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Article

Responses of *invitro* Strawberry Plants to Drought Stress under the Influence of Nano-Silicon Dioxide

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Abstract: Drought is an important factor seriously affects agricultural production worldwide. Agricultural practices that can increase resistance to drought are gaining importance. In this study the role of nano-silicon dioxide ($NaSiO_2$) in countering drought stress in *invitro* strawberry plantlets were investigated. In the experiment, the effects of PEG 6000 concentrations (0, 4, 8%) and $NaSiO_2$ concentrations (0, 50, 100 mg L^{-1}) on *invitro* strawberry plants were determined. Plantlets treated with PEG 6000 showed reduced vegetative growth parameters, but this decrease was reduced with $NaSiO_2$ application. $NaSiO_2$ at 50 mg L^{-1} induced the maximum shoot and root fresh weight (1.20 g, 1.24 g, respectively) and length (40.09 mm, 34.26 mm, respectively), leaves number (16.67 pieces/plant) and SPAD index 53.57 among 4% and 8% PEG applications. When the SOD and CAT activities were examined, the results showed that application of $NaSiO_2$ enhancement drought stress tolerance by promoted certain antioxidant response by increasing SOD and catalase (CAT) activities under drought stress. According to the results $NaSiO_2$, limited the devastating impact of drought stress and markedly enhanced all the examined parameters.

Keywords: abiotic stress; nanoparticles; drought; strawberry; silicon dioxide; tissue culture

1. Introduction

Climate crisis has a significant impact on agricultural production and reported that its effects will increase significantly in the next 20 years [1]. The most influential factor on agricultural production is drought, which affects plant growth, causes serious decreases in yield and negatively affects sustainable productivity [2–4]. Considering that approximately 80% of water resources are currently consumed by irrigated agriculture [5,6], it is seen that agricultural production is under serious threat due to increasingly arid conditions. In order to minimize the effects of agricultural drought and to avoid future risks, it is necessary to take measures and develop appropriate strategies as soon as possible. Drought reduces leaf relative water content, membrane stability index, net CO_2 assimilation rate, stomatal conductivity, transpiration and chlorophyll content [7–9] and so decreases; root and leaf growth [10–13], fresh and dry masses, leaf area and number [14] in plants.

Strawberry (*Fragaria* x *ananassa* Duch.), which is defined as a functional food source beneficial to human health due to its rich content of antioxidants, polyphenols, fiber, vitamins and many nutritional elements [15–17], is the most consumed berry fruit in the world [18]. This herbaceous plant is affected by environmental factors and frequently exposed to abiotic stress [8,19]. In arid and semi-arid areas, problems such as low precipitation, high evaporation and temperature, negatively affect strawberry cultivation [20,21]. It has been reported in various studies that the growth and development of strawberry plants are affected by drought [8–12,22].

The measures that can be taken to eliminate the drought, are limited. In recent years, various exogenous applications that allow the increase of strawberries' tolerance to abiotic stress conditions have attracted attention [3,8,10,11,23–25]. Some nanoparticle applications, which have less harmful environmental impact compared to many conventional products, are known to have the potential to increase tolerance to drought [10,23,26,27]. External applications of nanoparticles (NPs) contribute to

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strong root tissue formation, regulation of antioxidant enzyme activity and cellular water balance [7,23,26,28,29]. One of the precursors of these nanoparticles is SiO_2, which has recently been shown to play an effective role in regulating various mechanisms involved in abiotic stress in plants [30–34]. Silicon (Si) is recognized as one of the most valuable elements for plant life [35]. NaSi supplementation affects the activation of photosynthetic enzymes, the activation of antioxidant defense systems, increases water use efficiency, root growth and hydraulic conductivity, thus it can be effective in eliminating the negative effects of abiotic stress conditions and contribute to vegetative growth [7,10,36,37]. In this study, it was aimed to determine the effectiveness of $NaSiO_2$ on the tolerance of 'Albion' strawberry cultivar exposed to drought stress *invitro* conditions.

2. Materials and Methods

2.1. Material

The experiment was conducted in the tissue culture laboratory of Akdeniz University in 2019-2020. $NaSiO_2$ (Nanografi Nano Technology) average diameter 15-35 nm and with purity of 99.5% was used in the study. Morphological study of nanoparticles was done by scanning electron microscope (SEM) (ZEİSS-LEO 1430) (Figure 1). Drought stress was imposed using polyethylene glycol (PEG 6000, Sigma Aldrich Company Ltd.).

