BMC Plant Biology

RESEARCH Open Access

Enhancing salt stress tolerance in *Carthamus tinctorius* L. through selenium soil treatment: anatomical, biochemical, and physiological insights

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Abstract

Selenium (Se) plays a crucial role in ameliorating the negative impact of abiotic stress. The present study was performed to elucidate the efficacy of soil treatment of Se in reducing salt-induced stress in *Carthamus tinctorius* L. In this study, three different levels of Na_2SeO_4 (0, 0.01, and 0.02 g kg^{-1}) and four levels of NaCl (0, 0.5, 1.5, and 2.5 g kg^{-1}) were applied. The findings revealed that while NaCl decreased seed germination parameters, growth characteristics, K^+ content, relative water content (RWC), and photosynthetic pigments, it increased Na^+ content, soluble carbohydrates, H_2O_2 content, and malondialdehyde (MDA) level. The application of Se showed a positive effect on seed germination and growth characteristics under salinity conditions, which is linked to alterations in anatomical, biochemical, and physiological factors. Anatomical studies showed that treatment with Se led to increased stem diameter, cortical parenchyma thickness, and pith diameter under salinity stress. However, variations in the thickness of the xylem and phloem did not reach statistical significance. The application of Se (0.02 g kg^{-1}) raised Na^+ content (7.65%), K^+ content (29.24%), RWC (15%), Chl *a* (17%), Chl *b* (21.73%), Chl a + b (16.9%), Car (4.22%), and soluble carbohydrates (11%) in plants subjected to NaCl (2.5 g kg^{-1}) stress. Furthermore, it decreased H_2O_2 (25.65%) and MDA (11.9%) in the shoots. The findings of the current study advocate the application of the Se-soil treating technique as an approach for salt stress mitigation in crops grown in saline conditions.

Keywords Abiotic stress, Asteraceae, Germination, NaCl, Oxidative stress

Introduction

Salinity on a global scale is a major abiotic stress that is very challenging to the agricultural sector, causing huge losses in crop production [1]. Soil quality has been negatively affected over time through the extensive use of chemicals, fertilizers, and other factors related to alterations in rainfall patterns [2]. A large section of land is turning to saline soils over time, and it is projected that the current area experiencing salinity stress in South Asia will nearly triple by 2050 [2].

Although plant species differ in their mechanisms of salinity tolerance, salinity stress eventually reduces plant growth and development [3]. The production of many plant species declines when exposed to excessive salinity, which is generally linked to a drop in photosynthetic capability. A decrease in chlorophyll formation can also cause a decrease in photosynthesis when the environment is salinized [4]. Salt stress causes nutrient

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ion imbalance, a decrease in stomatal conductance [5, 6], morphological alteration, and secondary metabolites changes [7].

Germination plays a crucial role in the plant life cycle, which consequently has an impact on growth and development as well as the outcomes of crops. Salinity causes a reduction in osmotic potential hence affecting cell turgor pressure; besides enzymes' activity which in turn interferes with metabolic processes leading to poor establishment as well as productivity rates [8]. Seedlings are particularly vulnerable to stress as some may die under saline conditions. Tonguç, et al. [9] found that salinity decreases the germination of safflower cultivars. Salt stress reduced spikelet number, grain weight, and biomass production in wheat [10]. Roots and shoots are also adversely impacted by salt stress [11].

Studies and various techniques have been explored to enhance crop tolerance to salinity. Researchers have observed positive results from using external phytoprotectants to alleviate salt stress. Different substances have been used to counteract the harmful effects of salt on plants, with microelements being particularly significant and recognized for their role in helping plants adapt to salt stress [12]. Selenium (Se), a crucial micronutrient, has an important role in promoting plant growth and development, even in small amounts. Moreover, it protects against various environmental stresses, such as high temperatures, low temperatures, and drought by functioning as an antioxidant or stimulant in a concentration-dependent manner [13]. Selenium can be absorbed by plants because it has the same molecular structure as sulfur, which they take up through root plasma membrane transporters. It is taken up by the roots from the ground solution, transformed through the sulfur metabolism cycle, and released into the atmosphere. High doses of Se can lead to oxidative stress and disrupt protein structure and function, causing toxicity in plants [14]. In plant cells, selenium is crucial in regulating internal processes to enable their proper growth and development. It stimulates the development of shoots and roots, helps regulate water balance in plants, facilitates the absorption of essential nutrients like nitrogen, and defends against pathogens and pests that cause harm to plants [15]. Mushtaq, et al. [16] revealed that salt stress negatively impacted the physiological parameters of *Panicum miliaceum* L., while the addition of Se improved these parameters. The morphological and biochemical assessments showed that Se mitigated the effects of NaCl in foxtail millet. This was achieved by improving morphological traits, increasing the expression of the APX gene, boosting enzyme activity, and significantly decreasing the levels of H_2O_2 [17]. Du, et al. [18] studied the impact of sodium selenate on rice and found that it had a beneficial effect on seedling growth.

In many developing countries, medicinal plants are a vital component of traditional healthcare systems. These plants not only provide important curative medicines but also generate major economic benefits [19, 20]. With the rising demand for medicinal plants, innovative approaches are emerging to cultivate these valuable crops in challenging environments, such as salt-affected soils unsuitable for most conventional crops. This strategy serves as an attractive option for farmers in arid and semiarid regions to grow salt-tolerant medicinal plants. This choice could ensure high profits for them [21].

Safflower (*Carthamus tinctorius* L.) is an edible medicinal plant that belongs to the Asteraceae family. Primarily cultivated in semi-arid regions, it serves as a natural dye source [22] and is prized for its high oil content in its seeds, which can reach up to 27–32% [23]. With a wide range of applications in pharmaceuticals, cosmetics, and industries, safflower is considered a domesticated crop of great value [24]. Since it's commonly grown in hot and dry climates where soil salinity is a significant concern [25], our investigation focuses on exploring the effects of sodium selenate on the germination, and physiological, biochemical, and anatomical responses of safflower under salt stress conditions. The innovation of the study is its integrative approach that combines soil treatment with selenium, detailed physiological and anatomical assessments, and practical applications for agricultural resilience against salinity.

Materials and methods

Time and location of the experiment

This study was conducted in two separate experiments to evaluate the effect of Se on *C. tinctorius* L. under Salinity stress, one in the laboratory to study germination and the other in the research greenhouse of the Faculty of Biological Sciences, Kharazmi University, Tehran, Iran in 2023–2024.

Seed germination

C. tinctorius L. seeds obtained from the Pakan Bazr Company. In preliminary experiments, different concentrations of salt and sodium selenate were used based on the literature. Based on these experiments, four concentrations of NaCl and three concentrations of Na_2SeO_4 were selected. After sterilization, seeds were submerged into NaCl (0, 0.5, 1.5, and 2.5 g L^{-1}) and sodium selenate (Na_2SeO_4; 0, 0.01, and 0.02 g L^{-1}) treatment solutions for two hours. The treated seeds were then placed in sterile petri dishes in the dark and monitored daily at regular intervals. Three plates were used for each treatment and ten seeds were placed in each plate. After 7 days, the length, fresh, and dry weight of the seedlings were measured to calculate the desired parameters.

Germination Percentage (GP) = (seeds germinated / total seeds) × 100 [26].

Germination Rate (GR) $= \frac{\sum n}{\sum D.n}$ [27].

D: number of days passed.

n: number of germinated seeds on the desired day.

Seedling Length Vigour (SLV) = GP × Seedling length [28].

Seedling Weight Vigour (SWV) = GP × Seedling dry weight (mg) [28].

