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ORIGINAL PAPER

Effects of Foliar Spraying Nano and Ionized Silicon on Physiological Characteristics and Yield of Potato (*Solanum tuberosum* L.) Mini-tuber

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Abstract

Purpose The role of protecting and structure-stabilizing silicon (Si) has been demonstrated on different plant species. However, it has not been used in potato seed production under a soilless culture system. Therefore, this experiment was conducted to evaluate the ionized –Si and nano-Si particles on physiological characteristics and yield of potato mini-tuber.
Methods A greenhouse experiment under a soilless culture system was performed as a randomized complete block design (RCBD) arranged in 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.
Results 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, shoot dry weight, Si concentration in shoot, mean weight mini-tuber, and yield, whereas transpiration rate in Si-treated plants decreased. Moreover, the highest positive influence of Si was observed at 3.2 mmol L^{-1}. The effect of nano-Si was higher than ionized-Si at all Si concentrations. The results revealed improved physiological characteristics and yield of potato plantlets under nano-Si treatments compared to ionized-Si treatments. However, these relations were not significant under ionized treatment.
Conclusions This study indicated that the application of Si (nano and ionized) for potato growing and mini-tuber production has positive effects. Generally, under a soilless culture system, Nano-Si has higher efficiency than ionized-Si in mini-tuber production.

Keywords Antioxidant capacity · Chlorophyll content · Nano-Si · Soilless culture system

Abbreviations

Cd	Cadmium
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Cart	carotenoids
DPPH	DPPH radical scavenging
MC	Mesophyll conductance
Pn	Net photosynthetic rate
TW	mini-tuber weight
RCBD	randomized complete block design
Si	Silicon
Tr	Transpiration rate
T.phenol	Total phenol
SiS	Si concentration in shoot
TY	mini-tuber yield
WUE	Water use efficiency

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1 Introduction

Potato (*Solanum tuberosum* L.) is one of the important food crop in the world. In 2019, the potato was planted on 17,340,986 and 104,192 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 free from pathogens, especially viruses, potato plantlets are propagated from meristem culture and transferred to the greenhouse [3, 4].

Si is the second most (27.7%) abundant mineral element in the earth crust and mineral substrate for the most plants. Many researchers have shown that Si supply improved the growth and development of crop plants under normal [5–7] and stressful conditions such as salinity [8, 9], drought [9, 10], and toxic heavy metals [11–13]. Therefore, it has taken into consideration as a quasi-essential element [14]. Asmar et al. (2013) [15] reported that using Si 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 [16, 17] and non-enzymatic reactive oxygen species (ROS) scavenger such as phenolic compounds [18, 19]. Zhang et al. (2017) [20] found that the application of silicate fertilizer increased fruit yield, cluster weight, berry weight, and berry size of grape (*Vitis vinifera* L.) cultivars. Despite the positive effects of Si on plant growth, it has not been included in the formulation of nutrient solutions. However, not all plants are unable to absorb the Si actively through the root except plants in Poaceae and Cyperaceae families [14]. Therefore, foliar application of this element may be an effective strategy for Si plant nutrition [12, 17].

Recently, nanotechnology has revolutionized the world with tremendous developments in many fields of science [21, 22]. Engineered nanoparticles (ENPs), with sizes smaller than 100 nm in at least one dimension, have taken consideration [13, 17]. Haghighi et al. (2012) [22] 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) [23] also mentioned that improved chlorophyll content and concentration of macro (N and P) and micro (Zn and Cu) elements in Perennial ryegrass (*Lolium perenne*) shoot under water stress by nano-Si application. 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 demonstrated non-significant differences between nano and bulk Si application. Wang et al. (2014) [17] also found that the amelioration cadmium (Cd) toxicity by foliar application nano-Si (prepared by using sodium silicate) in rice (*Oryza sativa* L.) seedling. Moreover, nano-Si declined Cd accumulation and malondialdehyde (MDA) and improved micro-element content in rice seedling. Similar results have been reported in pea *(Pisum sativum* L.) seedlings growth under chromium stress by Si nanoparticles [13]. Considering that in the soilless greenhouse production of seed potato, the soilless substrate as growth media, and nutrition solutions do not have Si sources [4]. The application of Si has not been investigated yet. Therefore, this experiment was conducted to evaluate Si supplementation effects through the foliar application on potato-transplanted plantlets, characteristics, and mini-tuber yield. It is also tried to find that the effects of Si particle size on physiological characteristics and mini-tuber yield of potato.

