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ORIGINAL PAPER

Impact of Silicon Foliar Application on the Growth and Physiological Traits of *Carthamus tinctorius* L. Exposed to Salt Stress

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Abstract
Althought safflower is a tolerant crop against many environmental stresses, but its yield and performance reduce under stress. The aim of this experiment was to investigate the effect of silicon (Si) application on the possibility of increasing salinity resistance and related mechanisms in safflower. A greenhouse experiment was conducted to investigate the effects of Si spraying (0, 1.5 and 2.5 mM) on safflower plants grown under salt stress condition (non-saline and 10 dS m^{-1}). Salinity reduced seedling emergence percent and rate, growth parameters and disrupted ion uptake but increased emergence time and specifc leaf weight. Spraying of Si increased plant height, fresh and dry weight, leaf area, relative water content (RWC), potassium, calcium and silicon content, while sodium absorption was decreased. As a result, the K^+/Na^+ and Ca^{2+}/Na^+ ratios were increased. Elevated ion contents and ratios indicate an enhanced selectivity of ion uptake following silicon application and may increase ion discrimination against Na^+. Treatment with 2.5 mM Si showed the most positive effect on the measured growth traits. Decrement in leaf area ratio under salinity indicates a more severe effect of salinity on leaf area compared to biomass production. On the other hand, silicon reduced the specific leaf weight under stress and non-stress conditions, which revalues the positive effects of silicon on leaf area expansion. Improvement of RWC may a reason for the icrease in leaf area and biomass production. Data shows that spraying with Si especialy with 2.5 mM can reduce salinity stress damage to safflower and increase biomass production.

Keywords Dry weight · Leaf area ratio · Relative water content · Specific leaf area · Ionic relations

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1 Introduction

Soil salinization can reduce and limit agricultural lands and adversely affect crop plant growth and yield production. Salinity disturbs metabolism and plant structure through a combination of complex reactions. Salinity impacts normal plant growth and development through osmotic stress, ion toxicity, nutrient imbalance, and oxidative stress [1–4]. In green tissues, the accumulation of Na^+ causes a wide range of osmotic and metabolic dysfunction in normal plant performance, so damage to the leaves is always greater than that to the roots. The accumulation of sodium in leaves is known as the main reason for this problem. A disorder in nutrient uptake in response to salinity stress may directly be due to the disturbance in the uptake of other essential elements by interfering with the transporters localized in the cell membrane and the inhibition of root growth by the destructive effects of sodium on soil structure and osmotic stress [1, 5–8].

One approach to reduce the harmful effects of salinity stress is supplementation of plant nutrition with trace elements such as silicon. Studies have shown that the application of silicon can significantly reduce damage from salinity and drought stresses and has beneficial effects on plant growth and yield [9–11]. Mechanisms such as deposition of toxic sodium ions [12], a decrease in sodium absorption, an increase in potassium uptake [9, 10], and an increase in the selectivity of potassium/sodium [13] have been suggested for the application of silicon in salinity resistance. The element uptake and transport due to the application of silicon may be affected through two different mechanisms. The first mechanism is deposition of silicon in the cell wall, which can reduce apoplastic uptake of some elements through the roots and decrease their transport in the transpiration stream. Therefore, silicon may reduce the uptake of some elements that mainly use the apoplastic pathway [14]. The second mechanism states that silicon may improve the function and integrity of cell membranes and result in increased nutrient uptake and transport [12].

Carthamus tinctorius L. (*C. tinctorius,* safflower) is cultivated as an important oilseed crop in arid and semiarid regions. This crop is known as a salt-tolerant plant. Therefore, it is planted in areas where salinity is a major threat to crop production [15]. It has been shown that silicon foliar application improved some growth parameters, such as plant height, canopy dimension, stem diameter, and number of seeds per capitulate, in safflower [16]. The beneficial effects of silicon on increasing salinity stress tolerance in sorghum [17], dill [18], and canola [19] have also been reported.

Despite the studies on salinity effects on safflower and evaluating the possible mechanisms of salt tolerance in some safflower cultivars, it seems that the effect of silicon foliar application as a trace element in safflower salt tolerance has not been studied so far. Therefore, the current experiment was planned under greenhouse condition with the objective to explore the ameliorating effects of Si on plant growth and growth indexes, morphophysiological attributes and mineral uptake along with ions homeostasis in safflower (cultivar Goldasht) challenged with salt stress.

