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Salt-induced Effects on Physiological and Biochemical Characteristics of Quinoa (*Chenopodium quinoa* L.) Native of Salt Lake Urmia Coasts

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Abstract

In order to manage water and saline lands, cultivation of salt-tolerant plant species such as quinoa (*Chenopodium quinoa* L.), besides other strategies, is a very suitable option. Quinoa has a good capacity to grow in different environments, including saline soils, and also has valuable medicinal and nutritional properties. Effects of different salinity treatments (0, 100, 200, 300, and 400 mM NaCl) on quinoa plants grown in perlite under greenhouse conditions were studied using a factorial experiment with a completely randomized design and four replications. The content of Na^+ and Cl^- simultaneously increased with incremental salinity levels, and the maximum content was observed at 400 mM NaCl. Conversely, the highest content of potassium was determined in the control plants, and it decreased with the increase in salinity stress. Soluble sugars, total protein, and malondialdehyde contents, and guaiacol peroxidase enzyme activity showed a significant increase with the increase of salinity stress levels. Also, the highest content of phenol, hydrogen peroxide, and catalase enzyme activity was observed in 200 and 300 mM NaCl, and the expression of the *NHX* gene showed a significant increase one day after the application of 400 mM NaCl compared to the control. Leaf *SOS1* gene expression showed a 4-fold increase on the first day after the application of 400 mM NaCl treatment. The highest expression of the gene was observed on the fourth day, which shows the key role of Na^+/H^+ antiporters and resistance to salt stress. Of course, tolerance to salinity can be seen on this plant in higher amounts; But the effects of salinity stress can be observed to some extent at a concentration of 400 mM in some physiological and biochemical indicators. In general, the cultivation of this plant can be guaranteed by applying proper management and its valuable benefits can be used by cultivating it in saline soils, and quinoa can be a good choice for salty and low-yielding lands.

Keywords: catalase enzyme, hydrogen peroxide, malondialdehyde, *NHX*, quinoa, SOS1, salt stress.

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Introduction

Quinoa (*Chenopodium quinoa* L.) is a facultative halophyte plant and can tolerate salinity up to 40 ds/m (~ 400 mM) [1]. This plant belongs to the Amaranthaceae family and the Chenopodiaceae subfamily and is an annual plant with a height of 0.5 to 2 meters. The size of quinoa seeds is two millimeters and it has a high variation in terms of adaptability to different climates. This plant is mostly cultivated in South American countries, but its cultivation has also been reported in countries such as China, Canada, USA and India [1]. The Quinoa seed has a very high nutritional value and due to its production in unfavorable environmental conditions, it has been the subject of more studies with critical attention. In addition to being gluten-free, the seeds of this plant are also medicinally valuable; Because it is a good source of antioxidants and rich in protein (13.8 to 21.9 percent) and it is the sole plant that can provide all the essential amino acids for the human body [2]. Quinoa is rich in vitamin E and Omega-3, and its protein and minerals are much higher than wheat and barley. Since it contains a significant amount of iron, folic acid, lysine and sulfur-containing amino acids, it is a very suitable source for people who have low access to animal protein. In addition, its unsaturated fat content is also higher compared to corn [3].

Todays, salinity is considered as an important limiting factor in plant production. This abiotic stress affects different stages of plant growth and development and different morphological, physiological and biochemical changes from the germination stage to the production of plant seeds [4]. Salinity tolerance in plants has different degrees and has different manifestations in different stages of plant growth [5]. High concentrations of Na^+ and Cl^- ions in the soil affect the absorption of minerals by plants because sodium ions competitively inhibit the absorption of cations such as potassium and calcium, and chloride ions inhibit the absorption of anions (especially nitrate). On the other hand, the absorption and transport of sodium ions in high amounts cause replacement of this ion by calcium ions in the apoplastic space and cause ion imbalance

following the depolarization of the cell membrane and the inability of the membranes to absorb and transport some ions [6]. In addition, following salinity stress, the production of reactive oxygen species increases, which cause oxidative stress and damage to macromolecules such as lipids, proteins, nucleic acids, and biological membranes [7].

Quinoa has the ability to adapt to a wide range of agricultural soils such as soils with high salinity and those prone to drought. Among the morphological traits of quinoa against salt stress are epidermal bladder cells and stomatal density. Epidermal bladder cells accumulate 1000 times more Na^+ ions compared to the vacuoles of normal leaf cells. Also, other mechanisms such as efficient control of sodium ion sequestration in leaf vacuoles, Na^+ ion loading in xylem, high tolerance against ROS, better retention of K^+ ion, maintenance of low cytosolic Na^+ levels, high amount of H^+ pump in mesophyll cells and the accumulation of compatible solutes such as proline, total phenolics, polyamines and the activity of antioxidant enzymes like CAT, SOD, POX, APX and etc. [8]. Na^+ exclusion primarily is encoded by two genes: SOS1 (Salt overly sensitive 1) and NHX1 (Na^+/H^+ exchanger 1). Cloning, sequencing and characterization of two homologous SOS1 loci (cqSOS1A and cqSOS1B) was conducted in the study by Maughan et al. (2009) in saline condition (300 mM NaCl) in the *Quinoa salares* variety 'Ollague' [9]. Similar results were reported for other quinoa varieties when plants were grown in 300 and 450 mM NaCl [10-12]

Considering that quinoa is very important in human nutrition and considering its unique characteristics in production in adverse environmental conditions and having high nutritional and medicinal value, the present study was conducted in the greenhouse of Biology department, Faculty of Science, Urmia University, to investigate tolerance rate of quinoa against salinity and feasibility of its cultivation in saline soils.

Materials and Methods

Plant cultivation

In order to investigate the physiological effects of salinity stress on the quinoa plant, a research was conducted based on pot cultivation in the plant physiology laboratory, faculty of Science, Urmia University in 2016. This research was conducted as a factorial experiment in the form of a completely randomized design with five salinity (NaCl) treatments (0, 100, 200, 300 and 400 mM) and in four replications. Quinoa seeds were obtained from the shores of Lake Urmia and were disinfected with 5% sodium hypochlorite for three minutes and then washed several times with distilled water. After sterilization, 50 seeds were placed in each petri dish and after germination, they were transferred to pots containing Hoagland solution (1/4 strength). Cultures were incubated at a temperature of 23-25°C with a light and a darkness period of 16/8 hours at a photon flux density of 200 µM m^{-2} s^{-1} using white fluorescent light. About 10 to 15 seeds were placed in each pot and 20-day-old seeds were treated with considered salinity stress. Sampling was done two weeks after applying salt stress. At the time of sampling, at least 5 plants were harvested from each pot. The samples were immediately frozen in liquid nitrogen and kept in a -80 freezer until physiological and biochemical tests.

Sodium, potassium and chloride contents

To measure sodium, potassium and chloride contents of the samples, 100 mg of dry and powdered plant material (leaf and root) of all the treatments in 15 ml plastic test tubes (Falcon) Liter were weighed, and after adding 10 ml of deionized water, tubes were heated at a temperature of 100 ˚C for one hour. Then the samples were centrifuged at 5000 g. The content of chloride in 500 microliters of the extract was measured using a Corning 926 chloride analyzer, and the content of sodium and potassium in the extracts was measured using a Fater 405 flame photometer [13].

