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**Research Article** 

# Exogenous Silicon Dioxide Treatment in Carrot (*Daucus carota* L.) Under Salt Stress Conditions

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#### Abstract

TÜRK

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DERGİSİ

In this study, the effects of exogenous silicon dioxide applications (0.5, 1, 1.5 mM SiO<sub>2</sub>) on the germination parameters of carrot seeds were investigated under salt stress conditions (150, 200 mM NaCl). For this purpose, germination percentage (GP), mean germination time (MGT), germination rate coefficient (CVG), germination rate index (GRI), germination index (GI), seedling viability index (SVI), leaf number, shoot and root lengths (mm) and plant fresh weight (g) were determined. Germination parameters of yellow carrot, sensitive to salt stress, were adversely affected by 150 mM NaCl application and shoot and root lengths were reduced by 80%. Especially SiO<sub>2</sub> applications had a positive effect on germination parameters for the cultivar. In 150 mM NaCl application, the germination of the seeds treated with 0.5 mM SiO<sub>2</sub> increased approximately two times (23%), and 15% germination was obtained in 200 mM NaCl, which did not germinate in the control. Although the black carrot cultivar tolerated salt stress, silicon dioxide treatments had a stimulating effect on germination and development. It was determined that especially 1.5 mM SiO<sub>2</sub> application in black carrot seeds had a positive effect on germination percentage increased to 93% in seeds treated with 1.5 mM SiO<sub>2</sub> in 150 mM NaCl, and 86% at 200 mM NaCl concentration, reaching higher values compared to the control group. The results showed that pre-treatment with appropriate silicon concentrations significantly improved germination performance and promoted tolerance to the salt stress.

#### Key words: Carrot, Germination, Salt Stress, Silicon dioxide

# Tuz Stresi Koşullarında Havuçta (Daucus carota L.) Eksojen Silikon Dioksit Uygulamaları

Öz

Bu çalışmada, tuz stresi koşullarında (150, 200 mM NaCl) havuç tohumlarının çimlenme parametreleri üzerine dışarıdan silikon dioksit uygulamalarının (0.5, 1, 1.5 mM SiO<sub>2</sub>) etkileri araştırılmıştır. Bu amaçla çimlenme yüzdesi (ÇY), ortalama çimlenme süresi (OÇS), çimlenme oranı katsayısı (ÇOK), çimlenme hız indeksi (ÇHI), çimlenme indeksi (ÇI), fide güç indeksi (FGI), yaprak sayısı, sürgün ve kök uzunluğu (mm) ve bitki taze ağırlığı (g) belirlenmiştir. Tuz stresine duyarlı sarı havucun çimlenme parametreleri 150 mM NaCl uygulamasından olumsuz etkilenmiş, sürgün ve kök uzunlukları %80 oranında azalmıştır. Özellikle SiO<sub>2</sub> uygulamaları bu çeşit için çimlenme parametrelerini olumlu etkilemiştir. 150 mM NaCl uygulamasında 0,5 mM SiO<sub>2</sub> ile muamele edilen tohumların çimlenmesi yaklaşık iki kat (%23) artmış ve kontrol grubunda çimlenmenin gerçekleşmediği 200 mM NaCl'de %15 çimlenme elde edilmiştir. Siyah havuç çeşidi tuz stresini tolere etmesine rağmen, silikon dioksit uygulamalarının çimlenme ve gelişme üzerinde teşvik edici bir etkisi olmuştur. Siyah havuç tohumlarında özellikle 1.5 mM SiO<sub>2</sub> uygulamasının çimlenme parametrelerine olumlu etki yaptığı belirlenmiştir. 150 mM NaCl'de 1.5 mM SiO<sub>2</sub> ile muamele edilen tohumlarda çimlenme yüzdesi %93'e, 200 mM NaCl konsantrasyonunda ise %86'ya çıkarak kontrol grubuna göre daha yüksek değerlere ulaşmıştır. Sonuçlar, uygun SiO2 konsantrasyonları ile ön işlemin çimlenme performansını önemli ölçüde iyileştirdiğini ve tuz stresine toleransı desteklediğini göstermiştir.

Anahtar kelimeler: Çimlenme, Havuç, Silikon dioksit, Tuz Stresi.

