

Silicon nutrition counteracts salt-induced damage associated with changes in biochemical responses in apple

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ABSTRACT: Salt stress is an environmental challenge that adversely influences plant responses. Silicon (Si) nutrition plays critical roles in plant tolerance to salt stress. Apple (*Malus domestica* Borkh.), a salt sensitive fruit species, was used in the present experiment to investigate the influences of Si on salt stress as well as on alterations of biochemical responses. Apple cv Fuji grafted on M9 clonal rootstock was exposed to salt stress for 4 months with 35 mmol·L⁻¹ NaCl. CaSiO₃ doses (0.5, 1 and 2 mmol·L⁻¹) were applied to the roots of the salt-stressed apple plants except control. Si application resulted in mitigation of salt stress in apple plants. The highest chlorophyll a, b and a + b were obtained from the 1 mmol·L⁻¹ Si treatment (5.37, 2.41 and 7.78 μg·g⁻¹ fw, respectively). Moreover, Si treatment had higher chlorophyll content compared to the control as well as salt exposed plants. Silicon applications led to a reduction in malondialdehyde (MDA) content even lower than control. The 0.5 mmol·L⁻¹ Si treatment had the highest values of ascorbate peroxidase (APX) and phenolic content. The results show that Si nutrition plays important roles in apple salt tolerance via biochemical mechanisms and that it can be used in areas subject to salt stress for apple growing.

Key words: *Malus domestica* Borkh., plant behavior, rootstock, salinity.

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INTRODUCTION

Plants endure several environmental stressors and salt stress is one of the most common challenges limiting plant growth and development. Excessive fertilizer application with poor irrigation causes soil salinity, which may reach toxic levels for plants. Excessive salinity can cause oxidative stress and the formation of reactive oxygen species (ROS), thus resulting in lipid peroxidation, decrease in photosynthesis, and inhibition of enzymatic activities (Bressan et al. 1990). Temperate fruit trees, including apples, are salt-sensitive plants (Maas 1986). Apple cultivation under salt stress may result in a decline of fruit yield and quality. Salt stress impedes apple plant growth by decreasing water uptake, inhibition of photosynthesis, and by closing stomatal apertures (Yin et al. 2010; Fu et al. 2013).

One technique to promote plant tolerance against salt stress is utilization of some amendments (e.g., osmoprotectants, beneficial nutrients). In recent years, many experiments revealed that the application of silicon (Si) plays critical roles in plant tolerance to salt stress (Coskun et al. 2016; Aras and Eşitken 2018a). Silicon application has been observed to enhance tolerance of many stressors such as drought, and alkaline conditions (Abdel Latif and Tran 2016; Ma et al. 2016). Silicon is the second most abundant element on earth and plays an important role in stress tolerance, flowering and fruit quality (Dehghanipoodeh et al. 2016; Kim et al. 2017). Furthermore, Si is involved in cell wall reinforcement due to deposition of Si in epidermal cells to form a barrier (Trenholm et al. 2004; Romero-Aranda et al. 2006). Several experiments have documented the mitigation effects of Si application under salt stress on many plants. Conceição et al. (2019) examined the

protective role of Si in sunflower when grown under salinity conditions. According to these authors, Si mitigated adverse effects of salt stress by inducing antioxidant systems and modulating nitrogen metabolism. Moreover, Haghghi and Pessarakli (2013) revealed that Si could increase plant tolerance against salt stress by protecting cell membrane stability in tomato. Hashemi et al. (2010) reported that Si nutrition mitigated salt damage in canola evidenced by declined lipid peroxidation and enhanced antioxidant enzymes.

Apple (*Malus domestica* Borkh.) is an important temperate zone fruit species. As a salt sensitive fruit species, it may suffer from salinity where excessive fertigation is practiced. Little information is available on Si mitigation of salt damage to woody plants at a biochemical level. In the current experiment, the biochemical effects of Si nutrition on mitigating damage from salt stress were studied in apple plants.

