

Physiological and biochemical responses of Damask rose (*Rosa damascena* Miller) to potassium silicate application under water deficit stress

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Abstract

In this field experiment, the effect of potassium silicate (PS) on the physiological and biochemical responses of Damask rose was investigated under the water deficit stress. The treatments were four levels of irrigation water application including 100, 75, 50 and 25% plant water requirement (PWR) and potassium silicate at three rates (0, i.e., just pure water, 0.2 and 0.4%), once (in spring or summer) or twice (once in spring and once in summer) during the plant growth. The results showed that with irrigation of 75% of plant water requirement significantly reduced the concentration of chlorophyll *a* (Chl *a*, 170%), chlorophyll *b* (Chl *b*, 163%) and carotenoids (91%), the leaf relative water content (RWC, 14.8%) and the total flower yield (20%) as compared to control. The elevated malondialdehyde (MDA) content and ion leakage, as two indicators of oxidative damage, were observed in the plants subjected to the water deficit stress. In response to oxidative stress induced by water deficit stress, the leaf catalase (CAT, 59.5%) activity and concentration of proline (64.8%) as compared to control increased. The foliar-applied Si at two rates of 0.2 and 0.4% in spring and summer resulted in a higher concentration of Chl *a* (57.3% and 61.7%), Chl *b* (31% and 24.6%) and carotenoid content as compared to control, respectively. The increased concentration of proline and higher activity of CAT in the plants supplied with Si led to the higher leaf RWC and less intensity of oxidative damage, namely ion leakage and MDA content. According to the results, with the potassium silicate spraying in 0.2 or 0.4% both in spring and summer at the irrigation level equal to 50% of the PWR, the optimum flower yield was achieved.

Keywords: antioxidant; plant pigments; potassium silicate; *Rosa damascena* Miller; water deficit stress

Abbreviations: CAT: Catalase; MDA: Malondialdehyde; PS: Potassium silicate; PWR: Plant water requirement; RWC: Relative water content; SOD: Superoxide dismutase

Introduction

The flower of Damask rose (*Rosa damascena* Miller.) belongs to the *Rosaceae* family. Damask rose flower has been greatly cultivated in Iran during the recent years mainly due to the adaptation of this plant to different climate conditions, low cost of production, and considerable profitability expected from the production of this plant. The essential oil of Damask rose flower is one of the most valuable materials used in the perfume industry (Baydar and Baydar, 2004). The small industries associated with the production of Damask rose play an important role in economy and job creation in the rural areas (Kodori and Tabaei-Aghdai, 2007).

Plants are often subjected to the environmental stresses such as salinity, drought, high and low temperatures, which greatly reduce the growth and yield (Ahmad and Prasad, 2012). Water deficit is an important environmental stress limiting the production of crops in the arid and semi-arid regions of the world including Iran (Amin *et al.*, 2015). Plants have evolved several physiological and biochemical mechanisms to cope with the biotic and abiotic stresses (González-Orenga *et al.*, 2020). Plants use enzymatic and non-enzymatic antioxidant mechanisms to alleviate the oxidative stress induced by the excess accumulation of reactive oxygen species (ROS) (Hasanuzzaman *et al.*, 2014). It has been reported that the activity of superoxide dismutase and the content of malondialdehyde in maize increased under the water deficit stress, but the grain yield was reduced (Sajedi *et al.*, 2011).

Silicon (Si) is a beneficial nutrient element whose positive effect on the plant tolerance to the environmental stresses has widely been reported (Torabi *et al.*, 2013; Gengmao *et al.*, 2015). It has been reported that the application of silicon enhances the plant tolerance to stress salinity, drought, thermal, and heavy metal etc, through the regulation of free oxygen radicals, decrease of ion leakage, reduction of malondialdehyde content, and decline in sodium adsorption (Kim *et al.*, 2017). Scavenging free radicals is one of the most important defense mechanisms in plants for the tolerance to stress conditions (Baxter *et al.*, 2014). A possible mechanism by which Si enhances plant tolerance to biotic and abiotic stresses is the increase in the activity of antioxidant enzymes and the content of osmolytes (Liang *et al.*, 2007). Silicon improves the plant ability to scavenge free oxygen radicals via increasing the activity of antioxidant enzymes (Tripathi *et al.*, 2017). The application of Si in the form of potassium silicate improved the activity of superoxide dismutase and catalase in honeysuckle medicinal plant under the salinity conditions (Gengmao *et al.*, 2015). It has been reported that the application of silicon enhances the Chl *a* and Chl *b* contents in maize cultivars compared to the NaCl-treated plants and found that the application of silicon can moderate the salinity stress without decreasing the growth attributes in maize (Reza *et al.*, 2019). Pereira *et al.* (2013) reported that the use of silicon in pepper plants subjected to the water deficit stress resulted in the higher proline synthesis.

