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Research paper

Silicon nanoparticles alleviate arsenic toxicity in rice (*Oryza sativa* L.)

Tahereh Kiany[1], Leila Pishkar[1*], Nasrin Sartipnia[1], Alireza Iranbakhsh[2], and Giti Barzin[1]

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[1]Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran
[2]Department of Biology, Science and Research Branch, Islamic Azad University, Teheran, Iran
*Corresponding author; Email: Pishkar@iiau.ac.ir

Abstract
Arsenic (As) is one of the most hazardous metalloids for plants, however, little is understood about the role of silicon nanoparticles (Si-NPs) in improving rice tolerance under As toxicity. An experiment was conducted in 2020 at Islamshahr Branch, Islamic Azad University, Islamshahr, Iran, to examine the impacts of As (50 M) and Si-NPs (50 and 100 mg/L) on rice growth, chlorophyll and proline metabolism, antioxidant defense system, ionic homeostasis, and expression of Si/As transporters under hydroponic conditions. The results showed that Si-NPs by boosting the activities of antioxidant enzymes, diminished hydrogen peroxide and superoxide anion, and hence, protected the photosynthetic apparatus and enhanced plant growth during As toxicity. Si-NPs increased Si uptake and declined As uptake in As-treated seedlings by adjusting the relative expression of Si/As transporters (*Lsi1*, *Lsi2*, *Lsi6*). Si-NPs maintained ionic homeostasis under As stress by increasing the uptake of mineral nutrients. In general, Si-NPs increased rice growth and biomass during As toxicity, which might be exploited to develop effective fertilizers to improve crop growth and yield in As-contaminated areas.

Keywords: arsenic; nanoparticles; oxidative stress; rice; Si/As transporters

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Introduction

Many abiotic and biotic factors in the environment can have a negative impact on the growth and yield of crops by affecting the metabolism and vital processes of plants (Otero *et al.* 2016). One of these dangerous factors is arsenic (As), which is known as a metalloid with high toxicity for humans and plants (Ramezani *et al*. 2021). Arsenic-contaminated irrigation water with the continuous and gradual accumulation of As in the soil, causes a decrease in the yield of crops, a negative impact on sustainable agriculture, and contamination of the food chain (Ruíz-Huerta *et al*. 2017; Ghorbani *et al.* 2021).

Rice (*Oryza sativa* L.), one of the most important grains, is the staple food of more than half of the world's people, and has the ability to accumulate As in high concentrations (Ghorbani *et al.* 2009), which will decrease the biomass and yield of rice.

Nowadays, nanotechnology is employed for various objectives in agriculture to promote plant growth (De la Rosa *et al.* 2017; Ghorbani *et al*. 2020). It has been found that nanomaterials with sizes less than 100 nm can be considered bio-stimuli due to their effects on improving the growth and yield of plants, mainly in low concentrations (Juárez-Maldonado *et al*. 2019). It has been demonstrated that nanoparticles (NPs) exhibit unique optical effects, size-dependent behavior, and high surface area, thus displaying a significant function in plant nutrition and protection (Nair *et*

al. 2010). Likewise, the application of nanoparticles in the remediation of heavy metal-contaminated soils has attracted attention worldwide. Silicon nanoparticles (Si-NPs) could be useful in the phytoremediation of toxic metals-polluted areas. Due to the limited bioavailability of fertilizers containing Si, Si-NPs application may be the best alternative for crops as Si accumulators in metal-polluted soils (*Ali et al.* 2019). Tripathi *et al.* (2016) showed that Si-NPs reduced As absorption and increased maize growth and biomass under As toxicity more efficiently, demonstrating greater bioavailability of Si-NPs than conventional Si fertilizer. Rizwan *et al.* (2019) indicated that the application of Si-NPs reduced cadmium absorption and boosted the antioxidant defense system in rice under cadmium phytotoxicity, which was linked to higher rice growth and biomass. However, other reports have shown that the use of NPs can decrease the accumulation of heavy metals and, consequently, reduce plant growth under heavy metal toxicity (Ali *et al*. 2019).

Although a few investigations have indicated that Si-NPs can reduce As uptake and accumulation in rice plants during As toxicity, a better understanding of the function of Si-NPs in the molecular and biochemical pathways of lowering As accumulation in plants is needed. Aside from Si-NPs' beneficial effects on plant defense systems, the function of Si-NPs in the transcription of genes involved in As absorption and translocation, as well as regulating proline and chlorophyll metabolism, has been identified as a novel target for Si-NP application in rice plants under As toxicity. This study aimed to study the possible effect of silicon nanoparticles in alleviating the arsenic toxicity in rice.