Greenhouse study

The safflower seeds were initially planted in a culture tray filled with Cocopeat. After 15 days, once true leaves had emerged, they were transplanted into plastic pots. The used soil was taken from a research greenhouse of Kharazmi University. Soil characteristics analysis was done according to the procedures explained in the Methods of Soil Analysis Sect. [29] (Table 1). The dry soil turned artificially contaminated through the application of NaCl (0, 0.5, 1.5, and 2.5 g kg^{-1}), and Na_2SeO_4 (0, 0.01, and 0.02 g kg^{-1}). Then the soil was allowed to equilibrate for two weeks in the greenhouse condition. Experimental pots with a diameter of approximately 20 cm and a depth of 20 cm were each filled with 4 kg soil. These pots were then placed in a greenhouse with temperature conditions maintained at 28 ± 2 °C and a photoperiod of 16 h of light followed by 8 h of darkness. To mitigate microclimate effects on plant growth, the pots were repositioned and moved randomly daily. Sampling was conducted after 30 days.

Growth parameters

Assessment of shoot and root length, fresh and dry weight of shoot and root, and leaf area was done after one month of treatment at the vegetative stage. The determination of plant height was done by measuring from the shoot tips up to the collar. Plants were cut 2 cm above the ground and extracted from the pots. Fresh weight was determined by weighing the shoot on a balance. Samples were then dried in an oven at 70 °C for 72 h until a constant weight was achieved. Leaf area (cm^2) was calculated as x/y, where x represents the graph paper weight covered by the leaf outline (g) and y is the weight (g) of the cm^2 area of the graph paper. The outlined area of the graph paper was cut and weighed, along with one cm^2 section of millimeter graph paper for comparison [30].

Table 1 Physical and chemical characterization of the experimental soil

Parameter	Value
pH	7
EC (ds m^{-1})	0.73
Total lime (%)	9.9
Organic carbon (%)	5.15
Total Nitrogen (%)	0.37
Soil texture	loam
Cu (mg kg^{-1})	2.3
P (mg kg^{-1})	190
K (mg kg^{-1})	2780

Anatomical studies

Shoot samples were collected and kept in glycerin and ethanol (70%). Free-hand sections were made from the samples and stained with Methylene Blue and Carmine for lignin and cellulose identification, respectively [31]. Thin sections were subsequently examined under a light microscope, and cell and tissue measurements were done through ImageJ software. The impact of sodium selenate at concentrations of 0 and 0.02 g kg^{-1} on the anatomical features of safflower was investigated under conditions of saline stress with levels of 0 and 2.5 g kg^{-1}.

Ion concentration analysis

The dried shoot was powdered, then hydrogen peroxide was added to it with nitric acid, and it was digested. Na^+ and K^+ concentrations of different samples were determined by ICP (RCOS A Spectro) [32]. The effect of sodium selenate at concentrations of 0 and 0.02 g kg^{-1} on Na^+ and K^+ content of Safflower under salinity stress with levels of 0, 0.5, and 2.5 g kg^{-1} was measured.

Relative water content (RWC)

The fresh weight (FW) of leaves was measured, followed by immersing all samples in distilled water at 4 °C for 24 h. After this time, the turgid weight (TW) of the leaves was measured, and the leaves were then dried in an oven at 70 °C for 24 h to obtain the dry weight (DW) of each sample. The relative water content was calculated using the following equation:

RWC = (FW – DW)/(TW – DW) × 100 [33].

Soluble carbohydrates

Carbohydrate contents were determined using the phenol-sulfuric acid method. Dry leaves (0.05 g) were mixed with 5 mL of ethanol (70%), and after a week, 500 µL of the upper portion of the solution was extracted and diluted to 2 mL with distilled water. Subsequently, 1 mL of 5% phenol was added and thoroughly mixed, followed by the addition of 5 mL of sulfuric acid. Absorbance was measured using a spectrophotometer (Unico model 2150) at a wavelength of 485 nm after the solution had completely cooled for approximately 30 min. A standard curve prepared with glucose was utilized to quantify the sugar content [34].

Photosynthetic pigments

Acetone (80%) was used to quantify the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl b), total chlorophylls (T Chl), and carotenoids (Car). Leaf samples were collected during the vegetative stage from the fourth and fifth leaves at the apex. The absorption readings at 646.8 nm, 663.2 nm, and 470 nm were recorded using a spectrophotometer. The photosynthetic content (milligrams per gram of fresh weight) in each sample was determined using the provided formulas [35].

$$\text{Chl } a = 12.25\ A_{663.2} - 2.79\ A_{646.8}$$
$$\text{Chl } b = 21.51\ A_{646.8} - 5.1\ A_{663.2}$$
$$\text{Total Chl} = \text{Chl } a + \text{Chl } b$$
$$\text{Car} = (1000\ A_{470} - 1.8\ \text{Chl } a - 85.02\ \text{Chl } b) / 198$$

H_2O_2 and MDA content

To evaluate lipid peroxidation in plants, the measurement of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentrations are used. The levels of hydrogen peroxide (H_2O_2) were determined using potassium iodide (KI) [36]. To summarize, the leaf tissues (0.1 g) were grounded in liquid nitrogen and then mixed with 3 mL of 0.1% (w/v) trichloroacetic acid (TCA) to homogenize them thoroughly. The resulting homogenate was centrifuged for 15 min at 12,000 rpm. 0.5 mL extract supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL KI (1 M). The absorbance of the reaction was measured at 390 nm, and the concentration of H_2O_2 was determined using a standard curve created with known H_2O_2 concentrations.

Membrane lipid peroxidation was evaluated through the thiobarbituric acid test (TBAT) to quantify the MDA content. A 0.1 g sample of fresh leaves was ground in 3 mL of 0.1% trichloroacetic acid (TCA) and homogenized. The resulting extract was then centrifuged at 6000 g for 5 min. Subsequently, 1 mL of the supernatant solution was mixed with 20% trichloroacetic acid containing 0.5% thiobarbituric acid. This mixture was warmed in a water bath at 95 °C for 30 min then cooled down using ice cold bath and centrifuged at 6000 g for 10 min. Absorbance readings were taken at 532 and 600 nm, with the final absorbance calculated by subtracting the value at 600 nm. The MDA concentration was determined using extinction coefficient of 0.155 μM cm^{-1} and expressed in micromoles per gram of fresh weight (μmol g^{-1} FW) [37].

Statistical analysis

All treatments were replicated 3 times in the experiments. The experiment followed a factorial design and statistical analysis was carried out using one-way analysis of variance (ANOVA) with the support of SPSS statistical software. The mean values were compared by Duncan's comparison test. Graphs were created using GraphPad Prism 9.0 software. Heat map analysis was performed using CIMMiner online from https://discover.nci.nih.gov/cimminer/home.do.

Results

Germination parameters

After seven days, measurements were taken for shoot and radicle length, fresh and dry weight of seedlings, germination percentage (GP), germination rate (GR), seedling length vigor (SLV), and seedling weight vigor (SWV) of Safflower using appropriate calculations. The results of the statistical analysis showed that Se had a significant impact on the shoot and radicle length, fresh weight of seedlings, GP, GR, SLV, and SWV ($P \leq 0.01$) under salinity conditions. The combined effect of NaCl and Se on seedling dry weight was not found to be significant. As the salt concentration increased, the shoot and radicle length, the fresh weight of seedlings, GP, GR, SLV, and SWV decreased. Selenium was found to enhance the shoot and radicle length, fresh weight of seedlings, GP, GR, SLV, and SWV in the presence of salinity. The measurements for shoot and radicle length, fresh weight of seedlings, GP, GR, SLV, and SWV at a 2.5 g kg^{-1} salt concentration were 3.33 cm, 4.08 cm, 0.057 g, 50%, 1.72, 370.67, and 0.48 respectively. After the application of 0.02 g kg^{-1} Se, these measurements increased to 4.5 cm, 6.19 cm, 0.06 g, 73.33%, 1.83,781.07, and 0.75 respectively (Tables 2 and 3; Fig. 1).