Despite extensive reports on the positive effect of Si on the tolerance to the stress in several plants, little information is available on the role of Si in Damask rose under the water deficit conditions. Therefore, this field study aimed to investigate the physiological and biochemical responses and flower yield of Damask rose in the foliar-applied Si under water deficit conditions.

Materials and Methods

Experimental procedure

This field experiment was conducted in 2018 as split plot based on the randomized complete block design with three replications at Karimah Zaer research station located in Qom province, Iran. The annual rainfall of the area is 155 mm and the average annual temperature is 24 °C. Table 1 shows the selected physicochemical characteristics of the soil.

Table 1. Physical and chemical soil properties of the experimental site

Soil depth (cm)	EC (dS m ⁻¹)	pH	Absorption available P (mg kg ⁻¹)	Absorption available K (mg kg ⁻¹)	N (%)	Absorption available Si (mg kg ⁻¹)	OC (%)	Sand (%)	Silt (%)	Clay (%)
0-60	1.2	7.9	17	225	0.07	0.07	0.71	36	44	20

EC: Electrical conductivity, OC: organic carbon

The studied factors included the water deficit stress at four levels of 100 (well-watered), 75, 50 and 25% of plant water requirement. These levels of irrigation water were used throughout the growing season. Potassium silicate was used at three rates (0, i.e., just pure water application, 0.2 and 0.4% of potassium silicate solution) once (in spring or summer) or twice (once in spring and once in summer) during the plant growth. Each plot consisted of one row with a distance of 2 meters between the shrubs and a distance of 4 meters between the replications. In each treatment, five shrubs were considered. The study was conducted on 4-year-old flower garden shrubs. The irrigation was performed using the drip irrigation system. The evapotranspiration in the area was calculated through the report No. 56 of FAO and Cropwat software (Bidabadi *et al.*, 2013; Barzegari and Malekinezhad, 2016). The evapotranspiration of Damask rose was calculated according to the following formula (Sharifi Ashoorabadi *et al.*, 2015).

$$ET_c = ET_o \times K_c$$

Where: (ET_o) evapotranspiration of reference plant (alfalfa) based on standard FAO method; (K_c) Damask rose coefficient at each growth stage; (ET_c) evapotranspiration of Damask rose in mm/day.

After calculating the ET_c values with applying plant coefficients in each growth period and summing the daily evapotranspiration of plant growth period (230 days) based on the meteorological data, soil and plant characteristics and planting intervals counting water requirement for leaching and net water and gross water requirement, the water requirement of *Rosa damascena* was determined to be 4500 cubic meters per hectare according to Cropwat software. According to the above calculations, the irrigation was done for 4, 3, 2 and 1 hours once a week in treatments of 100, 75, 50 and 25% PWR, respectively. The irrigation was done from ice water in winter towards the end of growth period using the water counter. The volume of water used for treatments of 100, 75, 50 and 25% PWR was 4500, 3375, 2250 and 1125 m³, respectively. Potassium silicate was prepared from Biniz Tajhiz Company which contained 20% SiO₂ and 10% K₂O₅. The foliar application was carried out in the beginning of April after the leaves were fully expanded and in the end of the flowering stage in the beginning of July. The foliar application of potassium silicate was performed in two stages at a 15-day interval on the aerial parts of the shrubs. In order to measure the physiological and biochemical traits, the samples were collected at fully expanded young leaves on July 25, 2018.

Measurement of plant pigments

For the extraction and measurement of plant pigments, one gram of leaves was ground with 5 mL of 80% acetone in porcelain mortar and centrifuged at 8000 rpm for 10 min. The light absorption rate by the extract was measured at 645, 663, 470, 480, and 510nm wavelengths using the spectrophotometer (Varian 300 Scans, USA). The concentrations of plant pigments were calculated in terms of mg/g of fresh weight (Arnon, 1949; Dhopte and Manuel, 2002).

Determination of cell ion leakage

Ion leakage was measured according to Lutts *et al.* (1996). For this purpose, the discs of leaves were prepared. The discs were washed with distilled water and placed in a tube. They were added 20 mL of distilled water and placed on a rotary shaker at 25 °C for 24 h. Electrical conductivity (EC) was measured using an EC meter (EC₁). In order to measure the total electrolyte leakage, the specimens were placed in an autoclave at 120

°C for 20 min. The EC was measured again (EC_2). Ionic leakage (%) was calculated based on the following equation:

$$EL(\%) = \frac{EC_1}{EC_2} \times 100$$

Where EC_1 is the electrical conductivity of the solution 24 hours after the samples are placed in distilled water and EC_2 is the second reading 20 minutes after the autoclave.