2 Materials and Methods

2.1 Plant Materials and Growth Conditions

Safflower seeds (cultivar Goldasht) were obtained from Seed and Plant Research Institute, Oil Seeds Department (Karaj, Iran). Seeds were disinfected by carboxythyram fungicide and sown in individual pots at a density of 15 seeds per pot. Each pot was filled with 10 kg of soil in proportions of 6:3:1 of field soil, sand, and manure. The soil was a silty clay type and contained pH 7.5, EC 2.19 dS m^{-1}, available P 17.5 mg kg^{-1}, available K 182 mg kg^{-1}, Na 21.85 mg kg^{-1}, Ca 14 mg kg^{-1}, total N 0.069%, cation exchange capacity (CEC) 19.81 $cmol^+$ kg^{-1}. The needed salt was calculated and added to each pot from NaCl. Finally, salted pot's EC checked with an EC meter. The pots were irrigated with purified water with an EC of 380 µs.cm^{-1}. Seedlings were thinned after establishment, and five plants were kept in each pot. During the growing season, the soil moisture of the pots was maintained at 85–90% of the field capacity. Light intensity during the experiment was adjusted using natural and artificial light in the range of 10 to 14 klux and photo periods of the 16/8 day/night regime. The greenhouse temperature ranged from 25 to 28 °C during the day and 14 to 16 °C at night.

2.2 Experimental Design and Treatments

This study was conducted in a factorial experiment based on a randomized complete block design with three replications under greenhouse conditions (Fig. 1). Factors include salinity with two levels of 2.19 as a control and 10 dS.m^{-1} were provided using NaCl salt and spraying with silicon at three levels of 0.0, 1.5, and 2.5 mM in the form of potassium silicate. The pH of potassium silicate was adjusted to 7.0 using HCl (1 M) and NaOH (1 M), which could favor chemical compatibility if mixed with plant protection products. Foliar spraying with different concentrations of silicon was performed at the 3- to 4- leaf stage. At the 12-leaf stage and before the stem jointing stage, plants were harvested for further analysis.

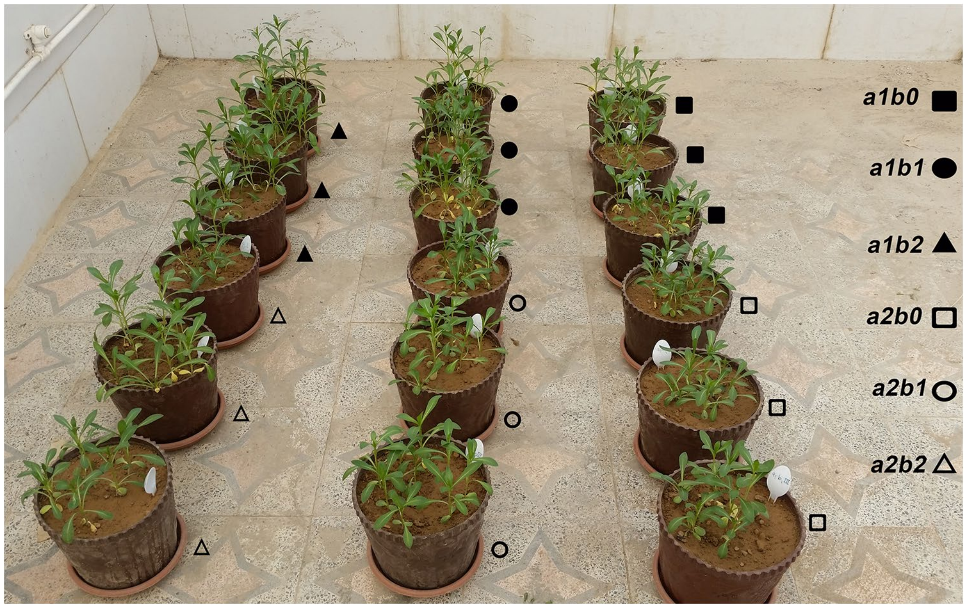

Fig. 1 Potted cultures of *Carthamus tinctorius* L. a1, control (no salt stress); a2, saline stress. b0, b1, and b2 correspond to the treatments with 0, 1.5, and 2.5 mM potassium silicate, respectively

2.3 Seedling Emergence Parameters

The number of emerged seedlings was counted daily. Counting of each pot was completed when the number of emerged seedlings remained constant for five consecutive days. The emergence rate was calculated using Eq. 1 as follows:

$$ER = N/\sum(ni \times di) \tag{1}$$

where ER is the emergence rate, ni is the number of emerged seedlings on day di, and N is the total number of emerged seedlings.

The final emergence percent (EP) was obtained from the last number of emerged and established seedlings to the total sown seeds.

$$EP = (ni/N) \times 100 \tag{2}$$

ni is the number of emerged seedlings, and N is the total number of sown seeds.
Emergence index and mean emergence time:

The emergence index (EI) was calculated by the modified equation of Benech Arnold and colleagues [20] for germination (Eq. 3):

$$EI = (dn - 1 \times E1) + (dn - 2 \times E2) + (dn - 3 \times E3) + \ldots \tag{3}$$

where dn is the last day on which all seedlings emerged and En is the number of seedlings that emerged on the n^{th} day.

Mean emergence time (MTE) was calculated by the equation for germination (Eq. 4).

$$MTE = \sum(nd)/\sum n \tag{4}$$

In Eq. 4, n is the number of emerged seedlings on the d^{th} day, d is the number of days from the beginning of emergence, and $\sum n$ is the total number of emerged seedlings.

