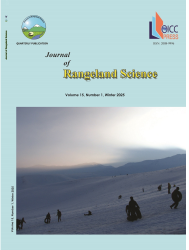

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Effect of salinity stress and salicylic acid on morpho-physiological and growth characteristics of *Satureja mutica* Fisch. & C. A. Mey

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Original Research

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Abstract:

Satureja mutica Fisch. & C. A. Mey is a wild and perennial species which grows in the northwestern, north, and northeastern of Iran, and it is used in health industries and food products. In order to investigating the moderating effect of Salicylic Acid (SA) on morpho-physiological traits of the *S. mutica* under salinity stress conditions, a pot experiment was performed as a factorial experiment based on a Completely Randomized Design (CRD) and three replications at the Kermanshah Agricultural & Natural Resources Research & Education Center, Iran, in 2019. Factor A was NaCl in four levels (0, 50, 100, and 150 mM) and Factor B was salicylic acid at two levels (0 and 2 mM). The results obtained from the analysis of variance showed a significant difference in the studied traits. The salinity stress reduced plant height, leaf area, leaf fresh and dry weight, root fresh and dry weight, shoot fresh and dry weight, relative water content, chlorophyll index, and maximum quantum yield of Photosystem II (PSII) (Fv/Fm value), but increased leaf Electrical Conductivity (EC), and proline content. Soluble protein increased at 50 and 100 mM NaCl, but it significantly decreased at 150 mM NaCl. Salicylic acid caused an increase in plant height (17.54%), leaf area (24.62%), leaf fresh weight (12.41%), leaf dry weight (8.23%), shoot fresh weight (25.87%), shoot dry weight (13.75%), root fresh weight (70.99%), root dry weight (72.38%), relative water content (32.85%), maximum quantum yield of PSII (Fv/Fm value) (10.42%), and chlorophyll index (11.36%) compared to non-SA-treated plants. SA reduced proline content (12.07%) and leaf electrical conductivity (2.37%) in the SA-treated plants compared to non-SA-treated plants. The tolerance threshold for salinity of *S. mutica* was less than 100 mm and in salty soils more than this value, its growth and yield significantly decreases. Foliar spraying with 2 mM salicylic acid in *S. mutica* plants exposed to salt stress, in the early stages of vegetative growth, enhanced the plant's tolerance to salinity and increased plant yield.

Keywords: Chlorophyll index; Photosynthesis; Proline; Maximum quantum yield of photosystem II; Salt stress

Introduction

Salinity decreases leaf water potential, disturbs ion homeostasis, and enhances reactive oxygen species (ROS). Consequently, salinity leads to osmotic and ionic stress (Arif et al., 2020), and salinity-induced oxidative stress that reduces the production of plant dry matter (Mushtaq et al., 2022). Under salinity stress, the osmotic potential of plant cells becomes more negative due to the presence of a high salt concentration in the soil, which creates an osmotic gradient which reduces water absorption to the plant and decreases turgor pressure (Betzen et al., 2019). Leaf relative water content (RWC) is an important indicator of water status in plants. In many plants, a decrease in the relative water content of leaves under different salt concentrations has been reported (Menezes et al., 2017; Arvin and Firuzeh, 2021). Salinity can reduce the photosynthetic pigments, degradation of chlorophyll, decrease in net photosynthesis, and stunted growth (Mahajan and Pal, 2023). The maximum quantum yield of photosystem II (Fv/Fm) and chlorophyll index (SPAD) are useful variables to evaluate photosynthetic activities under stress conditions. Fv/Fm value is a sensitive indicator for the early detection of plant responses to environmental stresses (Bertamini et al., 2019), that provides valuable information for evaluating plant physiological changes under drought stress. Also, chlorophyll index (SPAD) can provide key information regarding photosynthesis and stress resistance (Yang et al., 2023). Fv/Fm shows the electron transfer capacity of photosystem II and also exhibits the quantum efficiency of pure photosynthesis. Photosynthetic performance, Fv/Fm, and SPAD are usually reduced under stress conditions (Wang et al., 2022).

Under osmotic stress, the accumulation of organic osmolytes such as carbohydrates, protein, and proline regulates the osmotic pressure of the cell (Rad et al., 2021). Proline prevents electrolyte leakage, stabilizes protein structure and activity, regulates reactive oxygen species concentration, protects membrane integrity and stabilizes the subcellular structure in stressed plants (Zuo et al., 2022). Accumulation of proline under salt stress conditions has been reported in many plants such amaranth (Menezes et al., 2017). Several proteins accumulate in plants in response to salinity stress. Plant tissues normally respond to salt stress by degrading proteins or producing abundant salt stress related proteins (Athar et al., 2022). Severe stress causes the production of ROSs which leads to destruction of some enzymatic and structural proteins (Wang et al., 2015). Therefore, the change in leaf soluble protein is a proper indicator for investigating the intensity of salt stress on plants. Salicylic acid is one of the plant growth regulators (PGRs) that regulate plant growth and development by triggering many physiological and metabolic processes (Kaya et al., 2023).

