Modulation of Physiological and Biochemical Traits of Two Genotypes of Rosa Damascena Mill. By Nano Silicon Under in Vitro Drought Stress

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Abstract

Drought is a major abiotic stress that prevents plant growth and efficiency. Silicon increases drought tolerance by regulating the biosynthesis and acumulation of some osmolits.This study was conducted to modulate dought stress induced by Polyethylene glycol (PEG) in two genotypes of damasks by nano silicon dioxide ($nSiO_2$). The experiment included three levels of $nSiO_2$ (0, 50 and 100 mg L^{-1}) and PEG (0, 25, 50, 75 and 100 g L^{-1}) added to culture medium. Drought stress decreased protein content while Maragheh genotype under normal conditios and treating with 100 mg L^{-1} $nSiO_2$ had the highest protein content. Under severe drought stress Maragheh genotype had stronger membrane stability index (MSI) than Kashan genotype and explants treated with 100 mg L^{-1} $nSiO_2$ had the highest MSI in control plants. Contrary to the negative effects of drought, plants treated with 100 mg L^{-1} $nSiO_2$ maintained more of their photosynthetic parameters in comparison with other treatments and showed higher amount of protein and proline in Maragheh rather than kashan genotype. Drought stress reduced the values of Fm, Fv/Fm, and Fv. In general, under drought stress, treatment with $nSiO_2$ increased the mentioned characteristics before. It also improved water deficit tolerance through enhancing in the activity of antioxidant enzymes such as catalase, peroxidase, guaiacol peroxidase and superoxide dismutase while the amount of lipid peroxidation and hydrogen peroxide decreased. The results showed that Maragheh genotype may be more stronger in counter with water deficit by improving in water balance, antioxidant enzyme activities, and membrane stability.

Introduction

Rose is one of the most important commercial flowers among ornamentals. It is very popular as an ornamental garden plant, cut flower, potted plant and also medicinal plant[1]. *Rosa damascena Miller var. trigintipetala Dieck* is a pink rose which is a hybrid flower called *Rosa × damascena*[2]. It is suggested that damask rose developed in Iran by hybridaion between *R. moschata* Benth., *R. gallica* L. and *R. feldschenkoana* Regel[3] and therefore it is native to Iran[4] which is developed in the Lyzangan Valley, Fars province originally[5]. The growth and development of damask rose is affected by a variety of agricultural agents[6]. It can be propagated by sucker, cutting, budding and grafting techniques[7] which all of them are the common vegetative methods of propagating *R. damascena* Mill. since the mentiond techniques are time consuming, the use of micropropagation can be useful for producing a lot of genetically similar plants at the same time. Today, using of *invitro* culture systems to investigate the effect of abiotic stress on plant has been introduced. The study of abiotic stress under *invitro* experiment is considered perfectly acceptable because it simulates the field environment in which plants are exposed to adverse conditions in a controlled manner. On the other hand screen of tolerant plant for selection will not be so time consuming.

Drought stress is the most prevailing abiotic stress limits plant growth and efficiency. So, there are remarkable veriety between species for their sensitivity to hard environmental factors and one of the most critical breeding ideas could be improve in plant tolerance to water deficit. limiting in crop productivity by drought stress is due to photosynthesis inhibition through decreases in the level of photosynthetic pigments[8] as well as inhibition of photochemical activity[9]. It negatively affects plant hydraulic balance represented by a decrease in relative water content of leaf (RWC), stomatal conductance and the amount of transpiration with increasing in temperature of canopy and leaf that is possitively correlated to increase in drought intensity[10]. Diminished photosynthesis and respiration lead to generation and accumulation of active oxygen species (AOS) and subsequently oxidative damage of cell compartments including lipid peroxidation, denaturation of proteins and obstruction of nucleic acids[11].

Silicon has not a major role in plant growth and development as an essential element[12]. But lots of researchs have been demonstrated that adding several forms of silicon to the soil enhanced plant efficiency and cause to plant tolerance to a variety of biotic and abiotic stresses[13,14]. It is assumed that nanoparticles are an essential tool to overcome different challenges in crop productivity in all aspects of plant growth and development such as increasing in quantitative and qualitative factors of different crops either in stress or without stress conditions. There are lots of studies demonstrating the possitive effects of nano silicon on plants under drought stress. In fact cell-absorbed nano particles of silicon and increases tolerance to stress [15]. On the other hand, silicon dioxide also increases plant growth and development encuntring with environmental stresses including exteme temperatures and drought[16–18] by enhancing cell wall rigidity[19]. According to the findings of Hajizadeh et al. $nSiO_2$ could improve the growth, biochemical and physiological traits of *Gerbera jamesonii* under salinity (30 mM) by increased the adsorb of Ca and K and decreased absorption of Na[20]. Nano silicon application in strawberry supposed to salt[21] and dryness[22] stress had potential in modulatatin

stress by increasd antioxidant enzyme activities such as CAT, APX, GPX and SOD and decreased MDA and H_2O_2 content. Avestan et al. suggested that addition of $nSiO_2$ to MS culture medium cause to improve in proliferation and growth of apple explants[23].

Based on results form Al-Yasi et al. damask roes is a plant with moderate tolerance to drought stress because it can not be tolerate under severe water deficit[24]. They also concluded that under drought conditions fresh and dry weights of damask roes and all photosynthetic pigments, ecxept for leaf temperature decreased[24]. Al-Yasi et al. suggested two major mechanisms for drought tolerance in damask rose including osmotic and elastic adjustment in 25% FC[24]. Use of 50 or 100 mg L^{-1} nanosilicon dioxide cause to increase in growth of apple explants under water deficit (9 g L^{-1} agar) [24]. Reults of the same experiment showed that application of nanosilicon dioxide at 50 or 100 mg L^{-1} cause to increase in apple explants proliferation in control plants[25]. Under 15% PEG the growth and the amount of protein and chlorophyll concentration of *Phoenix dactylifera* explants decreased but adding of 3.6 mM Si to the medium increased all mentioned parameters and also catalase and superoxide dismutase activity increased[26], however the amount of proline decreased by adding Si to the culture medium.

Although, the interaction between silicon and plant antioxidative status under osmotic stress remains poorly understood, more experiments are necessary to detect the mechanism mediating of plant drought tolerance by means of silicon. Also there is no liturater in damask rose, so the aim of present experiment was to study and screen drought tolerance of two genotypes of *Rosa damascena* by adding nano silicon dioxide under *invitro* culture and to evaluate the potential of nano silicon in modulation of drought stress by measuring physiological and biochemical traits.