2.4 Phenotypic and Physiological Trait Measurements

2.4.1 Growth Parameters

Plant height, fresh and dry weight of shoots (cotyledons, leaves, and stems) and roots were measured for harvested plants. After harvesting, the contents of the pots were transferred to a five mm sieve, and roots were gently separated from the soil and washed with tap water to remove soil residue from the roots. Towel paper was used to absorb excess moisture from the surface of the roots. The fresh weight of roots was measured and incubated at 75 °C for 48 h to determine their dry weight.

2.4.2 Physiological Parameters

Relative leaf water content (RWC): At the 12-leaf stage, fully developed penultimate leaves were sampled. Leaf samples were weighed, and their fresh weight was recorded. Leaf samples were immersed in distilled water for 12 h to calculate turgid weight. Then, they were dried in an oven at 70 °C for 24 h, and the RWC was calculated according to Eq. 5 [21]:

$$RWC = [(FW - DW)/(TW - DW)] \times 100 \quad (5)$$

Chlorophyll content index (CCI): In all pots, the CCI of penultimate leaves from all five plants of pots was measured with a manual chlorophyll meter (SPAD model 502 Minolta, Japan).

Leaf area: After harvesting, cotyledon and rosette leaves were detached from the stems, their area was scanned with a scanner (HP-LaseJet Pro MFP), and then their area was calculated with ImageJ software. The leaf area ratio and specific leaf weight were calculated according to Eqs. 6 and 7 [22]:

$$\text{Leaf Area Ratio (LAR)} = \text{Leaf Area (cm}^2\text{)}/\ \text{Total Dry Weight (mg)} \quad (6)$$

$$\text{Specific Leaf Weight (SLW)} = \text{Leaf weigh (mg)}/\text{Leaf Area (cm}^2\text{)} \quad (7)$$

2.4.3 Mineral Elements

Leaves of each treatment were carefully washed after harvest and dried in an oven at 55 °C to obtain dry matter. Elements were measured by wet digestion with sulfuric acid, salicylic acid, and hydrogen peroxide. Eighteen milliliters of distilled water was poured into a 250 cc Erlenmeyer flask, and 100 ml of sulfuric acid concentrate (96%) was added frequently in small volumes. Then, 6 g of salicylic acid was added to the solution. A total of 0.3 g of ground plant sample was weighed and poured into a digested flask, and 2.5 ml of the acid mixture was added. The balloons were heated to 120 °C for one hour. The samples were cooled to room temperature, and 0.5 ml of hydrogen peroxide was added. This procedure was continued until the sample became transparent. Samples were transferred to a 50 ml volumetric balloon, and the volume was adjusted with distilled water. The potassium, calcium, and sodium contents were measured by a flame photometer (Jenway PFP7, England). The Si content was measured with the molybdo silicic acid method [23] and by an atomic absorption device.

2.5 Statistical Analysis

Statistical analyses were performed after normalization and data transformation using SAS software (version 9.1) and MSTATC software. It is worth noting that as explained in the above section, salt was added to the pots of salt treatments before the experiment was started, but silicon was sprayed after the establishment of seedlings. Therefore, data of emergence percent, emergence rate, and emergence index were analyzed based on a randomized complete block design with 9 replications. Mean comparisons were performed with Tukey's test at the 5% probability level.

3 Results and Discussion

Salinity is considered the major threat to agriculture and impacts crop production. Salt stress can inhibit or delay seed germination and seedling establishment [24–28]. It has been reported that seed germination and seedling establishment are the most critical stages of safflower and affected by salinity [29, 30].

Analysis of variance of emergence percent, emergence rate, emergence index and mean emergence time showed that salinity stress had a significant effect ($P \leq 0.001$) on the mentioned traits (Table 1). Comparison of the mean values showed a significant decrease in the percent, rate, and emergence index of seedlings under salinity stress. The seedling emergence percentage decreased by 38.5% under salinity stress. The emergence rate and index showed 54.4% and 53% decreases under salinity stress, respectively. In contrast, the mean time of emergence under salinity stress increased by 38.4% (Table 2).

Salinity stress at all stages of plant growth can be detrimental, but the early establishment of the plant has a decisive effect on the final yield. Therefore, salinity stress at the seedling stage can be very unfavorable for plant establishment [31] and the final plant population. As the osmotic potential of soil under salinity conditions decreases, seed water uptake decreases during the imbibition phase. Salinity can also affect seed performance by the toxic effects of

Table 1 Analysis of variance of emergence and establishment traits in safflower under salinity stress

Mean of squares					
S.O.V	df	Emergence Percent	Emergence Rate	Emergence Index	Mean time of emergence
Rep	8	28.39 ns	0.00069 ns	162.88 ns	1.479 ns
NaCl	1	3380.24 ***	0.0375 ***	6123.55 ***	26.33 ***
Error	8	35.80	0.00078	179.55	1.513
CV (%)	-	7.05	13	15.1	22.4