Satureja mutica Fisch. & C. A. Mey is a native plant in the Caucasus region, Central Asia and Iran (Karimi et al., 2021) which is distributed in the pastures of the north, northeast, and northwest of Iran. *Satureja mutica* is used in traditional medicine to treat rheumatic pain, migraine, toothache and diarrhea. The phenolic monoterpenes are widely used constituents in pharmaceuticals, cosmetics, and food products (Nieto, 2020). *Satureja* genus is an important commercial source of phenolic monoterpenes (Stahl-Biskup, 2022). Thymol and carvacrol are the best known phenolic monoterpenes. These aroma compounds are employed in medicine for their antibacterial, anti-spasmodic, antioxidant, and anticancer properties (Krause et al., 2021). They also serve as additives to cosmetics and are used in aromatherapy, because of their spicy, warm, and aromatic odors (Krause et al., 2021). Recently, the essential oil of this plant has been used as a food additive in animal feed (Imani et al., 2021). Domesticating of *S. mutica* (figure 1) has attracted the attention of medicinal plant researchers in recent years. The aim of this research was to investigate the ameliorating role of salicylic acid in some morphological, physiological, and photosynthetic traits of *S. mutica* exposed to salt stress.

Materials and methods

The seeds were collected in North Khorasan province, Iran (37°04″ E; 57°29″ N; 1610 m altitude; average rainfall was 347.7 mm; average annual temperature was 13.°C).

Research method

A factorial experiment based on a Completely Randomized Design (CRD) with four levels of salinity (0 – 50 – 100 – 150 mM) and 2 levels of salicylic acid (0 and 2 mM)

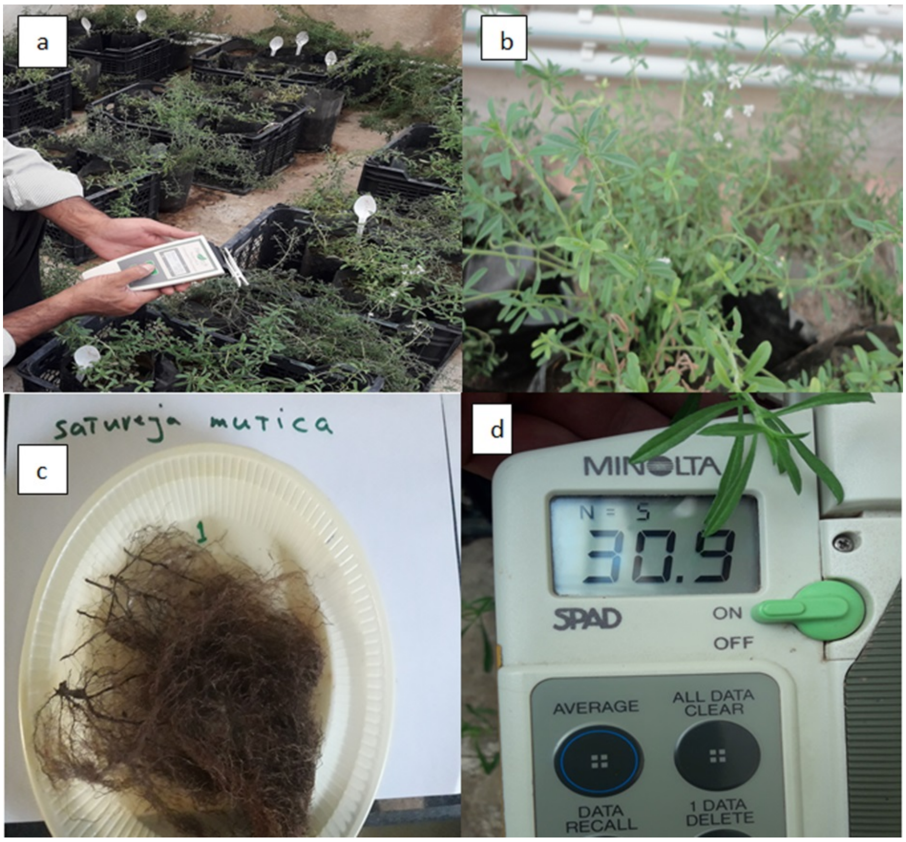

Figure 1. Fv/Fm measurement by chlorophyll fluorimeter (a), *S. mutica* plant (b), and root of *Satureja mutica* exposed to 2 mM SA + 50 mM NaCl (c), and SPAD measurement by chlorophyll meter (d).

was conducted (2019) under control conditions in the Kermanshah Agricultural & Natural Resources Research & Education Center.

Seeds of *Satureja mutica* were obtained from the Research Institute of forests and Rangelands, Tehran, Iran. The seeds were disinfected with 0.5% sodium hypochlorite and washed and dried with distilled water. The seeds were treated with Mancozeb fungicide (2%) and sown in a mixed bed of cocopeat and peat moss (1:1) in a tray. The seeds were irrigated once a day. After seed germination, the seedlings were irrigated every three days by misting. Individual seedlings (four week olds) at 4 to 6-leaf stage (Ou et al., 2018) were moved in the main plastic pots which were filled with 4.5 kg of agricultural soil, sand, and rotted manure (1:1:1).

The plants were subjected to 17 hours of light (300 μmol/m^2/s) and 7 hours of darkness, and relative humidity of $50-60\%$. Before the implementation of salt treatments, the plants were irrigated (once every three days) with 2500 mL of water. In order to adapt plants to salinity, the plants (except salt control plants) were irrigated twice with 20 mM NaCl before performing salt treatments.

