

Effect of SiO₂ nanoparticles on drought resistance in hawthorn seedlings

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Abstract. Drought is a significant factor limiting crop production in arid regions while hawthorns (*Crataegus* sp.) are an important component of such region's forests. Therefore, treatments that increase hawthorn drought resistance may also increase transplanting success. Thus, the physiological and biochemical responses of hawthorn seedlings to a factorial combination of different concentrations of silica nanoparticles (SNPs at 0, 10, 50 and 100 mg L⁻¹) and three soil moisture treatments (without stress, moderate stress and severe stress) were investigated. Seedlings were irrigated with one of the four concentrations of SNPs for 45 days before exposing them to drought stress. Photosynthesis parameters, malondialdehyde (MDA), relative water content (RWC), membrane electrolyte leakage (ELI) as well as chlorophyll, carotenoid, carbohydrate and proline content were determined. At the end of the experiment, positive effects by SNP pre-treatment on physiological indexes were observed during drought stress. Under drought conditions, the effect of SNPs on photosynthetic rate and stomatal conductance was evident. Although the SNPs increased plant biomass, xylem water potential and MDA content, especially under drought conditions, RWC and ELI were not affected by the SNP pre-treatments. Seedlings pre-treated with SNPs had a decreased carbohydrate and proline content under all water regimes, but especially so under drought. Total chlorophyll content and carotenoid content did not change among the treatments. Generally, the findings imply that SNPs play a positive role in maintaining critical physiological and biochemical functions in hawthorn seedlings under drought stress conditions. However, more studies are needed before the physiological and biochemical basis of induced drought resistance can be determined.

Keywords: Drought stress, Hawthorn, Nanoparticles, Silica

1. Introduction

After oxygen, silicon (Si) is the most abundant element in the earth's crust (Ma, 2004). The biological role of Si in plants has not been deeply studied by plant physiologists because it has not been classified as essential plant element (Ma & Yamaji 2006). Nevertheless, many researchers believe that Si is an important element for plants (Siddiqui & Al-Whaibi 2013; Epstein 2009; Gong & Chen 2012; Currie & Perry 2007). Si, as a physicomechanical barrier, is part of the epidermal cell walls and vascular tissues in stems, pods, leaves and bark (Siddiqui & Al-Whaibi 2013). Many beneficial effects have been reported. Liang et al. (2007), Ma (2004) and Pei et al. (2010) indicated that Si might decrease the negative effects of oxidative stress and offer slight resistance to some

abiotic and biotic plant stressors. Thus, using Si instead of herbicides and pesticides could reduce harmful environment effects (Vasanthi et al. 2012; Balakhnina and Borkowska 2013; Karmollachaab et al. 2013).

The positive effects of the Si (in bulk size) have been demonstrated in plants by investigators; however, compared with Si bulk size, absorption of Si in plants is greater when nanoparticles of silicon are used (Suriyaprabha et al. 2012b). Nanomaterials consist of particles smaller than 100 nm. The small size of Si particles implicates new physical, chemical and biological properties (Monica and Cremonini 2009).

At the global level, the use of nanotechnology in agriculture is at a nascent stage, yet it is increasing. Nanosciences led to the development of a wide range of applications for enhancing plant growth (Nair et al. 2010). In recent years,

effects of Si in nanoscale on plants have received increased attention, but research results are limited. Haghighi et al. (2012) applied nanosilica to tomato seeds and seedlings under salt stress and concluded that nanosilica application reduced the deleterious effects of salinity on germination; root length and plant dry weight. Bao-shan et al. (2004) immersed the roots of changbai larch (*Larix olgensis*) seedlings in 62–2000 $\mu\text{l.l}^{-1}$ concentrations of nanosilica for 6 hours. Their results clearly showed positive effects of silica nanoparticles (SNPs) on growth and quality of the seedlings. Suriyaprabha et al. (2012b) demonstrated greater absorption of silica in nanoscale in maize roots and seeds treated with nano-SiO₂, than when treated with micro-SiO₂, Na₂ SiO₃, and H₄ SiO₄; also SNPs have been proposed as an immediately utilisable form for plants. Also, the recent finding showed that irrigation of pear seedling with high concentrations of Nano-SiO₂ did not have any toxic effect on the plant biology (Zarafshar et al. 2015).

Water is a vital resource for plant survival and is also needed for transport of nutrients, so when the drought period and water stress emerged, vitality of plants was weakened (Martinez-Vilata & Pinol 2002), their growth reduced (Bigler et al. 2006), and mortality increased (Rebetez & Dobbertin, 2004). Drought stress, as a multidimensional abiotic stress, strongly effects growth, development and yield of plants (Mahajan and Tuteja 2005). Under drought condition, the plants initiate two strategies for survival - avoidance or tolerance; the strategies include morphological and/or physiological adjustments (Bassett, 2013). Finding resistance genotypes to biotic and abiotic stress is very important for plant research. The role of silica in plants under stress conditions is more pronounced, but there is no report for using of silica as nanoparticles in plant drought stress.