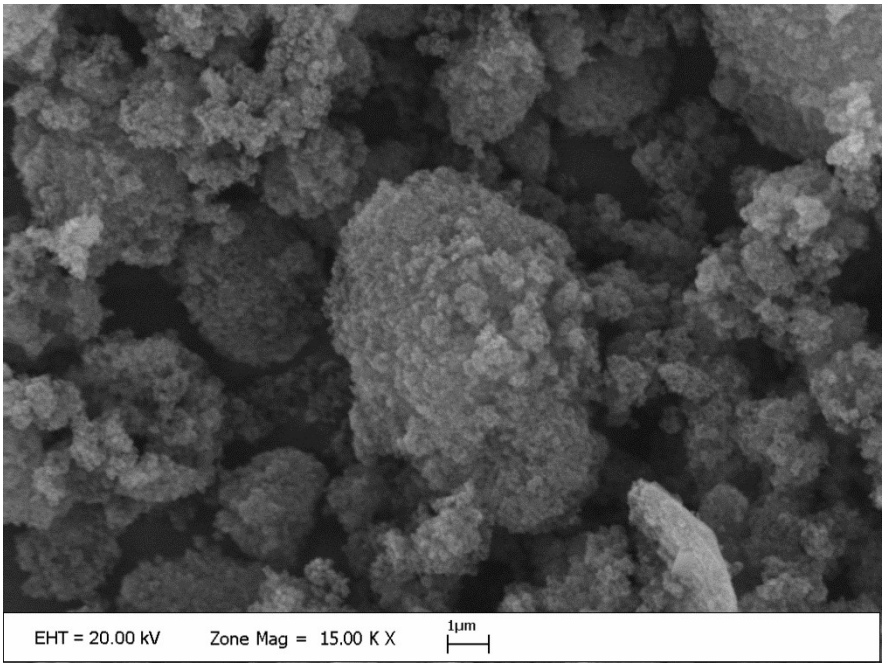

Figure 1. Imaging of the $NaSiO_2$ Scanning electron microscopy (SEM).

'Albion' strawberry variety was used as the donor plant in the experiment. The plants used in the experiment were grown in the glass greenhouse located in the Akdeniz University Research and Application Area. About 2-3 cm long runner tips were collected from donor plants and excised for aseptic sterilization (Figure 2a). Explants were washed with tap water for 2 hours, then surface sterilized with 70% ethanol solution for 30 s. and washed with sterilized distilled water twice. After that samples were soaking in 10% sodium hypochlorite solution for 10 min then vigorously rinsed with sterilized double-distilled water. The leaves, scales and hairs on the disinfected explants were cleaned under aseptic conditions under a binocular microscope and meristems were obtained [38]. Meristems were transferred to full strength MS starting medium containing 1.5 mg/l IBA and 1 mg/l IAA and cultured in a climate chamber (25±2 °C, 16/8 photoperiod, 40 µmol m^{-2} s^{-1} irradiance intensity) [38] (Figure 2b). These plantlets subcultured for three cycles on a 4-weekly interval before the drought and $NaSiO_2$ treatments (Figure 2c). The media pH was adjusted to 5.7 before autoclaving at 121 °C for 20 min.

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(a) (b) (c)

Figure 2. Different steps during in vitro propagation of strawberry cultivar 'Albion' (**a**) explants used for meristem isolation; (**b**) plantlet shooting from an isolated meristem; **(c)** in vitro plants subcultured for three cycles on a 4-weekly interval.

2.2. Preparation of Treatments

Within the scope of the study, different PEG 6000 (0, 4, 8%) $NaSiO_2$ concentrations (0, 50, 100 mg L^{-1}) were used to determine the effects of $NaSiO_2$ application on the tolerance of strawberry plants exposed to drought stress in vitro. Combinations of $NaSiO_2$ and PEG concentrations are given in Table 1.

Table 1. This is a table. Tables should be placed in the main text near to the first time they are cited.

PEG Concentration (%)	$NaSiO_2$ Concentration (mg L^{-1})	Treatments	
0	0	T1	0% PEG+0 mg L^{-1} SiO_2
	50	T2	0% PEG+50 mg L^{-1} SiO_2
	100	T3	0% PEG+100 mg L^{-1} SiO_2
4	0	T4	4% PEG+0 mg L^{-1} SiO_2
	50	T5	4% PEG+50 mg L^{-1} SiO_2
	100	T6	4% PEG+100 mg L^{-1} SiO_2
8	0	T7	8% PEG+0 mg L^{-1} SiO_2
	50	T8	8% PEG+50 mg L^{-1} SiO_2
	100	T9	8% PEG+100 mg L^{-1} SiO_2

Deionized water was used to prepare suspensions at different concentrations (50 and 100 mg L^{-1}) of $NaSiO_2$. These mixtures were homogenized with a sonicator (JL-360, Shanghai, USA) for 30 minutes. The obtained homogenized mixture was used in the preparation of MS medium containing PEG at different concentrations without waiting. Similar trained plantlets were sub-cultured into new MS medium which contain different doses of $NaSiO_2$ and PEG-6000 and cultured for 35 days (25±2 °C, 16/8 photoperiod, 40 µmol m^{-2} s^{-1} irradiance intensity).

