Effects of nano and ionized silicon on physiological and biochemical characteristics of potato (*Solanum tuberosum* L.)

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Research Article

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Abstract

The role of protecting and structure stabilizing effects of silicon (Si) has been demonstrated on different plant species. Still it has not been used in potato seed production under a soilless culture system. Furthermore, particle size is very important in particle adhesion and interactions with biological reactions. Therefore, the use of nano-Si particles may be more efficient than ionized -Si. For this purpose, a greenhouse experiment under a soilless culture system was performed as a randomized complete block design (RCBD) arranged in a factorial with three replications. In this study, Si concentration (distilled water (Control), 0.8, 1.6, 2.4, and 3.2 mmol Si L⁻¹) and Si type at two levels (nano and ionized Si-based in sodium silicate) were tested. The results revealed that foliar application of Si significantly improved the net photosynthesis rate, water use efficiency, mesophyll conductance, Chl *a*, Chl *b*, carotenoids, Chl *a/b* ratio, DPPH radical scavenging, total phenol, mean weight mini-tuber, and yield, whereas transpiration rate in Si-treated plants decreased. Moreover, the greatest positive influence of Si was observed at 3.2 mmol L⁻¹. The effect of Nano-Si was greater than ionized-Si at all Si concentrations. The results revealed that improved biochemical and photosynthetic characteristics of potato plantlet under Nano-Si treatments compared to ionized-Si treatments. However, these relations were not significant under ionized treatment. This study indicated that the application of Si (nano and ionized) for potato growing and mini-tuber production has positive effects. Generally, under soilless culture system, Nano-Si have higher efficiency than ionized-Si in mini-tuber production.

1 Introduction

Potato (*Solanum tuberosum* L.) is the world's fourth most important food crop after wheat, rice, and maize. In 2019, the potato was planted on 17340986 and 104192 ha with attainable yields of 21.3619 and 33.4324 ton ha⁻¹ in the world and Iran, respectively [1]. It is widely used in the food industry such as chips and starch. Moreover, it is operated as biofuel and natural polymers [2]. The maintenance of potato production systems is closely dependent on a sufficient and continuous supply of high-quality seed tubers [3]. To produce healthy potato seeds from pathogens and especially viruses, potato plantlets are propagated from meristem culture and transferred to the greenhouse [3, 4].

Silicon (Si) is the second most (27.7%) abundant mineral element in the earth crust and mineral substrate for most of the plants. Many researchers have shown that Si supply improved growth and development of crop plants under normal [5, 6, 7] and stressful conditions such as salinity [8], drought [9] and toxic heavy metals [10, 11, 12]. Therefore, it can be considered as a quasi-essential element [13]. Asmar et al. (2013) [14] reported that using silicon salts in micropropagation of banana (*Musa* spp.) plantlets can improve photosynthesis rate and chlorophyll content. It has been found that Si has beneficial effects in the antioxidant capacity [15, 16] and non-enzymatic reactive oxygen species (ROS) scavenger such as phenolic compounds [17, 18]. Zhang et al. (2017) [19] found that application of silicate fertilizer increased fruit yield, cluster weight, berry weight, and berry size of grape (*Vitis vinifera* L.) cultivars. Despite the positive results observed in many prior studies, Si has not been included in the formulation of nutrient solutions. However, not all plants are unable to absorb actively the silicon through the root except plants in Poaceae and Cyperaceae families [13]. Therefore, foliar application of this element may be an effective strategy for Si plant nutrition [11, 16].

Recently, nanotechnology has revolutionized the world with tremendous developments in many fields of science [20, 21]. Engineered nanoparticles (ENPs), with sizes smaller than 100 nm in at least one dimension, have received a lot of attention [12, 16]. Haghighi et al. (2012) [21] reported that the application of 1mM nano-Si led to improved germination rate, root length and dry weight of tomato (*Lycopersicum esculentum*) seedlings. Mahdavi et al. (2016) [22] also mentioned that application of nano Si enhanced chlorophyll index and concentration of essential elements such as N, P, Zn, and Cu in Perennial ryegrass (*Lolium perenne*) shoot under water stress. Although, Haghighi and Pessarakli (2013) [8] indicated that nano and bulk Si particles improved photosynthesis, mesophyll conductance, and plant water use efficiency under saline stress conditions in cherry tomato (*Solanum lycopersicum* L.), but they did not found a significant differences between nano and bulk Si application. Wang et al. (2014) [16] also found that the foliar application of nano-Si, prepared by using sodium silicate, alleviated cadmium (Cd) toxicity in seedling rice (*Oryza sativa* L.) by decreasing Cd accumulation and malondialdehyde (MDA) and increasing essential element contents of Mg, Fe, and Zn and antioxidant capacity. Similar results are reported the interaction between Si nanoparticles and chromium stress in pea (*Pisum sativum* L.) seedlings [12].

Considering that in soilless greenhouse production of seed potato, the soilless substrate as growth media, and nutrition solutions do not have silicon sources [4]. The application of silicon has not been investigated yet. Therefore, this study was conducted to assess Si supplementation effects through the foliar application on potato transplanted plantlets, potato growth, photosynthetic parameters, biochemical characteristics, and mini-tuber yield. It is also tried to find that the effects of Si particle size on growth and biochemical characteristics.