The Merck NaCl was used for preparation of 50, 100, and 150 mM NaCl. The plants were irrigated by 250 mL of different NaCl treatments (8 weeks and twice a week). The control plants were irrigated by 250 mL distilled water. After every 4 times irrigation by salt treatments, the plants were irrigated once with distilled water to remove the salts accumulated in the pots. Salicylic acid (HOC_6H_4COOH; CAS: $69-72-7$; Merck; Germany) was used to prepare a concentration of 2 mM. The plants were treated (8 weeks and once a week) with 100 mL of 2 mM SA (foliar spraying). The non-SA-treated plants were sprayed by 100 mL distilled water.

Measurement of traits

The list of trait names with their abbreviation and measurement method presented in Table 1.

Before harvesting the plants, the plant height (cm) was measured. To measure the leaf area, the 30 leaves were separated from each plant and were measured by a leaf area meter, then the average leaf area (mm^2) was calculated (Andalibi et al., 2021). After harvesting the plants, the aerial part was cut from the collar and the shoot fresh weight (g) was determined by weighing. The 30 leaves of each plant were separated and immediately weighed on a scale and the average leaf fresh weight was calculated (LFW). The leaves were dried in an oven (70 °C, 48 h) and the leaf dry weight (LDW) was weighed. The leaves were dried in an oven (70 °C, 48 h) and the leaves dry weight was measured. Shoot dry weight (g) was measured after drying up plant aerial parts at 70 °C, 48 h. The roots were gently washed, and its surface water dried with paper towels, and then the root's fresh weight (g) was measured. The root samples dried at 75 °C, 48 h and root dry weight (g) was measured.

For measurement of Fv/Fm value, 20 leaves from each pot were covered with aluminum foil and were adapted in the dark for 30 minutes. Fv/Fm was estimated using chlorophyll fluorimeter (Hansatech Pocket PEA, UK) at 695 nm. The light level (PFD photon flux level) was 400 μmol/m^2 and the light radiation time was 5 seconds.

Leaf chlorophyll index (SPAD) was measured using a SPAD-502Plus, Minolta, Japan.

Proline content was measured based on (Bates et al., 1973). The OD of plant samples and proline standard was read at 520 nm by Spectrophotometer. Then the OD of each sample was put into the standard equation and was reported as μg/g FW.

Soluble protein concentration (mg/g FW) was measured based on the method of Brdford (Bradford, 1976). 1 μL of the crude leaf extract was added to 200 μL of Coomassie Brilliant Blue. After fifteen minutes, the OD of samples was read at 595 nm Spectrophotometer. The concentration of soluble protein was obtained according to the absorption of the samples and using the Bovine Serum Albumin (BSA) standard curve.

For measurement of Relative water content (RWC), 30 leaves of each plant (approximately isodiametric) were separated and immediately weighed and the average leaf fresh weight was calculated (FW). Then the leaves were immersed in double-distilled water for 16 to 18 hours (22 °C). The surface of leaves was dried with filter paper and the turgor

Table 1. List of trait names with their abbreviation and measurement method.

Name of Traits	Abbreviation	Unit	Measurement method
Plant height	PLH (cm)	Cm	Using a roller
Leaf area	LA (cm^2)	Mm^2	(Andalibi et al., 2021)
Leaf fresh weight	Leaf FW	Mg	Weighing
Leaf dry weight	Leaf DW	Mg	(Vaieretti et al., 2007)
Shoot fresh weight	Shoot FW	G	Weighing
Shoot dry weight	Shoot DW	G	(Vaieretti et al., 2007)
Root fresh weight	Root FW	G	Weighing
Root dry weight	Root DW	G	(Vaieretti et al., 2007)
Maximum quantum yield of photosystem II	Fv/Fm	Unit	Chlorophyll fluorimeter
Chlorophyll index	SPAD	Unit	Spad-502plus
Relative water content	RWC	%	(Bian and Jiang, 2009)
Leaf electrical conductivity	Leaf EC	(ds/m)	(Blum and Ebercon, 1981)
Proline	Proline	Mg/g fw	(Bates et al., 1973)
Protein	Protein	Mg/g fw	(Bradford, 1976)

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Table 2. Analysis of variance of morphological traits in *S. mutica* under different salinity and SA treatments.

Treatments	df	PLH	LA	Leaf FW	Leaf DW	Shoot FW	Shoot DW	Root FW	Root DW
Salinity	3	375.3 **	0.08 **	9.38 **	1.96 **	133.21 **	23.04 **	173.74 **	4.91 **
SA	1	5607 **	0.12 **	8.75 **	0.18 *	68.85 **	1.43	52.16 **	2.45 **
Salinity×SA	3	73.78	0.02	6.05 **	0.12 *	7.23 *	2.37 *	1.16	0.13 *
Error	6	34.13	0.01	1.00	0.03	2.16	0.45	0.58	0.024
CV (%)		11.35	15.24	9.60	10.88	9.70	17.96	9.64	9.56

* and **= significant at 5 and 1% probability levels, respectively. The full name of the abbreviations is presented in Table 1.

weight of leaves was weighed (TW). The leaves were dried in an oven (70 °C, 48 h) and the leaf dry weight (DW) was weighed (Vaieretti et al., 2007). The RWC was calculated from equation (1) (Bian and Jiang, 2009).