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 (each experimental unit has five plantlets) (Fig. 1). Foliar application of Si concentrations (distilled water (control), 0.8, 1.6, 2.4, and 3.2 mmol Si L^{-1}) and the Si particle size (nano and ionized) was considered as experimental factors, respectively. Treatments were sprayed using an overhead trolley sprayer (Matabi 121,030 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 Si Nanoparticles

Nanoparticles of sodium silicate produced in Central Lab of the Ferdowsi University of Mashhad. At the elementary phase, 50 g of Na_2SiO_3 (Sigma Aldrich, code number product: 307,815, 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 6 µm to 1 nm) and Scanning Electron Microscope (SEM, model 1450VP, LEO, Germany, magnification ranging from 20x to approximately 300000x, and 2 nm resolution) (Fig. 2a and b). The average diameter of milled particles was 68 nm (Fig. 2c).

2.3 Preparation of Nano and Ionized Solutions

The preparation of each concentration level (nano or ionized), Citogate (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 0.01 N HCl. Then, the solutions shacked at 250 rpm in darkness at 20 °C for 2 h.

Fig. 1 The pots used for experiment (each experimental unit include five plantlets)

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 [3]. After subculture, the plantlets were grown in a culture room with 60±5% RH, 16 h light/8 h dark period (approximately 400 μmol photons m^{-2} s^{-1}) at 24±2 °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 (pH: 7.02, SiO_2: 74%, Al_2O_3: 12%, Fe_2O_3: 1.8%, CaO: 1.2%, K2O: 2%, Na2O: 1%, and other chemicals: 8%). 50% of growth medium was added in transplanting time and the other was added after four weeks.

2.5 Experimental Conditions

During the experiment, the mean of irradiance was approximately 1000 μmol photon m^{-2} 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±10%. Irrigation was applied equally (200 mL per plantlet) twice a week. The plantlets were nourished with 100 mL of complete Hoagland nutrient solution [24] once a week. The pH was measured with portable equipment and adjusted for 5.5±0.1 using HCl and NaOH 2 N solutions. To prevent the salt accumulation, every 15 days, 1200 mL of distilled water was added to each pot to wash the medium.

2.6 Physiological and Biochemical Assay

2.6.1 Gas Exchange Parameters

One week after second Si application spraying, leaf gas exchange parameters including net photosynthetic rate (Pn, μmol CO_2 m^{-2} s^{-1}), substomatal CO_2 concentration (Ci, CO_2 ppm), transpiration rate (Tr, mmol m^{-2} s^{-1}) and stomatal conductance (Gs, mmol m^{-2} 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 (MC, mmol CO_2 m^{-2} s^{-1}) was calculated as the net photosynthetic rate divided by the substomatal CO_2 concentration. Water use efficiency (WUE, μmol CO_2 $mmol^{-1}$ H_2O) was calculated as the net photosynthesis rate divided by the transpiration rate [8]. To assess these

