



Silicon Improves Strawberry Ability to Cope with Water Deficit Stress

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ABSTRACT

Strawberry is one of the most important commercial fruits. This research aimed to ameliorate the negative effects of water deficit stress using silicon application on strawberry. The experiment was conducted as a factorial in a completely randomized design with three factors including cultivar ('Selva' and 'Gavioita'), Na₂SiO₃ (Sodium silicate) concentrations [0, 3, and 6 mM], as the source of silicon, and water deficit stress (40%, 60%, 80%, and 100% of field capacity (FC)), in three replications. 'Gavioita' cultivar showed the highest superoxide dismutase enzyme activity in leaves under severe water deficit stress (40% of FC) following Na₂SiO₃ (3 and 6 mM concentrations) application. Application of Na₂SiO₃ at concentration of 6 mM increased the catalase enzyme activity significantly in the leaves of 'Gavioita' cultivar under severe water deficit stress (40% of FC). The activity of peroxidase enzyme was enhanced considerably under 80% of FC and lower water deficit in the fruits of both cultivars. The fruit total anthocyanin level was increased significantly by application of Na₂SiO₃ at 3 and 6 mM concentrations under water deficit stress (60% of FC). Also, Na₂SiO₃ (at concentrations of 3 and 6 mM) enhanced the proline level significantly in 'Gavioita' cultivar compared to the control treatment under normal conditions (100% of FC). In conclusion, the results of present study indicated that application of Na₂SiO₃ at 6 mM concentration is useful treatment to cope with severe water deficit stress (40% of FC) especially in 'Gavioita' cultivar of strawberry.

Introduction

The strawberry from Rosacea family has narrow creeping stems lying on the ground (Gasemi et al., 2018). This plant is valuable from the economic and nutritional perspectives. It is one of the important commercial fruits of the world due to its aroma, taste and rich content of vitamins (Ameli et al., 2016). According to Food and Agriculture Organization (FAO) statistics, worldwide production of strawberries is estimated to be 8885028 tons (FAO, 2019). Water deficit stress is one of the most important

factors that decrease crop production in arid and semiarid areas of the world (Bukhari et al., 2015). The plants undergo morphological and metabolic changes under water deficit stress, which make them more adaptable to such conditions. The water deficit stress response level depends on factors such as species, growth status, duration, and severity of water deficit stress (Sayfzadeh and Rashidi, 2011). Plants have enzymatic and non-enzymatic antioxidant systems to cope with water deficit stress and preventing of photooxidative damage (Ahmad and Haddad, 2011; Aliniaefard et al., 2016a,b; Shomali and Aliniaefard, 2020; Shomali et al., 2021; Susarai et al., 2021). Plants with significant level of silicon storage can survive

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long under water stress due to utilization of efficient substances (Malhotra et al., 2016). Silicon is one of the essential elements for growth of plants under different environmental conditions (Chanchal, 2016). The protective function of silicon in plants is mainly due to the accumulation of polycyclic acids in cells (Balakhnina and Borkowska, 2011). Despite the abundance of silicon, it is not found freely in the soil and is mainly absorbed by the plant in the form of monocyclic acid (El-Shazoly, 2019). Silicon is always bonded to two oxygen molecules and forms the SiO_2 compound in the nature, which is widely consumed for plant nutrition. In the biosphere, silicon is found mainly in three forms of Amorphous (Si), Silica gel and Ortho silicic acid (Afeef et al., 2016; Esmaili et al., 2021). The biochemical, physiological and photosynthetic processes are affected by silicon that causes resistance to water deficit stress (Mauad et al., 2016). Previous studies reported the beneficial effects of silicon on plants exposed to different abiotic stresses. For instance, treating Hawthorn (*Crataegus microphylla*) plants with silicon nanoparticles had positive effects on physiological parameters under water deficit stress conditions (Ashkavand et al., 2015). The application of silicon improved plant growth parameters and the chlorophyll pigments concentration in wheat plants under water deficit stress conditions (Maghsoudi et al., 2015). Production of the activated oxygen species, and oxidative damage was increased in potato plants exposed to water deficit stress, whereas silicon had adverse effect in this regard (Shi et al., 2016). Silicon is beneficial element for human health, bone formation (Marta et al., 2020), skin health, and hair care (Charles et al., 2013). Therefore, silicon deficiency can be partially compensated by consuming silicon treated fruits. This research aimed to ameliorate the negative effects of water deficit stress on two cultivars of strawberry by application of silicon.

Materials and Methods

Plant material and experimental design

This research was conducted in a smart greenhouse located in the Aras Greenhouse Town in Jolfa City in 2019. The strawberry's 'Selva' and 'Gavioita' cultivars were provided from the Royal Green Agricultural Company of Kurdistan, and Na_2SiO_3 was purchased, as the source of silicon fertilizer, by the brand of Sigma. Soil with sandy loam texture (suitable for strawberry cultivation based on soil test data

according to Table 1) was used as culture medium. Each replication was composed of one plant per pot, in total 72 experimental parcels were examined. At first, a weighted amount of soil was poured in to each pot. Then 4 pots were randomly selected and saturated. The pots were weighted after 48 h of drainage, and the soil was dried for 24 h at 105 °C. According to Equation (1), the soil moisture content was measured at 100% of FC based on the difference between the soil weight after drainage (FCW), and soil weight after drying (DW). Finally, fresh seedlings were planted. Application of silicon under water deficit stress conditions has a significant economic effect, which improves the quality of strawberry fruits. Given that sodium silicate is used in the minimum concentration (mM), so its use is economically justified.

