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Silica Application Facilitates Vegetative Growth of Winter Squash (*Cucurbita maxima* L.) Under Water-Saving Irrigation

Li-Cheng Huang†, Fu-Yu Yang, Yu Sung†, *

Department of Horticulture, National Chung Hsing University, Taichung, Taiwan

Email address:
yusung@dragon.nchu.edu.tw (Yu Sung)
*Corresponding author
† Li-Cheng Huang and Yu Sung are co-first authors.

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Abstract: Silicon (Si) is used to alleviate abiotic stress in plants. Applying siliceous fertilizer in combination with regulated deficit irrigation may reduce water consumption during plant vegetative growth period. In this study, winter squash 'East Elite' was irrigated with 0.5, 1.0, and 1.5 mM SiO_2 under a field capacity—that is, the water content of 60% (C60, water-saving irrigation). The SiO_2 treatments promoted plant growth including stem diameter, plant height and leaf number; however, the plant growth under water-saving irrigation was slightly (but significantly) lower than that under regular irrigation (C_{80}). Silicon application in the water-saving irrigation can increase the accumulation of fresh and dry weight in the aboveground and underground of plant, but there is no significant difference between the treatments with different concentrations of silicon. Silicon application treatments was significantly higher chlorophyll content (SPAD) than C_{60}, and followed by C_{80}. The SiO_2 in the plants increased with increasing SiO_2 treatment concentration; however, the difference was nonsignificant. The 1.0 and 1.5 mM treatments increased the leaf transpiration rate and stomatal conductance. The growth of the plants treated with 1.5 mM SiO_2 was greater than that of the plants left untreated. The 1.5 mM SiO_2 treatment increased the activity of leaf catalase and peroxidase and reduced the leaf malondialdehyde content of the mild water stressed plants. Irrigation with a SiO_2 solution in a water-saving irrigation system can stabilize plant growth and increase water use efficiency.

Keywords: Winter Squash, Water-Saving Irrigation, Silicon Application, Plant Growth, Antioxidant Enzyme Activity, Water Use Efficiency

1. Introduction

Although Si is the second most abundant element on Earth, pure Si crystals are rarely discovered in nature. Si typically exists in the form of silicate [24]. According to the essential elements for plants proposed by Epstein and Bloom (2005) [6], Si is an essential element for higher plants because plants grow abnormally in the absence of Si and are able to grow normally with Si supplementation [2]. Nevertheless, some researchers doubt the necessity of Si for higher plants because of the lack of evidence that Si exerts a direct effect on plant metabolism and the synthesis of Si-containing organic compounds in plants [4].

Guntzer *et al*. (2012) [9] reported that some dicotyledons, such as Cucurbitaceae, Asteraceae, and legumes, have a relatively high Si content in their shoots. Crop growth is inhibited under drought, and the damage caused by water stress can be alleviated through siliceous fertilizer supplementation. Si is used to alleviate abiotic stress in plants through the following mechanisms: (1) SiO_2 precipitates in plant tissues provide mechanical reinforcement for leaves, enabling them to stand upright; (2) Si increases the amount of light required for photosynthesis and regulates the mobility of nutrients and water; (3) Si boosts the plant antioxidant system; and (4) Si facilitates the complexation and precipitation of toxic metals in plants and soil [19].

Under water stress, decreases in the water content of leaves result in lower turgor pressure, and the stomata close up to reduce evapotranspiration. Under mild water stress, the closing of the stomata reduces the intercellular CO_2 concentration to levels insufficient for photosynthesis. This is referred to as the stomatal limitation to photosynthesis [13]. The ability of Si to promote plant growth under water stress is related to changes in transpiration [24]. Si supplementation can reduce leaf transpiration and water loss under water stress. Studies have suggested that the cuticle bilayer of SiO_2 precipitates formed in the epidermal tissue of leaves may be responsible for the decrease in leaf transpiration after Si supplementation [17, 21, 22].

Photosynthetic pigments are also affected by Si supplementation. Lobato *et al*. (2009) [14] discovered that under water stress, the content of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoid) in peppers could be maintained through Si supplementation. Ma *et al.* (2004) [16] explored the effect of Si treatment on cucumbers under water stress and reported that Si supplementation reduced the decomposition of chlorophyll in cucumber leaves, the permeability of the cell membrane, and malondialdehyde (MDA) level in leaves; maintained superoxide dismutase (SOD) activity; and increased catalase (CAT) activity.

