



## Assessment of The Effects of Silicon Application on Growth Parameters and Some Bioactive Components in Hungary vetch (*Vicia pannonica* Crantz)

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### ABSTRACT

The objective of this study was to investigate the effect of 10 mM silicon (Si) application on salt stress (50, 100, and 200 mM NaCl) tolerance in Hungarian vetch seedlings (Ege Beyazi-79) based on growth parameters (shoot length and fresh weight, organic dry matter, and moisture), bioactive compounds as chlorophyll, carotenoid, proline, protein, nitrate, and nutrient status. Silicon was applied to the seedlings from the leaves and NaCl from the salt soil, both of which were dissolved in Hoagland solution. The results revealed that the highest shoot length Si+100 mM NaCl was recorded and the highest fresh and dry weight was recorded with length Si+50 mM NaCl. The highest dry matter was obtained from Si and 200 mM NaCl. The highest chlorophyll, carotenoid, proline, protein, and nitrate were obtained from 100 mM NaCl+Si, while the lowest chlorophyll, carotenoid, and nitrate were obtained from 200 mM NaCl and the lowest proline and protein from 100 mM NaCl, respectively. The amount of K, Mg, and S were low in NaCl doses but high in Si and Si + NaCl doses, and P was higher in all groups compared to the control. The content of Na, Co, and Se were high in all groups except Cl 50 NaCl, while Mn, Zn, and Ni were higher in Si and Si + NaCl doses. Silicon content of samples was high at higher NaCl + Si doses. The results provided by this investigating indicated that silicate concentrations positively affected the parameters examined in Hungarian seedlings, and increased the tolerance of seedlings to salt stress.

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## Introduction

Salinity is one of the most significant problems which reduce the yield and quality of agricultural products. It has been reported that 20% of the total areas and 33% of irrigated agricultural lands are on saline-alkali soil in all parts of the world. Even more, if no measures are taken, this rate is estimated to reach 50% by 2050 (Fidan and Ekinci, 2020). Salt stress prevents some physiological processes such as water and minerals uptake from the soil (Chen et al. 2016), stomatal movements, photosynthesis metabolism, synthesis of pigments (Fidan and Ekinci, 2017) nitrogenous compounds (Conceição et al., 2019), and carbohydrates (Kuşvuran, 2015) by disturbing the physical and chemical properties of root cells, all of which result in reduced growth and development and lost yield (Khan et al., 2014). This situation leads to nutritional deficiency in ruminants, one of the most significant employment resources of our country, as well as an economic loss since it decreases productivity (Kılıçalp et al., 2020; Saçlı, 2020). Although the sowing rate of feed plants has reached up to 8% with the supports implemented

in Turkey since the 2000s, it is far from meeting the quality roughage deficit. For this reason, it is significant to select feed sources that can be produced at low costs and do not disturb the digestive activities of animals and to increase their quantities to a sufficient level (Kılıçalp et al., 2020; Yılmaz et al., 2020). Hungarian vetch (*Vicia pannonica* Crantz) is the only annual fodder plant that can grow in every region of Turkey without having any damage even in the harshest winter cold, and which is preferred as the main product or second product in production (Kılıçalp et al., 2020). Its high protein content makes it to be among the products sought as roughage and silage in terms of animal nutrition. Furthermore, it is employed as a green fertilizer in agriculture inasmuch as it enriches the nitrogen and organic matter content of the soil. Bovine and ovine breeding is quite common in Kastamonu province and its surroundings. Climatic characteristics of Kastamonu are suitable for forage crop cultivation in Kastamonu's agricultural lands (Karakule and Tüzemen, 2020). High amounts of rainfall, high humidity and minimum summer-

winter temperature changes provide optimal conditions for Hungarian vetch production. Much as animal breeding is very common in the Kastamonu region, the number of studies on the production of food resources, which have an important place in animal nutrition, the basic issues faced in production and the solution of the issues is almost non-existent. In this study, the effect of silicon application on tolerance to salt stress was investigated for the Ege Beyazı-79 variety.

## Material and Methods

### Experimental Design

This study was carried out between 20 November 2020 and 20 March 2021. The seeds of Ege Beyazı-79 were sterilized with % sodium hypochlorite for 10 min and cleaned four times thoroughly with distilled water. The sterilized seeds were planted in plastic trays (outer size 51 x 32 cm, rim diameter 7 cm and depth 7 cm) containing peat and (3: 1) perlite which had 4 rows with 7 cells on each row. 4-5 seeds were sown in each cell and one tray was used for each application group.

As seen in the Table 1, the soil pH was 6.24. Soil N, K and P (macroelements) concentrations were 14%, 10%, and 18% respectively, while, soil Fe, Mn and Zn concentrations (microelements) were 2195 mg kg<sup>-1</sup>, 38 mg kg<sup>-1</sup> and 32 mg kg<sup>-1</sup>, respectively. As for the applications to the seedlings, 10 mM silicon (Si: NaSiO<sub>3</sub>) and salt concentrations (50, 100, and 200 mM NaCl) were separately dissolved in Hoagland nutrient solution (Hoagland and Arnon, 1950). Silicon solution was applied to the leaves and NaCl concentrations to the soil and 10 ml were applied to the seedlings at the 4-5 leaf stage. The control group seedlings were only irrigated with nutrient solution. The nutrient solution consisted of 2.5 mM NO<sub>3</sub><sup>-</sup>, 0.5 mM NH<sub>4</sub><sup>+</sup>, 2 mM K<sup>+</sup>, 1 mM Ca<sub>2</sub><sup>+</sup>, 0.5 mM Mg<sub>2</sub><sup>+</sup>, 0.05 mM Fe-EDTA, 5 mM Mn<sup>2+</sup>, 0.5 mM Zn<sup>2+</sup>, 0.5 mM Cu<sub>2</sub><sup>+</sup>, 1 mM Cl, 0.55 mM SO<sub>4</sub><sup>2-</sup>, 0.5 mM PO<sub>4</sub><sup>3-</sup>, 1.5 mM BO<sub>3</sub>, 0.1 mM MoO<sub>4</sub>. The applications were made twice a week for four weeks. After the four weeks, 15 seedlings were randomly selected from each treatment group for seedling length measurements and their heights were measured by a ruler. The seedlings' fresh weight was determined by weighing on a precision scale. 6 seedlings (10 replicates) selected randomly from each group were used to determine the total dry matter and moisture content in the plant samples. Subsequently, the seedlings were cut into small pieces and fresh weights were recorded by a digital scale. Then the samples were dried in a 105 ± 2°C oven for 4 hours until they reached constant weight. The quantity of dry matter (%) in the samples was determined by calculating from the dry weight loss (James, 1995). Moisture content was determined based on the dry weight and total weight values of the samples, according to the wet base (Işık et al., 2000; Cemeroglu, 2013).