Materials and Methods

Materials and the experimental design

The present study was conducted in 2020 in the greenhouse of Islamic Azad University, Ayatollah Amoli Branch, Amol City, Mazandaran Province as a factorial experiment based on a completely randomized design with five replications. In the present study, rice (*Oryza sativa* L. cv. IR64) seeds were provided by the Rice Research Institute, Iran. After surface sterilization and washing with 1% NaOCl and distilled water, the seeds germinated. Then the rice seedlings were transplanted to containers containing 50% Hoagland medium (pH 6.0) after 10 days from the start of germination (Hoagland and Arnon 1941). Seedlings grew at 25-22°C and 16 h light (humidity: 60-70%; illumination intensity: 350-400 µmol m^{-2} s^{-1}) (Ghorbani *et al.* 2018). Arsenic ($NaAsO_2$, 0 and 50 µM) and Si-NPs (0, 50, and 100 mg/L) treatments were applied to the Hoagland solution 10 days after seedling transfer to pots. Si-NPs (99% purity, 50 nm ≥ size, 80-100 m^2/g surface area) were obtained from USA-Nano. Sampling was performed after 21 days. Total dry weight was obtained by incubating the samples at 65 °C for 48 h (Ghorbani *et al*. 2011).

Total chlorophyll and chlorophyll fluorescence

After homogenization of fresh leaves of rice plants using 3% acetone (v/v), the total chlorophyll content was quantified as Sharma *et al*. (2012). Fv/Fm values were assessed by a PAM fluorometer (Walz; PAM 2500).

Shoot and root contents of As, Si, and mineral nutrient

Shoot and root tissues were digested in an acidic combination of H_2O_2:HNO_3 (4:1 ratio), and then, their Si and As content was quantified by an ICP-MS (Agilent 7500 cx). The leaf content of Ca, Mg, and K was measured by PFP7 model flame-photometry. The leaf content of P and N was measured by the phosphomolybdate blue and Kjeldahl, respectively.

Leaf hydrogen peroxide (H_2O_2), superoxide anion, and proline

To assess H_2O_2 concentration, fresh leaves were homogenized by 1% trichloroacetic acid (1%, w/v). After centrifugation, the supernatant was blended with 1 M KI and potassium-phosphate buffer (1 mM, pH 6.8), and was calculated as the method previously described by Sinha *et al.* (2005) with a reading of 390 nm. After extracting fresh leaves with 65 mM potassium phosphate buffer (pH 7.8), the assay mixture containing supernatant, phosphate buffer (pH 7.8, 65 mM), hydroxylamine hydrochloride (10 mM), α-naphtylamine (7 mM) and sulphanilamide (17 mM) was read at 530 nm and the leaf content of superoxide anion was obtained by the procedure of Elstner and Heupel (1976). The leaf proline content was quantified using sulfosalicylic acid and measurements at 520 nm, as described by Bates *et al.* (1973).

Enzyme assay

The extraction solution (50 mM K-P buffer (pH 6.8), potassium chloride (100 mM), ascorbate (1 mM), glycerin (10%), and β-mercaptoethanol (5 mM)) was used to extract fresh leaves of rice. After centrifugation, supernatants were used to quantify the leaf activity of enzymes.

The leaf activities of monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), and peroxidase (POX) enzymes were quantified according to Miyake and Asada (1992), Nakano and Asada (1981), Nakano and Asada (1981), and Chance and Maehly (1955) methods and readings at 340, 265, 290, and 470 nm, respectively.

The methods depicted by Sumithra *et al.* (2006), Charest and Phan (1990), Costa *et al.* (2005), and Jain and Gadre (2004) were utilized to measure the leaf activity of proline dehydrogenase (PDH), Δ1-pyrroline-5-carboxylate synthetase (P5CS), chlorophyllase (Chlase), and δ-aminolevulinic acid dehydratase (ALAD) enzymes, respectively.

Expression of genes in roots and leaves

Total RNA extraction and cDNA synthesis were performed using TRIzol reagent (Invitrogen, USA) and RevertAid™ Reverse Transcriptase kit (Fermentas, Germany), respectively. RT-PCR reactions were conducted using Thermo Scientific Maxima SYBR Green qPCR Master Mix. *Actin* control gene was utilized for normalization. The primers of *Actin* and target genes are documented in Table 1, which were created by Primer3 program.

Statistics analysis

The data were analyzed using SAS 9.1.3 software. Means were compared according to the LSD test (at 0.05 probability level). Five independent replications were used to calculate the means (± SD) of morphological and biochemical traits (gene expression was calculated based on three biological replications).

Table 1. The sequences of primers used in qPCR reactions

Gene name	5′-primer-3′	Accession No.
Lsi1	F: GTTGCTCAGGCTTCTCAACC R: AGTTGTTGCTGGCCATTTCT	XM_015770687
Lsi2	F: CTCGCTGCTCGTCTTCTTCT R: GGTACGTTTGATGCGAGGTT	XM_015776731
Lsi6	F: GTCCGTTGATTCGTTGTCCT R: TCACGAACACAAGCAGGAAC	XM_015788648
Actin	F: TCCTCCGTGGAGAAGAGCTA R: GCAATGCCAGGGAACATAGT	XM_015774830

Results

Plant growth, chlorophyll content, and chlorophyll fluorescence

Analysis of variance (ANOVA) revealed significant differences ($p \leq 0.05$) in plant height, total dry weight, total chlorophyll content, chlorophyll fluorescence, and proline under As and Si-NPs application and their interaction (except for total dry weight under As × Si-NPs) (Table 2). Arsenic (50 µM) application reduced the height and total dry weight as compared to the control. However, the application of 100 mg/L Si-NPs, significantly boosted total dry weight as compared to the control. In the As-treated plants, the addition of Si-NPs enhanced the height and total dry weight (Table 2).