The experimental design was 2 factorial, arranged in a completely randomized design with 3 replications. Fifteen jars were used as each replicate and one plantlet were transferred in each jar. All measurements were performed 35 after transferring the plants to the described media.

2.3. Growth measurement

At the end of the experimental period, growth measurements were recorded on five randomly selected plants per treatment. Healthy plantlets were removed from glass jars and gently washed with tap water to remove culture medium attached to the roots. Root and shoot length and shoot diameter were measured a digital caliper. Fresh weight of shoot and roots were measured using a digital balance (Kern ABJ 320-4NM) with a sensitivity of 0.0001g. Dry weights were determined by drying the samples at 65 °C for 48 h in an oven. SPAD index was measured with a non-destructive

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field chlorophyll meter (SPAD–502, Konica Minolta Sensing, Inc., Tokyo, Japan) by the average of twenty readings per sample. Measurements were taken between 10:00 a.m. - 12:00 p.m., on the three most light-exposed leaves.

The leaf relative water contents (LRWC) was determined on three leaves of the same age and following the method of Sanchez et al., (2004) [39]. The LRWC was calculated as follows:

LRWC=(FW-DW)/(TW-DW)x100FW: Fresh weight, DW: Dry weight, TW: Turgid weight

2.4. Enzyme Activities Assay

The procedure of enzyme extraction was done under 4 °C. Enzymes were extracted 1 g leaf sample kept at -80 °C with 5 ml of extraction buffer, with a mortar and pestle, containing 0.1 M phosphate buffer (pH 7.8). The homogenized suspension was obtained by centrifuging at 10 000 rpm for 10 min at 4°C. The supernatant was used as assaying SOD and CAT. Spectrophotometric analysis was conducted on a Shimadzu 2401 UV/visible light spectrophotometer.

Catalase activity (CAT) was determined by the disappearance of H_2O_2 by measuring the decrease in an absorbance at 240 nm for 1 min due to H_2O_2 [40]. The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and enzyme.

SOD activity was defined as the photochemical reduction of nitro blue tetrazolium (NBT), according to Bayer and Fridovich (1987) [41]. One-unit SOD activity was defined as the amount of SOD required for 50% inhibition of NBT (*p*-nitro blue tetrazolium chloride) reduction measured at 560 nm of the reaction mixture containing 50 mM phosphate buffer (pH 7.8), 33 µM NBT, 10 mM l-methionine, 0.66 mM EDTA and 3.3 µM riboflavin.

2.5. Statistical Analysis

Analysis of variance (ANOVA) was applied to data using SPSS/PC+ Statistics software package (SPSS 23.0, SPSS, Inc. Chicago, Ill, USA). Differences among treatments were analyzed taking $P \leq 0.05$ as significance according to Duncan's multiple range test. Pearsion correlation were done using SPSS 23.0 to demonstrate the correlation between the various measurements and their relationship with the different treatments.

3. Results

3.1. Growth measurement

Mean squares due to different concentrations of $NaSiO_2$, PEG and their interaction were significant for root and shoot growth of *invitro* strawberries (Table 2).

Table 2. Influence PEG and $NaSiO_2$ treatment on vegetative growth of strawberry plants.

PEG (%)	$NaSiO_2$ (mg L^{-1})	Treatments	SW (g)	SL (mm)	SD (mm)	RW (g)	RW (mm)
0	0	T1	0.95 ab	44.88 abc	7.36	1.23 ab	36.22 a
	50	T2	1.21 a	57.85 a	9.52	1.57 a	34.00 a
	100	T3	1.23 a	53.55 ab	9.06	1.53 a	38.01 a
4	0	T4	0.63 b	27.84 de	7.09	0.83 bc	16.69 c
	50	T5	1.20 a	40.09 bcd	8.26	1.24 ab	34.26 a
	100	T6	1.00 ab	39.88 bcd	8.71	1.21 ab	30.01 ab
8	0	T7	0.62 b	18.53 e	6.46	0.47 c	14.32 c
	50	T8	0.70 b	32.42 cde	7.58	0.84 bc	23.08 bc
	100	T9	0.90 ab	29.77 cde	7.90	0.79 bc	27.53 ab

SW: shoot fresh weight, SL: shoot length, RW: root fresh weight, RL: root length, SD: shoot diameter, LN: number of leaves, LW: leaf width, LL: leaf length. * Values are the mean ±S.D. of three replicates (n=3). Treatments with

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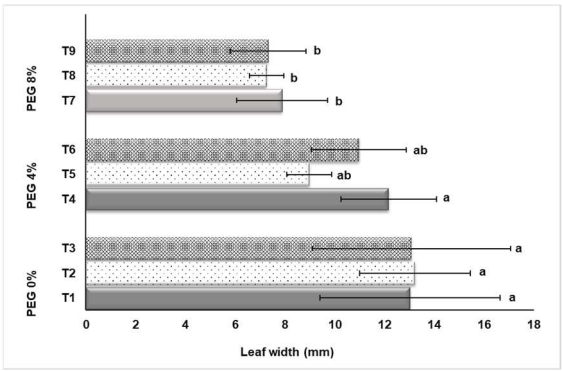
the same lower-case letters were not significantly different based on mean comparison by Duncan's test at $p > 0.05$.