2 Materials And Methods

2.1 Experiment

This greenhouse experiment was conducted at the College of Agricultural, Ferdowsi University of Mashhad (FUM), Khorasan Razavi Province, Mashhad (36° 27' N, 59° 63' E), Iran, in 2015. The experiment was conducted as a randomized complete block design (RCBD) with a factorial arrangement in three replications (experimental unit has five plantlets). Foliar application of silicon (Si) concentrations (distilled water (control), 0.8, 1.6, 2.4, and 3.2 mmol Si L⁻¹) and the silicon (Si) particle size (nano and ionized) were considered as experimental factors, respectively. Treatments were sprayed using an overhead trolley sprayer (Matabi 121030 Super Agro 20 L sprayer; Agratech Services-Crop Spraying Equipment, Rossendale, UK) at two growth stages: stolon (21 days after transplanting) and tuber (32 days after transplanting) initiation stages. At each time of application, 25 ml of Si solution was sprayed for each plantlet. This experiment was repeated twice under the same condition.

2.2 Synthesis of silicon nanoparticles

Nanoparticles of sodium silicate produced in Central Lab of Ferdowsi University of Mashhad. At the elementary phase, 50 g of Na_2SiO_3 (Sigma Aldrich, code number product: 307815, 99.9% purity, white color, and granule) were heated in an oven at 75°C for 5 h and was ground in a mixer mill (MM 400, Retsch, Germany) by 1680 rpm for 50 min with 1:10 of ratio sample to balls (w/w). Finally, bulk and milled particles size were measured with Particle Size Analyzer (PSA) (VASCO3, Cordouan Technologies, France, ranges of measurement 6µm to 1 nm) and Scanning Electron Microscope (SEM, model 1450VP, LEO, Germany, magnification ranging from 20x to approximately 300000x, and 2 nm resolution). The average diameter of milled particles was 68 nm (Figs. 1 and 2).

Please insert Figs. 1 and 2 near hear

2.3 Preparation of nano and ionized solutions

For preparation of each concentration level (nano or ionized), Citogait (a nonionic surfactant, 100% alkyl aryl polyglycol ether, Zarnegaran Pars, Iran) was added at 0.2% (v/v) to 500 mL of particle solution and its pH was adjusted to 5 ± 0.1 with 0.01N HCl. Then, the solutions shacked at 250 rpm in darkness at 20°C for 2 h.

2.4 Providing plantlets

To prepare the potato plantlets (Agria cv.), stem segments free from pathogens (approximately 15 mm length with one leaf node) were grown *in vitro* by using MS medium with 3% sucrose and 0.7% agar (Ma et al., 2015). After subculture, the plantlets were grown in a culture room with $60 \pm 5\%$ RH, 16 h light/8 h dark period (approximately 400 µmol photons $\text{m}^{-2} \text{s}^{-1}$) at $24 \pm 2^\circ\text{C}$ for 21 days. Afterward, each uniformed plantlet was gently washed with distilled water to remove agar and transplanted into a plastic pot (30 and 12 cm depth and diameter, respectively) filled with a 1:1:1 perlite, cocopite, and sand media. Fifty percent of growth medium was added in transplanting time and the rest was added after four weeks.

2.5 Experimental conditions

During the experiment, the mean of irradiance was approximately 1000 µmol photon $\text{m}^{-2} \text{s}^{-1}$ with 14 h light/10 h dark photoperiod, and the maximum and minimum air temperatures were 26°C and 18°C, respectively. Mean relative humidity was also $40 \pm 10\%$. Irrigation was applied equally (200 mL per plantlet) twice a week. The plantlets were nourished with 100 mL of complete Hoagland nutrient solution [23] once a week. The pH was measured with portable equipment and adjusted for 5.5 ± 0.1 using HCl and NaOH 2N solutions. To prevent the accumulation of salt every 15 days, 1200 mL of distilled water was added to each pot to wash the medium.

2.6 Gas exchange parameters

One week after second silicon application spraying, leaf gas exchange parameters including net photosynthetic rate (P_n , µmol $\text{CO}_2 \text{m}^{-2} \text{s}^{-1}$), substomatal CO_2 concentration (C_i , CO_2 ppm), transpiration rate (T_r , mmol $\text{m}^{-2} \text{s}^{-1}$) and stomatal conductance (G_s , mmol $\text{m}^{-2} \text{s}^{-1}$) were recorded from 10:00 to 11:00 a.m. in the clear and sunny sky by using a compact portable photosynthesis measurement system (LCi leaf chamber Analysis, ADC Bio Scientific Ltd. Hoddsdon, UK). Mesophyll conductance (M_C , mmol $\text{CO}_2 \text{m}^{-2} \text{s}^{-1}$) was calculated as the net photosynthetic rate divided by the substomatal CO_2 concentration. Water use efficiency (WUE , µmol $\text{CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$) was calculated as the net photosynthesis rate divided by the transpiration rate [8]. To assess these variables, an average of eight fully developed potato leaves was measured from each replication.

2.7 Biochemical assay

One week after the second Si application, chlorophyll (Chl), carotenoids (Cart), total phenol and DPPH radical-scavenging were measured from the second youngest fully developed leaves.