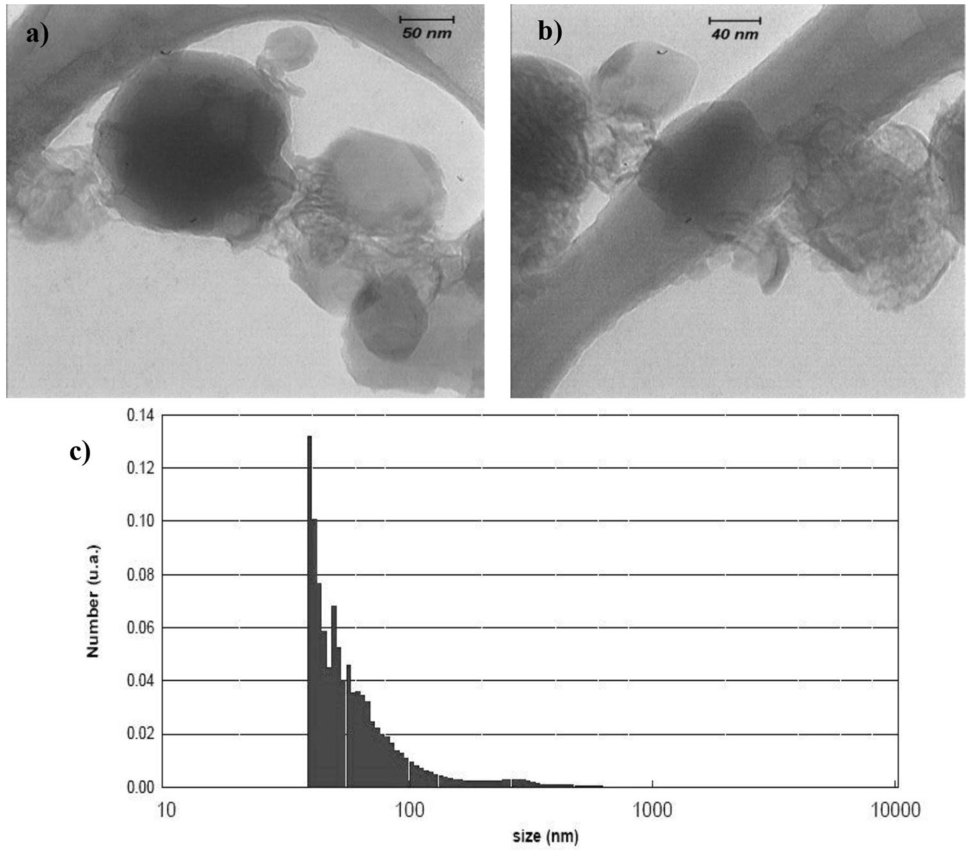

Fig. 2 Scanning Electron Ionized scope (SEM) ionized graph of nano-Si particles with magnification of 40000X (**a**), 50000X (**b**) and particle size analyzer (PSA) nano Si particles (**c**)

variables, an average of eight fully developed potato leaves was measured from each replication.

2.6.2 Pigments

Chlorophyll *a* and *b* and carotenoids contents were extracted based on Arnon (1949) [25] 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 (JENEWAY, 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) [25].

2.6.3 Total Phenols

The total phenol content was determined according to the Folin–Ciocalteu reagent method proposed by Singleton and Rossi (1965) [26] with slight modification. Briefly, the alcoholic extract (20%, w/v) was diluted with distilled water and Folin–Ciocalteau was added to the mixture. After five minutes, the sodium carbonate (20%, w/v) was added. 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.

2.6.4 DPPH Radical Scavenging

The DPPH radical scavenging activity of leaf extracts was measured according to the modified Abe et al. (1998) [27] method. The alcoholic extract (20%, w/v) was mixed with alcoholic DPPH solution (8%, w/v). The reaction mixture was shaken vigorously and hold 30 min in dark condition. The absorbance was measured by spectrophotometer (JENWAY, model 6305, UK) at 517 nm. DPPH radical scavenging was expressed as mg of ascorbic acid per g fresh weight.

2.7 Silicon Detection

Si in each plant sample was determined by Elliot and Snyder (1991) [28] method. Briefly, plant samples were dried and ground. 100 mg dry powder from plant samples were wetted by 2 mL H_2O_2 (50%) + 4.5 g NaOH in 100-mL polyethylene tubes at ambient temperature and each tube was gently vortexed. The samples were placed in an autoclave at 130 kPa for 1 h. After cooling samples at ambient temperature deionized water at to samples to get 10 mL solution. Then 35 mL

CH_3COOH (20%) + 5 mL tartaric acid (20%) were added to the samples. Finally, Si concentration was measured at 650 nm with a UV/Vis spectrophotometer (JENEWAY, model 6305, UK).

2.8 Harvest

After 95 days of transplanting, economic yield, including the mean mini-tuber weight and yield per plant was measured. Also, the shoots of potato plantlets were harvested and weighed after being exposed to 72 °C for three days.