The level of silicon (in the form of Na_2SiO_3) was weighted, and dissolved in 10 liters of water in each treatment. Na_2SiO_3 was sprayed in two stages (one week after transplanting, and then at the beginning of flowering). Water deficit stress (40%, 60%, 80%, and 100% FC) was studied since first stage of sodium silicate spray until the fruit harvest stage. When the weight of the pot containing soil and plants were lower than a certain level (measurements were performed daily on all pots based on the FC assigned for each treatment) irrigation was repeated.

Equation 1:

$$\text{Field capacity (FC)} = \frac{\text{soil weight in field capacity (FCW)} - \text{weight of dry soil (DW)}}{\text{weight of dry soil (DW)}} \times 100$$

The experiment was conducted as a factorial in a completely randomized design with three factors including cultivar ('Selva' and 'Gavioita'), Na_2SiO_3 concentrations (0, 3, and 6 mM), as the source of silicon, and water deficit stress at all levels (40%, 60%, 80%, and 100% of FC), and three replications. This experiment had 12 treatments (T_1 - T_{12}) as indicated in the following:

$T_1 = 0 \text{ mM Na}_2\text{SiO}_3 + 40 \text{ \% of FC.}$

$T_2 = 3 \text{ mM Na}_2\text{SiO}_3 + 40 \text{ \% of FC.}$

$T_3 = 6 \text{ mM Na}_2\text{SiO}_3 + 40 \text{ \% of FC.}$

$T_4 = 0 \text{ mM Na}_2\text{SiO}_3 + 60 \text{ \% of FC.}$

$T_5 = 3 \text{ mM Na}_2\text{SiO}_3 + 60 \text{ \% of FC.}$

$T_6 = 6 \text{ mM Na}_2\text{SiO}_3 + 60 \text{ \% of FC.}$

$T_7 = 0 \text{ mM Na}_2\text{SiO}_3 + 80 \text{ \% of FC.}$

$T_8 = 3 \text{ mM Na}_2\text{SiO}_3 + 80 \text{ \% of FC.}$

$T_9 = 6 \text{ mM Na}_2\text{SiO}_3 + 80 \text{ \% of FC.}$

$T_{10} = \text{Control: } 0 \text{ mM Na}_2\text{SiO}_3 + 100 \text{ \% of FC.}$

$T_{11} = 3 \text{ mM Na}_2\text{SiO}_3 + 100 \text{ \% of FC.}$

$T_{12} = 6 \text{ mM Na}_2\text{SiO}_3 + 100 \text{ \% of FC.}$

Table 1. Different characteristics of the soil used for the experiment

Soil Texture									
Type of texture	Clay %	Silt %	Sand %	Absorbable potassium ppm	Absorbable phosphorus ppm	Organic carbon %	T.N.V.*	pH	EC (ds/m)
Sandy loam	14	22	64	353	8	0.6	4	7.51	1.083

* Total Neutralizing Value

Determination of antioxidant enzymes activity

Superoxide dismutase enzyme activity in the leaves and fruits was assessed by Tris-Calcodylic sodium salt buffer, pH 8.2, containing 0.1 mM EDTA. The reaction mixture included 1.42% Triton X-100, 0.055 mM nitroblue tetrazolium (NBT), 16 mM pyrogallol and enzyme extract (50 mg protein). The measurement unit was defined as the enzyme level required to inhibit the reduction of NBT by 50% per 1 min. The leaves and fruits peroxidase enzyme activity was determined by application of t-butyl hydroperoxide as a substrate. The reaction mixture included 50 mM potassium phosphate buffer, pH 7.0, 2 mM EDTA, 0.28 mM NADPH, 0.13 mM GSH, 0.16 U GR, 0.073 mM t-butyl hydroperoxide and enzyme extract (50 mg protein). One unit of GSH-Px was defined as the level of enzyme catalyzing NADPH oxidation. The leaves and fruits catalase enzyme level was measured by consumption of H₂O₂ (extinction coefficient 0.0394 mM⁻¹ Cm⁻¹) at 240 nm for 1 min. The titratable mixture contained 100 mM potassium phosphate buffer (pH 7.0), 15 mM H₂O₂ and 50 µL of the leaves or fruits extract in a level of 3 mL. A unit of catalase activity (U) was specified as enzyme level that decomposed 1 µmol H₂O₂ mg⁻¹ soluble protein per min (Hajiboland et al., 2018).

Total phenolics, flavonoid, and anthocyanin estimation

The total phenols were determined according to the Folin-Ciocalteu's procedure (Carneiro et al., 2017). The absorption of these metabolites was determined at 725 nm. Flavonoids levels were spectrophotometrically determined by modified method of Upadhyay and Maier (2016). The fruits samples were prepared with 2 mL acidic methanol (80% methanol with 1% HCl) in the mortar at room temperature for 2 h. The extracts were centrifuged at 1000 × g for 15 min. The absorption recorded at 560 nm, and 0.2 g of fruit tissue was removed and its anthocyanin was extracted using 3 mL of 1% acidic methanol overnight for examining the anthocyanin

content. The constituted phase was isolated using chloroform (3 mL) and water (2 mL) after 24 h. The aqueous phase absorption was measured in the absorption bands of 530 and 657 nm. Anthocyanin content was obtained using the formula $\lambda_{530} - \lambda_{657}$ and expressed per g⁻¹ fresh weight (Laxmi et al., 2004).

Proline determination

To measure the leaves and fruits free proline level, 0.1 g of wet tissue (leaves and fruits) was placed in 10 mL of 3% solution of sulphosalicylic acid, and a uniform mixture was prepared. The resulting mixture was placed in a centrifuge at 10,000 rpm for 10 min. Ninhydrin reagent was used in this procedure. The solution adsorption colored phase, which contains toluene and proline, was set at 520 nm. Each specimen proline content was determined using a standard curve in mmol g⁻¹ Fwt (Bates et al., 1974).

