SA × Time interactions.

SA (mM)	Time (Days)	NaCl mM				
		0	50	100	150	200
0	15	8.36^{i}	12.75^{efghi}	19.67^{cd}	19.59^{cd}	17.33^{cde}
	30	10.61^{fghi}	16.25^{cdef}	14.79^{defgh}	13.03^{efghi}	12.83^{efghi}
0.1	15	10.14^{ghi}	11.68^{efghi}	10.36^{fghi}	11.53^{efghi}	19.21^{cd}
	30	15.31^{cdefgh}	16.05^{cdefg}	12.13^{efghi}	9.63^{hi}	0^{j}
0.5	15	13.89^{defghi}	10.22^{fghi}	11.88^{efghi}	21.18^{c}	0^{j}
	30	33.27^{b}	39.36^{a}	19.21^{cd}	0^{j}	0^{j}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test, $p \leq 0.05$.

3.5. Effect of NaCl, SA, and time on protein (mg/g FW):

The impact of sodium chloride (NaCl) and salicylic acid (SA) on protein content in parsley plants was evident and statistically significant. Increasing salinity levels generally led to a notable reduction in protein content.

As illustrated in Figure 2e, the highest protein content (23.17 mg/g FW) was observed in plants treated with 100 mM NaCl, while the lowest value (10.4 mg/g FW) was recorded in plants exposed to the highest salinity level (200 mM NaCl).

The application of SA also significantly influenced protein levels, as indicated by an *F*-probability value of <0.001. Protein content increased notably with higher SA concentrations, demonstrating that the application of 0.5 mM SA was more effective in enhancing protein levels compared to the lower concentration of 0.1 mM SA (Figure 3e).

Additionally, the time factor had a significant effect on protein content. The highest protein level (18.12 mg/g FW) was recorded during the first measurement period (15 days), while the lowest value (14.41 mg/g FW) was observed during the second measurement period (30 days) (Table 2).

Interactions among all studied factors (NaCl, SA, and time) significantly affected protein content in parsley plants, with probability values of <0.001 for all interactions (Table 2).

From Table 7, the highest protein content (38.87 mg/g FW) was observed in plants treated with 0 mM NaCl, 0.5 mM SA, and 15 days, highlighting the optimal conditions for protein synthesis in the absence of salinity stress. In contrast, the lowest protein content was recorded in plants treated with 200 mM NaCl and 0.5 mM SA during both measurement periods (15 and 30 days), resulting in a zero value due to plant mortality.

Notably, the lower SA concentration (0.1 mM) under the same high saline conditions (200 mM NaCl) sustained plant survival and maintained protein content at 30.00 mg/g FW at 15 days, demonstrating the dose-dependent protective role of SA under severe salinity stress. However, at 30 days under 200 mM NaCl and 0.1 mM SA, protein content dropped to 0, indicating that prolonged exposure to high salinity eventually exceeds

Table 7. mean protein (mg/g FW) in parsley plants under NaCl × SA × time interactions.

SA (mM)	Time (Days)	NaCl mM				
		0	50	100	150	200
0	15	6.64^{j}	6.57^{j}	25.65^{cd}	13.03^{fghi}	14.04^{fghi}
	30	10.66^{hij}	16.26^{efgh}	14.8^{fghi}	13.14^{fghi}	18.47^{efg}
0.1	15	15.07^{fghi}	11.56^{hij}	22.1^{de}	21.6^{de}	30^{bc}
	30	8.9^{ij}	12.57^{ghij}	19.2^{ef}	19.14^{ef}	0^{k}
0.5	15	38.87^{a}	16.13^{efgh}	22.05^{de}	28.45^{c}	0^{k}
	30	21.86^{de}	25.91^{cd}	35.25^{ab}	0^{k}	0^{k}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test, $p \leq 0.05$

the plant's tolerance threshold, even with SA application (Table 7).

Control plants, which received neither SA nor NaCl, exhibited significantly lower protein content (6.64 mg/g FW at 0 mM NaCl and 15 days) compared to those treated with SA alone or in combination with NaCl. For example, at 100 mM NaCl and 15 days, protein content increased from 25.65 mg/g FW under 0 SA to 22.1 mg/g FW under 0.1 mM SA and 22.05 mg/g FW under 0.5 mM SA, demonstrating SA's ability to enhance protein accumulation under moderate salinity(Table 7).