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 Physiological Traits

Si 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 CO_2 concentration (Ci) (Table 1). Whereas, Si 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).

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 ($P \leq 0.05$) 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 difference 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 the tuberization stage, and all Si concentration levels, a significant difference with control was recorded ($P<0.05$)

Table 1 Analysis of variance for the effect of particle size and Si concentration levels on physiological and biochemical characteristics and tuber yield of potato plantlets in greenhouse conditions

Source of variation	d.f	[a]Pn	Tr	Ci	MC	Gs	WUE	Chl *a*	Chl *b*	Chl *a*/*b*	Cart	DPPH	T. phenol	SD	SiS	TW	TY
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}	0.011^{*}	0.006^{NS}	0.01^{NS}	36.4^{***}	408^{***}
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}	0.006^{NS}	0.002^{**}	2.46^{***}	0.9^{*}	47^{***}
Si concentration (C)	4	2.6^{***}	1.76^{***}	$15{,}199^{***}$	0.0000091^{***}	234.1^{NS}	4.61^{***}	0.039^{***}	0.00200^{**}	0.138^{*}	0.0004^{**}	0.000206^{***}	0.043^{***}	0.254^{***}	6.34^{***}	0.3^{NS}	84^{***}
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^{***}	0.016^{***}	0.013^{NS}	0.20^{***}	0.1^{NS}	4^{NS}
CV (%)	-	7.1	11.3	7.3	9.3	8.2	13.1	6.8	6.5	8.8	7.3	15.2	11.2	7.7	4.8	6.0	5.1

[a]Net photosynthetic rate (Pn), Transpiration rate (Tr), Substomatal CO_2 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), shoot dry weight (SD), Si concentration in shoot (SiS), 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.

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 (μmol CO_2 m^{-2} s^{-1})	Ci (CO_2 ppm)	MC (mmol CO_2 m^{-2} s^{-1})	WUE (μmol CO_2 $mmol^{-1}$ H_2O)
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≤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, shoot dry weight and mini-tuber potato yield

Si concentrations (mmol L^{-1})	Traits				
	Tr (mmol m^{-2} s^{-1})	Chl *a/b* ratio	Carotenoids (mg g^{-1} FW)	shoot dry weight (g per plant)	Mini-tuber yield (g per plant)
Control	2.47±0.39	2.51±0.14	0.110±0.007	1.101d±0.073	28.1±5.8
0.8	2.22±0.21	2.53±0.21	0.112±0.009	1.184c±0.070	29.4±5.4
1.6	2.10±0.24	2.49±0.15	0.116±0.007	1.293b±0.110	30.8±6.1
2.4	1.70±0.17	2.59±0.18	0.125±0.013	1.405a±0.129	32.9±7.1
3.2	1.53±0.15	2.76±0.32	0.115±0.011	1.446a±0.139	34.6±7.0
LSD (P≤0.05)	0.18	0.19	0.007	0.082	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 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).

The foliar application of nano and ionized Si levels imposed different effects on substomatal CO_2 concentration. At 2.4 and 3.2 mmol Si L^{-1} treatment, the substomatal CO_2 concentration increased significantly compared with control (P<0.05). 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. In contrast, 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. These changes differed under the influence of the particle size. The significant effect of ionized particles on WUE was observed with an increase of 56.59% and 124.03% compared with control at 2.4 and 3.2 mmol Si L^{-1} levels, respectively. Generally, nano-Si levels had a significant effect on the WUE of potato leaves at the tuberization stage by increasing concentration. 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).