Statistical analysis

The data were analyzed using ANOVA. The means difference of the treatments was compared by Duncan's multiple range test, and the differences were considered significant at P ≤ 0.05. The statistical analysis was performed by SPSS version 16.0 software.

Results

Silicon application improved antioxidant enzymes activity in the strawberry leaves

The results of the data analysis of variance showed significant differences in antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase) in the leaves of 'Selva' and 'Gavioita' cultivars. Antioxidant enzymes activity (superoxide dismutase, peroxidase, and catalase) in the leaves of the 'Selva' and 'Gavioita' cultivars was increased under water deficit stress (40%, 60%, and 80% of FC) compared to their activities under well irrigated condition. The highest activity of the superoxide dismutase enzyme in the leaves was recorded in 'Gavioita' cultivar under severe water deficit stress (40% of FC) following application of Na₂SiO₃ (3 and 6 mM concentrations) and in 'Selva' cultivar under

water deficit stress at 40% of FC by spraying Na_2SiO_3 at concentration of 6 mM (Fig. 1). The peroxidase enzyme activity was increased in the leaves of both 'Selva' and 'Gavioita' cultivars compared to the control treatment under severe water stress conditions (40% of FC) regardless of Na_2SiO_3 foliar application at any concentration (0, 3, and 6 mM). The peroxidase enzyme activity in the leaves of the 'Gavioita' cultivar increased compared to the control treatment under water deficit stress up to 80% of FC (Fig 2). The catalase enzyme activity increased in the leaves

of the 'Gavioita' cultivar under severe water deficit stress (40% of FC) by foliar application of Na_2SiO_3 at a concentration of 6 mM. The lowest catalase activity was observed in the leaves of 'Selva' cultivar under normal moisture conditions (100% of FC) without considering the application of foliar or lack of Na_2SiO_3 at any concentration (0, 3, and 6 mM). The activity of the catalase enzyme in the leaves of both 'Selva' and 'Gavioita' cultivars increased significantly compared to the control treatment under water deficit stress up to 80% of FC (Fig. 3).

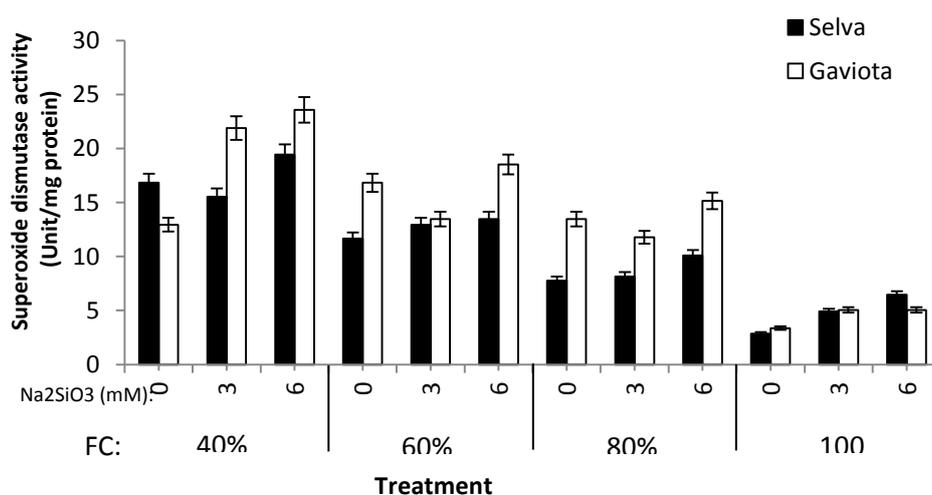
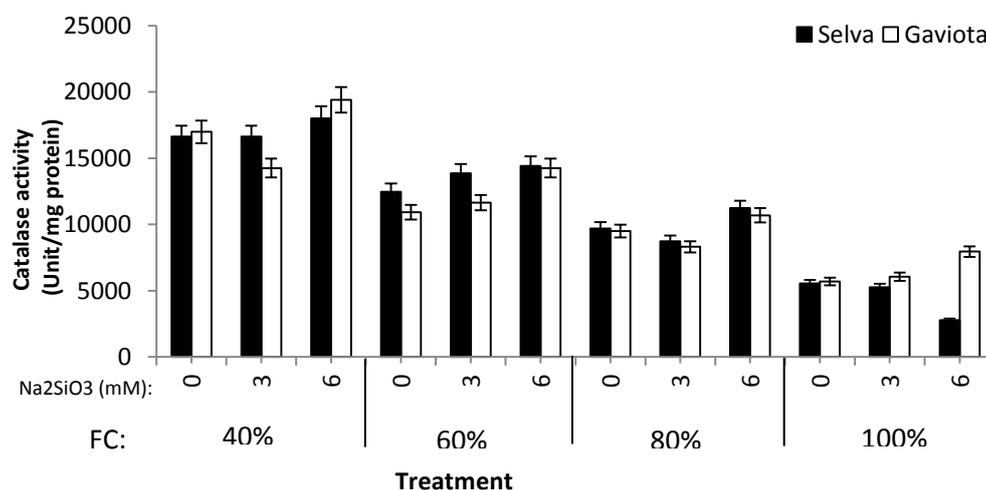


Fig. 1. Effects of silicon on superoxide dismutase activity in the leaves of 'Selva' and 'Gavioita' strawberry cultivars under water deficit stress. 'Selva' and 'Gavioita' cultivars were grown under three concentrations of Na_2SiO_3 (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC))].