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide content was determined based on the reaction of H_2O_2 with potassium iodide (KI) and by Alexieva's (2001) method. In this method, 0.5 grams of fresh plant leaf and root tissue were ground in 0.1% TCA. The resulting extract was centrifuged at 5000 g for 15 minutes. Then, 500 microliters of 100 mM potassium phosphate buffer (pH=7) and two milliliters of potassium iodide (1 M) were added to 500 microliters of the supernatant solution. The reaction mixture was placed in the dark at room temperature for one hour, and then the absorbance of the samples was evaluated by a spectrophotometer UV-Visible (WPA, S2100, UK) at 390 nm. The standard curve was used to calculate the concentration of hydrogen peroxide [14].

Malondialdehyde (MDA)

The amount of 0.2 g of fresh plant tissue was ground in 5 ml of 0.1% trichloroacetic acid. The resulting extract was centrifuged at 5000g for 5 minutes. 5 ml of 20% TCA solution and 0.5% thiobarbituric acid (TBA) were added to one milliliter of the supernatant solution obtained from centrifugation. The resulting mixture was heated for 30 minutes at a temperature of 95 ˚C in a hot water bath. Then the samples were immediately placed in an ice bath and cooled, and the mixture was again centrifuged at 5000 g for 10 minutes. The absorption intensity of

this solution was read using spectrophotometer at a wavelength of 532 nm. The desired substance for absorption in this wavelength is the red MDA-TBA complex. The absorbance of the rest of the non-specific pigments was determined at 600 nm and subtracted from this value. To calculate MDA concentration, it was calculated from the extinction coefficient equal to 155 mM^{-1} cm^{-1} and using the following formula [15].

$$\text{MDA (}\mu\text{mol / gFW)} = [A532 - A600/155] \times 1000$$

Total phenol

Total phenolic compounds were measured based on Folin-Ciocalteu colorimetric method. 0.5 grams of the fresh tissue of the plant were ground with 10 ml of 80% methanol in a mortar. The resulting mixture was centrifuged for 15 minutes at 10,000 g at 4°C. 9 ml of distilled water was added to one milliliter of extracts. Next, one milliliter of Folin-Ciocalteu reagent was added and mixed. After 5 minutes, 10 ml of 7% sodium carbonate was added and the samples were incubated for 90 minutes at laboratory temperature. After the mentioned time, the absorption of each sample was read using a spectrophotometer at a wavelength of 725 nm, and the total phenol content was calculated in terms of milligrams of gallic acid per gram of fresh weight. To determine the amount of total phenol, a standard curve was prepared using concentrations of 0 to 500 mg/liter of gallic acid [16].

Soluble sugars

For this purpose, the phenol-sulfuric acid method was used, which is based on the acid hydrolysis of soluble sugars and produces a colored complex while creating a compound of furfural with phenol. The amount of 0.5 g of fresh plant (leaf and root) weight from each treatment was weighed and ground with a fine mortar along with 5 ml of distilled water, then it was filtered. One milliliter of the obtained extract was transferred into a test tube and 1.5 milliliters of distilled water, one milliliter of phenol (5% V/W) and three milliliters of 98% sulfuric acid were added to it. The tubes were kept at laboratory temperature for one hour to get the final color. The intensity of the resulting color was read with a spectrophotometer at 485 nm. In order to determine the concentration of soluble sugars, a standard curve was prepared using concentrations of 0 to 200 mg/ml of glucose [17].

Total protein

Preparation of Bradford solution

Total protein was measured according to Bradford (1976) method [18]. First, Bradford's reagent was prepared by mixing 100 mg of Coomassie Brilliant Blue G250 and 50 ml of pure ethyl alcohol and adding 100 ml of phosphoric acid. After adding 100 ml of distilled water to the above mixture and stirring, the mixture was filtered. The solution obtained by filtering with distilled water was brought to 1000 ml.

Preparation of protein extract

The amount of 0.5 g of fresh plant tissue (leaf and root) was grinded in 5 ml of 50 mM potassium phosphate buffer (pH=7) containing 1% PVP and 1 mM EDTA. All extraction steps were done on ice. Then, the extracts were centrifuged for 20 minutes at 5000 g and 4 ˚C. The clear supernatant solution was used to measure the soluble protein content and enzyme activity.

Soluble protein assay

In order to measure the soluble proteins content, five milliliters of Bradford's reagent were added to 100 microliters of the resulting extract, and the absorbance of the solution was immediately measured at 595 nm. The Albumin standard curve was used to determine total protein.

Catalase activity (CAT) (EC 1.11.1.6)

The activity of catalase enzyme was measured by calculating the decrease in H_2O_2 absorption (decrease in the content of H_2O_2) at 240 nm and by the method of Dhindsa et al. (1981) The reaction mixture included 50 mM potassium phosphate buffer with pH=7 and 15 mM hydrogen peroxide. By adding 100 microliters of enzyme extract to the mentioned mixture, the reaction started. The decrease in the absorption of hydrogen peroxide was measured in 30 seconds at a wavelength of 240 nm. An enzyme unit is the content of an enzyme that decomposes one mM of hydrogen peroxide in

30 seconds. The content of H_2O_2 present in the reaction mixture after 30 seconds was calculated using the extinction coefficient ε=40 mM^{-1} cm^{-1} and formula A = εbc, which indicates the activity of catalase enzyme [19].

$$\text{Units (mM/min)} = \frac{\frac{\text{doD}}{\text{min (slop)}} \times \text{Vol. of assay}}{\text{Extinction coefficient}}$$

Guaiacol peroxidase (GPX) activity (EC 1.11.1.7)

Guaiacol peroxidase enzyme activity was measured using guaiacol precursor. In this method, 3 ml of the reaction mixture contained 2.77 ml of 50 mM potassium phosphate buffer (pH=7), 100 µl of 1% hydrogen peroxide, 100 µl of 4% guaiacol and 30 µl of enzyme extract. The increase in absorbance due to guaiacol oxidation was measured at 470 nm wavelength for 3 minutes. The content of tetraguaiacol formed was calculated using the extinction coefficient of tetragaiacol (ε=25.5 $mM^{-1}cm^{-1}$) and the formula A=εbc [20].

RNA extraction and cDNA synthesis

Total RNA was extracted from plant samples by the method of Louime et al. (2008) [21], with slight modifications. The extraction buffer included Tris-HCl M 0.5 (pH= 8), EDTA M 0.5 (pH= 8), NaCl 2 M, CTAB 2%, PVP 3%, β-mercaptoethanol 10% and sodium sulfite 1%. From each of the samples that were previously powdered in liquid nitrogen, 0.1 g was transferred to 2 ml tubes and one ml of fresh extraction solution was added to it and immediately vortexed and stored in a water bath and placed for one hour. Then, 800 microliters of chloroform were added to the sample tubes and centrifuged for 15 minutes at 13,000 g and at a temperature of 4 ˚C. The supernatant solution was transferred to a 2 ml tube and 800 microliters of phenol:chloroform was added at a ratio of 1 to 1 and centrifuged again for 10 minutes at 13000 g at 4 ˚C, the supernatant solution was transferred to a 2 ml tube and 800 microliters of isoamyl alcohol:chloroform at a ratio of 1 to 24 was added and centrifuged for 15 minutes at 13,000 g at 4°C. The supernatant solution was transferred to a new tube, one tenth of the sodium acetate (3 M), from supernatant solution was added to it, and shaken well, then cold isopropanol was added in an equal proportion to the volume of the supernatant solution. The tube containing the sample was incubated for 16 hours at 4 °C and then centrifuged for 20 minutes at 13000 g at 4 °C. The supernatant solution was discarded and the visible white RNA pellets were dissolved in 20 microliters of deionized water after washing twice with 70% ethanol. After the completion of RNA extraction, DNAase I was used to remove DNA. A mixture of 1 microgram RNA, 1 microliter reaction buffer, DNAase I enzyme in the amount of 1 microliter and 10 microliters of water were prepared and mixed for half an hour at 37 °C or the enzyme to work. Then one microliter of EDTA was added and then vortexed and placed at 65°C for ten minutes to inactivate the DNAase enzyme. RNA concentration was measured using a nanodrop device (Model ONE made by thermo USA) for each sample. The purity of RNA was also determined through the ratio of 260/280 and 230/260 nm.