Crataegus aronia L. is one of 21 hawthorn species native to Iran. It is used in reforestation of semi-arid sites and the leaves are a traditional medicine (Kumar et al. 2012). This study was conducted to determine the effects of SNPs on hawthorn seedling physiology and biochemistry under soil moisture stress. For this purpose, we irrigated hawthorn (*Crataegus aronia* L.) seedlings with different concentrations of nanosilica for 45 days and then subjected the treated seedling to soil moisture stress. Finally, the following hypotheses were considered; (1) the SNPs have a positive impact on physiological indices in hawthorn seedlings; (2) the SNPs can delay and dampen the deleterious effects of drought in all stress treatments equally.

2. Materials and methods

Preparation of materials

One-year-old bare root hawthorn seedlings were purchased from an Iranian forest nursery and transferred to the experimental garden facility at the Faculty of Natural Resources and Marine Sciences of Tarbiat Modares University, Noor, Mazandaran, Iran. A total of 108 uniformly sized hawthorn

seedlings were transplanted to plastic pots (7 l) containing a mixture of forest brown soil, river sand and clay (2:1:1, v/v/v) and grown in a greenhouse with day/night average temperatures of 30/21°C. The seedlings were irrigated to field capacity and grown for 2 months before doing any treatment.

Powdered SiO₂ nanoparticles were purchased from TECNAN (Tecnología Navarra de Nanoproductos S.L., Spain). The estimated size ranged from 10 to 30 nm. Particle size distribution was confirmed with X-ray diffraction (XRD) measurement and clearly showed that SiO₂ NPs were amorphous. The purity of SiO₂ NPs was calculated by the inductively coupled plasma mass spectrometry (ICP-MS) technique to be 99.999%.

Nanoparticle pre-treatments and drought stress treatments

A factorial combination of four SNPs concentrations and three watering treatments were imposed. SNPs were soil applied in four concentrations (0, 10, 50 and 100 mg.l⁻¹) for 45 days. The seedlings were irrigated to field capacity (300 ml per pot) with SNP suspensions every 3 days. There were 27 seedlings in each SNPs treatment. At the end of the SNPs treatments (day 45), seedlings in each SNPs treatment were randomly separated into one of three soil moisture stress groups. Seedlings were then irrigated with tap water every 3 days with 300 ml per pot (approximately to field capacity, low stress), 150 ml per pot of tap water every 3 days (moderate stress) and no water (severe stress). All seedlings were harvested after 19 days when leaf rolling appeared in seedling under the severe stress treatment.

Measurement of the plant physiological parameters

Net photosynthesis (A, $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (gs, $\text{mmol m}^{-2} \text{s}^{-1}$) and transpiration rate (E, $\text{mmol m}^{-2} \text{s}^{-1}$) were measured during the 19 days following the water stress treatment (at days 7, 14 and 19) on 2–3 leaves (per plant) of six randomly selected plants in similar positions on the plants, outside on sunny days (between the hours 09:00 and 11:00) at temperatures ranging from 22 to 28°C, using a portable infrared gas analyser (Model LCpro+, ADC BioScientific Ltd., Hertfordshire, UK). Average of leaf temperature and internal CO₂ concentration was $29.2 \pm 4.8^\circ\text{C}$ and 320 ± 17.4 v.p.m, respectively.

Predawn xylem stem potential (ψ stem, MPa) was measured with a pressure chamber system (Skye, SKPM 1400, UK) on day 19. Leaf relative water content (RWC) was determined according to following equation.

$$\text{RWC} = \frac{W_i - W_d}{W_f - W_d} \times 100$$

Four leaves (from similar positions) were removed from randomly selected plants in each treatment, immediately weighed (W_i), placed in deionised water for 24 h at room temperature under low-light condition. After that, individu-

al leaves were reweighed to determine their turgid weights (W_f). Finally, the samples were placed in an oven at 60°C for 48 h and then reweighed to obtain their dry weights (W_d).

Measurement of the plant morphological parameters

Primary shoot length and collar diameter of all plants were measured again at the end of the investigation period. At the end of the experiment, all plants were harvested and the longest root and root volume were measured. In this regard, root length using a scaled ruler and root volumes were measured using water displacement in graduated cylinders. Root, leaves and stem were oven dried for 48 h at 70°C and dry weights recorded for individual plants.

Determination of malondialdehyde and membrane electrolyte leakage

Thiobarbituric acid reaction (TBA), as described by Heath and Packer (1968), was measured. Leaf fresh mass (200 mg) was homogenised in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA), followed by centrifugation at $12,000 \times g$ for 20 min. The supernatant (1 ml) was mixed with an equal volume of TCA (10%) containing 0.5% (w/v) TBA or no TBA as the blank and heated at 95°C for 30 min and then cooled in ice. The reaction product was centrifuged at $12,000 \times g$ for 15 min and the supernatant absorbance was measured at 400, 532 and 600 nm. The malondialdehyde (MDA) equivalent was derived from the absorbance according to Hodges et al. (1999).

Seedling leaves were cut into 1–2 cm² pieces and placed in test tubes with 20 ml deionised distilled water (0.5–0.8 g fresh leaf tissue per sample). After vortexing the samples for 3 s, the initial electrical conductivity (EC0) of each sample was measured. The samples were stored at 4°C for 24 h and conductivity (EC1) was measured again. Samples were then autoclaved for 15 min, cooled to room temperature and conductivity (EC2) was measured for the third time. The relative permeability (RP) of cell membranes was calculated using a modification of the method of Zhao et al. (1992) as:

$$RP (\%) = ((EC1 - EC0) / (EC2 - EC0)) \times 100.$$

Measurements of biochemical parameters

At the end of the experiment, fresh leaf samples were covered with aluminum foil, frozen in liquid nitrogen and stored at –85°C until used for biochemical analysis. Chlorophylls and carotenoids were extracted from leaf samples in 80% acetone and their contents were determined by spectrophotometry according to Gholami et al. (2012). Free proline content in leaves was quantified following the procedure of Bates et al. (1973) as cited by Nikolaeva (2010).