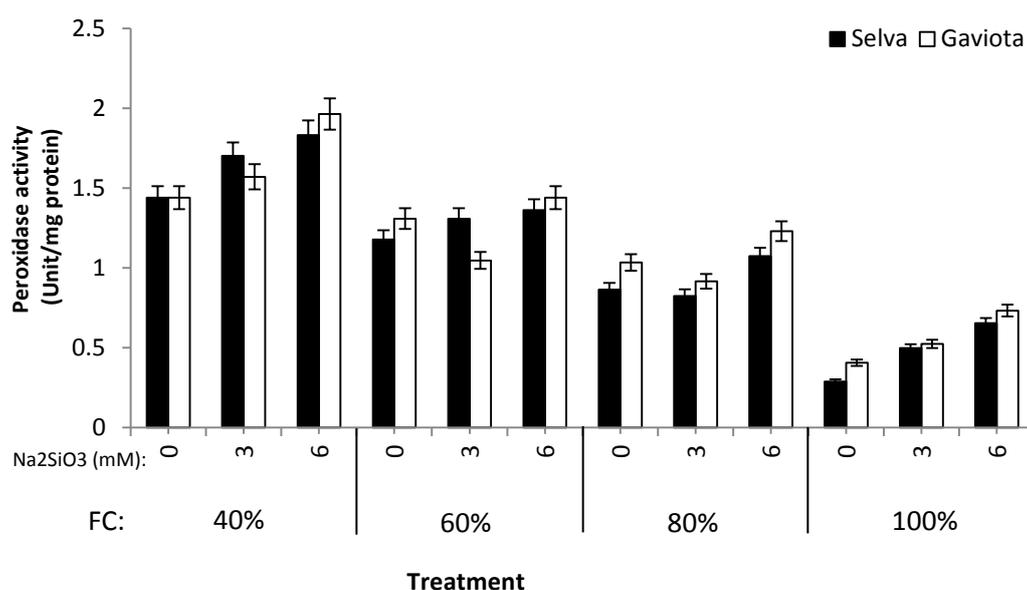


Fig. 2. Effects of silicon on peroxidase activity in the leaves of 'Selva' and 'Gavioita' strawberry cultivars under water deficit stress. 'Selva' and 'Gavioita' cultivars were grown under three concentrations of Na₂SiO₃ (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC)]. Fig. 3. Effects of silicon on catalase activity in the leaves of 'Selva' and 'Gavioita' strawberry cultivars under water deficit stress. 'Selva' and 'Gavioita' cultivars were grown under three concentrations of Na₂SiO₃ (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC))]

Silicon application improved antioxidant enzymes activity in the strawberry fruits

The results of data analysis of variance showed significant differences in antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase) in fruits of 'Selva' and 'Gavioita' cultivars. The fruits of both 'Selva' and 'Gavioita' cultivars showed the highest level of superoxide dismutase enzyme activity under severe water deficit stress conditions (40% of FC) with or without Na₂SiO₃ application and also under water deficit stress conditions at 60% of FC level using Na₂SiO₃ in concentrations of 3 and 6 mM (Fig. 4). The fruits of the 'Selva' cultivar depicted the highest peroxidase enzyme activity under

severe water deficit stress (40% of FC) without Na₂SiO₃ foliar application. The highest peroxidase enzyme activity in the fruits of the 'Gavioita' cultivar was observed by foliar application of 6 mM Na₂SiO₃. The activity of the peroxidase enzyme increased significantly under 80% of FC and lower water deficit levels in the fruits of both cultivars. The activity of peroxidase enzyme significantly increased in fruits of both cultivars of the 'Selva' and 'Gavioita' under normal moisture conditions (100% of FC) with the application of Na₂SiO₃ at concentration of 6 mM compared to the other treatment (Fig. 5).

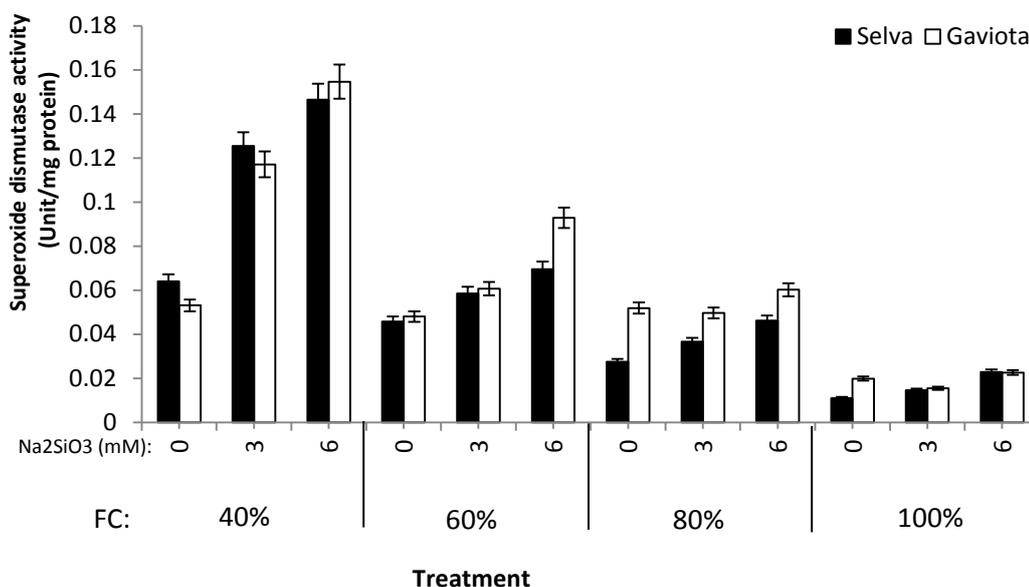


Fig. 4. Effects of silicon on superoxide dismutase activity in the fruits of ‘Selva’ and ‘Gaviota’ strawberry cultivars under water deficit stress. ‘Selva’ and ‘Gaviota’ cultivars were grown under three concentrations of Na₂SiO₃ (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC))]

The highest level of fruit catalase enzyme activity was detected in the ‘Selva’ cultivar under the most severe water deficit stress (40% FC) following Na₂SiO₃ treatment at concentration of 6 mM. The activity of catalase enzyme was significantly increased in the fruits of both

cultivars under water deficit stress up to 80% of FC and lower compared to the control treatment (Fig. 6).