cDNA synthesis

The first-strand cDNA was synthesized by means of High-Capacity cDNA Kit (Applied Biosystems) using random primers. The materials required for the test were poured into a special tube free of nuclease enzyme and placed on ice, including one microgram sample RNA and one microliter dT Oligo, then DEPC water was also added to the amount that the total content in the tube reaches 12 microliters. If the template RNA is rich in cytosine and guanine nucleotides or has a secondary structure, it is shaken well and then centrifuged a little and finally placed at 65°C for 5 minutes. After PCR, 4 microliters of reaction buffer (5X) one microliter inhibiting the activity of RNAase enzyme, two microliters of Deoxynucleotide triose phosphate (dNTP) and one microliter of Reverse Transcriptase enzyme were added to the tube. The tubes were placed at a temperature of 42 °C for one hour in a PCR machine made by Eppendorf, Germany, to synthesize cDNA. For sequences rich in CG, the temperature of the device can be increased up to 45 °C. The reaction was finished by

heating the desired mixture for 5 minutes at 70 °C.

RT-PCR reaction

The reaction mixture for the RT-PCR analysis was made in 25 microliters of a solution containing 12.5 microliters of Mastermix, 0.5 micromoles of each of the primers, 0.5 microliters of cDNA and 11 microliters of water. This reaction started at 95°C for 3 minutes (separation of two DNA strands) and with 26 cycles of 95°C periods of 30 seconds (the end of each cycle), continued at 54°C for 30 seconds (primer connection to the target sequence) and at 72°C for 30 seconds (extension and new strand construction) and finished at 72°C for 10 minutes (final extension) (ABI PRISM 7000 Real-Time PCR, USA). After the completion of the reaction, the product was transferred to a freezer at -20 °C. 18s rRNA gene was used as an internal reference gene. The sequences of specific primer pairs of genes used for PCR are listed in table 1.

Results and discussion

Na^+, Cl^- and K^+ contents

Different levels of salinity stress had significant ($p<0.05$) effects on sodium, potassium and chloride ions content of leaf and root compared to the control plants, as NaCl salinity led to an increase in the content of Na^+ and Cl^- in leaf and root tissue of quinoa plant with increasing salinity (Figures 1, 2, and 3). Increasing salinity levels up to 400 mM increased sodium and chloride ions approximately 7 and 5 times (400 mM NaCl) in the leaf tissue and 12 and 3 times (400 mM NaCl) in the root tissue compared to the control sample, respectively. Untreated plants showed a higher content of potassium ion, and with increasing salinity, the amount of this ion decreased significantly in leaf and root samples.

Accumulation of Na^+ and Cl^- in Quinoa leaves at high levels of salinity stress indicates a mechanism for osmotic adjustment in quinoa plant. In many halophyte plants, the accumulation of mineral ions for osmotic adjustment requires less energy compared to the new synthesis of organic solutes [22]. However, excess accumulation of salt more than the required amount can lead to toxicity and imbalance. It seems that this strategy is for the purpose of adequate tolerance or stress avoidance mechanism by regulating the internal concentrations of Na^+ and Cl^- [23, 24]. The ability of quinoa to grow even at a concentration of 400 mM of NaCl indicates the fact that this species has a great control over the absorption, transport and organization of ions inside the plant, and avoiding the continuous accumulation of NaCl in the tissue with active metabolic processes [25].

A number of previous studies on quinoa indicate that ion accumulation in different parts of the plant shows a significant difference. According to the study of Eisa et al. (2012), mature leaves are used as storage sites for Na^+ ions; because ion accumulation in these leaves is quantitatively higher than in young leaves and this shows the extraordinary organization of the quinoa plant in Na^+ ions sequestration rather than the accumulation of these ions in photosynthetically young and active parts. Also, the study of these researchers showed that bladder hairs on quinoa leaves act as salt removers [26].

Table 1. Primers used in RT-PCR experiments in quinoa plant (*Chenopodium quinoa* L.)

Genes	Forward Primer (5'→3')	Reverse Primer (5'→3')
18s rRNA	5′-ATGATAACTCGACGGATCGC-3′	5′-CTTGGATGTGGTAGCCGTTT-3′
NHX	5′-TTCTGGATTGCTCAGTGCTT-3′	5′-CAGCCAGCATGTAAGAGAGG-3′
SOS	5′-GGAAGGTTTGGGGATGGTAT-3′	5′-GTCCAGCAAGCAAACCATT-3

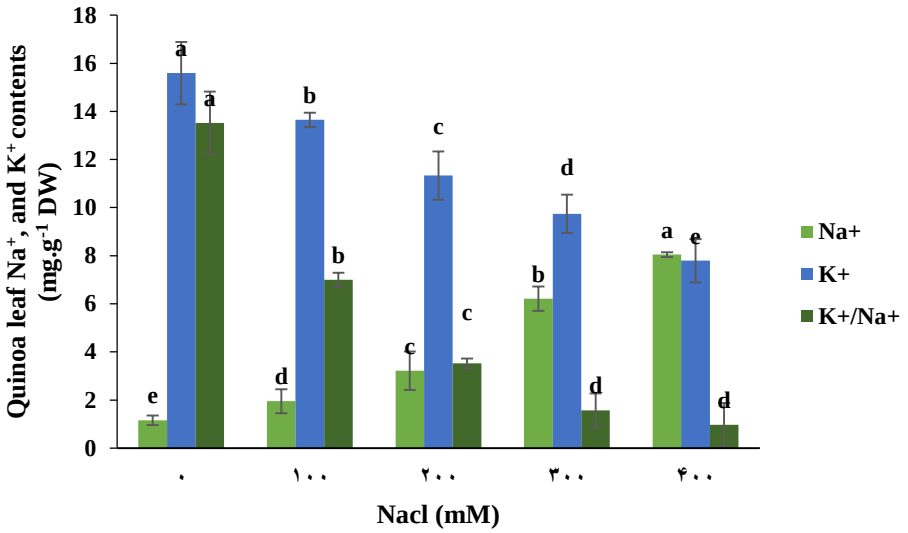

Figure 1. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on the content of sodium, and potassium ions and the ratio of K^+/Na^+ in quinoa leaves. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