Microscopic observation

At the end of the experiment, the fresh root sections were taken for microscopic analysis. The adsorption of SiO₂ to

fresh roots was observed by scanning electron microscopy (SEM) (KYKY-EM3200) in the laboratory of Tarbiat Modares University.

Statistical analysis

The experiment was done in randomised complete design with three replications and three plants per replication for each treatment combination. Statistical analyses were performed using SPSS (IBM SPSS Statistics 19.lnk) software. The data were tested on normality, homogeneity and Mauchly's test before analysis of variance (ANOVA). Leaf photosynthesis parameters data were subject to ANOVA using repeated measures. ANOVA was conducted using two-way ANOVA in a fixed factor with full factorial. For comparison between groups, Duncan's test was applied at a 0.05 probability level. In case of percentage data, Arcsin transformation was applied before ANOVA was used.

3. Results

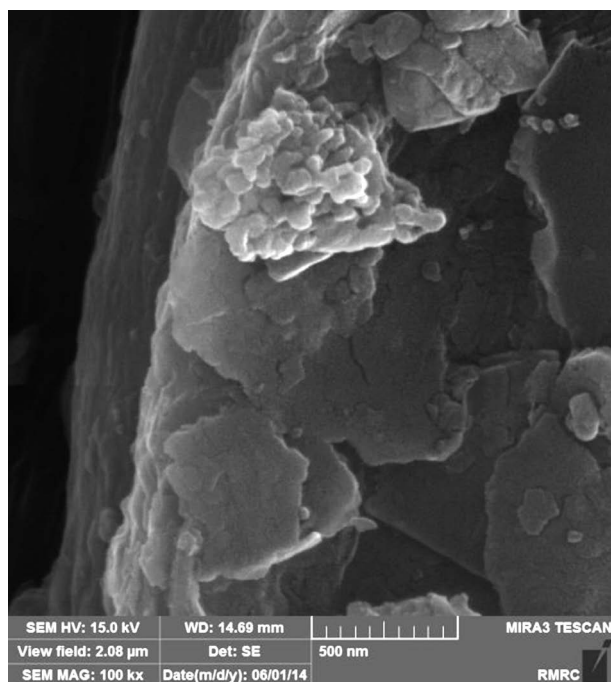
Microscopic analysis

Figure 1 shows photographs of hawthorn roots and SiO₂ aggregates on the root surface. The roots were exposed to different concentrations of SiO₂ NPs in the range 0–100 mg.l⁻¹. In particular, for the higher concentrations (100 mg.l⁻¹) nanoparticles attached to the roots could be observed. On the other hand, only a very small amount of particles were found to be attached to the roots for SNPs 10 and 50 mg.l⁻¹. There was no sign of nanoparticles on roots of control plants. Clearly, the particles on the root surface are in the nanometre range (for comparison: a bar is 500 nm in length).

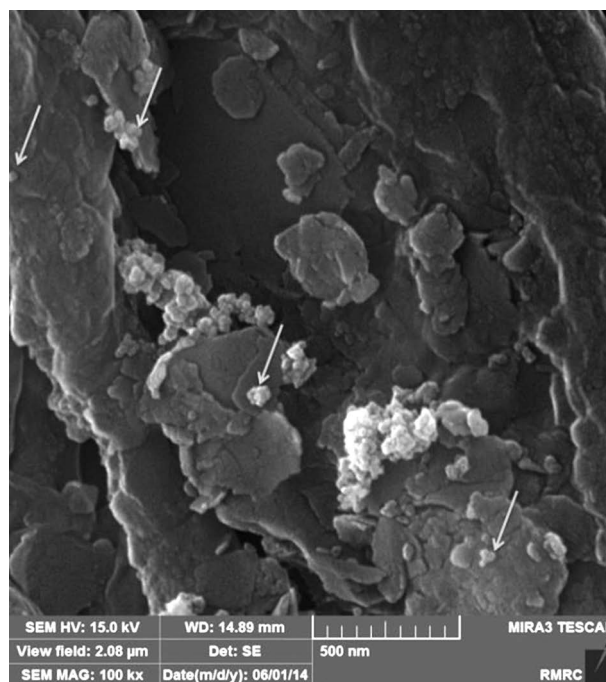
Effect of SiO₂NPs pre-treatments on photosynthesis parameters

There were no differences in photosynthetic parameters between SiO₂NPs treated and untreated seedlings when they were not exposed to drought stress. All photosynthesis parameters in the SNPs treated seedlings declined under moderate and severe drought stress (Figure 2), with the decline in A delayed about 7 days in both water stress treatments at the highest SNPs concentration. Under severe stress, A at 14 days in SNPs treated seedlings was higher than in non-SNPs treated seedlings.