***, $P \leq 0.001$; ns, not significant

Table 2 Mean comparison of evaluated traits in safflower under salinity stress

Stress level	Emergence Percent (%)	Emergence Rate	Emergence Index	Mean time of emergence (day)
Control	98.5 ± 0.98	0.2591 ± 0.0039	106.7 ± 1.054	3.9 ± 0.06
Salt stress	71.1 ± 2.48	0. 1678 ± 0.0122	69.8 ± 6.07	6.3 ± 0.57

Data represents the average of nine replicates (n = 9) ± standard error. Mean comparison was performed using Tukey's test at 5% probability level

sodium and chloride ions on seed viability and vigor [32]. Under abiotic stresses, lipid peroxidation may be one of the most important factors leading to the inhibition of seed germination [16]. Experiments showed that germination percent, germination index, and seed vigor decreased under salinity stress, while the mean time of germination increased significantly in different safflower cultivars [29, 33].

3.1 Phenotypical and Physiological Traits of Safflower Plants

3.1.1 Growth Parameters

Salinity and silicon application alter plant height and stem dry weight. Salinity stress, silicon foliar application and their interaction significantly affected plant height (Table 3). Salinity stress caused a 46.6% reduction in plant height compared to the control treatment. However, silicon foliar application significantly compensated for plant height reduction under stress (Table 4). The highest plant height was observed under non-stress conditions and in foliar spraying with 2.5 mM silicon (20.7 cm), which was significantly different from other treatments. The lowest height was related to non-sprayed plants under stress conditions (Table 5).

Under salinity stress, photoassimilates are mainly allocated to the pathways that are necessary to cope with the stress. Therefore, the normal development of plants is compromised, and as a result, various plant characteristics, such as plant height, are affected [1]. Decreased plant height due to salinity stress has been reported in safflower [15] and wheat [34]. However, the application of silicon compensated for the negative effects of salinity stress, and plant height improved with increasing silicon concentration compared to the untreated plants [34].

Salinity stress also significantly reduced stem dry weight. Application of silicon compensated for the reduction in stem dry weight under stress conditions (Table 6). The highest and lowest dry weights of the stem were observed in the 2.5 mM silicon treatments under non-stress conditions (21 mg/plant) and without spraying of silicon under stress conditions (10.3 mg/plant), respectively (Table 6). Salinity stress and silicon foliar application and their interaction on root fresh and dry weight were also found to be significant (Table 3). Foliar application with 1.5 mM silicon exhibited the highest fresh and dry weight of roots under non-stress conditions. Under salinity stress, the highest fresh and dry weight of roots was recorded in the 2.5 mM silicon treatment, which was significantly different from the other two levels of silicon (Table 6). Foliar application with 2.5 mM silicon increased root fresh and dry weight by 53.4% and 61.2%, respectively, compared to the non-treated plants under stress conditions. In general, salinity stress reduced the fresh and dry weight of roots. Therefore, 68.7 and 96.2% decreases in the fresh and dry weights of roots were observed in plants under salinity stress and without silicon spraying compared to the control (Table 6).

Salinity stress severely inhibits normal root and shoot growth [35]. There is evidence that suggests that the application of silicon plays an important role in growth, mineral nutrition, mechanical strength, and resistance to various stresses [11]. Reduction of root and stem dry weight in the presence of sodium chloride due to sodium ion toxicity has been reported [36, 37]. The application of silicon reduced the harmful effects of sodium chloride and improved the growth of rapeseed due to the reduction of sodium uptake and transport to the shoot, maintaining the integrity of the root cell membrane and reducing lignification [38]. It has been shown that the addition of silicon to the nutrient solution under salinity stress improved the growth and development of tomato plants [39]. In line with the observed positive effects of silicon application, our results showed that foliar application of silicon significantly reduced the detrimental effects of salinity stress and improved many growth-related traits under normal and salinity stress conditions (Tables 5, 6 and 7).

3.1.2 Phenotypical Effects on Leaves

Cotyledons of dicotyledonous plants play a dual functional role during early seedling establishment. They function as sources of food reserves and perform photosynthesis [40]. Upon germination, extension of the axial hypocotyl forces out the cotyledons above ground where they unfold, expand, and establish photosynthetic machinery and hence play an important role in the transition from the heterotrophic to photoautotrophic stage in plants. The results from different species showed that removal or damage to cotyledons during

Table 3 Analysis of variance of growth traits of safflower, cv Goldasht, under salinity stress and silicon foliar application. Independent ANOVAs were performed for each trait