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \tag{1}$$

where:
RWC = Relative water content,
FW = Fresh weight,
DW = Dry weight,
TW = Turgor weight.
For electrical conductivity (EC), 30 leaves from each plant were separated and washed with distilled water. The leaves were immersed in 25 mL of double-distilled water (22 °C and 24 h) and the EC (μS/cm) was measured with EC meter (Blum and Ebercon, 1981).

Statistical analysis

Analysis of variance (factorial) and LSD comparison of means (Means ± SD) were performed using IBM SPSS Statistics 26 software. The graphs were drawn using Excel software.

Results

Morphological traits

Results of analysis of variance showed significant differences (P<0.01) between different salinity treatments for all studied traits (Table 2). Effect of SA treatments was significant for plant height, leaf area, leaf fresh weight, shoot fresh weight, root fresh weight, root dry weight (P<0.01) and Also, significant for leaf dry weight (P<0.05) (Table 2). The salinity × salicylic acid interaction effect was significant for leaf fresh weight (P<0.01). Also, this interaction effect was significant (P<0.05) for leaf dry weight, shoot fresh weight, shoot dry weight, and root dry weight (Table 2).

Plant height

The increase in salinity levels caused a decrease in plant height. The plant height was decreased by 2.05%, 8.05%, and 31.53%, respectively, in salt-treated plants with 50, 100, and 150 mM salinity treatments compared to the control plants. The highest plant height (78.33 cm) was obtained in the 2 mM SA + 100 mM NaCl, and the lowest plant height value (45.67 cm) was observed in the 150 mM NaCl (figure 2 (a)). SA had increased the plant height in all the NaCl treatments (figure 2 (a)).

Leaf area

The salinity levels of 100 and 150 mM significantly decreased the leaf area. The average leaf area was significantly decreased by 23.87% and 26.86% in NaCl-treated plants with the 100 and 150 mM salinity treatments, respectively, compared to the control plants (Table 3). The highest leaf area (0.91 cm^2) was observed at 50 mM NaCl + 2 mM SA and the lowest leaf area (0.49 cm^2) was observed at 150 mM NaCl (figure 2 (b)). Salicylic acid was significantly increased in the leaf area in the SA-treated plants compared to non-SA-treated plants by 4.83, 35.62, 43.17, and 14.85% in the 0, 50, 100, and 150 mM NaCl concentrations, respectively (figure 2 (b)).

Leaf fresh weight and leaf dry weight

Leaf fresh weight was significantly decreased by 12.50% at 150 mM NaCl compared to the control plants (Table 3). The highest leaf fresh weight (12.97 mg) was obtained at 50 mM NaCl+ 2 mM SA and the lowest leaf fresh weight (8.67 mg) was observed at 150 mM NaCl (figure 2 (c)). Salicylic acid had increased leaf fresh weight in the SA-treated plants

Table 3. Means ± standard deviation of morphological traits in *S. mutica* plants under different salinity and SA treatments.

Treatments		PLH (cm)	LA (cm^2)	Leaf FW (mg)	Leaf DW (mg)	Shoot FW (g)	Shoot DW(g)	Root FW (g)	Root DW (g)
SA	0 mM	59.7±9.99 b	0.58±0.11 b	9.83±1.01 b	1.53±0.42 b	13.45±4.08 b	3.98±1.87 a	6.41±0.05 b	1.30±0.45 b
	2 mM	69.4±8.50 a	0.72±0.16 a	11.04±2.15 a	1.71±0.66 a	16.84±4.98 a	4.47±1.01 a	9.36±3.27 a	1.94±0.71 a
NaCl	0 mM	66.6±7.37 a	0.67±0.12 a	9.91±1.19 a	1.90±0.24 a	17.81±1.69 a	6.73±0.59 a	14.52±0.84a	2.77±0.17 a
	50 mM	65.3±5.77 a	0.67±0.06 a	10.43±0.52 a	1.92±0.05 a	16.23±2.56 a	3.86±0.88 b	5.42±0.89 b	1.04±0.18 b
	100 mM	61.3±6.11 a	0.51±0.08 b	10.31±0.18 a	1.20±0.10 b	9.57±1.64 b	3.26±0.50 bc	3.21±0.33 c	0.81±0.01bc
	150 mM	45.6±2.08 b	0.49±0.03 b	8.67±1.01 b	1.11±0.11 b	10.2±0.96 b	2.06±0.59 c	2.51±0.24 c	0.58±0.16 c
Mean		59.7±9.99	0.59±0.11	9.83±1.01	1.53±0.42	13.45±4.08	3.98±1.87	6.41±5.05	1.62±0.88
LSD		5.60	0.07	0.74	0.13	1.61	0.58	0.57	0.10

Means of columns with the same letters are not significantly different based on LSD test.
The full name of the abbreviations is presented in Table 1.