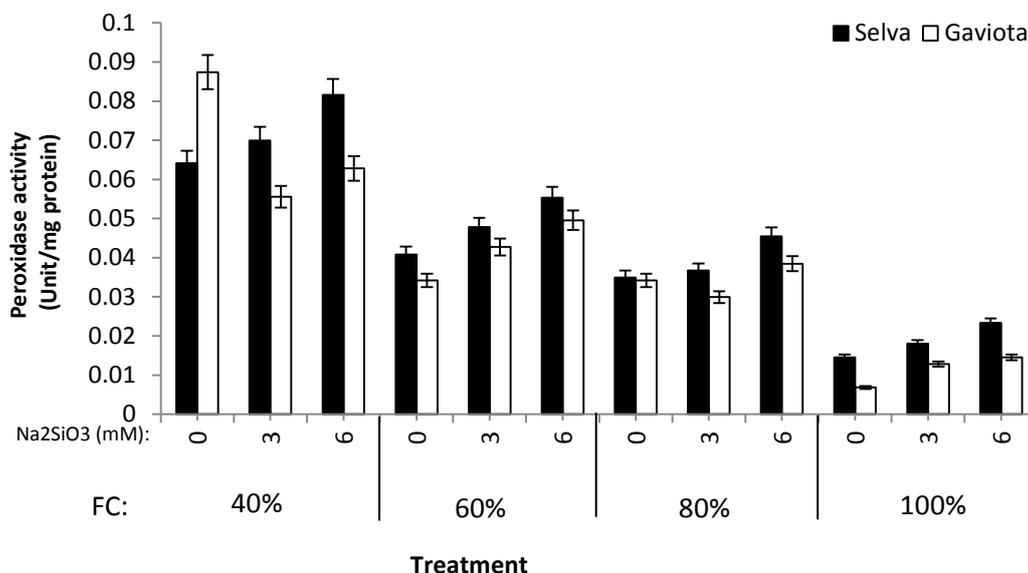


Fig. 5. Effects of silicon on peroxidase activity in the fruits of ‘Selva’ and ‘Gaviota’ strawberry cultivars under water deficit stress. ‘Selva’ and ‘Gaviota’ cultivars were grown under three concentrations of Na₂SiO₃ (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC))].

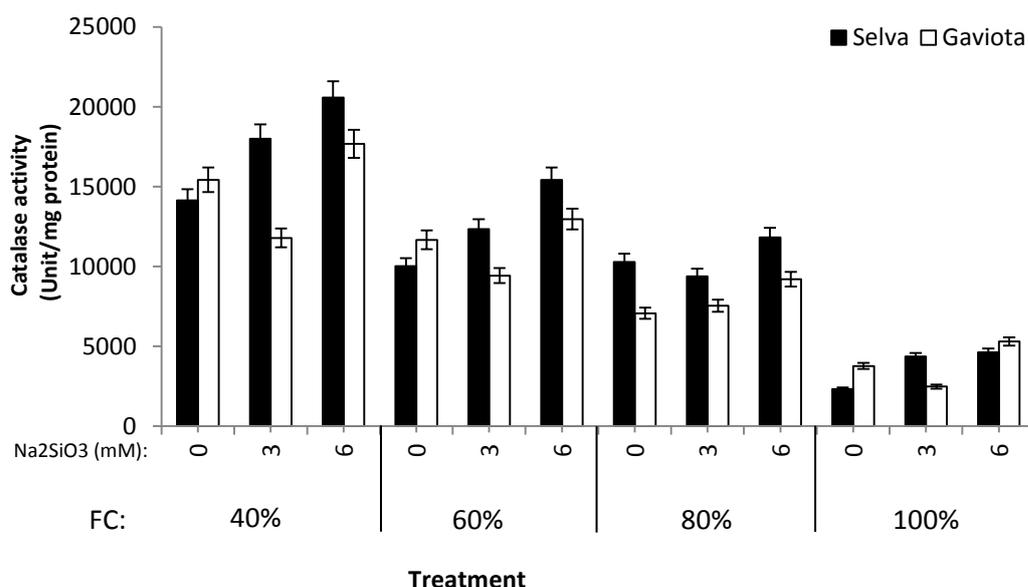


Fig. 6. Effects of silicon on catalase activity in the fruits of 'Selva' and 'Gavioita' strawberry cultivars under water deficit stress. 'Selva' and 'Gavioita' cultivars were grown under three concentrations of Na₂SiO₃ (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC))]

Silicon application elevated total phenolics, flavonoid and anthocyanin levels in the strawberry fruits

The highest total phenolics level was obtained in 'Selva' cultivar by application of 3 and 6 mM Na₂SiO₃ under water deficit stress condition at 40% of FC. The flavonoid contents in 'Selva' cultivar were significantly increased under water deficit stress condition at 40% of FC with application of all Na₂SiO₃ concentrations and also under water deficit stress condition at 60% of FC using Na₂SiO₃ at 6 mM concentration compared to the control. Flavonoid content was enhanced significantly in 'Gavioita' cultivar only under water deficit stress condition at 40% of FC by spraying Na₂SiO₃ at 3 mM concentration

compared to control treatment. The 'Selva' cultivar had the highest flavonoid content under water deficit stress condition at 40% of FC following spraying Na₂SiO₃ at 6 mM concentration. Total anthocyanin level in both 'Selva' and 'Gavioita' cultivars was significantly increased under water deficit stress condition at 60% of FC using Na₂SiO₃ at 3 and 6 mM concentrations compared to the control. Spraying Na₂SiO₃ at 6 mM concentration in the 'Selva' cultivar increased the anthocyanin level significantly in the treatment under water deficit stress condition at 40% of FC (Table 2).