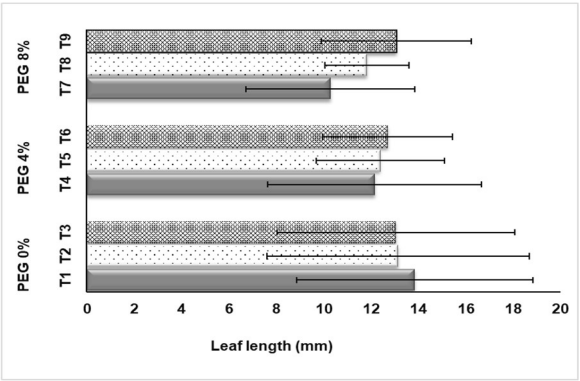
The highest responses of shoot and root fresh weight, shoot and root length, were obtained on the medium supplemented with 50 and 100 mg L^{-1} $NaSiO_2$ among all treatments (Table 2). Plants had water stress induced by PEG application, the increased PEG in the medium exerted a negative influence on the vegetative growth of shoots, whereas, $NaSiO_2$ treatment supplemented in the growth medium, stimulated a positive response in growth and development parameters. $NaSiO_2$ at 50 mg L^{-1} induced the maximum shoot fresh weight (1.20 g) and length (40.09 mm) among 4% and 8% PEG applications (Table 2). As seen in Table 2, the average highest root weight was similarly measured in T2 (1.57 gr) and T3 (1.53 gr) applications. The lowest average value was determined in the T7 (0.47 gr). The effect of Si had a similar effect on almost all vegetative parameters and it was also found to be effective on root length in tolerating the adverse conditions created by drought. The highest mean root lengths among all treatments were obtained in the control group (T1, T2, T3) and T5 group. An average of 34.26 mm root length was obtained from 50 mg L^{-1} $NaSiO_2$ treatments, among 4% PEG applications in which the drought effect was created. In the 4% PEG application, which $NaSiO_2$ did not apply, an average root length of 16.69 mm was obtained, which is even less than half of this value.

Considering all the data obtained, it has been proven that the addition of $NaSiO_2$ is effective in *invitro* strawberry cultivation. On the other hand, it was determined that the addition of 50 mg L^{-1} $NaSiO_2$ to the medium was effective in improving shoot and root growth parameters at 4% PEG concentrations, while adding 100 mg $NaSiO_2$ gave better results when the PEG concentration increased to 8%.

We documented the growth of plantlets in terms of leaf growth (Figure 3). Responses to drought conditions differed according to the doses of PEG application and $NaSiO_2$ treatment. It was determined that the effects of PEG and $NaSiO_2$ applications on leaf width were significant (Figure 3a). The highest average leaf width values among the different treatments were obtained in plants without PEG application (T1, T2, T3). In the groups in which 8% PEG was applied, drought stress was effective and thus leaf width values decreased. However, as seen in Figure 3a, the use of $NaSiO_2$ had a positive effect in control group (T1, T2, T3) on the increase in leaf width. According to the results obtained, leaf length was not affected statistically ($p \leq 0.05$) by the PEG and $NaSiO_2$ applications. When Figure 3b is examined, it is seen that; while the highest value was seen in the 0% PEG + 0 mg L^{-1} $NaSiO_2$ with 13.85 mm, the lowest value was observed with 10.30 mm in the application of 8% PEG +0 mg L^{-1} $NaSiO_2$. According to the evaluated results, the highest average leaves number was seen in the 0% PEG + 0 mg L^{-1} $NaSiO_2$ application (17 pieces/plant), followed by 4% PEG + 50 mg L^{-1} $NaSiO_2$ application (16.67 pieces/plant) (Figure 3c). The lowest value was obtained from plants (8% PEG+0 mg L^{-1} $NaSiO_2$; 13 pieces/plant) in which the highest PEG dose was applied and $NaSiO_2$ was not applied. It was observed that 4% and 8% PEG application decreased number of leaves, while $NaSiO_2$ treatment were found to improve the number of leaves (Figure 3c).



