Journal of Nuts

Journal homepage: ijnrs.damghan.iau.ir

ORIGINAL ARTICLE

Impact of Salinity on Growth Rate, Physiology, Elemental Composition, and *NHX1* Gene Expression of Almond (*Prunus dulcis*) Cultivars

Ghader Amani[1], Mansoore Shamili[*2], Ali Imani[3], Amir Mousavi[4], Hamed Rezai[5]

[1]*Department of Horticultural Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Hormoz Research Institute, University of Hormozgan, Hormozgan, Iran*

[3]*Horticultural Department of Seed and Plant Improvement Institute (SPII), P.O. Karaj, Iran*

[4]*National Institute of Genetic Engineering and Biotechnology (NIGEB), Iran*

[5]*Soil and Water Research Institute Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*

K E Y W O R D S

Chlorophyll;
Growth;
Macro elements;
Micro elements;
NHX1 gene expression;
Osmolites;
Photosynthesis

A B S T R A C T

In almonds (*Prunus dulcis*), selecting salt-tolerant rootstocks and genotypes is an appropriate breeding strategy. In the present research, we grafted two commercial almond cultivars ('Sahand' and 'TS3') on the 'GF677' rootstock. Then, we monitored the impact of salinity (0.5, 6.5, and 8.5 dS m^{-1}) on the morphological, physiological, and molecular characteristics of the Sahand and TS3 cultivars. The photosynthetic rate, chlorophyll (a, b and total) content, and carotenoid content decreased with increasing salinity levels in both cultivars, with the least decrease observed in TS3. Under a salinity level of 8.5 dS/m, Sahand exhibited the lowest growth (8.9 cm), leaf area (5412.5 mm^2), Chl_a, Chl_b, Chl_{total} and carotenoid contents (0.58, 0.15, 0.74 and 0.31 mg g^{-1}FW, respectively). Additionally, Sahand had a Fm/Fv (0.75), N content (1.33%) and Ca, B, Mg, S, Fe and Zn values of 1654.55, 1.64, 395.28, 168.6, 10.35 and 3.05 mg L^{-1}, respectively. Furthermore, Sahand exhibited the highest MDA level (25.17 nmol g^{-1}FW), TFC (2.95 mg GA g^{-1}FW), Na content (649.84 mg L^{-1}) and Cl content (3.52%). the lowest TFC (1.75 mg GA g^{-1}FW) and the highest *NHX1* expression, photosynthesis rate (5.65 µmol m^{-2} s^{-1}), gs (0.1 mol m^{-1} s^{-1}) transpiration rate (6.08 mmol m^{-1} s^{-1}), Ca, S and B content (1903.63, 196.9 and 2.09 mg $L^{-1,}$ respectively) were belonged to TS3 under 0.5 dS/m salinity. Higher levels of Mg and Fe in the TS3 cultivar resulted in the stablization of photosynthetic pigments. Compared to Sahand, TS3 had a higher nitrogen content, and its greater *NHX1* expression was a molecular confirmation of its salt tolerance.

Introduction

Climate change and biodiversity loss are severe concerns on the global scale (Anonymus, 2022; Vahdati *et al.*, 2019). Irrigated lands in semi-arid regions face salinity as one of the main climate change factors (Lotfi

[*]**Corresponding author**: Email address: shamili@ut.ac.ir
Received: 16 June 2023; Received in revised form: 5 August 2023; Accepted: 9 September 2023
DOI: 10.22034/jon.2023.1988992.1226

et al., 2009). It is estimated that 0.25 to 0.5 million hectares become less productive every year because of salt accumulation (FAO 2002). Salinity reduces plant growth, development, and productivity (Tunuturk *et al*., 2011). It causes nutrient deficiency due to the competition of Na^+ and Cl^- with Ca^{2+}, K^+ and NO_3^- (Alshaal *et al*., 2017; Forni *et al*., 2017). The major strategies of salinity tolerance in plants consist of alternation in morphological characteristics such as longer root length and dense hairy roots (Krasilnikoff *et al*., 2003), as well as physiological and biochemical responses, including the compartmentalization of salts in vacuoles, cytosolic osmotic regulation (Munns and Tester, 2008; Behzadi *et al.*, 2021), enzyme activities' changes (Helaly *et al*., 2018), potassium homeostasis, and salt elimination from shoot to root (Zhu *et al*., 2008; Huang *et al*., 2009). Some plants transport cytosolic Na^+ via the Na^+/H^+ antiporter group (encoded by *NHX* genes) (Nass *et al*., 1997). Some fruit tree species are sensitive to saline soil and water. The growth of pistachios (Adish *et al*., 2010; Gharibiyan *et al.*, 2023) and peaches (Tattini, 1990) is infulenced by soil salt concentrations (3 dS/m).

Almonds, *Prunus dulcis* (Mill.) D.A. Webb, a member of the Rosaceae family, are grown worldwide (Kester and Gradziel, 1996). Almonds possess 16 (2n = 2x = 16) small and discrete chromosomes (Corredor *et al*., 2004). Almond production had an increase of 1.6-fold from 2010 to 2020 (https://www.fao.org/faostat/en/#compare). According to FAO reports (2021), total world almond production reached nearly 4 million tons in 2021, with Iran ranked 6th worldwide (163,568 tons; cultivation area of 75,553 ha) after the United States (2,189,040 tons), Spain (365,210 tons), Australia (285,605 tons), Turkey (178,000 tons), and Morocco (169,255 tons) (FAO 2021).

Almonds are nut crop that consumed raw or roasted to prepare almond milk, oil, butter, flour, and paste (Ansari and Gharaghani, 2019). Almond's nut has high nutritional value that essential oil has been applied to treat neurotic disorders and respiratory problems. In addition, it has numerous biological activities such as probiotic, antimicrobial, antioxidant, anti-inflammatory, anti-cancer, laxative, and cardiometabolic protective impacts (Ansari and Gharaghani, 2019; Karimi *et al*., 2021).

Almond is one of the main fruit trees that it can thrive in semi-arid areas and calcareous soils (Ansari and Gharaghani, 2019; Imani *et al*., 2021). Almonds are introduced as salt-sensitive crops (Zrig *et al*., 2011). Na and Cl are the most toxic ions for almond and *Prunus* rootstocks (Sandhu *et al*., 2020). Treating with 3 dS/m of NaCl reduced gas exchange and increased the root-to-shoot ratio in almonds (Zrig *et al*., 2016). Increasing the salt concentrations from 1 to 5 dS/m resulted in leaves falling and reduced bitter almonds' vegetative growth and chlorophyll and carotenoid contents (Badran and Savin, 2019).

Salinity considered as one of the most serious threats limiting plant growth and development therefore in almonds, selecting salt-tolerant rootstocks and scions is an appropriate breeding strategy (Montaium *et al*., 1994; Robatjazi *et al*., 2020). Long-term (20 years) salt exposure on six almond rootstocks revealed that trees grafted on peach-almond hybrid rootstocks were larger than those on peach rootstocks. Also, peach-almond hybrid rootstocks had lower Na and Cl contents (Doll *et al*., 2014).

Plants acclimate to abiotic stresses through rootstock to scion molecular signaling and transcriptional alterations in both parts (He *et al*., 2009; Li *et al*., 2016). Investigations on the effect of abiotic stresses on different rootstock-scion combinations in citrus (Balfagón *et al*., 2021), walnut (Vahdati *et al.*, 2021) and tomato (Rouphael *et al*., 2017) have revealed the impact of each grafted unit, besides their interactions. Studies indicate lower accumulation of Na^+ and/or Cl^-

in the shoots and higher translocation of K^+, Ca^{2+} and Mg^{2+} in the leaves of grafted citrus, *Prunus*, and cucumber cultivars (Moya *et al*., 1999; Massai *et al*., 2004; Zhu *et al*., 2008; Zrig *et al*., 2011). Also, cultivar-dependent responses such as altered sodium and potassium contents (Shibli *et al*., 2000; Zrig *et al*., 2015a), declined shoot development (Zrig *et al*., 2016), and modified chlorophyll contents (Momenpour *et al*., 2018) were observed in the aerial parts of salt-exposed almond scion/rootstock combinations. Salt-tolerant almonds upregulate genes related to cation transporters, stress signaling, and biopolymer synthesis (Shao *et al*., 2021). Also, gene expression analyses of 14 almond genotypes showed higher expression of *P5CS1*, *SLAH3*, *NHX2*, and *NHX1* in the salt-tolerant genotypes (Sandhu *et al*., 2020). Also BADH gene increased salt tolerance of walnut through catalyzing the last, irreversible step in the synthesis of the osmoprotectant glycine betaine from choline (Rezaei Qusheh Bolagh *et al.*, 2020).

Since the plant growth and physiology is always affected by abiotic stress, mainly salinity, it is essential to estimate plants responses, understand the molecular involved-mechanisms and introduce stress-tolerant genotypes (Lotfi *et al.*, 2019; Gharaghani *et al*., 2018). In this research, we aimed to reveal the expression profiles of growth rate, physiology, elemental compositions and gene expression in two selective candidate almond cultivars under different salt levels to estimate their potential usage as pre-selective candidates for future salt breeding programs. So, we grafted two commercial almond cultivars 'Sahand' and 'TS3') on the 'GF677' rootstock. GF677, the natural hybrid between almond and peach, is widely utilized due to its tolerance to iron-induced deficiency. This vigorous rootstock has been recommended for dry, low drainage, and saline soils (Momenpour *et al*., 2018). Sahand is a high-yielding cultivar native to Iran, introduced by the Azarshahr Research Station (Iran). This low-branched cultivar has the high pruning capability. Also, it forms fruits on spores. This late bloom cultivar bears about 15-20% of double kernel nuts (Eskandari and Majidazar, 2009). TS3 is a late-blooming autogamous hybrid of Ferragnes × Tono (Eskandari and Majidazar, 2009). Ferragnes is a vigorous, semi-late blooming, late bearing, woody shell, semi-stone fruit, light brown kernel and self-incompatible almond (Eskandari and Majidazar, 2009; Grasselly and Duval, 1997). However, Tono is an autogame and double kernel cultivar. TS3 has intermediate characteristics of the parents (Eskandari and Majidazar, 2009). Then, we monitored the impact of salinity on the morphological, physiological, and molecular behaviors of Sahand and TS3. In particular, plant growth, leaf area, photosynthetic pigments, photosynthetic parameters, chlorophyll fluorescence, malondialdehyde content, glycine betaine content, total phenol content, leaf elements, and *NHX1* expression were evaluated. Compared with Sahand, TS3 had a higher photosynthesis rates, transpiration rate, N, Ca, S and B contents, and its greater NHX1 expression was a molecular confirmation of its salt tolerance.

Material and Methods

This experiment was carried out at the Temperate Fruits Research Center, Karaj, Iran {51°E, 35° 48′N, average annual temperature: 13.7°C, average rainfall: 254.5 mm/year, 1320 m elevation} during 2018-2020.

The factorial experiment involved a randomized complete block design with six replicates. Factors were almond cultivars 'Sahand'and 'TS3') grafted on six months old 'GF677' rootstock with different salt (NaCl, Merk) levels (0.5, 6.5, and 8.5 dS/m). The 0.5 dS/m was assumed as the control (the tap water used for regular irrigation had the same electrolyte conductivity).

Tissue cultured GF677 rootstocks were planted in 20 kg plastic pots containing loamy soil (44% of sand, 33% of silt, and 23% of clay) in September 2018. The soil moisture was determined using a pressure plate device

(Model F1 of the US Soil Moisture Equipment Corporation). The cultivars 'Sahand' and 'TS3') were grafted (T budding) at the beginning of September 2019. The soil moisture were kept under field conditions. Following the appropriate growth of plants and considering pests, diseases, and proper nutrition practices, salinity treatments started in June 2020 and lasted for ten weeks.

The salinity treatment was applied gradually to avoid sudden shock. Hence, the treatments were initiated with 0.5 dS/m and then 3.25 dS/m of salt. Finally, the salt concentration rose to 6.5 and 8.5 dS/m.

The following assays were evaluated ten weeks after the application of salinity treatment. To prepare the leaf samples for these assays, five leaves harvested from each plant unit, washed, dried, and ground in a pre-cooled pestle and mortar containing liquid nitrogen. The samples were then transferred to plastic tubes and kept frozen at -80°C.

Plant growth

Plant height was measured before the beginning of salinity treatment and re-measured at the end of the experiment (Rahmani *et al*., 2006). The difference was recorded as plant growth and expressed in cm.

Leaf area

Mature leaves were detached, and the leaf area was measured using an Area Meter device (Leaf Area Meter, Am 200, England). The leaf area was expressed in mm^2.

Photosynthetic pigments: chlorophyll (a, b, and total) and carotenoids

In order to measure chlorophyll (a, b, and total) and carotenoid contents, 0.5 g of fresh leaf sample was powdered. Then, 20 ml of 80% acetone was added and centrifuged (6000 rpm, 10 min). The optical absorption of the supernatant was recorded at 663, 645, and 470 nm by a spectrophotometer (Canada BT600 Plus). Pigment contents were expressed in mg g^{-1}FW (Lichtenthaler and Wellburn, 1983).

Photosynthetic parameters: photosynthetic rate (A), stomatal conductance of CO_2 (gs), and transpiration rate (E)

The fully expanded upper leaves were used to measure the photosynthetic rate (μmol m^{-2} s^{-1}), stomatal conductance of CO_2 (mol m^{-2} s^{-1}) and transpiration rate (mmol m^{-2} s^{-1}) (Bastam *et al*., 2013) using a portable photosynthesis meter (LCi model, ADC Bioscientific Ltd, England).

Chlorophyll fluorescence

Mature leaves were attached to a chlorophyll fluorescence device (OS-30p model, made in Opti-Sciences Company, USA). The leaves were then incubated under darkness for 30 min (Baker and Rosenqvist, 2004). Eventually, the Fv/Fm parameter was recorded.