Relative water content of leaves

A number of fully developed leaves were taken from each experimental plot and immediately transferred to the laboratory and weighted. Five discs with a diameter of 1 cm were prepared from each leaf and weighted (fresh weight). Then, the discs were submerged in distilled water for 24 h at 4 °C, the distilled water was discharged and the excess moisture was removed using the filter paper. The discs were weighted (saturated weight) and dried inside the oven at 65 °C for 72 h. The RWC of leaves was calculated from the following equation (Dhopte and Manuel, 2002).

$$\text{Relative water content}(\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \times 100$$

Proline content

To measure free proline, 0.5 g of intact leaves were ground in the porcelain mortar. Then, 10 mL of 3% sulfosalicylic acid was added and the contents of the mortar were stirred. Then, the contents of the mortar were filtered and 2 mL of this solution was added to 2 mL of ninhydrin acid + 2 mL of acetic acid in a test tube and the tube ends were closed. Then, the test tubes were placed in a boiling bath at 100 °C for one hour. Immediately afterwards, the tubes were placed inside the ice and 4 mL of toluene was added to each tube and stirred for 20 sec. The temperature was then allowed to reach the laboratory temperature. Then, the soluble colored part in toluene was isolated and its concentration was read by a spectrophotometer at the wavelength of 520 nm (Bates *et al.*, 1973).

Antioxidant enzyme assay

The Catalase activity was measured using the method described by Siminis *et al.* (1994) at 25 °C with a spectrophotometer. At first, 150 mg of ground leaf samples were mixed with 1.5 mL of extract buffer and centrifuged at 13000 rpm for 20 min. Potassium phosphate buffer and hydrogen peroxide were then added to the solution. The samples were read at time steps of 0, 20, 40, and 60 seconds with a spectrophotometer at a wavelength of 240 nm.

To measure the activity of SOD, 150 mg of ground fresh leaf sample was mixed with 1.5 mL of buffer. The solution was centrifuged for 20 min at 1300 rpm and then the calibration was performed with the control solution containing 2 μ M riboflavin, 134.03 mM betanin, 2.4 mM nitro yellow tetrazolium, 5.372 mM EDTA, and 50 mM potassium phosphate buffer. Then, using the spectrophotometer at a wavelength of 560 nm, it was read at 25 °C in a lightbox at four-time steps of 0, 4, 8 and 12 min after applying 40 watts of fluorescent light (Giannopolitis and Ries, 1977).

Malondialdehyde content

To measure the MDA content, 0.5 g of fresh leaf samples were ground in liquid nitrogen in the mortar for 1 min. Then, the powdered leaves were poured into the test tube and mixed with 5 mL of potassium phosphate buffer (pH = 7). The sample was centrifuged at 1400 rpm for 30 min and then added to 1 mL of the solution after the centrifuge, 1 mL of thiobarbituric acid containing trichloroacetic acid (20%). The solution was heated in a hot water bath at 95 °C for 30 min. In order to stop the reaction, the solution container was heated and quickly transferred to the ice bath and allowed to remain in the bath for 30 min, and then it

was centrifuged at 10,000 rpm for 10 min. The absorption of the solution was measured by UV-VIS spectrophotometer at two wavelengths of 532 nm and 600 nm (Aravind and Prasad, 2005).

Statistical analysis

This experiment was conducted as the split plot based on the randomized complete block design with three replications. The statistical analysis of data was performed using SAS software and the comparison of the means was made using the Duncan's multiple range test (DMRT) at the 5% probability level.

Results

The results showed that the effect of irrigation treatments on the traits of chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids and RWC content, leaf proline, and activity of CAT were significant at the 1% probability level, and the effect on the traits of ion leakage, activity of SOD, and flower yield were significant at the 5% probability level. The effect of foliar application of Si on the traits of carotenoid content was significant at the 5% probability level, and the effect on the traits of chlorophyll *a*, chlorophyll *b*, total chlorophyll, RWC content, ion leakage, leaf proline, concentration of malondialdehyde, activity of CAT, and SOD was significant at the 1% probability level. The interaction between potassium silicate spraying and irrigation treatments affecting the carotenoid content, ion leakage, leaf proline, concentration of MDA, activity of CAT, and SOD was significant at the 1% probability level (Table 2).

With the irrigation of 75, 50 and 25% of plant water requirement, the chlorophyll *a* (13.2, 103.8 and 170%), chlorophyll *b* (42.8, 102.9 and 163%), chlorophyll *a+b* (23.6, 103.4 and 164.3%) and carotenoids (34.8, 77.6 and 91%) contents decreased significantly compared to the normal irrigation conditions. The foliar application of Si at the both levels in summer and spring as well as in summer increased the plant pigments compared to the control (Table 3). Under the well-watered conditions (100% of plant water requirement), the foliar-applied Si at the both rates used in summer or spring as well as in summer improved the chlorophyll *a* (43.4 and 44.5%), chlorophyll *a+b* (31.4 and 28.3%) contents. Reducing the irrigation water from normal to 75% of the plant water requirement with the spraying of Si at different concentrations in spring and in summer increased the plant pigments compared to the control (Table 4). With the irrigation of 50% of plant water requirement, the spraying with different amounts of Si in spring or summer did not affect the carotenoid content, but improved the chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents (Table 5). Potassium silicate spraying at a concentration of 0.4% in spring, spraying of Si with a concentration of 0.2 and 0.4% in spring and in summer, and spraying at a concentration of 0.2% in summer with the irrigation of 25% of plant water requirement significantly increased the carotenoid content (Table 5).