Mean of squares											
S. O. V	df	Height	Fresh weight of cotyledon	Dry weight of cotyledon	Cotyledon area	Fresh weight of leaf	Dry weight of leaf	Leaf area	Dry weight of Stem	Fresh weight of root	Dry weight of root
Replication	2	0.291 ns	19.77 ns	1.97 ns	0.313*	765.15 ns	4.584 ns	5.26 ns	0.315 ns	220.5 ns	0.356 ns
NaCl	1	162.0***	7775.04***	98.300***	16.051***	124,861.14***	2152.96***	732.86***	125.98***	109,294.2***	1333.6***
Silicon	2	32.66***	4932.07***	28.722***	2.04***	62,917.08***	542.3***	146.42***	42.77***	10,023.53***	109.07***
interaction	2	2.66*	191.32 ns	4.50 ns	0.410*	35,610.15***	107.06***	10.80*	0.129 ns	12,520.34***	34.97**
Error	10	0.575	92.71	1.225	0.069	765.49	1.816	2.68	0.129	650.3	5.53
CV(%)	-	5.17	8.56	9.78	6.31	3.87	1.62	4.81	2.31	6.14	7.46

*, ** and *** represent significant differences at the probability level ($P \leq 0.05$), ($P \leq 0.01$), ($P \leq 0.001$) respectively. ns; not significant

Table 4 Analysis of variance of physiological traits and ions content of safflower, cv. Goldasht, under salinity stress and silicon foliar application

Mean of squares											
S. O. V	**df**	SLW	LAR	RWC	CCI	K	Ca	Na	Si	K^+/Na^+	Ca^+/Na^+
Replication	**2**	0.0109 ns	0.00035 ns	10.52 ns	0.313 ns	0.00307 ns	0.00000057 ns	0.00000006 ns	0.000018 ns	3.199 ns	0.0017 ns
NaCl	**1**	0.379***	0.0175***	707.31***	330.116***	2.172***	0.000322***	0.000755***	0.0278***	10,435.15***	1.212***
Silicon	**2**	0.085***	0.00146***	273.94***	103.76***	1.129***	0.000089***	0.000119***	0.0462***	3294.67***	0.321***
interaction	**2**	0.045*	0.00356***	53.41**	44.97***	0.0561**	0.0000297***	0.0000019*	0.00146*	295.98***	0.125***
Error	**10**	0.0108	0.00016	9.61	5.44	0.00763	0.0000034	0.00000038	0.00029	17.20	0.00604
CV(%)	-	4.17	4.11	4.09	4.66	4.51	10.54	3.88	4.12	6.29	12.86

*, ** and *** were significant at the probability level ($P \leq 0.05$), ($P \leq 0.01$), ($P \leq 0.001$) respectively, and ns not significant. SLW, Specific leaf weight; LAR, Leaf area ratio; RWC, Relative water content; CCI, Chlorophyll index

Table 5 Mean comparison of growth traits of safflower, cv. Goldasht, under salinity stress and foliar spraying with silicon

Stress levels	Silicon (mM)	Height (cm)	Cotyledon fresh weight (mg)	Cotyledon dry weight (mg)	Cotyledon area (cm^2)	Leaf fresh weight (mg)	Leaf dry weight(mg)	Leaf area (cm^2)
Control	0	14.66 ± 0.167 c	101.88 ± 6.45 c	10.98 ± 0.498 bc	4.156 ± 0.211 b	670.39 ± 18.98bc	85.41 ± 1.28 b	34.56 ± 1.89 c
	1.5	17.66 ± 0.67 b	135.0 ± 3.91 b	13.03 ± 1.33 b	5.446 ± 0.318a	720.17 ± 23.3 b	87.23 ± 1.008 b	39.64 ± 0.482 b
	2.5	20.66 ± 67 a	162.83 ± 5.34 a	16.92 ± 0.28 a	5.745 ± 0.053 a	1003 ± 2.52 a	110.01 ± 0.47 a	46.94 ± 1.26 a
Salt stress	0	10.000 ± 0 e	59.63 ± 4.43 d	7.48 ± 0.3 d	2.854 ± 0.08 c	609.72 ± 7.97 c	68.27 ± 0.797 d	24.70 ± 0.74 d
	1.5	11.66 ± 0.333 de	105.05 ± 3.05 c	9.18 ± 0.56 cd	3.38 ± 0.14c	624.44 ± 21.06 c	70.38 ± 0.581 d	26.38 ± 0.25 d
	2.5	13.33 ± 0.167 cd	110.33 ± 6.84 bc	10.25 ± 0.442 bcd	3.439 ± 0.215 bc	659.67 ± 10.66 bc	78.392 ± 0.843 c	31.78 ± 0.492 c

Data represents the average of three replicates (n = 3) ± standard error. Mean comparisons were performed using Tukey's test at 5% probability level. In each column same letter(s) indicate no significant difference at 5% probability level

Table 6 Means comparison of physiological traits of safflower, cv. Goldasht, under salinity stress and foliar spraying with silicon