At 30 days, protein content under 100 mM NaCl and 0.5 mM SA reached 35.25 mg/g FW, further emphasizing SA's role in sustaining protein synthesis under prolonged moderate stress.

However, at 150 and 200 mM NaCl, protein content declined to 0 under 0.5 mM SA at 30 days, confirming that prolonged high salinity exceeds the plant's tolerance threshold, regardless of SA application (Table 7).

3.6. Effect of NaCl, SA, and time on proline (mg/g DW):

In general, Proline accumulation in parsley exhibited a concentration-dependent response to NaCl stress, with significantly higher levels under elevated salinity (Figure 2f). The 200 mM NaCl treatment yielded peak proline content (11.04 mg/g DW), representing a 69% increase relative to non-saline controls (6.53 mg/g DW). Quantitative analysis revealed proline accumulation followed a biphasic pattern: (i) linear increase (1.210 mg/g DW per mM NaCl, R^2 = 0.957) across all concentrations, and (ii) an additional 12.3% boost per 50 mM above 100 mM.

SA treatment dose-dependently reduces proline accumulation (y = -3.270x + 15.41; R^2 = 0.970), with 0.5 mM SA reducing levels by 62.3% versus untreated controls. This response was temporally invariant (P = 0.674 over 15-30 days; Table 2).

However, significant differences were observed in the second- and third-order interactions (Table 2). The overall three-way interaction (NaCl × SA × Time) analysis exposed in Table 8 revealed significant differences, with a probability value of <0.001 (Table 2).

Plants treated with 200 mM NaCl and 0 SA accumulated the highest proline content (18.8 mg/g DW) after 15 days, demonstrating that high salinity stress significantly increases proline accumulation without SA (Table 8).

Conversely, the application of 0.5 mM SA at 200 mM NaCl during both measurement periods (15 and 30 days) resulted in zero proline content due to plant mortality (Table 8). Similarly, at 150 mM NaCl and 0.5 mM SA, proline content dropped to 0 at 30 days, further confirming the lethal effects of prolonged high salinity under elevated SA levels (Table 8).

Notably, under moderate salinity (100 mM NaCl), proline content varied with SA application and time, for instance, at 100 mM NaCl and 0 SA, proline content increased from 10.9 mg/g DW at 15 days to 11.0 mg/g DW at 30 days. However, with 0.1 mM SA, proline content decreased to 8.2 mg/g DW at 15 days and 8.7 mg/g DW at 30 days, suggesting that SA moderates proline accumulation under moderate stress. At 0.5 mM SA, proline content further decreased to 9.9 mg/g DW at 15 days and 7.8 mg/g DW at 30 days (Table 8).

Control plants (0 mM NaCl and 0 SA) exhibited the lowest proline content (6.6 mg/g DW at 15 days and 7.1 mg/g DW at 30 days), confirming that proline accumulation is primarily a stress-induced response (Table 8).

3.7. Effect of NaCl, SA, and time on APX (nmol/min/mg of protein):

Overall, the application of sodium chloride (NaCl) significantly influenced the levels of ascorbate peroxidase (APX) in parsley plants, as demonstrated in Table 2 and Figure 2g.

Salinity treatments did not result in significant varia-

Table 8. Mean proline content (mg/g DW) of parsley plants under NaCl × SA × time interactions.

SA (mM)	Time (Days)	NaCl mM				
		0	50	100	150	200
0	15	6.599^{jkl}	6.81^{jkl}	10.938^{fg}	16.369^{bc}	18.788^{a}
	30	7.082^{ijkl}	8.494^{hi}	11.002^{fg}	14.348^{d}	17.689^{ab}
0.1	15	$6.093l^{m}$	4.883^{m}	8.181^{ij}	10.373^{g}	13.601^{de}
	30	7.113^{ijkl}	7.725^{ijk}	8.669^{hi}	12.386^{ef}	0^{n}
0.5	15	6.15^{klm}	8.235^{ij}	9.862^{gh}	7.817^{ij}	0^{n}
	30	6.162^{klm}	6.693^{jkl}	7.793^{ij}	0^{n}	0^{n}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test, $p \leq 0.05$.

tions in APX levels compared to the control at concentrations up to 150 mM NaCl. However, plants treated with 100 mM NaCl exhibited the highest APX activity, which differed significantly from all other salinity treatments (Figure 2g). In contrast, plants treated with 200 mM NaCl registered the lowest APX levels, reaching zero values within 30 days due to plant mortality.