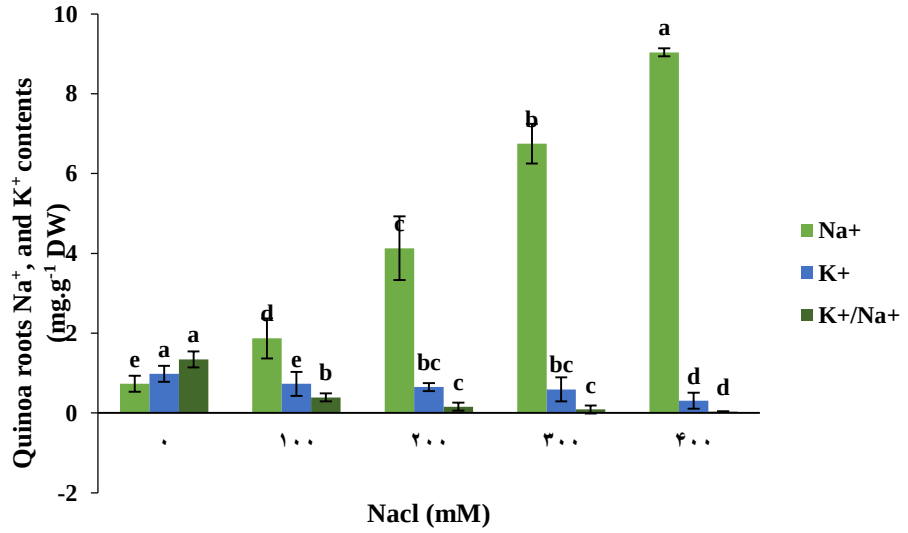

Figure 2. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on the content of sodium, and potassium ions and the ratio of K^+/Na^+ in quinoa plant roots. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

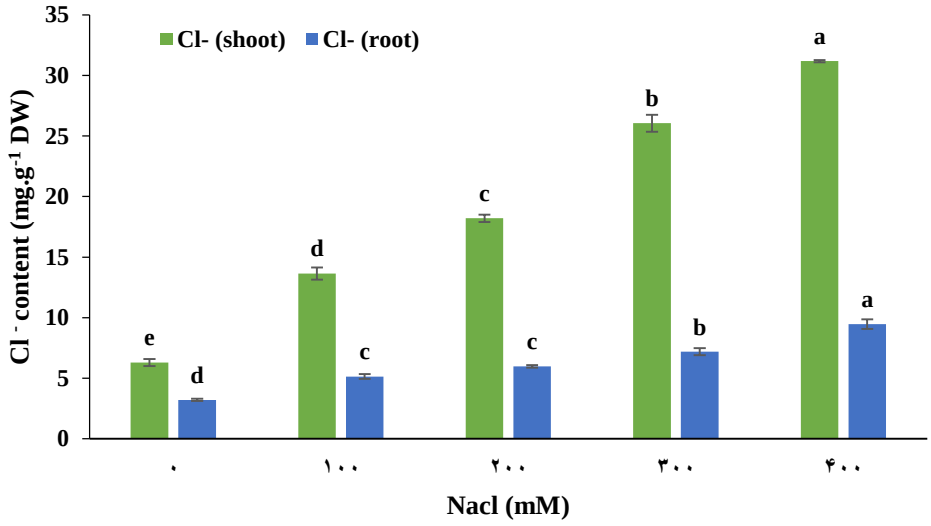

Figure 3. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on the content of chlorine in quinoa plant shoots and roots. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

Sodium accumulation was associated with a significant decrease in K^+ levels (Fig 1 and 2). Similar results have been reported before [6, 27-29], which could be due to the competition between Na^+ and K^+ in the ion absorption sites or due to the change in the integrity of the membrane, which also could be due to the replacement of Na^+ ions instead of Ca^{2+} [29]. Although this reduction in K^+ by inducing salt stress does not necessarily mean potassium deficiency and in most dicotyledonous plants, the osmotic function of potassium, calcium or magnesium in the vacuole can be shifted without any effect on growth [30].

Nutritional disorders are one of the specific effects of salinity on plants, which occur due to the high concentration of one ion compared to other ions in the soil solution, and the created ion competition prevents the absorption of some ions by the plant. Among these ions, sodium and potassium for example compete for diffusion into the cell [31], because they are transported into the cell by common proteins. Since potassium is an essential cofactor for many enzymes, in saline conditions and in the transport competition between sodium and potassium through the same transporters, potassium deficiency is observed in the plant, which can be seen both in the leaf and the root tissues. The accumulation of sodium in the concentration of 400 mM NaCl in the root was higher compared to the leaf, which is probably due to returning of this element from the shoot to the root under high level of stress. However, it can be attributed to the restricted sodium transport to shoot and keeping it in root, which is one of the resistance mechanisms of the plant against salt stress [32].

By comparing the content of chlorine accumulated in the roots and leaves, it is found that the transfer of ions to the leaves is high, and it can be due to the ion balance and creating an osmotic gradient in the direction of transferring more water to the aerial organs, as in all halophytes. Also by comparing The content of potassium contents with chlorine and sodium ions, it is found that the lowest content of potassium was observed in root samples, which could be due to the competition between sodium and potassium to enter the cell, which indicates the greater abundance of sodium in the root environment [32]. The results of the studies of Barhoumi, et al. (2008) also showed that with the increase of NaCl content in the root environment of the *Atriplex* halophyte plant, the amount of sodium ions in the shoot and root increases and potassium also decreases [33].

Hydrogen peroxide content

The results of investigating different levels of salinity stress on the content of hydrogen peroxide showed that with the increase of salinity stress, the amount of hydrogen peroxide increased significantly up to 300 mM and after that showed a decrease in the concentration of 400 mM of salinity (Figure 4). One of the important biochemical changes during salinity stress is the increase in the production of reactive oxygen species, which in the next stage causes oxidative stress in the plant and may cause cell membrane damages. Electrons leaked from the electron transport chain by reacting with oxygen can produce a variety of active species such as hydrogen peroxide and superoxide radicals, which are very reactive and toxic, and if there is no protective system, biomolecules such as proteins, nucleic acids and lipids will damage by creating oxidative stress [7]. Compared to other free radicals, such as superoxide and hydroxyl, hydrogen peroxide is the most stable and least reactive radical and can easily pass through the membrane and lead to cell damage. Of course, this substance acts as a secondary messenger in small amounts and is necessary for cell metabolism; But it is harmful to the cell in high concentrations, and its excessive accumulation must be prevented with plants antioxidant defense [34]. Iran-Bakhsh and Daneshmand (2021) showed that the increase in the content of hydrogen peroxide, as an index of oxidative stress, at all levels of salinity stress (0, 8, 16 and 32 ds/m) were observed in Q18 and Rosada cultivars, especially at 32 ds/m in Sajama of quinoa cultivars [35], which is consistent with the results of the present study. Also in the study by Singha and Choudhuri (1990), on effect of salinity stress on H_2O_2 metabolism, it was observed that the level of H_2O_2 was increased in *Vigna* and *Oryza* seedlings [36]. Also, Mosayebi et al. (2022), showed that salinity had a significant effect on the amount of hydrogen peroxide in the treatments, so that the highest amount of hydrogen peroxide was observed in the treatment of 400 mM salt on Salsola halophyte [37].