Growth parameters

The impact of Se on the growth factors of Safflower cultivated in mediums with various levels of NaCl was significant ($P \leq 0.01$). The length of the shoot and root, the fresh and dry weight of shoots and roots, and the leaf area, decreased as the NaCl concentrations increased. The lowest measurements for these parameters were observed in the 2.5 g kg^{-1} concentration treatment, with values of 26.03 cm, 10.23 cm, 1.93 g, 0.66 g, 0.18 g, 0.05 g, and 5.81 cm^2, respectively. On the other hand, the results in Table 4 indicated that Safflower growth parameters were enhanced by applying Se under NaCl stress. In the group treated with 2.5 g kg^{-1} NaCl and 0.02 g kg^{-1} Se, there was an increase in the length of shoot and root, fresh and dry weight of shoots and roots, and leaf surface area by 13.33%, 22.18%, 21.24%, 50%, 22.72%, 64.81%, and 13.94%, respectively (Table 4; Fig. 2).

Anatomical modifications

Cross sections of the stem of Safflower were analyzed to assess the anatomical effects of NaCl and Se (Fig. 3). The transverse section of the stem showed a significant increase in stem diameter, the thickness of cortical parenchyma, and pith diameter at Se (0.02 g kg^{-1}) and NaCl (2.5 g kg^{-1}) treatment ($P \leq 0.01$). In contrast, there were no significant changes in the thickness of the xylem and

Table 2 Effect of sodium selenate (0, 0.01, and 0.02 g L^{-1}) on seedling growth of Safflower under salinity stress (0, 0.5, 1.5, and 2.5 g L^{-1})

Treatment (g L^{-1})		Shoot length (cm)**	Radicle length (cm) **	Fresh weight (g) **	Dry weight (g) ns
NaCl	Se				
	0	4.16 ± 0.08^{bcd}	10.10 ± 0.08^{ab}	0.07 ± 0.002^{c}	0.018 ± 0.0004^{b}
0	0.01	5.55 ± 0.13^{a}	10.07 ± 0.08^{ab}	0.10 ± 0.01^{b}	0.019 ± 0.0004^{b}
	0.02	5.93 ± 0.03^{a}	10.30 ± 0.01^{a}	0.14 ± 0.004^{a}	0.020 ± 0.001^{a}
	0	4.12 ± 0.02^{bcd}	9.33 ± 0.16^{c}	0.063 ± 0.0004^{def}	0.010 ± 0.001^{ef}
0.5	0.01	4.31 ± 0.10^{bc}	9.93 ± 0.07^{b}	0.066 ± 0.0003^{de}	0.012 ± 0.0004^{de}
	0.02	4.53 ± 0.10^{b}	10.15 ± 0.08^{ab}	0.068 ± 0.0002^{cd}	$0.013^{c} \pm 0.0001c$
	0	3.73 ± 0.27^{de}	8.69 ± 0.12^{d}	0.060 ± 0.0001^{ef}	0.011 ± 0.0001^{efg}
1.5	0.01	3.73 ± 0.23^{de}	9.58 ± 0.13^{c}	0.062 ± 0.0001^{def}	0.012 ± 0.0002^{e}
	0.02	3.83 ± 0.23^{cde}	9.99 ± 0.02^{b}	0.063 ± 0.00004^{def}	0.013 ± 0.00003^{cd}
	0	3.33 ± 0.16^{e}	4.08 ± 0.02^{g}	0.057 ± 0.0004^{f}	0.0095 ± 0.0003^{h}
2.5	0.01	3.67 ± 0.29^{de}	5.37 ± 0.09^{f}	0.058 ± 0.0001^{f}	0.0099 ± 0.00003^{gh}
	0.02	4.50 ± 0.29^{b}	6.19 ± 0.15^{e}	0.060 ± 0.0003^{ef}	0.010 ± 0.00002^{fgh}

** Significant differences with the control group ($P \leq 0.01$)

ns no significant differences with the control group

Lowercases letters show significant statistical differences between treatments. Data was presented in means ($n = 3 \pm$ S.E.)

Table 3 Effect of sodium selenate (0, 0.01, and 0.02 g kg^{-1}) on germination parameters of Safflower under salinity stress (0, 0.5, 1.5, and 2.5 g kg^{-1})

Treatment (g kg^{-1})		GP (%)**	GR**	SLV**	SWV**
NaCl	Se				
	0	100 ± 0^{a}	1.5 ± 0^{bc}	1422 ± 14.47^{b}	1.86 ± 0.03^{a}
0	0.01	100 ± 0^{a}	1.5 ± 0^{bc}	1562.7 ± 9.15^{a}	1.91 ± 0.04^{a}
	0.02	86.67 ± 3.33^{bc}	1.0 ± 0^{d}	1406.9 ± 55.16^{b}	1.75 ± 0.13^{a}
	0	76.67 ± 3.33^{de}	1.50 ± 0^{bc}	1032.8 ± 57.21^{d}	0.85 ± 0.05^{d}
0.5	0.01	83.33 ± 3.33^{bcd}	1.33 ± 0.16^{c}	1187.8 ± 58.76^{c}	1 ± 0.06^{bc}
	0.02	86.67 ± 3.33^{bc}	1.33 ± 0.16^{c}	1272.7 ± 50.55^{c}	1.16 ± 0.04^{b}
	0	66.67 ± 3.33^{f}	1.72 ± 0.1^{ab}	826.8 ± 30.53^{e}	0.73 ± 0.03^{de}
1.5	0.01	80 ± 0^{cde}	1.50 ± 0^{bc}	1065.3 ± 26.97^{d}	0.92 ± 0.01^{c}
	0.02	90 ± 0^{b}	1.83 ± 0^{a}	1243.5 ± 22.6^{c}	1.16 ± 0.003^{b}
	0	50 ± 5.8^{g}	1.72 ± 0.1^{ab}	370.67 ± 43.72^{g}	0.48 ± 0.07^{f}
2.5	0.01	66.67 ± 3.33^{f}	1.83 ± 0^{a}	602 ± 29.14^{f}	0.66 ± 0.03^{e}
	0.02	73.33 ± 3.33^{ef}	1.83 ± 0^{a}	781.07 ± 9.1^{e}	0.75 ± 0.03^{de}

** Significant differences with the control group ($P \leq 0.01$)

Lowercases letters show significant statistical differences between treatments. Data was presented in means ($n = 3 \pm$ S.E.)

phloem. The stem diameter, thickness of cortical parenchyma, and pith diameter increased by 7.65%, 13.64%, and 5.97% in 0.02 g kg^{-1} Se and 2.5 g kg^{-1} NaCl treated plants as compared to 2.5 g kg^{-1} NaCl ones (Table 5).

Na^+ and K^+ uptake by plant tissues

The study revealed a significant increase in Na^+ and a decrease in K^+ accumulation in safflower under salinity stress. However, applying Se resulted in a change in Na^+ and K^+ accumulation ($P \leq 0.01$). In plants exposed to NaCl (2.5 g kg^{-1}), the use of Se (0.02 g kg^{-1}) led to an increase in Na^+ accumulation by 7.65%, compared to control (2.5 g kg^{-1}NaCl and 0 g kg^{-1} Se) (Fig. 4a). An increase in K^+ contents in shoots was observed using of Se (0.02 g kg^{-1}) by 29.24% relative to plants under NaCl (2.5 g kg^{-1}) conditions (Fig. 4b).

RWC

The findings showed that the stress experienced by plants from NaCl caused a significant decrease in their RWC. Nevertheless, there was an increase in RWC when Se was used under salinity stress ($P \leq 0.05$). The RWC values at NaCl concentrations of 0.5, 1.5, and 2.5 g kg^{-1} were 82.47%, 76.76%, and 61.1%, respectively. In contrast, plants treated with 0.02 g kg^{-1} of Se exhibited RWC levels of 84.09%, 84.20%, and 70.25% under these NaCl conditions (Fig. 5a).