Winter squash (*Cucurbita maxima* L.) is a popular type of squash mainly cultivated in autumn and winter in Taiwan. In Taiwan, winter is a dry season. In recent years, extreme weather has occurred more frequently because of climate change. The probability of drought and the average temperature in winter have increased. Some studies have reported that applying siliceous fertilizer in combination with regulated deficit irrigation may reduce water consumption during the vegetative period and minimize the effects of water deficit. In the present study, we explored the effect of SiO_2 supplementation on plant growth, photosynthesis, and antioxidant capacity during the early growth stage of winter squash under water-saving irrigation, with the expectation that it would stabilize plant growth under water-saving irrigation.

2. Materials and Methods

2.1. Experimental Materials

(1) Test plant: winter squash 'East Elite' (Known-You Seed Company).
(2) Cultivation medium: coir fiber (Bos'n International, Taoyuan, Taiwan).
(3) Cultivation container: 8-L plant pot (24 × 24 cm).

2.2. Experimental Methods

(1) Cultivation site and period: The experiment was divided into two parts and conducted in the greenhouse of the vegetable laboratory at National Chung Hsing University. The experimental period was from March to May.
(2) Experimental treatment:

Experiment 1: 'East Elite' winter squash seedlings were planted in 8-L pots when the primary leaf had fully expanded and started the treatment 1 week later. The field capacity of the water-saving irrigation group was set to be 60%, and that of the control group was set to be 80%. The field capacity was measured using a soil moisture sensor (WET Sensor Kit, Delta-T Devices, UK). In the water-saving irrigation group, modified Yamazaki nutrient solutions containing 500 mL of 0.5, 1.0, and 1.5 mM SiO_2 were manually added to different pots when the field capacity was below 55%. In the control group, the original nutrient solution was applied for irrigation. The treatment codes are listed in Table 1. The experiment contained 3 replicates per treatment, with 3 plants per replicate, for a total of 25 days.

Table 1. *Different treatment codes of water-saving experiment of winter squash.*

Treatment codes	Treatment
C_{80}	80% FC
C_{60}	60% FC
$C_{60}Si_{0.5}$	60% FC+0.5 mM SiO_2
$C_{60}Si_{1.0}$	60% FC+1.0 mM SiO_2
$C_{60}Si_{1.5}$	60% FC+1.5 mM SiO_2
$C_{80}Si_{1.5}$	80% FC+1.5 mM SiO_2

Experiment 2: We planted winter squash 'East Elite' in 8-L pots when the primary leaf had fully expanded, applied 300 mL of 1.5 mM SiO_2 5 days later, and implemented water-saving irrigation. The field capacity of the mild water stress treatment was set to be 60%, and that of the control group was set to be 80%. In the mild water stress group, 500 mL of nutrient solution containing 1.5 mM SiO_2 was manually added to the pots when the field capacity was lower than 55%. In the control treatment, the original nutrient solution was applied for irrigation. The experiment contained 4 replicates per treatment, with 3 plants per replicate, for a total of 15 days.

(3) Cultivation management: The plants were grown through vertical (creeping) cultivation in greenhouse plant beds. Single-stem pruning was applied. The plant spacing was 40 cm.

2.3. Investigated Items

In Experiment 1, we investigated the plant traits (i.e., stem diameter, plant height, and number of leaves), chlorophyll content, net photosynthesis rate of the leaves, stomatal conductance, transpiration rate, intercellular CO_2 concentration, and silicic acid and Si content in the leaves 20 days after the treatment began. In Experiment 2, we examined the plant traits; chlorophyll content; leaf area; fresh weight (FW) and dry weights (DW) of the shoots; leaf total soluble sugar; starch; specific leaf area (SLA); MDA content; and activity of SOD, CAT, ascorbate peroxidase (APX), and peroxidase (POD) 15 days after the treatment began.

(1) Silicic acid and Si content in leaves:

Modifying the method of Yoshida *et al.* (1962) [23], we selected the fourth fully expanded leaf from the growth point, dried it at 80°C, and ground it into powder. We weighed out 0.2 g of the sample (A), placed it into a 100-mL digestion test tube, and added 10 mL of three-acid solution (HNO_3: H_2SO_4: 62% $HClO_4$; 5:1:2 v/v/v). After 12 h, the tube was put into a decomposing furnace at 60°C for 1 h, heated at 180°C for 2.5 h, and removed for cooling. Subsequently, 50 mL of the solution was obtained and filtered with Whatman No. 42 filter paper. The filter paper was then placed into a crucible of known weight (B), dried in an oven at 80°C, and placed into an ashing furnace. It was heated in the furnace at 200°C for 2 h, at 400°C for 1 h, and at 550°C until it was completely ashed. The crucible was removed for cooling and weighed (C). The silicic acid and Si contents were calculated using the following formulae: silicic acid content (%) = (C − B) / A × 100%; Si content (%) = silicic acid content × 0.4677.