(**a**)



(**b**)

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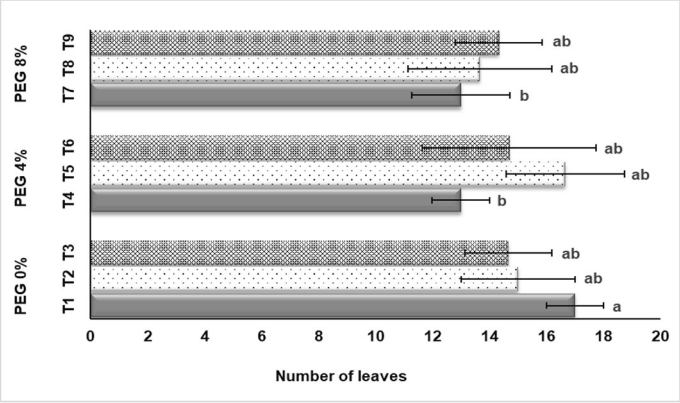
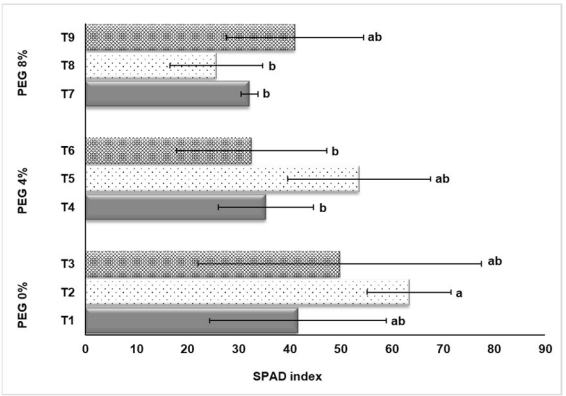


(c)

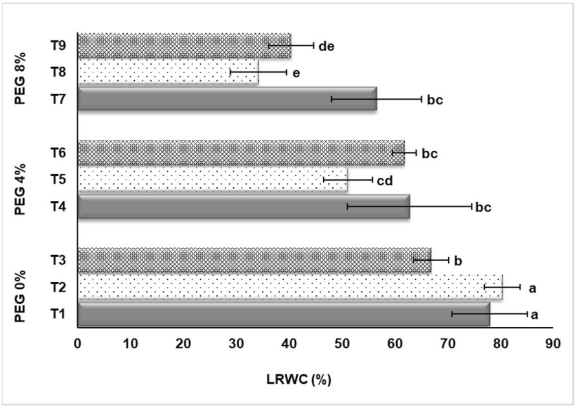
Figure 3. Influence of PEG and $NaSiO_2$ treatment in *invitro* strawberry leaves: (**a**) Leaf width (mm); (**b**) Leaf length (mm); (**c**) Number of leaves. (T1) 0% PEG+0 mg L^{-1} $NaSiO_2$; (T2) 0% PEG+50 mg L^{-1} $NaSiO_2$; (T3) 0% PEG+100 mg L^{-1} $NaSiO_2$; (T4) 4% PEG+0 mg L^{-1} $NaSiO_2$; (T5) 4% PEG+50 mg L^{-1} $NaSiO_2$; (T6) 4% PEG+100 mg L^{-1} $NaSiO_2$; (T7) 8% PEG+0 mg L^{-1} $NaSiO_2$; (T8) 8% PEG+50 mg L^{-1} $NaSiO_2$; (T9) 8% PEG+100 mg L^{-1} $NaSiO_2$. Values are the mean ±S.D. of three replicates (n=3). Common letters are not significant ($p < 0.05$).

3.2. SPAD index and Leaf relative water content (LRWC)

As seen in Figure 4 (a), four treatment groups showed a decreasing trend in SPAD index due to drought stress caused by PEG application. However, T2 group had the highest SPAD value (63.37) among the control groups without PEG application. When control the group (T1, T2 and T3) was examined; it is observed that the SPAD index increases significantly with $NaSiO_2$ application. In this case, it can be said that silicon application has a significant effect on the SPAD index in strawberry cultivation *invitro*. A similar situation can be observed when looking at the SPAD values of the plants in the group T4, T5 and T6 in which drought stress is created by 4% PEG application. The average SPAD values of the T5 plants (4% PEG + 50 mg L^{-1} $NaSiO_2$), were determined as 53.57. This value was determined as 35.34 in the T4 group without $NaSiO_2$ application. In the T7, T8, and T9 groups in which 8% PEG was applied; higher SPAD index values were obtained from T9 application compared to T7 and T8 applications, and this group was statistically in the same group as the T1 group in which drought stress was not applied (Figure 4a).



(a)



(b)

Figure 4. Effect of PEG and $NaSiO_2$ treatment on SPAD index (a) and LRWC (b) in strawberry leaves under *invitro* conditions. (T1)) 0% PEG+0 mg L^{-1} $NaSiO_2$; (T2) 0% PEG+50 mg L^{-1} $NaSiO_2$; (T3) 0% PEG+100 mg L^{-1} $NaSiO_2$; (T4) 4% PEG+0 mg L^{-1} $NaSiO_2$; (T5) 4% PEG+50 mg L^{-1} $NaSiO_2$; (T6) 4% PEG+100 mg L^{-1} $NaSiO_2$; (T7) 8% PEG+0 mg L^{-1} $NaSiO_2$; (T8) 8% PEG+50 mg L^{-1} $NaSiO_2$; (T9) 8% PEG+100 mg L^{-1} $NaSiO_2$. Values are the mean ±S.D. of three replicates (n=3). Common letters are not significant ($p < 0.05$).