The application of salicylic acid (SA) did not lead to significant differences in APX levels, as indicated by a probability value of approximately 0.529 (Table 2 and Figure 2g). Similarly, the time factor did not demonstrate a significant effect on APX activity, with an *F*-probability value of approximately 0.067 (Table 2).

The overall three-way interaction (NaCl × SA × Time) was not statistically significant, as indicated in Table 2, however, the second interaction between SA and NaCl revealed that SA significantly modulated the effect of NaCl, particularly at concentrations up to 100 mM. Specifically, 0.5 mM SA was more effective than other concentrations in influencing APX levels (Figure 4). At 0.5 mM, SA significantly increased APX activity in plants exposed to 50 mM and 100 mM NaCl, while it decreased APX levels in plants subjected to NaCl concentrations exceeding 100 mM (Figure 4).

Columns with different letters indicate significance at a level less than *0.05*

3.8. Effect of NaCl, SA, and time on catalase (nmol/min/mg of protein):

In general, sodium chloride (NaCl) significantly reduced catalase (CAT) activity compared to control plants, particularly under 50 mM and 200 mM NaCl treatments, while the reduction was statistically significant but less pronounced under 100 mM and 150 mM NaCl. Control plants (non-saline conditions) exhibited the highest catalase activity, averaging approximately 20.7 nmol per minute per gram of protein (nmol/min/mg protein). In contrast, plants treated with 200 mM NaCl showed the lowest catalase levels, averaging 10.16 nmol/min/mg protein (Figure 2h).

The application of salicylic acid (SA) significantly influenced catalase activity, as indicated by an *F*-probability value of less than 0.001 (Table 2). Plants treated with 0.5 mM SA exhibited higher catalase levels compared to those treated with 0.1 mM SA, suggesting that the higher concentration was more effective in enhancing catalase activity (Figure 3h). Additionally, catalase levels varied significantly over time, with the highest activity recorded during the first measurement period (15 days) (Table 2).

Significant differences were observed in all second- and third-order interactions among the studied factors, except for the interaction between salicylic acid (SA) and time, which did not yield a significant effect (*F*-probability > 0.05, 0.219) (Table 2).

Analysis of the three-way interaction (NaCl × SA × Time) revealed that the highest catalase activity (48.7 nmol/min/mg protein) was observed in control plants (0 mM NaCl) treated with 0.5 mM SA at 15 days (Table 9).

This value showed no significant difference compared to the same SA concentration at 30 days (38.1 nmol/min/mg protein) or with 150 mM NaCl × 0.5 mM SA × 15 days (40.8 nmol/min/mg protein), indicating that elevated SA levels significantly enhance catalase activity in the absence of salinity or under moderate stress. Conversely, the lowest catalase activity (8.5–8.7 nmol/min/mg protein) was recorded in plants treated with 50 mM NaCl × 0 or 0.1 mM SA × 15 days (Table 9).

Notably, the application of 0.5 mM SA to plants treated with 200 mM NaCl resulted in zero catalase activity due to plant mortality, highlighting the lethal effects of prolonged high salinity under elevated SA levels (Table 9).

Under moderate salinity (100 mM NaCl), catalase activity varied with SA application and time. For instance, at 100 mM NaCl and 0 SA, catalase activity increased from 21.8 nmol/min/mg protein at 15 days to 18.3 nmol/min/mg protein at 30 days. However, with 0.1 mM SA, catalase activity decreased to 5.6 nmol/min/mg