(2) Specific leaf weight (SLW): The SLW was measured 15 days after the treatment began and was calculated using the following formula: SLW = leaf DW/leaf area (mg/cm^2).

(3) MDA content:

Modifying the method of Heath and Packer (1986) [10], we selected 0.2 g of the fourth fully expanded leaf from the growth point, added 4 mL of 5% trichloroacetic (TCA), placed the leaf in an ice bath, and ground it into a powder. Subsequently, the solution was centrifuged with a centrifugal force of 10,000 g at 4°C for 5 min and filtered using Whatman No. 42 filter paper. We added 4 mL of 0.5% thiobarbituric acid (TBA; 0.5%TBA dissolved in 20% TCA) to 1 mL of supernatant. The solution was heated in a hot bath at 95°C for 15 min and placed in ice water for 5 min to stop the reaction. A spectrophotometer (U-2900, HITACHI) was used to measure the absorbance at 532 and 600 nm. The product extinction coefficient was 155 mM^{-1} cm^{-1}.

(4) SOD activity:

Modifying the method of Beyer and Fridovich (1987) [3], we selected 0.2 g of the fourth fully expanded leaf from the growth point, added 5 mL of phosphate buffer (PB) (50 mM, pH = 7.8), placed the leaf into an ice bath, and ground it into a powder. Afterwards, the solution was centrifuged with a centrifugal force of 12,000 g at 4°C for 5 min. We then added, in the following order, 1.5 mL of sodium phosphate (50 mM, pH = 7.8), 0.3 mL of methionine (130 mM), 0.3 mL of nitro blue tetrazolium (750 μM), 0.3 mL of EDTA-Na_2 (100 μM), 0.3 mL of riboflavin (20 μM), and 0.25 mL of deionized water to 0.05 mL of supernatant. After 30 min under 4000-lux light, the absorbance at 560 nm was measured using the spectrophotometer (U-2900, HITACHI).

(5) CAT activity:

Modifying the method of Aebi (1974) [1], we selected 0.2 g of the fourth fully expanded leaf from the growth point, added 4 mL of PB (50 mM, pH = 6.8), placed the leaf into an ice bath, and ground it into a powder. Afterwards, the solution was centrifuged with a centrifugal force of 12,000 g at 4°C for 20 min. We then added 2.7 mL of PB (100 mM, pH = 7.0) and 0.1 mL of H_2O_2 (0.1 M) to 0.2 mL of supernatant before quickly placing it into the spectrophotometer (U-2900, HITACHI) to measure the changes in absorbance at 240 nm within 1 min. The product extinction coefficient was 40 mM^{-1} cm^{-1}.

(6) APX activity:

Modifying the method of Kato and Shimizu (1987) [12], we selected the fourth fully expanded leaf from the growth point, added 4 mL of PB (50 mM, pH = 6.8), placed the leaf into an ice bath, and ground it into a powder. The solution was centrifuged with a centrifugal force of 12,000 g at 4°C for 20 min. Subsequently, 0.1 mL of supernatant was retrieved; mixed with 1 mL of potassium PB (0.15 M, pH = 7.0), 1 mL of 1.5 mM sodium L-ascorbate, 0.4 mL of 0.75 mM EDTA, and 0.5 mL of 0.006 M H_2O_2; and quickly placed into the spectrophotometer (U-2900, HITACHI) to measure the changes in absorbance at 290 nm within 1 min. The product extinction coefficient was 2.8 mM^{-1} cm^{-1}.

(7) POD activity:

Modifying the method of Curtis (1971) [5], we selected the fourth fully expanded leaf from the growth point, added 4 mL of PB (50 mM, pH = 5.8), placed the leaf into an ice bath, and ground it into a powder. The solution was centrifuged with a centrifugal force of 12,000 g at 4°C for 20 min. Afterwards, 0.1 mL of supernatant was retrieved; mixed with 1 of mL PB (50 mM, pH = 6.8), 1 mL of 21.6 mM guaiacol, and 0.9 mL of 39 mM H_2O_2; and quickly placed into the spectrophotometer (U-2900, HITACHI) to measure the changes in absorbance at 470 nm within 1 min. The product extinction coefficient was 26.6 mM^{-1} cm^{-1}.