### Chemical Analyses

To determine the chlorophyll content, 0.5 g of the fresh leaf was crushed in liquid nitrogen and homogenized by adding 10 ml of 80% acetone in an ice bath (Lichtenthaler and Wellburn, 1983). After, the mixture was centrifuged for 10 minutes at 3.000 rpm, and triplicate spectrophotometric (Shimadzu UV-260) readings of the

supernatants noted were obtained at values of 652 and 450. The proline level of samples was estimated according to Bates et al. (1973), and the Bradford method (1976) was used to measure the soluble protein content. The amount of nitrate was determined using the rapid colourimetric method according to Cataldo et al. (1975).

### Element Analysis

Powdered samples were used to determine the mineral content of the samples. After the length and weight measurements, the seedling samples, which were dried in a 65°C oven until they reached a constant weight, were pulverized and sent to the Kastamonu University Central Research Laboratory for elemental analysis.

### Statistical Analysis

Analysis of variance (ANOVA) was applied for analysing the differences in some bioactive compounds and nutrients of Hungarian vetch samples using the SPSS program ver. 11.0 for Windows. Following the results of ANOVAs, Tukey's honestly significance difference (HSD) test ( $\alpha = 0.05$ ) was used for testing differences between group means.

## Results and Discussion

The growth parameters, the quantities of some bioactive chemical components and nutrients showed a significant variation depending on the concentration and application type ( $P < 0.001$ ) in this study, in which the effect of Si (10 mM) application on salt stress (50, 100 and 200 mM NaCl) tolerance in Hungarian vetch under salt stress was investigated.

### Variation of Some Growth Parameters in Hungarian vetch seedlings

The effects of Si on growth parameters in common Hungarian vetch under salt stress are shown in Table 2. The differences between the applications in terms of seedling length, seedling fresh weight and dry matter quantity were seen to be significant at the level of  $P < 0.001$ , whereas no significant difference was noted in terms of moisture content in the seedlings ( $P < 0.6$ ). The seedling lengths varied between 21.80 cm and 27.67 cm and the highest seedling length was recorded for 100 mM NaCl + Si (27.67 cm) and 200 mM NaCl + Si (27.20 cm) applications compared to the control (23.6 cm) (Table 2).

For these concentrations, the length of the seedlings was 16.90% and 14.93% higher than the control. The fresh weight increased compared to the control as the concentration increased in the other application groups except for 50 mM NaCl. The highest fresh weight was 50 mM NaCl + Si (41.12%), 100 mM NaCl + Si (37.70%), 200 mM NaCl + Si (29.29%) and 50 mM NaCl (19%) and the lowest fresh weight was 50 mM NaCl (2.18%) was determined (Table 2). Salt applications decreased the quantity of organic dry matter in the samples compared to the control with 50 mM NaCl, 100 mM NaCl + Si and 200 mM NaCl + Si applications. The highest dry matter was observed at 11.23% with 10 mM Si application and the lowest dry matter at 59.03 % with 50 mM NaCl application (Table 2). The applications did not have a significant effect on the moisture content of the samples. However,

compared to the control, the lowest moisture content was determined in the application of 50 mM NaCl, whereas the highest moisture content was recorded with 200 mM NaCl + Si (Table 2). The results are in agreement with other studies, all of which revealed that when Si is applied to plants, the damages caused by salt stress are eliminated and the resistance of plants to salty increases. Torabi et al. (2015), Wu et al. (2017) and Aras (2020) determined that salt stress reduced leaf height, shoot length, fresh/dry weight, and leaf water content in wheat, in the stevia plant, in the borage and the common sainfoin under salty conditions and the apple, respectively, whereas these negativities decreased in Si + NaCl. Most researchers have suggested that Si strengthens the wall structure in the leaves by the building up on the walls under the cutin layer and also plays an important role in the functioning of turgor and osmosis events in the leaf (Abbas et al., 2015; Adelaal et al., 2020). Thus, shoot growth, fresh/dry weight are stimulated, water content is controlled and yield increases (Chen et al., 2016; Adelaal et al., 2020). Much as the water content in the seedlings did not show a significant change, the decrease in salt concentrations compared to the control and the increase in Si+NaCl confirmed this result (Adelaal et al., 2020; Cui et al., 2021). However, fresh/dry weight, root/stem length, quality and yield are closely related to the ratio of photosynthetic pigments and nitrogenous compounds in plants (Markovich et al., 2017; Adelaal et al., 2020).

### Variation of Some Bioactive Compounds in Hungarian Vetch Seedlings

Chlorophylls and carotenoids are essential compounds in photosynthesis (Adelaal et al., 2020), and nitrogenous compounds are the basic building blocks of essential compounds for growth and development such as amino acids, proteins, enzymes, and chlorophyll (Mao et al., 2015). The effects of Si application on total chlorophyll, total carotenoid, proline, total soluble protein and nitrate quantities in Hungarian vetches under salt stress are provided in Figure 1, Figure 2, Figure 3 and Figure 4, respectively. The applications according to the data led to statistically significant differences in the quantity of measured values (P<0.001).

Total chlorophyll, proline and total soluble protein quantity decreased with the 100 and 200 mM NaCl applications compared to the control and other groups. The highest chlorophyll content was recorded with the 100 mM NaCl + Si and 50 mM NaCl + Si (0.327 mg-0.317 mg) applications, respectively, while the lowest chlorophyll content was detected with the 0.241 mg and 200 mM NaCl applications (Figure 1). The carotenoid content did not differ significantly between the groups, while the highest carotenoid was determined with the 100 mM NaCl + Si application and the lowest value was determined with the 200 mM NaCl application (Figure 2).

Table 1. Soil properties used in the trial setup (in 1.2 kg m<sup>3</sup>)

	pH	uS/CM		%			mg kg <sup>-1</sup>			
		Conductivity	Organic dry matter	Moisture	N	K	P	Fe	Mn	Zn
Imported peat	6.24	270 - 330	96-99	33-35	14	10	18	2195	38	32

Table 2. Variation of some growth parameters in Hungarian vetch seedlings treated with NaCl and Si.

	Treatment	Length (cm)	Fresh weight (g)	Dry matter (%)	Moisture (%)
1	Control	23.67±0.42 <sup>b*</sup>	0.214±0.010 <sup>a</sup>	10.18±0.7 <sup>b4</sup>	89.82±0.74 <sup>b</sup>
2	50 mM NaCl	24.87±0.62 <sup>b</sup>	0.210±0.014 <sup>a</sup>	9.03±0.32 <sup>a</sup>	84.67±0.77 <sup>a</sup>
3	100 mM NaCl	25.27±0.77 <sup>b</sup>	0.236±0.011 <sup>b</sup>	10.46±0.67 <sup>b</sup>	89.55±0.67 <sup>b</sup>
4	200 Mm NaCl	21.80±0.41 <sup>a</sup>	0.240±0.012 <sup>b</sup>	10.53±0.49 <sup>c</sup>	89.47±0.49 <sup>b</sup>
5	10 mM Si	24.67±0.94 <sup>b</sup>	0.255±0.012 <sup>c</sup>	11.23±0.31 <sup>c</sup>	88.79±0.31 <sup>b</sup>
6	50 mM NaCl+Si	26.40±0.82 <sup>c</sup>	0.302±0.015 <sup>d</sup>	10.32±0.27 <sup>b</sup>	89.69±0.27 <sup>b</sup>
7	100 mM NaCl+Si	27.67±0.67 <sup>c</sup>	0.295±0.011 <sup>d</sup>	9.40±0.38 <sup>a</sup>	90.60±0.38 <sup>b</sup>
8	200 mM NaCl +Si	27.20±0.70	0.277±0.014 <sup>d</sup>	9.23±0.10 <sup>a</sup>	90.78±0.10 <sup>b</sup>
F.		7.683	6.735	2.769	0.787
Sig.		<0.001	<0.001	<0.011	<0.599

\*: Means within a group that has a different small letter are significantly different from each other (P<0.01).