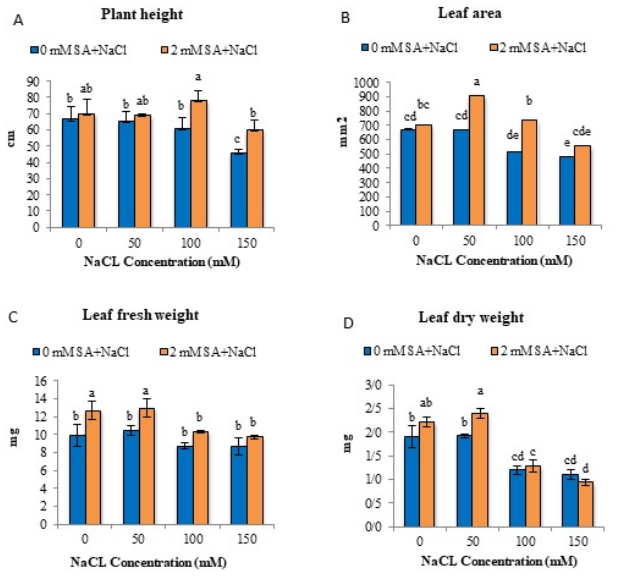

Figure 2. Means of plant height (a), leaf area (b), leaf fresh weight (c), and leaf dry weight (d) of *S. mutica* plants under different treatments of NaCl×SA.
Columns with the same letters are not significantly different based on LSD test (P<0.05).

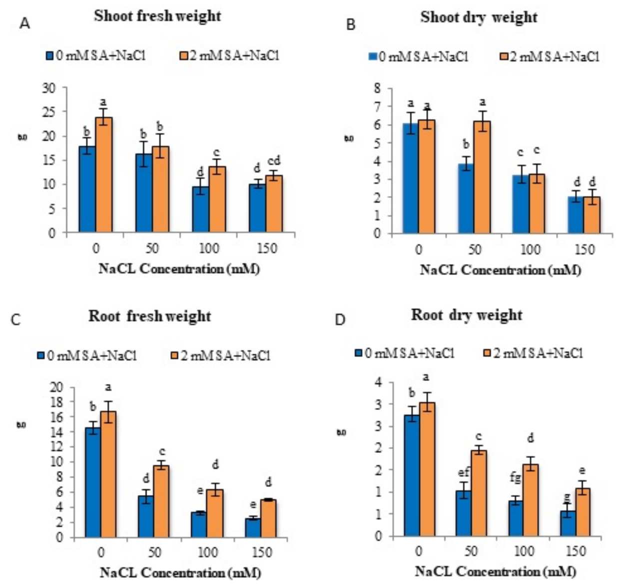

Figure 3. Means of shoot fresh weight (a), shoot dry weight (b), root fresh weight (C), and root dry weight (d) of *S. mutica* plants under different treatments of NaCl×SA.
Columns with the same letters are not significantly different based on LSD test (P = 0.05).

compared to non-SA-treated plants by 28.24, 26.34, and 12.34%, respectively, in the 0, 50, and 150 mM NaCl concentrations. SA decreased the leaf fresh weight by 15.32% in the 100 mM NaCl concentration compared to the non-SA-treated plants. Leaf dry weight was decreased by 36.84 and 41.58% in the 100 and 150 mM NaCl concentrations compared to the salt control plants, respectively, compared to the salt control plants (Table 3). The highest leaf dry weight (2.39 mg) was obtained at 50 mM NaCl + 2 mM SA and the lowest leaf dry weight (1.11 mg) was observed at 150 mM NaCl (figure 2 (d)). Salicylic acid increased leaf dry weight in the SA-treated plants compared to non-SA-treated plants by 16.75, 24.45, and 7.38%, respectively in the 0, 50, and 100 mM NaCl concentrations, but it decreased leaf dry weight by 15.66% in the 150 mM NaCl concentration.

Shoot fresh weight and shoot dry weight

The shoot fresh weight was decreased by 8.82, 46.22, and 42.69% at 50, 100, and 150 mM NaCl respectively, compared to NaCl control (Table 3). The highest shoot fresh weight (24.01 g) was observed in the treatment of 0 mM NaCl + 2 mM SA, and the lowest value (9.57 g) was observed at 100 mM NaCl (figure 3 (a)). Salicylic acid increased the shoot fresh weight in the SA-treated plants by 34.87, 10.35, 42.58, and 15.69%, respectively, in the 0, 50, 100, and 150 mM NaCl concentrations, compared to the non-SA-treated plants. The shoot dry weight was decreased by 42.34, 51.34, and 69.15%, respectively, in the plants treated with the 50, 100, and 150 mM NaCl compared to the salt control plants. The highest shoot dry weight (6.73 g) was obtained at 0 mM NaCl (salt control) and the lowest it (2.03 g) was obtained in the treatment of 150 mM NaCl + 2 mM SA (figure 3 (b)). Application of salicylic acid did not have significant effect on the shoot dry weight in salt control (0 mM NaCl) and high salinity treatments (100 and 150 mM NaCl), but SA increased shoot dry weight by 60.74% in the mild salt stress conditions (50 mM NaCl).