2.4. Statistical Analysis

The experiments were conducted using a completely randomized design. The data were processed through one-way ANOVA by using SAS 9.4 software (SAS Institute, Cary NC, USA; $\alpha = 0.05$). Fisher's least significant difference was used to compare the mean values of the treatment groups.

3. Results

3.1. Effect of Different SiO_2 Concentrations on Winter Squash Cultivated Under Water-Saving Irrigation

The effects of SiO_2 on the growth of winter squash under water-saving irrigation were presented in Table 2. At 20 days after the treatment period began, the control (C_{80}) had the largest stem diameter (9.34 mm), whereas the water-saving irrigation treatment (C_{60}) had the smallest (7.90 mm). Under water-saving irrigation, Si supplementation had no significant effect on stem diameter, which ranged between 8.46 and 8.97 mm. The plant height of the control was the highest (172 cm), whereas that of the C_{60} was the lowest (97.9 cm). Under water-saving irrigation, Si supplementation had no significant effect on plant height, which ranged between 123.9 and 126.2 cm. The control had the highest number of leaves (16.3), whereas the water-saving irrigation treatment had the lowest (12.3). No statistically significant differences were observed among the other treatments (which each had 14.1–14.7 leaves). The water-saving irrigation + Si supplementation treatment had the highest chlorophyll contents (SPAD; 44.42–44.87), followed by the control (43.39), and C_{60} treatment had the lowest chlorophyll content (41.20). The FWs of the leaves, stem, and roots of the control were the highest, whereas those of the water-saving irrigation (C_{60}) treatment were the lowest. Under water-saving irrigation, Si supplementation increased the cumulative FW and DW of the shoot and root biomass. However, the cumulative weights of the different Si treatments did not differ significantly.

Table 2. *The effect of deficit irrigation with different SiO_2 concentration on plant growth of winter squash 'East elite' after treatment for 20 days.*

Treatment	Stem diameter (mm)		Plant height (cm)		Leaves number (No.)		Chlorophyll content (SPAD)	
C_{80}	9.34	a[z]	172.0	a	16.3	a	43.39	ab
C_{60}	7.90	c	97.9	c	12.3	c	41.20	b
$C_{60}Si_{0.5}$	8.97	b	126.2	b	14.1	b	44.42	a
$C_{60}Si_{1.0}$	8.82	b	123.9	b	14.7	b	47.07	a
$C_{60}Si_{1.5}$	8.48	b	124.6	b	14.2	b	44.87	a

[z]Means in a column with the same letter are not significantly different by Fisher's LSD teat at 5% level.
Note: C subscripts are treatments with different field water capacity (FC %); Si subscripts are SiO_2 concentrations (mM).

The silicic acid and Si contents in the leaves after 20 days of treatment were presented in Table 3. The leaf silicic acid content and Si content of the control were 0.60% and 0.28%, respectively; those of the water-saving irrigation (C_{60}) treatment were 0.56% and 0.26%, respectively. No significant differences were observed between the two treatments. Under water-saving irrigation, the addition of 0.5, 1.0, and 1.5 mM Si resulted in silicic acid contents of 0.88%–1.10% and Si contents of 0.41%–0.51%. The silicic acid and Si contents of the three Si supplementation treatments were significantly higher than those of the control and water-saving irrigation treatments. However, no significant differences were identified among the three Si supplementation treatments.

Table 3. *The effect of deficit irrigation with different SiO_2 concentration on vegetative growth of winter squash 'East elite' after treatment for 25 days.*

Treatment	Leaf area (cm^2)	Plant fresh weight (g)						Plant dry weight (g)					
		Leaves		Stem		Root		Leaves		Stem		Root	
C_{80}	3166.9a[z]	69.97	a	163.82	a	42.57	a	10.44	a	12.19	a	2.53	a
C_{60}	1015.2c	20.75	c	82.68	c	21.33	c	3.02	c	7.02	b	1.45	b
$C_{60}Si_{0.5}$	1812.8b	40.35	b	110.23	b	25.13	bc	5.14	b	8.44	b	2.12	a
$C_{60}Si_{1.0}$	1855.1b	39.13	b	101.50	bc	30.01	b	6.34	b	8.06	b	2.17	a
$C_{60}Si_{1.5}$	1872.6b	39.25	b	97.83	bc	29.61	b	6.17	b	7.62	b	2.34	a

[z]Means in a column with the same letter are not significantly different by Fisher's LSD teat at 5% level.
Note: C subscripts are treatments with different FC (%); Si subscripts are SiO_2 concentrations (mM).