Fig. 1 Effect of sodium selenate (0, 0.01, and 0.02 g L^{-1}) on germination of Safflower under salinity stress (0, 0.5, 1.5, and 2.5 g L^{-1})

Soluble carbohydrates

Exogenous Se application significantly influenced the soluble carbohydrates content in safflower leaves under salinity conditions ($P \leq 0.01$). The findings indicated a significant rise in soluble carbohydrates when plants were subjected to NaCl treatments. Plants treated with 0.5. 1.5 and 2.5 g kg^{-1} of NaCl exhibited a 50.69, 88.90, and 132.16% increase in soluble carbohydrates relative to the control group (0 g kg^{-1} NaCl). However, the application of Se resulted in an improvement in soluble carbohydrates under NaCl conditions. The 0.02 Se showed the highest increase in soluble carbohydrates under NaCl treatments, with a 14.17%, 6.16%, and 11.02% enhancement compared to plants that did not receive Se treatments (Fig. 5b).

Photosynthetic pigments

The impact of Se on the photosynthetic pigments content of safflower under salinity conditions has been displayed in Fig. 6a-d. The application of Se led to an enhancement in Chl *a*, Chl *b*, T Chl ($P \leq 0.01$), and Car ($P \leq 0.05$) under salinity conditions. Following the application of 0, 0.5, 1.5, and 2.5 g kg^{-1} NaCl, the levels of Chl *a* in safflower were measured at 0.62, 0.51, 0.49, and 0.47 mg g^{-1} FW, respectively. These values were 0.65, 0.63, 0.54, and 0.55 mg g^{-1} FW in the group treated with Se (0.02 g kg^{-1}) and NaCl. After the application of 0.02 g kg^{-1} Se, the levels of

Table 4 Effect of sodium selenate (0, 0.01, and 0.02 g kg^{-1}) on growth parameters of Safflower under salinity stress (0, 0.5, 1.5, and 2.5 g kg^{-1})

Treatment (g kg^{-1})		Shoot length (cm)	Root length (cm)	Shoot fresh biomass (g)	Root fresh biomass (g)	Shoot dry biomass (g)	Root dry biomass (g)	Leaf area (cm^2)
NaCl	Se							
	0	43.23 ± 0.15 [a]	16 ± 0.5 [c]	3.51 ± 0.18 [b]	1.5 ± 0.08 [b]	0.16 ± 0.02 [f]	0.087 ± 0.001 [bc]	9.83 ± 0.4 [c]
0	**0.01**	44.5 ± 0.28 [a]	17.63 ± 0.28 [b]	3.32 ± 0.05 [b]	1.58 ± 0.07 [b]	0.27 ± 0.007 [b]	0.086 ± 0.002 [bc]	10.83 ± 0.4 [b]
	0.02	45 ± 0.15 [a]	19 ± 0.58 [a]	3.91 ± 0.02 [a]	1.86 ± 0.02 [a]	0.29 ± 0.003 [ab]	0.11 ± 0.005 [a]	11.67 ± 0.2 [a]
	0	35.33 ± 0.33 [e]	15.27 ± 0.12 [c]	2.78 ± 0.02 [de]	1.05 ± 0.02 [de]	0.21 ± 0.02 [cd]	0.081 ± 0.002 [bcd]	7.93 ± 0.1 [de]
0.5	**0.01**	36.67 ± 0.17 [d]	16.27 ± 0.32 [c]	2.9 ± 0.03 [cd]	1.18 ± 0.02 [cd]	0.28 ± 0.007 [ab]	0.087 ± 0.001 [b]	8.51 ± 0.2 [d]
	0.02	38.13 ± 0.19 [c]	17.63 ± 0.19 [b]	2.99 ± 0.003 [c]	1.27 ± 0.01 [c]	0.31 ± 0.004 a	0.089 ± 0.001 [b]	8.33 ± 0.2 [d]
	0	31.67 ± 0.44 [f]	12.63 ± 0.41 [d]	2.38 ± 0.09 [f]	0.95 ± 0.03 [e]	0.2 ± 0.003 [de]	0.071 ± 0.006 [e]	7.06 ± 0.4 [fg]
1.5	**0.01**	34.6 ± 0.49 [e]	15.5 ± 0.58 [c]	2.66 ± 0.01 [e]	1.01 ± 0.01 [e]	0.22 ± 0.007 [cd]	0.077 ± 0.002 [cde]	7.43 ± 0.1 [ef]
	0.02	36.47 ± 0.32 [d]	17.73 ± 0.28 [b]	2.74 ± 0.02 [de]	1.08 ± 0.02 [de]	0.23 ± 0.002 [c]	0.082 ± 0.002 [bcd]	7.98 ± 0.04 [de]
	0	26.03 ± 0.42 [h]	10.23 ± 0.15 [f]	1.93 ± 0.02 [g]	0.66 ± 0.01 [g]	0.18 ± 0.009 [e]	0.054 ± 0.002 [f]	5.81 ± 0.2 [h]
2.5	**0.01**	29.4 ± 0.38 [g]	11.3 ± 0.15 [e]	2.01 ± 0.02 [g]	0.69 ± 0.1 [fg]	0.19 ± 0.003 [de]	0.075 ± 0.002 [de]	5.98 ± 0.2 [h]
	0.02	29.5 ± 0.76 [g]	12.5 ± 0.29 [d]	2.34 ± 0.06 [f]	0.81 ± 0.02 [f]	0.27 ± 0.003 [b]	0.089 ± 0.002 [b]	6.62 ± 0.2 [gh]

Lowercases letters show significant statistical differences between treatments ($P \leq 0.01$). Data was presented in means ($n = 3 \pm$ S.E.)

Chl *b* in safflower reached 0.32, 0.31, 0.27, and 0.28 mg g^{-1} FW under salinity stress respectively. These values were 0.31, 0.25, 0.24, and 0.23 mg g^{-1} FW in the groups without Se application. In plants exposed to NaCl (2.5 g kg^{-1}), the use of Se (0.02 g kg^{-1}) led to an increase in T Chl and Car content by 16.9% and 4.22%, compared to control (2.5 g kg^{-1}NaCl and 0 g kg^{-1} Se).

H_2O_2 and MDA content

H_2O_2 and MDA content increased significantly under salinity-stressed conditions than under the control treatment (Fig. 7). The H_2O_2 values gradually increased with increasing salinity levels, with the maximum values being obtained under 2.5 g kg^{-1} NaCl (44%). Plants treated with Se (0.02 g kg^{-1}) significantly ($P \leq 0.05$) decreased H_2O_2 content by 44.59%, 9.43%, 16.48%, and 25.78% under NaCl treatments of 0, 0.5, 1.5, and 2.5 g kg^{-1}, respectively (Fig. 7a).

Plants exposed to 2.5 g kg^{-1} NaCl recorded an increase of 103% in their levels of MDA which was highly remarkable compared with the control group. The application of Se resulted in lower MDA levels under salt stress conditions. The use of 0.01 and 0.02 g kg^{-1} Se in plants exposed to 2.5 g kg^{-1} NaCl led to a 5.64% and 11.9% decrease in MDA content, respectively, compared to plants that did not receive Se treatments ($P \leq 0.01$, Fig. 7b).

Heat map analysis and cluster analysis of studied parameters of *C. Tinctorius* at different salinity and Se treatments

The heat map analysis identified clear patterns associated with the variability of H_2O_2, MDA, and soluble carbohydrates under different treatments. These parameters demonstrated the highest level of variability among the traits assessed, reflecting their sensitivity to the treatment conditions. On the other hand, a cluster consisting of carotenoids and total chlorophylls showed minimal variability, indicating a more consistent response to the treatments (Fig. 8). Three primary clusters were identified based on the treatments. The classification of plant samples into these clusters was mainly influenced by the concentrations of NaCl and Se in the soil (Fig. 8, at the left). The first cluster comprised control plants and those treated with Se. The second cluster included plants at 0.5 NaCl concentrations along with Se treatments and plants treated with 1.5 g kg^{-1} NaCl and Se. The third cluster included plants at 2.5 g kg^{-1} NaCl and Se concentrations and those exposed to only 1.5 g kg^{-1} NaCl. Overall, these findings highlighted the differing sensitivities of various plant traits and their responses to the treatments.