Glycine betaine assay

Leaf samples (0.5 g) were homogenized in 5 ml of toluene (0.5%) and shaken well (25 °C, 24 h). Following the filtration, 1 ml of 2 N hydrochloric acid and 0.1 ml of potassium tri-iodide (containing 7.5 g of iodine and 10 g of potassium iodide in 100 ml of 1 N of hydrochloric acid) were added to 0.5 ml of the extract. It was then shaken in a cold water bath (90 min). Afterward, 10 ml of 1, 2 dichloroethane (cooled at -10°C) and 2 ml of cold water were added. The absorption of the lower phase was recorded at 365 nm. Betaine (0, 2.5, 5, and 10 ppm) was used to draw the standard curve (Grieve and Grattan, 1983). Glycine betaine content was expressed in μg g^{-1}FW.

Malondialdehyde (MDA) assay

Fresh leaf samples (0.2 g) were ground and

homogenized in 5 ml of 0.1% trichloroacetic acid and then centrifuged (10000 rpm, 5 min). Afterward, 5 ml of 20% trichloroacetic acid solution (containing 0.5% thiobarbituric acid) was added to 1 ml of the supernatant. The reaction mixture was allowed to react in a boiling water bath (95°C, 30 min) and placed in cold water to terminate the reaction. The mixture was re-centrifuged (10000 rpm, 10 min), and the absorption was recorded at 532 and 600 nm. The extinction coefficient of 155 mM^{-1} cm^{-1} was used to calculate the malondialdehyde concentration. Malondialdehyde content was expressed in nmol g^{-1}FW (Heath and Packer, 1968).

Total phenol assay

The leaf total phenol content was measured using the Folin-Siocalcu procedure (Singleton *et al*., 1999). In brief, 1 g of leaf sample was homogenized with liquid nitrogen, then 10 ml of methanol was added. Following the filtration, 200 µl of the extract was mixed with 300 µl of distilled water and 2500 µl of foline. Then 2000 µl of sodium carbonate (7.5%) was added, and samples were incubated under laboratory conditions (5 min). Subsequently, the samples were kept under dark conditions (90 min). Ultimately, the absorption was recorded at 760 nm. Gallic acid (0, 10, 15, 20, 25, and 50 µl) was used to draw the standard curve. Total phenol content was expressed in mg GA g^{-1}FW (Gallic acid).

Preparation of leaf powder for elements measurement

In order to evaluate elemental content, leaves were collected, washed, and dried in an oven (75°C, 48h) and finally powdered. The powder was used for the following assays:

Cl measurement

Briefly, 25 ml of hot distilled water was added to 1 g of leaf powder and shaken (120 rpm, 1 h). The extract was then filtered. Afterward, potassium dichromate (4 drops) was added to 10 ml of the extract, which was then titrated with 0.05 N silver nitrate until the appearance of red color. The Cl content was expressed in % (Benton, 2001).

Na, K, Ca, Mg, P, B, S, Fe, Zn, and Mo measurement

Leaf powder (0.5 g) was mixed with 7 ml of 65% HNO_3 and 1 ml of H_2O_2 and then digested (95°C, 10 min) using a Microwave Digestion device (Ethos Plus model, Italy). Following the acidic digestion, Na, K, Ca, Mg, P, B, S, Fe, Zn, and Mo were measured using an inductively coupled plasma-optical emission spectrophotometry device (ICP-OES 730-ES, Varian, USA). The concentrations of the elements were expressed in mg L^{-1} (Eça *et al*., 2014).

N measurement

Leaf powder (0.25 g) was mixed with 10 ml of sulfosalicylic acid (50 mg of salicylic acid per one liter of sulfuric acid), 2 g of catalyst (100 g of potassium sulfate, 10 g of copper sulfate, and 1 g of selenium) and 20 ml of distilled water. The solution was heated (420 °C, 3 h), then distilled water (20 ml) was added. Finally, the titration and distillation were performed using a kajledal device (K1100, Hanon Company, China). The nitrogen content was expressed in % (Chapman and Pratt, 1961).

Assessment of expression of target gene (NHX1)

Assessment of NHX1expression was carried out in National Institute of Genetic Engineering and Biotechnology (Iran). The frozen composite leaf samples (at -80°C) were used to extract the total RNA. Total RNA was extracted as described by Rubio-Piña and Zapata-Pérez (2011). Briefly, the leaf sample (200 mg) was homogenized in liquid nitrogen, then 900 µl of extraction buffer (containing 0.1 M Tris-HCl, 20 mM EDTA, 1.4 M NaCl, CTAB 2%, Polyvinylpirolidone 2%, pH:8) and 100 µl of beta mercaptoethanol were

added to each sample. The incubation (10 min, 65°C) was followed by adding 800 µl of phenol-chloroform (1:1), vortexing (30 sec) and centrifuging (10000 rpm, 10 min). The Supernatant then mixed with chlorophytum- isoamyl alcohol (24:1), vortexed 30 more seconds and re-centrifuged (10000 rpm, 4°C, 10 min). The next steps were adding LiCl (8 M) to supernatant, re-incubation (-20°C, 4 hours) and centrifuging (10000 rpm, 4°C, 10 min). The sediment washed with absolute and then 70% ethanol, dissolved in 250 µl of membrane desalting buffer, 3M acetate and absulture ethanol. The incubation (-20°C, 2 hours), centrifuging (13000 rpm, 4°C, 15 min), washisng with 70% ethanol, air-drying, dissolving in 30 µl of membrane desalting buffer, storing (-80°C) and treating by DNase were the last steps. Agarose gel, spectrophotometric measurement and NanoDrop (Thermo 2000C, USA) verified the quality and quantity of RNA. The primers were designed using NCBI, GenSCAN and Oligo 7 software. After primer BLAST using NCBI databank, the primers were synthesized by

TAG Copenhagen A/S (Frederiksberg, Denmark). The forward and reverse sequences (5'-3') of designed primers for the NHX1 gene were GCA TCA TAA TTG GTC ATT TGT and GGA AGT AGA TAG AGG AAG AAC, respectively (Genbank Accession No. XM_007208394.2). Moreover, GGT GTG ACG ATG AAG AGT GAT G and TGA AGG AGA GGG AAG GTG AAA G were the forward and reverse sequences designed for the housekeeping gene, TEF2 (Genbank Accession No. JQ_7321808.1). In this experiment, a mi-1STEP RT-qPCR kit (Ampliqon Real Q Plus 2x Master Mix, Denmark) was applied to investigate the transcription of the target gene using a real-time PCR cycler (CORBETT RESEARCH Rotor-Gene RG-6000).

The program was structured as reverse transcription (50℃, 15 min), initial denaturation (95 ℃, 15 min), denaturation (95 ℃, 15 sec), annealing, and elongation (58°C,1 min). Equation of $2^{-\Delta\Delta CT}$ (the delta-delta Ct method) was used to calculate the relative fold expression of the *NHX1* gene using LinRegPCR and REST 2009 softwares (Livak and Schmittgen, 2001). Three biological samples were considered for each experimental unit.

Data analysis

The data analysis was done using SAS version 9.1.3 ("SAS Institute Inc. Cary, NC, USA," 1990). The factorial experiment involved a randomized complete block design with six replications. The factors were cultivars and NaCl levels. Shapiro-Wilks test confirmed the data normality (SAS: PROC UNIVARIATE). Multivariate analysis of variance was accomplished for the dependent variables (SAS: PROC GLM). Pillai's trace test confirmed the variance homogeneity (SAS: PROC GLM). The Tukey test was used to compare means (SAS: PROC GLM, $P < 0.01$).

Results

Based on the analysis of variance, NaCl levels significantly affected all the evaluated characteristics except for peroxidase activity. The cultivar did not significantly affect Mg, P, and total phenolic content. Also, the interaction effect of NaCl level and cultivar on all traits was statistically significant ($P<0.01$) (Tables 1 and 2).

Table 1. The analysis of variance of the effects of NaCl levels and cultivars on growth rate and physiology of almonds

S.V	D.F	growth	Leaf area	Chl_a	Chl_b	Chl_{total}	Carotenoids	A	gs	E	Fv/Fm	Glycine betaine	MDA	Total phenolic content	NHX1
Cultivars	1	†511.6*	3481281*	0.06	0.003*	0.09*	0.01*	5.2*	0.02*	2.19*	0.01*	0.16*	6.5*	0.04	22.2*
Block	5	0.6*	181864*	0.02	0.001*	0.02*	0.01*	0.2*	0.01*	1.03*	0.003*	0.001*	7.9*	0.07*	1.9*
NaCl	2	66.1**	16703858*	0.2	0.05*	0.17*	0.02*	8.6*	0.04*	12.02	0.08*	6.97*	165.1*	1.38*	8.8*
NaCl * Cultivars	2	5.1**	930603*	0.03	0.01*	0.19*	0.12*	0.7*	0.07*	0.32*	0.006*	0.02*	49.5*	0.08*	2.3*
Error	25	0.4	169647*	0.01	0.03	0.01	0.01	0.3*	0.01	1.25	0.005	0.0006	7.4	0.02	2.2*

†The mean square values are given., * and ** state significance at 5% and 1%, respectively, NaCl * Cultivars: states the interaction of NaCl and cultivars

Table 2. The analysis of variance of the effects of NaCl levels and cultivars on ions content and gene expression of almonds

S.V	D.F	Na^+	Cl^-	K^+	N	Ca^{2+}	Mg	P	S	Fe	Zn	Mo	B
Cultivars	1	150582*	2.89*	138900*	0.03	14747*	9.25	19725	329.9*	74.40*	0.85*	0.012*	0.007*
Block	5	2133*	0.05*	4385*	0.001*	1682*	576.31*	1.2*	1.3*	0.36*	0.001*	0.00006*	0.002*
NaCl	2	408561**	6.65**	59840*	0.19*	42910*	847.07*	16582.1*	452.4*	3.12*	1.57*	0.005*	0.19*
NaCl * Cultivars	2	39668**	1.47**	77125*	0.07*	13466*	1238.05*	9*	89.1*	26.81*	1.86*	0.005*	0.013*
Error	25	1556	0.09	5467	0.0002	1477	706.06	4.6	2.2	0.57	0.1	0.0001	0.003

†The mean square values are given.,* and ** state significance at 5% and 1%, respectively,NaCl * Cultivars: states the interaction of NaCl and cultivars

Impact of salinity on plant growth and leaf area

Declined plant growth was observed in both cultivars with increasing NaCl concentrations. Fig 1 elucidates the impact of 0.5, 6.5, and 8.5 dS/m of salt on the Sahand (Fig. 1A) and TS3 (Fig. 1B) cultivars.

Although the highest plant growth was observed in the TS3 cultivar (25.90 cm) under 0.5 dS/m salinity, it showed a negligible decline (reached 24.75 cm) in the 6.5 dS/m of salt. However, under this condition, Sahand expressed a remarkable reduction from 17.24 to 13.67 cm. Moreover, given the significant reduction in both cultivars under 8.5 dS/m salinity, the lowest plant growth belonged to Sahand (8.9 cm) (Fig. 2A).

Similarly, salinity stress reduced leaf area in both cultivars, reaching significance in the Sahand cultivar. Thus, the largest leaf area under 0.5 dS/m salt treatment (9316.33 mm^2) was recorded in the Sahand plants, while the highest values under both 6.5 and 8.5 dS/m belonged to TS3 (7371 and 6901.5 mm^2, respectively). The smallest leaf area (5412.5 mm^2) belonged to the Sahand cultivar exposed to 8.5 dS/m of salt (Fig. 2B).

Fig. 1. Sahand (A) and TS3 (B) almonds treated with 0.5 (1), 6.5 (2), or 8.5 (3) dS/m of salt.

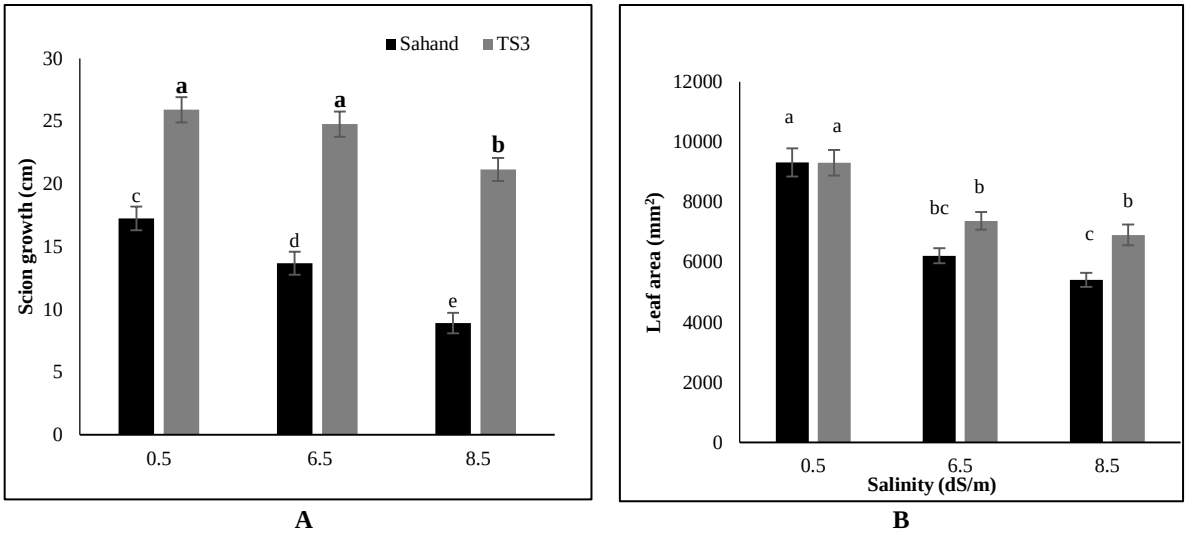

Fig. 2. Impact of salinity on growth (A) and leaf area (B) of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, p<0.01).