Materials And Methods

Experiment design

The purpose of this work was to stimulate drought stress using polyethylene glycol 6000 and modulate it by nano silicon dioxide in two Iranian damask rose genotypes under *invitro* conditions. In this case, according to our preliminary experiments, two genotypes of damask rose were choosed for more experiments according to their reaction to drought stress (data not published yet). Explants from the mid-stem region of one-year stemes (0.4-0.6 cm in diameter) of two local Damask rose (*R. damascena* Mill) from University of Maragheh in west north of Iran (37.3892° N, 46.2534° E) and Kashan (33.9850° N, 51.4100° E) in central region of Iran were chosed. The plant material and seeds for wild collections were obtained under the supervision and permission of Maragheh University guidelines and according to national guidelinesand all authors comply with all the local and national guidelines.The central part of vegetative shoots of damask rose having axillary buds were chosed for the experiment. At the first Each of 1.5 to 2 cm shoot explants were washed with a commercial disinfectant solution for 20 min and then rinsed with running tap water. The mentiond explants were sterilized by 10% chlorox solution for 15 min and then washed 3 times in ddH_2O. Finally they were planted in culture bottles having 25 ml of MS culture medium salts[68] and vitamins plus 30 g L^{-1} agar and 360 μg^{-1} L benzyl adenine and 30 µg L^{-1} NAA. pH of the media was adjusted to 5.7 using NaOH or HCl. All jars containing explants were placed in a germinator with a temperature of 25 °C and 8 hours of darkness, 16 hours of light and 60-70% humidity. After transferring the explants to the germinator, screen them daily and, if there is fungal or bacterial contamination, remove the glasses and autoclave to remove the contamination. Approximately seven days after the establishment of the explants, the first traces of bud growth appeared, and finally, after four to five weeks, when the explants had grown sufficiently, they were taken out of the germinator to be filled and placed in a proliferating environment. For the experiment we used of regenerated plants (~4 cm) after 35 days as an experiment explants (Figure 9) and transferred to sterile bottles including 25 ml of medium (five shoots per bottle), as experiment materials.

Preparing the treatment medium including PEG and $nSiO_2$

Polyethylene glycol was used to induce drought stress. For this purpose, treatments applied at five levels (0, 25, 50, 75 and 100 g L^{-1}) or with osmotic pressure of 0, -0.2, -0.5, -0.7 and -0.9 MPa on two genotypes was applied. After preparing the propagation medium, the shoots were placed in culture medium. After preparing the mentioned concentrations and complete dissolution of PEG in water and adjusting the pH, it was added to the culture medium so that it is one centimeter higher than the medium, then five shoots were placed in each bottle and transferred to the germinator and the level of proliferation of explants, was evaluated fter 4 weeks. Nano particles of silicon (size≤50 nm) were used in our experiment were bought from the NANOSANY Corporation (Mashhad, Iran) the same as our last work[20], and prepared at three levels (0, 50 and 100 mg L^{-1}) and then suplemented to the culture medium in phase

suspension in MS medium[68]. Then five shoots were placed in each glass and transferred to the germinator, after About 14 days collect them and measured the traits. All *invitro* culturs were maitaned at 23 ± 2ºC under a 16/8 h day/night photoperiod provided by coolwhite fluorescent lamps at 40 µmol m^{-2} s^{-1} (Philips TLD 36W/95). After About 14 days collect them and measured the traits.

Mesurment of physiological traits of Rosa damascena

Leaf relative water contents. The amount of RWC of leaf was determined in the fully expanded topmost leaf of explants. At the first the fresh weight of the leaves was recorded and then they were plunged in ddH_2O in a Petri dish. After 2 h and removing the surface water of samples, their turgid weight were recorded. The sample leaves were then placed in an oven at 70°C and deried to reach a stable weight. The amount of relative water content of leaves was calculated as the methods described by Turner[69].

Membrane Stability Index. The leaves were cut into the small samples with the same size. Then leaf discs were weighed and transferd to the test tubes having 10 mL of ddH_2O. The mentined tubes were transferd to a water bath with 40°C for 30 min and then the EC of the samples was recorded. The samples were placed in to the other test tubes and incubated at 100°C in the boiling water bath for 15 min, and their EC was recorded as mentined before. The amount of MSI was evaluated by means of the following formula[70]:

EL%= [EC1/EC2)] x100

Measurement of photosynthetic pigments and chlorophyll fuorescence of leaf. Chlorophyll a, Chlorophyll b, total Chlorophyll and carotenoids were evaluated in leaves of explants according to the method of Arnon [71] by means of spectrophotometer (Shimadzu, Model UV 1800, Kyoto, Japan) at 470, 663 and 645 nm, respectively and calculated as mg g Fw^{-1}. The Chlorophyll parameters of *Rosa damascena* explants were measured using a portable photosynthesis meter (Walz GmbH Eichenring, 691090 Efeltrich, Germany) at the end of experiment. Minimal fuorescence, F0, was evaluated in leaves after 30 min dark-incubation and then for measuring the maximal fuorescence, Fm, we used of the mentined leaf samples under full light conditions. Maximal variable fuorescence (Fv) and the photochemical efficiency of PSII (Fv/Fm) were then evaluated from the recorded parameters[72].

Mesurment of biochemical traits of Rosa damascena

Hydrogen peroxide (H_2O_2) determination. The amount of hydrogen peroxide in explants was carried out following a previously established method by Liu et al.[73]. In this case, 0.5 g of leaf tissues were ground in liquid nitrogen and a potassium phosphate buffer (KPB) (pH 6.8). the grounded leaf sampls were centrifuged at 7000 rpm for 25 min at 4°C. A 100-µL aliquot of the supernatant was added to 1 mL of xylenol solution, completley mixed and let the solution to rest for 30 min. the amount of hydrogen peroxide which is related directly to the intensity of the color and represents ts amount in the samples, was evaluated by spectrophotometer (Shimadzu, Japan) at 560 nm and recorded as µmol gFw^{-1}.

Malondialdehyde (MDA) determination. MDA was determined as 2-thiobarbituric acid (TBA) reactive metabolites[74]. About 1.5 mL extract of each samples were homogenized in 2.5 mL of 5% TBA made in 5% trichloroacetic acid (TCA). The solution was warmed at 95°C for 15 min, and then cooled on ice, quickly. After centrifugation at 5000 rpm for 10 min, the amount of the supernatant absorbance was recorded at 532 nm. The level of malondialdehyde was measured as nmol gFw^{-1} according to the following equation.

MDA=1000× [(532nm – 600nm) × 1/049]/155

Proline determination. The amount of proline was measured by homogenizing 0.2 g fresh weight of leavs in 2 mL of 3% aqueous sulfo salicylic acid and then centrifuged at 10000 rpm for 30 min. The supernatant was removed and the pellet was washed with 3% aqueous sulfo salicylic acid for two times. The supernatant was pooled and the amount of proline was evaluated using ninhydrin reagent and toluene extraction[75] and the protocol for each determination was calibrated with standard curve of proline solution within the detection range of the method (0-39 µg mL^{-1}).

Protein determination. The amount of protein was measurd following the Bradford method[76] and the method was calibrated for each determination with bovine serum albumin standard curve. In this case, 100 mg treated explants were placed in a test tube with 2 mL of 50 mM potassium phosphate buffer at pH 7.0. The solution were centrifuged at 7000-12000 rpm. Then supernatant was recovered

and centrifuged at 3000 rpm for 15 min at 4°C. Samples were praeperd with 1:100 dilution ratio and measured at 595 nm and recorded in terms of mg g Fw^{-1}.