The irrigation of 50% and 25% of plant water requirement increased ion leakage (13.7 and 24.8%) respectively, as compared with 25% of plant water requirement, but spraying with Si at different concentrations reduced ion leakage (19.9, 19.9, 13.8, 22, 17.7 and 10.4%) as compared with control, significantly (Table 3). With the irrigation of 100% of the plant water requirement, the spraying at the 0.4% Si concentration in summer significantly reduced ion leakage. With the irrigation of 75% of the plant water requirement, the spraying with different concentrations of Si either in spring or summer or in both spring and summer significantly decreased ion leakage compared to the control (Table 4). With the irrigation of 50% and 25% of plant water requirement, the ion leakage rate was significantly reduced by spraying with different amounts of Si except for 0.4% concentration in summer (Table 5).

With irrigation of 50% and 25% of plant water requirement, leaf water relative content decreased by %8 and %14.8, compared to the control, respectively (Table 3). The spraying of Si at the concentrations of 0.2 and 0.4% either in spring or summer, spraying at the concentrations of 0.2 and 0.4% in both spring and summer

increased the leaf water relative content relative to the control by 4.4%, 7%, 9.8%, 12.4%, 10.5% and 15%, respectively (Table 3).

Table 2. Analysis of variance for different plant parameters as affected by different experimental treatments

SOV	DF	Mean Square											Essence yield
		Chl <i>a</i> content	Chl <i>b</i> content	Chl <i>a+b</i> content	Carotenoid content	Electrolyte leakage	Relative water content	Proline content	CAT	SOD	MAD	Flower yield	
Replication (R)	2	0.088*	0.002 ^{ns}	0.078*	0.0037**	13.30 ^{ns}	85.58**	0.22 ^{ns}	248.60 ^{ns}	20.06 ^{ns}	11417.80**	37272.91 ^{ns}	0.052*
Irrigation (I)	3	1.59 ^{ns}	0.370**	3.350**	0.44**	287.01*	472.91**	10.12**	37042.36**	2.86*	3122.30 ^{ns}	503938.01*	0.0038 ^{ns}
Ea	6	0.02	0.003	0.019	0.0096	53.97	17.23	0.08	222.30	0.49	6241.16	52512.96	0.018
Potassium silicate (PS)	6	0.14 ^{ns}	0.013**	0.212**	0.222*	79.19**	142.48**	1.88**	8363.26**	8.72**	13862.20**	1951456.00 ^{ns}	0.011 ^{ns}
I×PS	18	0.017 ^{ns}	0.0013 ^{ns}	0.007 ^{ns}	0.010**	69.79**	15.56 ^{ns}	1.14**	2930.44**	4.78**	5965.30**	5622.75 ^{ns}	0.002 ^{ns}
Eb	48	0.023	0.003	0.013	0.0004	24.28	11.45	0.08	126.50	1.08	1531.30	62617.20	0.016
CV (%)		24.07	17.20	12.26	4.54	13.80	4.62	8.32	7.10	29.50	10.77	12.49	0.13

Source of variation (S.O.V), Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), Superoxide dismutase (SOD); Catalase (CAT), Malondialdehyde (MDA)

ns, * and **: not significant, significant at 5 and 1 % level of probability, respectively.

Table 3. Comparisons of the simple effects of water deficit stress and potassium silicate on the measured traits in Damask rose

Experimental treatment	Traits												Essence yield (kg/h)
	Chl <i>a</i> content (mg/g fw)	Chl <i>b</i> content (mg/g fw)	Chl <i>a+b</i> content (mg/g fw)	Carotenoid content (mg/g fw)	Electrolyte leakage (%)	Relative water content (%)	Proline content (µg g ⁻¹ fw)	CAT (U.g fw)	SOD (U.g fw)	MAD (µmol g ⁻¹ fw)	Flower yield (g/plant)		
Water deficit (WD)													
WW	0.909a	0.487a	1.396a	0.666a	32.37b	77.28a	2.59d	103.96c	3.93a	346.39a	2137.68a	0.945a	
WD1	0.803a	0.341b	1.129b	0.494b	33.19b	76.81a	3.42c	157.58b	3.22b	366.71a	2058.65a	0.937a	
WD2	0.446b	0.240c	0.686c	0.375c	36.83ab	71.57bc	3.64b	206.06a	3.18b	375.39a	2035.75a	0.967a	
WD3	0.336b	0.185d	0.528d	0.348b	40.42a	67.28c	4.27a	165.85b	3.7ab	364.78a	1780.1b	0.941a	
Potassium silicate (PS)													
PS1	0.457c	0.276d	0.733c	0.450c	40.84a	67.5e	2.76e	126.93d	2.68b	428.19a	1932.2a	0.905 ^a	
PS2	0.530bc	0.284d	0.814bc	0.420d	34.05b	70.52d	3.34d	161.78b	4.13a	364.11bc	1938.6a	0.929a	
PS3	0.566bc	0.295cd	0.860b	0.436cd	34.04b	72.27cd	3.61bc	163.37b	3.82a	376.91b	1997.7a	0.962a	
PS4	0.719a	0.362a	1.051a	0.540a	35.86b	74.59bc	3.8ab	205.82a	4.63a	365.33bc	2049.9a	0.958a	
PS5	0.739a	0.344ab	1.084a	0.506b	33.46b	77.75a	4.01a	170.37b	4.08a	349.76bd	2048.9a	0.998a	
PS6	0.655ab	0.336abc	0.990a	0.494b	34.69b	74.16bc	3.4cd	147.17c	2.44b	314.12d	2001.8a	0.937a	
PS7	0.713a	0.302bcd	1.013a	0.451c	36.99ab	75.88ab	3.46cd	133.15d	2.64b	334.8cd	2007.2a	0.942a	

Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), Superoxide dismutase (SOD); Catalase (CAT), Malondialdehyde (MDA)

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Duncan's multiple rang test.

Well-watered (WW), Irrigation equal to 75% PWR (WD1), irrigation equal to 50% PWR (WD2), irrigation equal to 25% PWR (WD3), without spraying of potassium silicate i.e just absolute water application (PS1), spraying of PS with concentration of %0.2 in spring (PS2), spraying of PS with concentration of %0.4 in spring (PS3), spraying of PS with concentration of %0.2 in spring along with summer (PS4), spraying of PS with concentration of %0.4 in spring along with summer (PS5), spraying of PS with concentration of %0.2 in summer (PS6) and spraying of PS with concentration of %0.4 in summer (PS7)

Table 4. Comparisons of the interaction effects of potassium silicate application under well-watered (100 % PWR) and irrigation level of 75% PWR on the measured traits in Damask rose

Experimental treatment	Traits											Essence yield (kg/h)
	Chl a content (mg/g fw)	Chl b content (mg/g fw)	Chl a + b content (mg/g fw)	Carotenoid content (mg/g fw)	Electrolyte leakage (%)	Relative water content (%)	Proline content ($\mu\text{g g}^{-1}\text{fw}$)	CAT (U.g fw)	SOD (U.g fw)	MAD ($\mu\text{mol g}^{-1}\text{fw}$)	Flower yield (g/plant)	
WWPS1	0.721 b-c	0.473a	1.194de	0.689b	35.13c-g	76.97a-e	2.32p	127.87jkl	4.25c-h	390.96bcd	2085ab	0.916 a
WWPS2	0.782a-c	0.475a	1.257cd	0.537d	34.92c-g	75.803a-g	2.42op	117.97klm	4.54b-f	385.45bcd	2121ab	0.920 a
WWPS3	0.821 a-d	0.471a	1.292bcd	0.578c	34.27c-g	77.103a-c	2.54nop	101.64mno	5.13a-d	370.97b-c	2193.9a	0.990 a
WWPS4	1.034a	0.535a	1.569a	0.688b	34.18c-g	75.2b-g	2.6m-p	94.19no	2.72f-j	360.65b-f	2185a	0.992a
WWPS5	1.042a	0.49a	1.532a	0.723ab	33.91c-g	79.8ab	2.7l-p	94.36no	3.79c-i	360.05b-f	2121ab	0.983a
WWPS6	0.952ab	0.498a	1.45abc	0.714ab	32.687d-g	77.9a-d	2.72l-p	108.68l-n	2.96c-j	299.35c-h	2120.2ab	0.930a
WWPS7	1.010a	0.473a	1.483ab	0.735a	21.51h	78.2abc	2.85k-p	83.03op	4.16c-h	278.29h	2137ab	0.935 a
WD1PS1	0.551 d-i	0.306b-c	0.857fg	0.429f	37.89b-g	71.3d-h	2.85k-p	136.85jk	2.65f-j	416.32abc	2004ab	0.893a
WD1PS2	0.654 c-h	0.319b-d	0.973f	0.427f	28.89fgh	73.0c-g	2.95i-o	131.747jk	4.96a-c	275.13gh	2020ab	0.909 a
WD1PS3	0.682 b-g	0.338bc	1.02ef	0.432f	36.68c-g	74.8b-g	3.36g-k	142.22ij	4.43b-g	380.74bcd	2036ab	0.938 a
WD1PS4	1.053a	0.374b	1.31bcd	0.690b	32.69d-g	79.0abc	3.5c-i	215.12d	2.85f-j	387.42bcd	2133.9ab	0.989a
WD1PS5	1.013a	0.350bc	1.363a-d	0.564cd	28.09gh	82.3a	3.6d-g	170.48gh	2.84f-j	377.03bcd	2067ab	0.963a
WD1PS6	0.819 a-d	0.364b	1.183de	0.485e	31.32c-g	78.0abc	3.79d-g	177.48f-h	2.44g-j	367.1b-c	2086ab	0.943a
WD1PS7	0.855 a-c	0.342bc	1.197de	0.434f	36.78c-g	79.3abc	3.9d-g	129.2jk	2.51f-j	363.23b-f	2063.7ab	0.922 a

Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), Superoxide dismutase (SOD); Catalase (CAT), Malondialdehyde (MDA)

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Duncan's multiple rang test.

