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ORIGINAL PAPER

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SILICON NUTRITION IN ALLEVIATING SALT STRESS IN APPLE PLANT

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ABSTRACT

Salinity is one of the major environmental stresses that adversely affect fruit yield and quality. Thus, finding an effective way of ameliorating salinity damage is important for sustainable fruit production. Silicon (Si) treatment effectively counteracts the effects of many abiotic stress factors such as salinity, drought, cold, iron deficiency. To probe into the potential alleviating salinity malignant effects, we investigated the protective roles of Si. An apple plant (*Malus domestica* Borkh.) cv. 'Fuji' grafted on M9 clonal rootstock was chosen for the experiment and imposed to salinity stress for 4 months with 35 mM NaCl. Si with different three doses (0.5, 1 and 2 mM) was applied to the roots of the salt-stressed apple plants except control. Si treatments inhibited apple plants growth depression through increasing stomatal conductivity, chlorophyll and decreasing electrolyte leakage. 0.5 mM Si improved root : shoot dry weight under salinity condition. The lowest values of membrane permeability were found in 0.5 and 2 mM Si treatments (21.45 and 21.55%, respectively) while salt had the highest value (48.43%). Salt exhibited a rapid decrease in stomatal conductivity by 49% compared to the control. We hypothesis that Si treatment contributed to cell walls, involving membrane stabilizing and fortification. Our findings showed that Si increased apple plant tolerance against salinity.

Key words: calcium silicate, *Malus*, silicon, physiology

INTRODUCTION

Salinity is one of the major environmental stresses that adversely affect crop yield and quality. It is estimated that there are approximately 830 million hectares of salinised soils in world [Martinez-Beltran and Manzur 2005]. The problem of soil salinity is increasing due to the poor drainage, excessive fertilization and growing near seashore lands [Chinnusamy et al. 2005, Aras and Eşitken 2018]. Temperate fruit trees are salt-sensitive plants [Maas 1986]. Among horticultural plants, apple trees have been suggested to be salt-affected and can result diminish in apple fruit yield and quality [Fu et al. 2013]. Among soluble salts, sodium chloride (NaCl) is the major salt type with detrimental effects on plant body [Pessarakli and Szabolcs 2010]. Salinity may have deleterious effects on plant growth, crop production, seed germination, protein synthesis [Levitt 1980, Bressan et al. 1990]. Moreover, salinity stress is shown to reduce nutrient uptake, photosynthesis, metabolism and increase in membrane permeability [Yin et al. 2010, Aras et al. 2015]. In short term salinity, the responses to salt stress are reduction in stomatal conductance and chlorophyll and leaf relative content, increase in membrane permeability [Aras and Eşitken 2018].

One strategy to increase apple plant growth under salinity condition is utilization of salt-tolerant rootstocks. Apple cultivars can be grafted onto many apple rootstocks such as M9, MM106, MM111. However, using different rootstocks may be not sufficient for apple growing under salinity. Therefore, some

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beneficial mineral nutrients can be applied to plants in order to maintain better fruit yield and quality. More recently, silicon (Si) nutrition has been used to mitigate many abiotic stress factors such as drought [Chen et al. 2011], cold [Zhu et al. 2006], iron deficiency [Pavlovic et al. 2013]. In addition, many studies showed exogenous Si could alleviate the salinity damage in strawberry [Dehghanipoodeh et al. 2016], tomato [Romero-Aranda et al. 2006], cucumber [Zhu et al. 2004]. In recent years there has been evidence that Si is involved many physiological processes of plants, such as plant water relations [Zhu and Gong 2014], photosynthesis [Zuccarini 2008], mineral uptake and mobility inside plants [Guntzer et al. 2012]. Si treatment has shown to increase plant growth of tomato plant under salt stress [Romero-Aranda et al. 2006]. Furthermore, Si could increase chlorophyll content and maintain the stability of cell membrans, which was benefical to plants, especially under salinity condition [Haghighi and Pessarakli 2013]. Liang et al. [2003] suggested that Si can enhance salt tolerance by increasing antioxidant enzyme activity and decreasing lipid peroxidation. Zhang et al. [2018] reported that Si may decrease the malondialdehyde (MDA) content in *Glycyrrhiza uralensis* under salt stress.