2.7.1 Pigments

Chlorophyll *a* and *b* and carotenoids contents were extracted based on Arnon (1949) [24] method. Samples of 100 mg fresh leaves were homogenized with 80% methanol at 4°C in a micro-tube and centrifuged at 3000 g for 15 min at 4°C and put in the refrigerator for 24 h. Then, the upper extract absorbance was measured at 653, 666, and 470 nm with a UV/Vis spectrophotometer (JENWAY, model 6305, UK). Chlorophyll content was calculated using the formula and expressed in mg per g fresh weight. The chlorophyll *a/b* ratio was also calculated as described by Arnon (1949) [24].

2.7.2 Total phenols

The total phenol content was determined according to the Folin–Ciocalteu reagent method proposed by Singleton and Rossi (1965) [25] with slight modification. Briefly, the alcoholic extract (20%, w/v) was diluted with distilled water and Folin–Ciocalteu was added to the mixture. After five minutes, the sodium carbonate (20%, w/v) was added and the mixture was kept in the dark for 30 min. The absorbance was measured at 765 nm (JENWAY, model 6305, UK), and total phenols were expressed as mg of gallic acid per g fresh weight.

2.7.3 DPPH radical scavenging

The DPPH radical scavenging activity of leaf extracts was measured according to the modified Abe et al. (1998) [26] method. The alcoholic extract (20%, w/v) was mixed with alcoholic DPPH solution (8%, w/v). The reaction mixture was shaken vigorously and left to stand in darkness for 30 min and absorbance was measured at 517 nm (JENWAY, model 6305, UK). DPPH radical scavenging was expressed as mg of ascorbic acid per g fresh weight.

2.8 Harvest of mini-tuber

The economic yield, including the mean mini-tuber weight and yield per plant was measured after 95 days of transplanting.

2.9 Statistical analysis

The study was arranged in a pooled two factorial experiments design with six replications. The data were analyzed with SAS 9.1 (SAS, Institute Inc., NC). Significant differences between the treatments were determined using Fisher's Least Significant Difference (FLSD) test at 0.05 probability level. Pearson correlation coefficient was also used to determine the correlation between traits.

3 Results

3.1 Gas exchange parameters

Silicon particle size (P) significantly affected net photosynthetic rate (Pn), Mesophyll conductance (MC), and water use efficiency (WUE) in potato plantlets, but no significant effect was observed on transpiration rate (Tr) and substomatal CO₂ concentration (Ci) (Table 1). Whereas, silicon concentration levels (C) showed a significant effect on all measured gas exchange parameters. A significant interaction between P and C was also observed on Pn, Ci, MC and WUE (Table 1).

Table 1
Analysis of variance for the effect of particle size and Si concentration levels on gas exchange parameters, pigment contents, biochemical traits, and tuber yield under greenhouse conditions.

Source of variation	d.f	^a P _n	Tr	Ci	MC	Gs	WUE	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a/b</i>	Cart	DPPH	T. phenol
Replication	5	^b 0.05 NS	0.04 NS	2391*	0.0000009 NS	12.3*	0.03 NS	0.004 NS	0.0009 NS	0.024 NS	0.0001 NS	0.000015 NS	0.000001 NS
Si particle size (P)	1	0.74**	0.05 NS	1706 NS	0.0000028*	276.7 ^{NS}	0.24*	0.066***	0.00090***	0.001 NS	0.0006**	0.000003 NS	0.000001 NS
Si concentration (C)	4	2.6***	1.76***	15199***	0.0000091***	234.1 NS	4.61***	0.039***	0.00200**	0.138*	0.0004**	0.000206***	0.000001 NS
P×C	4	0.2*	0.05 NS	5668***	0.0000022*	369 NS	0.17*	0.009*	0.00114*	0.008 NS	0.0001 NS	0.000240***	0.000001 NS
CV (%)	-	7.1	11.3	7.3	9.3	8.2	13.1	6.8	6.5	8.8	7.3	15.2	11.1

^a Net photosynthetic rate (Pn), Transpiration rate (Tr), Substomatal CO₂ concentration (Ci) Mesophyll conductance (MC), Gs (stomatal conductance), water use efficiency (WUE), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (Cart), DPPH radical scavenging (DPPH), total phenol (T. phenol), mini-tuber weight (TW), mini-tuber yield (TY).

^b NS: not significance at *P*> 0.05; *, **, *** significance level at *P*< 0.05, 0.01 and 0.001.

Please insert Table 1 near here

Foliar application of nano-Si and ionized-Si improved the net photosynthetic rate, mesophyll conductance, and water use efficiency (Table 2). The increasing Si concentrations, increased net photosynthesis rate, but the different sizes of Si had no similar effects. Net photosynthesis rate was significantly increased (*P* ≤ 0.05) by 23.84% in nano-Si treated plants compared with ionized-Si particles at 1.6 mmol Si L⁻¹ whereas in other concentration levels, the different between Si particle sizes was not significant. In nano and ionized Si treatments, the highest Pn of leaves was observed at 3.2 mmol Si L⁻¹ level, as 36.54% and 33.02% increment compared with control, respectively (Table 2). Although the interaction between particle size and Si concentration level was not significant on leaf transpiration rate (Table 1), transpiration rate distinctively decreased by increasing Si concentration in tuberization stage, and all Si concentration levels, a significant difference with control was recorded (*P*< 0.05) (Table 3). Furthermore, the highest value of transpiration rate was also obtained at 3.2 mmol Si L⁻¹ level with an increase of 38.06% compared with control (Table 4).