Arsenic stress significantly lowered the total chlorophyll content compared to the control plants. However, the application of 50 and 100 mg/L Si-NPs raised the total chlorophyll content by 61.8 and 111 %, respectively, compared to As stress alone (Table 2). As application declined Fv/Fm over control plants, however, the supplementation of Si-NPs notably boosted Fv/Fm value in the As-treated plants (Table 2). As toxicity significantly enhanced the leaf proline accumulation compared to the control plants. When As-stressed plants were treated with Si-NPs, the leaf proline content was raised more over the plants treated with As alone (Table 2).

Table 2. Analysis of variance and mean comparison of plant height, total dry weight, total chlorophyll, and chlorophyll fluorescence (Fv/Fm) of rice seedlings under arsenic (As, 0 and 50 µM) and silicon nanoparticles (Si-NPs, 50 and 100 mg/L) treatments

Comparison of means					
Treatments	Height (cm)	Total dry weight (g)	Total chlorophyll (mg g^{-1} FW)	Fv/Fm	Proline (mM g^{-1} FW)
Control	35.19 ± 1.25a	3.77 ± 0.09b	4.71 ± 0.04a	0.685 ± 0.011bc	2.33 ± 0.11d
50 mg/L Si-NPs	37.02 ± 0.77a	4.03 ± 0.11ab	4.72 ± 0.25a	0.712 ± 0.011ab	2.52 ± 0.14d
100 mg/L Si-NPs	37.09 ± 1.01a	4.23 ± 0.23a	5.03 ± 0.11a	0.716 ± 0.007a	2.59 ± 0.12d
50 µM As	22.59 ± 1.49c	2.23 ± 0.08e	1.91 ± 0.15d	0.397 ± 0.006g	6.64 ± 0.43c
50 µM As + 50 mg/L Si-NPs	28.94 ± 1.01b	2.74 ± 0.07cd	3.09 ± 0.14c	0.529 ± 0.009e	8.78 ± 0.21b
50 µM As + 100 mg/L Si-NPs	30.93 ± 1.07b	2.87 ± 0.06c	4.03 ± 0.16b	0.624 ± 0.011d	10.8 ± 0.24a
Analysis of variance					
As	361**	8.8**	14.8**	0.16**	176**
Si-NPs	44**	0.5**	2.3**	0.03**	7**
As × Si-NPs	17**	0.03^{ns}	1.3**	0.02**	6**
Error	1.8	0.04	0.05	0.0003	0.11
CV (%)	4.3	6.3	7.3	2.7	4.6

Means with different letters in each column are significantly different at $p \leq 0.05$ (LSD test).
^{ns}non-significant; * and ** significant at the $p \leq 0.05$ and $p \leq 0.01$ levels, respectively

Root and shoot concentrations of As and Si

The effect of As, Si-NPs, and their interaction on the root and shoot concentrations of As and Si were significant ($p \leq 0.01$) (except the effect of As × Si-NPs on the shoot concentration of As) (Table 3). As treatment increased the root and shoot accumulation of As. However, Si-NPs application decreased the shoot and root contents of As in the As-subjected plants (Table 3). As stress declined the shoot and root contents of Si as compared with the non-stressed plants. However, the application of Si-NPs increased the root and shoot levels of Si in both As-treated and non-treated seedlings (Table 3).

Mineral nutrients

The results of ANOVA showed that the effect of As, Si-NPs, and their interaction on the leaf concentrations of P, N, Ca, Mg, and K was significant ($p \leq 0.01$) (Table 4). As stress significantly reduced the leaf accumulation of P, N, and Ca by 55, 42, and 38%, respectively, over control plants. In the As-stressed plants, the application of Si-NPs significantly improved the leaf accumulation of P, N, and Ca over their controls, with the highest increase observed at a concentration of 100 mg/L Si-NPs (Table 4). Decreases of 49 and 21% were observed in the leaf content of Mg and K in the As-stressed plants, respectively, compared to the control plants. The exogenous application of Si-NPs restored the leaf contents of Mg and K in the As-stressed plants compared to the As-stressed plants alone (Table 4).