Impact of salinity on chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (Chl_{total}), and carotenoid content

According to the results, the interaction of salinity and cultivar was statistically significant on Chl_a, Chl_b, Chl_{total}, and carotenoids (P<0.01). Declined Chl_a and Chl_b contents were observed in both cultivars. However, the reduction severity was greater in Sahand. The highest Chl_a and Chl_b contents (0.99 and 0.25 mg g^{-1}FW, respectively) belonged to the TS3 cultivar under 0.5 dS/m of salt (Fig. 3A and B). However, the lowest Chl_a and Chl_b contents (0.58 and 0.15 mg g^{-1}FW, respectively) belonged to Sahand under 8.5 dS/m salinity (Fig. 3A and B).

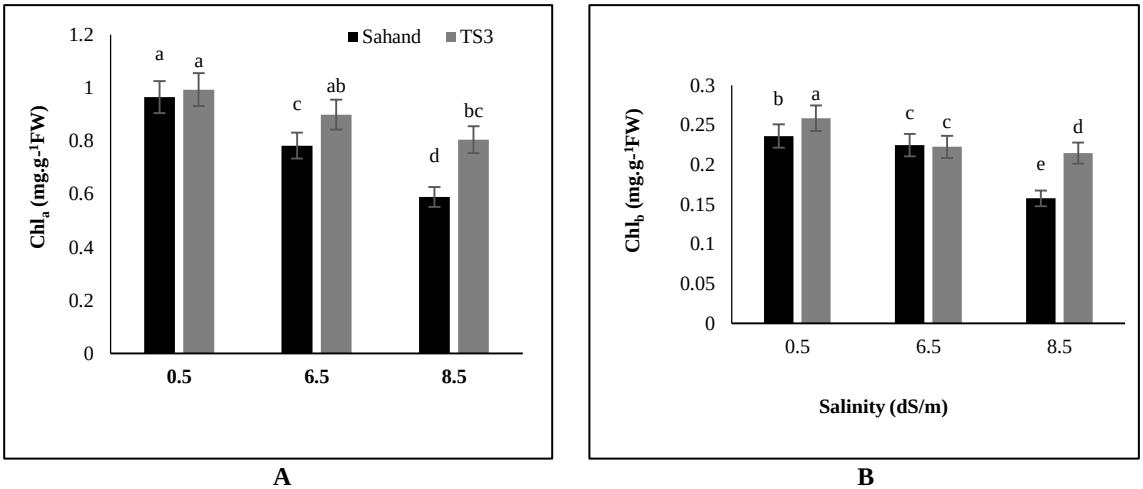

Fig. 3. Impact of salinity on chlorophyll$_a$ (A) and chlorophyll$_b$ (B) of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, p<0.01).

The Chl_{total} content followed a similar trend with increasing salt levels. The highest Chl_{total} content (1.25 mg g^{-1}FW) was observed in TS3 plants treated with 0.5 dS/m salinity, and the lowest Chl_{total} content (0.74 mg g^{-1}FW) was observed in Sahand plants treated with 8.5 dS/m of salt (Fig. 4A).

The carotenoid content in Sahand featured a significant difference at 0.5 dS/m salinity compared with other levels. The highest (0.53 mg g^{-1}FW) and lowest (0.31 mg g^{-1}FW) carotenoid contents belonged to the Sahand cultivar under 0.5 and 8.5 dS/m salinity, respectively. In TS3, there were no significant differences in carotenoid contents between 0.5 and 6.5 dS/m salinity levels. However, 8.5 dS/m salinity significantly reduced the carotenoid content in this cultivar (Fig. 4B).

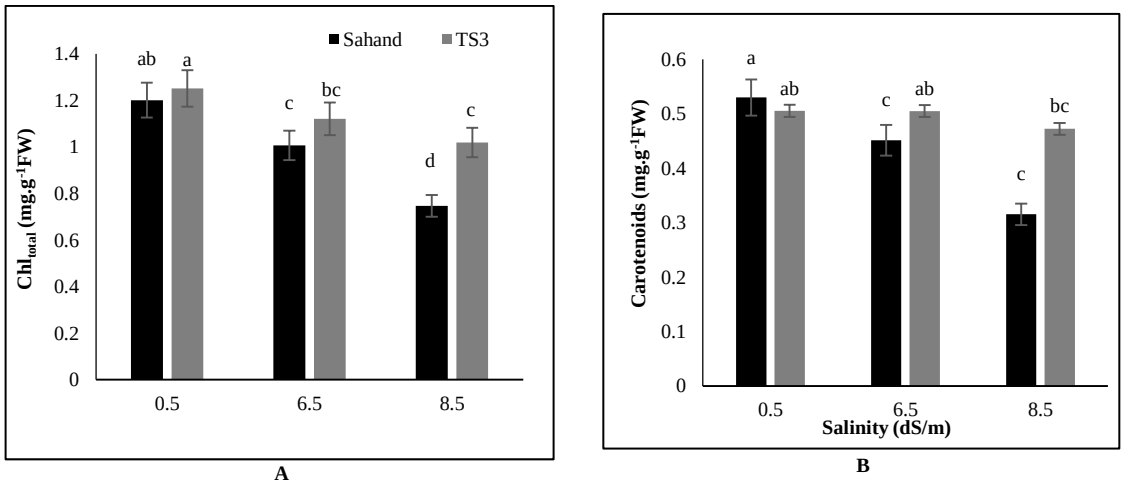

Fig. 4. Impact of salinity on $chlorophyll_{total}$ (A) and carotenoids (B) of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, $p<0.01$).

Impact of salinity on photosynthesis rate (A), stomatal conductance (gs), transpiration rate (E), and Fm/Fv

The salt treatments caused a significant decline in photosynthesis rate, stomatal conductance (gs), and transpiration rate (E) in both cultivars, with TS3 featuring higher values (Figs. 5A, 5B, 6A).

The highest and the lowest photosynthesis rates (5.65 and 2.14 µmol m^{-2} s^{-1}, respectively) belonged to TS3 under 0.5 dS/m salinity and Sahand under 8.5 dS/m salinity, respectively (Fig. 5A). The highest gs (0.1 mol m^{-1} s^{-1}) belonged to TS3 under 0.5 dS/m of salt. In Sahand, the lowest gs value (0.03 mol m^{-1} s^{-1}) was recorded in response to both 6.5 and 8.5 dS/m salinity (Fig. 5B). The highest transpiration rate was observed in TS3 (6.08 mmol m^{-1} s^{-1}) under 0.5 dS/m salt treatment, while the minimum (2.91 mmol m^{-1} s^{-1}) was observed in Sahand at 8.5 dS/m salinity (Fig. 6A).

The impact of salinity on chlorophyll fluorescence was not significant in the TS3 cultivar. That is, it exerted no significant effect on this parameter at 0.5 or 6.5 dS/m of salt. However, in the Sahand cultivar, Fm/Fv decreased significantly at 8.5 dS/m salinity. Therefore, the lowest Fm/Fv (0.75) was observed in Sahand at the 8.5 dS/m salt level. In contrast, the highest value (0.85) was recorded in TS3 under 6.5 dS/m salinity (Fig. 6B).

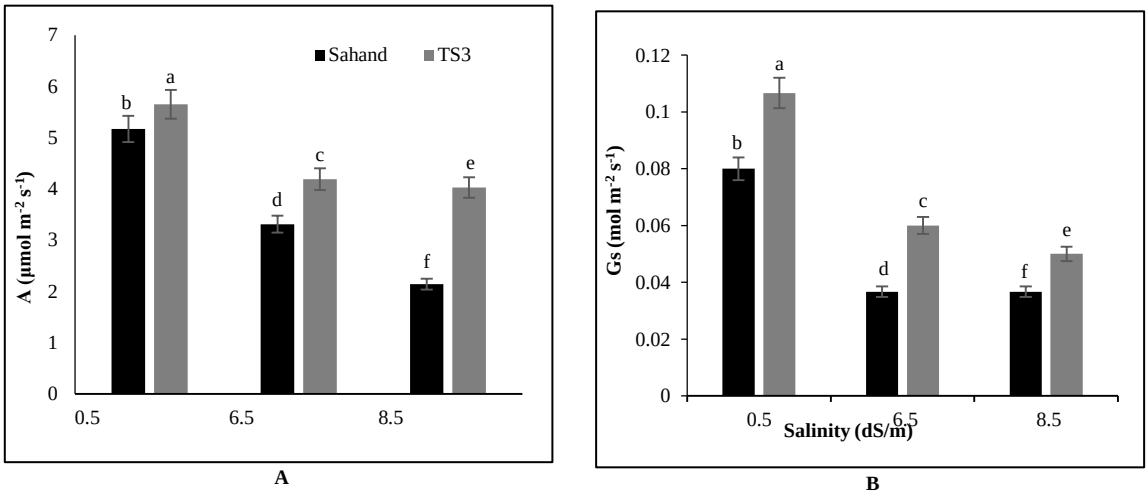

Fig. 5. Impact of salinity on photosynthesis rate: A (A) and stomatal conductance: G_s (B) of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, p<0.01).

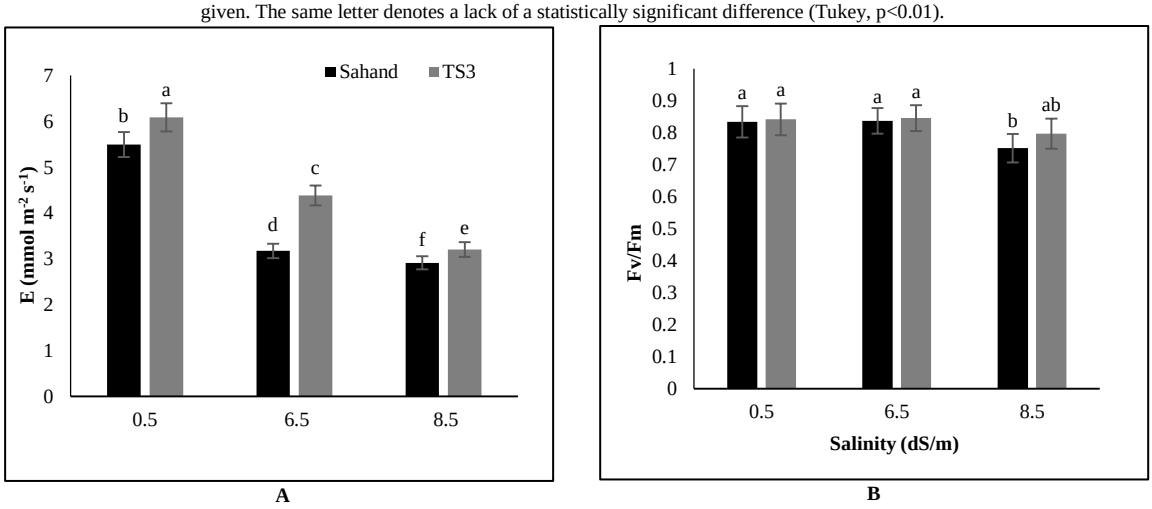

Fig. 6. Impact of salinity on transpiration rate: E (A) and chlorophyll fluorescence (B) of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, p<0.01).

Impact of salinity on glycine betaine (GB), malondialdehyde (MDA), and total phenolic content (TFC)

Both cultivars indicated a remarkable rise in GB value under 6.5 dS/m of salt. Hence, the highest GB (2.81 µg g^{-1}FW) was demonstrated in Sahand at 6.5 dS/m salinity. There was a slight decline in GB value in both cultivars under 8.5 dS/m of salt. However, the lowest GB (0.61 µg g^{-1}FW) was recorded in TS3 under 0.5 dS/m salinity (Fig. 7A).

Salt treatments increased the MDA content in both cultivars. TS3 demonstrated the least MDA (10.58 nmol g^{-1}FW) under 0.5 dS/m of salt, ascending with increasing salinity. In Sahand, the MDA increment was insignificant up to 6.5 dS/m of salt. However, the highest MDA value (25.17 nmol g^{-1}FW) was recorded in Sahand exposed to 8.5 dS/m salinity (Fig. 7B).

Higher salinity levels boosted the TFC in both cultivars. The TPC was not significantly different in TS3 under 6.5 and 8.5 dS/m salinity. However, the lowest TFC (1.75 mg GA g^{-1}FW) was recorded in TS3 under 0.5 dS/m ^{of} salt. In contrast, the highest TFC (2.95 mg GA g^{-1}FW) belonged to the Sahand cultivar at 8.5 dS/m salinity (Fig. 7C).