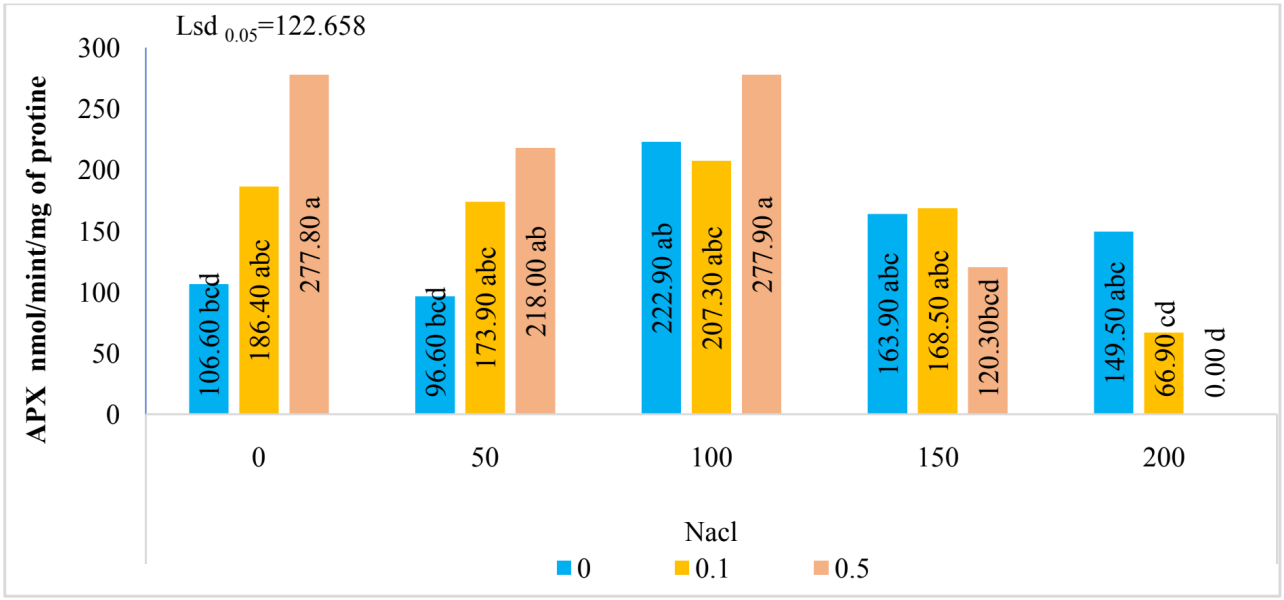


Figure 4. Interaction effects of NaCl and salicylic acid (SA) on ascorbate peroxidase (APX) activity (nmol/min/mg protein) in parsley plants

Table 9. Catalase activity (nmol/mg protein/min) in parsley plants under NaCl × SA × time interaction.

SA (mM)	Time (Days)	NaCl				
		0	50	100	150	200
0	15	7.81^{de}	8.69^{de}	21.77^{cd}	12.33^{cde}	19.76^{cd}
	30	10.22^{cde}	10.26^{cde}	18.28^{cd}	5.93^{de}	19.92^{cd}
0.1	15	11.07^{cde}	8.5^{de}	5.64^{de}	20.55^{cd}	21.31^{cd}
	30	8.37^{de}	9.24^{de}	18.78^{cd}	14.06^{cde}	0^{e}
0.5	15	48.73^{a}	11.85^{cde}	16.2^{cd}	40.78^{a}	0^{e}
	30	38.09^{ab}	12.07^{cde}	25.55^{bc}	0^{e}	0^{e}

Different lowercase letters within a row and column indicate significant differences among the treatments according to Duncan's multiple range test, $p \leq 0.05$.

protein at 15 days but increased to 18.8 nmol/min/mg protein at 30 days, suggesting that SA modulates catalase activity dynamically under moderate stress. At 0.5 mM SA, catalase activity remained relatively high (16.2 nmol/min/mg protein at 15 days and 25.6 nmol/min/mg protein at 30 days), further emphasizing the role of SA in enhancing antioxidant defense under moderate salinity (Table 9).

When comparing the effects of SA and NaCl, catalase levels increased significantly at the second measurement period (30 days) for all SA concentrations across different NaCl levels, except for 150 mM NaCl, where the trend was reversed. For example, at 150 mM NaCl and 0.5 mM SA, catalase activity dropped from 40.8 nmol/min/mg protein at 15 days to 0 at 30 days, indicating that prolonged exposure to moderate-high salinity under elevated SA levels can overwhelm the plant's antioxidant defense system (Table 9).