Table 3. Variation of the amount of K, Mg, Ca, P, S, Na and Cl in Hungarian vetch seedlings treated with salt and silicon (mg kg<sup>-1</sup>).

	K	Mg	Ca	P	S	Na	Cl
1	44840±40 <sup>d*</sup>	3074±47 <sup>b</sup>	15160±20 <sup>d</sup>	8872±10 <sup>a</sup>	7314±7 <sup>d</sup>	6100±390 <sup>a</sup>	12360±10 <sup>b</sup>
2	43820±40 <sup>c</sup>	3463±49 <sup>e</sup>	12720±20 <sup>a</sup>	9805±11 <sup>d</sup>	7516±7 <sup>f</sup>	8600±400 <sup>b</sup>	12030±10 <sup>a</sup>
3	41440±40 <sup>b</sup>	3020±54 <sup>b</sup>	14620±20 <sup>c</sup>	9061±11 <sup>c</sup>	6733±7 <sup>a</sup>	18880±490 <sup>d</sup>	21230±10 <sup>e</sup>
4	37730±40 <sup>a</sup>	2562±58 <sup>a</sup>	14340±20 <sup>c</sup>	9245±12 <sup>e</sup>	7205±8 <sup>c</sup>	20529±580 <sup>e</sup>	23890±20 <sup>g</sup>
5	44820±40 <sup>d</sup>	3140±54 <sup>c</sup>	14270±20 <sup>c</sup>	8988±11 <sup>b</sup>	7060±8 <sup>b</sup>	18910±510 <sup>d</sup>	22900±20 <sup>f</sup>
6	45620±40 <sup>e</sup>	3290±51 <sup>d</sup>	13870±20 <sup>b</sup>	9920±11 <sup>f</sup>	7487±7 <sup>e</sup>	14110±450 <sup>c</sup>	16950±10 <sup>c</sup>
7	46300±40 <sup>f</sup>	3453±51 <sup>e</sup>	15220±20 <sup>d</sup>	10590±10 <sup>g</sup>	7963±8 <sup>g</sup>	14990±460 <sup>c</sup>	17410±10 <sup>d</sup>
8	49070±50 <sup>g</sup>	3655±78 <sup>f</sup>	15282±30 <sup>e</sup>	20140±20 <sup>h</sup>	15940±20 <sup>h</sup>	24380±630 <sup>f</sup>	25110±10 <sup>h</sup>
F	24326614	202057	28726614	38785239	25080148	17.9	53207741
Sig.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

\*: Means within a group that has a different small letter are significantly different from each other (P <0.01). 1: Control; 2:50 mM NaCl; 3: 100 mM NaCl; 4: 200 mM NaCl; 5: 10 mM Si; 6: 50 mM NaCl+ Si; 7: 100 mM NaCl + Si; 8: 200 mM NaCl + Si

Table 4. Variation of the amount of Al, Si, Mn, Fe, Co, N Cu, and Zn in Hungarian vetch seedlings treated with salt and silicon (mg kg<sup>-1</sup>).

	Al	Si	Mn	Fe	Co	Ni	Cu	Zn
1	593.3±7.6 <sup>f*</sup>	1627±7 <sup>f</sup>	59.1±0.5 <sup>c</sup>	239.3±1.4 <sup>h</sup>	35.8±3.2 <sup>a</sup>	52.9±0.6 <sup>b</sup>	17.7±0.3 <sup>b</sup>	90.5±0.4 <sup>c</sup>
2	541.3±7.3 <sup>e</sup>	1514±7 <sup>e</sup>	46.2±0.5 <sup>a</sup>	179.2±1.1 <sup>e</sup>	39.2±3.1 <sup>b</sup>	51.5±0.6 <sup>a</sup>	14.6±0.3 <sup>a</sup>	78.2±0.4 <sup>a</sup>
3	383.8±7.1 <sup>b</sup>	642.3±6.1 <sup>a</sup>	57.7±0.5 <sup>c</sup>	154.2±1.1 <sup>c</sup>	40.9±3.2 <sup>bd</sup>	51.6±0.6 <sup>a</sup>	15.6±0.3 <sup>a</sup>	81.6±0.4 <sup>b</sup>
4	495.4±9.1 <sup>d</sup>	790.8±7.2 <sup>b</sup>	54.1±0.4 <sup>b</sup>	167.6±1.1 <sup>d</sup>	43.3±3.3	50.9±0.7 <sup>a</sup>	15.3±0.3 <sup>a</sup>	81.5±0.4 <sup>b</sup>
5	465.5±8.4 <sup>c</sup>	1085±7 <sup>d</sup>	59.6±0.6 <sup>c</sup>	122.7±0.9 <sup>a</sup>	43.4±3.4 <sup>d</sup>	61.9±0.6 <sup>c</sup>	15.5±0.4 <sup>a</sup>	92.5±0.5 <sup>d</sup>
6	313.7±5.8 <sup>a</sup>	923.8±6.4 <sup>c</sup>	60.2±0.6 <sup>d</sup>	126.1±0.9 <sup>b</sup>	37.5±3.4 <sup>b</sup>	63.4±0.7 <sup>d</sup>	17.9±0.4 <sup>b</sup>	90.7±0.5 <sup>c</sup>
7	600.4±13.7 <sup>g</sup>	2818±10 <sup>h</sup>	64.6±0.6 <sup>e</sup>	219.9±1.3 <sup>g</sup>	44.7±3.3 <sup>e</sup>	64.8±0.6 <sup>d</sup>	18.5±0.4 <sup>b</sup>	96.4±0.5 <sup>e</sup>
8	607.2±11 <sup>h</sup>	2513±15 <sup>g</sup>	79.80.6 <sup>f</sup>	209.1±1.3 <sup>f</sup>	41.3±3.4 <sup>c</sup>	79.3±0.7 <sup>e</sup>	19.5±0.4 <sup>c</sup>	100.7±0.5 <sup>f</sup>
F	119652.4	4227165.6	2636072.5	47403121.3	117829.73	1323397.8	63.53	1622526.3
Sig.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

\*: Means within a group that has a different small letter are significantly different from each other (P<0.01). 1: Control; 2:50 mM NaCl; 3: 100 mM NaCl; 4: 200 mM NaCl; 5: 10 mM Si; 6: 50 mM NaCl+ Si; 7: 100 mM NaCl + Si; 8: 200 mM NaCl + Si