Table 4. *The effect of deficit irrigation with different SiO_2 concentration on leaf SiO_2 and Si content of winter squash 'East elite' after treatment for 20 days.*

Treatment	SiO_2 (%)		Si (%)	
C_{80}	0.60	b[z]	0.28	b
C_{60}	0.56	b	0.26	b
$C_{60}Si_{0.5}$	0.88	a	0.41	a
$C_{60}Si_{1.0}$	0.97	a	0.45	a
$C_{60}Si_{1.5}$	1.10	a	0.51	a

[z]Means in a column with the same letter are not significantly different by Fisher's LSD teat at 5% level.
Note: C subscripts are treatments with different FC (%); Si subscripts are SiO_2 concentrations (mM).

The effects of SiO_2 on net photosynthesis rate (Pn) of the winter squash cultivated under water-saving irrigation were illustrated in Table 4. The photosynthesis rate of C_{60} treatment was the lowest (7.40 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). Under water-saving irrigation, Si supplementation in different concentrations increased the net photosynthesis rate of the leaves (9.49–9.63 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). However, no significant differences were observed among the different Si treatment treatments. The water-saving irrigation (C_{60}) treatment had the lowest intercellular CO_2 concentration (304.0 $mg \cdot L^{-1}$). No significant differences were identified among the other treatment treatments (which had intercellular CO_2 concentrations of 329.2–341.0 $mg \cdot L^{-1}$). The stomatal conductance (g_s) under water-saving irrigation (C_{60}) significantly declined to 0.17 mol $H_2O \cdot m^{-2} \cdot s^{-1}$. Following Si supplementation, the stomatal conductance (g_s) of $C_{60}Si_{1.0}$, $C_{60}Si_{1.5}$, and $C_{60}Si_{0.5}$ significantly rose to 0.33, 0.33, and 0.25 mol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively. The water-saving irrigation (C_{60}) treatment had the lowest transpiration rate (Tr) (5.22 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$). Following Si supplementation, the transpiration rate (Tr) of $C_{60}Si_{1.0}$, $C_{60}Si_{1.5}$, and $C_{60}Si_{0.5}$ increased to 9.92, 9.33, and 7.79 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$, respectively.

Table 5. *The effect of deficit irrigation with different SiO_2 concentration on photosynthesis rate of winter squash 'East elite' after treatment for 20 days.*

Treatment	Pn (μmol$CO_2 \cdot m^{-2} \cdot s^{-1}$)		g_s (mmol$H_2O \cdot m^{-2} \cdot s^{-1}$)		Ci ($mg \cdot L^{-1}$)		Tr (mmolH2O$\cdot m^{-2} \cdot s^{-1}$)	
C_{80}	11.65	a[z]	0.46	a	341.0	a	10.75	a
C_{60}	7.40	b	0.17	d	304.0	b	5.22	d
$C_{60}Si_{0.5}$	9.51	ab	0.25	cd	329.2	ab	7.79	c
$C_{60}Si_{1.0}$	9.63	ab	0.33	b	339.5	a	9.92	ab
$C_{60}Si_{1.5}$	9.49	ab	0.33	bc	334.8	a	9.33	b

[z]Means in a column with the same letter are not significantly different by Fisher's LSD teat at 5% level.
Note: Natural light source was used as the light source, and there was not significantly different in PAR among the treatments of 500-600 μmol·m-2·s-1; C subscripts are treatments with different FC (%); Si subscripts are SiO_2 concentrations (mM).