The positive effect of SNPs pre-treatments was observed under severe stress because photosynthesis rate and stomatal conductance in the treated plants decreased with a milder slope comparing with control plants. The photosynthesis rate (A), stomatal conductance (gs), and transpiration (E) were affected by SNPs treatments and drought stress (main effects) and time of testing (internal effects) (repeated measures ANOVA and treatment effect: $P < 0.001$) (data not shown). Under moderate stress, trend of photosynthesis and transpiration rate were similar for all



The absence of nanoparticles on root epidermis in control seedlings



The presence of nanoparticles on root epidermis in 100 mg.l⁻¹ seedling SiO₂ treatment.

Figure 1. Scanning electron microscopy (SEM) images of root surface. The absence of nanoparticles on root epidermis in control seedlings (left). The presence of nanoparticles on root epidermis in 100 mg.l⁻¹ seedling SNPs treatment (right).

SNPs pre-treatments. Although, treated seedlings with SNPs had a similar trend for stomatal conductance under moderate stress, stomatal conductance of control seedlings (0 mg.l⁻¹) decreased more rapidly. The silica treated plants had declining E during the experiment, especially after day 14, but the decline was similar regardless of the SNP concentration.

Effect of SiO₂NPs pre-treatments on root morphology and biomass allocation

At the beginning of the experiment, the seedlings' main stem height and root collar diameter averaged 46.89 ± 7.5 cm and 8.08 ± 1.3 mm (at 1 cm above soil surface), respectively. Irrigation with SNPs did not affect hawthorn seedling height or root collar diameter (data not shown). The root length was greater in SNPs treated seedlings because the highest values were recorded for seedlings irrigated with 100 mg.l⁻¹ during 45 days. The shortest roots were observed in control seedlings without stress. On the other hand, the positive effects of SNPs on root volume were evident because root volume progressively increased with increasing the concentration of SNPs at pre-treatment. Generally, positive effects of SNPs on the whole dry weight of the plant under three irrigation regimes were recorded. Among three plant components, stem biomass was changed slightly by SNPs pre-treatments in comparison with root and leaf biomass (Figure 3).

Effect of SiO₂NPs pre-treatments on water relations and biochemical parameters

Relative water content was significantly affected by water stress, while, SNPs supply did not improve RWC of the stressed plants (Figure 4). On the other hand, the xylem water potential was significantly affected by SNPs pre-treatments, water stress and their interaction. Particularly, in severe stress, with increasing SNPs concentrations, the negative effects of drought stress on xylem water potential were reduced (Figure 4). The effect of nanosilica pre-treatments on electrolyte leakage index was not significant, while drought stress significantly affected electrolyte leakage index. The effects of SNPs pre-treatments and drought stress on the level of lipid peroxidation (MDA content) in leaves of hawthorn seedlings are shown in Figure 4. Although, the effects of SNPs pre-treatments on MDA content under moderate and without stress were not tangible, SNPs pre-treatments could decrease MDA content in severe stressed seedlings. Compared with control plants without stress (0 mg.l⁻¹), MDA content went up 38.6% in control plants in severe stress (0 mg.l⁻¹), while there was no significant difference between without stress and moderate stress with respect to MDA. Compared with control plants under severe stress (0 mg.l⁻¹), MDA content went down by 14.8, 20.2 and 32.7% in 10, 50 and 100 mg l⁻¹, respectively, nano Si pre-treatments. Thus, it seems the positive effects of SNPs pre-treatments on severe stressed seedlings are considerable.