Stress levels	Silicon (mM)	Stem dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)	SLW (mg/cm^2)	LAR (cm^2/g)	RWC (%)	CCI
Control	0	15.44 ± 0.19 c	464 ± 15.84 b	36.3 ± 0.87 b	2.483 ± 0.112 abc	0.309 ± 0.014 bc	76.82 ± 2.36 b	48.22 ± 0.68 cd
	1.5	18.04 ± 0.36 b	540 ± 13.63 a	42.2 ± 1.52 a	2.202 ± 0.051c	0.371 ± 0.007 a	79.83 ± 1.56 b	59.04 ± 1.57 a
	2.5	21.06 ± 0.092 a	475 ± 12.16 ab	41.96 ± 2.13 a	2.347 ± 0.055 c	0.339 ± 0.0087 ab	89.38 ± 0.73 a	55.72 ± 1.23 ab
Salt stress	0	10.38 ± 0.132 e	275 ± 21.78 c	18.5 ± 0.811 d	2.77 ± 0.056 a	0.252 ± 0.007 d	60.08 ± 0.55 c	42.44 ± 1.63 d
	1.5	12.85 ± 0.19 d	313 ± 8.67 c	20.43 ± 0.64 d	2.667 ± 0.0133 ab	0.274 ± 0.004 cd	74.13 ± 1.3 b	44.16 ± 1.33 cd
	2.5	15.43 ± 0.31 c	422 ± 4.84 b	29.83 ± 0.817 c	2.467 ± 0.0135 bc	0.305 ± 0.0014 bc	74.20 ± 2.99 b	50.67 ± 0.55 bc

Data represents the average of three replicates (n = 3) ± standard error. Means in each column followed by the same letter(s) are not significantly different at 5% probability level, using Tukey's test. SLW, Specific leaf weight; LAR, Leaf area ratio; RWC, Relative water content; CCI, Chlorophyll index

Table 7 Means comparison of ions contents of safflower, cv. Goldasht, under salinity stress and foliar spraying with silicon

Stress levels	Silicon (mM)	K (%)	Ca (%)	Na (%)	Si (%)	K^+/Na^+	Ca^+/Na^+
Control	0	1.83 ± 0.04 d	0.0173 ± 0.0013 bc	0.0307 ± 0.00027 d	0.356 ± 0.005 c	59.6 ± 1.42 c	0.57 ± 0.04 bc
	1.5	2.36 ± 0.03 b	0.0192 ± 0.0004 b	0.0259 ± 0.00059 e	0.492 ± 0.0142 ab	91.3 ± 3.13 b	0.742 ± 0.03 b
	2.5	2.66 ± 0.06 a	0.0287 ± 0.0018 a	0.0223 ± 0.00015 f	0.523 ± 0.004 a	118.9 ± 3.63 a	1.29 ± 0.087 a
Salt stress	0	1.204 ± 0.03 e	0.0120 ± 0.0002 d	0.0436 ± 0.0003 a	0.289 ± 0.0127 d	27.6 ± 0.926 d	0.275 ± 0.0019 d
	1.5	1.45 ± 0.02 e	0.0127 ± 0.00038 cd	0.0399 ± 0.0003 b	0.378 ± 0.0049 c	36.2 ± 0.337 d	0.317 ± 0.01 d
	2.5	2.110 ± 0.08c	0.0151 ± 0.0008 bcd	0.0342 ± 0.00018 c	0.468 ± 0.0085 b	61.7 ± 1.933 c	0.442 ± 0.024 cd

Data represents the average of three replicates (n = 3) ± standard error. Means in each column followed by the same letter(s) are not significantly different at 5% probability level, using Tukey's test

the early growth phase has detrimental effects on plant size and total physiological performance [41].

The effect of salinity stress and silicon spraying on cotyledon fresh and dry weight and area was found to be significant (Table 3). Salinity stress caused a significant decrease in the fresh and dry weight and area of safflower cotyledons. Silicon spraying improved these growth characteristics under stress and non-stress conditions (Table 5). The highest fresh and dry weight and cotyledon area under stress and non-stress conditions were found for the treatment of 2.5 mM silicon. Spraying with 2.5 mM silicon increased the dry weight of cotyledons by 37% compared to non-application of silicon under stress conditions (Table 5). However, a 38.2% increase in cotyledon area was observed after foliar application of 2.5 mM silicon under control conditions.

Salinity stress, silicon spraying, and their interaction showed significant effects on leaf fresh and dry weight and area (Table 3). The highest fresh and dry weights of leaves were observed after treatment with 2.5 mM silicon under control conditions (1003 and 110 mg/plant). In contrast, the lowest leaf fresh and dry weight was observed in the stress treatment without silicon foliar application (609 and 68 mg plant^{-1}). The highest leaf area under both stress and non-stress conditions was related to 2.5 mM silicon spraying

(Table 5). Spraying of 2.5 mM silicon under control conditions increased leaf area by 35.8% compared to the control treatment (Table 5).