## Introduction

The salinity tolerance levels of plants vary according to their developmental stages. For this reason, the tolerance levels of genotypes should be examined at every stage from germination to harvest. The germination stage is very important in that it constitutes the beginning of seedling development. Abiotic stress factors such as salt stress may cause longer germination time or even germination failure depending on the species and genotype. Alongside starting cultivation with tolerant genotypes, increasing seed germination or existing tolerance with exogenous applications is one of the methods that can be applied to deal with salt stress (Hubbard et al., 2012; Haghighi et al., 2012; Bolton and Simon, 2019; Shi et al., 2014). Carrot, a glycophytic plant, is one of the vegetable species that is sensitive to salt stress (Shannon and Grieve, 1998; Bolton and Simon, 2019). Bolton and Simon (2019) determined salt stress tolerance in 294 carrot accessions. According to the absolute decrease values based on parameters such as germination percentage, salt stress index, they stated that 150 mM NaCl application is suitable for screening the salt stress tolerance in carrots. Similarly, Jahan et al (2019) found that 100 mM and 150 mM NaCl concentrations significantly declined the vegetative parameters. In addition to the negative effects of salt stress on vegetative parameters, it was determined that the activities of peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) and total soluble proteins contents decreased in the edible part of carrots, so the nutritional quality was reduced (Bano et al., 2014). This is a very limiting effect in terms of carrot production, which is widely consumed all over the world due to its rich nutrient content.

Silicon, which has positive effects on plant growth and development, is also an element that provides tolerance to different abiotic stresses such as salinity, drought, and heavy metal stress (Ma and Yamaji, 2008). Despite its known benefits in agriculture, agricultural applications of silicon are not common. However, silicon is very important in terms of sustainable agriculture applications and is an alternative to conventional applications (Guntzer et al., 2012). In order to overcome the salt stress, which has a limiting effect on agricultural production, exogenous silicon application has been tried in different plant species and at different stages of development. It has been determined that exogenous silicon application in soybeans under salt stress reduces the negative effects caused by salt stress by regulating the relationship between antioxidants and nitric oxide scavengers. This effect was occurred by regulating the gene activities associated with reactive oxygen and nitrogen derivatives and by reducing the levels of enzymatic and non-enzymatic antioxidants (Chung et al., 2020). Parallel results were also reached in studies conducted on tomato (Al-Aghabary et al., 2005), barley (Liang et al., 2003), and grapevine rootstock (Soylemezoglu et al., 2009), and the negative effect of salt stress could be partially balanced. The effects of silicon dioxide on germination were mostly determined by using nano-silicon dioxide, but silicon dioxide applications were very limited. Silicon dioxide is known to positively affect the germination performance of the seeds of Glycyrrhiza uralensis (Zhang et al., 2015). The positive effects of SiO<sub>2</sub> application on rice germination and growth under drought stress conditions were found. It was also found that antioxidant enzyme activities increased 2-fold compared to control plants (Ali et al., 2021). There is no study in the literature regarding external applications of silicon dioxide in carrots. In this study, the effects of silicon dioxide on germination performance under salt stress conditions were determined in two different varieties of carrot, which is a glycophytic plant.

## Materials and Methods

This research was carried out in the laboratory of Akdeniz University Vocational School of Technical Sciences, Department of Plant and Animal Production in 2021. Two different carrot cultivars (black, yellow) supplied from a commercial company were used as plant material in the research. For surface sterilization, the seeds were kept in 10% sodium hypochlorite solution for 10 minutes and then rinsed with sterile distilled water 3 times for 5 minutes. In order to determine the effects of silicon dioxide (SiO<sub>2</sub>) on germination under salt stress conditions, seeds were kept in solutions prepared at 0.5, 1 and 1.5 mM SiO<sub>2</sub> concentrations for 6 hours in dark condition. Then it was placed on double-layered sterile blotting papers placed on petri plates (90 x 15 mm). The seeds were exposed to salt stress by watering with 150 mM NaCl, which was determined as the threshold value in a previous study (Nasircilar et al., 2019), and 200 mM NaCl concentrations to

evaluate the effect of more severe salt stress. Control groups were formed by keeping them in distilled water. Experiments were conducted in 4 repetitions and 25 seeds were placed in petri dishes per treatment. The petri plates were maintained to germinate under 24±1ºC temperature and dark conditions. The number of germinated seeds in the days following the experiment was recorded daily and at the end of the experiment, germination percentage (GP), mean germination time (MGT), coefficient of velocity of germination (CVG), germination rate index (GRI), germination index (GI), seedling vigor index (SVI) were calculated. Germination tests were completed in 14 days by applying ISTA rules (ISTA 1985).

GP (%) = Number of germinated seeds / Total number of seeds X 100 (Gosh et al. 2014)

MGT:  $\Sigma$  Dn /  $\Sigma$ n D = days counted from the beginning of the test, n = number of seeds germinated on day D (Ellis and Roberts, 1981; Sivritepe, 2012).