Root fresh weight and root dry weight

Root fresh weight was decreased by 62.60, 77.88, and 82.71%, respectively in the plants treated with 50, 100, and 150 mM NaCl compared to the control (Table 3). The highest root fresh weight (16.68 g) was obtained in the treatment of 0 mM NaCl + 2 mM SA and the lowest value (2.51 g) was observed at 150 mM NaCl (figure 3 (c)). Salicylic acid increased root fresh weight by 15.01, 76.34, 95.53, and 97.07%, respectively, in the 0, 50, 100, and 150 mM NaCl concentrations compared to the non-SA-treated plants. Root dry weight decreased by 62.98, 71.07, and 79.17%, respectively, in the plants treated with 50, 100, and 150 mM NaCl compared to the control plants. The highest root dry weight (3.73 g) was obtained in the treatment of 0 mM NaCl + 2 mM SA and the lowest (2.07 g) was obtained at 150 mM NaCl (figure 3 (d)). Salicylic acid increased the root dry weight by 9.29, 88.10, 104.11, and 88.02%, respectively in the 0, 50, 100, and 150 mM NaCl concentrations compared to the non-SA-treated plants (figure 3 (d)).

Physiological traits

Results of analysis of variance showed significant differences (P<0.01) between salinity treatments for all studied traits (Table 4). Significant differences between SA treatments (P<0.01) were observed for Fv/Fm, RWC, leaf electric conductivity, proline and protein content. Also, significant differences (P<0.05) were observed between SA treatments for SPAD (Table 4). The salinity by salicylic acid interaction effect were significant for SPAD, Fv/Fm, RWC, leaf electrical conductivity, proline, and soluble protein content (P<0.01) (Table 4).

Table 4. Analysis of variance of physiological traits in *S. mutica* under different salinity and SA treatments.

Treatments	df	Fv/Fm	SPAD	RWC	Leaf EC	Soluble protein	Proline
Salinity	3	0.01 **	271.80 **	553.63 **	194526.21 **	386.66 **	0.1**
SA	1	0.03 **	36.75 *	463.75 **	245863.54 **	55.71 **	0.01 **
Salinity × SA	3	0.01 **	76.72 **	185.37 **	48962.21 **	42.11 **	0.1 **
Error	6	0.0001	5.19	8.72	83.29	2.36	0.002
CV (%)		0.41	6.59	3.53	3.57	5.99	24.96

* and **= significant at 5 and 1% probability levels, respectively. The full name of the abbreviations is presented in Table 1.

Maximum quantum yield of Photosystem II (Fv/Fm value)

Fv/Fm value was decreased by 4.56, 10.59, and 16.75%, respectively in the salt-treated plants with 50, 100, and 150 mM NaCl concentrations compared to control plants (Table 5). The highest Fv/Fm (0.81 units) was observed at 0 mM NaCl (control) and the lowest value (0.66 units) was observed at 150 mM NaCl (figure 4 (a)). Salicylic acid increased Fv/Fm by 2.24, 4.86, 12.93, and 21.64%, respectively, at 0, 50, 100, and 150 mM NaCl concentrations compared with non-SA-treated plants (figure 4 (a)).

Leaf chlorophyll index (SPAD)

Increase in salinity up to 100 mM increased the amounts of chlorophyll index. SPAD was decreased by 12.7, 21.48 and 31.90%, respectively in the 50, 100, and 150 mM NaCl concentrations compared to the control plants (Table 5). The highest SPAD (36.63 units) was observed at 100 mM NaCl + 2 mM SA and the lowest value (25.87 units) was observed at 150 mM NaCl (figure 4 (b)). Salicylic acid enhanced leaf chlorophyll index by 19.20 and 28.6, and 23.1%, respectively, in the 50, 100, and 150 mM NaCl concentrations compared to the non-SA-treated plants (figure 4 (b)).

Leaf proline content

Increase of salinity up to 100 mM, sharply increased the proline content, but 150 mM NaCl did not show any trend in the increase or decrease in proline content. The proline content was increased by 483.46, 976.92, and 976.92%, respectively in the salt-treated plants with 50, 100, and 150 mM NaCl compared with the non-NaCl-treated plants (Table 5). The highest level of proline content (14.00 μg/g) was observed at 150 mM NaCl and the lowest proline content (1.00 μg/g) was observed in the 2 mM SA without salt (figure 4 (c)). Salicylic acid decreased the proline content

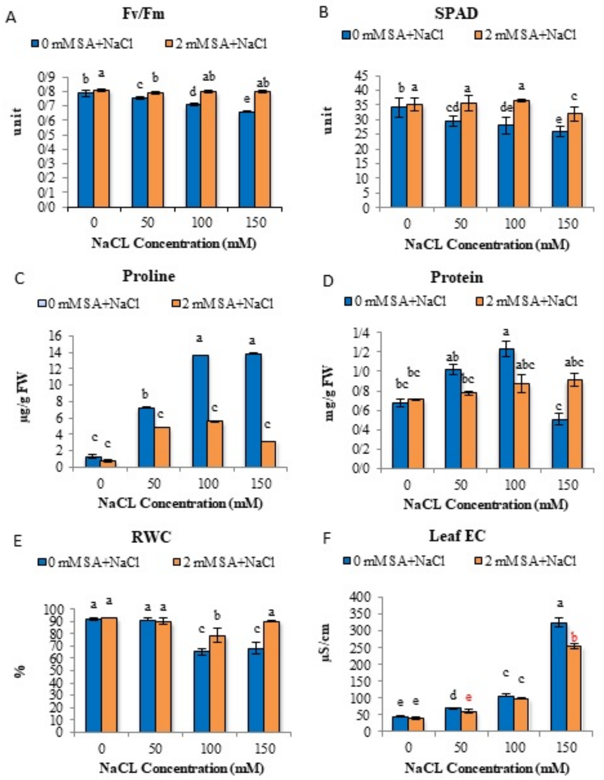

Figure 4. Means of Fv/Fm (a), SPAD (b), proline content (c), soluble protein content (d), RWC (e), and leaf electric conductivity (f) of *S. mutica* plants under different treatments of NaCl×SA.
Columns with the same letters are not significantly different based on LSD test (P= 0.05).