H_2O_2 and superoxide anion contents and antioxidant enzymes

The results of ANOVA showed that the effect of As, Si-NPs, and their interaction on H_2O_2 and superoxide anion**,** and the activity of DHAR, MDHAR, APX, and POX enzymes were significant ($p \leq 0.01$) (Table 5). As stress significantly enhanced the leaf accumulation of H_2O_2 over the control seedlings. However, the supplementation of Si-NPs declined the accumulation of H_2O_2 in the As-subjected seedlings, with 100 mg/L Si-NPs having the most impact (Figure 1A). The leaf content of superoxide anion was significantly elevated in the seedlings subjected to As as compared to the control seedlings. However, in the As-stressed seedlings, 50 and 100 mg/L Si-NPs treatments diminished leaf superoxide anion levels by 23.1 and 34.8%, respectively, over seedlings treated with only As (Figure 1B). As stress enhanced the leaf activities of DHAR, MDHAR, APX, and POX by 13, 19, 37, and 70% over the controls. However, Si-NPs treatments further enhanced the activities of DHAR, MDHAR, APX, and POX over seedlings treated with the As alone (Figures 2A, 2B, 2C, and 2D).

Enzymes involved in chlorophyll and proline metabolism

The results of ANOVA showed that the effect of As, Si-NPs, and their interaction on the activities of chlorophyllase, aminolevulinic acid dehydratase, proline dehydrogenase, and pyrroline-5-carboxylate synthase was significant (except the effect of Si-NPs and As × Si-NPs on the activity of proline dehydrogenase) (Table 6). As treatment upregulated the activity of chlorophyllase enzyme in rice leaves over the control plants. The exogenous application of Si-NPs further upregulated chlorophyllase activity in As-stressed plants, which was most induced under 100 mg/L Si-NPs (Figure 3A) A significant reduction in the leaf activity of aminolevulinic acid dehydratase was found by 32.8% under As stress compared to the control. However, Si-NPs upregulated the activity of aminolevulinic acid dehydratase in the As-stressed plants (Figure 3B). As stress significantly diminished the leaf activity of

Table 3. Analysis of variance and mean comparison of the root and shoot concentrations of arsenic (As) and Si of rice seedlings under arsenic (0 and 50 µM) and silicon nanoparticles (Si-NPs, 50 and 100 mg/L) treatments

Comparison of means				
Treatments	As in root	As in shoot	Si in root	Si in shoot
	mg/kg DW		g/kg DW	
Control	--	--	3.98 ± 0.07d	4.56 ± 0.13d
50 mg/L Si-NPs	--	--	6.12 ± 0.12b	6.38 ± 0.16b
100 mg/L Si-NPs	--	--	8.97 ± 0.13a	7.77 ± 0.12a
50 µM As	508.7 ± 16.1a	90.17 ± 7.07a	1.81 ± 0.11f	1.73 ± 0.11f
50 µM As + 50 mg/L Si-NPs	379.3 ± 14.2c	46.57 ± 4.22d	3.37 ± 0.13e	3.55 ± 0.12e
50 µM As + 100 mg/L Si-NPs	273.0 ± 14.3d	36.00 ± 4.21e	4.94 ± 0.12c	5.13 ± 0.15c
Analysis of variance				
As	40**	35**	673960**	14918**
Si-NPs	25**	16**	20893**	1237**
As × Si-NPs	1.4**	0.02^{ns}	20893**	1237**
Error	0.03	0.04	180	19
CV (%)	3.7	4	6.9	15

Means with different letters in each column are significantly different at $p \leq 0.05$ (LSD test).
^{ns}non-significant; * and ** significant at the $p \leq 0.05$ and $p \leq 0.01$ levels, respectively

Table 4. Analysis of variance and mean comparison of the leaf concentrations of mineral nutrients of rice seedlings under arsenic (0 and 50 µM) and silicon nanoparticles (Si-NPs, 50 and 100 mg/L) treatments
Means with different letters in each column are significantly different at $p \leq 0.05$ (LSD test).

Comparison of means					
Treatments	P	N	Ca	Mg	K
Control	1.95 ± 0.05a	13.92 ± 0.22a	10.45 ± 0.15a	6.79 ± 0.15a	5.44 ± 0.17b
50 mg/L Si-NPs	1.94 ± 0.08a	14.02 ± 0.21a	10.49 ± 0.21a	6.74 ± 0.16a	5.43 ± 0.15b
100 mg/L Si-NPs	1.91 ± 0.06a	13.87 ± 0.21a	10.58 ± 0.25a	6.89 ± 0.23a	5.48 ± 0.14b
50 µM As	0.87 ± 0.05d	8.09 ± 0.32d	6.51 ± 0.16d	3.49 ± 0.17d	4.30 ± 0.15c
50 µM As + 50 mg/L Si-NPs	1.13 ± 0.07c	10.14 ± 0.19c	7.87 ± 0.21c	4.18 ± 0.15c	5.25 ± 0.07b
50 µM As + 100 mg/L Si-NPs	1.54 ± 0.06b	11.53 ± 0.15b	9.44 ± 0.24b	5.49 ± 0.21b	6.13 ± 0.12a
Analysis of variance					
As	2.5**	73**	29**	25**	0.2*
Si-NPs	0.2**	5**	4**	3**	1.3**
As × Si-NPs	0.2**	5**	3**	2**	1.**
Error	0.01	0.1	0.08	0.08	0.05
CV (%)	4.2	5.7	3.2	4.1	5.2