CVG: N1+N2+....+Nx/100xN1T1+.....+NxTx (Kotowski, 1926)

T: number of days corresponding to N, N: number of seed germinated each day

GRI (%/day): G1/1+G2/2+.....+Gx/x (Esechie, 1994)

G1: Germination percentage x100 at the first day after sowing, G2: Germination percentage x100 at the second day after sowing, GI:  $(14xn1) + (13xn2) + \dots + (1xn14)$ 

n1, n2,..., n14: number of germinated seeds on the first, second and subsequent days until the 14th; 14, 13...., and 1 are weights given to the number of germinted seeds on the first, second and subsequent days respectively (modified from Benech et al., 1991). SVI = [Seedling length (cm) x GP (%)] (Baki and Anderson, 1973)

The emergence of the radicle (1mm) from the testa was taken as a basis for germination, and after germination, the experiments were continued in the same temperature plant growth room with a light/dark photoperiod of 16/8 hours. During the experiment, 3 ml irrigation was done each time considering the drying condition of the filter papers. Trials for vegetative parameters were continued for 21 days, and at the end of this period, shoot length (cm), root length (cm), plant fresh weight (g) were measured and the number of leaves was determined.

The obtained data were evaluated to statistical analysis with the MINITAB 17 package program, vegetative growth parameters were evaluated by analysis of variance, and differences were determined by Tukey test. The evaluation and comparison of the confidence intervals of the mean of the data of germination parameters were made with Interval Plot (Minitab).

#### **Results and Discussion**

Germination, which is the first stage of plant growing, is adversely affected by abiotic stress factors, including salt stress. Therefore, in this study, the effects of silicon dioxide pre-treatments were investigated to reduce the effects of salt stress on the carrot which is a glycophytic plant (Bolton and Simone, 2019) during the germination period. The variance analysis results regarding the number of leaves, shoot length, root length and plant fresh weight were given in Table 1. As seen, sources of variation on shoot length, root length, plant weight and leaf number were found statistically significant at 0.01 level (Table 1). Only the effect of the cultivar on the leaf number was 0.05 determined to be significant level

Variation source	df	Leaf number	Shoot length (mm)	Root length (mm)	Plant weight (g)				
Cultivar (C)	1	*	**	**	**				
Salt (S)	2	**	**	**	**				
SiO <sub>2</sub>	3	**	**	**	**				
CxS	2	**	**	**	**				
Cx SiO <sub>2</sub>	3	**	**	**	**				
Sx SiO2	5	**	**	**	**				
CxSx SiO <sub>2</sub>	11	**	**	**	**				

Table 1. Variance analysis result with respect to the number of leaves, shoot length, root length and plant fresh weight of carrot cultivars

Distinct letters in the row indicate significant differences according to Tukey's test (p < 0.05). \*, \*\*Significant at p < 0.05 and p < 0.01, respectively. ns: Nonsignificant. df: degrees of freedom

Table 2 was represented the effects of salt stress and  $SiO_2$  applications on the number of leaves, shoot and root length, and plant weight of carrot cultivars. It was determined that the Yellow

cultivar, which did not germinate at 200 mM NaCl, was more sensitive to salt stress. Germination occurred in the seeds treated with  $SiO_2$  at 200 mM NaCl, and the plants developed tolerance to salt

stress. It was determined that 0.5 and 1 mM SiO<sub>2</sub> application results gave approximately the same results as the control group, and 1 mM  $SiO_{\rm 2}$ application gave better results than the control group in terms of shoot length (21.43 mm) and plant weight (0.0963 g). When the results of plants exposed to salt stress were compared, SiO<sub>2</sub> applications at 150 mM NaCl were included in the same statistical group in terms of leaf number, shoot and root length, on the other hand, it was determined that plant fresh weights increased with SiO2 application and 1.5mM SiO2 gave the best results (0.0945 g). Under more severe salt stress conditions (200 mM NaCl), the best results were obtained in the 0.5 mM SiO<sub>2</sub> treated group. The fact that the results obtained are in the same

statistical group as the control group or have reached very close values shows that 0.5 mM SiO<sub>2</sub> application at 200 mM salt stress is quite effective.