Analysis of Antioxidant enzyme activities. One gram of leaf samples was weighted and quickly homogenized in 5 mL of 50 mM K–phosphate buffer (pH 7.0), brought to 5 mM Na–ascorbate and 0.2 mM EDTA by the addition of concentrated stocks. The homogenated samples were centrifuged at 10000 rpm for 15 min at 4°C. Then the supernatants were used for enzyms assays and were carried out at 4°C. The activity of SOD, POD and CAT was measured, as previously established by Li et al.[77] Fresh leaf samples (0.5 g FW) were chosed from 2-week-old treated explants, harvested and ground in liquid nitrogen and extracted with following described method: 100 mM potassium phosphate buffer (pH 7.8) including 0.1 mM EDTA, 1% (w/v) PVP and 0.1% (v/v) Triton x100. The extracted solution was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant was collected and used for measuring the activity of enzyms. Guaiacol peroxidase activity was assayed by monitoring the increase in absorbance at 470 nm (ε = 26.6 $mM^{-1}cm^{-1}$) during polymerization of guaiacol. One unit of activity was defined as the amount of enzyme producing 1 µmol of tetraguaiacol per min at 25°C.

Statistical analysis

The experiment was conducted as a completely randomized design with 3 replications and five explants in each bottle. Data were statistically analyzed by MSTAT-C software and the means were compared using LSD method and at the level of five percent error probability

Results

physiologycal triats of Damask in response to $nSiO_2$ treatment under in vitro droght stress

Drought treatment decreased the leaf water content of the Maragheh and Kashan genotypes up to 57% and 51% respectively. However treatment with $nSiO_2$ increased the percentage of leaf water content in both control and drought stress conditions as plants exposed to 100 g L^{-1} PEG and without $nSiO_2$ treatment had the lowest leaf water content compared to the same explants treated with 100 mg L^{-1} $nSiO_2$. The comparsion between two genotypes showed that Maragheh genotype had the highest leaf water content (35.57%) in severe drought stress and treatment with 100 mg L^{-1} $nSiO_2$ compared to Kashan genotype (26.68) by 1.3 fold at the same conditions (Figure 1).

In our experiment, the effect of drought stress in damask rose caused to damage on membrane stbility index (MSI). The maximum (37.3%) and minimum decrease (28.9%) in MSI were observed in Kashan and Maragheh genotype, respectively, in control conditons. Increasing in drought stress caused to decrease in MSI in bouth genotypes. However as well as increasing in concentration of $nSiO_2$, the stability of the membranes were increased by 17% and 19% in Maragheh and Kashan, respectively at 100 g L^{-1} PGE and 100 mg L^{-1} $nSiO_2$ compared to controls (Fig. 2).

Drought stress caused to decrease in Chla, Chlb and total chlorophyll by 32.5%, 46.1% and 38%, respectively while exposure to $nSiO_2$ increased all chlorophyll components by about 26% (Fig. 3). Although the interaction between two genotypes and Chla, Chlb and total chlorophyll in response to drought and $nSiO_2$, was not significantly different but decrease in carotenoid content was more in kashan genotype (39%) compared to Maragheh genotype (33%) (Table 1).

Table 1. Effect PEG and $nSiO_2$ on carotenoid, Fv and Fv/Fm content of Maragheh and Kashan genotypes

Treatment		Maragheh	Kashan	Maragheh	Kashan	Maragheh	Kashan
PEG (g L^{-1})	$nSiO_2$ (mg L^{-1})	carotenoid (mg g^{-1} FW)	carotenoid (mg g^{-1} FW)	Fv	Fv	Fv/Fm	Fv/Fm
	0	2.939d	3.032d	3.913ab	3.095jk	0.7823a-c	0.7480g-k
0	50	3.039d	3.94b	3.977a	3.286gh	0.7913ab	0.762d-g
	100	3.483c	4.432a	4.004a	3.723cd	0.7980a	0.7927ab
	0	2.485gh	2.406h-k	3.543e	2.816l	0.7657d-f	0.733j-n
25	50	2.32i-l	2.769e	3.841bc	2.981k	0.7713c-e	0.749g-j
	100	2.579fg	2.926d	3.920ab	3.104jk	0.7767b-d	0.7607d-g
	0	2.124no	2.161m-o	3.5ef	2.625m	0.7507f-i	0.7323k-n
50	50	2.296j-l	2.417h-j	3.683d	2.744lm	0.7653d-f	0.7227l-o
	100	2.519f-h	2.62f	3.841bc	2.756l	0.7703c-e	0.7380i-l
	0	2.104op	1.985pq	3.277gh	2.283op	0.7347i-m	0.7053pq
75	50	2.258lm	2.249l-n	3.478ef	2.495n	0.7477g-k	0.7183n-p
	100	2.442hi	2.411h-j	3.673d	2.695lm	0.7563e-h	0.7327k-n
	0	1.969q	1.825r	3.143ij	2.169p	0.7217m-o	0.6957q
100	50	2.154m-o	2.111o	3.24hi	2.306o	0.733j-n	0.702q
	100	2.323i-l	2.279k-m	3.381fg	2.472n	0.7427h-k	0.7090o-q
Significance							
Drought (a)		**	**	**	**	**	**
Treatment (b)		**	**	**	**	**	**
ab		**	**	ns	ns	**	**
Genotype (c)		**	**	**	**	**	**
ac		**	**	**	**	ns	ns
bc		**	**	ns	ns	**	**
abc		**	**	**	**	**	**

The different letter in attributes were not significant at P< 0.05 by LSD test

Water deficit had no difference on F0 but significantly reduced the Fm parameter in damask as the lowest Fm was observed under 75 and 100 g L^{-1} PEG without $nSiO_2$ treatment while treatment with 100 mg L^{-1} $nSiO_2$ in controls had the highest amount of Fm (Figs. 4a and b). Furthermore, water-deficit reduced Fv and the maximum quantum yield of PSII (Fv /Fm) in both genotypes (Table 1) by

treatment with $nSiO_2$ led to increase in the mentioned factors of drought-stressed plants compared to the same plants without $nSiO_2$ treatment. However Fv and Fv /Fm were higher in the leaves of Maragheh than the other one either with $nSiO_2$ or without $nSiO_2$.