Restriction in water uptake under salt stress may reduce turgor pressure, which inhibits leaf expansion mainly through repression of *EXPANSIN* genes. EXPANSINS are cell wall-loosening proteins engaged in many developmental and physiological processes, including stem and leaf growth [42]. Several studies have shown that *EXPANSIN* genes are regulated by environmental cues and modulate plant tolerance to various abiotic stresses, including drought [43] and salinity [44]. The results showed that under salinity stress, leaf area was negatively affected compared to normal conditions. Moreover, another harmful effect of salinity on the growth rate may be due to a reduction in plant photosynthesis and resource allocation towards the regulation of osmotic potential. Therefore, the accumulation of dry matter in plants may be reduced. Studies have shown that a decrease in leaf area is an immediate response of plants to salinity stress, which leads to the inhibition of leaf expansion with increasing salinity concentrations [1, 45]. Application of silicon in basil decreased sodium uptake and transport to aerial parts of plants and therefore reduced its toxicity and increased plant growth indexes such as fresh and dry weight of shoots [46].

3.2 Physiological Traits

Application of silicon improved the negative effects of salinity stress on physiological traits. Salinity stress, silicon foliar application and their interaction exhibited significant effects on the relative water content (RWC) (Table 4). RWC was reduced under salt stress conditions, while silicon foliar application improved RWC under stress and non-stress conditions. The highest and lowest RWC values were recorded after foliar application with 2.5 mM silicon under non-stress conditions and non-spraying of silicon under stress conditions, respectively (Table 6).

In rocket plants, RWC significantly decreased under salinity stress conditions. There was a positive correlation between RWC and osmotic potential. In addition, the study showed that any change in osmotic potential is a coping mechanism against stress through the accumulation of osmolytes and is considered a defense mechanism against salinity stress [47]. It is suggested that the increasing water content of the plant after the application of silicon is due to silicon precipitation in the form of silica in the apoplast of the epidermal cell wall of leaf tissue and may reduce water loss through the stomata [12].

The chlorophyll content index (CCI) was also affected by salinity and silicon spraying and their interaction (Table 4). Salinity decreased CCI, while silicon spraying increased this index under stress and non-stress conditions. Mean comparisons showed that CCI was improved after silicon foliar application even under non-stress conditions (Table 6). The lowest CCI was related to the treatment without silicon foliar application under stress conditions. However, foliar application with 2.5 mM silicon under stress conditions improved CCI by 5.08% compared to the control (Table 6).

Two mechanisms are proposed to decrease chlorophyll concentration under salinity stress conditions: chlorophyll degradation due to oxidative damage and reduced nitrogen uptake as the most important element for chlorophyll synthesis. Application of silicon can improve plant defense systems and increase the photochemical efficiency of photosystem II by detoxifying free radical species induced under salinity stress [12]. The negative effect of salinity stress on chlorophyll content in safflower has been reported [15]. Salinity stress reduced the chlorophyll and carotenoid content of tomato plants compared to the control, but the treatment of stressed plants with silicon was shown to improve plant performance and increase the amount of chlorophyll and carotenoids [39, 48, 49].

Analysis of variance revealed that the leaf area ratio (LAR) was affected by salinity stress, silicon spraying and their interactions (Table 4). Salinity caused a negative effect on LAR, but spraying silicon recovered this effect (Table 6). Since a direct relationship exists between LAR and leaf area [22], any decrease in LAR and dry matter accumulation under salinity stress can be due to a decrease in leaf area. Although both leaf area and dry matter were negatively affected under salt stress, LAR measurements showed that the reduction in leaf area was more pronounced than that in dry matter production. Plant height, stem diameter, leaf number, leaf dry weight, specific leaf area, and leaf area ratio in eggplant plants irrigated with saline water were affected by salinity, but the greatest effects were observed for the leaf number and area [25, 50, 51].

Among the other physiological traits, specific leaf weight (SLW) was also found to be affected by salinity, silicon spraying, and their interaction (Table 4). Salinity stress led to an increase in SLW. The highest SLW was observed in the plants under salt stress without silicon treatment, which was not significantly different from 1.5 mM silicon. Silicon foliar application caused a reduction in SLW under both stress and non-stress conditions. The reduction in SLW with the spraying of silicon under control conditions can explain the greater effect of this element on leaf area expansion than on leaf dry weight (Table 6).

An explanation for the increase in SLW under salinity stress is the decrease in leaf area and the accumulation of sodium and chloride ions in the leaves. Furthermore, an elevated SLW value is correlated with increasing numbers of mesophilic layers, which increases leaf thickness. The results showed that spraying silicon can improve the leaf area, leaf number, leaf thickness, and water content of leaves to some extent, which is probably due to less accumulation of toxic ions in leaves. Since LAR is explained as the division of leaf area to total plant weight and SLW is the division of leaf weight to leaf area, a decrease in LAR and an increase in SLW under salinity conditions was reasonable. Reduction in leaf area and increment of SLW is reported in soybean leaves under salt stress [52]. It has also been shown that the application of silicon increased leaf area and SLA in canola [19].