To the best of our knowledge, no reports have examined the effects of Si nutritient on salt stress for deciduous plants. Apple (*Malus domestica* Borkh.) is sensitive to salt, and as a perennial woody plant the mechanism of salt stress adaption and response to Si application could be different from that of annual plants. The aims of this study are to clarify the involvement of exogenous Si in apple plant growth and physiology under salt stress.

MATERIAL AND METHOD

Pot trials and experimental design

The study was conducted in 2014 in a heated greenhouse of Department of Horticulture at Selcuk University in Turkey. An apple plant (*Malus domestica* Borkh.) cv. 'Fuji' grafted on M9 clonal rootstock was chosen for the experiment with following a randomized plot design involving three replications, with three plants per replication and was planted in pots in March. Up until the start of the experiment, all plants were irrigated with tap water and 1 month later (in April) plants were watered with 35 mM NaCl solution. Two months after the salt stress, three different $CaSiO_3$ doses (0.5, 1 and 2 mM) were treated twice a month interval (in June and July) to plant rhizosphere as solution except control. Control and salt plants were not applied with $CaSiO_3$, salt plants were watered with NaCl solution compared to control. Four months after the salinity (in August), many morphological and physiological properties were evaluated.

Growth measurements and physiological determinations

The growth promoting effects of $CaSiO_3$ treatments were evaluated by many morphological properties. Relative growth rate measurement was taken for plant biomass. The weights of plant biomass were taken and recorded. The relative growth rate (RGR) of plant biomass was calculated using the equation given below [Del Amor and Marcelis 2003]:

$$RGR = 100\ [(\ln Xt_2 - \ln Xt_1)/(t_2 - t_1)]$$

with t_2 – end of the NaCl treatment period, t_1 – start of the NaCl treatment period, Xt_2 – final plant biomass weight, Xt_1 – initial plant biomass weight.

Root and shoot dry weights were measured after drying the plant material at 70°C for 48–72 h. The value was calculated as dry weights of root/shoot.

The growth promoting effects of Si treatments were also evaluated by determination of rootstock and scion diameter and shoot length. Tolerance indices (TI) of rootstock and scion diameter and shoot height were determined according to Shetty et al. [1995] with some modifications and calculated as follows: TI = 100 (rootstock and scion diameter and shoot length of SNP + salt applied plant/ rootstock and scion diameter and shoot length of control plant).

Relative chlorophyll (SPAD) value was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co, Ltd, Osaka, Japan). Stomatal conductivity was conducted on the youngest fully expanded leaves on upper branches of the plants with leaf porometer.

The procedure of membrane permeability (electrolyte leakage) based on Lutts et al. [1996] was used to assess membrane permeability. Electrolyte leakage was measured using an electrical conductivity

(EC) meter. Mature leaves per plant were taken and cut into 1 cm segments. The leaf samples were then placed in individual stoppered vials containing 10 mL of distilled water after three washes with distilled water for removing surface contamination. These samples were incubated at room temperature (25°C) on a shaker (100 rpm) for 24 h. Electrical conductivity of bathing solution (EC_1) was measured after incubation. The same samples were then placed in an autoclave at 120°C for 20 min and the second measurement (EC_2) was taken after cooling solution to room temperature. The electrolyte leakage was calculated as EC_1/EC_2 and expressed as percent.

In order to determine leaf relative water content (LRWC), the leaves were collected from the young fully expanded leaves of three plants per replicate. The individual leaves first detached from the stem and then weighted to determine fresh weight (FW). In order to determine turgid weight (TW), the leaves were floated in the distilled water inside a closed petri dish. The leaf samples were weighted periodically, after gently wiping the water from the leaf surface with the tissue paper until a steady state was achieved. At the end of imbibition period, the leaf samples were placed in a preheated oven at 72°C for 48 h, in order to determine dry weight (DW). Values of FW, TW, and DW were used to calculate LRWC using the equation given below [Smart and Bingham 1974]:

$$LRWC(\%) = 100[(FW - DW)/(TW - DW)]$$

Table 1. Effect of $CaSiO_3$ on RGR and root : shoot dry weight

Treatments	RGR		Root : shoot dry weight	
	Mean ±s.d.	*V*	Mean ±s.d.	*V*
Control	4.11 ±0.13 a	0.031	0.490 ±0.11	0.224
Salt	3.88 ±0.10 b	0.025	0.622 ±0.05	0.080
0.5 mM $CaSiO_3$ + salt	3.99 ±0.01 ab	0.002	0.435 ±0.05	0.114
1 mM $CaSiO_3$ + salt	3.98 ±0.15 ab	0.037	0.545 ±0.16	0.293
2 mM $CaSiO_3$ + salt	3.99 ±0.04 ab	0.010	0.508 ±0.03	0.059

Means separation within column by Duncan's multiple range test, $P < 0.05$. Data are presented as mean, standard deviation (s.d.) and coefficient of variation (*V*)

Table 2. Effect of $CaSiO_3$ on rootstock and scion diameters and shoot length

Treatments	Rootstock diameter (mm)		Scion diameter (mm)		Shoot length (cm)	
	Mean ±s.d.	*V*	Mean ±s.d.	*V*	Mean ±s.d.	*V*
Control	20.99 ±2.86	0.136	17.47 ±2.52 a	0.144	44.29 ±5.58 a	0.125
Salt	18.90 ±0.78	0.041	13.17 ±0.32 b	0.024	32.73 ±2.78 b	0.084
0.5 mM $CaSiO_3$ + salt	19.96 ±1.78	0.089	14.40 ±0.92 b	0.063	33.15 ±9.42 b	0.284
1 mM $CaSiO_3$ + salt	19.62 ±2.20	0.112	13.79 ±0.22 b	0.015	34.76 ±1.70 ab	0.048
2 mM $CaSiO_3$ + salt	22.52 ±1.59	0.070	15.50 ±1.86 ab	0.120	42.60 ±5.23 ab	0.122

Means separation within column by Duncan's multiple range test, $P < 0.05$. Data are presented as mean, standard deviation (s.d.) and coefficient of variation (*V*)

Table 3. Effect of $CaSiO_3$ on TI of rootstock and scion diameters and shoot length

Treatments	Rootstock diameter TI		Scion diameter TI		Shoot length TI	
	Mean ±s.d.	*V*	Mean ±s.d.	*V*	Mean ±s.d.	*V*
Salt	90.01 ±3.71	0.041	75.40 ±1.87	0.024	73.90 ±6.27	0.084
0.5 mM $CaSiO_3$ + salt	95.08 ±8.48	0.089	82.46 ±5.26	0.063	74.84 ±21.27	0.284
1 mM $CaSiO_3$ + salt	93.44 ±10.50	0.112	78.93 ±1.29	0.016	78.47 ±3.84	0.048
2 mM $CaSiO_3$ + salt	107.28 ±7.60	0.070	88.74 ±10.69	0.120	96.21 ±11.82	0.122

Means separation within column by Duncan's multiple range test, $P < 0.05$. Data are presented as mean, standard deviation (s.d.) and coefficient of variation (*V*)

Table 4. Effect of $CaSiO_3$ on SPAD and LRWC

Treatments	SPAD		LRWC (%)	
	Mean ±s.d.	*V*	Mean ±s.d.	*V*
Control	48.68 ±1.65 ab	0.033	71.61 ±4.63 a	0.064
Salt	44.48 ±1.02 b	0.022	65.36 ±0.78 b	0.011
0.5 mM $CaSiO_3$ + salt	52.61 ±2.32 a	0.044	67.71 ±2.55 ab	0.037
1 mM $CaSiO_3$ + salt	52.50 ±3.37 a	0.064	67.98 ±0.87 ab	0.012
2 mM $CaSiO_3$ + salt	48.73 ±4.15 ab	0.085	64.01 ±3.54 ab	0.055

Means separation within column by Duncan's multiple range test, $P < 0.05$. Data are presented as mean, standard deviation (s.d.) and coefficient of variation (*V*)