3.2. Effects of Water-Saving Irrigation and SiO_2 Supplementation on Plant Growth and Antioxidant Enzyme Activity

3.2.1. Effect of Water-Saving Irrigation and SiO_2 Supplementation on Plant Growth

The plant growth in each group after 15 days of treatment was presented in Table 6. The stem diameters of the control (10.58 mm) and the $C_{60}Si_{1.5}$ treatment (10.26 mm) were significantly higher than those of the other treatments, followed by the $C_{60}Si_{1.5}$ treatment (9.97 mm). That of the C_{60} treatment was the lowest (9.52 mm). Plant height, number of leaves, and leaf area followed identical trends. No significant difference was observed between the plant heights of the control and the $C_{60}Si_{1.5}$ treatment (169.0–177.2 cm). The plant height of the C_{60} treatment was the lowest (128.4 cm). The control and the $C_{60}Si_{1.5}$ treatment had significantly higher numbers of leaves (16.0–16.4) than the other treatments, whereas the C_{60} treatment had the fewest leaves (13.6). The C_{60} treatment had the smallest leaf area (2167.8 cm^2). Under water-saving irrigation, Si supplementation significantly increased leaf area. The SLWs of the different treatment groups did not differ significantly (3.58–3.89 mg/cm^2).

Table 6. *The effect of deficit irrigation and SiO_2 on plant growth, leaf soluble sugar and starch content of winter squash 'East elite' after treatment for 15 days.*

Treatment	Stem diameter (mm)	Plant height (cm)	Leaves number (No.)	Leaf area (cm^2)	SLW (mg/cm^2)	Leaf total soluble sugar (mg/g DW)	Leaf starch (mg/g DW)
C_{80}	10.58 a^z	169.0 a	16.0 a	3454.0 a	3.89 a	9.65 a^z	7.97 a
$C_{80}Si_{1.5}$	10.26 ab	177.2 a	16.4 a	3786.3 a	3.67 a	9.97 a	7.59 a
C_{60}	9.52 c	128.4 c	13.6 c	2267.0 c	3.58 a	7.59 b	3.01 b
$C_{60}Si_{1.5}$	9.97 bc	153.8 b	15.4 b	2717.6 b	3.83 a	8.99 ab	2.21 b

^{z}Means in a column with the same letter are not significantly different by Fisher's LSD teat at 5% level.
Note: C subscripts are treatments with different FC (%); Si subscripts are SiO_2 concentrations (mM).

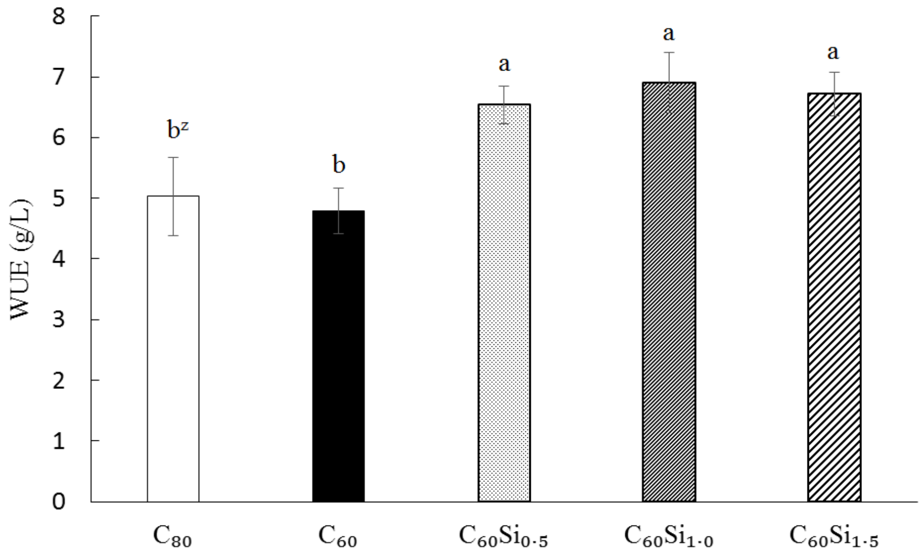

Figure 1. *The effect of deficit irrigation with different SiO_2 concentration on water use efficiency of winter squash 'East elite' after treatment for 25 days I represent standard error ±SE (n=3). ^{z}Mean different letters above the bar indicate a significantly difference by Fisher's LSD test at 5% level.*

Note: WUE = dry weight of whole plant (g)/water consumption per plant (L); C subscripts are treatments with different FC (%); Si subscripts are SiO_2 concentrations (mM).

3.2.2. Effect of Water-Saving Irrigation and SiO_2 Supplementation on Sugar, Starch, and Antioxidant Enzyme Activity

The contents of soluble sugar and starch in the leaves after 15 days of treatments were presented in Table 6. No significant differences were observed between the content of soluble sugar in the control and the Si supplementation treatments (9.65–9.97 mg/g DW). The C_{60} treatment had the lowest content of soluble sugar (7.59 mg/g DW). The content of starch in the leaves in the control was significantly higher than that in the water-saving irrigation treatment (under water stress). Si supplementation had no significant effect on the starch content in the leaves.