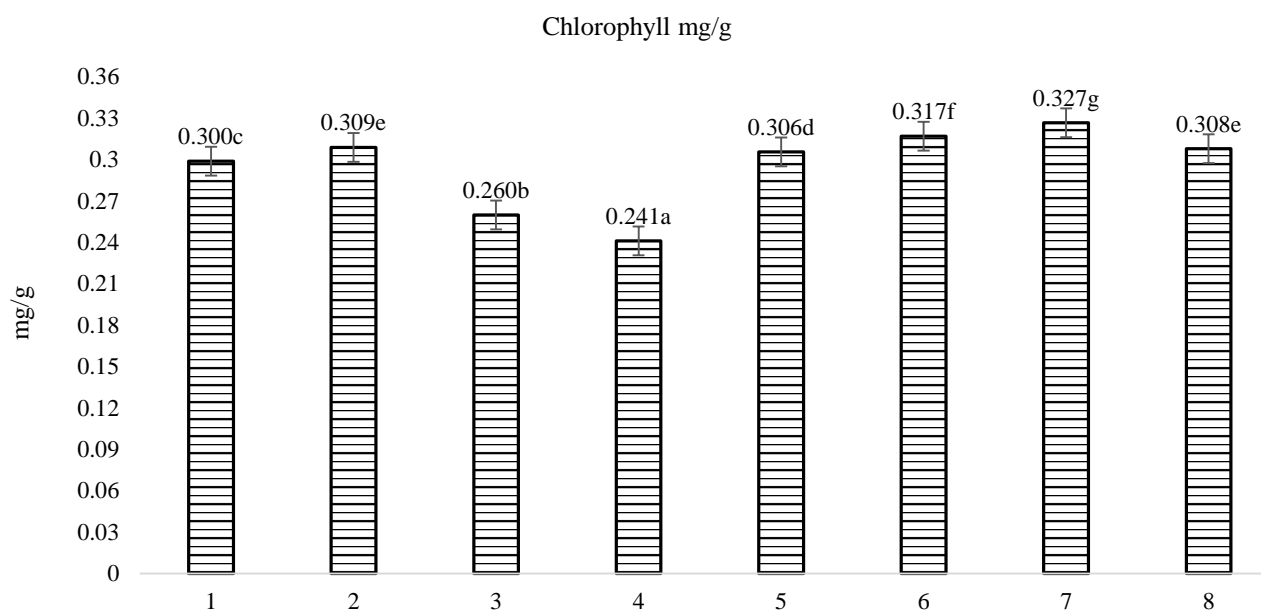


Figure 1. Variation of the amount of total chlorophyll in Hungarian vetch seedlings treated with NaCl and Si. 1:Control; 2:50 mM NaCl; 3: 100 mM NaCl; 4: 200 mM NaCl; 5: 10 mM Si; 6: 50 mM NaCl+ Si; 7: 100 mM NaCl + Si; 8: 200 mM NaCl + Si

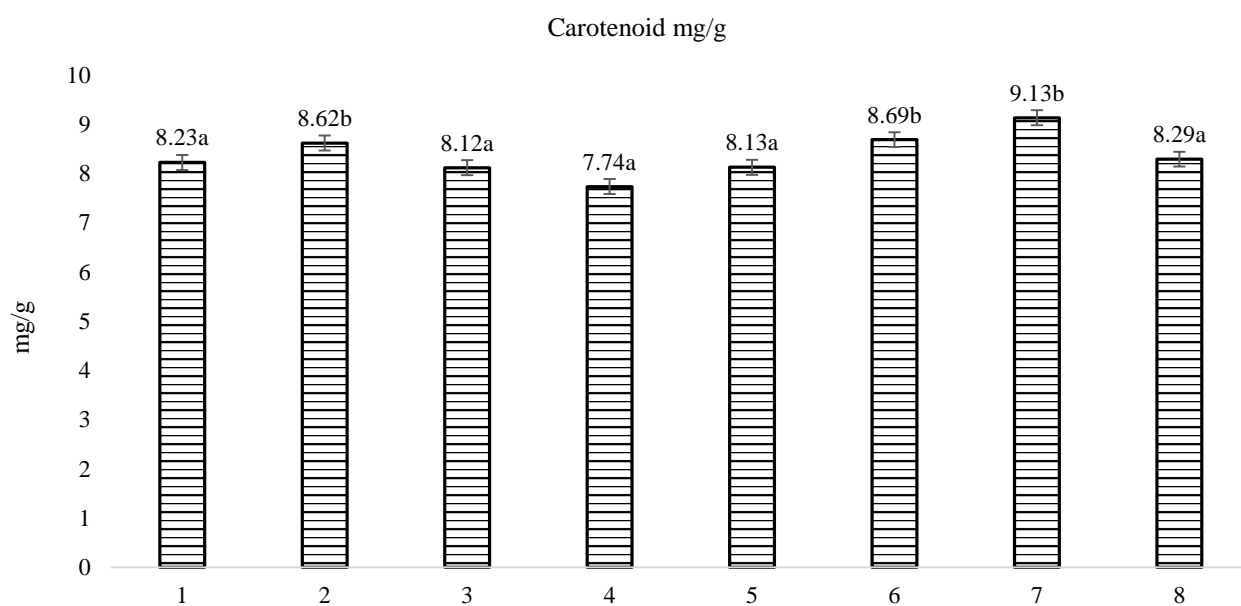


Figure 2. Variation of the amount of total carotenoid in Hungarian vetch seedlings treated with salt and silicon. 1: Control; 2:50 mM NaCl; 3: 100 mM NaCl; 4: 200 mM NaCl; 5: 10 mM Si; 6: 50 mM NaCl+ Si; 7: 100 mM NaCl + Si; 8: 200 mM NaCl + Si