3.3 Leaf Mineral Content

Salinity stress and silicon foliar application affected mineral concentrations in safflower. Salinity stress, silicon foliar application and their interaction revealed a significant effect on leaf sodium, potassium, calcium, and silicon content as well as K^+/Na^+ and Ca^{2+}/Na^+ ratios (Table 4). Salinity stress increased the sodium ion concentration. Although the Na^+ concentration increased significantly under salinity stress, the K^+, Ca^{2+} and Si concentrations decreased (Table 7). Mean comparison of mineral contents showed that foliar application with 2.5 mM silicon significantly increased K, Ca, Si and K^+/Na^+ and Ca^{2+}/Na^+ ratios of safflower plants under stress and non-stress conditions. The lowest amount of K, Ca, Si and the ratio of K^+/Na^+ and Ca^{2+}/Na^+ were related to the non-foliar treatment under stress conditions (Table 7). Spraying with 2.5 mM silicon increased the K^+/Na^+ ratio compared to the control under non-stress conditions. However, under stress conditions, an increase in the Ca^{2+}/Na^+ ratio was observed after foliar application with 2.5 mM silicon compared to non-foliar application (Table 7).

By increasing the salinity levels in the rhizosphere, the absorption and transfer of toxic ions to plant tissues will be increased, which in turn negatively affects the absorption of other essential elements. Decreased absorption of essential elements will result in disturbance of ionic balance and toxicity due to accumulation of sodium and chloride ions [1, 53]. Potassium plays an important role in the regulation of osmotic potential in root tissue, which is necessary for the maintenance of cell turgor pressure, transport of xylem sap, and balancing plant water relations [9, 10]. Exclusion or inhibition of sodium uptake by roots are suggested mechanisms to reduce the rate of salt accumulation in leaf tissue. Depending on the species, the toxic effects of sodium due to the inability to exclude or excrete this element became visible in leaves after days or weeks [5].

Decreased levels of calcium and potassium in the aerial part are prominent effects of salinity, which causes symptoms of calcium deficiency and impaired protein synthesis under salinity stress [54]. Calcium is an important element in maintaining the structure and function of cell membranes in the maintenance of cell wall integrity, selective regulation of ion transport and ion exchange and initiation of signal transduction cascades during stress conditions [55–57]. Competition between potassium and sodium under salinity stress significantly reduced the potassium content in safflower leaves. In addition, safflower dry weight shows a negative correlation with leaf and root sodium concentrations and a positive correlation with potassium content [58]. Leaf sodium concentration and K^+/Na^+ ratio were suggested as the most important indicators to identify safflower tolerant cultivars against salinity stress [59]. According to Gengmao and colleagues, the calcium content in tolerant safflower roots increased significantly because of salinity stress [15]. The authors stated that more calcium accumulation in the roots under saline conditions could be considered one of the most important factors to reduce the accumulation of sodium in the roots and increase potassium content in the shoot, which will result in more selective uptake and transfer of potassium versus sodium. With increasing NaCl concentration in two cultivars sensitive and resistant to salinity of chickpea, the calcium and silicon content of roots and leaves decreased significantly [60].

It has been reported that silicon treatment can moderate the uptake and transport of heavy metals and salts from roots to shoots by reducing the apoplastic pathway [12]. Application of silicon increased the concentration of silicon in the shoots of *Zea mays* L. [61]. Foliar application with silicon was effective in increasing the accumulation of silicon in the shoots of cotton [62]. In barley, the application of silicon increased potassium transport and improved the K^+/Na^+ ratio by activating the H^+-ATPase pump [10]. In addition, silicon can reduce osmotic stress by inhibiting sodium uptake as well as reducing its transport to the shoot [63]. Sodium uptake and transport by roots were greatly reduced by the application of silicon under salinity stress. Therefore, an increase in salinity tolerance by silicon spraying is attributed to the selective uptake and transport of potassium versus sodium by plants. Under salinity stress conditions, foliar application of silicon decreased sodium and increased potassium content in the shoots of wheat plants [64]. Salinity stress induced Na^+ uptake and a decrease in K^+ and Ca^{2+} contents and in K^+/Na^+ ratio in the leaves of sorghum. The application of silicon decreased the Na concentration and increased the K and Ca contents and the K^+/Na^+ ratio [17]. Application of silicon increased the silicon content of chickpea leaves and roots under salt stress and non-stress conditions [60].

4 Conclusions

Salinity stress negatively affected all evaluated traits and indexes in safflower. Silicon foliar application improved plant functions under stress and non-stress conditions. Spraying silicon increased the plant height and leaf area of the treated plants. It is reasonable that this increase related with increasing the relative water content. Furthermore, upon silicon spraying, biomass production was improved, which indicates the positive effect of this element on plant performance. On the other hand, increasing the ratio of K^+/Na^+ and Ca^{2+}/Na^+ indicates that silicon may increase plant selectivity during salt stress. Altogether, our data showed that foliar application with 2.5 mM silicon improved the metrics of all analyzed traits under both stress and non-stress conditions in safflower plants. The outcomes of the study provide useful information to get minimum damage and maximum establishment, resulting in the better production from salt-affected land under current global warming predictions.

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Declarations

Conflict of Interest The authors report that there are no competing interests to declare.

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