Table 5. Means comparison of physiological traits in *S. mutica* plants under different salinity and SA treatments.

Treatments		Fv/Fm (unit)	SPAD (unit)	RWC (%)	Leaf EC (μS/cm)	Proline (μg/g FW)	Protein (mg/g FW)
SA (mM)	0	0.72±0.05 b	29.56±7.12b	79.2±13.18b	137.5±11.59a	9.75±0.18 a	0.86±0.05 a
	2	0.79±0.01 a	33.35±3.42a	88.01±6.36a	113.7±8.82 b	2.00±0.08 b	0.81±0.03 b
NaCl (mM)	0	0.79±0.02 a	34.10±1.59a	91.66±0.88a	45.4±1.98 e	1.28±0.02 c	0.68±0.04 c
	50	0.75±0.01 b	30.27±2.92a	91.56±0.88a	70.2±1.97 d	7.00±0.04 b	1.02±0.05 b
	100	0.71±0.01 c	28.07±3.17b	65.51±2.67b	108.1±3.57c	13.99±0.03a	1.23±0.09 a
	150	0.66±0.003d	25.86±1.80b	68.13±4.52b	324.7±14.14a	14.00±0.02a	0.51±0.06 d
Mean		0.76±0.05	29.56±7.12	83.61±11.07	125.3±101.38	4.28±0.50	0.84±0.24
LSD		0.002	1.39	1.81	9.24	0.08	0.03

Means of columns with the same letters are not significantly different based on LSD test.
The full name of the abbreviations is presented in Table 1.

by 23.08, 71.43, 271.43, and 257.14% respectively in the 0, 50, 100, and 150 mM NaCl concentrations compared to non-SA-treated plants (figure 4 (c)).

Soluble protein content:

By increasing salinity up to 100 mM NaCl, the amount of leaf soluble protein increased, but it was decreased at 150 mM NaCl. Leaf soluble protein was increased by 50.00 and 80.88% in the NaCl-treated plants with 50 and 100 mM NaCl, respectively, but it decreased by 25% in the NaCl-treated plants with 150 mM NaCl compared to the non-NaCl-treated plants (Table 5). The highest soluble protein was found at 100 mM NaCl (1.23 mg/g) and the lowest value (0.51 mg/g) was observed at 150 mM NaCl (figure 4 (d)). Application of salicylic acid in non-Salt stress conditions (0 mM NaCl) increased the soluble protein by 5.88%. SA decreased the soluble protein by 26.47, 29.27, and 78.43%, respectively at 50, 100, and 150 mM NaCl concentrations compared to non-SA-treated plants (figure 4 (d)).

Relative water content (RWC)

The relative water content was decreased by 28.57, and 25.70%, respectively in the 100 and 150 mM NaCl concentrations compared to non-NaCl-treated plants (Table 5). The highest RWC (93.00%) was observed at 2 mM SA without salt and the lowest RWC (65.51%) was observed at 100 mM NaCl (figure 4 (e)). Salicylic acid did not have a significant effect on the RWC in the salt control plants and plants treated with 50 mM NaCl. SA increased the RWC by 20.15 and 32.85%, respectively, in the 100 and 150 mM NaCl concentrations compared with the non-SA-treated plants (figure 4 (e)).

Leaf electrical conductivity

The leaf electrical conductivity was increased by 56.01, 140.37, and 621.74%, respectively in the 50, 100, and 150 mM NaCl concentrations compared to the non-NaCl-treated plants (Table 5). The lowest EC (40.88 μS/cm) was observed in 2 mM SA without salt and the highest EC (324.78 μS/cm) was observed at 150 mM NaCl (figure 4 (f)). Salicylic acid decreased the EC by 38.71, 14.28, 8.92, and 21.37%, respectively in the 0, 50, 100, and 150 mM NaCl concentrations compared to the non-SA-treated plants (figure 4 (f)).

Discussion

Morphological traits were affected by salt stress. Salt stress negatively affected the leaf area and plant height earlier than other morphological traits (Su et al., 2013). In *S. mutica* plants an increase in salinity levels caused a decrease in plant height. In line with this finding, salinity stress has reduced the plant height in *Satureja khuzestanica* plants (Saadatfar and Jafari, 2022). In this study, Salicylic acid increased plant height in the SA-treated plants compared to non-SA-treated plants. In a recent study, the application of SA had increased the plant height in *Lantana camara* (Ardakani et al., 2021) at different salinity levels. In present research, salinity decreased leaf area and salicylic acid increased it. Similar to our result, salinity stress had reduced the leaf area in *Thymus vulgaris* (Harati et al., 2015). SA had increased the leaf area in *S. hortensis* (Pourghadir et al., 2021).