Table 5. Effect of $CaSiO_3$ on membrane permeability and stomatal conductance

Treatments	Membrane permeability (%)		Stomatal conductance (mmol $m^{-2}s^{-1}$)	
	Mean ±s.d.	*V*	Mean ±s.d.	*V*
Control	28.14 ±3.68 b	0.130	345.50 ±78.1 a	0.226
Salt	48.43 ±3.80 a	0.078	175.06 ±20.7 b	0.118
0.5 mM $CaSiO_3$ + salt	21.45 ±2.39 b	0.111	205.36 ±46.2 b	0.224
1 mM $CaSiO_3$ + salt	25.82 ±4.47 b	0.173	246.93 ±62.0 ab	0.251
2 mM $CaSiO_3$ + salt	21.55 ±4.69 b	0.217	270.26 ±90.5 ab	0.334

Means separation within column by Duncan's multiple range test, $P < 0.05$. Data are presented as mean, standard deviation (s.d.) and coefficient of variation (*V*)

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

RESULTS

In the current study, the apple plants were exposed to moderate salinity at 35 mM for 4 months at the vegetative stage and relative damage to the indices of stress and the role of exogenously supplied Si were evaluated.

Exposure of apple plants to salt stress significantly decreased plant growth, while application of exogenous silicon significantly increased relative growth rate. With prolonged stress, salt treatment limited RGR and root : shoot dry weight and Si treatment provided better plant growth (Tab. 1). RGR was significantly affected with NaCl addition and the lowest value was found in salt plant (3.88). To estimate salt-induced relative inhibition on plant biomass, the ratio between dry weight of roots and shoots was calculated. There were no significant differences in the root : shoot dry weight between salt and Si treatment. 0.5 mM Si improved root : shoot dry weight under salinity condition. Moreover, salt decreased root : shoot dry weight by 27% compared to the control.

Si application alleviated salinity damages in salt plants while salt treatment decreased rootstock and scion diameter and shoot length compared to control (Tab. 2). 2 mM Si application provided higher rootstock and scion diameter and shoot length compared to control, while salt treatment remarkably caused decrease in the parameters. Similar with rootstock and scion diameter and shoot length, tolerance indices of the parameters were also significantly affected by Si applications (Tab. 3). 2 mM Si application had the highest values among applications. TI of shoot length was increased by 30% through 2 mM Si application compared to control.

Silicon treatments significantly influenced the physiology of salinity exposed apple plants (Tab. 4). Salt treatment caused sigfinicant recution in SPAD value. Moreover, Si treatments increased SPAD value even more than control plants. Si treatments affected significantly LRWC. The lowest values of membrane permeability were found in 0.5 and 2 mM Si treatments (21.45 and 21.55%, respectively) while salt had the highest value (48.43%). Furthermore, salt increased membrane permeability by 72% compared to the control. Salt exhibited a rapid decrease in stomatal conductivity by 49% compared to the control. The highest stomatal conductance was obtained from control (345.50 mmol $m^{-2} s^{-1}$) (Tab. 5).

DISCUSSION

Salinity is an important environmental stress factor that could influence the plant growth and physiological traits of plants [Fu et al. 2013, Zrig et al. 2016, Aras and Eşitken 2018]. In the current study, apple plants were treated by 35 mM NaCl solution at moderate salinity stress. Beside that, we treated the calcium silicate with different three doses in order to overcome the malignant effects of salt stress.