Discussion

Salt toxicity and various environmental stresses have a negative impact on the morphological and physiological characteristics of plants [38]. The productivity of a crop is influenced by the physical and chemical properties of the soil, in addition to other environmental conditions. Soil alkalinity has been reported to continuously rise due to salt stress, which is a major cause of reduction in plant growth and yield [39]. Selenium is a crucial inorganic plant stimulant known for its stress-alleviating functions. Selenium exhibits both beneficial and harmful effects on plant life. High doses of Se can lead to various negative outcomes, impacting plant growth and physiology. On the other hand, at lower concentrations, Se acts as a stimulant, enhancing plant growth and yield [40, 41].

The germination process is the beginning of a plant's life cycle as it affects later growth, development, and yield aspects. In areas prone to salinity, seed germination and early seedling stages are particularly vital for plant establishment and overall growth, determining the plant's abilities for salinity stress [8, 42]. Based on the information

Fig. 2 Effect of sodium selenate (0, 0.01, and 0.02 g kg^{-1}) on growth of Safflower under salinity stress (0, 0.5, 1.5, and 2.5 g kg^{-1})

provided, it seems that salinity hurt the germination and growth of seedlings. When Se was used in this experiment, germination characteristics and seedlings' growth improved. This suggests that sodium selenate may have a positive effect on overcoming the negative effects of salinity on seed germination.

The reduced germination of seeds under salinity stress could be due to the delayed uptake of water and decreased activity of α-amylase, which breaks the starch down. Compared with the internal osmotic potential of the seeds, salinity decreases their soil's osmotic potential, thus hindering the uptake of water during seed germination [43]. Therefore, the germination rate declines, and the period for germination increases. Additionally, salinity can affect the viability of embryos even after germination, as it may lead to the excessive accumulation of Na^+ and Cl^- ions [44]. Following seed germination, embryonic growth, seedling development, and overall vigor are influenced by both ionic and osmotic stress [45]. The saline stress restricted the germination of seeds causing a considerable decrease in the wheat yield [8]. Salt stress negatively influenced germination percentage and rate, shoot and root length, seedling growth, root-to-shoot length ratio, seed vigor, germination index, and germination time for the six safflower genotypes [46]. Salinity stress hindered the germination of *Brassica rapa*

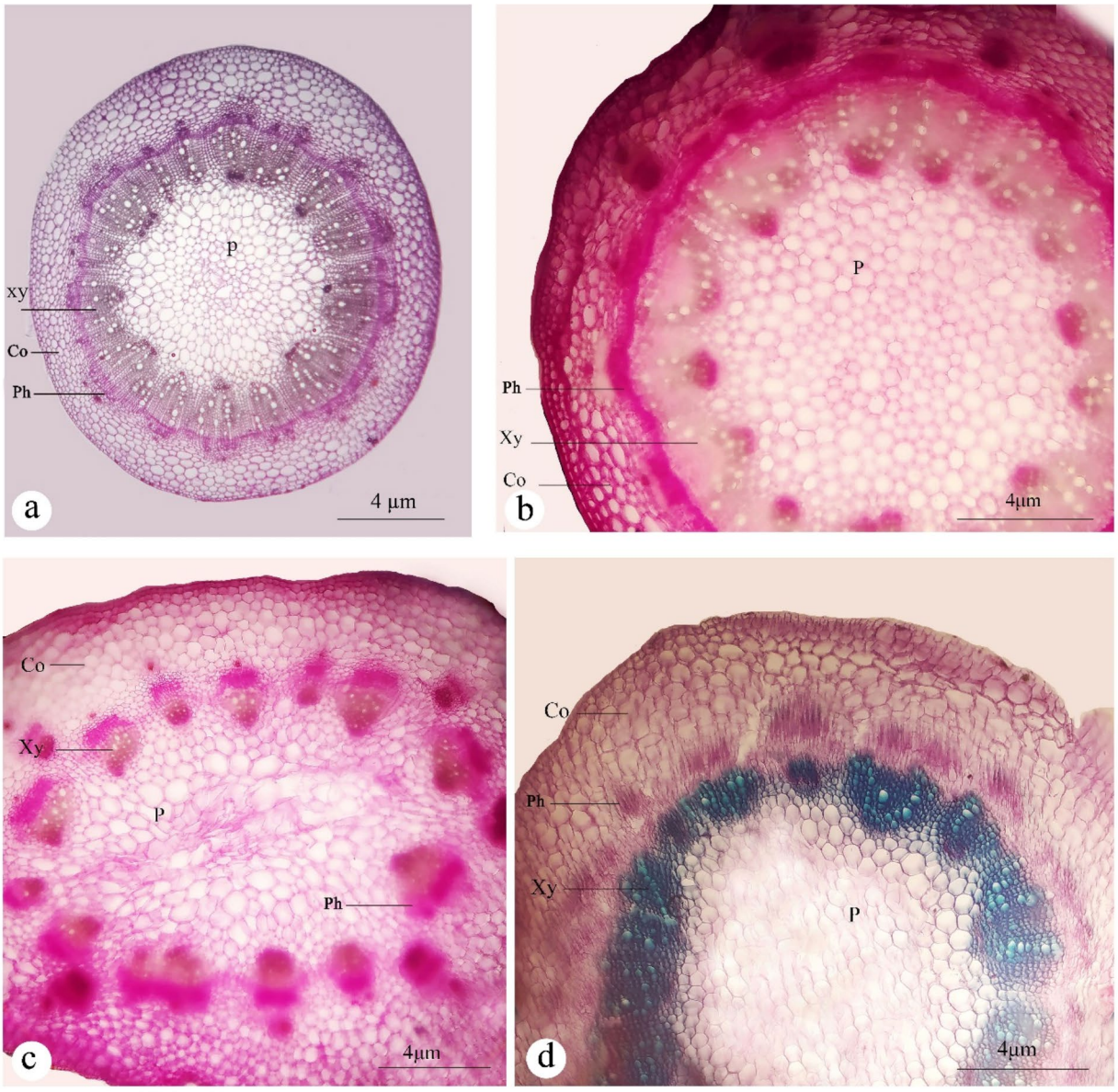

Fig. 3 Effect of sodium selenate (0 and 0.02 g kg^{-1}) on stem anatomy of Safflower under salinity stress (0 and 2.5 g kg^{-1}). (**a**) control treatment (0 NaCl and 0 Se); (**b**) 0.02 g kg^{-1} Se treatment; (**c**) 2.5 g kg^{-1} NaCl treatment; (**d**) 2.5 g kg^{-1} NaCl with 0.02 g kg^{-1} Se treatment. Co, cortex; P, pith; Ph, phloem; Xy, xylem

Table 5 Effect of sodium selenate (0 and 0.02 g kg^{-1}) on anatomical parameters of Safflower under salinity stress (0 and 2.5 g kg^{-1})

Treatments (g kg^{-1})		Stem dimeter (µm)**	Pith diameter (µm)**	Cortical parenchyma diameter (µm) **	Xylem diameter (µm)^{ns}	Pholem diameter (µm)^{ns}
NaCl	Se					
0	**0**	5.64 ± 0.06^{c}	3.44 ± 0.03^{d}	1.81 ± 0.02^{c}	0.72 ± 0.02^{d}	0.26 ± 0.04^{c}
	0.02	16.87 ± 0.12^{b}	9.34 ± 0.08^{c}	7.25 ± 0.10^{ab}	1.27 ± 0.06^{c}	0.48 ± 0.06^{c}
2.5	**0**	17.10 ± 0.05^{b}	10.88 ± 0.02^{b}	6.57 ± 0.23^{c}	1.63 ± 0.08^{b}	0.82 ± 0.02^{b}
	0.02	18.41 ± 0.14^{a}	11.53 ± 0.32^{a}	7.47 ± 0.36^{a}	2.18 ± 0.13^{a}	1.17 ± 0.10^{a}

** Significant differences with the control group ($P \leq 0.01$)

^{ns} no significant differences with the control group

Lowercases letters show significant statistical differences between treatments. Data was presented in means ($n = 3 \pm$ S.E.)