Malondialdehyde (MDA) content

Malondialdehyde is a compound that shows the level of lipid peroxidation and is used as an indicator to determine the membrane destruction degree caused during stress. According to the results, by increasing the stress level up to a concentration of 200 mM, the content of malondialdehyde did not show a significant change, but with an increase in the concentration of salinity up to 300 mM and then 400 mM, the amount of malondialdehyde increased. Esfandiari et al. (2011) showed that increasing salinity levels increased the amount of malondialdehyde in both wheat cultivars [38]. In the study of Bhattacharjee and Mukherjee (2002), the concentration of malondialdehyde in leaves under salt stress increased in salt sensitive and tolerant rice seedlings, which is consistent with the results of the present study. The results of this study showed that the destruction of cell membranes under the influence of salt stress, and the production of malondialdehyde in the leaves, which is caused by the destruction and breakdown of cell membrane fats, can be used as a suitable measure to investigate the reaction of plant tolerance against salinity (Bhattacharjee and Mukherjee, 2002). Salt stress causes the destruction of cell membranes and increases the content of malondialdehyde, which is higher in the tissue of sensitive plants than in tolerant plants. The present study shows that the content of malondialdehyde in the concentration of 100 and 200 mM did not increase significantly compared to the control; However, at higher concentrations, there was a small but significant increase in the content of this index compared to the control (Figure 5), which indicates that the quinoa plant is tolerant to salt stress.

Total phenol content

Phenolic compounds in plants are synthesized from phenyl-propanoid pathway and using the key enzyme of this pathway, that is, phenyl-alanine-ammonialyase. These compounds can act as scavengers of oxygen free radicals. In most studies, an increase in the content of these compounds has been reported during stress [39-41]. These compounds, with their protective role, increase the plant's resistance against stress; Also, by accumulating in the epidermal tissue of the plant, they can protect the photosynthetic tissues against excess radiation.

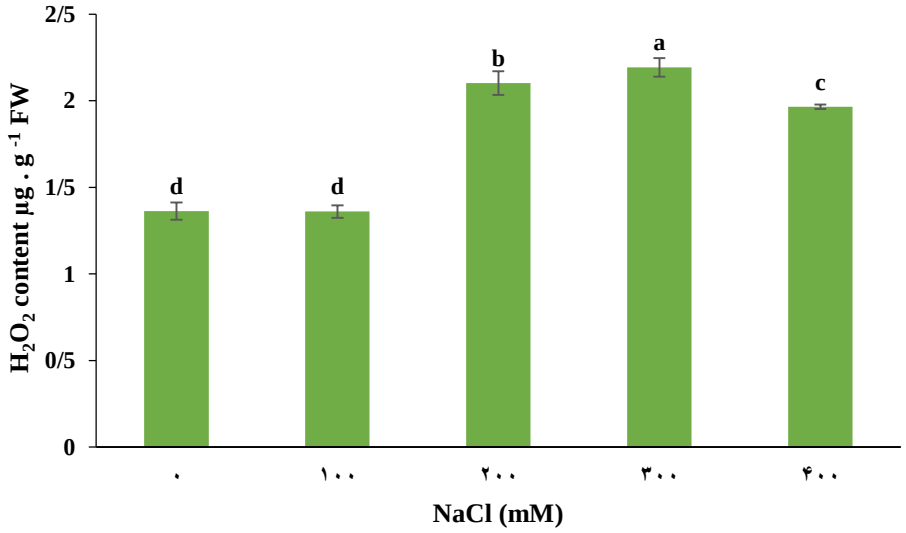

Figure 4. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on the hydrogen peroxide content of quinoa plant leaves. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

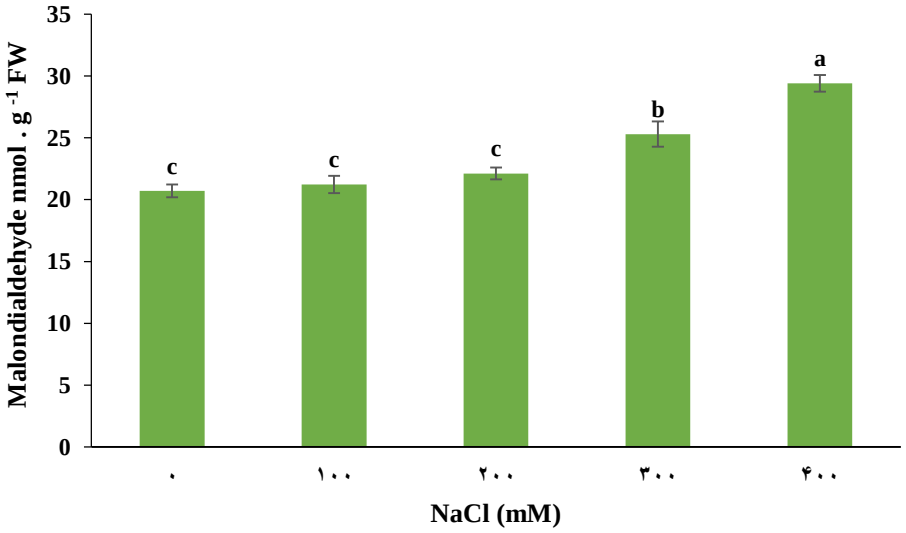

Figure 5. Effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on malondialdehyde content of quinoa plant leaves. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

In order to protect themselves against salt stress, plants need to use mechanisms to reduce the toxicity caused by salt stress. In the present study, with increasing salinity levels, the amount of total phenol also increased. However, at a concentration of 400 mM of NaCl, a decrease in the content of phenol was observed (Figure 6). It is possible that the plant resisted against oxidative stress by using other antioxidant mechanisms. In a study on the content of quinoa seeds under salinity stress conditions, it was observed that the phenol content of quinoa seeds increased with increasing salinity levels [42], which is consistent with the results of the present study.

Soluble sugar content

According to the obtained results, it can be seen that with increasing levels of salinity stress, the content of soluble sugar in the leaves also increased. So that the highest content of Soluble sugar (49.6225 µg/g of fresh weight) was observed in the concentration of 400 mM of Nacl (Figure 7). The most important groups of compatible solutes studied in quinoa plant are carbohydrates. Accumulation of soluble sugars has been repeatedly reported as a response to salinity stress and plays an important role in osmotic adjustment of most salinity-tolerant plants (Murakeözy et al., 2003). In addition to the important role of these compounds in osmotic adjustment, they may also play a role as molecular chaperones (Liu et al., 2008). There are many similar observations that show an increase in the content of soluble sugar with increasing levels of salinity stress, which is observed in this study too. For example, Nemati et al. (2011), by investigating the effect of salinity stress on *Oryza sativa* L., observed that with the increase of stress, the content of soluble sugar increases, which they are attributed to the potential of osmotic adjustment of the plant and improvement of water absorption [43]. Also, Molazem's study (2022), by investigating the physiological response and antioxidant defense mechanism of corn cultivars (*Zea mays* L.) to salt stress showed that resistant cultivars tolerate induced salt stress by increasing osmolality and antioxidant enzyme activity [44].