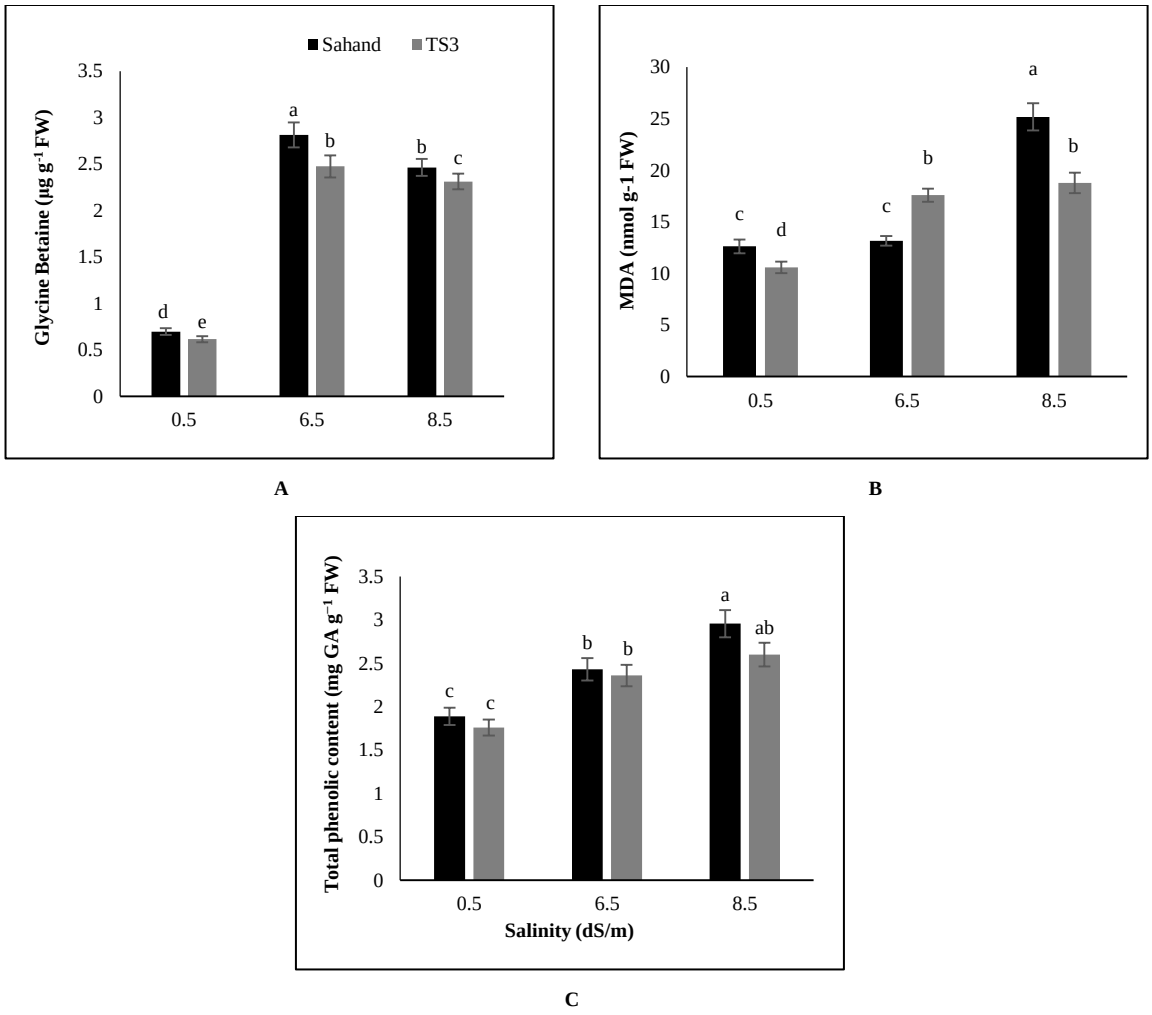

Fig. 7. Impact of salinity on glycine betaine (A), malondialdehyde (B), and total phenol contents (C) of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, $p<0.01$).

Impact of salinity on leaf Cl, Na, K, Ca, Mg, N, P, B, S,

Fe, Zn, and Mo content

In the present study, Na and Cl levels significantly increased in both cultivars with rising salt levels. The highest Na (649.84 mg L^{-1}) and Cl (3.52%) levels were observed in Sahand at 8.5 dS/m salinity (Fig. 8 A and B).

Salt treatment increased the K content in both cultivars; the highest K content (1575.8 mg L^{-1}) was recorded in TS3 under 8.5 dS/m salinity. In Sahand, the K content increased from 1157.55 under 0.5 dS/m of salt to 1317.03 mg L^{-1} under 6.5 dS/m of salt, before declining to 1138.36 mg L^{-1} at 8.5 dS/m salinity (Fig. 8C).

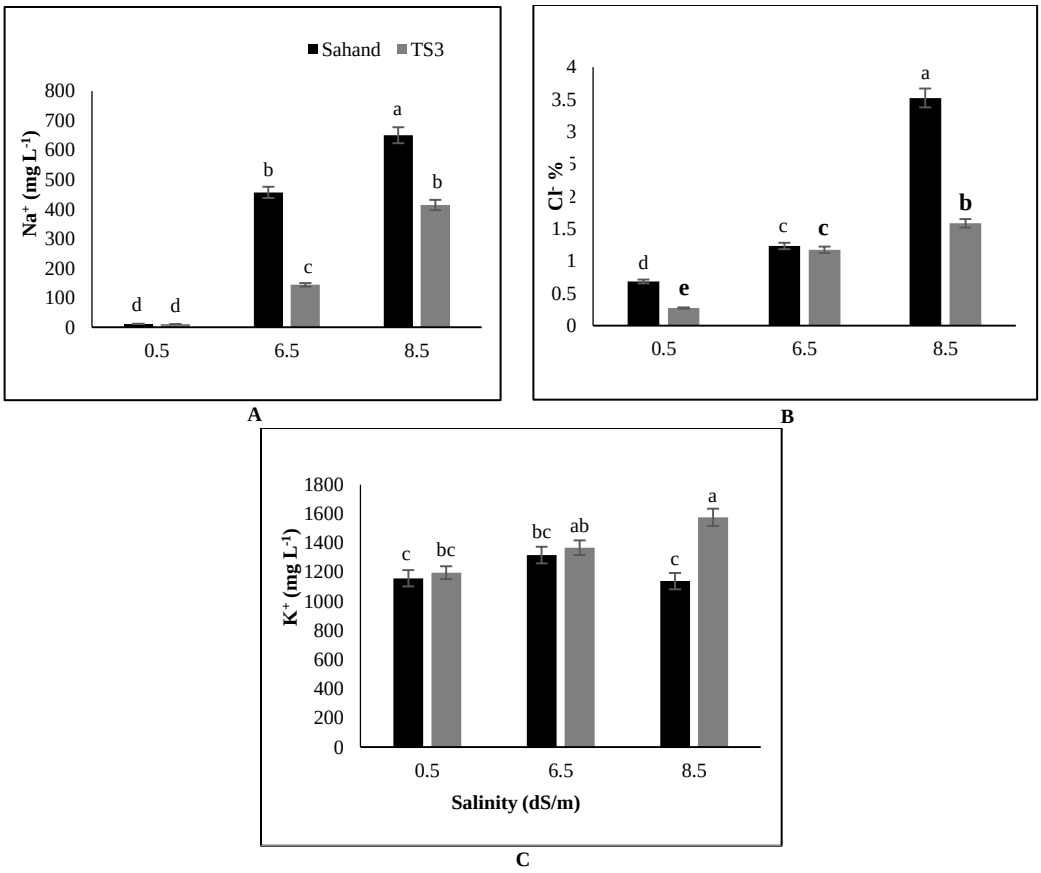

Fig. 8. Impact of salinity on Na (A), Cl (B), and K (C) content of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, $p<0.01$).

The N content significantly declined in both cultivars with rising salt levels, with Sahand demonstrating a greater reduction. Thus, the maximum (1.90%) and minimum (1.33%) N contents were recorded in Sahand plants exposed to 0.5 and 8.5 dS/m NaCl levels, respectively (Fig. 9A).

Salinity led to a reduction in Ca in both cultivars. However, this decrement was significant in Sahand plants under 8.5 dS/m of salt. The highest (1903.63 mg L^{-1}) and lowest (1654.55 mg L^{-1}) Ca concentrations were observed in TS3 under 0.5 and Sahand under 8.5 dS/m salinity (Fig. 9B).

The Mg content was not influenced significantly by salt levels, though a slight decline was observed. The highest (441.79 mg L^{-1}) and lowest (395.28 mg L^{-1}) Mg contents were observed in Sahand under 0.5 and 8.5 dS/m salinity, respectively (Fig. 9C).

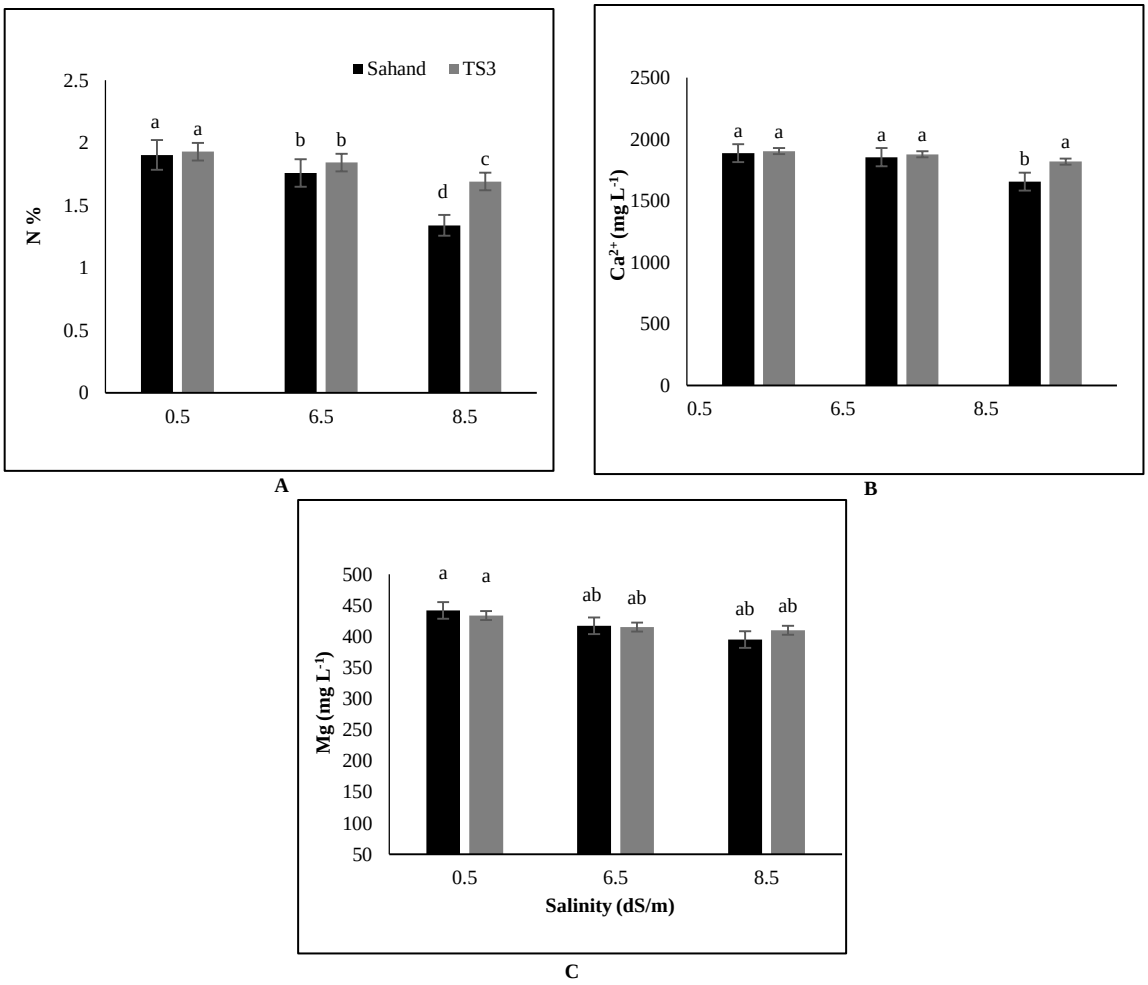

Fig. 9. Impact of salinity on N (A), Ca (B), and Mg (C) of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, $p<0.01$).

Similar to N, significant reductions occurred in leaf P contents in both cultivars in response to rising salt levels. The highest P concentration (163.12 mg L^{-1}) was recorded in Sahand under 0.5 dS/m of salt. The lowest P content (138.5 mg L^{-1}) was observed in the TS3 cultivar under 8.5 dS/m salinity (Fig. 10A).

Reduced S concentrations were recorded in both almond cultivars with rising salt levels. The minimum S level (168.6 mg L^{-1}) was observed in Sahand under 8.5 dS/m salinity, while the highest S content (196.9 mg L^{-1}) was recorded in TS3 under 0.5 dS/m salinity (Fig. 10B).

Fe concentrations indicated different trends in Sahand and TS3 cultivars. In Sahand, declining Fe concentration was observed with increasing salt levels and reached the minimum value (10.35 mg L^{-1}) under 8.5 dS/m salinity. Conversely, in TS3, increasing salinity augmented the Fe concentration; the highest Fe content was recorded in TS3 under 8.5 dS/m of salt (Fig. 10C).

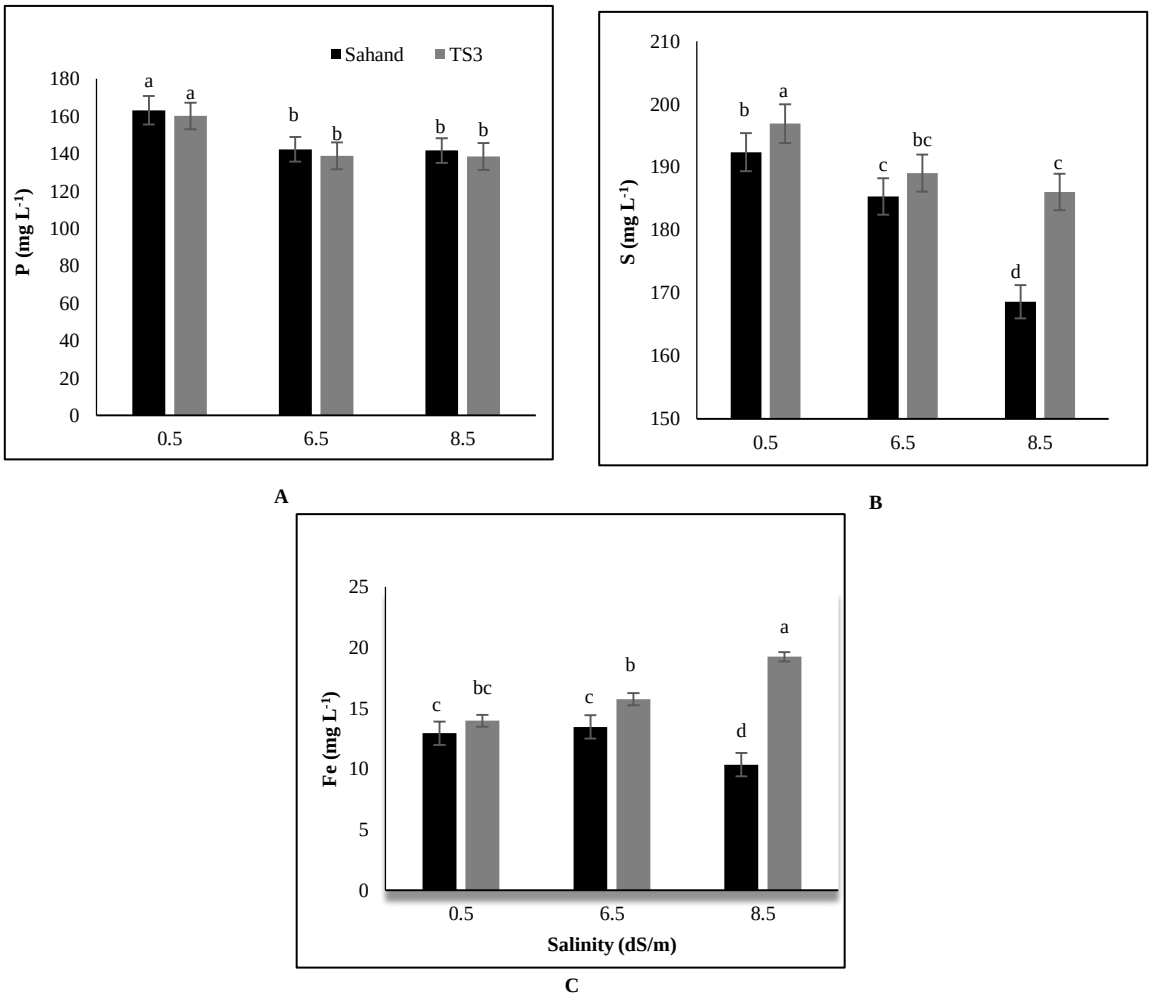

Fig. 10. Impact of salinity on k and Ca of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, p<0.01).