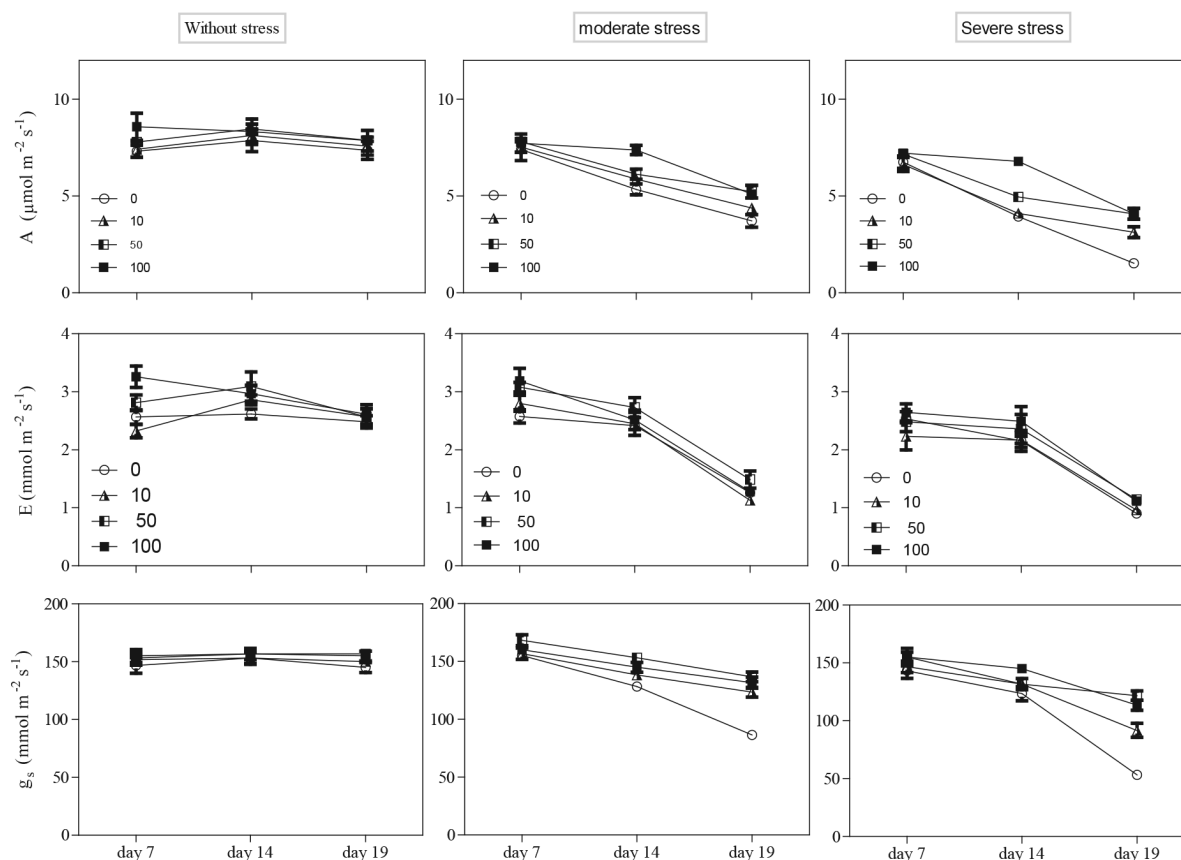


Figure 2. The effect of SNPs pre-treatment and drought stress on photosynthesis rate (A), stomatal conductance (g_s) and transpiration (E). The gas exchange was assayed with a portable infrared gas analyzer (Model LCpro+, ADC BioScientific Ltd., Hertfordshire, UK), at days 7, 14, and 19 (during water regime period) on the recent fully expanded leaves of six randomly selected plants in similar positions on the plant (multiple leaves per plant) in hawthorn seedlings, (Mean \pm SE; $n = 6$). SNPs: silica nanoparticles.

Under moderate and severe drought stresses, total chlorophyll content in the control plants (0 mg.l^{-1}) did not change. The treated seedlings with 10 and 50 mg.l^{-1} under moderate stress showed the highest value for total chlorophyll content. Although, statistical analysis proved a significant effect for drought stress and SNPs pre-treatments on carotenoid content, but we could not find any significant trend between all treatments. Soluble carbohydrate and proline content in stressed leaf (0 mg.l^{-1} in moderate and severe stress) slightly increased. Interestingly, soluble carbohydrate and proline content progressively decreased with increasing SNPs concentration in pre-treatments. Fertilising the seedlings with nano Si for 45 days led to decrease in the soluble carbohydrate and proline content under drought stress.

4. Discussion

Some plant physiologists have shown that silica applications have increased abiotic and biotic stresses plant defence systems, including water stress (Epstein 1994; Zhang and Schmidt 1999). The use of nano-compound materials has been given much attention by plant biological researchers (Pourkhaloee et al. 2011; Haghghi 2012). In the current

research, we studied the combined advantages of silica as nanoparticles for improving of deleterious effects of moderate and severe water stress in hawthorn seedlings. As already reported (Farooq et al. 2012; Pessaraki, 2014), moderate and severe stress disrupted stomatal conductance and photosynthesis process of the seedlings and this change over time was so much more intuitive. Under drought stress, silica treated plants showed similar transpiration patterns to non-treated ones, but A and g_s were mostly higher, particularly at higher concentrations of SNPs. Nevertheless, no influence was observed when SNPs were applied to non-stressed plants, similar, to the results of Matoh et al. (1986) and Zuccurini (2008) in rice and *Phaseolus vulgaris*, respectively. Recent reports indicate that the bulk silica could improve photosynthesis parameters of some plants under drought stress conditions (Ma, 2009; Pei et al. 2010; Zhang et al. 2013), but there is no evidence for effects of SNPs on plants under drought stress. According to Haghghi and Pessaraki (2013), SNPs did increase photosynthesis parameters of cherry tomatoes (*Solanum lycopersicum* L.) under saline stress. Also, our results are in accordance with Zhang et al. (2013) on chestnut plants (*Castanea* spp.), who reported that Si application enhanced photosynthesis and stomatal conductance under drought