Protein content

The content of soluble protein showed a significant increase with increasing salinity stress levels (Figure 8). The change in the content of plant protein under salt stress depends on the intensity of the stress and the tolerance of the plant. In some quinoa genotypes, it has been observed that with the increase of salinity stress, the content of protein increases [45], which indicates the potential of osmotic adjustment of the plant to deal with the stress and requires energy spending. Protein accumulation under stress conditions in many plant species is related to stress tolerance, and generally high protein concentrations are found in salt-tolerant plants compared to sensitive plants [46]. In addition to helping the osmotic adjustment of the plant under stress conditions, protein accumulation also leads to membrane stability and cell survival. Moreover, under stress conditions, plants synthesize new proteins that help them grow and develop under stress conditions and lead to osmotic balance and decrease the toxicity of sodium ions in the cytoplasm compared to sensitive species, and preventing protein reduction under stress conditions [45]. In fact, an increase in the content of soluble protein indicates an increase in the plant osmotic adjustment power and an increase in salt tolerance.

Catalase and guaiacol-peroxidase activities

Against the oxidative stress caused by other stresses, plants initiate antioxidant defense systems by activating enzymatic and non-enzymatic antioxidants. Non-enzymatic antioxidants include ascorbate, tocopherol, carotenoid, glutathione, phenolic compounds, flavonoids, and anthocyanins, and enzymatic antioxidants include catalase, ascorbate peroxidase, polyphenol oxidase, and superoxide dismutase, which are oxygen free radicals protect the plant against oxidative damage [47]. The antioxidant enzyme guaiacol-peroxidase is responsible for the quenching of singlet oxygen, the level of its activity compared to different plants can show the plant's tolerance against different stresses. Of course, there are other various factors to determine the plant's tolerance to salinity, which must be taken into account. In the study of Goudarzi and Pakniyat (2009), the amount of peroxidase enzyme in wheat plants exposed to salinity increased [45], something that can be seen in this study as well.

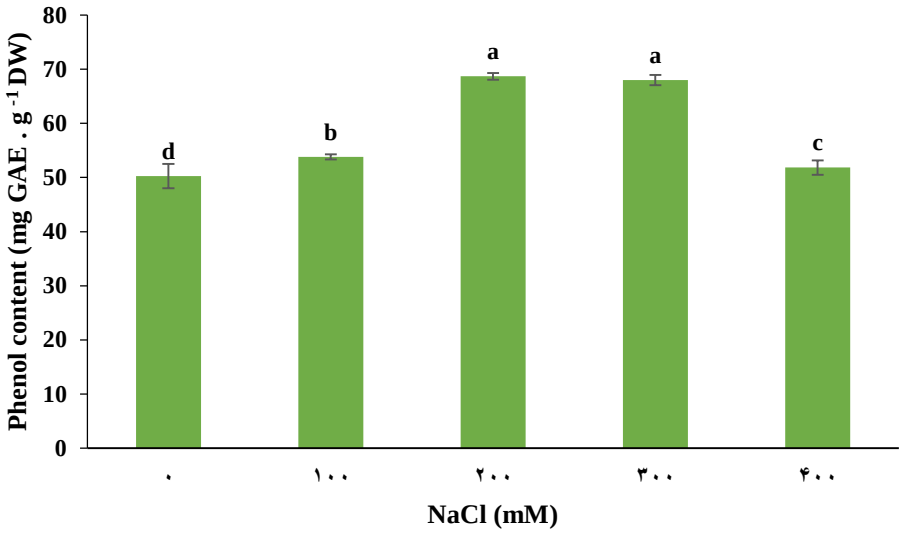

Figure 6. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on the total phenolic content of quinoa plant leaves. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

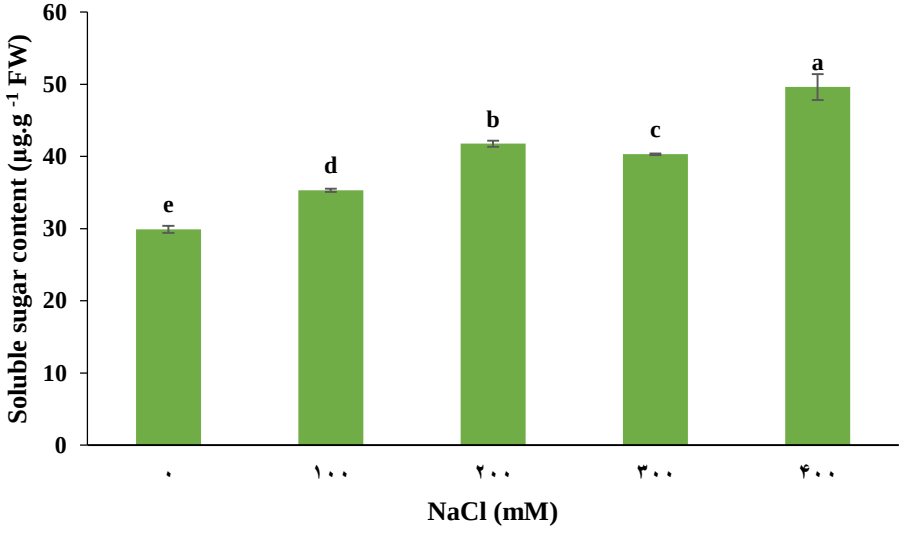

Figure 7. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on the sugar content of quinoa leaves. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

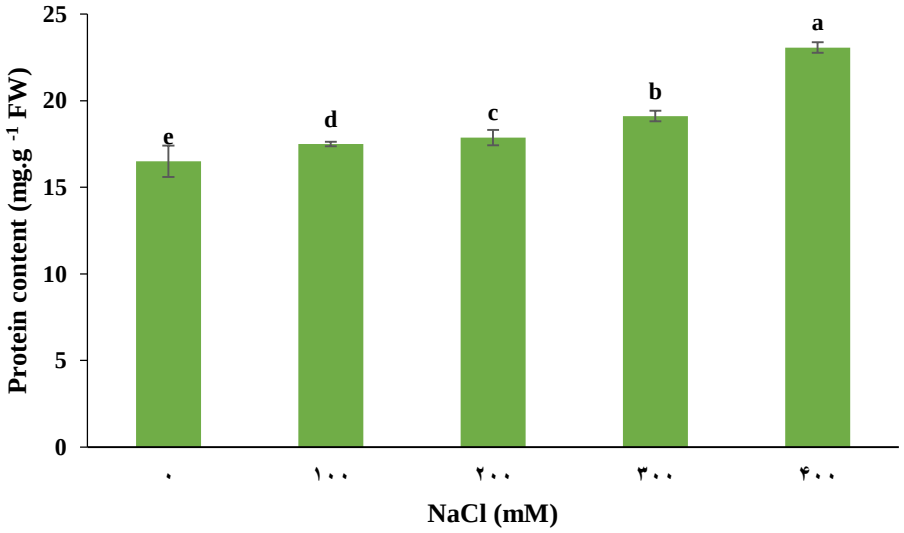

Figure 8. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on the soluble protein content of quinoa plant leaves. Values represent the mean ± SE and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

According to the results, it was observed that the amount of catalase activity showed a significant increase with the increase of salinity stress levels up to 200 mM. The highest activity of this enzyme was observed at a concentration of 200 mM; But at concentrations of 300 and 400 mM of NaCl, a significant decrease in its activity was observed (Figure 9). Catalase catalyzes the decomposition of H_2O_2 into water and oxygen, and peroxidase basically removes H_2O_2 from chloroplasts. Among the antioxidant enzymes, superoxide dismutase is in the first line of oxidative defense, and it is necessary to study the activity of all enzymes for comprehensively investigate the effect of stress on plant antioxidant changes and determine its tolerance level. Change in the activity of antioxidant enzymes in different concentrations can be attributed to genetic changes and difference in the kinetics of stress development. Considering the changes in the amount of H_2O_2 and against it, the amount of phenol and catalase enzyme, it seems that the plant can cope well with the use of antioxidant defense mechanisms in concentrations of 200 and 300 mM of NaCl to protection against salinity stress. Considering the increase in the content of malondialdehyde in the concentration of 400 mM NaCl, as well as the decrease in the content of phenol and the activity of the catalase enzyme, it is possible that the plant does not have enough protective power in this concentration, or it has been used other defense mechanisms like the activity of other antioxidant enzymes such as peroxidase. As peroxidase enzyme activity is observed, its highest activity was at 400 mM NaCl concentration (Figure 10).