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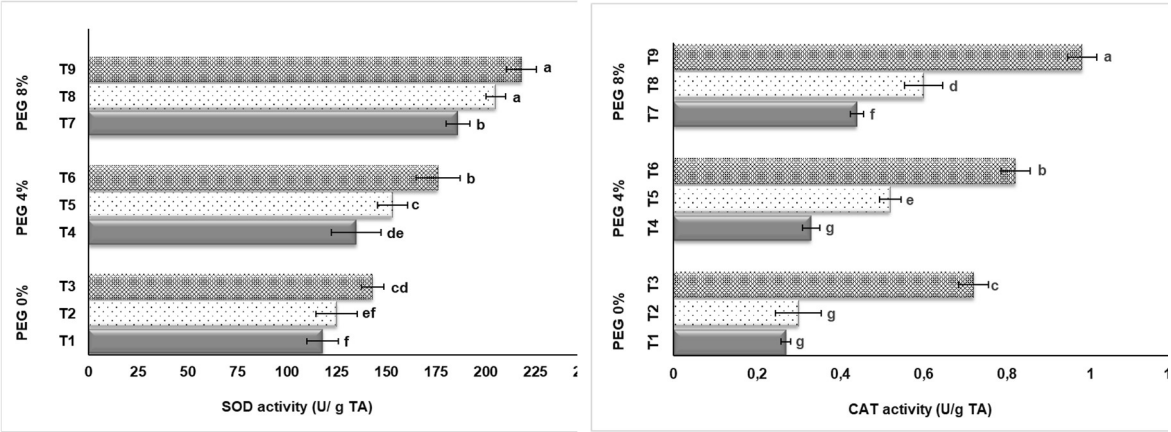
Drought stress significantly reduced LRWC content in *invitro* strawberries (Figure 4b). The LRWC of plants exposed to drought stress decreased by up to 57% (T1: 80.40%, T8: 34.21%). In the study, it is seen in Figure 4 (b) that the effect of $NaSiO_2$ on LRWC, which is aimed to determine its effectiveness in tolerating the effects of drought stress, is low. When the control group (T1, T2 and T3) in which drought stress was not applied was examined, there was no significant difference between T1 without $NaSiO_2$ application and T2 application with 50 mg L^{-1} $NaSiO_2$. The LRWC content of the T3 group, on which 100 mg L^{-1} $NaSiO_2$ was applied, remained lower than T1 and T2. A similar situation was also reflected in the results obtained from plants in the T4, T5, T6, T7, T8, T9 groups treated with 4% PEG and 8% PEG (Figure 4b).

3.3. SOD and CAT activity

As a result of our research, the effects of PEG and $NaSiO_2$ treatment on the SOD and CAT activities of strawberry leaves grown in *invitro* conditions were found to be statistically significant ($P < 0.05$). Figure 5 shows that strawberry plants grown in invitro conditions respond to drought stress and SOD and CAT activity values increase in direct proportion to with increasing PEG doses. Also, the addition of $NaSiO_2$ caused an increase in enzyme activities in all applications. 8% PEG application (T7, T8, T9) had higher enzyme activity than 4% and control group (T1, T2, T3). Our data showed that the highest SOD value was determined in application T9 (8% PEG+100 mg L^{-1} $NaSiO_2$) with 218 U/g TA, followed by application T8 (8% PEG+50 mg L^{-1} $NaSiO_2$) with value 205 U/g TA (Figure 5a). The lowest value was found in the control group (T1) with 118 U/g TA. 8% PEG+ 100 mg L^{-1} $NaSiO_2$ application up-regulated SOD activity. CAT activity values obtained from different applications are shown in Figure 5 (b). The highest value was observed with 0.98 U/g TA in T9 (8% PEG + 100 mg L^{-1} $NaSiO_2$) application, while the lowest value was found in the control group (T1) with 0.27 U/g TA.



(**a**) (**b**)

Figure 5. Effect of PEG and $NaSiO_2$ treatment on SOD (a) and CAT (b) activities in strawberry leaves under *invitro* conditions.) 0% PEG+0 mg L^{-1} $NaSiO_2$; (T2) 0% PEG+50 mg L^{-1} $NaSiO_2$; (T3) 0% PEG+100 mg L^{-1} $NaSiO_2$; (T4) 4% PEG+0 mg L^{-1} $NaSiO_2$; (T5) 4% PEG+50 mg L^{-1} $NaSiO_2$; (T6) 4% PEG+100 mg L^{-1} $NaSiO_2$; (T7) 8% PEG+0 mg L^{-1} $NaSiO_2$; (T8) 8% PEG+50 mg L^{-1} $NaSiO_2$; (T9) 8% PEG+100 mg L^{-1} $NaSiO_2$. Values are the mean ±S.D. of three replicates (n=3). Common letters are not significant ($P < 0.05$).