Table 2

Interaction effects of the Si particle size (P) and Si concentration levels (C) on the net photosynthetic rate (Pn), substomatal CO₂ concentration (Ci), mesophyll conductance (MC) and water use efficiency (WUE) of potato plantlet leaves at tuberization stage.

Treatments		Traits			
P	C	Pn	Ci	MC	WUE
		($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	(CO ₂ ppm)	($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$)
Nano-Si (mmol L^{-1})	Control	3.12 ± 0.25	395 ± 36	7.98 ± 1.14	1.28 ± 0.14
	0.8	3.36 ± 0.21	388 ± 32	8.69 ± 0.91	1.59 ± 0.06
	1.6	3.48 ± 0.22	372 ± 24	9.40 ± 1.11	1.69 ± 0.23
	2.4	3.78 ± 0.42	494 ± 45	7.70 ± 0.87	2.32 ± 0.28
	3.2	4.26 ± 0.14	399 ± 27	10.69 ± 0.57	2.68 ± 0.19
Ionized-Si (mmol L^{-1})	Control	3.15 ± 0.32	395 ± 41	8.01 ± 0.55	1.29 ± 0.20
	0.8	3.20 ± 0.19	398 ± 27	8.04 ± 0.44	1.39 ± 0.19
	1.6	2.81 ± 0.18	347 ± 20	8.11 ± 0.67	1.34 ± 0.13
	2.4	3.53 ± 0.23	417 ± 33	8.52 ± 0.99	2.02 ± 0.28
	3.2	4.19 ± 0.15	438 ± 26	9.61 ± 0.55	2.89 ± 0.43
LSD ($P \leq 0.05$)		0.29	34	0.90	0.28
The presented values are the means ± standard deviation from six replications (n = 6). LSD: The least significant difference at $P < 0.05$.					

Table 3

Effects of Si concentration levels on the transpiration rate (Tr), chlorophyll a/b ratio (Chl a/b) and carotenoids content of leaves at tuberization stage and mini-tuber potato yield.

Si concentrations (mmol L^{-1})	Traits			
	Tr	Chl a/b	Carotenoids	Mini-tuber yield
	($\text{mmol m}^{-2} \text{ s}^{-1}$)	ratio	($\text{mg g}^{-1} \text{ FW}$)	(g per plant)
Control	2.47 ± 0.39	2.51 ± 0.14	0.110 ± 0.007	28.1 ± 5.8
0.8	2.22 ± 0.21	2.53 ± 0.21	0.112 ± 0.009	29.4 ± 5.4
1.6	2.10 ± 0.24	2.49 ± 0.15	0.116 ± 0.007	30.8 ± 6.1
2.4	1.70 ± 0.17	2.59 ± 0.18	0.125 ± 0.013	32.9 ± 7.1
3.2	1.53 ± 0.15	2.76 ± 0.32	0.115 ± 0.011	34.6 ± 7.0
LSD ($P \leq 0.05$)	0.18	0.19	0.007	1.3
The presented values are the means ± standard deviation from twelve data (n = 12). LSD: The least significant difference at $P < 0.05$.				

Table 4
Interaction effects of the Si particle size (P) and Si concentration levels (C) on the Chlorophyll a (Chl a), chlorophyll b (Chl b), DPPH radical scavenging and total phenol of potato plantlet leaves at tuberization stage.

Treatments		Traits			
P	C	Chl a	Chl b	DPPH radical scavenging	Total phenol
		(mg g ⁻¹ FW)	(mg g ⁻¹ FW)	(Ascorbate mg g ⁻¹ FW)	(Galic acid mg g ⁻¹ FW)
Nano-Si (mmol L ⁻¹)	Control	0.743 ± 0.066	0.295 ± 0.018	0.0245 ± 0.0024	0.458 ± 0.028
	0.8	0.776 ± 0.068	0.308 ± 0.015	0.0243 ± 0.0029	0.493 ± 0.047
	1.6	0.804 ± 0.045	0.329 ± 0.021	0.0267 ± 0.0038	0.493 ± 0.050
	2.4	0.876 ± 0.077	0.336 ± 0.030	0.0366 ± 0.0039	0.624 ± 0.064
	3.2	0.952 ± 0.085	0.346 ± 0.027	0.0396 ± 0.0050	0.654 ± 0.111
Ionized-Si (mmol L ⁻¹)	Control	0.737 ± 0.031	0.296 ± 0.017	0.0243 ± 0.0017	0.479 ± 0.040
	0.8	0.729 ± 0.041	0.289 ± 0.015	0.0320 ± 0.0084	0.471 ± 0.047
	1.6	0.758 ± 0.034	0.301 ± 0.026	0.0357 ± 0.0062	0.570 ± 0.089
	2.4	0.800 ± 0.033	0.313 ± 0.016	0.0257 ± 0.0021	0.558 ± 0.063
	3.2	0.796 ± 0.039	0.292 ± 0.017	0.0317 ± 0.0069	0.543 ± 0.073
LSD ($P \leq 0.05$)		0.063	0.023	0.006	0.070
The presented values are the means ± standard deviation from six replications (n = 6). LSD: The least significant difference at $P < 0.05$.					