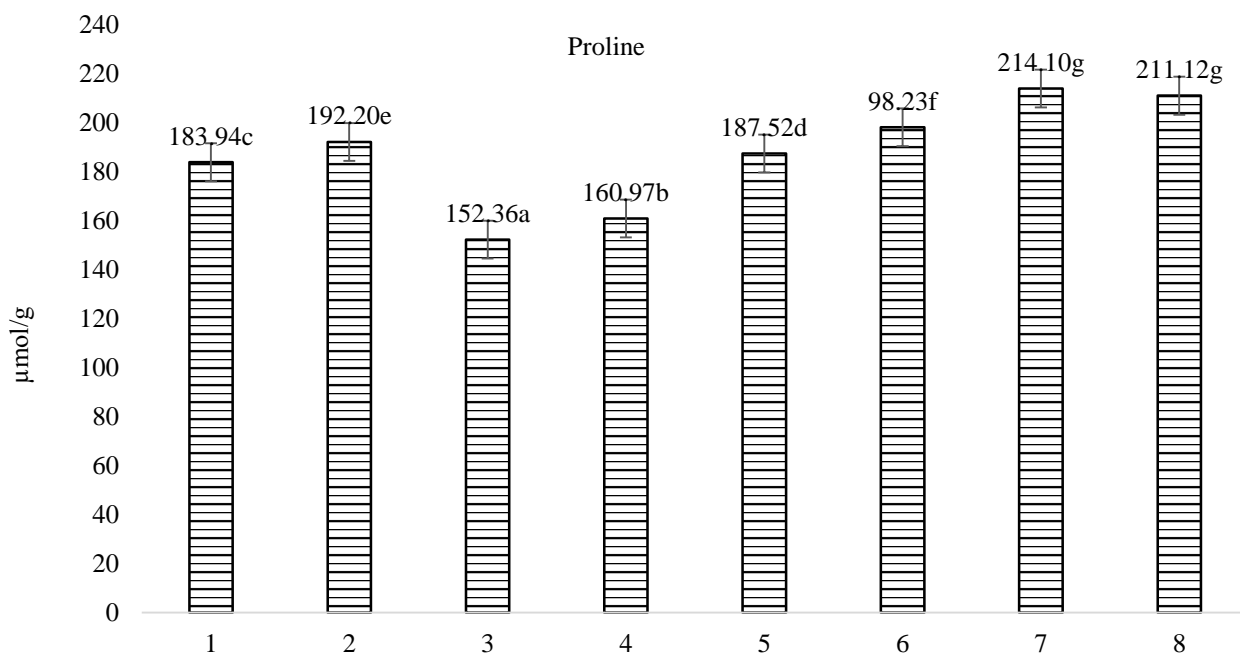


Figure 3. Variation of the amount of Proline carotenoid in Hungarian vetch seedlings treated with salt and silicon. 1: Control; 2:50 mM NaCl; 3: 100 mM NaCl; 4: 200 mM NaCl; 5: 10 mM Si; 6: 50 mM NaCl+ Si; 7: 100 mM NaCl + Si; 8: 200 mM NaCl + Si

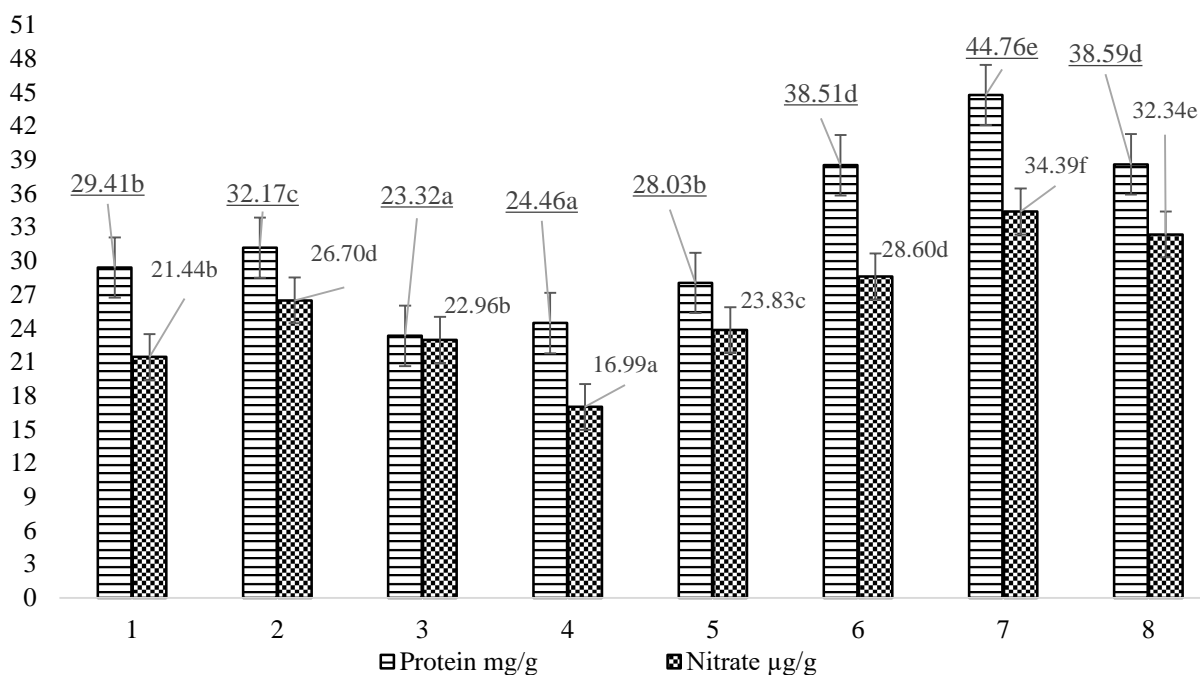


Figure 4. Variation of the amount of Protein and Nitrate in Hungarian vetch seedlings treated with salt and silicon. 1: Control; 2:50 mM NaCl; 3: 100 mM NaCl; 4: 200 mM NaCl; 5: 10 mM Si; 6: 50 mM NaCl+ Si; 7: 100 mM NaCl + Si; 8: 200 mM NaCl + Si

The quantity of proline and soluble protein (214.10 µmol- 44.79 mg) were found to be 16.40% and 52.20 % higher with the 100 mM NaCl + Si application compared to the control (183.94 µ mol - 29.41 mg) (Figure 3, Figure 4, respectively). Total nitrate content decreased compared to the control with the high salt concentrations. In the samples, the lowest nitrate value was recorded with the 100 mM NaCl and 200 mM NaCl applications, and the highest nitrate value was recorded with the 100 mM NaCl + Si and 200 mM NaCl + Si applications. The increase of nitrate with the NaCl + Si applications compared to the control was 33.42%, 60.43% and 50.88%, respectively (Figure 4).

According to the changes in the quantity of bioactive components, it can be said that while the amount of chlorophyll, carotenoid, proline, protein, and nitrate decrease with the high salt concentrations, Si applications cause an increase in the quantity of these compounds. These are consistent with the results from the other studies. It has been revealed by Meng et al. (2020) that salt stress inhibits nitrate uptake in plants by causing Cl ion accumulation in the soil, disrupting the functions of nitrate carrier proteins, and inhibiting the synthesis of amino acids and enzymes responsible for nitrate metabolism. Moreover, these negative effects of salinity also suppress chlorophyll biosynthesis (Markovich et al., 2017; Adelaal

et al., 2020). On the other hand, the application of Si elevated the amount of chlorophyll, proline and protein contents by increasing nitrate intake in this study. This may be due to the indirect preservation of the structure of ion channels and nitrate carrier proteins in the membranes since Si protects the membrane integrity (Conceição et al., 2019; Aras, 2020). Besides, Si may have also stimulated the synthesis of enzymes in nitrate metabolism (Cui et al., 2021). These results coincide with the literature. Salim (2014) observed that Si application in maize under salt stress leads to an increase in growth parameters such as shoot length, fresh and dry weight, and total amino acid and chlorophyll quantities in seedlings similar to our study (Table 2, Figure 1, Figure 2, Figure 3, and Figure 4). In another study, Abbas et al. (2015), Adelaal et al. (2020) found that total chlorophyll, carotenoid, proline, protein, amino acids and antioxidant enzyme activities were decreased in sensitive species in *Abelmoschus esculentus* varieties under salt stress and pepper respectively; and at the same time, they found that when Si was applied to varieties, the amount of these compounds increased and Si application eliminated the negative effects of salt stress. Similarly, Si applications to plants under salt stress have been shown to stimulate the accumulation of amino acids with osmolyte properties, proteins in cells and tissues by increasing nitrate uptake in tomatoes (Manivannan et al., 2016), Brassicanapus (Haddad et al., 2018), sunflower (Conceição et al., 2019) and Glycyrrhiza uralensis (Cui et al., 2021). Similarly, Khan et al. (2014), Torabi et al. (2015), Conceição et al. (2019) and Yin et al. (2019) found that the amount of chlorophyll pigments and osmolites such as proline, protein, polyamines and sucrose decreased in salty conditions, whereas these compounds increased in the Si treated groups, as well as the increase in nitrate enzyme activity and nitrate content.