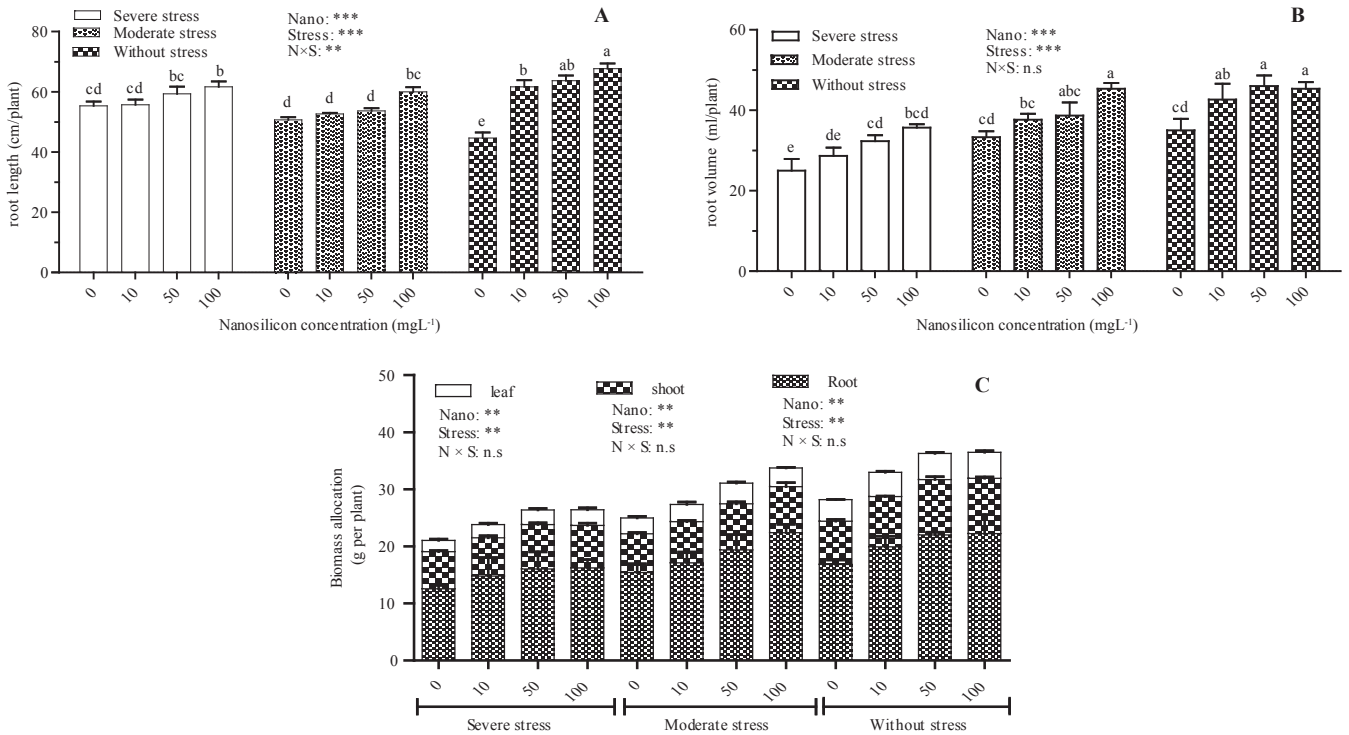


Figure 3. Root length (A), root volume (B), and biomass allocation (C) of hawthorn seedlings subjected to 45 days of SNPs pre-treatment followed by 19 days at control conditions (without stress), 19 days at moderate stress (50% field capacity) and 19 days at severe stress (Withholding). Different letters indicate significant differences ($p < 0.05$) among treatments based on the Duncan's test. (Mean \pm SE). SNPs: silica nanoparticles.