Well-watered (WW), Irrigation equal to 75% PWR (WD1), irrigation equal to 50% PWR (WD2), irrigation equal to 25% PWR (WD3), without spraying of potassium silicate i.e just absolute water application (PS1), spraying of PS with concentration of %0.2 in spring (PS2), spraying of PS with concentration of %0.4 in spring (PS3), spraying of PS with concentration of %0.2 in spring along with summer (PS4), spraying of PS with concentration of %0.4 in spring along with summer (PS5), spraying of PS with concentration of %0.2 in summer (PS6) and spraying of PS with concentration of %0.4 in summer (PS7)

The amount of leaf proline increased under the water deficit stress and spraying with Si (Table 3). The irrigation of 100% of plant water requirement did not affect the amount of leaf proline with the spraying of Si. With the irrigation of 75% and 50% of plant water requirement, the spraying with concentrations of 0.2 and 0.4% of Si in both summer and spring or in summer alone significantly increased the amount of leaf proline (Table 4). The spraying with different concentrations of Si at different times alone or in combination significantly increased the amount of leaf proline under the irrigation of 25% of plant water requirement (Table 5).

The leaf catalase activity increased in the water deficit stress and spraying with Si (Table 3). With the irrigation equal to 100% of plant water requirement, the leaf catalase activity decreased with the Si spraying. With the irrigation of 75% of plant water requirement, the spraying with concentrations of 0.2 and 0.4% of Si in both summer and spring or the spraying by 0.2% in summer significantly increased the catalase activity (Table 4). With the irrigation of 50% of plant water requirement, the spraying at the concentrations of 0.2 and 0.4% of Si in spring or in both summer and spring significantly increased the catalase activity. Also, with the irrigation of 25% of plant water requirement, the spraying with Si at different concentrations and times alone or in combination increased the leaf catalase activity (Table 5).

Water deficit stress did not affect the activity of superoxide dismutase, but the spraying with the concentration of 0.2 or 0.4% of Si in spring and the spraying at the concentration of 0.2 or 0.4% of Si in both spring and summer significantly increased the activity of superoxide dismutase (Table 3). With the irrigation of 100% of plant water requirement, the spraying of Si did not affect the activity of superoxide dismutase. The foliar application of Si with the concentration of 0.2 or 0.4% in spring increased the activity of superoxide dismutase by 87.2 and 67.2%, as compared to the control, under the irrigation of 75% of plant water requirement (Table 4). Therefore, with the irrigation of 50% of plant water requirement, the spraying at the concentrations of 0.2 or 0.4% of Si in both spring and summer increased the level of superoxide dismutase 4.9 and 3.3 times, respectively. Also, with the irrigation of 25% of plant water requirement, the spraying at the concentrations of 0.2 and 0.4% of Si in spring and also in summer increased the activity of superoxide dismutase by 63.8% and 97.8% compared to the control, respectively (Table 5).

Table 5. Comparisons of the interaction effects of potassium silicate application under irrigation level of 50 and 25% PWR on the measured traits in Damask rose