^{ns}non-significant; * and ** significant at the $p \leq 0.05$ and $p \leq 0.01$ levels, respectively

Table 5. Analysis of variance of hydrogen peroxide (H_2O_2) and superoxide anion, and activity of dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), ascorbate peroxidase (APX) and peroxidase (POX) enzymes of rice seedlings under arsenic (0 and 50 µM) and silicon nanoparticles (Si-NPs, 50 and 100 mg/L) treatments

	H_2O_2	Superoxide anion	DHAR	MDHAR	APX	POX
As	7173**	34**	93**	42**	89**	144**
Si-NPs	703**	2.9**	21**	8**	4.3**	4.6**
As × Si-NPs	876**	1.8**	22**	8**	3.7**	3.9**
Error	13	0.06	0.15	0.07	0.11	0.13
CV (%)	4.6	4.2	3.4	4.6	3.4	4.2

**Significant at the $p \leq 0.01$ probability level

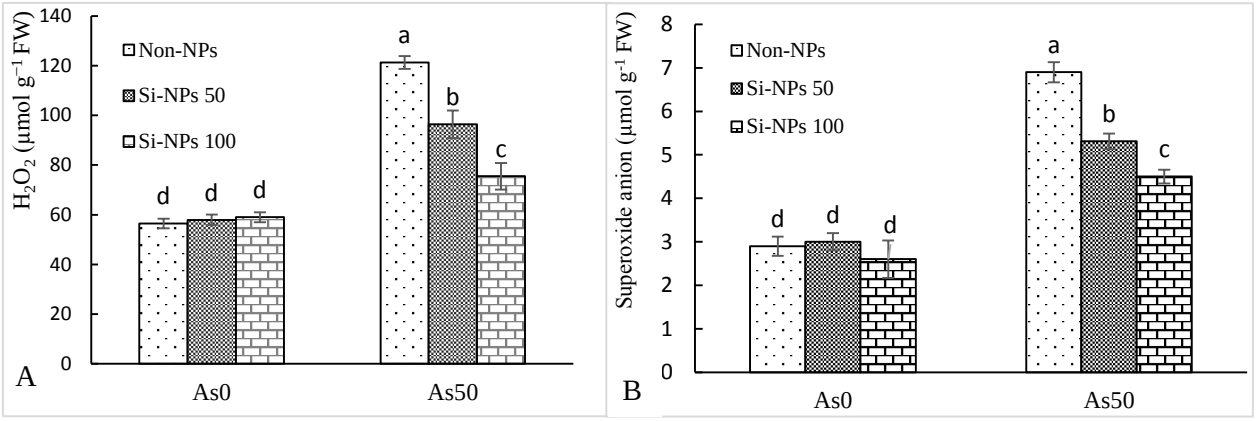

Figure 1. Effect of silicon nanoparticles (Si-NPs, 50, and 100 mg/L) on hydrogen peroxide (H_2O_2, A) and superoxide anion (B) of rice seedlings under arsenic (As, 0 and 50 µM) toxicity. Different letters above the bars (means ± SD, n = 5) indicate significant differences among the means at $p \leq 0.05$ (LSD test).

proline dehydrogenase and pyrroline-5-carboxylate synthase enzymes by 35.4 and 221.5%, respectively, over the control seedlings. The application of Si-NPs in the As-stressed plants did not induce a significant effect on proline dehydrogenase activity but significantly increased the pyrroline-5-carboxylate synthase activity compared to the As-stressed plants alone (Figures 3C and 3D).

Expression of As transporters

Arsenic stress improved the relative expression of *Lsi1* gene in the root and leaf by 6.6- and 2.1-fold, respectively over the controls. In the root, the application of Si-NPs downregulated the expression of *Lsi1* gene in the As-treated seedlings over the seedlings subjected to As only. In the leaf of the As-subjected seedlings, the addition of Si-NPs lessened the relative expression of *Lsi1* (Figure 4A). The As stress upregulated the expression of *Lsi2* and *Lsi6* genes in the root and leaf over the controls. In the As-treated seedlings, the supplementation of Si-NPs lowered the expression of *Lsi2* and *Lsi6* in both roots and leaves over the seedlings exposed to only As (Figure 4B and 4C).