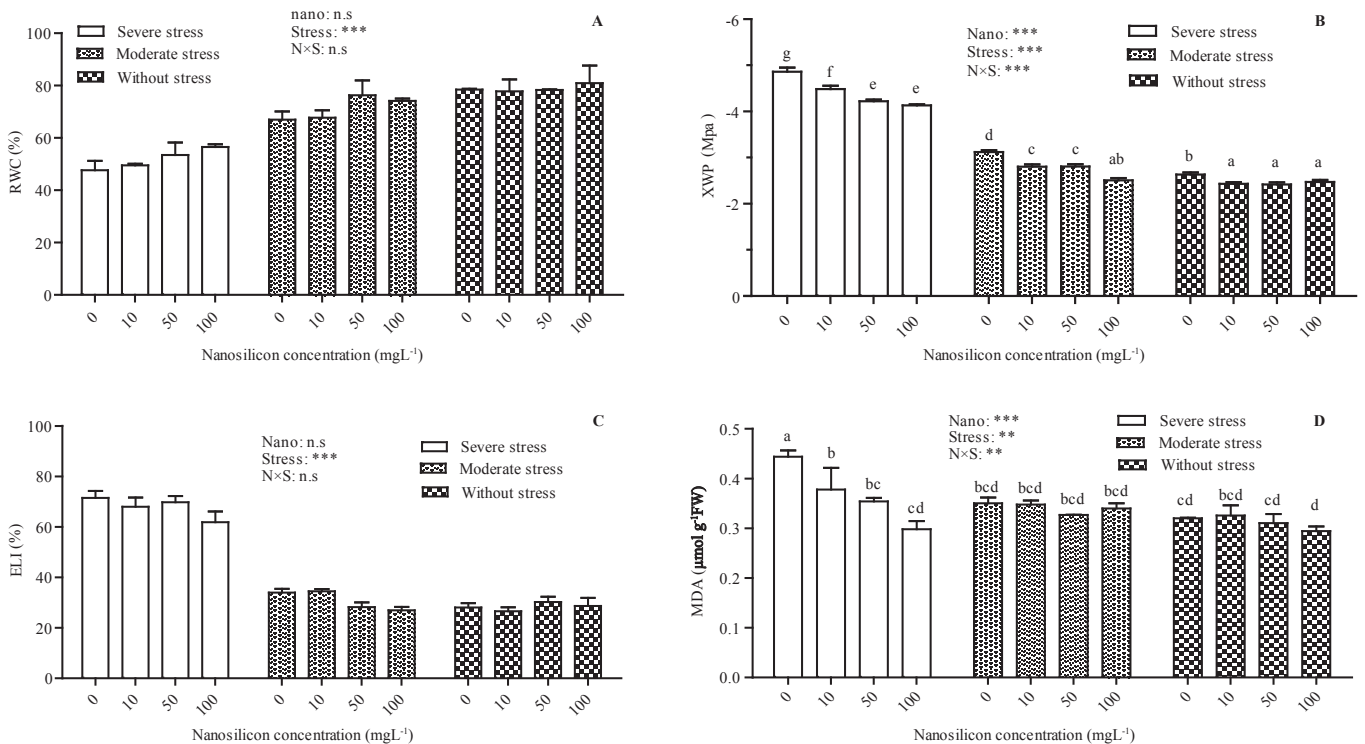


Figure 4. Water relations parameters (relative water content (A), xylem water potential (B)), electrolyte leakage index (C), and malondialdehyde (D) of hawthorn seedlings subjected to 45 days of SNPs pre-treatment followed by 19 days at control conditions (without stress), 19 days at moderate stress (50% field capacity) and 19 days at severe stress (Withholding). Different letters indicate significant differences ($p < 0.05$) among treatments based on the Duncan's test. (Mean \pm SE). SNPs: silica nanoparticles.

Table 1. The interactive effects of SNPs in different concentrations and drought stress on some biochemical characteristics of hawthorn seedlings

Treatment	Without stress	Moderate drought	Withholding
Chlorophyll a+b (mg/g fw)			
0 mg l ⁻¹	0.001 c ± 0.009	0.001 c ± 0.009	0.001 c ± 0.009
10 mg l ⁻¹	0.001 c ± 0.009	0.000 a ± 0.018	0.000 c ± 0.009
50 mg l ⁻¹	0.000 bc ± 0.009	0.001 ab ± 0.01	0.000 d ± 0.008
100 mg l ⁻¹	0.002 bc ± 0.01	0.001 c ± 0.009	0.002 cd ± 0.009
Carotenoid (mg/g fw)			
0 mg l ⁻¹	0.133 a ± 2.183	0.028 a-c ± 1.990	0.099 b-d ± 1.825
10 mg l ⁻¹	0.045 a-d ± 1.871	0.078 ab ± 2.108	0.013 a-d ± 1.895
50 mg l ⁻¹	0.122 b-d ± 1.793	0.151 a-d ± 1.863	0.058 cd ± 1.694
100 mg l ⁻¹	0.246 b-d ± 1.831	0.192 b-d ± 1.788	0.455 d ± 1.633
Carbohydrate (mg/g fw)			
0 mg l ⁻¹	0.0009 e ± 22.414	0.0000 b ± 22.420	0.0021 a ± 22.429
10 mg l ⁻¹	0.0002 ef ± 22.413	0.0016 d ± 22.415	0.0007 c ± 22.417
50 mg l ⁻¹	0.000 ef ± 22.412	0.0003 d ± 22.416	0.0010 g ± 22.410
100 mg l ⁻¹	0.0003 f ± 22.412	0.0003 c ± 22.419	0.00016 c ± 22.418
Proline content (mg/g fw)			
0 mg l ⁻¹	0.0002 d ± 0.056	0.0002 d ± 0.057	0.0001 a ± 0.063
10 mg l ⁻¹	0.0000 h ± 0.052	0.0003 ef ± 0.055	0.0003 b ± 0.061
50 mg l ⁻¹	0.0002 h ± 0.052	0.0004 fg ± 0.054	0.0009 d ± 0.057
100 mg l ⁻¹	0.0004 e ± 0.055	0.0003 g ± 0.054	0.0004 c ± 0.060

(Mean ± SD) Mean values indicated by the same letter in same column are not significantly different ($P < 0.05$) according to the Duncan's test. SNPs: silica nanoparticles.

stress conditions. The mechanism on how Si regulates stomatal response remains unclear and needs deeper investigation (Gao et al. 2005) to confirm Agarie et al's, (1998) finding of the positive effects of Si on the stomata light reaction (Agarie et al. 1998). The finding that SNPs did not have an effect on the transpiration rate contradicted previous reports, which showed that silica application led to a decrease (Yoshida, 1965, Matoh et al. 1991) in rice or an increase (Hattori et al. 2005) in *Sorghum bicolor*.

Undoubtedly, limitations of photosynthesis parameters during drought lead to a reduction in whole-plant biomass accumulation (Zarafshar et al. 2014). In the present work, SNPs applied as pre-treatment significantly improved the whole dry biomass of hawthorn seedlings grown under all three water regimes. In this regard, positive effects of SNPs pre-treatments, especially with high concentration on root growth and biomass were considerable. Si plays a significant role in water uptake and root growth under water stress (Ahmed et al. 2008). This finding also proven by Haghghi and Pessarakli (2013) for tomato, Suriyaprabha et al. (2012a) and Yuvakkumar et al. (2011) for maize and Bao-shan et al. for larch (2004). At the moderate and severe drought stress conditions,

the effect on photosynthesis and stomatal conductance is more pronounced compared with the effect on growth and improvement of biomass allocation. In case of normal irrigation (without stress), biomass allocation and root growth were extensively increased by SNPs pre-treatments, while gas exchange parameters remained unchanged. The mechanism for Si-induced seedling mass increase is unknown, but Si cell wall deposition (Gao et al. 2005) and nutrient absorption (Zarafshar et al. 2015) may be involved in biomass reallocation. Hattori et al. (2005) observed Si-induced acceleration of dry matter production in sorghum only when the plants were subjected to drought. Generally, positive effect of Si application in the plants is not too obvious under optimum condition, but it is most evident when the plant is under sub-optimal condition (Ahmed et al. 2008; Henri et al. 2006).