Regarding Zn content, in TS3, increased Zn content was obtained by exposure to higher salt concentrations. In the Sahand cultivar, this increment was observed under 6.5 dS/m salinity. However, the Zn level of Sahand decreased to 3.05 mg L^{-1} under 8.5 dS/m salinity (Fig. 11A).

Mo remained unchanged in Sahand up to 8.5 dS/m of salt, whereas a significant increase was observed in TS3 with increasing salinity up to 6.5 dS/m (0.2 mg L^{-1}) (Fig. 11B).

The B concentration in both cultivars showed a slight descending trend. However, the highest B content (2.09 mg L^{-1}) belonged to TS3 under 0.5 dS/m of salt. On the other hand, the lowest B (1.64 mg L^{-1}) content was recorded in Sahand under 8.5 dS/m salinity (Fig. 11C).

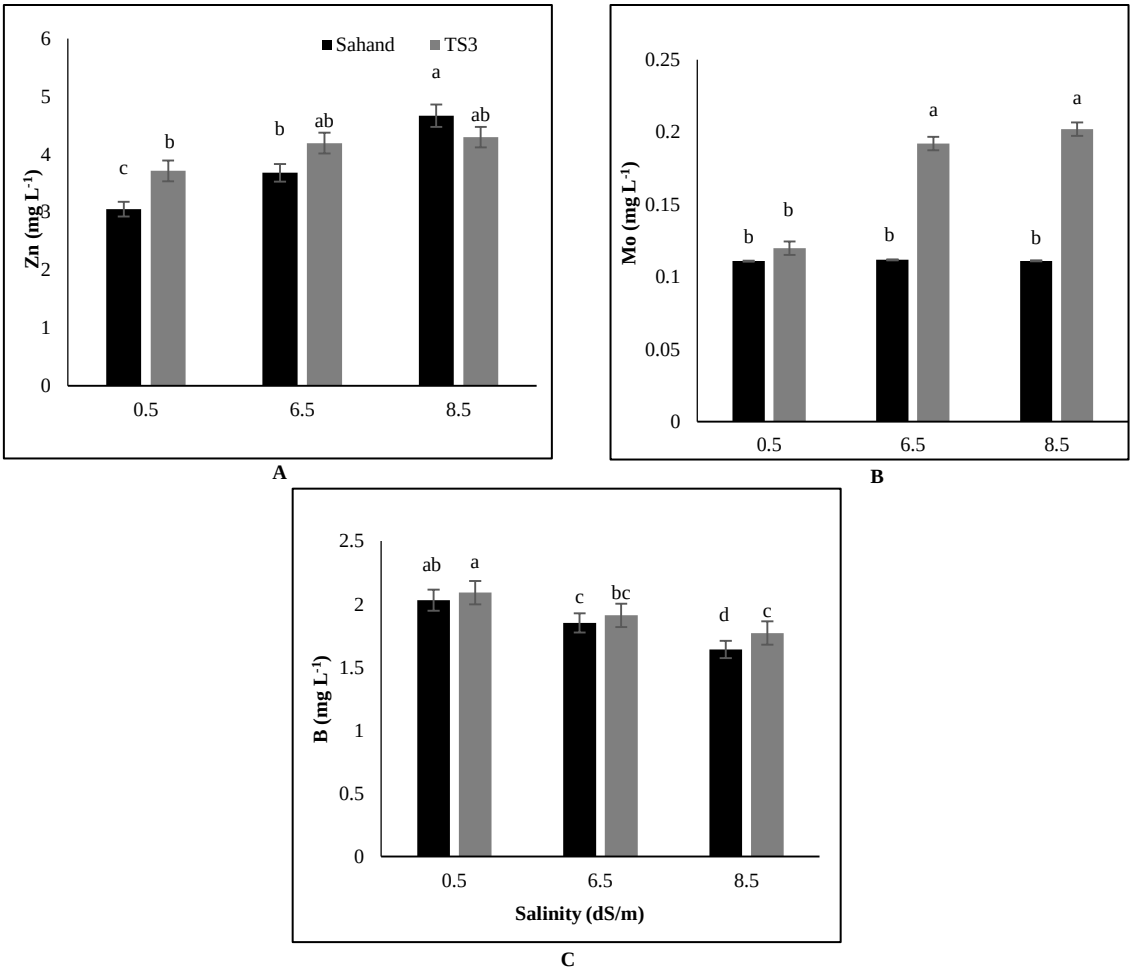

Fig. 11. Impact of salinity on Zn (A), Mo (B), and B (C) contents of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, $p<0.01$).

Impact of salinity on NHX1 expression

The *NHX1* gene showed different expression patterns in the two cultivars. There was a significant difference in the expression level of the gene under different salinity levels. The Sahand cultivar exhibited a slight but non-significant increase in gene expression under 8.5 dS/m salinity. Gene expression in TS3 significantly increased by raising the salt level to 6.5 and 8.5 dS/m (Fig. 12).

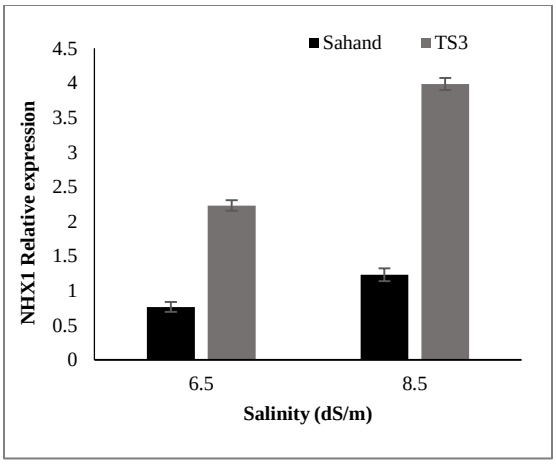

Fig. 12. Impact of salinity on the expression of the almond *NHX1* gene involved in Na+ transport of Sahand and TS3 cultivars. The mean ± SD of six replicates is given. The same letter denotes a lack of a statistically significant difference (Tukey, $p<0.01$).

Discussion

Salinity causes gradual cell water loss and reduces cell division and elongation, producing smaller and thicker leaves with lower leaf area (Tattini *et al*., 1995). Decreases in stem growth were found in salt-exposed pistachios (Adish *et al*., 2010) and almonds (Najafian *et al*., 2008; Shibli *et al*., 2003). It must be noted that salt-tolerant plants can retain their growth rate when exposed to saline conditions (Tester and Davenport, 2003). Significant differences were reported in the stem height and diameter of almond species and cultivars (Rahmani *et al*., 2006; Zrig *et al*., 2016). In this study, plant growth and leaf area were less influenced by salt levels in the TS3 cultivar.

Salinity decreases chlorophyll content by inducing its destruction, decreasing its synthesis (or both), and reducing the strength of the thylakoid membrane (Ashraf and Harris, 2013). Reduced chlorophyll levels have been reported in red raspberries (Necleous and Vasilakakis, 2007), pomegranates (Khayyat *et al*., 2014), and almonds (Zrig *et al*., 2011; Zrig *et al*., 2016) under saline conditions. Carotenoids protect chlorophylls from oxidative damage by inhibiting ROS and peroxide radicals (Stahl and Sies, 2005). NaCl (5 dS/m) reduced the carotenoid content of almonds (Badran and Savin, 2019). According to our findings, the chlorophyll (chl_a, chl_b, and chl_{total}) and carotenoid contents were reduced significantly in both TS3 and Sahand cultivars under different salt concentrations, with TS3 showing less of a drop.

Photosynthetic parameters such as photosynthetic rate [A], intercellular CO_2 [*Ci*], transpiration rate [E], stomatal conductance [gs], and maximum efficiency of photosystem II [Fv/Fm] are generally limited by salinity (Yang *et al*., 2008; Wei and Zimmermann, 2017). Such limitations are due to declined CO_2 permeability and conductance, increased transpiration rate, stomatal closure, cell membrane dehydration, cytoplasm structure changes, chlorophyll degeneration (Reza *et al*., 2006; Tabatabaei, 2006), photosynthetic electron transport chain disruption, and enzyme inhibition (such as phosphoenolpyruvate carboxylase, ribulose-5-phosphate kinase, and glyceraldehyde 3-phosphate dehydrogenase) (Parida and Das, 2005; Meloni *et al*., 2008). Some reports have illustrated the suppressive effects of salinity on the photosynthetic parameters of almonds (Isaakidis *et al*., 2004; Karimi *et al*., 2015; Zrig *et al*., 2015a, Zrig *et al*., 2016), the stomatal conductance of pistachios (Adish *et al*., 2010) and almonds (Wang *et al*., 2016; Mohammadi *et al*., 2020), and the leaf transpiration rate of pomegranates (Bhantana and Lazarovitch, 2010) and almonds (Amiri *et al*., 2016; Zrig *et al*., 2015b). Also, the Fv/Fm of almond

genotypes decreased from 0.79 to 0.55 when exposed to 10 dS/m of NaCl (Ranjbar, 2006). According to our data, the photosynthetic parameters and Fv/Fm were affected by higher levels of NaCl. The TS3 cultivar showed better photosynthetic parameters, indicating a preserved photosynthetic assimilation rate.

Glycine betaine is involved in the stability and synthesis of proteins and enzymes (Carillo, 2018). It maintains the photosynthetic rate (Yang *et al*., 2008; Fan *et al*., 2012), regulates ion channels (Wei and Zimmermann, 2017), and enhances plasma membrane H^+-ATPase activity (Li *et al*., 2019). According to our findings, the glycine betaine content of both cultivars was augmented by higher salt levels.

Salt-sensitive plants generally accumulate higher amounts of MDA due to lipid peroxidation (Ashraf and Harris, 2004; Sorkheh *et al*., 2012). In our research, the MDA content showed a greater increment in the Sahand cultivar under 8.5 dS/m of NaCl. This finding is in line with the results of Sorkheh *et al*. (2012) and Zrig *et al*. (2015a) on almonds. Also, the total phenolic content (TPC), which plays an important role against abiotic stresses (Lattanzio, 2013), varied significantly in leaves of different almond graft combinations (Zrig *et al*., 2016; Sandhu *et al*., 2020). According to our results, Sahand showed more phenol accumulation than TS3.

By increasing the sodium and chlorine content, soil salt accumulation decreases potassium availability, disturbs calcium absorption (Gcaber, 2010), and ultimately declines plant productivity (Grattan and Grieve, 1999). According to reports, salinity stress amplifies the sodium content in almond leaves (Zrig *et al*., 2016; Pitt *et al*., 2018). According to our results, Sahand cultivar showed higher leaf Na^+ content.

Potassium plays a major role in osmotic balance and ion homeostasis (Devi *et al*., 2012; Wang *et al*., 2013). Maintaining the K^+ balance is essential for plant adaptation to environmental stresses (Shabala and Pottosin, 2014). Lower K^+ levels in leaves lead to stomatal closure and transpiration inhibition (Hasanuzzaman *et al*., 2018). However, potassium and sodium have some similar bonding sites to plasma membrane carriers. Hence, the competition of the two ions causes a decline in K content (Gaber, 2010). Potassium loss in salt-sensitive crops is considerably higher than in salt-tolerant crops (Shabala and Pottosin, 2014). According to our findings, TS3 cultivar had higher leaf potassium content.

High levels of Cl^- interrupt K^+, $Ca^{2+,}$ and NO_3^- homeostasis; this reduces enzymes' activity, causes membrane destruction, and lessens plants' effectiveness (Ashraf and Ahmad, 2000). Salt-tolerant plants transfer less chlorine into aerial organs (Munns, 2002). Salinity tolerance in citrus is related to low transportation of Na and Cl into the shoot (Marschner, 2012); in grape rootstocks, this issue is essentially related to low Cl transfer (Luachli and Wteneke, 1979). According to reports, sodium chloride causes increases in the Cl content of the roots and aerial parts of almonds (Zrig *et al*., 2016; Pitt *et al*., 2018). In our case, TS3 had a lower leaf chlorine content than Sahand.

Nitrogen is essential to generate cellular elements such as RuBisCO, which assimilates carbon dioxide. Reduced N uptake under salinity stress is due to the antagonistic effects between Na^+ with NH_4^+ and Cl^- with NO_3^- (Parihar *et al*., 2015). Salinity stress disturbs photosynthesis by restricting N uptake, thereby decreasing both vegetative and reproductive growth (Marschner, 2012; ZarataValdez *et al*., 2015). Our results confirmed the decremental impact of salt on the leaf nitrogen content of both almond cultivars, agreeing with previous work on bitter almonds (Shibli *et al*., 2003).