4. DISCUSSION

Salinity poses a significant challenge to seed germination and plant growth, primarily by hindering water absorption due to osmotic stress caused by Na+ and Cl- ions. This stress reduces shoot length and overall development. Additionally, salt stress disrupts cell division, growth, and nutrient absorption, leading to ionic imbalances. Sodium accumulation can cause toxicity and compete with potassium ions, exacerbating these imbalances and hindering the uptake of essential nutrients such as calcium Ca and K [58, 10, 9, 22, 40] The detrimental effects of salinity on plant growth have been well-documented in previous studies [75, 65, 8, 61]. Furthermore, salinity-induced oxidative stress can significantly impact both root and vegetative growth [12, 61]. This reduction in plant growth

and development is often accompanied by an increase in lipid peroxidation and a decrease in malondialdehyde (MDA) levels, indicating a heightened vulnerability of plant membranes [5, 46]. In addition, ion toxicity, osmotic imbalance, and disruptions in nutrient and ion homeostasis adversely affect photosynthetic pigments, leading to a decline in the photosynthetic rate, as observed in rice [44], and in soybean [62].

Dry matter, a critical physiological parameter, represents the cumulative effect of photosynthesis by the leaf and ion absorption by the root. The alteration in cumulative shoot and root dry matter percentage, compared to control plants, indicates the plant's response to salinity. In this experiment, the response of the experimental plants to mitigate NaCl toxicity was observed.

The shoot and root dry matter percentage in plants treated with NaCl decreased as NaCl concentrations increased. This suggests that Na ions accumulate in the roots without significant transfer to the shoots, resulting in a notable reduction in dry matter compared to the control. Furthermore, the decrease in dry matter accumulation may be linked to the activity of photosynthesis. This is likely due to the suppression of chlorophyll production by the chlorophyllase enzyme, which accelerates pigment degradation. Additionally, ion toxicity and oxidative stress may also contribute to the decline in photosynthetic rate and dry matter [75, 25]. The impact of salinity on photosynthetic pigments is thought to be caused by damage and impaired biosynthesis [71], leading to a reduced carbohydrate content. A decrease in carbohydrates can positively influence salt stress tolerance [42]. Salinity also reduces the activity of ribulose-1,5-diphosphate (Rubisco) carboxylase, which affects carbohydrate synthesis and consequently reduces carbohydrate formation in leaves exposed to salt stress [77].

Moreover, the increase in salt concentration affects the process of photosynthesis and leads to stomatal closure, thereby inhibiting biochemical reactions. This feedback mechanism disrupts carbon metabolism and negatively impacts growth [68]. This may explain the observed reduction in dry matter associated with salinity.

The proline content of stressed plants increased gradually with the increase in salt concentration. Salinity causes a decrease in the total proteins when compared to the control, as indicated by [67] in the *Vicia faba* plant.

Also, the decrease in plant protein content when salt increases goes in the same direction as indicated by [76], and this is in contrast to what was stated by [43], who indicated that the protein content increased when the salt concentration increased, however, we found that the protein was increased under 100 mM only.

Under salt stress, proline accumulation is thought to mitigate the metabolic disturbances induced by salinity [78, 48]. Proline plays several critical roles in plant cells, including osmotic regulation, membrane stabilization, and the detoxification of harmful ions under salt stress conditions [20]. Initially, proline functions as an enzyme protector, safeguarding both enzymes and membrane integrity in plants exposed to stress [20].

The increase in proline content in stressed plants is primarily attributed to its role in counteracting osmotic stress and reactive oxygen species (ROS). Proline stabilizes cell structures and enzymes while maintaining cellular oxidation balance [63]. Notably, proline accumulation often exceeds that of other amino acids, making it a key indicator of stress in plants [27].

In contrast, the increase in certain proteins under stress conditions contributes to osmotic adjustment and plant adaptation [37]. However, the overall protein content in stressed plants often decreases due to the leakage of proteins into the surrounding medium caused by osmotic shock or a reduction in protein synthesis [27]. Thus, the increase in the proline content of the experimental plants can be explained by the fact that it is under the influence of salt stress.

Increased activity of catalase and peroxidase is considered one of the key defense mechanisms plants use to confront stress [9, 37]. Additionally, the elevation of antioxidant enzymes such as APX and CAT in plants is attributed to their role in enhancing resistance to harmful reactive oxygen species (ROS) generated by stress. Superoxide dismutase (SOD) accelerates the conversion of the superoxide anion to O_2 and H_2O_2, the latter of which can hinder natural processes by causing damage to various molecules [6]. However, in this study, the activity of APX increased in plants grown in 100 mM NaCl, while catalase (CA) activity did not show significant differences between the control and 100 or 150 mM NaCl treatments. These findings suggest that parsley may exhibit tolerance to salinity up to 100 mM.