Experimental treatment	Traits											
	Chl a content (mg/g fw)	Chl b content (mg/g fw)	Chl a+b content (mg/g fw)	Carotenoid content (mg/g fw)	Electrolyte leakage (%)	Relative water content (%)	Proline content ($\mu\text{g g}^{-1}$ fw)	CAT (U.g fw)	SOD (U.g fw)	MAD ($\mu\text{mol g}^{-1}$ fw)	Flower yield (g/plant)	Essence yield (kg/h)
WD2PS1	0.370h-j	0.175gh	0.547ij	0.371ghi	40.15b-c	65.33hi	2.900j-o	170.52gh	1.343j	436.13ab	1961ab	0.932 a
WD2PS2	0.409g-j	0.186gh	0.585ij	0.379gh	37/04c-g	69.20g-i	3.230h-l	209.95dc	2.83f-j	372.9b-c	2089ab	0.993 a
WD2PS3	0.433g-j	0.215c-h	0.647g-j	0.384gh	30.89c-fg	71.00e-h	3.430f-j	236.88bc	2.51f-j	327.42d-g	1969ab	0.974 a
WD2PS4	0.468f-j	0.283b-f	0.751ghi	0.395fg	35.71c-g	73.72b-g	3.840d-g	267.01a	6.54a	380b-d	2074ab	0.953 a
WD2PS5	0.492f-i	0.325bc	0.817fgh	0.382gh	33.3c-g	76.00a-f	4.120d	223.36dc	4.43b-g	370.97b-c	2067ab	1.014 a
WD2PS6	0.431g-j	0.274b-g	0.705g-i	0.38gh	33.75c-g	71.03e-h	3.960d-f	159.33hi	2.44g-j	347.74c-g	2010ab	0.936 a
WD2PS7	0.532c-i	0.222c-h	0.751g-i	0.335ij	46.99ab	74.7b-g	4.030dc	175.43fgh	2.19h-j	382.58b-d	2032ab	0.966 a
WD3PS1	0.184j	0.150h	0.334k	0.312j	50.2a	56.4j	2.990i-n	72.47p	3.21d-j	469.25a	1678.6ab	0.894 a
WD3PS2	0.284ij	0.156h	0.44jk	0.338ij	35.36c-g	64.067i	4.750c	187.46fg	4.18c-h	422.96abc	1704ab	0.946 a
WD3PS3	0.329ij	0.156h	0.482jk	0.352hi	34.32c-g	66.167hi	5.100bc	172.73gh	3.21d-j	418.49abc	1762ab	0.947 a
WD3PS4	0.32ij	0.254c-h	0.574ij	0.385gh	40.84bcd	70.433c-g	5.260ab	246.94b	6.35ab	333.26d-f	1806ab	1.033 a
WD3PS5	0.367h-j	0.210c-h	0.624h-j	0.356ghi	38.55b-f	72.9c-g	5.630a	193.260ef	5.26abc	290.97fgh	1923.1ab	0.939 a
WD3PS6	0.417g-j	0.206c-h	0.623h-j	0.395fg	41.03bcd	69.7f-i	3.120h-m	143.180ij	2.04ij	283.31gh	1791ab	0.945 a
WD3PS7	0.454f-j	0.169h	0.623h-j	0.300j	42.68abc	71.3d-h	3.060h-n	144.940ij	1.68j	336.11d-g	1796ab	0.922 a

Chlorophyll a (Chl a), Chlorophyll b (Chl b), Superoxide dismutase (SOD); Catalase (CAT), Malondialdehyde (MDA)

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Duncan's multiple rang test.

Well-watered (WW), Irrigation equal to 75% PWR (WD1), irrigation equal to 50% PWR (WD2), irrigation equal to 25% PWR (WD3), without spraying of potassium silicate i.e just absolute water application (PS1), spraying of PS with concentration of %0.2 in spring (PS2), spraying of PS with concentration of %0.4 in spring (PS3), spraying of PS with concentration of %0.2 in spring along with summer (PS4), spraying of PS with concentration of %0.4 in spring along with summer (PS5), spraying of PS with concentration of %0.2 in summer (PS6) and spraying of PS with concentration of %0.4 in summer (PS7)

The concentration of malondialdehyde significantly decreased with the spraying of Si in different concentrations and seasons (Table 3). With the irrigation of 100% of plant water requirement and the spraying of Si at the concentration of 0.2 or 0.4% in summer, the malondialdehyde content decreased by 30.6% and 40.4% compared to the control, respectively. With the irrigation of 75% of plant water requirement, the spraying of Si at the concentrations of 0.2 and 0.4% in spring or summer or in combination decreased the level of malondialdehyde compared to the control (Table 4). With the irrigation of 50% of plant water requirement and the spraying of Si at the concentrations of 0.4% in spring and at the concentration of 0.2% in summer, the

level of malondialdehyde decreased significantly compared to the control. Also, with the irrigation of 25% of plant water requirement and the spraying of Si at the concentrations of 0.2 and 0.4% in both spring and summer or in summer, the level of malondialdehyde significantly decreased compared to the control (Table 5).

The irrigation of 75 and 50% of plant water requirement compared to the irrigation of 100% of plant water requirement did not have a significant difference in flower yield, but with the irrigation of 25% of plant water requirement, the flower yield decreased by 20% compared to the control (Table 3). With the irrigation of 50% of plant water requirement together with the spraying of Si at the concentration of 0.2 or 0.4% in summer, the optimal flower yield can be achieved (Table 4).

The main effects of water stress and Si and their interaction effect on the essential yield was insignificant (Tables 2 and 3) but in each level of water stress, foliar spray of Si resulted in increase in the essential yield as compared with the control (Tables 4 and 5).