NHX gene expression

In plants, the main mechanism of transferring excess amounts of cytosolic sodium ion to vacuoles or tissues with lower sensitivity prevents the toxic effects of this ion. This action is performed by a group of Na+/K+ antiporters belonging to the IC-NHE/NHX subfamily located in the tonoplast. The transport of sodium ion by means of protons across the tonoplastic membrane is carried out by Na^+/K^+ antiporters, which are encoded by NHX genes. In the present study, the expression of NHX gene showed a significant increase one day after the application of 400 mM stress compared to the control, and the highest level of gene expression was related to this treatment (figure 11). In the treatment two days after the stress, the expression of this gene showed a fivefold increase. From the third day after application of salt stress and stable ratio of

sodium to potassium ions and establishment of ionic balance in quinoa, the expression level of this gene did not show a significant increase or decrease compared to the control, that such behavior can be observed in the high power of this plant to quickly establish ion balance in the plant (figure 10). An increase in NHX gene expression after salt stress has been reported in Arabidopsis, rice, tomato and bean plants [48, 49]. Also, the expression level of NHX gene in safflower plant during six, 12, 24 and 48 hours after application of salt stress showed a significant increase compared to the control samples [50], which indicates the importance of vacuolar membrane antiporter gene in the mechanism of plant salt tolerance, especially in salt-tolerant plants such as quinoa.

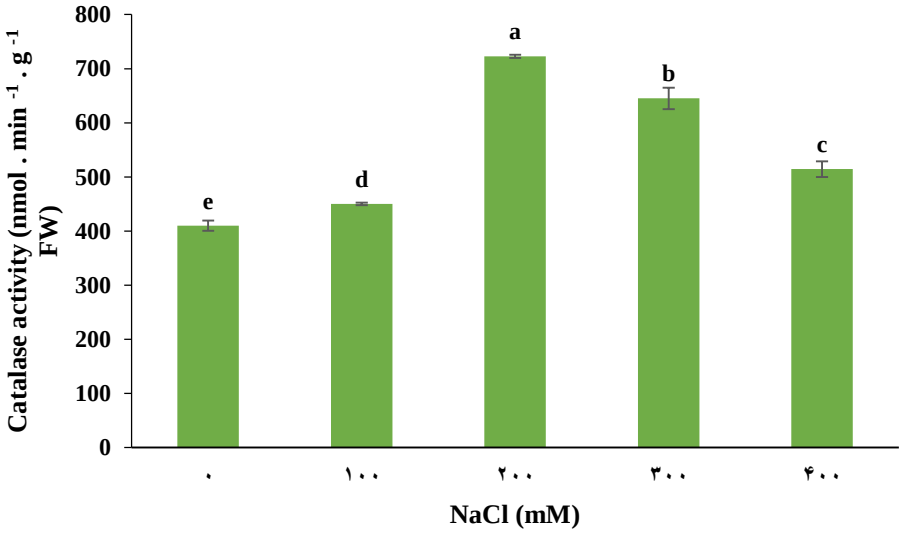

Figure 9. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on catalase enzyme activity of quinoa leaves. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

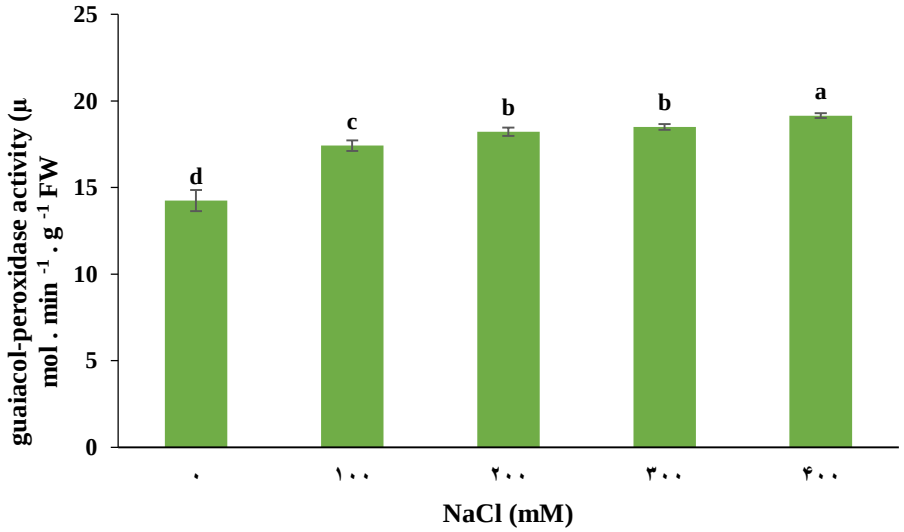

Figure 10. The effect of different levels of salinity stress (0, 100, 200, 300 and 400 mM) on the content of guaiacol peroxidase enzyme activity in quinoa plant leaves. The values represent the mean ± SE, and different letters in the columns indicate the significance of the means based on Duncan's test ($p<0.05$).

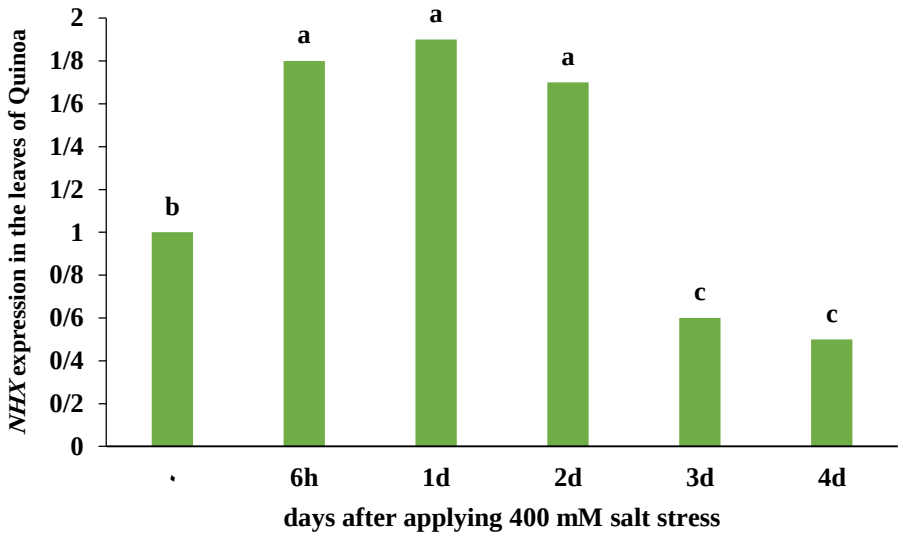

Figure 11. The relative expression changes of the *NHX* gene in the concentration of 400 mM NaCl in Quinoa leaves