Calcium is known for its metabolic and structural functions (Jin *et al*., 2007). Increased Na^+ concentrations not only reduce the availability of Ca^{2+} but also limit the transportation of extracellular Ca^{2+} (Hadi and Karimi, 2012). High salt levels induced Ca^{2+}

deficiency in *Vigna unguiculate* (Murillo-Amador *et al*., 2006), tomatoes (Tuna *et al*., 2007), and almonds (Zrig *et al*., 2015a, Zrig *et al*., 2016). We found decreases in the Ca content of both almond cultivars.

Magnesium, the central atom of chlorophyll, plays a role in the accurate functioning of carboxylases, ATPases, RNA polymerases, proteins kinases, and phosphatases (Shaul, 2002). Chlorophyll biosynthesis and Mg^{2+} concentrations decreased with higher salt levels in salt-sensitive rice cultivars (Lutts *et al*., 1996), *Atriplex* (Hussin *et al*., 2013), and almonds (Zrig *et al*., 2016). The lower transportation of Na^+ and Cl^- to the aerial parts and the higher K^+, Ca^{+2}, or Mg^{+2} translocation to the leaves are associated with the salt tolerance of *Prunus* cultivars (Dejampour *et al*., 2012; Soliman and Farhan, 2021). Our results indicated a slight decline in Mg^{+2} levels of both almond cultivars.

The phosphorus content of chloroplasts is essential for photosynthesis; its deficiency restricts carbon assimilation and stomatal function (Singh and Prasad, 2014; Zhang *et al*., 2014). NaCl treatment reduced the P content of bitter almonds (Shibli *et al*., 2003). Tolerant plants exhibit less decline in leaf P content when exposed to salt stress (Mahajan and Tuteja, 2005), explaining our results in almonds.

Sulfur improves photosynthesis by augmenting chlorophyll formation and promoting plant growth (Osman and Rady, 2012). In addition, S is involved in maintaining the K^+/Na^+ balance (Abdelhamid *et al*., 2013). It has been suggested that SO_4^{2-} may be involved in Cl homeostasis in salt-exposed almonds (Sandhu *et al*., 2020). Boron maintains the membrane structure and protects the cell membrane against ROS (Yu *et al*., 2002). Boron deficiency alters the membrane potential and H^+ efflux, resulting in membrane dysfunction (Archana *et al*., 2017). Our findings exhibited declined S and B with rising salt levels in both almond cultivars, though the decrement was less in the TS3 cultivar.

Iron is involved in mitochondrial respiration, photosynthesis, nitrogen assimilation, phytohormones' biosynthesis, osmotic protection, and ROS suppression (Hansch and Mendel, 2009). Salinity disturbs iron absorption (Munns and Tester, 2008). We found increased Fe levels in Sahand up to 6.5 dS/m salinity and then declined levels under 8.5 dS/m salinity. However, in TS3, the levels were boosted by salt.

Zinc reduces cell damage caused by the over-generation of ROS (Cakmak, 2000; Pandey *et al*., 2002). The effect of Zn deficiency on photosynthesis may include changes in chloroplast structure, electron transfer, CO_2 stabilization ability, and the photochemical function of membranes (Salama *et al*., 2006) and the photosystem II complex (Chen *et al*., 2008). Ascending Zn levels were observed with salt treatment, reported previously by Shibli *et al*. (2003). TS3 had the highest Zn content in comparison to Sahand at all salt levels. This may be due to the activity of carbonic anhydrase, a zinc metalloenzyme that buffers the pH in the stroma, thereby preventing the denaturation of chloroplast proteins.

Members of the molybdenum-containing aldehyde oxidase multigene family (aldehyde oxidase, xanthine dehydrogenase, and nitrate reductase) are involved in plant adaptation to environmental stresses (Brychkova *et al*., 2008; Ventura *et al*., 2010) via the synthesis of abscisic acid (involved in the response of plants to freezing, cold, drought, and salinity) and indole-3-acetic acid (involved in the response of plants to water deficits and salinity) (Koshibu *et al*., 1996). According to our results, salinity caused a significant enhancement in Mo content, and TS3 exhibited greater Mo accumulation.

The genetic diversity in plants' salt tolerance is connected to a variation in Na^+ secretion by retaining high K^+ and low Na^+ levels in the cytosol (Rai *et al*., 2011) or Cl^- excluding strategies (Murkute *et al*., 2005; Tester and Davenport, 2003). Na^+/H^+ vacuolar antiporters attached to tonoplasts (NHX transporter family) play an important role in the distribution of Na

inside the vacuole (Chakraborty *et al*., 2018), prevention of Na-induced cell toxicity, and maintenance of the intracellular osmotic potential (Bassil *et al*., 2012; Pasapula *et al*., 2011). Overexpression of *NHX1* in salt-exposed rice (Ohta *et al*., 2002), maize (Yin *et al*., 2004), and *Rosa rugos* (Feng *et al*., 2015) enhanced the salt tolerance responses. Also, salt-tolerant cultivars of almonds ('Empyrean 1' and 'Cornerstone') showed high *NHX2*, *NHX1*, *AVP1*, *SERF1*, and *SAL1* expression, while salt-sensitive cultivars ('Guardian' and 'Lovell') had low *NHX1*, *NHX2*, and *HKT1* expression (Sandhu *et al*., 2020). According to our expression analysis, the *NHX1* gene was expressed more in leaves of TS3 under salinity, which represents its capability to endure under salinity stress.

Under salinity stress, excess Na^+ and Cl^- lead to cell membrane destruction, osmotic stress, reactive oxygen species (ROS) accumulation, photosynthesis malfunction, cell division prevention, and growth decline (White and Broadley, 2001; Volkov and Amtmann, 2006; Kusunoki, 2007; Amtmann, 2009). Plants' ability to efflux chlorine into roots (Melgar *et al*., 2008) or allocate sodium into vacuoles (Munns and Tester, 2008) is diverse due to their differences in growth habits, root morphology, elemental absorption, and molecular basis (Fernandez-Ballester *et al*., 2003). We found that Sahand, as a salt-sensitive cultivar, could not inhibit the sodium flux from root to shoot, explaining why it accumulated more Na in the foliar part. In addition, we suggest the higher potassium content of the salt-tolerant cultivar, TS3, may be motivated by an increase in selective uptake of potassium, a rise in sodium removal from the root, better activity of Na^+/H^+ carriers, and greater activity of *NHX1* (Chakraborty *et al*. 2016).

Conclusions

The current research was conducted to estimate the responses of two selected candidate almond genotypes to different salinity levels. The aim was to understand the growth, physiology, and molecular mechanisms involved, as well as to introduce a stress-tolerant genotype. Salinity significantly affects the metabolic processes and nutritional status, leading to a reduction in the growth of Sahand (a salt-sensitive cultivar). The photosynthetic rate, chlorophyll (a, b, total) content, and carotenoid content decreased with increasing salinity levels in both cultivars, with the decrease being less pronounced in TS3 (a salt-tolerant candidate cultivar). Sahand could not inhibit the sodium flux from the root to the shoot and accumulated more Na in the leaves. TS3 had a higher leaf potassium content and efficiently transported sodium from the root to the shoot. Higher levels of Mg and Fe (structural elements of chlorophyll) in TS3 resulted in the stability of photosynthetic pigments. Compared to Sahand, TS3 had a higher nitrogen (N) content, and its increased expression of NHX1 served as molecular evidence of its salt tolerance.

Acknowledgments

This experiment was supported by the Horticultural Division of the Tehran University of Science and Research, Temperate Fruits Research Center, Karaj, Iran. The authors thank Hamid Haji Seyed Javadi (Tehran University of Science and Research) for laboratory assistance. This manuscript was reviewed in terms of language and grammar by a native English-speaking language editor, Dr. Seyed Ali Hosseini (Native Editor Co., Shiraz, Iran).

Conflicts of interest

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

Authorship contribution statement

GA contributed in preparing the resources, methodology, formal analysis, writing- original draft; MS contributed in conceptualization, visualization, methodology, investigation, formal analysis, software analysis, writing- original draft, review, and editing; AI contributed in preparing the resources, conceptualization, methodology, investigation, review, and editing; AM contributed in preparing the resources, conceptualization, investigation, methodology, review, and editing; HR contributed in preparing the resources, conceptualization, investigation, methodology, review, and editing.

References

Abdelhamid M, Eldardiry E, El-Hady MA (2013) Ameliorate salinity effect through sulphur application and its effect on some soil and plant characters under different water quantities. Agricultural Sciences. 4(1), 39–47. doi: 10.4236/as.2013.41007

Adish M, Fekri M, Hokmabadi H (2010) Response of Badami-zarand pistachio rootstock to salinity stress. Journal of Nuts. 1(1), 4-14.

Alshaal T, El-Ramady H, Al-Saeedi AH, Shalaby T, Elsakhawy T, Omara AED, Gad A, Hamad E, El-Ghamry A, Mosa A, Amer M (2017) The rhizosphere and plant nutrition under climate change. In: Naeem M, Ansari A, Gill S (eds) Essential plant nutrients. pp. 275-308.

Amiri A, Baninasab B, Ghobadi C, Khoshgoftarmanesh AH (2016) Zinc soil application enhance photosynthetic capacity and antioxidant enzyme activities in almond seedlings affected by salinity stress. Photosynthetica. 54, 1–18. Doi: org/10.1007/s11099-016-0078-0

Amtmann A (2009) Learning from evolution: Thellungiella generates new knowledge on essential and critical components of abiotic stress tolerance in plants. Molecular Plant. 2(1), 3–12. Doi: 10.1093/mp/ssn094.

Ansari A, Gharaghani A (2019) A comparative study of genetic diversity, heritability and inter-relationships of tree and nut attributes between *Prunus scoparia* and *P. elaeagnifolia* using multivariate statistical analysis. International Journal of Horticultural Science and Technology. 6(1), 137-150.

Archana Pandey N, Verma P (2017) Boron deficiency and toxicity and their tolerance in plants: a review. Journal of Global Biosciences. 6, 4958–4965.

Ashraf M, Ahmad S (2000) Influence of sodium chloride on ion accumulation, yield components and fibre characteristics in salt-tolerant and salt-sensitive lines of cotton (*Gossypium hirsutum* L.). Fruit Crop Research. 66, 115–127. doi: org/10.1016/S0378-4290(00)00064-2

Ashraf MPJC, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Science. 166(1), 316. doi: 10.1.1.566.6053&rep=rep1&type=pdf

Ashraf MHPJC, Harris PJ (2013) Photosynthesis under stressful environments: an overview. Photosynthetica. 51(2), 163–190. DOI: 10.1007/s11099-013-0021-6

Badran AM, Savin IY (2019) Influence of salinity on vegetative growth and photosynthetic pigments of bitter almond rootstock. Rudun Journal of Agronomy and Animal Industries 14(4), 319—328.

Baker NR, Rosenqvist (2004) Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. Journal of Experimental Botany. 55(403), 1607–1621.

Balfagón D, Terán F, Oliveira TD, Santa-Catarina C, Gómez-Cadenas A (2021) Citrus rootstocks

modify antioxidant system under drought and heat stress combination. Plant Cell. Report 1–10. doi.org/10.1007/s00299-021-02744-y

Bassil E, Coku A, Blumwald E (2012) Cellular ion homeostasis: emerging roles of intracellular NHX Na^+/H^+ antiporters in plant growth and development. Journal of Experimental Botany. 63(16), 5727–5740. doi.org/10.1093/jxb/ers250

Bastam N, Baninasa B, Ghobadi C (2013) Improving salt tolerance by exogenous application of salicylic acid in seedlings of pistachio. Plant Growth Regulation. 69 (3), 275–284. doi.org/10.1007/s10725-012-9770-7.

Behzadi Rad P, Roozban MR, Karimi S, Ghahremani R, Vahdati K (2021) Osmolyte accumulation and sodium compartmentation has a key role in salinity tolerance of pistachios rootstocks. Agriculture. 11(8), 708.

Benton J (2001) Laboratory guide for conducting soil tests and plant analysis. Boca Raton, Fl.CRC Press. pp. 384.

Bhantana P, Lazarovitch N (2010) Evapotranspiration, crop coefficient and growth of two young pomegranates (*Punica granatum* L.) varieties under salt stress. Agriculture Water Manangment. 97(5), 715–722.

Brychkova G, Alikulov Z, Fluhr R, Sagi M (2008) A critical role for ureides in dark and senescence-induced purine remobilization is unmasked in the Atxdh1 Arabidopsis mutant. Plant Journal. 54, 496–509. DOI: 10.1111/j.1365-313X.2008.03440.x

Cakmak I (2000) Possible roles of zinc in protecting plant cells from damage by reactive oxygen spices. New Phytol. 146, 185–205. Doi: abs/10.1046/j.1469-8137.2000.00630.x

Carillo P (2018) GABA shunt in durum wheat. Frontiers in Plant Science. 9, 100. Doi: 10.3389/fpls.2018.00100

Chakraborty K, Basak N, Bhaduri D, Ray S, Vijayan J, Chattopadhyay K, Sarkar RK (2018) Ionic basis of salt tolerance in plants: nutrient homeostasis and oxidative stress tolerance. In: Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B. (eds) Plant nutrients and abiotic stress tolerance. Springer, Singapore. pp.325-362.

Chakraborty K, Bose J, Shabala L, Shabala S (2016) Difference in root K^+ retention ability and reduced sensitivity of K^+ -permeable channels to reactive oxygen species confer differential salt tolerance in three Brassica species. Journal of Experimental Botany. 67, 4611–4625. Doi:10.1093/jxb/erw236

Chapman HD, Pratt PF (1961) Methods of Analysis for Soils. Plants and Water. 93(1) , 68-70.