Table 2. Effects of silicon on some biochemical characteristics of 'Selva' and 'Gavioita' cultivars of strawberry fruits under water deficit stress conditions. 'Selva' and 'Gavioita' cultivars were grown under three concentrations of Na₂SiO₃ (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC))]

Treatment	Total Phenolics (μg g ⁻¹)		Flavonoid (μg g ⁻¹)		Total Anthocyanin (λ530 - λ 657 g ⁻¹)	
	'Selva'	'Gavioita'	'Selva'	'Gavioita'	'Selva'	'Gavioita'
0 mM Na ₂ SiO ₃ + 40 % of FC	618.20	579.49	12.42	12.75	17.36	13.43
3 mM Na ₂ SiO ₃ + 40% of FC	763.35	394.94	15.49	9.03	20.60	12.27
6 mM Na ₂ SiO ₃ + 40% of FC	666.58	508.37	20.45	12.60	36.23	19.36
0 mM Na ₂ SiO ₃ + 60% of FC	414.98	355.25	11.68	8.75	8.68	10.18

Treatment	Total Phenolics ($\mu\text{g g}^{-1}$)		Flavonoid ($\mu\text{g g}^{-1}$)		Total Anthocyanin ($\lambda 530 - \lambda 657 \text{ g}^{-1}$)	
	'Selva'	'Gavioita'	'Selva'	'Gavioita'	'Selva'	'Gavioita'
3 mM Na_2SiO_3 + 60% of FC	492.40	396.50	10.60	10.11	9.95	13.17
6 mM Na_2SiO_3 + 60% of FC	535.94	628.51	12.92	13.14	13.43	14.67
0 mM Na_2SiO_3 + 80% of FC	376.27	298.54	9.69	7.87	9.26	12.57
3 mM Na_2SiO_3 + 80% of FC	342.40	319.16	9.11	8.19	10.42	10.33
6 mM Na_2SiO_3 + 80% of FC	289.55	391.34	7.12	9.31	8.56	13.92
0 mM Na_2SiO_3 + 100% of FC	168.22	236.67	4.81	6.19	5.21	3.14
3 mM Na_2SiO_3 + 100% of FC	235.96	190.27	5.31	6.43	3.58	5.38
6 mM Na_2SiO_3 + 100% of FC	352.08	283.07	6.05	6.43	5.67	9.13
LSD (5%)	250		3.6		6.5	

Silicon application elevated proline levels in the strawberry leaves and fruits

The results of data analysis of variance showed significant differences in proline content in leaves and fruits of 'Selva' and 'Gavioita' cultivars. Spraying Na_2SiO_3 at 6 mM concentration increased the proline level significantly in the leaves of both 'Gavioita' and 'Selva' cultivars under water deficit stress conditions (40% and 60% of FC). The level of proline increased significantly in the leaves of 'Gavioita' and 'Selva' cultivars compared to the control treatment under water deficit stress up to 80% of FC (Fig. 7). The highest proline level

was reported in the fruit of the 'Selva' cultivar under water deficit stress condition (40% FC) by application of Na_2SiO_3 at 6 mM concentration. The highest level of the fruit proline was obtained in the treatments in which Na_2SiO_3 was applied at 3 mM concentration under water deficit stress condition (40% FC) in 'Gavioita' cultivar. Proline of fruits significantly increased compared to the control treatment in 'Gavioita' cultivar under normal moisture conditions (100% of FC) following the application of Na_2SiO_3 (at concentrations of 3 and 6 mM) (Fig. 8).

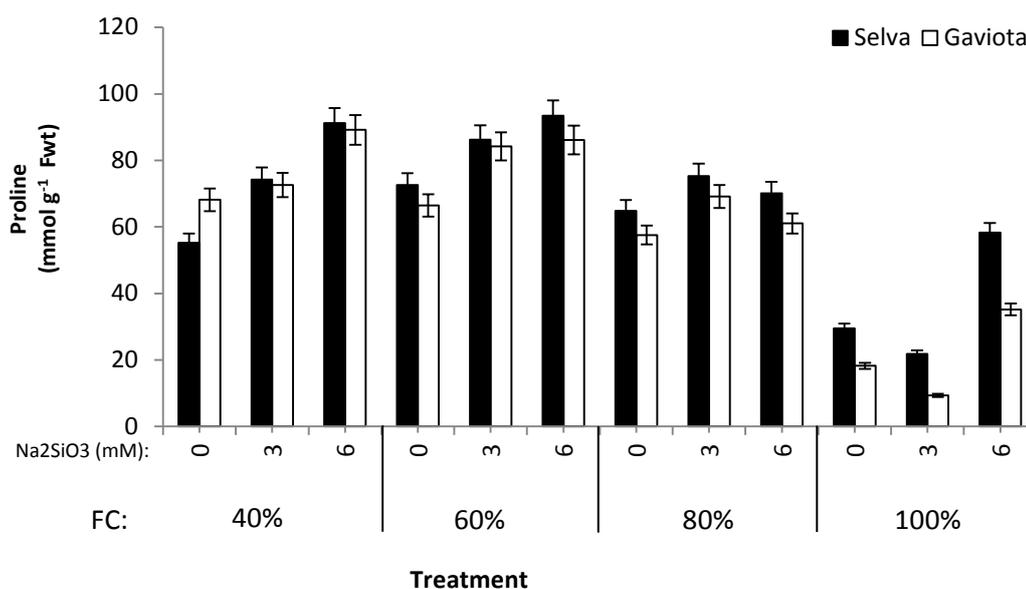


Fig. 7. Effects of silicon on proline content in the leaves of 'Selva' and 'Gaviota' strawberry cultivars under water deficit stress. 'Selva' and 'Gaviota' cultivars were grown under three concentrations of Na₂SiO₃ (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC))]