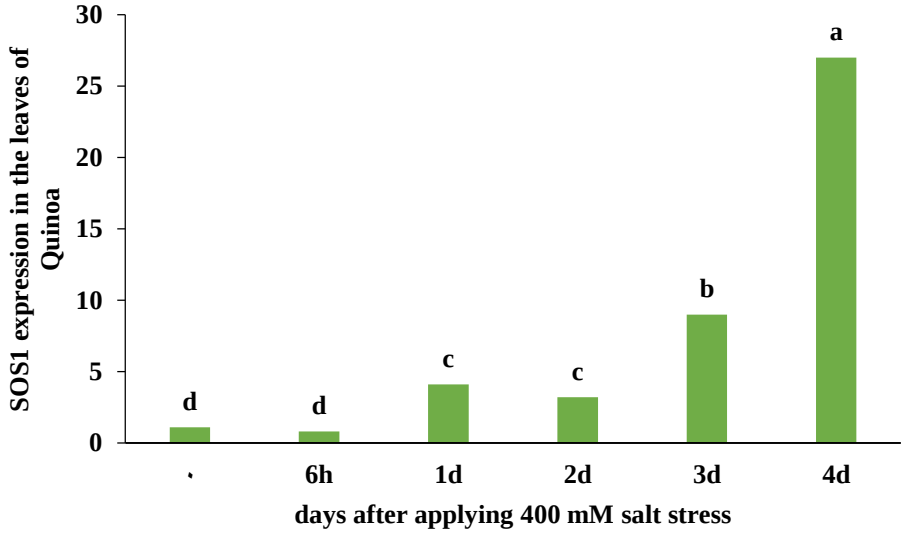

Figure 12. The relative expression changes of the SOS1 gene in the concentration of 400 mM NaCl in Quinoa leaves

SOS1 gene expression

One of the most important factors involved in reducing cytosolic sodium accumulation in plant cells, especially plants related to saline areas, is the regulation of Na^+ release using the transport processes of this ion and its vacuole compartmentation. Sodium excretion in higher plants is performed using plasma membrane antiporters (SOS1) [51].

Expression of SOS1 in leaf tissue and at a concentration of 400 mM of NaCl increased fourfold on the first day after applying (figure 12), and the highest expression of the gene was observed on the fourth day, which shows the key role of Na^+/H^+ antiporters, which shows resistance against salinity. Also, genes involved in sodium transport during salt stress indicates that plants can control the high concentration of potassium ion and low concentration of sodium

ion in the cytosol by regulating the expression and activity of sodium and potassium transporters and proton ion pumps that create proton driving force required for transportation processes [52]. In a study, the expression level of TaSOS1 gene reached its highest level after three hours of stress applying in the roots of wheat plants, and then returned to the normal state [53].

Conclusion

Quinoa, with its remarkable growth characteristics in unfavorable climates and due to the richness of the plant in terms of valuable nutritional and medicinal substances, can be a very suitable option for cultivation in low yielding and salty soils. The studies of changes in physiological and biochemical indicators of this plant against different levels of salinity showed that quinoa plant has shown a very good resistance to salinity stress up to 300 mM NaCl concentration. Salinity led to an increase in the content of Na^+ and Cl^- and a decrease in K^+ ions with an increase in salinity in the leaf tissue and root of quinoa plant, which is probably due to the transfer of sodium from the shoot to the root under high stress and limiting the transfer of sodium to the shoot and cause to its storage in the roots, which is one of the plant's resistance mechanisms against salt stress. Also, the amount of soluble sugars and total protein increased with increasing stress levels, which can be a sign of osmotic adjustment. Malondialdehyde and peroxidase enzyme activity also showed a significant increase with increasing levels of salinity stress. The highest amount of phenol, catalase enzyme activity and phenol content were observed in 200 and 300 mM of NaCl concentrations. Also, the expression of NHX gene showed a significant increase one day after the application of 400 mM NaCl compared to the control. Of course, tolerance to salinity can be seen in this plant in higher amounts; But the effects of salinity stress can be observed to some extent at a concentration of 400 mM in some physiological and biochemical indicators of the plant. In general, cultivation of this plant in the presence of water and saline soil can be guaranteed by applying proper management and its valuable benefits can be used by cultivating it in saline soils.

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مقاله پژوهشی، صفحات۲۱۱-۱۹۹

تأثیرات ناشی از شوری بر ویژگی های فیزیولوژیکی و بیوشمیایی گیاه کینوا (*Chenopodium quinoa* L.) بومی حاشیه دریاچه ارومیه

رز اقبالیان، ناصر عباس پور و لطیفه پوراکبر

ایران، ارومیه، دانشگاه ارومیه، دانشکده علوم، گروه زیست شناسی

تاریخ دریافت: ۱۴۰۲/۰۵/۱۹ تاریخ پذیرش:۱۴۰۲/۰۷/۰۳

چکیده

جهت مدیریت آب و زمین‌های شور، کشت گیاهان متحمل به شوری مانند کینوا گزینه‌ی بسیار مناسبی است که علاوه بر قابلیت رشد در محیط‌های مختلف، دارای خواص دارویی و تغذیه‌ای بسیار ارزشمند نیز می‌باشد. به منظور بررسی تأثیر سطوح مختلف شوری روی گیاه کینوا، آزمایشی بصورت فاکتوریل در قالب طرح کاملاً تصادفی با چهار تکرار و سطوح شوری ۰، ۱۰۰، ۲۰۰، ۳۰۰ و ۴۰۰ میلی‌مولار از نمک NaCl در آزمایشگاه و اتاق کشت دانشکده علوم، دانشگاه ارومیه، انجام شد. با افزایش تنش شوری، محتوای یون‌های سدیم و کلر افزایش نشان دادند و بیشترین میزان آنها) به ترتیب ۸/۰۴ و ۳۱/۱۸ (mg . g -1 DW در غلظت ۴۰۰ میلی–مولار مشاهده شد. همچنین بیشترین میزان پتاسیم (59/15 mg . g -1 DW) در نمونه‌های شاهد مشاهده شد و با افزایش میزان تنش محتوای آن کاهش یافت. میزان قند محلول، پروتئین کل، مالون‌دی‌آلدهید و فعالیت آنزیم پراکسیداز) به ترتیب µg.g -1 FW6/49، mg.g -1 FW 07/23، nmol.g -1 FW 4/29، µmol.min -1. g FW 1/19) با افزایش سطوح تنش شوری افزایش معنی‌داری نشان دادند. همچنین بیشترین میزان فنول، فعالیت آنزیم کاتالاز و محتوای پراکسیدهیدروژن) به ترتیب mg GAE.g -1 DW 7/68، nmol.min-1 . g -1 FW 9/722 و µg.g -1 FW 19/2) نیز در غلظت‌های ۲۰۰ و ۳۰۰ میلی‌مولار نمک مشاهده گردید. بطور کلی گیاه کینوا می‌تواند انتخاب مناسبی برای کشت در زمین‌های شور و کم بازده باشد.

واژگان کلیدی: آنزیم کاتالاز، پراکسیدهیدروژن، تنش شوری، کینوا، مالون‌دی‌آلدهید