MATERIAL AND METHODS

Pot trials and experimental design

The study was conducted in 2014 in a heated greenhouse of the Department of Horticulture at Selcuk University (lat 38°01'46"N, long 32°30'39"E) in Turkey and 1-year-old apple plants (*Malus domestica* Borkh.) cv. Fuji grafted onto M9 clonal rootstock were chosen for the experiment following a complete randomized plot design involving three replications, with three plants per replication. The plants were planted in March in 13 L pots with media consisting of soil, substrate and perlite (1:3:1). The plants were placed in a semicontrolled greenhouse where temperatures fluctuated between 25-35 °C and relative humidity range between 60-85% during the day. Up until the start of the experiment, all plants were irrigated with tap water and 1 month later (in April) saline-treatment plants were watered with 35 mmol·L⁻¹ NaCl solution which is considered to be a moderate level of salinity for temperate fruit tree species (Akçay and Eşitken 2017; Aras and Eşitken 2018b). Two months after the salt stress was initiated, three different CaSiO₃ doses (0.5, 1 and 2 mmol·L⁻¹) were applied twice a month (in June and July) to the plant rhizosphere as a solution, except the controls. Plants were watered three times a week and fertilized once a week with Hoagland's nutrient solution (Hoagland and Arnon 1950). Excess solution was allowed to drain from the pot. Control and salt-treated control plants were not treated with CaSiO₃, salt treated plants were watered with NaCl solution compared to the tap-water treated control. After 4 months of salinity treatments (in August), several biochemical properties were evaluated.

Matured leaves were used for protein content determination. The leaf segments were ground in cold phosphate buffer (pH 6.5) and then filtered. The filtrate was centrifuged at 4000 g for 20 min at 4 °C. The supernatant was decanted and then the Bradford Protein Kit was added. The mixture was vigorously shaken with vortex. The sample absorbance was read at 595 nm. The protein levels were estimated by the method of Bradford (1976) using bovine serum albumin as standard and expressed at mg protein g⁻¹ fresh weight (fw).

The proline content was estimated by the method of Bates et al. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. The supernatant was used for estimation of the proline content. The reaction mixture consisted of 2 ml supernatant, 2 ml acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100 °C for 1 h. After termination of the reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene and the absorbance was read at 520 nm.

For the determination of chlorophyll (a, b and a + b) content, fine powder (0.1 g) of the leaves was homogenized in 10 mL of 80% acetone, and then centrifuged at 12,000 g for 10 min. The chlorophyll (a, b, and a + b) content was spectrophotometrically determined by measuring absorbance at 663 and at 646 nm. The chlorophyll (a, b and a + b) content was calculated using the equations of Porra et al. (1989), as follows: (1) chlorophyll a (µg·mL⁻¹) = 12.25 A₆₆₃ - 2.55 A₆₄₆; (2) chlorophyll b (µg·mL⁻¹) = 20.31 A₆₄₆ - 4.91 A₆₆₃; and (3) chlorophyll a + b (µg·mL⁻¹) = 17.76 A₆₄₆ + 7.34 A₆₆₃.

The chlorophyll stability index (CSI) was calculated as follows (Sairam et al. 1997): CSI = (total chlorophyll under stress/total chlorophyll under control) × 100.

For the quantification of total phenols, methanol was used for extraction. Total phenolic content was assayed by A765 with Folin-Cocalteau reagent (Singleton and Rossi 1965). The results were expressed as μg of p-hydroxycinnamic acid (g fresh weight).

The lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in 1 g leaf fresh weight according to Madhava Rao and Sresty (2000). MDA is a product of lipid peroxidation by the thiobarbituric acid reaction. The concentration of MDA was calculated from the absorbance at 532 nm by using an extinction coefficient of $155 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{cm}^{-1}$.

The ascorbate peroxidase (APX) activity was estimated according to the method of Nakano and Asada (1981). The enzyme activity was determined by the decline in absorbance of ascorbate at 290 nm. The reaction mixture consisted of enzymatic extract, $50 \text{ mmol}\cdot\text{L}^{-1}$ sodium phosphate buffer, pH 7, $0.5 \text{ mmol}\cdot\text{L}^{-1}$ ascorbate, $0.5 \text{ mmol}\cdot\text{L}^{-1} \text{H}_2\text{O}_2$ and $0.1 \text{ mmol}\cdot\text{L}^{-1}$ EDTA, in a 0.3 ml final volume. The reaction started after the hydrogen peroxide addition. The molar extinction coefficient $2.8 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{cm}^{-1}$ was used to calculate APX activity. Enzyme activity was expressed at $\text{unit}\cdot\text{mg}^{-1}$ protein. One unit of enzyme was the amount necessary to decompose $1 \mu\text{mol}$ of the substrate per minute at 25°C .

Statistical analyses were performed with the statistical software package SPSS, version 20.0. The means were compared by the Duncan's multiple-comparisons test at 5%.

RESULTS

Salt stress and Si applications affected several biochemical responses in apple plants. There was a considerable increase in the chlorophyll content. The highest chlorophyll a, b and a + b were obtained from the $1 \text{ mmol}\cdot\text{L}^{-1}$ Si treatment (Table 1) increasing chlorophyll contents by 68, 32 and 55%, respectively. In addition to the chlorophyll content, $1 \text{ mmol}\cdot\text{L}^{-1}$ Si increased the chlorophyll stability index a, b and a + b by 75, 36 and 60%, respectively compared to the salt only treatment (Table 2).