In the Black cultivar, the control group was in the same statistical group with 150 and 200 mM NaCl applications, which shows that this cultivar is quite tolerant to salt stress. In addition, SiO<sub>2</sub> applications also increased the development of plants. The highest growth values under salt stress conditions were at both 150 mM (leaf number: 6, shoot length: 40.38 mm, root length: 79.00 mm, plant fresh weight: 0.1195 g) and 200 mM (leaf number: 6, shoots length: 39.80 mm, root length: 77.88 mm, plant fresh weight: 1.1218 g) was obtained in 1.5 mM SiO<sub>2</sub> treatment.

Table 2. Effect of salt stress and SiO<sub>2</sub> on the number of leaves, shoot and root length, and plant fresh weight of carrot cultivars.

Cultivar	Treatment	Leaf	Shoot length	Root length	Plant fresh
		ner plant	(IIIII)	(IIIII)	(g/nlant)
Yellow	Control	6 a	20.05 ab	79.78 a	0.0795  bc
	150mM NaCl	2 b	5.25 c	18.58 b	0.0770 c
	200mM NaCl	0	0.00	0.00	0.0000
	0.5 mM Si	6 a	21.08 ab	80.50 a	0.0890 abc
	0.5mM Si+ 150mM NaCl	2 b	5.43 c	18.70 b	0.0888 abc
	0.5mM Si+ 200mM NaCl	6 a	20.38 ab	80.68 a	0.0853 abc
	1 mM Si	6 a	21.43 a	81.43 a	0.0963 a
	1mM Si+150mM NaCl	2 b	5.10 c	18.98 b	0.0910 abc
	1mM Si+ 200mM NaCl	0	0.00	0.00	0.0000
	1.5 mM Si	2 b	5.43 c	19.25 b	0.0920 abc
	1.5mM Si+150mM NaCl	2 b	5.58 c	19.28 b	0.0945 ab
	1.5mM Si+200mM NaCl	2 b	4.50 c	14.48 b	0.0383 d
Black	Control	2 c	16.13 c	46.48 b	0.0723 b
	150mM NaCl	2 c	16.00 c	45.85 b	0.0710 b
	200mM NaCl	2 c	16.23 c	46.60 b	0.0680 b
	0.5 mM Si	6 a	41.18 a	78.25 a	0.1120 a
	0.5mM Si+150mM NaCl	4 b	19.58 b	79.15 a	0.8075 b
	0.5mM Si+ 200mM NaCl	4 b	10.13 d	29.75 с	0.0700 b
	1 mM Si	4.5 b	39.85 a	81.08 a	0.1070 a
	1mM Si+150mM NaCl	2 c	16.28 bc	45.73 b	0.0750 b
	1mM Si+ 200mM NaCl	2 c	15.30 c	46.23 b	0.0648 b
	1.5 mM Si	4 b	39.90 a	78.90 a	0.1188 a
	1.5mM Si+150mM NaCl	6 а	40.38 a	79.00 a	0.1195 a
	1.5mM Si+200mM NaCl	6 а	39.80 a	77.88 a	1.1218 a

Distinct letters in the row indicate significant differences according to Tukey's test ( $p \le 0.05$ ).

When the data in Table 2 were examined, it was determined that the values obtained as a result of SiO<sub>2</sub> applications were higher than the control and that the SiO<sub>2</sub> application stimulated plant growth in the severe salt stress conditions of the Black cultivar. The results obtained in maize (Sun et al., 2021) revealed that SiO<sub>2</sub> can have positive effects on some parameters that may negatively affect both germination and seedling growth such as breaking seed dormancy, restoring seed viability, and improving storage damage. The curative effect of SiO<sub>2</sub> on plant growth under plant salt stress conditions in soybean (Lee et al., 2010) was explained by increasing endogenous gibberellin levels and decreasing abscisic acid and proline levels as a result of SiO<sub>2</sub> applications. In addition, it has been determined that exogenous SiO<sub>2</sub> applications have an amelioration effect on plant development as a result of salt stress by increasing antioxidant enzyme activities and chlorophyll content in tomato (Al-aghabary et al., 2005), and by regulating photosynthetic parameters such as chlorophyll content, transpiration rate, and stomatal conductivity in two legume cultivars (Murillo-Amador et al., 2007).