Please insert Tables 2 and 3 near hear

The foliar application of nano and ionized silicon levels imposed different effects on substomatal CO₂ concentration. For instance at 2.4 and 3.2 mmol Si L⁻¹, the substomatal CO₂ concentration increased significantly compared with control ($P < 0.05$), and the highest amount was observed at 2.4 mmol nano-Si L⁻¹ with a difference of 25.06% compared with control. The highest effect of ionized-Si particle size was also observed at 3.2 mmol Si L⁻¹ with an increase of 10.89% compared to control (Table 2). The application of nano and ionized Si levels changed the mesophyll conductance and the influence of 3.2 mmol ionized-Si L⁻¹, with an increase of 19.97%, was significant compared with control treatment, whereas other ionized-Si levels did not have a significant effect on mesophyll conductance of potato leaves. At 1.6 and 3.2 mmol nano-Si L⁻¹, the mesophyll conductance showed a significant difference of 17.79% and 33.96% compared to control, respectively. The highest mesophyll conductance of both Si sizes was observed in 3.2 mmol Si L⁻¹ (Table 2).

Similar to the results of net photosynthetic rate, either ionized or nano Si application improved the water use efficiency of potato and these changes differed under the influence of the particle size. The significant effect of ionized particles on WUE was observed with the increase of 56.59% and 124.03% compared with control at 2.4 and 3.2 mmol Si L⁻¹ levels, respectively. The whole nano-Si levels had a significant effect on the WUE of potato leaves at the tuberization stage and by increasing concentration WUE changes were more noticeable. Furthermore, the influence of nanoparticles was significantly higher than ionized particles at both 1.6 and 2.4 mmol Si L⁻¹ levels, but this difference was not significant in the highest concentration level (Table 2).

3.2 Biochemical traits

The silicon particle size (P) significantly affected chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids (Cart) content of potato leaves, but no significant effect was observed on chlorophyll a/b ratio (Chl a/b), the capacity of DPPH radical scavenging and total phenol content. Si concentration levels (C) showed a significant effect on all measured biochemical traits. Although the interaction between P and C was not significant on chlorophyll a/b ratio and carotenoids content, these effects were significant in the other biochemical characteristics as well (Table 1).

The application of ionized-Si particles had no significant effect on chlorophyll a and b content, whereas the foliar application of nano-Si levels improved chlorophyll a and b contents in potato leaves. At 2.4 and 3.2 mmol nano-Si L⁻¹ levels, chlorophyll a content was increased by 17.90% and 28.13% compared with control, respectively (Table 4). Furthermore, the highest significance of chlorophyll a content was recorded at 3.2 mmol nano-Si L⁻¹. The significant influences of nanoparticles on chlorophyll b content were observed by 11.52, 13.90, and 17.29 % at 1.6, 2.4, and 3.2 mmol Si L⁻¹ compared with control, respectively. There was no significant difference between mentioned nano-Si levels (Table 4). Foliar application of 3.2 mmol Si L⁻¹ enhanced the chlorophyll a/b ratio compared with control in potato leaves, whereas the influence of other Si treatments was not significant on this ratio (Table 4). The results showed that the carotenoids content of potato leaves was higher by 6.25% under nano-Si application than ionized-Si (Table 5). In potato plantlets treated with 2.4 mmol Si L⁻¹, carotenoids content was also significantly higher compared with control ($P < 0.05$), whereas other Si treatments was not effective (Table 3).

Table 5

Effects of Si particle size on the carotenoids content of leaves at tuberization stage, mean mini-tuber weight and mini-tuber potato

Si particle	Traits		
	Carotenoids (mg g ⁻¹ FW)	Mean mini-tuber weight (g)	Mini-tuber yield (g per plant)
Nano	0.119 ± 0.012	7.299 ± 1.645	32.063 ± 6.684
Ionized	0.112 ± 0.008	7.059 ± 1.977	30.299 ± 6.447
LSD ($P \leq 0.05$)	0.004	0.224	0.800

The presented values are the means ± standard deviation from thirty data (n = 30). LSD: The least significant difference at $P < 0.05$.

Table 6. Correlation analysis of gas exchange parameters, pigment contents, biochemical traits and yield of potato plantlets.