The Si 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

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 and Si concentration in shoot and data of shoot dry weight

Treatments		Traits					
P	C	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	DPPH radical scavenging (Ascorbate mg g^{-1} FW)	Total phenol (Galic acid mg g^{-1} FW)	Si concentration in shoot (mg.g^{-1}DW)	shoot dry weight (g per plant)
Nano-Si (mmol L^{-1})	Control	0.743±0.066	0.295±0.018	0.0245±0.0024	0.458±0.028	1.449±0.028	1.108±0.076
	0.8	0.776±0.068	0.308±0.015	0.0243±0.0029	0.493±0.047	2.063±0.113	1.200±0.077
	1.6	0.804±0.045	0.329±0.021	0.0267±0.0038	0.493±0.050	2.796±0.168	1.360±0.067
	2.4	0.876±0.077	0.336±0.030	0.0366±0.0039	0.624±0.064	3.193±0.111	1.447±0.163
	3.2	0.952±0.085	0.346±0.027	0.0396±0.0050	0.654±0.111	3.541±0.095	1.532±0.134
Ionized-Si (mmol L^{-1})	Control	0.737±0.031	0.296±0.017	0.0243±0.0017	0.479±0.040	1.433±0.065	1.094±0.061
	0.8	0.729±0.041	0.289±0.015	0.0320±0.0084	0.471±0.047	1.796±0.117	1.168±0.066
	1.6	0.758±0.034	0.301±0.026	0.0357±0.0062	0.570±0.089	2.274±0.106	1.226±0.114
	2.4	0.800±0.033	0.313±0.016	0.0257±0.0021	0.558±0.063	2.533±0.123	1.364±0.078
	3.2	0.796±0.039	0.292±0.017	0.0317±0.0069	0.543±0.073	2.981±0.121	1.361±0.084
LSD ($P\leq0.05$)		0.063	0.023	0.006	0.070	0.131	ns

The presented values are the means ± standard deviation from six replications (n=6). LSD: The least significant difference at $P<0.05$.ns: is not significant difference

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 physiological characteristics (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 were not effective (Table 3).

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 had 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, DPPH radical scavenging 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

Table 5 Effects of Si particle size on the carotenoids content of leaves at tuberization stage, shoot dry weight, mean mini-tuber weight and mini-tuber potato yield

Si particle	Traits			
	Carotenoids (mg g^{-1} FW)	shoot dry weight (g per plant)	Mean mini-tuber weight (g)	Mini-tuber yield (g per plant)
Nano	0.119±0.012	1.329±0.189	7.299±1.645	32.063±6.684
Ionized	0.112±0.008	1.243±0.132	7.059±1.977	30.299±6.447
LSD ($P\leq0.05$)	0.004	0.052	0.224	0.800

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

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. 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).

3.1.1 Mini-tuber Yield and Shoot Dry Weight

The Si particle size (P) was significantly effects on the shoot dry weight (SD), mean of mini-tuber weight (TW) and mini-tuber yield (TY) (Table 1). Si concentration levels (C) also showed a significant effect on shoot dry weight and mini-tuber yield, but their simple and interaction effects were not significant on the shoot dry weight and mean mini-tuber weight. In comparison with the ionized-Si treatment, the nano-Si significantly increased the shoot dry weight (Table 5). A similar result was observed in the mean of mini-tuber weight and mini-tuber yield (Table 5). The shoot dry weight significantly improved by increasing the Si concentration levels, and all Foliar application of Si treatments showed a significant difference with control (P<0.05). However, there was not difference between 2.4 and 3.2 mmol Si L^{-1} concentrations. The potato mini-tuber yield was significantly improved by increasing Si levels and the highest of it was observed at 3.2 mmol Si L^{-1} (Table 3).

3.1.2 Si Concentration in Shoot

The main effects of Si particle size (P) and Si concentration levels (C) significantly affected Si concentration in the shoot. Moreover, the interaction between P and C was also significant (Table 1). Foliar application of nano and ionized Si treatments increased Si concentration in shoot potato plants. The influence of nano-Si was also significantly higher than ionized-Si by 14.86, 22.95, 26.06, and 18.78% at each of 0.8, 1.6, 2.4, and 3.2 mmol Si L^{-1} levels. (Table 4).