#### **Variation of Nutrient Status in Hungarian Vetch Seedlings**

Salt stress tolerance in plants is closely related to the absorption of nutrients from the roots and their transport to the leaves (Bityutskii et al., 2014; Chen et al., 2016; Greger et al., 2018). Nutrients are minerals that work in physiological processes having a role in plant growth and development. K is one of the essential elements in physiological processes such as opening and closing of stomata, carbohydrate metabolism, and regulation of osmotic potential in cells (Chen et al., 2016; Meng et al., 2020). It is a known fact that P and S join into the sulfhydryl groups of proteins and enzymes (Kostic et al., 2017), the structure of Mg chlorophyll and Ca wall structure (Morgan et al., 2014). The effects of Si on the quantity of essential and trace elements are given in Table 3 and Table 4. The amount of K ranged from 37730 to 49070 mg kg<sup>-1</sup>, Mg from 2562 to 3655 mg kg<sup>-1</sup>, Ca from 12720 to 5282 mg kg<sup>-1</sup>, P from 8872 to 20140 mg kg<sup>-1</sup>, and S from 633 to 5940 mg kg<sup>-1</sup> (Table 3). As seen in the Table 3, the Si application generally positively affected K, Mg, Ca, P and S content in the seedlings under the salt stress. The quantity of K, Mg, P and S was the highest with the 200 mM NaCl + Si and 100 mM NaCl + Si applications (P < 0.001). On the other hand, K and Mg were the lowest with the 200 mM NaCl applicatipn, while Ca quantity with the 50 mM NaCl and S quantity were the lowest with the 100

mM NaCl application (Table 3). It can be said that the Si applications (200 mM NaCl + Si and 100 mM NaCl + Si) depending on the quantity of macronutrients, stimulated the accumulation of K > Mg > P > Ca > S, and the 200 mM NaCl and 100 mM NaCl doses reduced the intake of the elements (Table 3). Na, one of the trace elements, was found to be higher with all treatment groups. Cl content was also found to be higher with all groups except with the 50 mM NaCl application compared to the control (Table 3). The average quantities of Al, Si, Mn, Fe, Co, N Cu, and Zn detected in the Hungarian vetch seedlings are shown in the Table 4.

The recorded values vary between 313.75 and 607.2 mg kg<sup>-1</sup> for Al, 642.3 and 2818 mg kg<sup>-1</sup> for Si, 46.2 and 79.80 mg kg<sup>-1</sup> for Mn, 122.7 and 239.3 mg kg<sup>-1</sup> for Fe, 35.8 and 44.7 mg kg<sup>-1</sup> for Co, 50.9 and 79.3 mg kg<sup>-1</sup> for Ni, 14.6 and 19.5 mg kg<sup>-1</sup> for Cu, and 78.2 and 100.7 mg kg<sup>-1</sup> for Zn, respectively (Table 4). As shown in the Table 4, the Si content increased with the 100 mM NaCl + Si and 100 mM NaCl + Si applications compared to the control and the other groups, especially with the high salt concentrations, which showed a decrease of 60.52% and 50.40% (Table 3). While Co quantity increased with all groups compared to the control, Fe content was lower with all groups. Besides, Al quantity increased only with the NaCl + Si application, and Cu content significantly increased with the Si + NaCl concentrations and Ni content significantly increased with the Si and Si + NaCl applications compared to the control, (Table 4).

Finally, Mn and Zn contents were lower than the control with only NaCl concentrations, and they were highest with the 200 mM NaCl + Si (79.8-100.7 mg) and 100 mM NaCl + Si (64.6-96.4 mg) applications compared to the control (59.1-90.5 mg) (Table 4). The results revealed that while the NaCl concentrations in the seedlings generally caused a decrease in the amount of micronutrients, the applications of Si and Si + NaCl caused an increase (Table 4). However, while the amount of Fe decreased with all treatment groups compared to the control, the amount of Co increased (Table 4). Considering all the elements examined in the Hungarian seedlings under salt stress, it could be said that the 200 mM NaCl + Si, 100 mM NaCl + Si, 50 mM NaCl + Si of the elements stimulated the salt stress tolerance, whereas the 200 mM NaCl and 100 mM NaCl reduced salt resistance. The results related to the nutrients are in agreement with the literature. Many studies have shown that K, Mg, Ca, P, S, and Na are required in larger quantities, whilst Cl, Co, Mn, Fe, Cu, Zn, Ni, and Mo are required in lower quantities in plants (Greger et al., 2018). However, the amount of these minerals in plant tissues is reduced, probably because salt suppresses mineral uptake by the roots. Although the root's properties are impaired due to ion toxicity at high salt concentrations and mineral deficiency or toxicity can occur in plants, especially in sensitive species, the applications like Si, Se, methyl jasmonate and melatonin remove adverse effects of salt stress (Chen et al., 2016; Kostic et al., 2017). Ibrahim et al. (2016) found that the N, P, K content of wheat seedlings under salt stress decreased, but the quantity of these elements increased when they applied Si to the plants. In another study, Greger et al. (2018), found in their study investigating the elemental uptake capacities of corn, lettuce, peas, carrots and wheat in different soil types, that Si application stimulated Ca, P and