Nano-Si treatment	Traits	^a Pn	Tr	Ci	MC	Gs	WUE	Chl a	Chl b	Chl a/b	Cart	DPPH	T.phenol
	Pn	1	-0.810	0.921 ^{ab}	0.934 [*]	0.123	0.958 [*]	0.684	-0.098	0.903 [*]	0.219	-0.164	0.152
	Tr	-0.932 [*]	1	-0.592	-0.913 [*]	0.235	-0.937 [*]	-0.932 [*]	-0.278	-0.878 [*]	-0.703	-0.155	-0.687
	Ci	0.312	-0.541	1	0.722	0.054	0.785	0.523	-0.074	0.673	-0.029	-0.449	-0.126
	MC	0.647	-0.407	-0.521	1	0.087	0.988 ^{**}	0.762	-0.067	0.985 ^{**}	0.395	0.109	0.412
	Gs	0.114	0.067	0.087	0.095	1	0.067	0.098	0.032	0.054	0.154	0.136	0.098
	WUE	0.986 ^{**}	-0.971 ^{**}	0.462	0.515	0.214	1	0.817	0.027	0.959 ^{**}	0.441	0.021	0.417
	Chl a	0.997 ^{***}	-0.939 [*]	0.370	0.598	0.101	0.992 ^{***}	1	-0.200	0.673	0.858	-0.086	0.773
	Chl b	0.934 [*]	-0.940 [*]	0.346	0.573	0.104	0.937 [*]	0.937 [*]	1	-0.200	0.815	-0.352	0.654
	Chl a/b	0.888 [*]	-0.760	0.321	0.525	0.118	0.875 [*]	0.890 [*]	0.675	1	0.308	0.246	0.362
	Cart	0.433	-0.709	0.904 [*]	-0.325	0.064	0.570	0.475	0.570	0.256	1	0.080	0.956 [*]
	DPPH	0.947 [*]	-0.920 [*]	0.560	0.400	0.157	0.974 ^{**}	0.968 ^{**}	0.5886 [*]	0.884 [*]	0.589	1	0.334
	T.phenol	0.953 [*]	-0.959 [*]	0.580	0.388	0.194	0.987 ^{**}	0.968 ^{**}	0.886 [*]	0.887 [*]	0.634	0.987 ^{**}	1
	TW	-0.705	-0.822	0.191	0.495	0.102	0.712	0.682	0.835	0.362	0.554	-0.560	0.633
	TY	0.982 ^{**}	-0.962 ^{**}	0.464	0.512	0.217	0.995 ^{***}	0.992 ^{***}	0.954 [*]	0.850 [*]	0.575	0.980 ^{**}	0.980 ^{**}

^a Net photosynthetic rate (Pn), Transpiration rate (Tr), Substomatal CO₂ concentration (Ci), Mesophyll conductance (MC), Stomatal conductance (Gs), Water potential (WUE), Chlorophyll a (Chl a), Chlorophyll b (Chl b), Carotenoids (Cart), DPPH radical scavenging (DPPH), Total phenol (T. phenol), Mini-tuber weight (TW), Mini-tuber yield (TY).

^b *, **, *** significance level at $P < 0.05$, 0.01 and 0.001, respectively.

Please insert Table 4 near here

Foliar application of nano and ionized Si treatments improved the capacity of leaf DPPH radical scavenging in potato plants, but the influence of particle size was different on each level. At 0.8 and 1.6 mmol Si L⁻¹, DPPH radical scavenging of nano particle treated plants did not significantly change, whereas the application of ionized showed a significant effect by increasing 31.69% and 46.91% compared with control, respectively. However, at 2.4 and 3.2 mmol Si L⁻¹, application of nanoparticles, it was increased by 49.39% and 61.63% compared with control, respectively. The influence of nano-Si was also significantly higher than ionized-Si by 42.41% and 24.92% at each of 2.4 and 3.2 mmol Si L⁻¹. Overall, the highest total antioxidant capacity was observed at 3.2 mmol nano-Si L⁻¹ (Table 4).

The effect of both particle sizes on total phenol content at 0.8 mmol Si L⁻¹, were not significant. The highest influence of ionized observed at 1.6 mmol Si L⁻¹ by 18.99% difference compared with control but, ionized-Si concentration increased to more than 1.6 mmol Si L⁻¹, phenol content decreased (Table 4). Increasing concentration of nano-Si particles also improved the total phenol content of potato leaves and the highest total phenol content was observed at 2.4 and 3.2 mmol nano-Si L⁻¹ by 36.24% and 42.79% difference compared with control, respectively (Table 4). The difference between 2.4 and 3.2 mmol nano-Si L⁻¹ levels was also not significant. The total phenol content of nanoparticle treated plants was significantly higher than ionized at 3.2 Si mmol L⁻¹ (Table 4).

3.3 Mini-tuber yield

The silicon particle size (P) was significantly effective on the mean of mini-tuber weight (TW) and mini-tuber yield (TY) (Table 1). Silicon concentration levels (C) also showed a significant effect on mini-tuber yield, but their sole and interaction effects were not significant on the mean mini-tuber weight. In comparison with the ionized-Si treatment, the nano-Si significantly increased the mean of mini-tuber weight (Table 5), and a similar result was observed in the mini-tuber yield (Table 5). The potato mini-tuber yield significantly improved by increasing the Si concentration levels, and all Si treatments showed a significant difference with control ($P < 0.05$). These changes increased by increasing Si levels and the highest mini-tuber yield was observed at $3.2 \text{ mmol Si L}^{-1}$ (Table 3).

Please insert Table 5 near here

3.4 Correlation results

The correlations among the different characteristics were calculated using Pearson's correlation coefficients (Table 6). As shown in Table 6, mini-tuber yield was positively and significantly correlated with Pn ($P < 0.01$), WUE ($P < 0.001$), Chl a ($P < 0.001$), Chl b ($P < 0.05$), Chl a/b ratio ($P < 0.05$), scavenging DPPH radical ($P < 0.01$) and total phenol ($P < 0.01$) in nano-Si treatments and negative significant relationship was observed between mini-tuber yield and Tr ($P < 0.01$). In ionized-Si treatment, mini-tuber yield was positively and significantly correlated with WUE ($P < 0.05$) and Chl a ($P < 0.05$). The Tr in both nano-Si and ionized-Si treatments showed negative relationship with other measuring characteristics.