Significant increases in protein and proline content were obtained with the salt treatment compared to the water-only control (Table 3). The 0.5 and $1 \text{ mmol}\cdot\text{L}^{-1}$ Si applications had lower protein and proline contents compared to saline-treated plant. Furthermore, $2 \text{ mmol}\cdot\text{L}^{-1}$ Si possessed lower proline content compared to saline-treated plant. Si applications led to a reduction in MDA content even lower than the control, while the salt treatment significantly increased the MDA content by 24% compared to the control (Table 3). The $0.5 \text{ mmol}\cdot\text{L}^{-1}$ Si treatment had the highest values of APX and phenolic content among all treatments (Table 3).

Table 1. Effect of CaSiO_3 on chlorophyll content on apple plants exposed to moderate levels of salinity ($35 \text{ mmol}\cdot\text{L}^{-1}$ NaCl).

Treatments	Chl a ($\mu\text{g}\cdot\text{g}^{-1}$ fw)	Chl b ($\mu\text{g}\cdot\text{g}^{-1}$ fw)	Chl a + b ($\mu\text{g}\cdot\text{g}^{-1}$ fw)
Control	3.20 d	1.82 c	5.03 d
NaCl	3.07 d	1.77 c	4.85 d
$0.5 \text{ mmol}\cdot\text{L}^{-1} \text{CaSiO}_3 + \text{NaCl}$	3.90 c	1.92 c	5.83 c
$1 \text{ mmol}\cdot\text{L}^{-1} \text{CaSiO}_3 + \text{NaCl}$	5.37 a	2.41 a	7.78 a
$2 \text{ mmol}\cdot\text{L}^{-1} \text{CaSiO}_3 + \text{NaCl}$	4.93 b	2.15 b	7.09 b

Means separation within columns by Duncan's multiple range test, $p < 0.05$.

Table 2. Effect of CaSiO_3 on chlorophyll stability index (CSI) a, b and a + b on apple plants exposed to moderate levels of salinity ($35 \text{ mmol}\cdot\text{L}^{-1}$ NaCl).

Treatments	Chl a CSI	Chl b CSI	Chl a + b CSI
NaCl	96.14 d	98.70 c	96.48 d
$0.5 \text{ mmol}\cdot\text{L}^{-1} \text{CaSiO}_3 + \text{NaCl}$	121.97 c	107.03 c	115.90 c
$1 \text{ mmol}\cdot\text{L}^{-1} \text{CaSiO}_3 + \text{NaCl}$	167.91 a	133.88 a	154.73 a
$2 \text{ mmol}\cdot\text{L}^{-1} \text{CaSiO}_3 + \text{NaCl}$	154.16 b	119.81 b	140.95 b

Means separation within columns by Duncan's multiple range test, $p < 0.05$.

Table 3. Effect of CaSiO₃ on protein, proline, malondialdehyde (MDA), ascorbate peroxidase (APX) and phenolic contents on apple plants exposed to moderate levels of salinity (35 mmol L⁻¹ NaCl).

Treatments	Protein (µg·g ⁻¹ fw)	Proline (µmol g ⁻¹ fw)	MDA (µmol g ⁻¹ fw)	APX (µmol g ⁻¹ fw min ⁻¹)	Phenolic (µg GAE 100 g ⁻¹ fw)
Control	0.0110 b	0.0120 ^{NS}	0.0021 ab	226.66 ab	0.091 bc
NaCl	0.0145 ab	0.0156	0.0026 a	281.66 a	0.105 b
0.5 mmol·L ⁻¹ CaSiO ₃ + NaCl	0.0144 ab	0.0130	0.0018 b	145.00 c	0.085 c
1 mmol·L ⁻¹ CaSiO ₃ + NaCl	0.0135 b	0.0146	0.0020 ab	193.33 bc	0.095 bc
2 mmol·L ⁻¹ CaSiO ₃ + NaCl	0.0175 a	0.0113	0.0018 b	226.66 ab	0.124 a

Means separation within columns by Duncan's multiple range test, $p < 0.05$. NS = nonsignificant.

DISCUSSION

Salt stress influences many biochemical responses in plants. In the current experiment, the protective effects of Si application in apple plant under salinity conditions were investigated. This work revealed that supplemental Si nutrition could effectively increase the tolerance of young apple plants to salt stress. Leaf-tip necrosis is utilized as an indicator of NaCl toxicity and remarkable necrosis in salt-treated control plants was observed. The Si treated plants showed less necrosis compared to the salt-treated control. Visual symptoms as leaf scorch were observed one month after the onset of saline stress. In the short term (for one month), there was no any symptom as reported in previous experiments (Aras and Eşitken 2018b).