Table 2. The Effect of PEG and $nSiO_2$ on H_2O_2, MDA and Protein content of Maragheh and Kashan genotypes

Treatmenr		Maragheh	Kashan	Maragheh	Kashan	Maragheh	Kashan
PEG ($g\ L^{-1}$)	$nSiO_2$ ($mg\ L^{-1}$)	H_2O_2 ($\mu G\ L^{-1}$)	H_2O_2 ($\mu g\ L^{-1}$)	MDA (nmol g^{-1} FW)	MDA (nmol g^{-1} FW)	Protein (mg g^{-1} FW)	Protein (mg g^{-1} FW)
	0	1.76o	1.647p	1.315q	1.765no	1.95d	1.8ef
0	50	1.403r	1.511q	1.021r	1.255q	2.017c	1.951d
	100	1.203s	1.274s	0.8767r	0.8823r	2.209a	2.116b
	0	2.371l	2.61ij	2.484hi	2.639gh	1.807ef	1.446jk
25	50	2.268m	2.465k	2.098kl	2.269jk	1.417kl	1.495j
	100	2.163n	2.308lm	1.508p	1.864m-o	1.647hi	1.656h
	0	2.919f	2.921f	2.780fg	3.015de	1.731g	1.251n
50	50	2.542jk	2.683hi	2.355ij	2.779fg	1.842e	1.327m
	100	2.279m	2.464k	1.714o	2.481hi	1.927d	1.436k
	0	3.071de	3.123d	3.039c-e	3.217c	1.603i	0.9903q
75	50	2.746gh	2.826g	2.52hi	2.893ef	1.645hi	1.17o
	100	2.606ij	2.585j	1.9mn	2.574h	1.797ef	1.231n
	0	3.893a	3.518b	3.817b	4.122a	1.381l	0.791s
100	50	3ef	3.215c	2.775fg	3.78b	1.626hi	0.8983r
	100	2.721h	3.012e	1.988lm	3.12cd	1.759fg	1.074p
Significance							
Drought (a)		**	**	**	**	**	**
Treatment (b)		**	**	**	**	**	**
ab		**	**	**	**	**	**
Genotype (c)		**	**	**	**	**	**
ac		**	**	**	**	**	**
bc		**	**	**	**	**	**
abc		**	**	**	**	**	**

The different letter in attributes were not significant at P< 0.05 by LSD test

Biochemical traits of Damask in response to $nSiO_2$ treatment under in vitro droght stress

H_2O_2 and MDA. According to the results in Table 2 as well as increasing in PEG level, the amount of H_2O_2 and MDA increased in bouth genotypes as the highest amount of H_2O_2 (3.89 $\mu g\ L^{-1}$) and MDA (4.12 nm g^{-1} FW) were related to Maragheh and Kashan genotype, respectively. While the lowest level of H_2O_2 and MDA under severe drought stress and application of 100 mg L^{-1} $nSiO_2$ were belonges to the Maragheh genotype. So treatment with 100 mg L^{-1} $nSiO_2$ decreased the level of H_2O_2 in Maragheh and Kashan by 30% and 14% and the level of MAD by 48% and 24% (Table 2) under 100 g L^{-1} PEG.

Proline and protein. The interaction between PEG- induced drought stress and $nSiO_2$ treatment was sinificantly different in terms of proline content. Increasing in drought severity caused to increase in proline content by 2.5 folded in 100 g L^{-1} PEG in control. However traetment with $nSiO_2$ decreased the amount of proline and in this case 100 mg L^{-1} $nSiO_2$ was more effective than 50 mg L^{-1} $nSiO_2$ in decreasing of proline production (21% and 35%, respectively) at severe drought stress compared to control (Fig. 5). The interaction between different genotypes of Damask and PEG- induced drought stress (Fig. 6) showed that only at 25 g L^{-1} PEG the amount of proline had more increase in Maragheh (8.6 µmol g^{-1} Fw) compared to other levels of PEG (Fig. 6). The effect of $nSiO_2$ in differenet genotypes of damask showed that proline content in Maragheh was more than Kashan by 9% at control level (Fig. 7) and there were no difference in other concentrations of $nSiO_2$ between two genotypes.

Protein content showed a significant difference between two genotypes and in response to $nSiO_2$ treatment under drought stress. According to the Table 2 protein content had a decreasing trend as long as increasing in PEG concentration in both genotypes. Maragheh damask explants treated with 100 mg L^{-1} $nSiO_2$ had the highest protein content (2.2 mg g^{-1} FW) and Kashan damask explants under control conditions without $nSiO_2$ had the lowest content of protein (0.7 mg g^{-1} FW). With increasing in PEG concentration from 0 to 100 g L^{-1}, protein content decreased by 29% and 61% in Maragheh and Kashan, respectively. However treatment with 50 and 100 mg L^{-1} $nSiO_2$ slowed down the reduction prossecc by 19 and 20% in Maragheh and 56 and 49% in Kashan genotype (Table 2). In general Maragheh genotype under severe drought stress had the highest protein content compared to Kashan.

Antioxidative enzymes. As increasing in the level of PEG, the activity of GPX and POD were increased in both genotypes (Table 3), although Maragheh genotype had the highest GPX activity in 100 g L^{-1} PEG. The interaction between drought stress and genotypes on POD activity was not significantly different. However treating explants with different levels of $nSiO_2$ up-regulated the activity of POD and GPX. According to Table 3 the effect of $nSiO_2$ specially at high level (100 mg L^{-1}) was very obvious in modulating the mentined enzyme activities in Maragheh genotype. Also increasing in the level of PEG, respectively caused to increased SOD and CAT activities and treatment with $nSiO_2$ were increased also the enzymes activity more (Table 3).

Table 3. Effect of PEG and $nSiO_2$ on SOD, POD, GPX and CAT activity of Maragheh and Kashan genotypes

Treatment		Maragheh	Kashan	Maragheh	Kashan	Maragheh	Kashan	Maragheh	Kashan
PEG ($g\ L^{-1}$)	$nSiO_2$ ($mg\ L^{-1}$)	SOD (Unit mg^{-1} protein)	SOD (Unit mg^{-1} protein)	POD (Unit mg^{-1} protein)	POD (Unit mg^{-1} protein)	GPX (Unit mg^{-1} protein)	GPX (Unit mg^{-1} protein)	CAT (Unit mg^{-1} protein)	CAT (Unit mg^{-1} protein)
	0	0.4154u	0.1762y	0.01323n	0.01283n	0.223t	0.1681u	0.1171q	0.1926o
0	50	0.498t	0.2552x	0.01537mn	0.0149mn	0.3114r	0.2693s	0.1575p	0.2010o
	100	0.525s	0.3344w	0.01907l-n	0.0231k-n	0.3634op	0.3087r	0.1965o	0.2205n
25	0	0.5152s	0.3517v	0.0238j-n	0.02553j-n	0.3765no	0.3446q	0.1966o	0.2259n
	50	0.567r	0.4168u	0.02863i-n	0.02873i-n	0.4187l	0.3965m	0.2491m	0.2629m
	100	0.6696p	0.4832t	0.0333h-l	0.0338h-l	0.5044i	0.4524jk	0.3652i	0.2884l
	0	0.8178l	0.5591r	0.02967i-m	0.03383h-l	0.4967i	0.3586pq	0.3452j	0.258m
50	50	0.9485h	0.602q	0.0357g-k	0.03957f-j	0.538h	0.4384k	0.3877h	0.3637i
	100	1.032e	0.7318o	0.04643d-h	0.04607e-h	0.6156f	0.5842g	0.4536f	0.416g
	0	0.8704k	0.7557n	0.03737f-k	0.04783c-h	0.5928g	0.3842mn	0.4293g	0.3087k
75	50	1.007f	0.7898m	0.0519c-g	0.0527c-f	0.675 e	0.5418h	0.4893e	0.3796hi
	100	1.169b	0.9131j	0.0632bc	0.05927b-e	0.761 c	0.7583c	0.5555c	0.4667f
	0	0.9298i	0.903j	0.0432e-i	0.05927b-e	0.7097 d	0.4655j	0.5353d	0.3671i
100	50	1.115d	0.9706g	0.0626b-d	0.06317bc	0.7833 b	0.5934g	0.5926b	0.4207g
	100	1.305a	1.153c	0.08167a	0.0704ab	0.885 a	0.7978b	0.6802a	0.5205d
Significance									
Drought (a)		**	**	**	**	**	**	**	**
Treatment (b)		**	**	**	**	**	**	**	**
ab		**	**	**	**	**	**	**	**
Genotype (c)		**	**	**	**	**	**	**	**
ac		**	**	ns	ns	**	**	**	**
bc		**	**	**	**	**	**	**	**
abc		**	**	**	**	**	**	**	**
The different letter in attributes were not significant at P< 0.05 by LSD test									