Leaf fresh and dry weights were increased in the low salinity levels, but they decreased in the high NaCl concentrations. In line with our findings, Leaf fresh and dry weight were decreased in *Pyrus betulifolia* Bunge under salt stress (Yu et al., 2019). Salicylic acid had increased leaf fresh and dry weights in the SA-treated plants compared to non-SA-treated plants. In some other species such *Solanum melongena* L. (Mady et al., 2023) salicylic acid had increased leaf fresh and dry weights.

We found that shoot fresh and dry weight decreased in the *S. mutica* plants in the salt stress conditions. Also, salicylic acid significantly increased shoot fresh and dry weight. Similar to our result, shoot dry weight was decreased in different salinity levels in *Amaranthus cruentus* (Menezes et al., 2017). Salicylic acid had improved the shoot dry weight in *Lantana camara* at high salinity levels compared to the control (Ardakani et al., 2021). These results were consistent with our observations.

The root fresh and dry weights were decreased in the plants treated with NaCl. Similarly, a decrease in root dry weight has been observed in *Amaranthus cruentus* plants treated with 50, 75, and 100 mM NaCl (Menezes et al., 2017). Salicylic acid increased root fresh and dry weight in the SA-treated plants under salt stress conditions. Similar to this, Salicylic acid has increased the root fresh and dry weight in *Thymus vulgaris* plants under salinity stress conditions (Harati et al., 2015).

Chlorophyll index is a proper factor to evaluate the plant's photosynthetic potential (Yang et al., 2023). In our research, chlorophyll index (SPAD) was significantly decreased in the high NaCl concentrations (Khattab 2007). In *Satureja khuzestanica* plants the chlorophyll content has significantly been reduced by increasing the salt concentration (Saadatfar and Jafari, 2023). Salicylic acid increased leaf chlorophyll index in the SA-treated *S. mutica* plants at all NaCl concentrations compared to the non-SA-treated plants. In some other plants such as St. John's wort, salicylic acid had increased the photosynthetic index under salt stress conditions (Kwon et al., 2023).

Fv/Fm value decreased in the NaCl-treated plants compared to control. High levels of salinity had decreased Fv/Fm in Nitere Bush (*Nitraria schoberi* L.) Plants (Ranjbar-Fordoei and Dehghani-Bidgoli, 2016). Salicylic acid increased Fv/Fm in the SA-treated plants compared with non-SA-treated plants in all NaCl concentrations. In line with our finding, salicylic acid had increased the Fv/Fm in *Hypericum perforatum* (Kwon et al., 2023) under salinity stress conditions.

The relative water content (RWC) was decreased in the salt-treated plants and SA increased its value in the SA-treated plants compared to the non-SA-treated plants. In *Trigonella foenum-graecum* L. (Arvin and Firuzeh, 2021) salinity had decreased RWC. Application of salicylic acid had increased the RWC in *Lantana camara* (Ardakani et al., 2021) under salinity conditions.

Salinity stress critically increased leaf electrical conductivity and salicylic acid decreased it under salt stress conditions. In line with this finding, salt stress had increased the relative electrical conductivity (REC) in *Dianthus superbus* plants, and the application of salicylic acid effectively reduced it (Ma et al., 2017).

In salinity stress, plants adjust their osmotic potential by accumulating osmolytes to escape from dehydration. In the present study, the proline content was increased significantly by salinity levels. Similarly, proline content was significantly increased by rising salt levels in *Satureja khuzestanica* (Saadatfar and Jafari, 2023).

Salicylic acid significantly reduced proline content in the *S. mutica* under severe salt stress conditions. Similar to our result, SA application had reduced proline content in *Nigella sativa* L. (Zarei et al., 2019) under salt stress conditions.

By increasing salinity up to 100 mM NaCl, the amounts of leaf soluble protein were increased, but it value was decreased at 150 mM NaCl concentrations. Protein content in *Thymus vulgaris* was increased in mild salt treatments and decreased in severe salt stress conditions (Harati et al., 2015). In our research, application of salicylic acid had increased the soluble protein content in control and low and moderate salt stress conditions, but it decreased the soluble protein in high salt stress conditions. Salicylic acid had increased leaf soluble protein under low and moderate salt stress conditions in *Thymus vulgaris* (Harati et al., 2015). These results confirm the validity of our findings.

Conclusion

Salinity stress decreased the relative water content, and increased the electrical conductivity of the cell, subsequently disturbed the physiological, photosynthetic and biochemical functions of the cell, and as a result, reduced the growth and yield of the *Satureja mutica* plants. Application of 2 mM salicylic acid modulated the effects of salinity stress by increasing the relative water content and improved the physiological, photosynthetic and biochemical functions of the cell, and subsequently increased plant growth and yield. The threshold of salinity tolerance of *Satureja mutica* was less than 100 mM, and in soils more salty than this value, its yield significantly decreased. Foliar spraying with 2 mM salicylic acid (four times) in the early stages of vegetative growth, increases the plant's tolerance to salinity and significantly increases its yield in low salinity and mild salinity soils.

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Authors contributions

Authors have contributed equally in preparing and writing the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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