3.4. Correlations

The correlations between the growth parameters and physiological traits was showed in Table 3. A significant relationship was observed between shoot and root growth. Among parameters that were measured, a positive correlation was found between RW and SL, RL, LW, LRWC and SPAD index. Also, SL was exhibited a positive correlation with RL, LW and LRWC. Enzymatic antioxidants (SOD and CAT) were negatively correlated with some growth parameters; RW, SL, LW and LRWC (Table 3).

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Table 3. Pearson's correlation coefficients between the growth parameters and physiological traits.

	S	R	SL	RL	SD	LN	LW	LL	LR	SP	SO	CA
SW	1	0.3	0.56	0.83	0.3	0.3	0.18	0.1	0.33	0.25	-	0.10
RW		1	0.65	0.48	0.3	0.2	0.51	0.3	0.38	0.69	-	-
SL			1	0.75	0.2	0.3	0.60	0.1	0.55	0.35	-	-
RL				1	0.3	0.4	0.31	0.1	0.36	0.22	-	0.10
SD					1	0.2	0.10	-	0.04	0.35	-	0.11
LN						1	0.15	0.1	0.25	0.16	-	-
LW							1	0.0	0.63	0.33	-	-
LL								1	0.24	0.13	-	0.09
LR									1	0.30	-	-
SPA										1	-	-
SO											1	0.71
CA												1

SW: shoot fresh weight, SL: shoot length, RW: root fresh weight, RL: root length, SD: shoot diameter, LN: number of leaves, LW: leaf width, LL: leaf length. * Correlation is significant at α 0.05. ** Correlation is highly significant at α 0.01.

4. Discussion

Strawberry is a major horticultural plant worldwide and the growth and development of this plant can be affected by different factors [42]. Responding to abiotic stresses has been one of the main subjects of research since it is very important in terms of plant productivity [43,44]. This research involved $NaSiO_2$ effect on invitro strawberries growth under various levels of PEG-induced drought stress. It has been reported in various previous studies that the application of different nanoparticles to strawberry plants *invitro* and *invivo* conditions has significant effects on plant growth, crop quality and yield, and also reduces the effect of abiotic stress factors [7,10,23,26,45,46]. One of the most important signs observed in plants exposed to abiotic stress is growth inhibition [20]. Our data's showed that, the increased PEG in the medium exerted a negative influence on the vegetative growth parameters, whereas, $NaSiO_2$ treatment supplemented in the growth medium stimulated a positive response in growth parameters. Additions of 50 or 100 mg L^{-1} $NaSiO_2$ increased the shoot and root growth of strawberry plants in the stress or non-stressed conditions. The significant ($p < 0.05$) decrease in growth parameters of in vitro plants (Table 2) in PEG applications could be due to the reduction of water absorption and consequently to decreased cell division and elongation [47]. Our study observed that $NaSiO_2$ at 50 mg L^{-1} induced the maximum shoot (1.20 g) and root (1.24 g) fresh weight also shoot (40.09 mm) and root (34.26 mm) length, among 4% and 8% PEG applications. These results are congruent with the results of Mozafari et al., (2018) [23] which reported the significant effect of iron nanoparticles (FeNPs) and salicylic acid (SA) on invitro strawberry plants under various concentrations of drought stress. Similar results were obtained in two different studies investigating the effects of FeNPs on strawberry plants grown in vitro under salt [45] and drought stress [26]. In both studies, it is reported that the application of FeNPs under stress conditions can reduce the negative effects of stress. Zahedi et al. (2020) reported that spraying solutions containing SiO_2, Se and Se/SiO_2 (50 and 100 mg L^{-1}) nanoparticles improved the growth parameters of strawberry plants grown under normal and drought stress conditions [10]. Our results agree with the previous studies reporting that $NaSiO_2$ enhanced shoot and root development of in vitro strawberries under drought stress conditions.

According to the findings of this study, leaf width and leaves number of *invitro* strawberries were significantly affected by the combined application of $NaSiO_2$ and PEG. Limitations in the number, width and length of leaves are one of the adaptations seen in plants to control water loss in arid environments. $NaSiO_2$ increases the leaf width of *invitro* strawberry plants in non-stress