Table 6. Analysis of variance of the leaf activities of enzymes involved in proline and chlorophyll metabolism in rice seedlings under arsenic (0 and 50 µM) and silicon nanoparticles (Si-NPs, 50 and 100 mg/L) treatments

	Chlorophyllase	Aminolevulinic acid dehydratase	Proline dehydrogenase (ProDH)	Pyrroline-5-carboxylate synthase
As	294**	1353**	17.9**	11.8**
Si-NPs	13**	146**	0.03^{ns}	0.6**
As × Si-NPs	12**	133**	0.2^{ns}	0.6**
Error	0.37	4.07	0.13	0.004
CV (%)	5.2	4.8	5.3	3.9

^{ns}not significant; **Significant at the $p \leq 0.01$ probability level

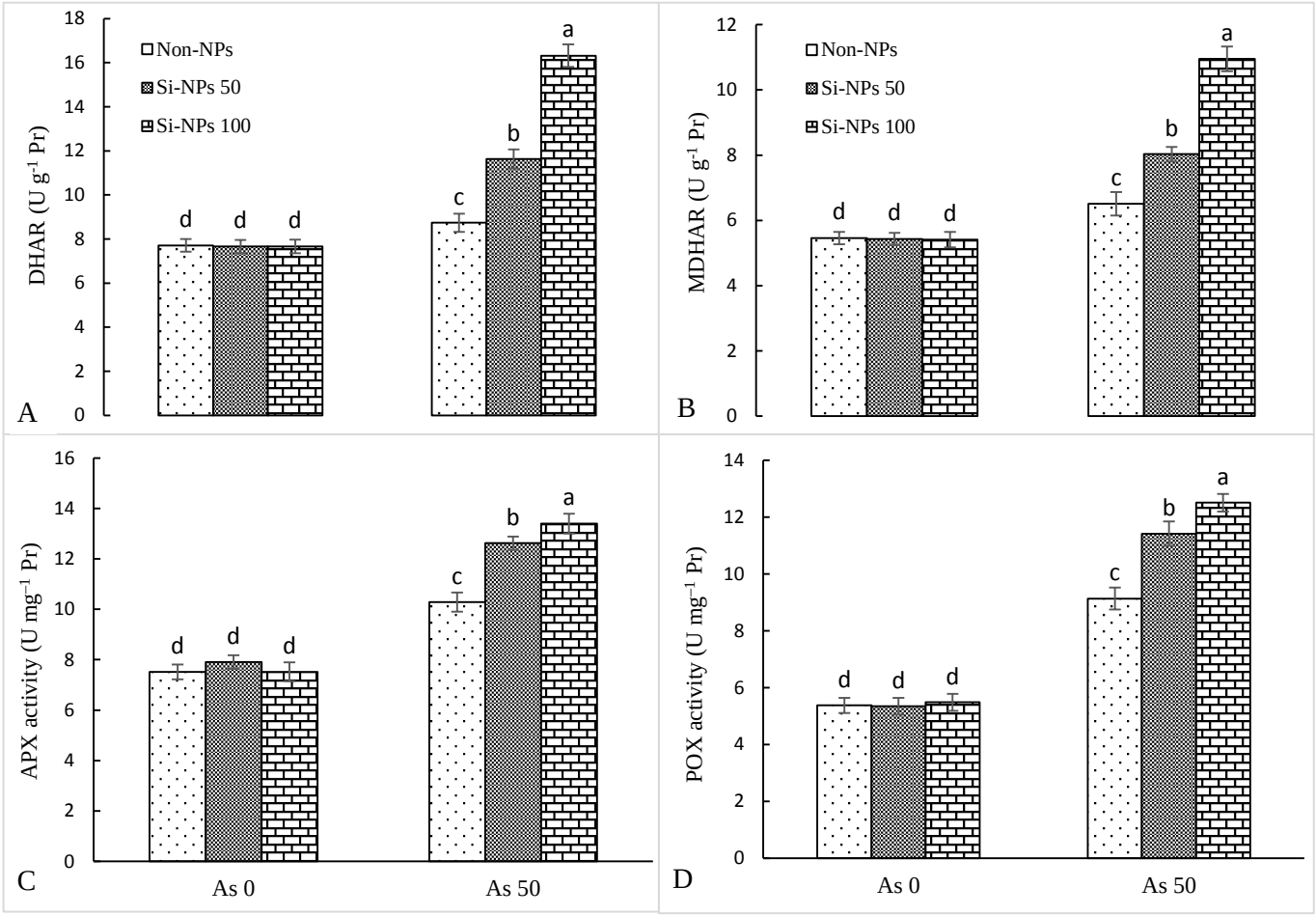

Figure 2. Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) on the leaf activities of dehydroascorbate reductase (DHAR, A), monodehydroascorbate reductase (MDHAR, B), ascorbate peroxidase (APX, C) and peroxidase (POX, D) enzymes of rice seedlings under arsenic (As, 0 and 50 µM) toxicity. Different letters above the bars (means ± SD, n = 5) indicate significant differences among the means at $p \leq 0.05$ (LSD test).