S uptake, but did not show a significant effect on K and Mg quantity. The high amount of K and Mg in the Hungarian vetch seedlings according to the data from this study (Table 3) was associated with the variety of the plant and the difference in the applied Si concentration (Neu et al., 2016; Meng et al., 2020). The increase in the quantity of P detected in Hungarian vetch seedlings was reported in the study of Neu et al. (2016) and Kostic et al. (2017) conducted with wheat. The researchers have suggested that the increase in the quantity of P in Si-treated plants is due to the replacement of Al and Fe phosphate anions from silicon sources with mono silicic acid. In this study, high Na accumulation may have decreased the uptake of Ca element forming silicate in the wall structure with Si and high Si doses may have suppressed Ca uptake. The high quantity of Na and Cl ions in Hungarian vetch seedlings confirms this result (Table 4). Similarly, Mehraban et al. (2015) reported that Si treatment caused an increase in the amount of K, P, Zn, and Fe in wheat, canola, and cotton while decreasing Ca level and this decrease was due to inhibition of apoplastic Ca uptake. It has been reported that Na accumulation in legume types is higher than in other species and varieties, and Cl inhibits the transport of other elements (Geilfus et al., 2015; Thor, 2019). Fe, Mn, Zn, Cu, Mo, Co are elements that play a role in the control of growth and development in plants as many enzyme cofactors (Lambers et al., 2015; Lange et al., 2016). Cobalt content in this study was higher with all groups than in the control, which was associated with the function of this element in legumes. Co has an important role in nitrogen fixation of nodules, leghemoglobin synthesis which plays a role in nitrogen fixation and development of stem and leaves in legume species (Lange et al., 2016). As seen in the Table 2 and the Table 3, the high quantity of Coin Hungarian vetch seedlings under the salt stress with all application groups and the higher shoot length, fresh weight and dry weight quantities in seedlings confirm the functions of Coin legume species. While Mn is essential in the breakdown of water in photosystems, activation of metalloenzymes, P uptake, and P metabolism in leaves, Zn is necessary for chlorophyll biosynthesis, participating in sulphhydryl groups of proteins, water uptake and tolerance to low temperatures (Bityutskii et al., 2014; Neu et al., 2016). As seen in the Figure 1, Figure2, Figure 3, and Figure 4, Si triggered salty tolerance by stimulating the accumulation of Mn and Zn in Hungarian vetch exposed to NaCl, and the high pigment, proline, protein and nitrate levels at these concentrations coincided with the functions of Mn and Zn (Bityutskii et al., 2014; Lambers et al., 2015). However, the quantity of micronutrients in vegetative tissue is closely related to the antagonistic interaction between the elements. The researchers reported that there was an antagonistic effect between Zn uptake and P, Cu, Fe, Mn and Ca; between Cu uptake and Fe, Mn, Zn and Ni; and between Mn uptake and Fe (Bityutskii et al., 2014; Lambers et al., 2015; Lang et al., 2016). In this study, P, Na and Cl content decreased Fe, Ca and Cu intake (Table 3, Table 4). In addition, the application of NaCl and Si reduced Al and Si accumulation. Similar to this study, Morgan et al. (2014), Meng et al. (2020) stated that Si facilitated the uptake of K and other major elements by giving high quantities of H<sup>+</sup> to the environment during the reduction of Al and Fe ions. Pontigo et al. (2017) reported which Si application prevented Al and Fe toxicity.

## Conclusion

With this study, the application of Si to Hungarian vetch under salinity demonstrated to have a great potential in increasing the resistance to salty. Considering shoot length, shoot fresh weight and dry matter values, the highest value was obtained with Si, Si + NaCl and 100 mM NaCl applications, while the lowest value was obtained with the 50 mM NaCl and 50 mM NaCl applications. As for chlorophyll, carotenoid, proline, protein and nitrate contents, the 200 mM NaCl + Si application reached the highest value with the 100 mM NaCl + Si, while it reached the lowest level with the 200 mM NaCl and 100 mM NaCl applications. However, these compounds also showed a significant increase with the lowest salt concentration compared to the control. On the other hand, P, Na, and Co contents were high in all application groups, while Fe contents were low. Ca and Al contents were high in seedlings and Si + NaCl Mn, Zn, K and Mg contents were higher in Si and Si + NaCl concentrations compared to the control. S content in seedlings was lower in high NaCl and Si concentrations and Cu was lower in NaCl concentrations and Si application compared to the control. Considering all the parameters examined, the highest value was determined with the applications of 200 mM NaCl + Si, 100 mM NaCl + Si, 50 mM NaCl + Si, Si, while the lowest value was determined with the concentrations of 200 mM NaCl, 100 mM NaCl and 50 mM NaCl, respectively. We have concluded that the most suitable doses increasing salt resistance are 100 mM NaCl + Si and 200 mM NaCl + Si. Secondly, the Hungarian variety can tolerate low doses of salt, is sensitive to high salt concentrations, and also Si applications induce salt tolerance. However, only a single dose of Si was applied in this study. Applying Si at different doses may have contributed to understand the exact results to the effect of Si application on salt tolerance in Hungarian vetch.

## Conflict of Interest

The authors declared no conflicts of interest for this work.

## References

- Abbas T, Balal RM, Shahid MA, Pervez MA, Ayyub CM, Aqueel MA, Javaid MM. 2015. Silicon-induced alleviation of NaCl toxicity in okra (*Abelmoschus esculentus*) is associated with enhanced photosynthesis, osmoprotectants and antioxidant metabolism. *Acta Physiologiae Plantarum*, 37(6):1-15.
- Adelaal KAA, Yasser SA, Mazrou YSA, Ha YM. 2020. Silicon Foliar Application Mitigates Salt Stress in Sweet Pepper Plants by Enhancing Water Status, Photosynthesis, Antioxidant Enzyme Activity and Fruit Yield. *Plants*, 9(6): 733.
- Aras S. 2020. Silicon Nutrition in Alleviating Salt Stress in Apple Plant. *Acta Scientiarum Polonorum-Hortorum Cultus*, 19(1):3-10.
- Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205-207.
- Bityutskii N, Pavlovic J, Yakkonen K, Maksimovic V, Nikolic M. 2014. Contrasting effect of silicon on iron, zinc and manganese status and accumulation of metal-mobilizing compounds in micronutrient-deficient cucumber. *Plant Physiology and Biochemistry*, 74: 205-211.

- Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.
- Cataldo DA, Haroon LE, Schrader LE, Youngs VL. 1975. Rapid colourimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis*, 6:71-80.
- Cemeroglu BS. 2013. *Gıda Analizleri*, 3. Baskı, Ankara, Bizim Grup Basımevi.
- Chen D, Cao B, Wang S, Deng X, Yin L, Zhang S. 2016. Silicon moderated K deficiency by improving the plant-water status in sorghum. *Scientific Reports*, 6:22882.
- Conceição SS, Oliveira Neto CF, Marques EC, Barbosa AVC, Galvão JR, Oliveira TB. 2019. Silicon modulates the activity of antioxidant enzymes and nitrogen compounds in sunflower plants under salt stress. *Archives of Agronomy and Soil Science*, 65:1237-1247.
- Cui J, Enhe Zhang E, Xinhui Zhang X, Wang Q. 2021. Silicon alleviates salinity stress in liquorice (*Glycyrrhiza uralensis*) by regulating carbon and nitrogen metabolism. *Scientific Reports*, 11(1):1115.
- Fidan E, Ekincialp A. 2020. Effect of Salt Applications on Plant Growth in Some Pole and Dwarf Bean Genotypes. *Turkish Journal of Agriculture - Food Science and Technology*, 8(5): 1074-1082.
- Geilfus CM, Mithöfer A, Ludwig-Müller J, Zörb C, Muehling KH. 2015. Chloride-inducible transient apoplastic alkalinizations induce stomata closure by controlling abscisic acid distribution between leaf apoplast and guard cells in salt-stressed *Vicia faba*. *New Phytologist*, 208:803-816.
- Greger M, Landberg T, Marek Vaculík M. 2018. Silicon Influences Soil Availability and Accumulation of Mineral Nutrients in Various Plant Species. *Plants*, 7(2):41.
- Haddad C, Arkoun M, Jamois F, Schwarzenberg A, Yvin J-C, Etienne P and Laîné P. 2018. Silicon Promotes Growth of *Brassica napus* L. and Delays Leaf Senescence Induced by Nitrogen Starvation. *Frontiers in Plant Science*, 9:516.
- Hoagland DR, Arnon DI. 1950. The water culture method for growing plants without soil. *California Agricultural Experiment Station Circular*, 347.
- Işık E, Alibaş İ. 2000. *Tarımsal Ürünlerin Kurutulmasında Kullanılan Yöntemler ve Kurutma Sistemleri*. Uludağ Üniversitesi Ziraat Fakültesi Yardımcı Ders Notu, No:3. Bursa. s.64.
- Ibrahim MA, Merwad AM, Elnaka EA, Burras CL, Follett L. 2016. Application of silicon ameliorated salinity stress and improved wheat yield. *Journal of Soil Science and Environmental Management*, 7(7): 81-91.
- James CS. 1995. *Analytical Chemistry of Foods*. Publisher Blacac Academic and Professional, 176p, London.
- Karakule S, Tüzemen N. 2020. Fertility Characteristics of Cattle Production in Enterprises Belonging to Cattle Breeding Association in Devrekâni. *Journal of Animal Science and Products*, 3(2):120-133.
- Khan NA, Khan MIR, Asgher M, Fatma M, Masood A. 2014. Salinity tolerance in plants: Revisiting the role of sulfur metabolites. *Journal of Plant Biochemistry and Physiology*, 2:120.
- Kılıçalp N, Özkurt M, Karadağ Y. 2020. The Effects of Hungarian Vetch (*Vicia pannonica* Crantz.) and Triticale (*x Triticosecale* sp. Wittmack) Sown in Different Seed Rates on Feed Value and Ruminant Degradability Characteristics of Nutrients. *Yuzuncu Yil University Journal of Agricultural Science*, 30(3):553-562.
- Kostic L, Nikolic N, Bosnic D, Samardzic, J, Nikolic M. 2017. Silicon increases phosphorus (P) uptake by wheat under low P acid soil conditions. *Plant and Soil*, 419:447-455.
- Kusvuran A. 2015. The effects of salt stress on the germination and antioxidative enzyme activity of Hungarian vetch (*Vicia pannonica* Crantz) varieties. *Legume Research*, 38: 51-59.
- Lambers H, Hayes PE, Laliberte E, Oliveira RS, Turner BL. 2015. Leaf manganese accumulation and phosphorus-acquisition efficiency. *Trends in Plant Science* 20: 83-90.
- Lange B, Pourret O, Meerts P, Jitaru P, Cancès B, Grison C, Faucon MP. 2016. Copper and cobalt mobility in soil and accumulation in a metallophyte as influenced by experimental manipulation of soil chemical factors. *Chemosphere*, 146: 75-84.
- Lichtenthaler HK, Wellburn AR. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 11(5): 591-592.
- Manivannan A, Soundararajan P, Muneer S, Ko C, Jeong BR. 2016. Silicon mitigates salinity stress by regulating the physiology, antioxidant enzyme activities, and protein expression in *Capsicum annuum* 'Bugwang'. *BioMed Research International*, 307.
- Mao ZX, Fu H, Nan ZB, Wan CG. 2015. Fatty acid, amino acid, and mineral composition of four common vetch seeds on Qinghai-Tibetan plateau. *Food Chemistry*, 171: 13-18.
- Markovich O, Steiner E, Kouřil S, Tarkowski P, Aharoni A, and Elbaum R. 2017. Silicon promotes cytokinin biosynthesis and delays senescence in *Arabidopsis* and *Sorghum*. *Plant Cell Environment*, 10:1189-1196.
- Mehraban S, Albdolzadeh H, Sadeghpour RH, Aghdası M. 2015. Silicon Affects Transcellular and Apoplastic Uptake of Some Nutrients in Plants. *Pedosphere*, 25(2):192-201.
- Meng Y, Yin Q, Yan Z, Wang Y, Niu J, Zhang J and Fan K. 2020. Exogenous Silicon Enhanced Salt Resistance by Maintaining K<sup>+</sup>/Na<sup>+</sup> Homeostasis and Antioxidant Performance in Alfalfa Leaves. *Frontiers in Plant Science*, 11:1183.
- Morgan SH, Maity PJ, Geilfus CM, Karl SL, Muehling H. 2014. Leaf ion homeostasis and plasma membrane H-ATPase activity in *Vicia faba* change after extra calcium and potassium supply under salinity. *Plant Physiology and Biochemistry*, 82: 244-253.
- Neu S, Schaller J, Dudel GD. 2016. Silicon availability modifies nutrient use efficiency and content, C: N:P stoichiometry, and productivity of winter wheat (*Triticum aestivum* L.). *Scientific Reports*, 7:1-8.
- Pontigo S, Godoy K, Jiménez H, Gutiérrez-Moraga A, Mora ML, Cartes P. 2017. Silicon-Mediated Alleviation of Aluminum Toxicity by Modulation of Al/Si Uptake and Antioxidant Performance in Ryegrass Plants. *Frontiers in Plant Science*, 8:642.
- Saçlı Y. 2020. The Factors Affecting the Beef Producer Price Formation in Turkey. *Turkish Journal of Agriculture - Food Science and Technology*, 8(3): 759-767.
- Salim BBM. 2014. Effect of Boron and Silicon on Alleviating Salt Stress in Maize. *Middle East Journal of Agriculture Research*, 3(4): 1196 -1204.
- Thor K. 2019. Calcium-Nutrient and Messenger. *Frontiers in Plant Science*, 10:40.
- Torabi F, Majd A, Enteshahari S. 2015. The effect of silicon on alleviation of salt stress in borage (*Borago officinalis* L.). *Soil Science and Plant Nutrition*, 61: 788-798.
- Wu GQ, Liu HL, Feng RJ, Wang CM, Du YY. 2017. Silicon ameliorates the adverse effects of salt stress on sainfoin (*Onobrychis viciaefolia*) seedlings. *Plant, Soil and Environment*, 63(12):545-551.
- Yılmaz H, Ayasan T, Sağlam C, Gül M. 2020. Socio-Economic Characteristics of Dairy Farms and Use Level of Feedstuff in the Eastern Mediterranean Region. *Turkish Journal of Agriculture - Food Science and Technology*, 8(1): 89-94.
- Yin J, Jia J, Lian Z, Hu Y, Guo J, Huo H, Zhu Y, Gong H. 2019. Silicon enhances the salt tolerance of cucumber through increasing polyamine accumulation and decreasing oxidative damage. *Ecotoxicology and Environmental Safety*, 169: 8-17.