The MDA content and antioxidant enzyme activity in the leaves were presented in Table 7. No significant differences were observed in terms of MDA contents under water-saving irrigation (19.15–19.76 μmol/g FW). The MDA content of the C_{60} treatment significantly increased to 29.64 μmol/g FW, whereas that of the $C_{60}Si_{1.5}$ treatment significantly decreased to 24.80 μmol/g FW. The SOD activity of the control was 316.02 U/g FW, and that of the $C_{60}Si_{1.5}$ treatment was 371.36 U/g FW, which was the highest among the treatments. Under water-saving irrigation, Si supplementation slightly increased SOD activity. No significant differences were identified in terms of CAT activity without water-saving irrigation (0.70–0.75 U/min·g FW). The CAT activity significantly declined to 0.22 U/min·g FW under water-saving irrigation, whereas the CAT activity of the $C_{60}Si_{1.5}$ treatment increased to 0.52 U/min·g FW under water-saving irrigation. The addition of 1.5 mM SiO_2 under water-saving irrigation had no significant effect on APX activity. POD activity under water-saving irrigation significantly declined to 2.20 U/min·g FW, whereas that of the plants treated with 1.5 mM SiO_2 under water-saving irrigation significantly increased to 3.34 U/min·g FW.

Table 7. *The effect of deficit irrigation and SiO_2 on leaf malondiadehyde content and antioxidant activity of winter squash 'East elite' after treatment for 15 days.*

Treatment	MDA (μmol·g^{-1} FW)	SOD (U·g^{-1} FW)	CAT (U·min^{-1}·g^{-1} FW)	APX (U·min^{-1}·g^{-1} FW)	POD (U·min^{-1}·g^{-1} FW)
C_{80}	19.15 c^z	316.02 b	0.75 a	3.54 a	3.72 a
$C_{80}Si_{1.5}$	19.76 c	336.41 ab	0.70 a	3.21 a	3.68 a
C_{60}	29.64 a	353.88 ab	0.22 c	1.07 b	2.20 b
$C_{60}Si_{1.5}$	24.80 b	371.36 a	0.52 b	1.45 b	3.34 a

^{z}Means in a column with the same letter are not significantly different by Fisher's LSD teat at 5% level.
Note: C subscripts are treatments with different FC (%); Si subscripts are SiO_2 concentrations (mM).

4. Discussion

4.1. Effect of Different Si Concentrations on the Growth of Winter Squash 'East Elite' Under Water-Saving Irrigation

We grew the winter squash through vertical (creeping) cultivation. In the early vegetative phase, plant growth under 60% FC decreased significantly (Table 2). Under water-saving irrigation, water stress decreased the leaf water potential and turgor pressure, which resulted in the stomata closing and hindered cell growth [11]. Under water-saving irrigation, the stem diameter, plant height, number of leaves, chlorophyll content, leaf area, and plant FW and DW of the three SiO_2 treatments were all significantly higher than those of the control. The increased chlorophyll content (SPAD) and leaf area under water-saving irrigation facilitated photosynthesis. According to the results, under water-saving irrigation, Si supplementation can alleviate the negative effects of water stress. In the experiment conducted by Ma *et al.* (2004) [16], 120 mg of SiO_2 ·kg^{-1} soil was used to grow cucumbers under moderate water stress (55%–70% FC) accelerated the photosynthesis rate and the accumulation of weight in the shoot.