Please insert table 6 near here

4 Discussion

In this experiment, the foliar application of silicon concentrations enhanced gas exchange parameters, pigment contents, antioxidant capacity, and mini-tuber yield of potato which are consistent with previous reports regarding the beneficial influences of Si supplementation on antioxidant activities, growth and yield of other crops including cucumber [5], barley [27], soybean [29, 30], banana [14] and tomato [8].

In this study, the increasing Pn and pigment content was apparent at concentrations above 1.6 mmol L^{-1} of both Si sizes. These results were in agreement with studies on tomato, cucumber, and soybean which indicated the positive influence of Si supplement on the Pn and Chl content of leaves in hydroponic conditions [5, 8, 30]. Furthermore, our results indicated that DPPH radical scavenging was clearly increased by Si particles in the tuberization stage of potato. There might be numerous mechanisms involved in the influence of silicon on net photosynthesis however, an increase in antioxidant capacity as a result of Si induction may be the possible mechanism. Feng et al. (2010) [10] showed that Si could promote net photosynthesis, which is related to the individual role of Si in protecting photosynthesis apparatus from ROS damages. Furthermore, in similar previous reports it was proposed that silicon helps to improve the stability of cell plasma membranes [29], the integrity (thylakoids, grana lamellae) and function of chloroplast [8, 9, 10] and following that the electron transport chain in thylakoid membranes for the production of ATP and NADPH will be protected against ROS [9] by promoting antioxidant system for detoxifying reactive oxygen species [10, 30]. Our results also showed that total phenol content in potato leaves was enhanced significantly by Si at above 0.8 mmol L^{-1} levels. These observations are in agreement with the findings of Gagoonani et al. (2011) [17], who applied 1.5 mM Si which improved phenolic components of leaves at the control and Al toxicity treatments in *Brago officinalis* L. seedling was grown in hydroponically medium. Moreover, Shetty et al. (2011) [18] reported that the application of 3.6 mM Si stimulated phenolic acids and flavonoids in rose (*Rosa hybrida*). They suggested that Si can promote the expansion of genes encoding enzymes and transcript levels in the phenylpropanoid pathways compared with untreated Si plants. Phenolic components can impress plant development via lignin and pigment biosynthesis or accumulation in the subepidermal layers of plant tissues [30] and the up-regulation biosynthesis of phenols in chloroplasts could enhance radiation intercept in leaves [31]. So, increasing phenol content induced by Si has influenced the structure, function, and protection system of potato leaves, especially in the chloroplast. Since increase of DPPH radical scavenging and total phenols content under the influence of silicon was simultaneously accompanied with improvement in photosynthetic pigments, the probable conclusion can be that partial increasing concentration of photosynthesis pigments (chlorophyll *a* and *b*) may depend on maintaining ultrastructure and orderliness of chloroplast or improving of chlorophyll biosynthetic pathways as a consequence of Si-related up-regulation of antioxidant system and phenol components.

Several previous reports have shown silicon can regulate the activities of main photosynthetic enzymes of Calvin cycle [5, 11]. Adatia and Bestford (1986) [5] reported that the Si addition to nutrient solution enhanced carboxylase activity (RubisCO) of cucumber leaves under normal conditions. Similar findings were reported for barley [27] and *Spartina densiflora* [11] under saline and Cu toxicity stresses. Silicon application increased the activity of phosphoenol pyruvate carboxylase in wheat under drought conditions [32]. Hence it seems that in this experiment, part of the Si influence has been linked with activity regulation of key enzymes in non-photochemical photosynthetic processes. Moreover, up-regulation endogenous phytohormones such as GAs, IAA, and cytokines in Si-treated plants as reported for mango under drought stress [33] or GA₁ in soybean leaves under normal hydroponically conditions [28] mentioned in previous studies. In conclusion, it is likely that enhance of chlorophyll content and Pn were correlated with an increase of growth regulators in Si-treated potato leaves.

Results of this study indicated that by increasing Si concentration, Chl *b* content was significantly increased at 1.6 and carotenoids increased at 3.2 mmol L^{-1} of Si. The Chl *b* and carotenoids are considered as an antenna and auxiliary pigments for Chl *a* reaction centers. Therefore, an increase in carotenoids and Chl *b* can be helpful for the absorption of light energy for electron transport photosystems in Chl *a* [34]. It is possible to suggest a positive role of Si in controlling photoinhibition of potato leaves. Since the absorption spectrum of Chl *a* and Chl *b* are different, it seems that an increase in the Chl *a/b* ratio can determine the quality of light-harvesting by leaf. Moreover, the finding of Kitajima and Hogan (2003) verified that improvement of Chl *a/b* accompanied by an increase in the electron transport rate in reaction centers of Chl *a* and rubisco carboxylation capacity which are in agreement with our results that shown a positive correlation between Pn with Chl *a* and Chl *a/b* in two scales of Si particle treatments (Table 6).