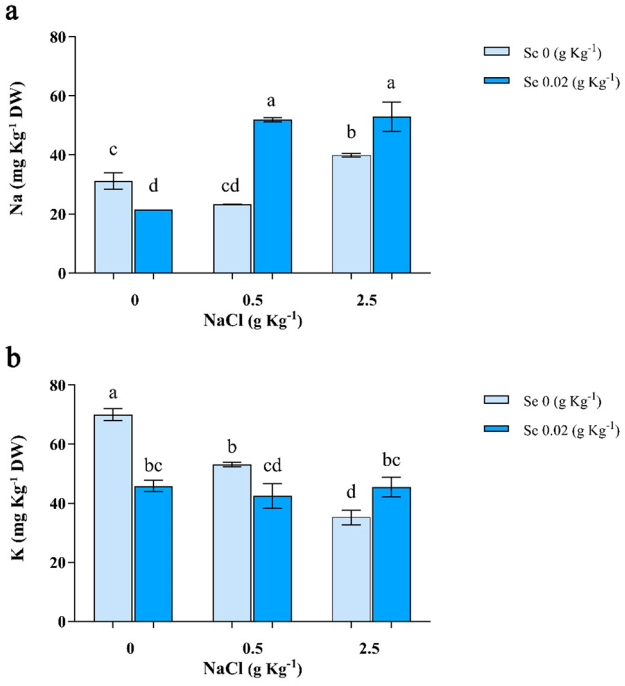

Fig. 4 Effect of sodium selenate (0 and 0.02 g kg^{-1}) on (**a**) Na^+ and (**b**) K^+ content of Safflower under salinity stress (0, 0.5, and 2.5 g kg^{-1}). Lowercases letters show significant statistical differences between treatments. Data was presented in means ($n = 3 \pm$ S.E.)

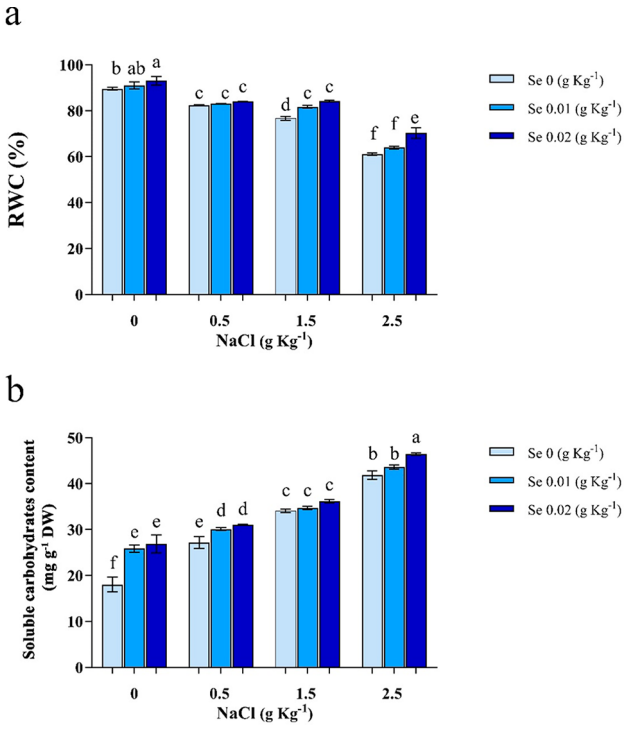

Fig. 5 Effect of sodium selenate (0, 0.01, and 0.02 g kg^{-1}) on some physiological parameters of Safflower under salinity stress (0, 0.5, 1.5, and 2.5 g kg^{-1}). (**a**) RWC; (**b**) soluble carbohydrates content. Lowercases letters show significant statistical difference between treatments. Data was presented in means ($n = 3 \pm$ S.E.)

seeds, as well as the length and fresh weight of seedlings when compared to seeds grown under normal conditions. However, stressed and non-stressed conditions responded positively to Se applications [39]. The results of the experimental study demonstrate that Se has a beneficial role in alleviating salt stress thereby proposing its use against salinity challenges in agricultural areas.

In this investigation, it was shown that the plants subjected to the Se treatment have increased shoot and root length, higher fresh and dry weight, and increased leaf area when exposed to salt stress. Salinity alters plant growth and development due to its multifaceted effects on physiological and biochemical factors including mineral ion homeostasis, water balance, osmolytes accumulation, antioxidant functions, nitrogen fixation, and photosynthetic capacity [47]. The enhanced growth of roots and shoots in Se-treated plants may have been partly due to their improved physiological and metabolic properties [48, 49]. Selenium functions as an anti-aging agent, supporting cellular components and functions, thereby enhancing plant performance [50]. Moreover, Se had positive impacts on the uptake and transport of essential nutrient elements by the plants from the growing medium, significantly enhancing their growth and development [14]. Wang, et al. [51] and Hemmati, et al. [52] asserted that the application of Se fertilizer in soil is a practical and effective approach to enhance the overall performance of plants. In the research, Se was used as a seed priming agent for seeds cultivated in salt - amended soils. Results demonstrated that seedlings originating from the Se-primed seeds showed increased root and shoot growth, fresh weight, and biomass production which confirms the current findings [39].

Anatomical modification of the stem was observed in Safflower treated with Se and NaCl. The stem diameter, thickness of cortical parenchyma, pith diameter, and thickness of xylem and phloem increased in salinity conditions which may be a defensive attribute under salinity conditions. Studies showed that the epidermal cells' diameter and thickness of the cortex of *Salvadora persica* decreased by salinity. In contrast, the thickness of the hypodermal layer, the diameter of the hypodermal cell, the pith area, and the pith cell diameter increased by high salinity. It also showed no significant changes in xylem vessel diameter in the stem of *S. persica* with increasing salinity [53]. An increase in the thickness of the xylem may be considered as an adaptive mechanism of Safflower to maintain a steady water flow in a shoot at saline conditions and with steady water flow may help in translocation of more mineral ions to the shoot. In contrast to our results, salinity-induced decreases in xylem vessel diameter causing a reduction in water and mineral conductivity have been reported in many plants [54, 55]. In this research, Se treatment increased stem diameter,