In Experiment 1 in the present study, we treated winter squash different concentrations of Si under water-saving irrigation (60% FC), which increased the stomatal conductance, transpiration rate, and the intercellular CO_2 concentration. Si treatment could reduce the stomata limitation to photosynthesis caused by water-saving irrigation. Ko (2016) [13] reported that the net photosynthesis rate decreased significantly under water-saving irrigation, and the photosynthesis rate could be increased by SiO_2 in various concentrations. In addition to decreasing the photosynthesis rate, water-saving irrigation reduces the intercellular CO_2 concentration, transpiration rate, and stomatal conductance of plant leaves. Under water-saving irrigation, the stomata close and the transpiration rate decreases, which reduced the intercellular CO_2 concentration to levels insufficient for photosynthesis. This was referred to as the stomata limitation to photosynthesis. In an experiment conducted by Gong and Chen (2012) [8], the intercellular CO_2 concentration, transpiration, and stomatal conductance of wheat significantly decreased under water stress, and the stomata limitation significantly increased. The researchers discovered that Si supplementation under water stress could reduce the stomata limitation and increase the photosynthesis rate. In one study, the benefits of Si for plant growth were reported to be related to changes in transpiration [24]. According to Wang *et al.* (2015) [20], Si supplementation under salinity stress could increase the transpiration rate of cucumbers by promoting water uptake by the roots. A possible reason why Si supplementation could maintain the stomatal conductance and transpiration rate and increase the photosynthesis rate of winter squash 'East Elite' to minimize the effect of water stress was that Si could increase water uptake by the roots. The SiO_2 content in the plants indicated that the treatments we applied were absorbed by the roots and transferred to the leaves. We observed no significant differences in the SiO_2 contents of the three Si treatment groups. However, we applied 1.5 mM of SiO_2 in Experiment 2 because it was the highest concentration among tested treatment.

4.2. Effect of Water-Saving Irrigation and SiO_2 Treatment on the Growth and Antioxidant Enzyme Activity of Winter Squash 'East Elite'

We applied 1.5 mM SiO_2 to the winter squash cultivated under C_{60}, and the plant height, number of leaves, leaf area, and FW and DW significantly increased. The plant DW under water-saving irrigation was 40% lower than that of the control (C_{80}). The plant DW under water-saving irrigation with the addition of 1.5 mM SiO_2 was 19% lower than that of the control. This suggests that Si may alleviate the negative effects of water-saving irrigation. Leaves are highly sensitive to water stress. Under water-saving irrigation, the leaf area and plant height both decreased significantly. Si supplementation attenuated the decrease in leaf area to 21.3%, and the C_{80} treatment increased the leaf area. The increase in leaf area facilitated photosynthesis and the accumulation soluble sugar in leaves and promoted plant growth. Si supplementation significantly attenuated the negative effects of water-saving irrigation.

Water deficit impedes cell enlargement, photosynthesis, and plant growth. Oxidative stress induced by water stress occurred because inhibition of photosynthesis leads to difficulty in light harvesting and the imbalanced use of light [7]. Chloroplast photochemical changes under water stress dissipate excess light energy and generate reactive oxygen species, such as O_2^-, $1O_2$, H_2O_2, and OH^- [18]. The accumulation of reactive oxygen species damages cell membranes and results in lipid peroxidation. MDA is an intermediate product of and can be used to measure lipid peroxidation. In this experiment, the leaf MDA content significantly increased under water-saving irrigation and significantly decreased under Si supplementation, indicating that Si could alleviate lipid peroxidation. In the experiment conducted by Ma *et al.* (2004) [16], Si (120 mg/kg) was added to cucumbers under moderate water stress, which reduced the leaf MDA content, indicating that under water-saving irrigation, Si could reduce the effect of oxidative stress.

Many studies have reported that Si promotes antioxidant enzyme activity and alleviates oxidative damage under stress. Liu *et al.* (2009) [15] reported that under low-temperature stress, Si supplementation could increase the activity of SOD, APX, glutathione peroxidase, and glutathione in cucumbers, thereby reducing the content of H_2O_2 and O_2^-. According to Zhu *et al.* (2004) [24], Si supplementation increased the activity of antioxidant enzymes (i.e., SOD and APX) in cucumbers under salinity stress, thereby reducing oxidative damage. In the present study, antioxidant enzyme activity was not significantly different under the 80% FC treatment. However, under the 60% FC treatment, Si supplementation increased the activity of antioxidant enzymes (i.e., SOD, CAT, and POD) in leaves. SOD can convert O_2^- into H_2O_2 and serves as the front line of the antioxidant defense system. CAT and POD can convert H_2O_2 into H_2O to protect against oxidative damage.

5. Conclusion

Melon plant growth was inhibited under drought. In the vegetative growth period, the addition of SiO_2 to winter squash 'East Elite' under water-saving irrigation increase plant growth, transpiration rate, and antioxidant enzyme activity. This increased the photosynthesis rate, reduces lipid peroxidation, and attenuated the negative effects of water-saving irrigation, thereby promoting both plant growth and water use efficiency. The SiO_2 in the plants increased with increasing SiO_2 treatment concentration. This approved the application method to be useful to alleviate the damage caused by water stress. The effect of SiO_2 supplementation could be expected to increase the ability of plants to respond physiologically to adversity in a future changing environment.

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