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conditions. In contrast, in drought stress conditions (4% and 8%PEG) $NaSiO_2$ had negative effects on this parameter (Figure 3). Using $NaSiO_2$ under drought stress or without stress, on the other hand, improved leaves number, which can be attributed to the important role of $NaSiO_2$ in drought conditions. Studies that fully demonstrate the effectiveness of silica nanoparticles are still limited. According to Sun et al. (2016) [48], application of 2000 mg L^{-1} mesoporous silica nanoparticles (Si NP) had no adverse effects in terms of oxidative status or cell membrane integrity in both wheat and lupine, while the same Si NP concentration decreased root and stem biomass and plant height in cotton (*Gossypium hirsutum* L.) [49]. The effect of Si NP application on plant growth and development is affected by the properties of the material such as; size and shape, as well as the application stage, biomechanical and physical factors [50]. One of the important effects of drought on plants is the production of fewer leaves or the production of leaves with a smaller surface area [51]. In previous studies, silicon application improved the biomass accumulation of the strawberry plant [7]; this may be correlated with higher leaves number during stress conditions. Under drought stress conditions, application of $NaSiO_2$ significantly increased leaves number of apple (*Malus domestica* Borkh.) [52], feverfew (*Tanacetum parthenium* L.) [53] and wheat [54].

In this study, 50 mg L^{-1} $NaSiO_2$ application significantly increased the SPAD index in the control group without drought stress (T1, T2, T3) and in the group with 4% PEG application (T4, T5, T6) (Figure 4a.). However, in the group where the drought level increased and 8% PEG was applied; it is seen that the highest mean value was determined in the T9 group, which was administered 100 mg L^{-1} of $NaSiO_2$. The decrease in the SPAD index, which is expected to be positively correlated with photosynthesis, leads to the limitation of plant biomass and yield [54,55]. Various studies have reported that the adverse effects of drought on the SPAD index were alleviated by silicon application [56–58]. Our study shows that LRWC decreases as PEG concentrations increase. Many studies have shown that RWC (Relative Water Content) decreases in response to drought stress [59]. As a result of the decrease in turgor pressure in drought conditions, cell division and cell elongation are restricted, and as a result of this situation, the development of plants and leaves decreases [54,60]. With the restriction of access to water, the loss of leaf water content occurs, followed by impaired cell growth and metabolism, which eventually leads to the development of secondary damage to the leaf due to drought [61–63]. Addition of 50 mg L^{-1} of $NaSiO_2$ increased LRWC under unstressed conditions. Under drought conditions where 4% PEG was applied, LRWC was not significantly changed in its presence compared to the absence of additional $NaSiO_2$. In the stress conditions created by 8% PEG application, $NaSiO_2$ application had a negative effect on LRWC. Some studies, in contrast to our findings, reported that silicon supplementation reduces the osmotic potential of strawberry leaves and increases turgor pressure under drought stress. Therefore, it has been suggested that silicon supplementation can increase the water content of plants [8,10]. On the other hand, in line with our study findings; Mali and Aery (2008) also reported that the RWC content decreased with silicon application [64]. Researchers speculated that this reduction in RWC may be due to a lack of water in the soil or root systems, which cannot compensate for water lost by transpiration through reduced absorbent surface [65]. In addition to all these, it can be thought that the effectiveness of silicone application varies in all studies, and this change may vary according to growing conditions and application dose.

Our results indicated that the application of $NaSiO_2$ elevated the CAT and SOD activity in stressed plants. 100 mg L^{-1} $NaSiO_2$ had the highest effect between all treatments. Silicon, which regulates endogenous plant hormones, resulted in improved tolerance to drought stress [66]. A number of ecological conditions can cause the production of reactive oxygen species (ROS), which is formed as a by-product of plant metabolic processes [67,68]. Plants need to balance/maintain ROS levels within the cell through enzymatic and non-enzymatic activities to cope with oxidative damage under conditions of abiotic stress [69–73]. Antioxidant enzyme activities can be improved by CAT activation, which scavenges ROS, participates in the defence mechanism against H_2O_2 increase, and controls H_2O_2 levels in cells [67,74,75]. Also, if the activation of SOD is combined with other ROS scavenging enzymes, defence strategies can be provided to attenuate the oxidative burst in plants under arid conditions [76,77]. It has been reported that nanosilicone has a protective effect on plants

through regulation of antioxidant systems, and phytohormone regulation induced by Si addition results in improved tolerance to drought stress [78,79]. In a recent study, SiNPs were shown to increase the tolerance of rose (*Rosa damascena* Mill.) to PEG-induced drought stress by decreasing the H_2O_2 concentration and increasing the activity of antioxidant enzymes [80]. Therefore, from this point of view, silicon supplementation is thought to be responsible for increased tolerability in drought-stressed plants.

5. Conclusions

The current study investigated the effects of $NaSiO_2$ application on drought tolerance under in vitro culture induced by PEG and its effects on strawberry growth and development. Drought stress leads to adverse effects in plants, which is associated with the reduction of growth parameters. According to results addition of $NaSiO_2$ improved water stress tolerance in strawberry plants in vitro by increasing shoot weight and length, root weight and length, number of leaves, SPAD index, SOD and CAT activity. Hence $NaSiO_2$ application could alleviate the oxidative damage of in vitro strawberry plants under drought conditions, which could contribute to improvement of drought tolerance.

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