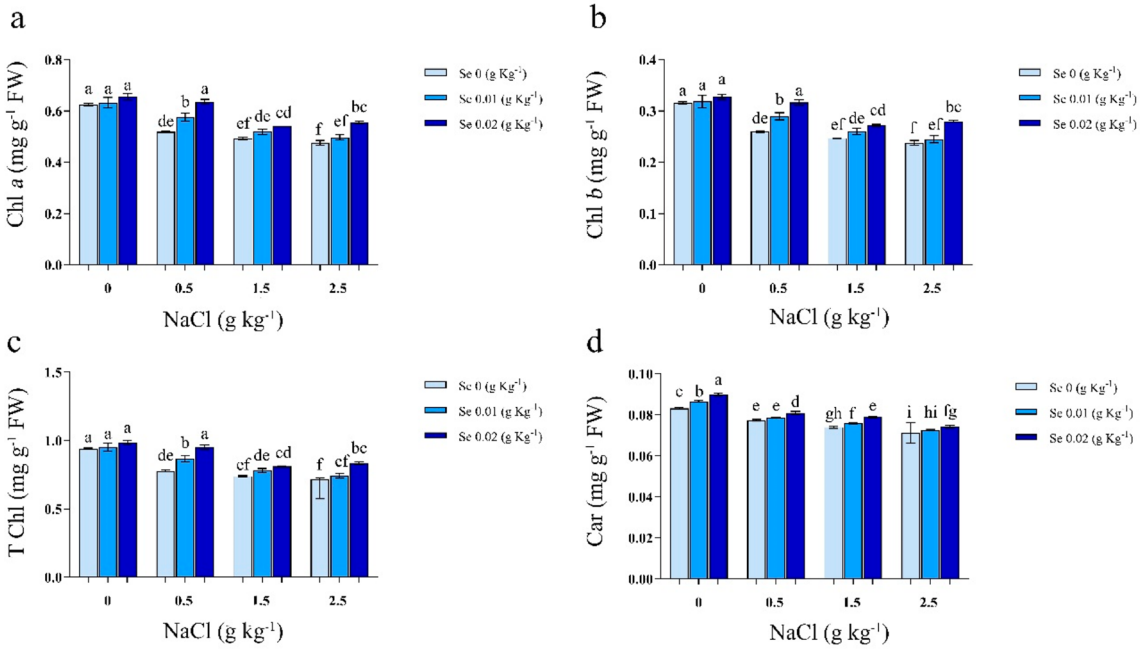

Fig. 6 Effect of sodium selenate (0, 0.01, and 0.02 g kg^{-1}) on photosynthetic pigments of Safflower under salinity stress (0, 0.5, 1.5, and 2.5 g kg^{-1}). (**a**) chlorophyll a; (**b**) chlorophyll b; (**c**) total chlorophyll; (**d**) carotenoid. Lowercases letters show significant statistical differences between treatments. Data was presented in means ($n = 3 \pm$ S.E.)

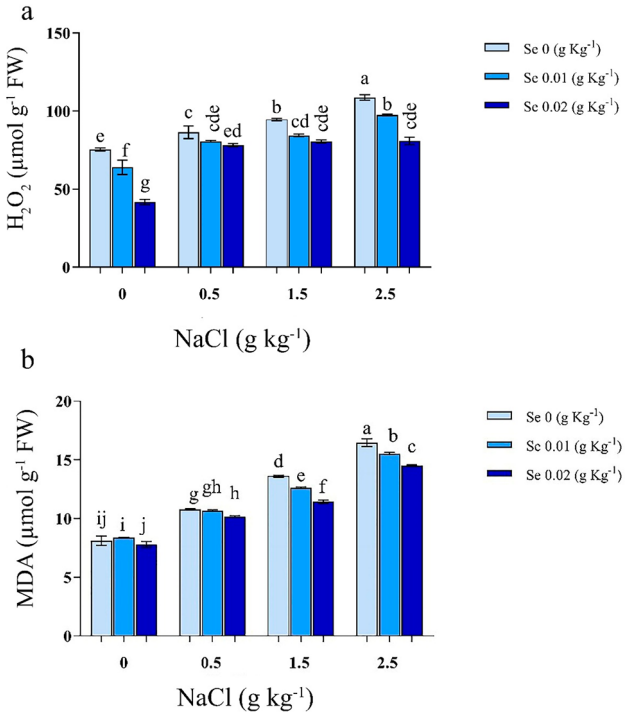

Fig. 7 Effect of sodium selenate (0, 0.01, and 0.02 g kg^{-1}) on (**a**) H_2O_2 and (**b**) MDA content of Safflower under salinity stress (0, 0.5, 1.5, and 2.5 g kg^{-1}). Lowercases letters show significant statistical differences between treatments. Data was presented in means ($n = 3 \pm$ S.E.)

the thickness of cortical parenchyma, and pith diameter. Several lines of evidence support Se-associated changes in signaling hormones [56–59] which may be responsible for differential growth, metabolism, tissue differentiation, and morphology.

In the present study, it was found salinity had an adverse effect on safflower's RWC whereas sodium selenate showed a contrary result. Salt in solution surrounding the root zone may have an adverse impact on plant growth through the osmotic effect that reduces water uptake resulting in reduced leaf and tissue water potential [60]. In salt-stressed conditions, there is an increase in chlorophyllase activity and cellular damage leading to chlorophyll depletion and decreased water content in plants [61]. The improved water content in tissues of plants treated with Se may be associated with enhanced root growth, which enables better water uptake [62]. It is also involved in maintaining the stability of membranes and cell turgor under NaCl stress [63]. According to Elkelish, et al. [64], a low concentration of Se (5 µM) reduced the adverse effects of salt stress. Plants treated with 5 µM Se showed increased RWC. Shekari, et al. [65] found that the application of 5 µM Se led to an increase in root length as well as a 13% enhancement in RWC for *Capsicum anuum* compared to control plants. Therefore, our results confirm that Se plays a role in improving RWC under salinity stress.

Our findings indicated that salinity decreased photosynthetic pigments such as Chl *a*, Chl *b*, T Chl, and Car in