Discussion

The current study found that the As stress lowered total chlorophyll content and Fv/Fm, inhibiting rice growth, which is similar to earlier findings on *Oryza sativa* (Pandey *et al.* 2020) and *Pteris cretica* (Zemanová *et al.* 2020). Arsenic toxicity has been shown to reduce plant biomass and growth by lowering chlorophyll content, causing oxidative stress, and restricting stomatal conductance (Ghorbani *et al.* 2020). However, Si-NPs treatments enhanced total chlorophyll content and restored the plant height and total dry weight of rice seedlings under As stress. Cui *et al.* (2017) indicated that Si-NPs by boosting the activity of antioxidant enzymes and declining the uptake of cadmium improved the growth of rice plants under cadmium stress. Most studies have found that the exogenous application of NPs under toxic metal stress raised plant biomass by increasing mineral nutrient accumulation and boosting antioxidant defenses (Chen *et al*. 2018; Khan *et al*. 2020). Si-NPs have been shown to improve chlorophyll contents in maize (Tripathi *et al.* 2016) and rice (Rizwan *et al.* 2019; Zhang *et al.* 2020) plants

during heavy metal phytotoxicity. The supplementation of Si-NPs has been demonstrated to protect the photosynthetic system from toxic metals by lowering harmful radical levels and upregulating antioxidant enzyme activities (Cui *et al.* 2017; Rizwan *et al*. 2019). Si-NPs improve chlorophyll content by promoting nutrient absorption (Bidi *et al.* 2021). As a result, Si-NPs improved rice height and biomass during the As stress by enhancing chlorophyll content and enhancing the photosynthetic system's function.

Stresses can induce oxidative stress in plants by increasing the accumulation of toxic compounds such as H_2O_2 and superoxide anion, which will be associated with damage to bio-macromolecules (Ghorbani *et al.* 2019; Ghasemi-Omran *et al*. 2021). Here, As stress increased the accumulation of H_2O_2 and superoxide in the leaves, revealing the induction of oxidative stress. The boosted levels of H_2O_2 and superoxide anion in mustard (Ahmad *et al.* 2021), *Pteris cretica* (Zemanová *et al*. 2020), and rice (Ghorbani *et al.* 2020; Pandey *et al.* 2020) have already been expressed. However, the supplementation of Si-

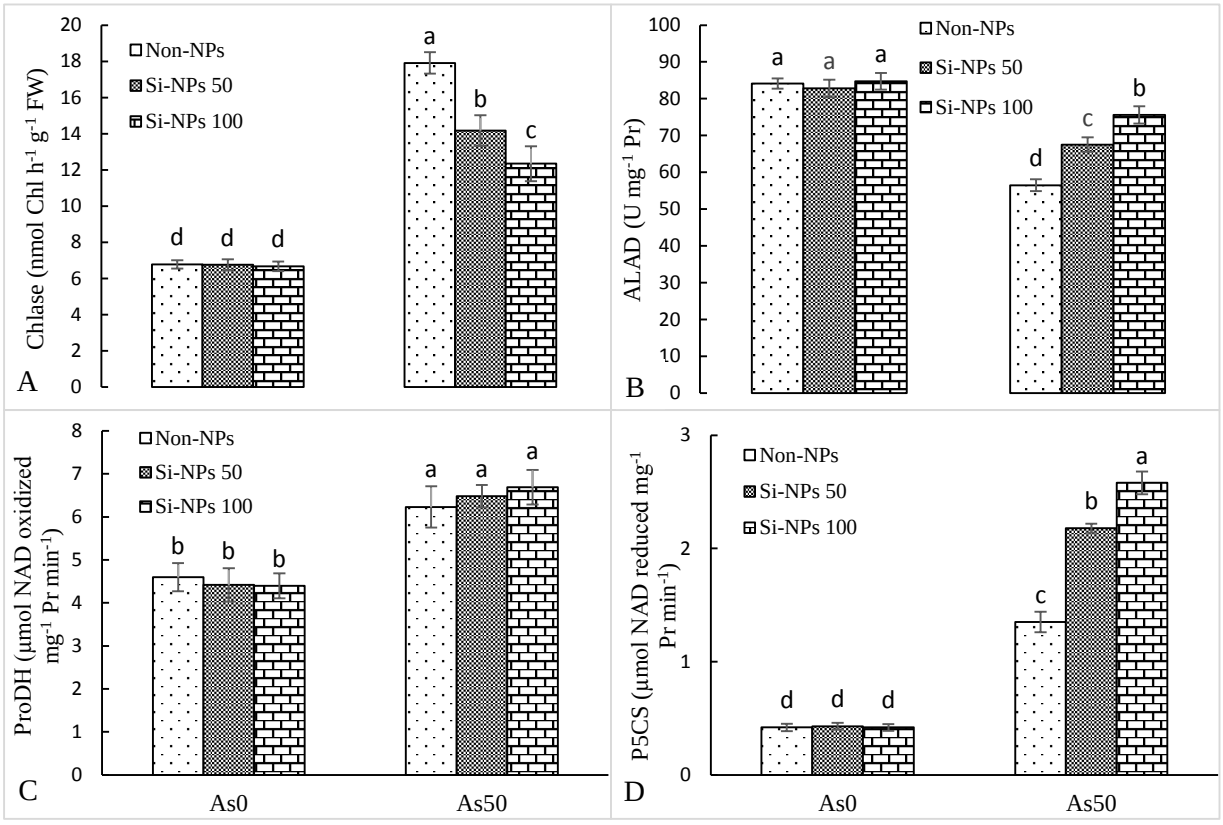

Figure 3. Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) on the leaf activities of chlorophyllase (Chlase, A), aminolevulinic acid dehydratase (ALAD, B), proline dehydrogenase (ProDH, C), and pyrroline-5-carboxylate synthase (P5CS, D) enzymes of rice seedlings under arsenic (As, 0 and 50 µM) toxicity. Different letters above the bars (means ± SD, n = 5) indicate significant differences among the means at $p \leq 0.05$ (LSD test).