Leaf water potential is a primary indicator of the degree of plant's stress under water deficit (McCutchan & Shackel, 1992). As expected, the lowest (-MPa) values of xylem water potential were recorded in severe stressed seedlings, while application of SNPs alleviated the effect of drought stress on xylem water potential. Also, under moderate stress and without stress, the positive effects of SNPs on xylem water poten-

tial were significant. Gao et al. 2005 believed that this process is related to a change in xylem hydraulic resistance. Zarafshar et al. 2015 reported that xylem water potential of wild pear seedlings decreased with increasing concentrations of NSiO₂ in irrigation from 10 to 1000 mg l⁻¹. RWC is a complement of xylem water potential to assess the water status of plants (Zarafshar et al. 2014). RWC was affected by irrigation regimes, but SNPs could not have positive effects on RWC. In agreement with our results, Zhang et al. (2013) demonstrated that under two irrigation treatments and Si application, there were no significant differences in the RWC for leaves of chestnut plants. Under water stress, cell membranes are subjected to changes such as increase of permeability and decrease of selectivity, which can be viewed through the increase in electrolyte leakage (Blokhina et al. 2003). We found a general increase of electrolyte leakage under severe drought stress, which suggest the occurrence of damage to cell membranes (see also Campos et al. 2003), but SNPs could not improve this negative effect.

MDA is a good indicator of oxidative damage to membrane lipids (Ozkur et al. 2009). The highest MDA value was recorded for 0 mg.l⁻¹ SNPs in severe drought. Positive effects of SNPs pre-treatments completely were clear on MDA content in severe drought stress because MDA values declined with increasing the SNPs concentration. Thus, it can be noted that SNPs concentrations could strengthen some antioxidant systems. Liang et al. (2003) and Zarafshar et al., 2015 believe that the exogenous Si significantly enhances the activity of superoxide dismutase, peroxidase and catalase. Before that, some researchers have shown that Si application leads to decrease in the electrolyte leakage (Karmollachaab et al. 2013) and MDA concentration (Pei et al. 2010) of plants in water stress conditions.

Typical decrease in the leaf pigment content is one of the symptoms of drought stress (Egert & Tevini 2002). In our experiment, total chlorophyll (a+b) and carotenoids were significantly changed by SNPs pre-treatments and water stress treatments. Total chlorophyll of moderate stressed seedlings increased with supplying of SNPs, but in the other water treated did not increase. Generally, there are contradictory reports in relation to effect of Si on chlorophyll content. SNPs application increased the chlorophyll content in the maize crop (*Zea mays* L.) (Yuvakkumar et al. 2011; Suriyaprabha et al. 2012a) and changbai larch (*Larix olgensis*) seedling (Bao-shan et al. 2004). Haghighi and Pessarakli (2013) proved that adding SNPs slightly increased the total chlorophyll content under salt stress. In another investigation, Wei et al. (2010) showed that the SNPs decreased chlorophyll content in *Scenedesmus obliquus*.

Plant physiologists revealed the positive role of proline and carbohydrates in plants resistance to drought stress (Farooq et al. 2012). Leaf proline and soluble carbohydrates content were greater in stressed plants grown without Si addition. Interestingly, SNPs pre-treatment significantly decreased leaf soluble carbohydrate and proline content at each stress level. Similar findings have been documented that Si is able to re-

duce the amount of proline in plants under salinity stress (Lee et al. 2010) and drought stress (Shen et al. 2010).

5. Conclusion

Generally, the role of nanosilica in plant biology is not well developed and the attempts to associate Si with metabolic or physiological activities have been inconclusive. In the current study, it has been demonstrated that SNPs can increase plant resistance to drought stress. It could be explained by improvement of photosynthesis rate and stomatal conductance by SNPs pre-treatments. On the other hand, the decrease of xylem water potential and MDA contents in response to severe and moderate stress support this hypothesis that silica has a promised potential in reduction of the deleterious effects of drought on plants. Results of this study can be used as a reference for predicting the effect of SNPs on plants response to drought stress. Surely, more complete studies using SNPs in the longer period can be contributory to more accurate results for the discovered mechanisms of woody plants in response to SNPs and finding of the molecular approach mechanism, especially about antioxidant enzymes is necessary.

Conflict of interest

None declared.

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Authors' contribution

The current paper has extracted from the master science thesis of the first author in Tarbiat Modares University (TMU). Dr. Masoud Tabari and Dr. Mehrdad Zarafshar was member of scientific committee in the thesis. Dr. Ivana Tomášková and Prof. Daniel Struve helped us to description of results and gave us a lot of valuable comments on the paper. Mr. Peyman Ashkavand collected the plant material and carried out all of the experiment. Dr. Masoud Tabari prepared experimental design and he was the project supervisor responsible for experimental design. Dr. Mehrdad Zarafshar provided chemical materials in laboratory and helped to interpretation of all results. Dr. Ivana Tomášková and Prof. Daniel Struve helped to writing of the results and discussion.