On the other hand Maragheh had the highest level of enzyme activity compared to Kashan, so that under 100 g L^{-1} PFG and treatment with with 100 mg L^{-1} $nSiO_2$, SOD activity were 1.30 and 1.15 and CAT activity were 0.68 and 0.52 Unit mg^{-1} protein, respectively in Maragheh and Kashan genotypes. Application of 100 mg L^{-1} $nSiO_2$ under severe drought stress caused to up-regulation of SOD activity by 41% and 28% in Maragheh and Kashan and the effect of 100 mg L^{-1} $nSiO_2$ was better than 50 mg L^{-1} $nSiO_2$ in this case while CAT acivity increased up to 28% and 44% in Maragheh and Kashan at the same situation (Table 3).

Discussion

Physiological traits. One of the most suitable traits for measuring the plant hydraulic balance and subsequently physiological changes in water stressed cells could be likely leaf water content (RWC). According to the Figure 3 water status of Maragheh genotype was higher than Kashan genotype especially in highest drought stress intensity. Application of 100 mg L^{-1} $nSiO_2$ induced 51% and 29% increase in RWC under severe drought stress, respectively in Maragheh and Kashan genotype (Fig. 1). The same findings were demonstrated by Hajizadeh et al.[20] and Ahmadian et al.[27] under salinity and drought stress, respectively. Treatment with $nSiO_2$ under

water deficit conditions caused to increase in the leaf strength. In the leaf tissue, accumulation of silicon as silica form ($SiO_2.nH_2O$) in the cell wall apoplast led to strengths it[28] as the same occurs in our experiment but each of the genotypes had different reactions. Increase in the water uptake and/or decrease in the transpiration losses keep the relative leaf water content at high amount in the plant leaf, that is finally cause to increased photosynthesis and improved the plant tolerance under drought stress[29]. Modulation in the cell wall formation and also increasing the strength of individual organelles of plant cells by silicon increases tolerance to drought stress[30]. Under drought stress conditions, lipid peroxidation decreased the stability of cell membrane. On the other hand, increasing in membrane stability index by silicon treatment has also been reported in various studies[31,32]. Maragheh genotype had the strongest membrane stability index in control plants treated with 100 mg L^{-1} $nSiO_2$ (82.28%) while the lowest MSI was related to Kashan (36.91%) exposed to 100 g L^{-1} PEG without any treatment with $nSiO_2$ (Fig. 2). This result is confirmed by the amount of MDA which was the highest in Kashan control plants under severe water deficit.

Drought stress significantly reduced the content of photosynthetic parameters such as chla, chlb and total chlorophyll and also carotenoids in water stressed plants relative to control plants, especially for the 100 g L^{-1} PEG treatment where there was a 38% decrease in the total chlorophyll. The amount of carotenoid also showed the highest decline in Kashan genotype under severe drought stress and without $nSiO_2$ treatment (Table 1). Reduction of chlorophyll biosynthesis under water deficit stress can be because of the competition between glutamyl kinase (catalyzing enzyme of proline)[33] and glutamate ligase (the first enzyme in the biosynthetic pathway of Chlorophyll)[34] which caused to use of glutamate precursors for proline more than chlorophyll biosynthesis. Also, up-regulation of the Chlorophylase activity under drought stress can be the other reason for chlorophyll diminishes[35]. Treatment with $nSiO_2$ increased chla, Chlb and total chlorophyll and carotenoid content in comparison with control plants (no $nSiO_2$) under stress and non-stress condition (Fig. 3 and Table 1). Beneficial effects of Si or $nSiO_2$ in water-stressed plants could be because of the increased photosynthetic efficiency, stomatal conductivity by means of increased potassium uptake which is responsible for stomatal conductivity and translocation of potassium to the guard cells of stomata[36], and water use efficiency; traits which then led to improved plant tolerance[37]. The same results rely on the improvement in photosynthetic efficiency rate were observed in our experiment (Table 1). However carotenoid content of leaves were increased in severe drought stress by 18% and 24% in Maragheh and Kashan, respectively as treated with 100 mg L^{-1} $nSiO_2$ (Table 1). These findings were in agreement with Ghorbanpour et al.[38] results.

The Fv /Fm is generally an indicator for screening resistance to stress conditions and diminish of Fv /Fm values reveals serious damage to PSII and possible changes in plant photosynthetic rate exposed to stress conditions[39]. In the present work, fluorescence chlorophyll parameters decreased significantly under drought stress; however, treatment with $nSiO_2$ especially at 100 mg L^{-1} concentration decreased them at a lower rate. As increasing in drought stress from 0 to 100 g L^{-1}, the maximum PSII efficiency decreased by 7.6% and 6.7% in Maragheh and Kashan genotypes. However treatment with 100 mg L^{-1} $nSiO_2$ led to increase in the efficiency of photosynthesis so that Maragheh genotype had the higher photosynthetic efficiency compared to Kashan genotype. Decline of Fv/Fm in this study can be related to the chloroplasts damage which can be verified with the data related to the chlorophyll reduction in Table 1. Chlorophyll molecules dissipate within chloroplast and the integrity of thylakoid collapses because of ROS production under drought stress and earlier works showed that silicone increases the amount of photosynthetic pigments in various plants in stress and control conditions[40]. In this work, although drought stress led to reduce in Fv /Fm value, they were significantly higher in damask explants treated with Si under drought stress. The findings of this experiment indicated that drought stress led to decrease in Fm (Figure 4) and Fv (Table 1) values. Probably the reason for the positive effect of Si in maintaining plant hydraulic balance and increasing stomatal conductivity against to more water loss, because of higher water uptake as it is demonstrated by Shen et al.[32]. Silicon transmits light to the leaf mesophyll where the photosynthetic active center is and increases the photosynthesis rate[41]. Also, silicon preserves photochemical reactions from damage of stress by increasing of Fv/Fm values[42]. Using of silicon also