Chen YF, Wang Y, Wu WH (2008) Membrane transporters for nitrogen, phosphate and potassium uptake in plants. Journal of Integrated Plant Biology. 50, 835–848. Doi: 10.1111/j.1744-7909.2008.00707.x

Corredor E, Romßn M, Garcφa E, Perera E, Ars P, Naranjo T (2004) Physical mapping of DNA genes enables to establish the karyotype of almond. Annal Aplied Biology. 144, 219–222. Doi: 10.1111/j.1744-7348.2004.tb00336.x

Dejampour J, Aliasgarzad N, Zeinalabedini M, Rohani Niya M, Majidi Hervan E (2012) Evaluation of salt tolerance in almond [*Prunus dulcis* (L.) Batsch] rootstocks. African Journal of Biotechnology. 11, 11907-11912. DOI: 10.5897/AJB11.2996

Devi BSR, Kim YJ, Selvi SK, Gayathri S, Altanzul K, Parvin S, Yang DU, Lee OR, Lee S, Yang DC (2012) Influence of potassium nitrate on antioxidant level and secondary metabolite genes under cold stress in *Panax ginseng*. Russion Journal of Plant Physiology. 59, 318–

325. Doi: 10.1134/S1021443712030041

Doll DA, Duncan RA, Hendricks L, Micke W (2014) Performance of six almond rootstocks with long-term exposure to sodium and chloride. Acta Horticulturae. 1028, 273-278. Doi: 10.17660/ActaHortic.2014.1028.44

Eca GF, Brito GB, Barbosa IS, Korn VMGAH, Hatje V (2014) Microwave-Assisted Acid Digestion Procedure for Trace Elements Determination in Polychaete Chaetopterus Variopedatus. Revista Virtual Quim. 6(4), 1047–1061.

Eskandari S, Majidazar M (2009) Introduction of new hybrid varieties of almond (*Prunus amygdalus Batsch*) for almond producing regions of Iran. World Applied Sciences Journal. 6(3), 323–330.

Fan W, Zhang M, Zhang H, Zhang P (2012) Improved tolerance to various abiotic stresses in transgenic sweet potato (*Ipomoea batatas*) expressing spinach betaine aldehyde dehydrogenase. PLoS One. 7 (5), 37344.

FAO (2002) Crops and drops. Making the best use of water for agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy. pp. 1-22

FAO STAT (2020) FAO Statistics Database on the World Wide Web (n.d.). Retrieved from. https://www.fao.org/faostat/en/#data/QCL and https://www.fao.org/faostat/en/#compare

FAO STAT (2021) FAO statistics database on the world wide web (n. d.). https://www.fao.org/faostat/en/#data/QCL (https://www. fao.org/faostat/en/#compare). Accessed the 31 of July 2023.

Feng L, Ding H, Wang J, Wang M, Xia W, Zang S, Sheng L (2015) Molecular cloning and expression analysis of RrNHX1 and RrVHA-c genes related to salt tolerance in wild Rosa rugosa. Saudi Journal of Biological Sciences. 22(4), 417–423. Doi:10.1016/j.sjbs.2015.01.008

Fernandez-Ballester G, Garcia–Sanchez F, Cerda A, Martinez V (2003) Tolerance of citrus rootstock seedling to saline stress based on their ability to regulate ion uptake and transport. Tree Physiology. 23, 265–271. Doi: 10.1093/treephys/23.4.265

Forni C, Daiana D, Bernard RG (2017) Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant Soil. 410, 335–356. Doi.org/10.1007/s11104-016-3007-x

Gaber MA (2010) Antioxidative defense under salt stress. Plant Signal Behaviour 5, 369–374. Doi: 10.4161/psb.5.4.10873

Gharaghani A, Mohammadi Javarzaria A, Vahdati K (2018) Kaolin particle film alleviates adverse eects of light and heat stresses and improves nut and kernel quality in Persian walnut. Scientia Horticulturae. 239, 35–40.

Grasselly C, Duval H (1997). *L'amandier*. CTIFL, Paris.

Grattan SR, Grieve CM (1999) Mineral nutrient acquisition and response by plants grown in saline environments. In: Pessarakli M (Ed.), Handbook of Plant and Crop Stress. Marcel Dekker, New York. pp. 203–229.

Grieve CM, Grattan SR (1983) Rapid assay for determination of water-soluble quaternary ammonium compounds. Plant and Soil. 70(2), 303–307. Doi.org/10.1007/BF02374789

Hadi MR, Karimi N (2012) The role of calcium in plants' salt tolerance. Journal of Plant Nutritient. 35, 2037–2054. Doi: abs/10.1080/01904167.2012.717158

Hansch R, Mendel RR (2009) Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). Current Opininion in Plant Biology. 12, 259–266. Doi: 10.1016/j.pbi.2009.05.006

Hasanuzzaman M, Bhuyan MHMB, Nahar K, Hossain MS, Mahmud JA, Hossen MS, Masud AAC,

Moumita, M Fujita (2018) Potassium: A Vital Regulator of Plant Responses and Tolerance to Abiotic Stresses. Agronomy. 8, 31. Doi: 10.3390/agronomy8030031

Hasanuzzaman M, Fujita M, Oku H, Nahar K, Hawrylak-Nowak B (2018) Plant nutrients and abiotic stress tolerance. Singapore: Springer. pp, 590.

He Y, Zhu Z, Yang J, Ni X, Zhu B (2009) Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. Environmental Experimental Botany. 66, 270–278. Doi:10.1016/j.envexpbot.2009.02.007

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 125 (1), 189–198. Doi: 10.1016/0003-9861(68)90654-1

Helaly MN, El-Sheery N, El-Hoseiny H, Rastogi A, Kalaji H (2018) Impact of treated wastewater and salicylic acid on physiological performance, malformation and yield of two mango cultivars. Scientia Horticulturae 233, 159–177. Doi: 10.1016/j.scienta.2018.01.001

Huang Y, Tang R, Cao Q, Bie Z (2009) Improving the fruit yield and quality of cucumber by grafting onto the salt tolerant rootstock under NaCl stress. Scientia Horticulturae. 122(1), 26–31. Doi: 10.1016/j.scienta.2009.04.004

Hussin S, Geissler N, Koyro HW (2013) Effect of NaCl salinity on (*Atriplex nummularia* L.) with special emphasis on carbon and nitrogen metabolism. Journal of Acta Physiolgy Plant 35, 1025–1038. Doi: 10.1007/s11738-012-1141-5.

Imani A, Amani G, Shamili M, Mousavi M, Rezai H, Rasouli M, José Martínez- García P (2021) Diversity and broad sense heritability of phenotypic characteristic in almond cultivars and genotypes. International Journal of Horticultural Science and Technology. 8(3), 281-289.

Isaakidis A, Sotiropoulos T, Almaliotis D, Therios I, Stylianidis D (2004) Response to severe water stress of the almond (*Prunus amygdalus*)'Ferragnès' grafted on eight rootstocks. New Zealand Journal of Crop and Horticultural Science. 32(4), 355–362. Doi: abs/10.1080/01140671.2004.9514316

Jin ZM, Wang CH, Liu ZP, Gong WJ (2007) Physiological and ecological characters studies on *Aloe vera* under soil salinity and seawater irrigation. Process Biochemestry. 42, 710–714. Doi:10.1016/j.procbio.2006.11.002

Karimi S, Yadollahi A, Arzani K, Imani A, Aghaalikhani M (2015) Gas-exchange response of almond genotypes to water stress. Photosynthetica. 53 (1), 29–34. Doi: 10.1007/s11099-015-0070-0

Karimi Z, Firouzi M, Dadmehr M, Javad-Mousavi SA, Bagheriani N, Sadeghpour O (2021) Almond as a nutraceutical and therapeutic agent in Persian medicine and modern phytotherapy: A narrative review. Phytotherapy Research. 1–16. Doi: 10.1002/ptr.7006

Khayyat M, Tehranifar A, Davarynejad GH, Sayyari-Zahan MH (2014) Vegetative growth, compatible solute accumulation, ion partitioning and chlorophyll fluorescence of Malase-e-Saveh and Shishe-kab pomegranate in response to salinity stress. Photosenthetica. 5, 301–312. Doi: 10.1007/s11099-014-0034-9

Kester DE, Gradziel TM (1996) Almonds (Prunus) In: Moore JN, Janick J (eds) Fruit Breeding. Wiley & Sons, New York, USA. pp.1–97

Koshiba T, Saito H, Ono N, Yamarnoto N, Sato M (1996) Purification and properties of flavin-and

molybdenum containing aldehyde oxidase from coleoptile of maize. Plant Biology. 110, 781–789. Doi:.org/10.1104/pp.110.3.781

Krasilnikoff G, Gahoonia T, Nielsen NE (2003) Variation in phosphorus uptake efficiency by genotypes of cowpea (*Vigna unguiculata*) due to differences in root and root hair length and induced rhizosphere processes. Plant and Soil. 251, 83–91. Doi: 10.1023/A:1022934213879

Kusunoki M (2007) Mono-manganese mechanism of the photosystem II water splitting reaction by a unique Mn_4Ca cluster. Biochim Biophys Acta. 1767, 484–492. Doi: 10.1016/j.bbabio.2007.03.012

Lattanzio V (2013) Phenolic Compounds: Introduction 50. Natural Products 1543–1580. Doi: 10.1007/978-3-642-22144-6_57

Li H, Ahammed GJ, Zhou G, Xia X, Zhou J, Shi K, Yu J, Zhou Y (2016) Unraveling main limiting sites of photosynthesis under below and above-ground heat stress in cucumber and the alleviatory role of Lufa rootstock. Front Plant Science. 7, 746. Doi: 10.3389/fpls.2016.00746

Li X, Luo M, Hei C, Song G (2019) Quantitative evaluation of debond in concrete-filled steel tubular member (CFSTM) using piezoceramic transducers and ultrasonic head wave amplitude. Smart Materials and Structures. 28(7), 075033. DOI:10.1088/1361-665X/ab1f27

Lichtenthaler H, Wellburn A (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions. 603, 591–592.

Livak KJ, Schmittgen T (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408. Doi: 10.1006/meth.2001.1262

Lotfi N, Soleimani A, Vahdati K, Çakmakçı R. (2019) Comprehensive biochemical insights into the seed germination of walnut under drought stress. Scientia Horticulturae 250, 329-43.

Lotfi N, Vahdati K, Kholdebarin B, Ashrafi EN (2009) Germination, mineral composition, and ion uptake in walnut under salinity conditions. HortScience. 44(5), 1352–1357.

Luachli A, Wteneke J (1979) Studies on growth and distribution of Na, K and CL soybean varieties differing in salt lolerance. Z. pflanenernacher. Bodenkd. 142, 3–13. Doi: 10.1002/jpln.19791420103

Lutts S, Kinet J, Bouharmont J (1996) NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Annal Botany. 78, 389–398.

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Archives of Biochemistry and Biophysics. 444 (2), 139–158. Doi: 10.1016/j.abb.2005.10.018

Marschner P (2012) Mineral nutrition of higher plants. Academic Press, Elsevier, Waltham, MA, USA. pp. 672.

Massai R, Remorini D, Tattini M (2004) Gas exchange, water relations and osmotic adjustment in two /rootstock combinations of prunus under various salinity concentrations. Plant and Soil. 259, 153–162. Doi: 10.1023/B:PLSO.0000020954.71828.13

Melgar JC, Syvertsen JP, Martinez V, Garcia-sanchez F (2008) Leaf gas exchange, water relations, nutrient content and growth in citrus and olive seedlings under salinity. Biologia Plantarum 52, 385–390. Doi: 10.1007/s10535-008-0081-9

Meloni DA, Gulotta MR, Martínez CA (2008) Salinity tolerance in Schinopsis quebracho colorado: Seed germination, growth, ion relations and metabolic responses. Journal of Arid Environments. 72 (10), 1785–1792.

Doi: 10.1016/j.jaridenv.2008.05.003

Mohammadi H, Imani A, Abdosi V, Asghari MR, Talaei AR (2020) Exogenous salicylic acid mitigates adverse effects of salinity on some photosynthesis-related parameters of almond. Journal of Agrculture Science and Technology 22(2), 519–534.

Momenpour A, Imani A, Bakhshi D, Akbarpour E (2018) Evaluation of salinity tolerance of some selected almond genotypes budded on GF677 rootstock. International Journal of Fruit Science. 18(4), 410–435. Doi: 10.1080/15538362.2018.1468850

Montaium R, Hening H, Brown PH (1994) The relative tolerance of six Prunus rootstocks to boron and salinity. Journal of the American Society for Horticultural Science. 6, 1169–1175.

Moya JL, Primo-Millo E, Talon M (1999) Morphological factors determining salt tolerance in citrus seedlings: the shoot to root ratio modulates passive root in roots of grafted and non-grafted tomato plants. Plant Growth Regulation. 56, 7–19. Doi: 10.1046/j.1365-3040.1999.00495.x

Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environmrnt. 25, 239–250. Doi: 10.1046/j.0016-8025.2001.00808.x

Munns R, Tester M (2008) Mechanisms of Salinity Tolerance. Annual Review of Plant Biology. 59, 651–681. Doi: 10.1146/annurev.arplant.59.032607.092911

Murillo-Amador B, Jones HG, Kaya C, Aguilar RL, Garcφa-Hernßndez JL, Troyo-DiΘguez E, Vila-Serrano NY, Rueda-Puente E (2006) Effects of foliar application of calcium nitrate on growth and physiological attributes of cowpea (*Vigna unguiculata* L. Walp.) grown under salt stress. Environmental Experimental Botany. 58, 188–196. Doi: 10.1016/j.envexpbot.2005.08.003

Murkute AA, Sharma S, Singh SK (2005) Citrus in term of soil and water salinity: a review. Journal of Science Industrial Research. 64, 393–402.