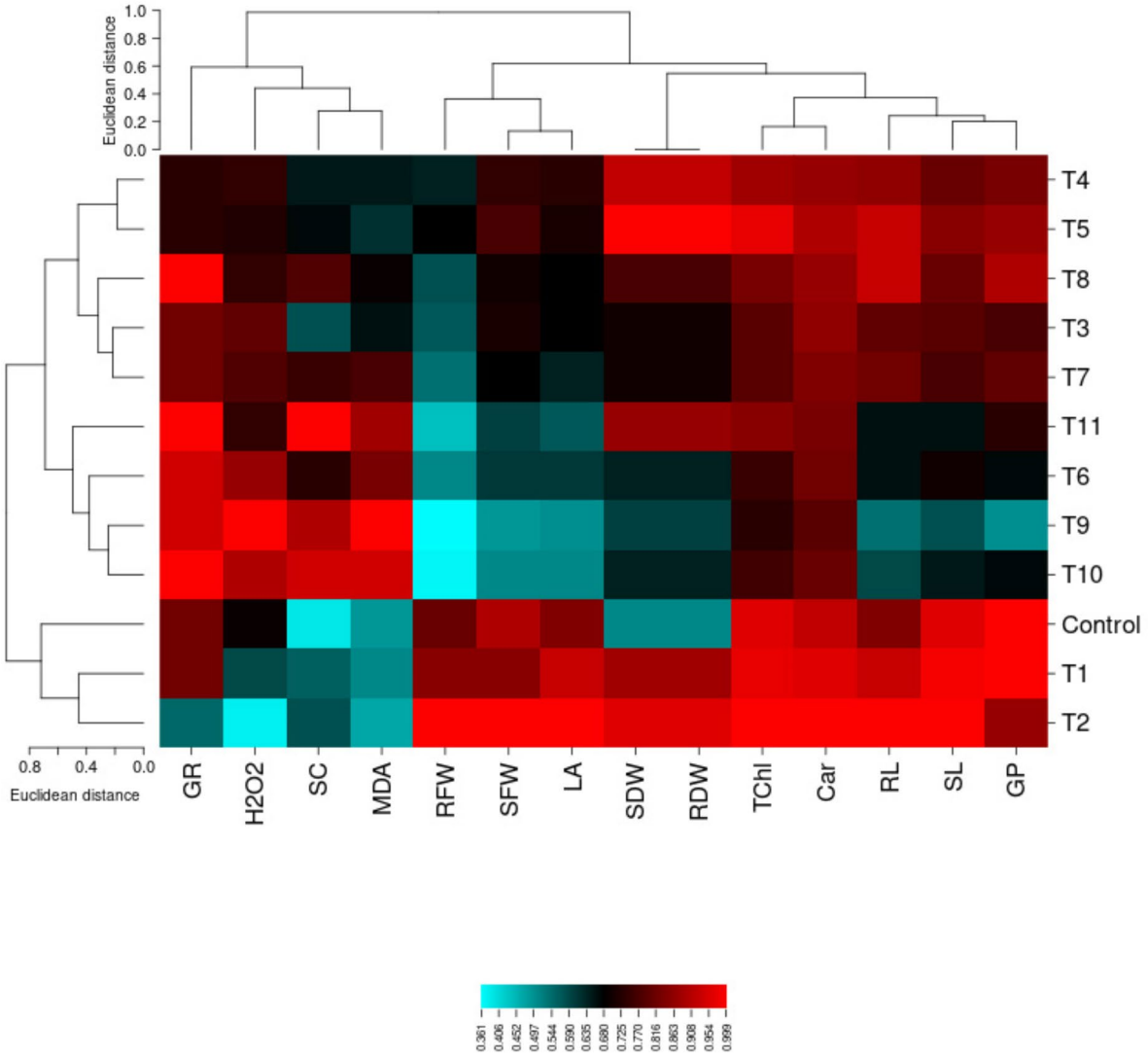

Fig. 8 Heat map of studied traits under NaCl and Se treatments. GR: germination rate, SC: soluble carbohydrate, RFW: root fresh weight, SFW: shoot fresh weight, LA: leaf area, SDW: shoot dry weight, RDW: root dry weight, TCh: total chlorophyll, CAR: carotenoids, RL: root length, SL: shoot length, GP; germination percentage. T1 = Se 0.01, T2 = Se 0.02, T3 = NaCl 0.5, T4 = NaCl 0.5 * Se 0.01, T5 = NaCl 0.5 * Se 0.02, 6 NaCl 1.5, T7 = NaCl 1.5 * Se 0.01, T8 = NaCl 1.5 * Se 0.02, T9 = NaCl 2.5, T10 = NaCl 2.5 * Se 0.01, T11 = NaCl 2.5 * Se 0.02

Safflower leaves. However, the treatment with Se significantly increased the content of photosynthetic pigments in plants under salt stress. The excessive accumulation of salt reduces photosynthetic pigment synthesis by decreasing the activity of enzymes such as 5-aminolevulinic acid dehydratase, porphobilinogen deaminase, coproporphyrinogen III oxidase, porphyrinogen IX oxidase, Mg-chelatase, and protochlorophyllide oxidoreductase [66]. This reduction in enzyme activity may be accompanied by an increase in chlorophyllase activity and cellular damage, as well as a decrease in leaf water potential, nitrogen uptake, and allocation for Rubisco synthesis, ultimately leading to a decline in photosynthetic efficiency [61, 67–69]. Selenium enhances the glutathione peroxidase (GSH-Px) activity and decreases the free radicals to support plants under stress. Replacing sulfur with Se enhances protein biosynthesis and increases chlorophyll content by influencing the formation of porphyrins [13, 70]. It has been reported in various researches that porphyrins are important in the production of photosynthetic pigments [71]. Hussain, et al. [72] demonstrated that seedlings treated with Se had significantly higher photosynthetic content than NaCl-spiked and control seedlings. The studies also showed that applying Se reduced the degradation of photosynthetic pigments when crops were exposed to abiotic stresses [39, 64, 73].

Therefore, it can be deduced that increased synthesis of photosynthetic pigments resulted in enhanced photosynthetic activity in Safflower plants treated with Se under salt stress.

The study findings indicated that NaCl increased the production of soluble carbohydrates in plants. The optimal levels of osmolytes, such as total soluble sugars, play a crucial role in alleviating plant stresses through osmoregulation [74, 75]. Osmoprotectants aid in preserving the synthesis of proteins and chlorophyll, maintaining membrane integrity, regulating redox balance, and detoxifying excess reactive oxygen species (ROS) in stressed plants [63]. Selenium may enhance the production of plant metabolites that help mitigate salt stress by sustaining osmotic balance. This equilibrium maintains cellular turgidity, provides membrane strength and protection, and thereby prevents oxidative stress and photooxidation in stressed plants [39]. In the current study, Se treatment led to an increase in the synthesis of total soluble sugars, probably due to starch hydrolysis into sugars and enhanced amylase activity. Furthermore, it has been suggested that Se may improve carbohydrate metabolism by stimulating fructose 1,6-bisphosphatase activity [76]. The results of this investigation are consistent with previous studies conducted by Põldma, et al. [77], Elkelish, et al. [64], and Hussain, et al. [72] who reported increased amounts of total soluble sugar in Se-treated plants under salt stress conditions.

Salt stress increases the production of H_2O_2, which plays a crucial role in cellular contraction, apoptosis, and DNA damage [78]. Enzymatic and non-enzymatic antioxidants can reduce excess H_2O_2 and diminish stress [79]. The results from using Se on plants indicate that it can significantly decrease the concentration of H_2O_2 in them which is consistent with the findings observed by Rady, et al. [80], Rehman, et al. [81] and Rasool, et al. [82]. Malondialdehyde serves as a crucial indicator of lipid peroxidation. There was a notable increase in the MDA content in leaves when exposed to salt stress. Nevertheless, applying Se reduced the MDA of plants exposed to salt stress. MDA is a byproduct resulting from the breakdown of polyunsaturated fatty acids, and its levels indicate the degree of lipid peroxidation due to oxidative stress [83]. Salinity causes osmotic and ionic stress, which leads to the production of ROS [84]. When the level of ROS exceeds a certain threshold, lipid peroxidation occurs in both the cell and organelle membrane [85]. Shekari, et al. [86] discovered that the use of Se led to a decrease in levels of ROS and MDA by enhancing the antioxidative activity of enzymes. Additionally, seleno-proteins are vital for stress tolerance by regulating redox processes [87]. Therefore, seleno-proteins induced by Se may alleviate oxidative damage caused by salinity stress by detoxifying ROS produced during stress [88]. Similarly, enhancing the coordination of antioxidative defense mechanisms in Se-treated plants led to a reduction in membrane damage and MDA levels under salinity conditions [59, 89, 90]. The present results are supported by other research indicating that the application of exogenous selenium reduced MDA levels in various plant species under stress conditions [91, 92].

Salt decreased K^+ absorption, while Se (0.02) increased K^+ uptake in plants exposed to salt stress (2.5 mg kg^{-1}). Typically, Elevated levels of Na^+ lead to a reduction in K^+ levels. It is crucial for tolerance to regulate cytosolic K^+ levels effectively to maintain homeostasis in the plant [93]. Plants under salinity stress had poorly developed root systems, resulting in low nutrient absorption. The root structure and development improved with Se which enhanced uptake of essential plant nutrients. Additionally, K^+ promotes growth by regulating stress-responsive metabolites production, enhancing nitrogen assimilation, and aiding the antioxidant system in plants [67, 94, 95]. The effects of Se on *Zea mays* salinity tolerance were investigated by Jiang, et al. [96]. According to their research, K^+ levels were increased and Na^+ levels were decreased with 1 µM of Se under salinity stress conditions. Research revealed that salinity stress had a detrimental impact on nutrient absorption in both the shoots and roots of *Brassica rapa*. In addition, it was shown that pre-treating seeds with Se minimizes the harmful impacts of salinity stress on plants since they can easily absorb essential nutrients [39].

Jawad Hassan, et al. [97] found that Se increased K^+ levels in salt-affected plants treated with Se. Conversely, Hawrylak-Nowak, et al. [98] indicated that Se does not affect the absorption of Na^+ or K^+ in the treated plants. As a result, the precise mechanism by which Se-treated plants mitigated Na toxicity remains unclear. It is suggested that changes in cellular processes induced by appropriate levels of Se may aid in the sequestration of Na^+ within root vacuoles. Furthermore, structural modifications have been observed as a strategy for alleviating salinity stress in plants [99].

Conclusions

The external application of Se has shown significant potential in relieving salt stress in safflower plants. The protective effects of Se may be attributed to the increase in osmoprotectants (like soluble sugars), increased photosynthetic efficiency, and alterations in anatomical characteristics. Consequently, the application of Se to soil could potentially serve as a future strategy for combating abiotic stresses and reducing the adverse impacts of salinity.

Acknowledgements

The authors would like to thank the research council of Kharazmi University, Tehran, Iran.

Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Fatemeh Fatahiyan and Farzaneh Najafi. The first draft of the manuscript was written by Zohreh Shirkhani and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding
Not applicable.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This article does not contain any studies involving humans and animals as research subjects.

Consent for publication
Not applicable.

Clinical trial number
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 30 October 2024 / Accepted: 7 January 2025
Published online: 24 January 2025

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