improves maximum performance Quantum PSII was subjected to environmental stress condition[43]. On the other hand, it is likely understood that Si helps to preserve the integrity of chloroplasts despite the severe oxidative stress including collapse of grana as well as stroma lamellae, and increase in the photosynthetic pigments biosynthesis through preserving photosynthetic enzymes. Si most likely has a cofactor role in most of enzymatic reactions involved in the biosynthetic pathways of the mesophyll[44]. Therefore, the Si-treated explants preserved a higher amount of chlorophyll under drought stress conditions which were in agreement with Maghsoudi et al.[41]. According to the results of Atal et al.[45] also a decrease in Fm was also observed. Toivonen and Dell[46] demonstrated that under appropriate supply of CO_2 for photosynthesis, photoinhibition may be prevented. This phenomena has been confirmed the higher Fv/Fm index in wheat. Kaufman et al[47] suggested that silicon settled in the epiderm of plant cells in form of silica and help to the photosynthetic efficiency by transferring light to the mesophyll as mentioned before.

Biochemical traits. One of the biochemical alterations in plants under drought conditions is accumulation of reactive oxygen species (ROS). Some literatures refer to increasing in ROS level under drought stress[48].

Plant cells are able to induce stress conditions by inducing the activity of antioxidative enzymes to overcome the oxidative damage[49]. Therefore, the potency to trap ROS molecules is a compromise approach in plants that plant species use to cope with oxidative stress[48]. To reduce and fix oxidative stress, plants have equipped with a complex antioxidative defense mechanism to preserve cell homeostasis by means of non-enzymatic and enzymatic antioxidants[50].

Water deficit is closely attributed to the production of reactive oxygen species, specially hydrogen peroxide and superoxide anion in water deficit conditions, which in turn caused to damage of membranes and leakage of electrolyte[51]. Under water deficit, accumulation of H_2O_2 and subsequently peroxidation of membrane lipids cause to decay in plasma membrane structure and integrity[52]. In the present study, against the increased levels of catalase and ascorbate peroxidase activities, higher levels of H_2O_2 and MDA accumulated in drought-stressed explants which most likely are due to enhanced photorespiration. However, application of $nSiO_2$ decreased the amount of H_2O_2 and MDA (Table 2) in Maragheh genotype more than the other one. Similar to our results, Gunes et al. demonstrated that the amount of MDA decreased in Si-treated sunflowers during water deficit stress[53], whilst, Shi at al. observed that adding silicon to the nutrient solution of tomato plants under water deficit stress led to plant resictance by means of increasing in the activity of superoxid dismutase and catalase and then improved the ability of roots in water uptake[54]. Having the stable amount of MDA in drought-stressed plants is the best indicator for the positive effects of antioxidative enzymes in maintaining membrane stability against oxidative damage and also the protective effect of $nSiO_2$ on the photochemistry of leaves which is observed in this study is likely another reason for that.

It is suggested that proline plays role as an osmo-protectant molecule and is accumulated under a variety of environmental conditions including water deficit and salinity[55], as seen in this work (Fig. 5). In our study leaf proline concentration significantly increased in drought-stressed plants (Figs. 5 and 6) but not in response to application of nano silicon which was not in agreement with some other scientists[56]. Although some reports showed a reduction in proline content as well as increasing in silicon concentration[57]. The highest amount of Leaf proline was recorded under severe drought stress (100 g L^{-1} PEG). According to the interaction between drought and $nSiO_2$, the lowest amount of proline was related to the control plants treated with 100 mg L^{-1} $nSiO_2$ which means that application of $nSiO_2$ decreased the proline content in plants.

Proline as an osmotic adjuster and antioxidant had the ability to enhance the stability and integrity of membranes[58]. Generally the amount of free amino acids in plants under extreme environments factors including water, salinity, and low or high temperatures were increased and free amino acids can participate in proline synthesis. On the other hand the level of proline degradation in plants under water deficit was decreased. So both mentioned factors can be the reasons of proline accumulation in drought-stressed plants[59]. In plants under stress conditions with inhibited growth, proline cause to hydration of biomolecules and serving energy as a nitrogen reserve source in this period, so it can be readily used for the plants[60]. In some plants, changes in proline levels have been shown to be related to their potency to resist or tolerate to stress conditions and will be used as an indicator for screening plants with resistance to stress[61]. So Maragheh genotype with high level of Proline (Fig. 6) and Protein (Table 2) seems to be more tolerate than Kashan genotype.

The plant protective systems for overcoming the harmful effects of ROSs derived from water deficit stress are the use of antioxidant enzymes including superoxide dismutase, glutathione peroxidase, and catalase[62]. It seems that the ability of each enzyme to trap and scavenge free radicals of oxygen is dependent to the level of enzymes activity[63].

The activity of antioxidant enzymes such as GPX, peroxidase, catalase and superoxide dismutase were high in Maragheh compared to Kashan genotype and also application of $nSiO_2$ caused to more up-regulation of them in Maragheh genotype. In the study of the effect of drought stress on sensitive and tolerant cultivars to drought stress of wheat was reported peroxidase activity increased and that was more in tolerable cultivars[64]. Considering to earlier studies, application of exogenous silicon, improves the ROS scavenging ability of antioxidant enzymes by regulation of their activities[65,66]. Superoxide dismutase initially removes free radicals of superoxide in chloroplasts, since the first place for their generation is photosystem I through light reactions. Catalase which is located in the peroxisome eliminates peroxide hydrogen which is preliminary produced during superoxide dismutase reactions[50]. Similar to Gong et al. treatment of wheat plants with silicon caused high drought tolerance plants through increasing in the activity of antioxidant enzymes including catalase, superoxide dismutase, and glutathione reductase[67]. Similarly, Shi et al. reported the increased activity of superoxide dismutase and catalase in Si-treated tomato plants under water deficit[54].

Pearson Correlation Analysis

The analysis of Pearson correlation demonstrated that photosynthetic pigments were positively correlated with RWC and MSI, chlorophyll florescence parameter and protein. Similarly, the correlation detected between MAD, proline and H_2O_2 were positive while they displayed a negative correlation with photosynthetic pigments, RWC, chlorophyll florescence parameters and protein. Antioxidant enzymes including SOD, POD, GPX and CAT had a significant positive correlation with each other which is illustrated in figure 8.