Studies on salt stress tolerance of carrots during germination are very limited and increasing salt concentrations result in a decrease in both the total number of germinated seeds and the germination rate (Bolton and Simone, 2019). In Figure 1 and 2, the GP, MTG, CVG, GRI, GI, and SVI of carrot varieties treated with salt stress and SiO<sub>2</sub> are presented as an interval plot. The highest germination percentage in both cultivars was obtained from the control group (yellow: 73%; black: 78%), and this rate increased with SiO<sub>2</sub> treatment in the black cultivar (97% at 0.5 mM SiO<sub>2</sub>). Salt stress caused a decrease in germination percentages. Any germination could not be obtained in the yellow variety at 200 mM, while this rate was 48% in the black variety at the same concentration. The increasing effect of SiO<sub>2</sub> treatments on germination percentages differed according to the cultivars. 0.5 mM SiO<sub>2</sub> application for yellow cultivar and 1.5 mM for black cultivar gave the best results at 150 mM salt concentration and 23% and 93% germination rates were obtained respectively. 1.5 mM SiO<sub>2</sub> application had a stimulating effect on germination for both cultivars at 200 mM salt concentration and germination rates were 15% in yellow and 86% in black cultivar. It was determined that the MGT value decreased

considerably as a result of SiO<sub>2</sub> applications in saline conditions. Especially in the presence of 200 mM NaCl, the MGT value was determined as 2.25 in yellow carrots and 7.22 in black carrots after 1.5mM SiO<sub>2</sub> application. It was found that 1.5 mM SiO2 application increased the CVG value in black carrot cultivar (38.73 in 150 mM, 33.54 in 200 mM NaCl). The GRI value, which reflects the germination percentage, increased with Si application. The application of 0.5 mM SiO<sub>2</sub> for the yellow cultivar and 1.5 mM SiO<sub>2</sub> for the black cultivar (93.00 at 150 mM NaCl, 86.00 at 200 mM NaCl) came forward. GI values, which are an indicator of germination rate and percentage, were also positively affected by SiO<sub>2</sub> treatment. While the results differed according to the severity of salt stress in the yellow cultivar, 1.5 mM SiO<sub>2</sub> (182.50 for 150 mM NaCl; 167.25 for 200 mM NaCl) was found to be effective in the black cultivar. SVI values, which decreased under salt stress, increased in SiO<sub>2</sub> applied groups. 0.5 mM SiO<sub>2</sub> (5.53, 150 mM NaCl; 15.25, 200 mM NaCl) in vellow cv and 1.5 mM SiO<sub>2</sub> application in black cv (111.05 for 150 mM NaCl; 101.18 for 200 mM NaCl) gave better results.

In maize (Sun et al., 2021), SiO<sub>2</sub> had an improving effect on the germination rate, germination potential, germination index, and vigor index, which are important germination parameters for all plants. Pre-treatment with SiO<sub>2</sub> significantly increased germination of rice under drought stress conditions. The applied SiO<sub>2</sub> had a stimulating effect on germination by regulating antioxidant enzyme activities, total soluble sugar and proline content (Ali et al., 2021). Similarly, it was stated that the application of SiO<sub>2</sub> in tomato (Shi et al., 2014) and lettuce (Alves et al., 2020) improved germination depending on the antioxidant enzyme activity. Supporting the results of this study, it was determined that SiO<sub>2</sub> applications promoted germination in wheat (Feghhenabi et al., 2020), maize (Khan et al., 2015) and cucumber (Gou et al., 2020) under salt stress conditions. In addition, studies on arsenic stress (Zia et al., 2017) and drought stress (Ali et al., 2021) are important in terms of showing the positive effect of silicon on germination under different stress conditions.



Figure 1. Interval plots of the effect of salt stress and  $SiO_2$  applications on germination parameters (GP, MGT, CVG, GRI, GI, SVI) of Yellow carrot cultivar.



Figure 2. Interval plots of the effect of salt stress and  $SiO_2$  applications on germination parameters (GP, MGT, CVG, GRI, GI, SVI) of Black carrot cultivar

## Conclusion

External application of SiO<sub>2</sub> had a positive effect on the germination parameters of the carrot, which is a glycophytic plant. It was determined that SiO<sub>2</sub> application increased germination percentage, decreased germination time and increased seedling strength depending on genotype. In the yellow cultivar, although no germination occurred in the control group at 200 mM NaCl, germination was also obtained at this concentration as a result of SiO<sub>2</sub> application. The application of 1.5 mM SiO<sub>2</sub> was effective on the germination parameters of the black carrot cultivar, which is more tolerant to salt stress. As a result, it has been revealed that the application of SiO<sub>2</sub> as a pre-treatment helps to alleviate the negative effects of salt stress in carrots. This study, in which exogenous SiO<sub>2</sub> application is made in order to develop tolerance to salt stress in carrots, is expected to be a resource for researchers who will work on this subject.

**Conflict of Interest Statement:** The authors of the article declare that there is no conflict of interest between them.

**Researchers' Contribution Rate Statement:** The authors declare that they have contributed equally to the article.

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