Chlorophyll biosynthesis is a conspicuous process that is necessary for photosynthesis. Chlorophyll is made from 5-aminolaevulinic acid (ALA) (Beale 1999) that was reported to decrease in salt stressed plant leaves (Santos 2004; Tavallali et al. 2008). Moreover, the decrease in chlorophyll content may be due to an increase in chlorophyll degradation and/or decrease in mineral acquisition needed for chlorophyll synthesis (El-Desouky and Atawia 1998). In the current study, salinity decreased chlorophyll content in apple plants. The highest contents of chlorophyll a, b and a + b were obtained with the 1 mmol·L⁻¹ Si treatment. Furthermore, the Si treatment considerably increased chlorophyll content in apple leaves, compared to the control as well as the salt treated plants.

Magnesium (Mg) is the key element of chlorophyll (Chen et al. 2017) and iron (Fe) is used for chlorophyll formation (Miller et al. 1984). Silicon may induce internal Fe and Mg transport, leading to the synthesis of chlorophyll. Similarly, as with the chlorophyll content, the Si treatment increased the CSI. Chlorophyll stability index can be used as a rapid method to estimate resistance to stresses (Rahbarian et al. 2011; Aras et al. 2019). The 1 mmol·L⁻¹ Si treatment increased CSI a, b and a + b compared to salt treated plants. These results are in agreement with Rahbarian's et al. (2011) findings who observed a decrease in CSI of chickpea genotypes under drought stress.

Salt damage in plants may result from the production of ROS. Reactive oxygen species formation may cause cellular damage, and was reflected in terms of the increased MDA content in salt-exposed leaves, in accordance with the results obtained by Zhang et al. (2018) and Liu et al (2014). Exogenously applied Si had a likely protective effect on salt induced membrane damage. Under salinity, Si is deposited on cell walls and such deposition strengthens the cell membranes (Liang et al. 2005; Zhang et al. 2018). Therefore, Si addition may have reinforced membranes of plant cells and prevented ion leakage from membranes as evinced by the observed decrease in lipid peroxidation. Silicon thus likely had antisalt stress effects due to its membrane stabilizing property. Moreover, Si may have provided better cell membranes against salinity, as Si competes with Na⁺ for membrane binding spots. A protective effect of Si on relative membrane injury under salt stress has been previously reported (Tuna et al. 2008; Hashemi et al. 2010). Silicon itself acts as an antioxidant and scavenges ROS, resulting in reduced MDA content.

Accumulation of protein and proline is a common physiological response against salt stress (Yoon et al. 2005; Khadri et al. 2006). Conversely, a decrease in protein and proline content in response to Si treatment, which was observed in our experiment, may indicate low salt stress damage. In a study by Lee et al. (2010), Si treatment decreased proline content in soybean under salt stress conditions. Similar results with protein and proline content were obtained with respect to phenolic

content and APX activity in the present study. Decreases in phenolic content and APX enzyme activity were detected in apple plants treated with Si under salt stress. Although phenolics and APX activity elevate as a defense mechanism, increased levels of phenolics and APX activity may demonstrate the level of stress damage, thus, it can be stated that low contents of phenolics and APX activity likely reflect less stress damage. Thus, this work postulate that Si treatment has beneficial influences by acting as an antioxidant and functioning in plant defense against salt stress. In a previous work with canola, it was proposed that a decrease in plant growth may have resulted from the increased phenolic content in plants exposed to salinity (Hashemi et al. 2010).

CONCLUSION

Taken together, the results of the current experiment attribute the protective role of Si in salt stress alleviation to its improvement of antioxidant activity, protection of cell membranes and enhancing chlorophyll content thus photosynthesis. The results suggest that Si nutrition can improve the establishment of apple cultivation in areas subject to salt stress. This work considers that the Si 1 mmol.L⁻¹ level was more effective than the other Si levels, the reason may be due to protecting chlorophyll and showing less protein content that is related with indicating less salt stress damage. The results indicate that Si utilization in the greenhouse on plants exposed to salinity also reflect the potential use of Si under field conditions.

AUTHORS' CONTRIBUTION

Conceptualization, Aras, S. and Eşitken, A.; Methodology, Aras, S. and Keles, H.; Investigation, Aras, S. and Eşitken, A.; Writing – Original Draft, Aras, S.; Writing – Review and Editing, Aras, S.; Resources, Aras, S., Keles, H. and Eşitken, A.

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