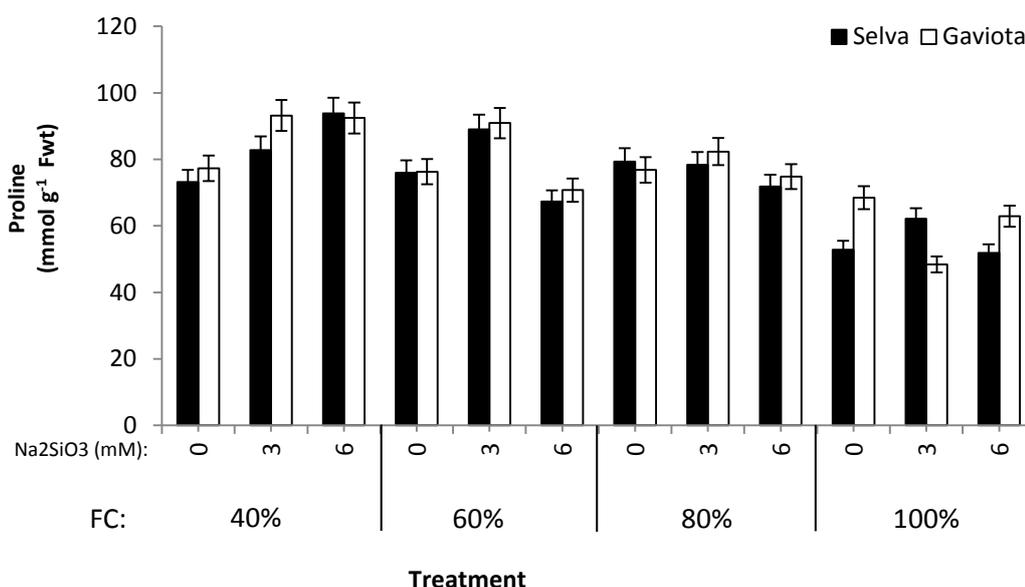


Fig. 8. Effects of silicon on proline content in the leaves of 'Selva' and 'Gaviota' strawberry cultivars under water deficit stress. 'Selva' and 'Gaviota' cultivars were grown under three concentrations of Na₂SiO₃ (0, 3, and 6 mM), and four levels of water deficit stress [(40%, 60%, 80%, and 100% of field capacity (FC))]

Discussion

Promoting effects of silicon on antioxidant enzymes activities in the leaves and fruits

Strawberry plant needs much water due to its superficial root system and large leaves, so it is more sensitive to water shortage (Dehghanipoodeh et al., 2018). Under such conditions, the quantitative and qualitative traits

of strawberry would be negatively influenced. Various methods have been used to cope with water deficit stress. The most important one is to elevate the antioxidant enzymes (superoxide dismutase, peroxidase, and catalase) activity, non-enzymatic antioxidants (total phenolics, flavonoid, and anthocyanin), and osmolytes (such as proline) under stress condition

(Seifikalhor et al., 2019a,b,c; Aliniaiefard et al., 2020a,b). The plants naturally produce numerous reactive oxygen species and oxidize proteins, lipids and nucleic acids, and eventually cause a number of abnormalities at the cellular level (Behnamnia, 2015). Antioxidant compounds, as the first defense mechanism coping with stresses, neutralize the toxic effects of the reactive oxygen species, and minimize the negative effects of stresses on plant quantitative and qualitative traits. Although silicon is not an essential element for the plants, it is very useful for the growth and performance of plant physiological processes and as the consequence tolerance to the environmental stresses such as water deficit stress. Biochemical reactions are directly influenced by silicon utilization under severe stresses (Balakhnina and Borkowska, 2011). The plants adopt to water deficit stress through mechanisms such as boosting antioxidants activities (Ahmad and Haddad, 2011). Silicon plays an important role in regulating the antioxidant defense system by enhancing the antioxidant enzymes activity (Al-Huqail et al., 2019). The results of present study revealed a significant effect of Na_2SiO_3 spraying on promotion of antioxidant enzymes activity (superoxide dismutase, peroxidase, and catalase) in 'Selva' and 'Gavioita' strawberry cultivars, resulted in amelioration of negative effects of water deficit stress (Figs. 1-6). Recent experimental results depicted that the antioxidant enzymes activity depends on various factors, including the levels of stress and the type of cultivar. Therefore, water deficit stress up to 60% FC increased the activity of the superoxide dismutase enzyme significantly in the leaves of 'Selva' cultivar compared to the control treatment. While, in 'Gavioita' cultivar, the activity of superoxide dismutase enzyme in the leaves was enhanced under stress up to 80% FC compared to the control treatment. It seems that superoxide dismutase enzyme activity is affected significantly under water deficit stress conditions in 'Gavioita' cultivar compared to 'Selva' cultivar. In other words, in 'Gavioita' cultivar, the activity of superoxide dismutase enzyme increases significantly under stress (80% FC). Our findings are in agreement to the studies reporting the increased antioxidant enzymes activity in response to water deficit stress in plant species such as pistachio (Habibi and Hajiboland, 2013), wheat (Ahmed et al., 2013), barley (Liang et al., 2005), and sunflower (Manivannan et al., 2008). Superoxide dismutase is the primary scavenger of the reactive oxygen species, which converts superoxide, a precursor of a highly reactive oxygen species, to hydrogen