Najafian SHO, Rahemi M, Tavallali V (2008) Growth and chemical composition of GF677 (*Prunus amygdalus* × *prunus persica*) influenced by salinity levels of irrigation water. Asian Journal of Plant Science. 7, 309313. Doi: 10.3923/ajps.2008.309.313

Nass R, Cunningham KW, Rao R (1997) Intracellular sequestration of sodium by a novel Na^+/H^+ exchanger in yeast is enhanced by mutations in the plasma membrane H^+- ATPase. Journal of Biological Chemistry. 272, 26145–26152. Doi: 10.1074/jbc.272.42.26145

Necleous D, Vasilakakis M (2007) Effects of NaCl stress on red raspberry (*Rubus idaeus* L. 'Autumn Bliss'). Scientia Horticulturae. 112, 282–289. Doi: 10.1016/j.scienta.2006.12.025.

Ohta M, Hayashi Y, Nakashima A, Hamada A, Tanaka A, Nakamura T, Hayakawa T (2002) Introduction of a Na^+/H^+ antiporter gene from Atriplex gmelini confers salt tolerance to rice. Federation of European Biochemical Societies Letters 532L 279–282. Doi: 10.1016/s0014-5793(02)03679-7

Osman AS, Rady MM (2012) Ameliorative effects of sulphur and humic acid on the growth, anti-oxidant levels, and yields of and pea (*Pisum sativum* L.) plants grown in reclaimed saline soil. Journal of Horticulture Science Biotechnology. 87, 626–632. Doi: 10.1080/14620316.2012.11512922

Pandey N, Pathak GC, Singh AK, Sharma CP (2002) Enzymic changes in response to zinc nutrition. Journal of Plant Physiology. 151, 1151–1153. Doi: 10.1078/0176-1617-00674

Parida AK, Dasa AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicology

Environment Safety. 60, 354–349. Doi: 10.1016/j.ecoenv.2004.06.010

Parihar P, Singh S, Singh R, Singh VP, Prasad SM (2015) Effect of salinity stress on plants and its tolerance strategies: a review. Environmental Science and Pollution Research. 22 (6), 4056–4075. Doi: 10.1007/s11356-014-3739-1

Pasapula V, Shen G, Kuppu S, Paez-Valencia J, Mendoza M, Hou P, Chen J, Qiu X, Zhu L, Zhang X, Auld D, Blumwald E, Zhang H, Gaxiola R, Payton P (2011) Expression of an Arabidopsis vacuolar H^+ pyrophosphatase gene (AVP1) in cotton improves drought- and salt tolerance and increases fibre yield in the field conditions. Plant Biotechnology Journal. 9(1), 88–99. Doi: 10.1111/j.1467-7652.2010.00535.x

Pitt T, Stevens R, Cox JW (2018) Assessing the salt sensitivity of mature almond trees by replacing the resident saline irrigation with fresh water at different growth stages. Acta Horticulture. 1219, 251-258.

Rahmani A, Sardabi H, Daneshvar A (2006) Responses of Cultivated and Wild Almonds to Salinity. Acta Horticulture. 726, 265-272. Doi: 10.17660/ActaHortic.2018.1219.39

Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan AK (2011) Developing stress tolerant plants through in vitro selection-An overview of the recent progress. Environmental Experimental Botany. 71, 89–98.Doi:10.1016/j.envexpbot.2010.10.021

Ranjbar A (2006) Using chlorophyll fluorescence to study photosynthetic activities in sweet almond (*prunus dulcis* mill.) in response to salinity stress. Acta Horticulturae 726, 343-349. Doi: 10.17660/ActaHortic.2006.726.55

Reza S, Heidari R, Zare S, Norastehnia A (2006) Antioxidant response of two salt-stressed barley varieties in the presence or absence of exogenous proline. Geneteic Appllied Plant Physiology 32, 233251. Doi.org/10.3389/fpls.2020.559876

Robatjazi R, Roshandel P, Hooshmand S (2020) Benefits of silicon nutrition on growth, physiological and phytochemical attributes of basil upon salinity stress. International Journal of Horticultural Science and Technology. 7(1), 37-50.

Rouphael Y, Venema JH, Edelstein M, Savvas D, Colla G, Ntatsi G, Ben-Hur M, Kumar P, Schwarz D (2017) Grafting as a tool for tolerance of abiotic stress. CAb International, Oxford Press, U.K. 171–215.

Rubio-Piña JA, Zapata-Pérez O (2011) Isolation of total RNA from tissues rich in polyphenols and polysaccharides of mangrove plants. Electronic Journal of Biotechnology 14(5), 11–11. Doi: 10.2225/vol14-issue5-fulltext-8

Salama ZA, El-Fouly MM, Lazova G, Popova LP (2006) Carboxylating enzymes and carbonic anhydrase functions were suppressed by zinc deficiency in maize and chickpea plants. Acta Physiology Plantearum. 28, 445–451. Doi:10.1007/BF02706627

Sandhu D, Kaundal A, Acharya BR, Forest T, Pudussery MV, Liu X, Ferreira JFS, Suarez DL (2020) Linking diverse salinity responses of 14 almond rootstocks with physiological, biochemical, and genetic determinants. Scientific Reports 10: 1-13. Doi: 10.1038/s41598-020-78036-4

Shao Y, Cheng Y, Pang H, Chang M, He F, Wang M, Davis D J, Zhang S, Betz O, Fleck C, Dai T, Madahhosseini Sh, Wilkop T, Jernstedt J, Drakakaki G (2021) Investigation of Salt Tolerance Mechanisms Across a Root Developmental Gradient in Almond Rootstocks. Frontiers in Plant Science. Article 595055. Doi:

10.3389/fpls.2020.595055

Shabala S, Pottosin I (2014) Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. Physiologia Plantarum. 151 (3), 257–279. Doi: 10.1111/ppl.12165

Shaul O (2002) Magnesium transport and function in plants: the tip of the iceberg. BioMetals. 15, 309–323. Doi: 10.1023/A:1016091118585

Shibli RA, Mohammad M. Abu-Ein A, Shatnawi M (2000) Growth and micronutrient acquisition of some apple varieties in response to gradual in vitro induced salinity. Journal of Plant Nutritient. 23, 1209–1215. Doi: 10.1080/01904160009382094

Shibli RA, Shatnawi MA, Swaidat IQ (2003) Growth, osmotic adjustment, and nutrient acquisition of bitter almond under induced sodium chloride salinity *in vitro*. Communications in Soil Science and Plant Analysis. 34 (13 & 14): 1969–1979 Doi:10.1081/CSS-120023231

Singh S, Prasad SM (2014) Growth, photosynthesis and oxidative responses of *Solanum melongena* L. seedlings to cadmium stress: mechanism of toxicity amelioration by kinetin. Scientia Horticulturae. 176, 1–10. Doi: 10.1016/j.scienta.2014.06.022

Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology. 299, 152–178. Doi: 10.1016/S0076-6879(99)99017-1

Soliman G M, Farhan SS (2021) Evaluation of salinity tolerance on new selected Almond × Peach hybrid rootstocks. Arab University Journal of Agricultural Science. 29(1), 419 – 436. Doi: 10.21608/AJS.2021.51647.1307

Sorkheh K, Shiran B, Rouhi V, Khodambashi M, Sofo A (2012) Salt stress induction of some key antioxidant enzymes and metabolites in eight Iranian wild almond species. Acta Physiologiae Plantarum. 34(1), 203–213. Doi.org/10.1007/s11738-011-0819-4

Stahl W, Sies H (2005) Bioactivity and protective effects of natural carotenoids. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 1740(2), 101–107. Doi: 10.1016/j.bbadis.2004.12.006

Tabatabaei SJ (2006) Effects of salinity and N on the growth, photosynthesis and N status of olive (*Olea europaea* L.) trees. Scientia Horticulturae. 108(4), 432–438. Doi: 10.1016/j.scienta.2006.02.016

Tattini M (1990) Effect of increasing nitrogen concentration on growth and nitrogen uptake of container-grown peach and olive. In: van Beusichem, M.L. (Ed.), Plant Nutrition-Physiology and Application. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp.515–518.

Tattini M, Gucci R, Coradeschi MA, Ponzio C, Everard JD (1995) Growth, gas exchange and ion content in Olea europaea plants during salinity stress and subsequent relief. Physiologia Plantarum. 95(2), 203–210. Doi: abs/10.1111/j.1399-3054.1995.tb00828.x

Tester M, Dovenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Annals of Botany. 91, 503–527. Doi: 10.1093/aob/mcg058

Anonymus 2022. The Global Risks Report 17th edn. World Economic Forum, Geneva, Switzerland. https://www.weforum.org/reports/global-risks-report-2022.

Tuna AL, Kaya C, Ashraf M, Altunlu H, Yokas I, Yagmur B (2007) The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under

salt stress. Environmental Experimental Botany. 59, 173–178. Doi: 10.1016/j.envexpbot.2005.12.007

Tunuturk M, Tuncturk R, Yildirim B, Ciftci V (2011) Effect of salinity stress on plant fresh weight and nutrient composition of some Canola (*Brassica napus* L.) cultivars. African Journal of Biotechnology. 10(10), 1827–1832.Doi: 10.5897/AJB10.1618

Vahdati K, Bavani AR, Khosh-Khui M, Fakour P, Sarikhani S. (2019) Applying the AOGCM-AR5 models to the assessments of land suitability for walnut cultivation in response to climate change: A case study of Iran. PloS ONE. 14(6). e0218725

Vahdati K, Sarikhani S, Arab MM, Leslie CA, Dandekar AM, Aletà N, Bielsa B, Gradziel TM, Montesinos Á, Rubio-Cabetas MJ, Sideli GM, Serdar Ü, Akyüz B, Beccaro GL, Donno D, Rovira M, Ferguson L, Akbari M, Sheikhi A, Sestras AF, Kafkas S, Paizila A, Roozban MR, Kaur A, Panta S, Zhang L, Sestras RE, Mehlenbacher S (2021) Advances in rootstock breeding of nut trees: objectives and strategies. Plants 10 (11), 2234.

Ventura Y, Wuddineh WA, Ephrath Y (2010) Molybdenum as an essential element for improving total yield in seawater-grown *Salicornia europaea* L. Scientia Horticulturae. 126, 395–401. Doi: 10.1016/j.scienta.2010.07.015

Volkov V, Amtmann A (2006) Thellungiella halophila, a salt-tolerant relative of *Arabidopsis thaliana*, has specific root ion-channel features supporting K^+/Na^+ homeostasis under salinity stress. The Plant Journal. 48(3), 342–353. Doi:10.1046/j.0016-8025.2003.01116.x

Wang M, Zheng Q, Shen Q, Guo S (2013) The critical role of potassium in plant stress response. International Journal of Moleculrat Science. 14, 7370–7390. Doi: 10.3390/ijms14047370

Wang XM, Huang T, Wu WL, Li WL, Zhu H (2016). Effects of salt stress on photosynthetic characteristics of beach plum and other Prunus species. Acta Horticulture. 1112, 233-240 DOI: 10.17660/ActaHortic.2016.1112.32

Wei R, Zimmermann W (2017) Biocatalysis as a green route for recycling the recalcitrant plastic polyethylene terephthalate. Microbial Biotechnology. 10(6), 1302–1307. Doi: 10.1111/1751-7915.12714

White PJ, Broadley MR (2001) Chloride in Soils and its Uptake and Movement within the Plant: A Review. Annual Botany. 88(6), 967–988. Doi: 10.1006/anbo.2001.1540

Yang Y, Iji PA, Kocher A, Mikkelsen LL, Choct M (2008) Effects of xylanase on groth and gut development of broiler chickens given a wheat-based diet. Asian-Australasian Journal of Animal Sciences. 21(11), 1659–1664. Doi: 10.5713/ajas.2008.80074

Yin XY, Yang A.F, Zhang KW, Zhang JR (2004) Production and analysis of transgenic maize with improved salt tolerance by the introduction of AtNHX1 gene. Acta Botanica Sinica 7, 12–20.

Yu Q, Baluska F, Jasper F, Menzel D, Goldbach E (2002) Short term boron deprivation enhances levels of cytoskeletal proteins in maize, but not in zucchini, root apices. Physiologia Plantearum 117, 270–278. Doi: 10.1034/j.1399-3054.2003.00029.x.

Zaratavaldez JL, Muhammad S, Saa S, Lampinen BD, Brown PH (2015) Light interception, leaf nitrogen and yield prediction in almonds. A case study. Europian Journal of Agriculture. 66, 17. Doi: 10.1016/j.eja.2015.02.004.

Zhang J, Lü F, Shao L, He P (2014) The use of biochar-

amended composting to improve the humification and degradation of sewage sludge. Bioresource Technology. 168, 252–258. Doi:10.1016/j.biortech.2014.02.080.

Zhu J, Bie ZL, Huang Y, Han XX (2008) Effect of grafting on the growth and ion concentrations of cucumber seedlings under NaCl stress. Soil Science and Plant Nutrition. 54, 895–902. Doi: 10.1111/j.1747-0765.2008.00306.x

Zrig A, Ben Mohamed H, Tounekti T, Ennajeh M, Valero D, Khemira H (2015a) A comparative study of salt tolerance of three almond rootstocks: contribution of organic and inorganic solutes to osmotic adjustment. Journal of Agricultural Science Technology. 17, 675-689.

Zrig A, Mohamed HB, Tounekti T, Ahmed SO, Khemira H (2015b) Differential responses of antioxidant enzymes in salt-stressed almond tree grown under sun and shade conditions. Journal of Plant Sciences and Research. 2, 117-119.

Zrig A, Mohamed HB, Tounekti T, Khemira H, Serrano M, Valero D, Vadel AM (2016) Effect of rootstock on salinity tolerance of sweet almond (cv. Mazzetto). South African Journal of Botany. 102, 50-59. Doi: 10.1016/j.sajb.2015.09.001

Zrig A, Tounekti T, Vadel AM, BenMohamed H, Valero D, Serrano M, Chtara C, Khemira H (2011) Possible involvement of polyphenols and polyamines in salt tolerance of almond rootstocks. Plant Physiology and Biochemistry. 49, 1313–1320. Doi: 10.1016/j.plaphy.2011.08.009