3.1.3 Correlation Results

The correlations among the different characteristics were calculated using Pearson's correlation coefficients (Table 6). As shown in Table 6, in nano-Si treatments, Si concentration in the shoot was positively correlated with Chl a (P<0.001), shoot dry weight (P<0.001), and mean of mini-tuber weight (P<0.01). The 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), total phenol (P<0.01), shoot dry weight (P<0.001), and Si concentration in shoot (P<0.05) and significant negative relationship was observed between Tr with Si concentration in the shoot (P<0.05) and mini-tuber yield (P<0.01). In ionized-Si treatment, Si concentration in shoot was positively a significantly correlated with Chl a (P<0.05) and shoot dry weight (P<0.05). Mini-tuber yield was positively and significantly correlated with WUE (P<0.05), Chl a (P<0.05), shoot dry weight (P<0.05), and Si concentration in shoot (P<0.001). The Tr in both nano-Si and ionized-Si treatments showed a negative relationship with other measuring characteristics.

4 Discussion

In this experiment, the foliar application of Si concentrations enhanced gas exchange parameters, pigment contents, antioxidant capacity, shoot dry weight, and mini-tuber yield of potato. These results are in accordance with previous reports regarding the beneficial influences of Si supplementation on antioxidant activities, growth, and yield of other crops including cucumber [5], barley [29], soybean [30, 31], banana [15] 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, 31]. 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 Si on net photosynthesis however, an increase in antioxidant capacity as a result of Si induction may be the possible mechanism. Feng et al. (2010) [11] showed that increase of Si concentration in plant 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 Si helps to improve the stability of cell plasma membranes [30], the integrity (thylakoids, grana lamellae) and function of chloroplast [8, 11, 32], and following that the electron transport chain in thylakoid membranes for the production of ATP and NADPH will be protected against ROS [32] by promoting antioxidant system for detoxifying reactive oxygen species [11, 31]. Our results also showed that total phenol

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

	Traits	[a]Pn	Tr	Ci	MC	Gs	WUE	Chl *a*	Chl *b*	Chl *a/b*	Cart	DPPH	T.phenol	SD	SiS	TW	TY	
Nano-Si treatment	Pn	1	-0.810	0.921^{*b}	0.934*	0.123	0.958*	0.684	-0.098	0.903*	0.219	-0.164	0.152	0.686	0.698	-0.386	0.702	Ionized-Sitreatment
	Tr	-0.932*	1	-0.592	-0.913*	0.235	-0.937*	-0.932*	-0.278	-0.878*	-0.703	-0.155	-0.687	-0.960**	-0.980**	-0.128	-0.978**	
	Ci	0.312	-0.541	1	0.722	0.054	0.785	0.523	-0.074	0.673	-0.029	-0.449	-0.126	0.517	0.436	-0.591	-0.447	
	MC	0.647	-0.407	-0.521	1	0.087	0.988**	0.762	-0.067	0.985**	0.395	0.109	0.412	0.770	0.858	-0.134	0.853	
	Gs	0.114	0.067	0.087	0.095	1	0.067	0.098	0.032	0.054	0.154	0.136	0.098	0.168	0.231	0.142	0.121	
	WUE	0.986**	-0.971**	0.462	0.515	0.214	1	0.817	0.027	0.959**	0.441	0.021	0.417	0.827	0.869	-0.156	0.867*	
	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	0.952*	0.893*	0.055	0.883*	
	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	0.469	0.269	0.155	0.258	
	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	0.724	0.844	-0.048	0.843	
	Cart	0.433	-0.709	0.904*	-0.325	0.064	0.570	0.475	0.570	0.256	1	0.080	0.956*	0.803	0.740	0.396	0.726	
	DPPH	0.947*	-0.920*	0.560	0.400	0.157	0.974**	0.968**	0.5886*	0.884*	0.589	1	0.334	0.120	0.333	0.834	0.341	
	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	0.746	0.768	0.553	0.751	
	SD	0.945*	-0.947*	0.375	0.558	0.148	0.950*	0.949*	0.998***	0.703	0.580	0.908*	0.906*	1	0.952*	0.246	0.956*	
	SiS	0.930*	-0.953*	0.372	0.549	0.186	0.939*	0.932*	0.998***	0.668	0.602	0.882	0.891	0.997***	1	0.317	0.999***	
	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	0.812	0.990**	1	0.329	
	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**	0.966***	0.952*	0.701	1	

[a] Net photosynthetic rate (Pn), Transpiration rate (Tr), Substomatal CO_2 concentration (Ci), Mesophyll conductance (MC), Stomatal conductance (Gs), Water use efficiency (WUE), Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), Carotenoids (Cart), DPPH radical scavenging (DPPH), Total phenol (T. phenol), shoot dry weight (SD), Si concentration in shoot (SiS), Mini-tuber weight (TW), Mini-tuber yield (TY).