peroxide (H_2O_2) and oxygen (Habibi and Hajiboland, 2013). Superoxide dismutase belongs to the group of metalloenzymes that react rapidly with spontaneous radicals' free superoxide (Balakhnina and Borkowska, 2011). Catalase and peroxidase along with superoxide dismutase play an important protective role in inhibition process (Zhang et al., 2008). Catalase, in conjunction with superoxide dismutase, converts the toxic superoxide radical to water and molecular oxygen (Ahmad and Haddad, 2011). Peroxidase by oxidation, co-substrates such as phenolic compounds or antioxidants, cause the breakdown of H_2O_2 (Zhang et al., 2008). As shown in Figures 1-3, it can be seen that the catalase and peroxidase enzymes activity also increased with superoxide dismutase enzymes activity in the leaves of 'Selva' and 'Gavioita' cultivars under water stress conditions (40-80% FC). Similar to our findings, catalase and peroxidase enzymes activity increased under drought stress in sunflower (Susaraei et al., 2021; Sayfzadeh and Rashidi, 2011). Peroxidase acts as a regulatory mechanism to cope with water loss under stress conditions by modification in cellular walls (Mauad et al., 2016).). As mentioned before, 'Gavioita' cultivar showed the highest enzymes activities, such as superoxide dismutase than 'Selva' cultivar under water deficit stress conditions. However, it can be concluded that 'Gavioita' cultivar is more resistant to water deficient stress.

Promoting effects of silicon on total phenolics, flavonoid and anthocyanin contents in the leaves and fruits

The non-enzymatic defense system produces compounds with water-soluble antioxidant properties, such as phenols and flavonoids (Racchi, 2013). These metabolites, which act as a second line of defense against ROS, are hydrophilic molecules found in high concentrations in various parts of the cell (Racchi, 2013). There is a direct relationship between total phenol content and their antioxidant activity because phenolic compounds act as a hydrogen donor due to their hydroxyl groups (Robatjazi et al., 2020). Silicon stimulates a series of biochemical reactions in plants leading to the production of phenolic compounds (Munaretto et al., 2018). In the present study, application of all concentrations of Na_2SiO_3 induced the phenolic compounds (total phenolic, flavonoid, and anthocyanin) of 'Gavioita' and 'Selva' cultivars under water deficit stress conditions compared to the control treatment (Table 2), which was probably due to

the high stimulation of the phenylpropanoid pathway under stress condition. Most of the genes encoding phenylpropanoid pathway enzymes are regulated by water deficit stress which stimulates the biosynthesis of phenolic compounds (Sharma et al., 2019). Phenylpropanoid is the main constituent of most phenolic compounds (Hajiboland et al., 2018). Silicon spray significantly induced the synthesis of these compounds in diverse plant species such as oat (Wahed et al., 2019) and maize (Kidd et al., 2001) under stress conditions, which are in consistence with our results. Flavonoids are derivatives of glycosylate, and mainly stored in vacuoles (Racchi, 2013). These compounds act as a defensive metabolite against environmental stress and as a secondary inhibitor for reactive oxygen species (Sharma et al., 2019). These substances are very effective in protection of plant against harmful effects (El-Shazoly, 2019). Anthocyanins can interact with protective molecules in the plant cells and minimize defects in the concentration of molecules under stress conditions. In other words, the anthocyanins in the plant act as free radical scavengers and protect plants against stress (Yosefi et al., 2013). According to the above findings, both 'Gavioita' and 'Selva' cultivars showed a relative tolerance to water deficit stress by elevating internal phenolic compounds.

Promoting effects of silicon on proline content of the leaves and fruits

Osmoregulation is as an efficient mechanism under water deficit stress conditions that preserve the cells by accumulating salts in them (Pang et al., 2019). Proline is one of the osmotic regulators that are effective in rapid elimination of the negative effects of stress. It establishes the membranes and some macromolecules; it is a free radical scavenger with sufficient amounts of carbon and nitrogen elements (Avestan et al., 2019). Proline is synthesized through two pathways of glutamate or ornithine in plants. In the first pathway, glutamate is converted to proline following two consecutive reductions. This process is catalyzed by two enzymes of pyrroline-5-carboxylate (P₅C) synthetase and P₅C reductase. Silicon improves the expression of genes involved in the synthesis of P₅C (Pang et al., 2019). Proline is a general osmoprotectant that plays an antioxidant role as a source of energy (Verma et al., 2019). Accumulation of osmolites such as proline and its osmotic potential is increased by silicon that influences water transport (Robotjazi et al., 2019). In the present study, the leaves and fruits were affected by intense water deficit stress along with foliar

application of higher concentrations of Na₂SiO₃ in both 'Selva' and 'Gavioita' cultivars. More proline was detected from these cultivars than other leaves and fruits (Fig. 7 and 8). In agreement with the results of present study, accumulation of proline has been also detected under water stress conditions by spraying silicon in strawberry plants ('Camarosa' cultivar) (Dehghanipoodeh et al., 2018), and sunflower (Gunes et al., 2008).

Conclusion

In the present study, Na₂SiO₃ increased the phenolic compounds in 'Selva' and 'Gavioita' strawberry cultivars under water deficit conditions. It seems that this enhancement is due to high activity of the phenylpropanoid pathway in both of these cultivars under water stress conditions. Na₂SiO₃ boosted antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase) in 'Selva' and 'Gavioita' cultivars and reduced the negative effects of water deficit. Na₂SiO₃ increased antioxidant enzymes activity in 'Gavioita' cultivar under water stress conditions. Therefore, it can be concluded that (mainly) response of 'Gavioita' cultivar is significantly affected by silicon application under water stress condition.

The leaves and fruits of 'Selva' and 'Gavioita' cultivars were affected by intense water deficit stress along with foliar application of higher concentrations of silicon, and much proline was extracted in these cultivars than other leaves and fruits. Our findings confirmed that application of Na₂SiO₃ at 6 mM is the most influential dosage on 'Gavioita' than 'Selva' cultivar under water stress conditions (40% of FC).

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