Discussion

The reduced chlorophyll content due to the water stress is associated with the increase in the production of oxygen radicals in cells, where the radicals cause the peroxidation and, as a result, decomposition of photosynthetic pigments (Sheteawi and Tawfik, 2007). The effect of potassium silicate on the stability of plant pigments can be attributed to the accumulation of silicate in epidermal cells, which has an indirect protective effect on the photosynthetic device and therefore, reduces the damage induced by the stress to the photosynthetic pigments (Klyngerda Silva *et al.*, 2013). Similar to our result, moderator effect of Si on biomass and chlorophyll content of corn plants subjected to the drought stress has been reported (Kaya *et al.*, 2006). Silicate reduces the damage to the carotenoids. The increased level of carotenoids with the use of silicate under the drought stress conditions has been reported in oxtongue (Torabi *et al.*, 2013).

Silicon (Si) plays an important role in the plant defense capacity under the environmental stress. Within the plant, silicate is a non-mobile element, which becomes a polymer gel and reduces ion leakage from the biomembranes after being deposited inside the cell (Liang *et al.*, 2007). The results of this study are consistent with the findings of Zhu *et al.* (2004) who stated that the increased stability of the cell membrane in the presence of silicon is due to the wall hardening and firmness. The reduced water loss in the plants supplied with Si may also be due to the lower transpiration from the plants. The accumulation of silicate in the lower epidermal cells reduces the water loss through the cuticle (Hattori *et al.*, 2008).

The results showed that the leaf proline concentration significantly increased with the water deficit and foliar-applied Si. Si may directly or indirectly induce proline biosynthesis (Liang *et al.*, 2006). The treatment of borage (*Borago officinalis* L.) with silica has been reported to increase the amount of leaf proline (Gagoonani *et al.*, 2011). In line with these results, Pereira *et al.* (2013) reported that the effect of silica application increased the proline content in pepper plants subjected to the drought stress.

An increase in the leaf CAT activity was observed under the both water deficit stress and foliar-applied Si. The elevated activity of CAT in plants is an adaptive mechanism, preventing cells from oxidative damage by reducing the hydrogen peroxide concentration produced from the cellular metabolism (Gill and Tuteja, 2010). By stimulating the activity of CAT through the detoxification of hydrogen peroxide, Si prevents the oxidative stress and inhibits the hydroxyl radical production (Rastgoo and Alemzadeh, 2011). Aligned with our results, Gong *et al.* (2005) reported that the use of silica increased catalase and superoxide dismutase in wheat grown under the drought stress.

Superoxide dismutase is an enzyme that catalyzes the free radicals of superoxide into hydrogen peroxide and oxygen and plays an important role in protecting the cells against the adverse effects of the radicals (Linag *et al.*, 2007). SOD is the first cell defense line against the attack of free radicals under the stress conditions (Tale Ahmad and Haddad, 2011). In this study, under the water stress conditions (application of irrigation water by 50 and 25% of the plant water requirement), the foliar-applied Si at the both rates of 0.2 and 0.4% in summer

led to the increased activity of SOD. The effect of Si nutrition on SOD activity and removed free radicals has been reported (Gong *et al.*, 2005). Taheri and Haghghi (2018) reported that the application of potassium silicate at 5 mM concentration increased the activity of antioxidant enzymes in pepper under the thermal stress conditions (35 °C).

Malondialdehyde is the peroxidation product of unsaturated fatty acids in phospholipids. Therefore, the production of malondialdehyde under the stress conditions is used as a marker to indicate the lipid peroxidation (Katsuhara *et al.*, 2005). Due to the dryness, the peroxidation of the glycopeptides of chloroplast thylakoid occurred followed by the production of diacylglycerol, triacylglycerol and free fatty acids, resulting in a higher level of malondialdehyde in the plant tissues (Smirnoff, 1993). Fatty acids and lipids are reported to be highly susceptible to oxygen species and are rapidly oxidized. Si removing the reactive oxygen species decreases the permeability of the cell membrane and increases the activity of catalase, peroxidase and superoxide dismutase, which indirectly reduces the peroxidation of cell membrane lipids and lowers the amount of malondialdehyde (Liang *et al.*, 2007). The results of this study are consistent with other findings indicating the positive effect of Si on the yield of corn (Kaya *et al.*, 2006) and barley (Liang *et al.*, 2003). By decreasing the vegetative growth and changing the anatomical structure of plant through the induction of secondary stresses such as oxidative stress, drought stress causes the changes in the synthesis pathways of secondary compounds and metabolites (Foyer *et al.*, 2012).

Conclusions

The results showed that under the water deficit stress conditions, the catalase activity, proline, and ion leakage increased and the leaf water relative content, plant pigments, and yield decreased in the Damask rose flower. The spraying with potassium silicate at the concentration of 0.2 or 0.4% in spring or summer alone or in combination increased the leaf water relative content, content of plant pigments, activity of catalase and superoxide dismutase, proline content, and flower yield and decreased the rate of ion leakage and malondialdehyde content. In general, the results showed that for the irrigation equal to 50% of the plant water requirement along with the potassium silicate spraying at the 0.2 or 0.4% level both in spring and summer, by improving the activity of the antioxidants and reducing the damage caused by the water stress conditions, the flower Damask rose optimum yield is achieved.

Authors' Contributions

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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