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

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) [18], 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) [19] 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 [31]. The up-regulation biosynthesis of phenols in chloroplasts could enhance radiation intercept in leaves [33]. Therefore, 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 Si 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 chlorophyll biosynthetic pathways as a consequence of Si-related up-regulation of antioxidant system and phenol components.

Several previous reports have shown Si can regulate the activities of main photosynthetic enzymes of Calvin cycle [5, 12]. 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* [32] under saline and Cu toxicity stresses. Si application increased the activity of phosphoenol pyruvate carboxylase in wheat under drought conditions [34]. 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 GA_1 in soybean leaves under normal hydroponically conditions [35] 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* [36]. It is possible to suggest a positive role of Si in controlling the 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) [37] 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* [12] (Mateos-Naranjo et al. 2015) and tomato [38] treated with Si under salinity stress in greenhouse conditions. Many current researches have described reduce water loss through the cuticles of plants by applying Si [12, 14, 39]. 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) [15] 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 [19]. 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 CO_2 diffusion into the sub-stomata chamber or CO_2 assimilation in chloroplasts. Accordingly, an increase of Pn with Si may associate with the photosynthetic enzymatic process and chloroplast function.

Overall, Si 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, 40]. 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 [38]. Si concentration in the shoot was more than in nano-Si compared to ionized-Si treatments due to higher absorption and biological activity of nano-Si than ionized-Si. In accordance with the results of our experiment higher absorption and biological activity of nano-Si was demonstrated [15, 36]. Also, according to the results of correlation relationships, it can be concluded that the superiority of nano-Si in improving photosynthesis, leaf biochemical properties, weight, and yield of potato tubers through higher concentrations of silicon in these treatments compared to ionized particles. Tripathi et al., (2015) [13] reported that the amelioration effects of nano-Si on heavy metals toxicity due to high biological activity. Higher biological activity of nano-Si in foliar spraying than ionized-Si is the main factor for improving potato growth.

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 effects on 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, 13, 39] and facility uptake by leaf stomata because of their smaller size. These observations are in agreement with Tripathi et al. (2015) [13], 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) [40] also confirmed that nano-Si increased the germination characteristics, which enhanced the dry seedling 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 Si 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-Haliem et al. (2017) [39] showed that application of nano and ions Si in rice seedling under saline conditions increased growth, antioxidant activity, and physiological traits such as soluble carbohydrates and amino acids, but the difference between particle sizes of Si was not noticeable.

Stomatal uptake can be the main pathway for the foliar uptake of mineral nutrients and other solutes [36] (Taiz et al., 2015), which cannot be 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 ectodesmota with an approximate density of 10^{10} per cm^{-2} leaf area. Moreover, inside of this pores are covered with polygalacturonic acids, which only allow positive particles to enter [41] (Marschner 1995). It can be concluded that since in our study Si solutions were acidic (pH=5) and ectodesmota diameter was nanoscale, it seems that the high influence of nano-Si treatments may be due to an increase of their foliar uptake via ectodesmota in potato leaves.

5 Conclusion

In this study, foliar application of Si particles imposed a remarkably positive role in the improving of photosynthetic, physiological characteristics, shoot dry weight, Si concentration in shoot and mini-tuber yield of potato. Moreover, the nanoparticles were more effective than ionized-Si in many measured traits due to higher foliar absorption. 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. According to the positive results of foliar application of nano and ionized silicon in improving the growth and yield of tissue culture potato seedlings in greenhouse conditions, it is suggested that additional studies on the application of silicon in hydroponic and aeroponic systems be used to produce potato mini-tubers.

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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 aval 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.

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Declarations

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Conflict of Interest None.

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