Conclusion

The aim of the present work was to investigate the physiological and biochemical responses of two genotypes of damask rose to different water deficit severity and compare their tolerance in response to $nSiO_2$. Two damask genotypes including Maragheh and Kashan where naturally distributed in different rainfall regions with annual rainfalls of 322.4 mm and 116 mm, respectively. Drought stress led to the oxidative damage in cell membrane, protein and nucleic acid and disrupts their functional efficiency. As long as increase in the level of water deficit, relative water content of leaf, chlorophyll pigments and protein values decreased, but proline, H_2O_2, MDA content and the activity of superoxide dismutase, peroxidase, guaiacol peroxidase and catalase increased in both genotypes. Application of $nSiO_2$ decreased the amount of proline and protein while the activity of antioxidant enzymes increased. Under moderate and severe water deficit and in presence of 100 mg L^{-1} $nSiO_2$, Maragheh genotype had higher RWC, Chla, Chlb, MSI, carotenoid, protein, Fv/Fm, SOD, GPX, CAT and POD and lower MDA and H_2O_2 in leaf than Kashan genotype. In the present experiment Changes in the amount of antioxidants and degradation Cell membranes markers were observed, which varied depending on the genotype and application of the $nSiO_2$. The findings of the present experiment demonstrated that water deficit affected the growth of damask differently in genotypes. Use of $nSiO_2$, especially at 100 mg L^{-1} concentration, declined the harmful effect of water deficit induces by PEG. In normal conditions also, there was significant difference in treatment with $nSiO_2$ in both genotypes as the positive effect of $nSiO_2$ was obvious in plants. It can be concluded that $nSiO_2$ may produce proline, activated so many antioxidative enzymes and preserve photosynthetic pigments that cause reduction in oxidative stress, improve photosynthesis rate and increase the efficiency of chlorophyll and phytochemicals of damask rose. Therefore, the findings suggest that application of $nSiO_2$, can be helpful to damask plants either in drought stress or without stress, although the effectiveness of $nSiO_2$ is related to the stress intensity. However, more studies are required to understand completely the mechanism of $nSiO_2$ in the plant growth and development improvement. These results suggested that Maragheh genotype may maintain stronger during water deficit through increase in water-conservation capacity, antioxidative activities, and cell membrane integrity.

Declarations

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Author Contributions

H.S.H. perceived the idea, S.A. conducted the field experiments, F.R. and S.A. data collection and analysis, H.S.H wrote first draft of manuscript, H.S.H. and V. O. reviewed and prepared final draft of manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Figures

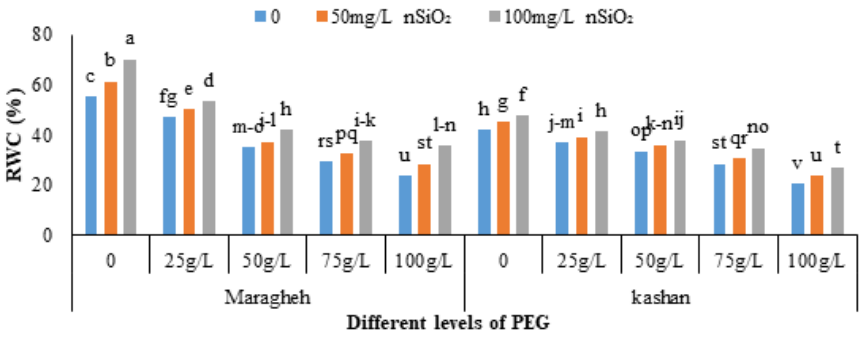

Figure 1

The interaction between drought stress and nSiO2 on leaf water content of two Damask genotypes

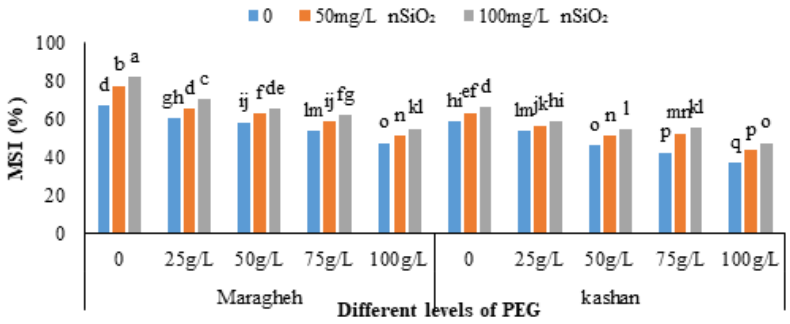

Figure 2

The interaction between drought stress and nSiO2 on leaf membrane stability index of two Damask genotypes

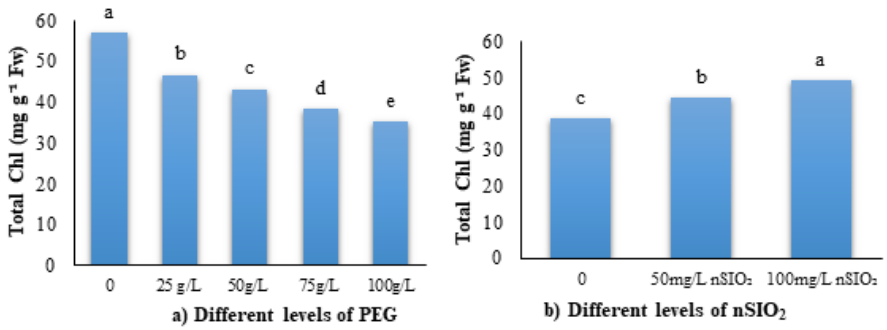

Figure 3

The effect of drought stress (a) and (b) nSiO2 on Chla,Chlb and total chlorophyll of two Damask genotypes

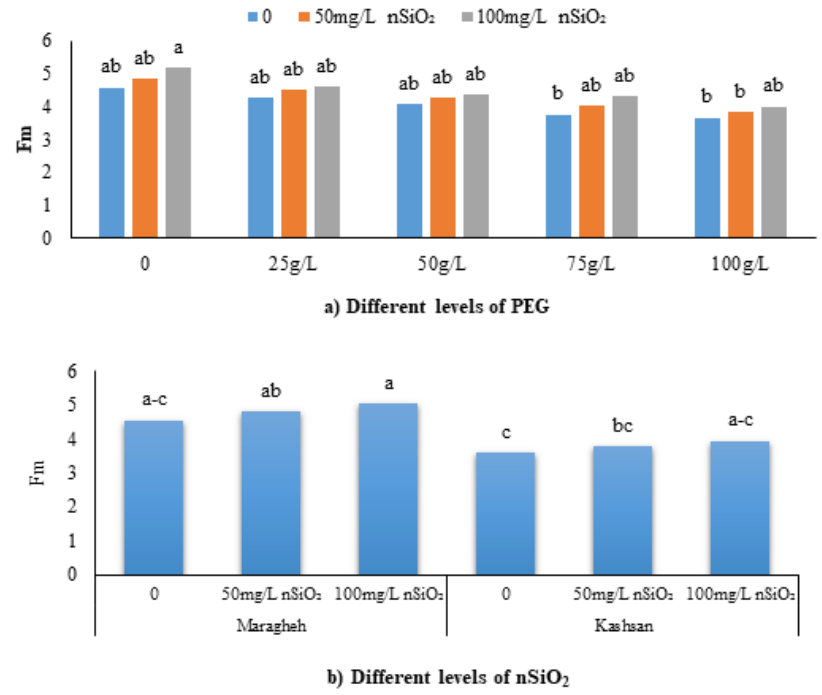

Figure 4

The (a) interaction between drought stress and nSiO2 and (b) differents levels of nSiO2 on Fm content in two Damask genotypes