Based on this study results, an increase of Pn was simultaneously accompanied with decrease of Tr in Si-treated leaves at the tuberization growth stage. Nevertheless, the Gs was not affected by Si treatments (Tables 1 and 2). If the limitation of Tr was due to stomatal closure, there should be a decrease in Ci. However, Ci had a slight increase in Si-treated leaves with 2.4 and 3.2 mmol L⁻¹. Therefore, the decrease in Tr may be due to nonstomatal restrictions which are in agreement with the results reported for *S. densiflora* [11] (Mateos-Naranjo et al. 2015) and tomato [35] treated with Si under salinity stress in greenhouse conditions. Various studies have described that by applying of silicon, the silica cuticle double layer was formed on the leaf epidermis which may reduce water loss through the cuticles of plants [11, 14, 36]. Although, the cuticular transpiration rate is lower than the stomatal transpiration rate, it can perform an important role in leaf water loss. Therefore, it seems that the decrease of Tr in Si-treated potato leaves most likely has been due to the reduction of cuticular Tr. Asmar et al. (2013) [14] suggested that the Si accumulation in epidermal tissue can have a positive effect on water relations in leaves during acclimatization under humidity changes in greenhouse conditions. Moreover, physical strength caused by Si deposition may develop mechanical protection to infection pathogens in crop leaves [18]. Therefore, it seems that foliar application of Si particles can be useful for the growth and health of potato plantlets are transferred to soilless culture. Our results also indicated that stomatal conductance did not change (Table 1) and it has not limited the CO₂ diffusion into sub-stomata chamber or CO₂ assimilation in chloroplasts. Accordingly, an increase of Pn with Si may associate with the photosynthetic enzymatic process and chloroplast function.

Overall, silicon particles levels improved water use efficiency. Previous studies are in agreement with these results indicated that Si can enhance water use efficiency at the normal conditions in tomato [8, 36]. Our results showed that a decrease in transpiration rate was accompanied by an increase of Pn in Si-treated leaves. Therefore, improvement of water use efficiency was predictable. A positive effect of Si application on mesophyll conductance of potato leaves was also in agreement with Haghighi and Pessarakli (2013) [8] results in cherry tomato. Our results showed that all Si levels had a positive effect on mini-tuber yield. The ability of Si to increase yield production has been demonstrated in cucumber [5] and tomato [35].

According to this study, although net photosynthesis, pigment content, and yield measured in Si treatments were enhanced. These changes in nano-Si treated plants were more than ionized-Si. These results also indicated that potato leaves could foliar uptake of Si and nanoscale particles showed more efficiency in response to this method. The higher influence of nanoparticles may be due to unique characteristics [8, 12, 37] and facility uptake by leaf stomata because of their smaller size. These observations are in agreement with Tripathi et al. (2015) [12], who achieved the beneficial influence of nano-Si on growth and dry weight of *Pisum sativum* in normal and Cr toxicity. Siddiqui and Al-Whaibi (2014) [36] also confirmed that nano-Si increased the germination characteristics, which enhanced the seedling dry weight of tomato. However, Haghighi and Pessarakli (2013) [8] showed that although Si addition mitigated adverse effects of salinity in gas exchange parameters and dry weight of cherry tomato, no difference between root application of nano and bulk Si was observed. The comparison of silicon nanoparticles and silicate in Fenugreek by Nazaralian et al. (2017) [6] also indicated that the influence of the added nanoparticles in nutrient solution declined over time. Moreover, the results of Abdel-Halim et al. (2017) [37] showed that application of nano and ions Si in rice seedling under saline conditions increased growth, antioxidant activity, and biochemical traits such as soluble carbohydrates and amino acids, but the difference between particle sizes of Si was not noticeable.

Stomatal uptake can be a main pathway for the foliar uptake of mineral nutrients and other solutes [34] (Taiz et al., 2015), which can not able to penetrate through the surface of the epidermal cells. There are very fine pores with a diameter on a nanoscale on adaxial and abaxial leaf surface which are called ectodesmata with an approximate density of 10¹⁰ per cm⁻² leaf area. Moreover, inside of this pores are covered with polygalacturonic acids, which only allow positive particles to enter [38] (Marschner 1995). It can be concluded that since in our study silicon solutions were acidic (pH = 5) and ectodesmata diameter was nanoscale, it seems that the high influence of nano-Si treatments may be due to an increase of their foliar uptake via ectodesmata in potato leaves.

Conclusion

In this study, foliar application of Si particles imposed a remarkably positive role in the improving of photosynthetic, biochemical characteristics, and mini-tuber yield of potato. Moreover, the nanoparticles was more effective than ionized-Si in many measured traits. As a result, it could be recommended that Si application improve the safety and tuber propagation of potato plantlets in soilless culture conditions. To maximize the influence of Si treatment, the use of nanoparticles will be a proper strategy.

Declarations

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Author contribution

Bijan Saadatian designed and performed the experiments, **Mohamad Kafi** was involved in planning and supervised the project, **Mohamad Banayan** conceived the original idea and supervised the project **Hossein Hammami** Processed the experimental data, performed the analysis, wrote the manuscript with support from all authors. All authors discussed the results and contributed to the final manuscript.

Data Availability Not applicable.

Compliance with Ethical Standards

Conflicts of Interest None.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Code Availability Not applicable.

Research involving Human Participants and/or Animals Not applicable.

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Figures

Figure 1

The result of particle size analyzer (PSA) nano Si particles.

Figure 2

Scanning Electron Ionizedscope (SEM) ionizedgraph of nano-Si particles with magnification of 40000X (a) and 50000X .