Leaf-tip necrosis can be used as an indicator of salt stress damages [Wahome et al. 2001]. In the present study, salt-stressed plants survived with slight leaf burn under 35 mM NaCl irrigation for four months. Salinity led to toxicity in the apple plant as evidenced by a remarkable reduction in growth. Si suppressed the reduction in plant growth of apple plant. Repression of plant growth is a consequence of salinity [Yin et al. 2010]. In the current study, the Si treatments decelerated RGR of plant biomass and root : shoot dry weight of the apple plants. The protective effect of Si on the plant growth under salinity was also reported by Zuccarini [2008]. De Lacerda et al. [2005] have reported that in sorghum plant, relative growth rates decreased by salinity. We did not find significant differences in root : shoot ratio between salinised plants treated with or without Si. However, root : shoot ratio determined at the end of the experimental period was considerably lower in salinised apple plants with Si. Current study showed that salt stress decreased relative growth rate and increased root : shoot ratio compared with control. Biomass partitioning between roots and shoots is influenced under stress conditions and root growth was less affected than shoot, so that the root : shoot ratio decreased in our study. In the plant growth model, carbon is supplied to the root from the shoot by phloem transport [Marschner et al. 1996]. In the current study, carbon may be allocated in roots and less transferred into shoots due to Si deposition in root cell walls. Dry weight distribution in apple plant between root and shoot can be an indicator in terms of salinity effect on plant tolerance. Furthermore, exogenous Si significantly mitigated the inhibition of apple rootstock and scion diameter and shoot lentgh induced by salt stress. Related with that, tolerance indices of these values were alleviated by Si applications. Moreover, salt treatment decreased scion diameter and shoot length by 25 and 26%, respectively compared to non-salted plants.

Chlorophyll biosynthesis is a very 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 Chl content may be due to an increase in Chl degradation and/or decrease in mineral acquisition needed for the Chl synthesis [El-Desouky and Atawia 1998]. In the current study, salinity decreased chlorophyll content (SPAD value) in apple plant. The highest contents of Chl were obtained by 0.5 and 1 mM Si treatments. Furthermore, Si treatment considerably increased chlorophyll content in apple leaves, whereas the treatment of Si increased it even further compared to the control as well as salt plants.

Reduction in LRWC due to salt stress has been stated in many papers [Massai et al. 2004, Erturk et al. 2007]. Some researchers suggest that silicate crystals deposition beneath the epidermal cells may reduce water loss through the cuticles [Trenholm et al. 2004, Romero-Aranda et al. 2006]. Moreover, reinforcement of the cell wall by silica deposition impedes excessive loss of water [Romero-Aranda et al. 2006]. In our study, LRWC dropped significantly with salinity and Si partially mitigated the drop in LRWC, indicating that the leaves of plants treated by Si were able to maintain turgor regardless of salt stress, consistent with previous findings for bean [Zuccarini 2008] and tomato [Haghighi and Pessarakli 2013].

Utilization of Si decreased membrane permeability under salt stress and this result was consistent with the findings of Zhu et al. [2004] research on cucumber. Exogenously applied Si had a protective effect on salt induced membrane damage. Under salinity, Si is deposited on cell walls and such deposition strengthen the cell membranes [Zhang et al. 2018]. Therefore, Si addition may have reenforced membranes of plant cells and prevented ion leakage from membranes. Si had anti-salt stress effect due to its membrane stabilizing property. Moreover, Si may have provided cell membranes against salinity, as Si competes with Na^+ for membrane binding spots. A protective effect of Si on relative membrane injury has been reported under salt stress [Tuna et al. 2008, Hashemi et al. 2010].

Stomatal conductance recorded on plants treated with NaCl was 49% lower than those recorded in control plants. The reduction in stomatal conductance is controlled by leaf water potential via turgor loss of guard cells [Savvas and Ntatsi 2015], thus Si treatment leads decrease in stomatal conductance and reduces transpiration via stomata presumably due to silica gel associated with the cellulose in the cell walls [Gong et al. 2003]. In our experiment, Si treatment improved water status and gas exchange for photosynthesis compared with salt treated plants. Furthermore, mitigation in plant growth may be attributed to improvement in leaf water status and higher stomatal conductance by Si treatment compared with salt plants. Similar results were noticed in previous studies [Zuccarini 2008, Ali et al. 2012].

CONCLUSION

Our research is the first to demonstrate that Si can mitigate the negative influence of salt stress in Malus. Salt stress limited plant growth and development by altering many physiological and biochemical processes. Reduction in stomatal conductance, LRWC and chlorophyll content are most likely responsible for reduction in plant growth. The promotion of plant growth by silicon under stress conditions may be attributed to reinforcement of the cell walls and membranes. Our findings showed that Si increased apple plant tolerance against salinity, which closely correlated with the increase in membrane stability.

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