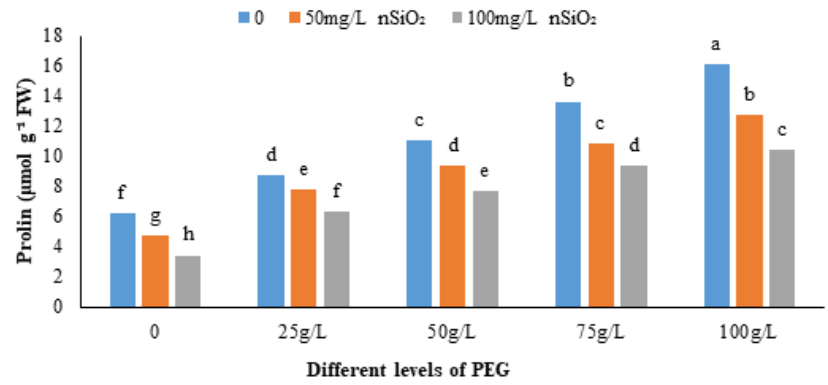

Figure 5

The interaction between drought stress and nSiO2 on leaf Proline content of damask

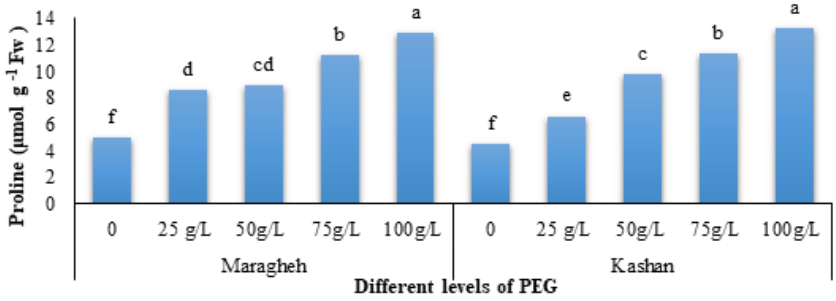

Figure 6

The interaction between drought stress and Damask genotypes on Proline content

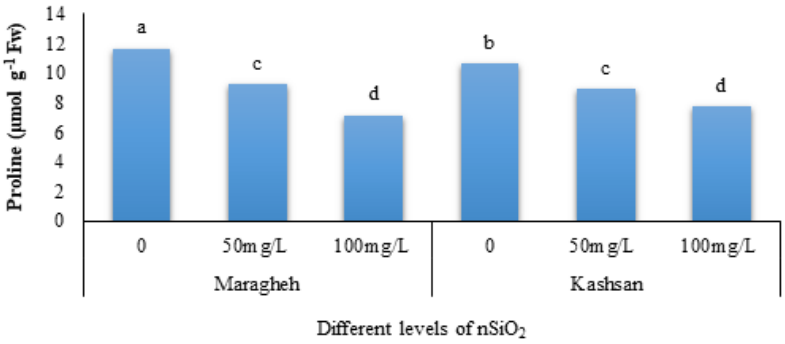

Figure 7

The interaction between nSiO2 and Damask genotypes on Proline content

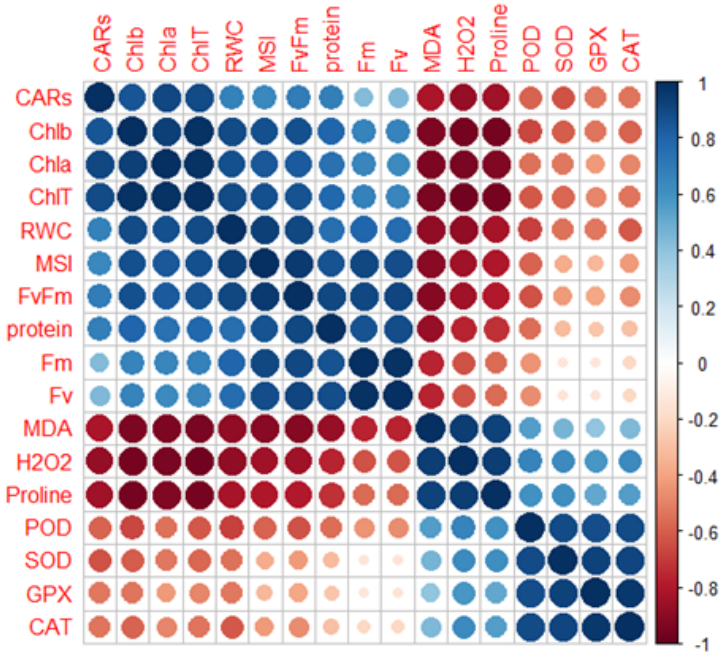

Figure 8

Pearson correlation analysis of nSiO2 treatment and variable trait relationship in damask under control and different drought conditions. Heatmap of Pearson correlation coefficient (r) values of variable traits, where the colored scale indicates the positive (blue) or negative (red) correlation and the 'r' coefficient values (r = -1.0 to 1.0). The tested variables included carotenoids, Cars; Chla, Chlb, ChlT, relative water content, RWC; membrane stability index, MSI; maximum PSII, Fv/Fm; protein, Pro; maximal fluorescence

from dark-adapted leaf, Fm; variable fluorescence, Fv; malondialdehyde, MDA; hydrogen peroxidase, H2O2; proline, Pro; peroxidase, POD; superoxide dismutase, SOD; guaiacol peroxidase, GPX and catalase, CAT.

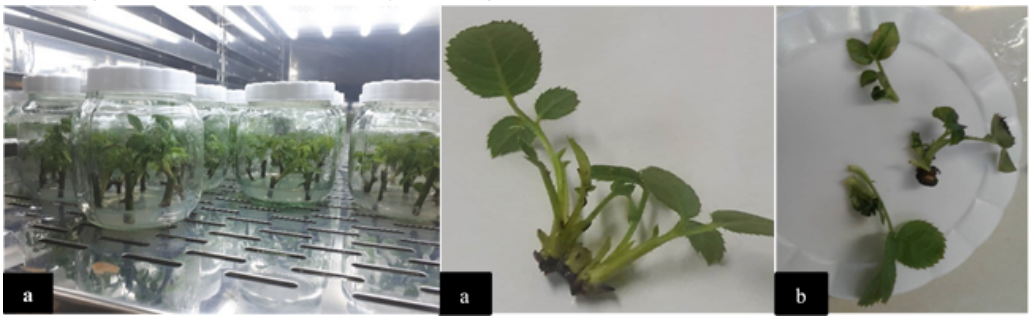

Figure 9

a; Invitro shoot proliferation of damask rose, b; the shoots regenerated from one explant