In the present investigation, the application of salicylic acid did not reduce the impact of salinity on root and shoot length characteristics. However, it resulted in root lengths similar to the control when exposed to 100 mM NaCl.

The application of salicylic acid (SA) has been shown to contribute to an increase in root dry matter in parsley under salt stress, particularly when treated with 0.5 mM SA. This suggests that SA enhances the plant's tolerance to salt stress by reducing membrane damage [66]. This effect may be attributed to improved membrane stability, which helps mitigate the deterioration typically observed in salt-stressed plants, thus preserving membrane function [80]. Additionally, this enhancement could be related to increases in soluble sugar content, proline accumulation [82], or the reduction of malondialdehyde (MDA) levels [51, 54]. Our findings are consistent with previous studies on fenugreek [61, 28], parsley, and chickpea [9].

In several plant species, the use of SA has been reported to reduce oxidative damage under salt stress. For instance, studies on *Brassica juncea* [33], Mungbean [70], and wheat [55] demonstrate that SA mitigates toxic

effects by enhancing the activity of antioxidant enzymes [5, 64, 74, 52], a pattern also observed in our study.

Moreover, the application of SA has been found to positively affect protein content under salt stress [72] and to stimulate the activity of antioxidant enzymes such as catalase (CAT) and peroxidase (POX) in plants exposed to salinity [3]. This increase in enzyme activity is a response to the formation of superoxide and hydrogen peroxide, which can be detrimental to plant growth and development.

The results of this study demonstrate the dual role of salicylic acid (SA) in mitigating salinity stress in parsley plants, with its efficacy varying significantly depending on NaCl concentration, SA levels, and exposure time. Under moderate salinity (100 mM NaCl) and shorter exposure (15 days), SA application enhanced growth. However, SA failed to prevent plant mortality, as indicated by zero growth values. This suggests that SA's protective mechanisms are overwhelmed under severe and prolonged stress, highlighting the importance of optimizing salinity levels and exposure duration, contrasting with studies on fenugreek [61], and highlighting the role of genetic factors. Despite reducing electrical conductivity (EC) as shown in Table 1, SA failed to sustain plant viability under high salinity, indicating parsley's salinity tolerance is limited to ~100 mM NaCl. Beyond this threshold, salt stress effects become irreversible.

The combination of SA + 200 mM NaCl caused plant death due to SA's dual role in stress responses. At low concentrations, SA mitigates stress by enhancing antioxidants and osmotic adjustment. However, under extreme salinity, SA acts as a stressor, exacerbating stress effects [36]. When applied with 200 mM NaCl, SA overstimulates stress responses, increasing reactive oxygen species (ROS), disrupting cellular homeostasis, and misregulating hormones. This overactivation, including programmed cell death (PCD), inhibits photosynthesis and depletes energy, pushing plants beyond their tolerance threshold. Additionally, SA combined with salt stress increases ROS, reduces nitric oxide (NO), and activates pathways like ethylene, accelerating cell death [73]. Thus, SA and high salinity create a synergistic stress effect that overwhelms the plant's survival capacity.

5. CONCLUSION

Understanding the tolerance of local plants to salt stress is essential for sustainable development and plant breeding programs. The local genotype of parsley is sensitive to salinity levels exceeding 100 mM. The application of salicylic acid (SA) has been shown to improve parsley plants by mitigating the adverse effects of salt stress and enhancing dry matter accumulation, antioxidant activity, and proline content. However, the combination of SA with high salinity levels did not sustain plant survival, suggesting that SA may not be effective under extreme salt stress conditions. This finding warrants further investigation into the biochemical compounds produced by the plant, such as ethylene and phenols, which may contribute to the observed mortality in SA-treated plants under high salinity, despite their survival under the same salinity levels without SA. Additionally, it may be beneficial to compare the effectiveness of the foliar application of SA with its incorporation into irrigation water to determine the most efficient method of application. We recommend expanding research on the effects of salt stress on various local plant species using modern techniques.

Authors' contributions:

This work was collaboratively conducted by all authors. E.M. designed and performed the experiments, collected data, and prepared the original manuscript draft. I.M. supervised the research, conducted formal analysis, performed software analysis, and contributed to manuscript writing, review, and editing. K.M. provided supervision and contributed to manuscript review and editing. All authors have read and approved the final version of the manuscript.

Competing Interests:

The authors indicate no conflict of interest in this work.

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