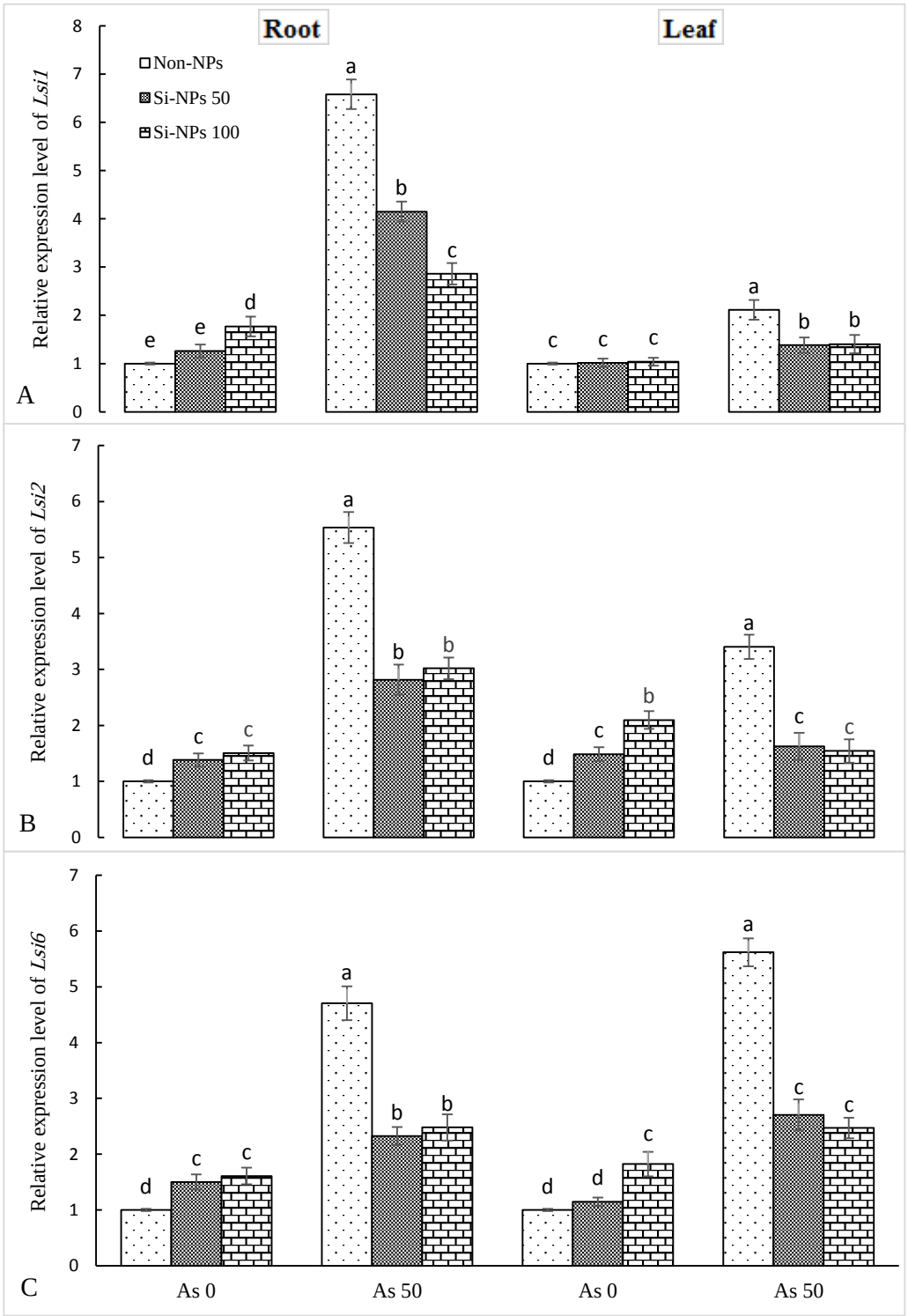

Figure 4. Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) on the expression of *Lsi1* (A), *Lsi2* (B), and *Lsi6* (C) genes of rice seedlings under arsenic (As, 0 and 50 µM) toxicity. Different letters above the bars (means ± SD, n = 5) indicate significant differences among the means at $p \leq 0.05$ (LSD test).

NPs lessened the contents of H_2O_2 and superoxide anion, demonstrating the affirmative impacts of Si-NPs in ameliorating the As-mediated oxidative stress. The decline in the contents of H2O2 and superoxide anion and thus the mitigation of oxidative stress mediated by Si-NPs has

previously been reported by Rizwan *et al.* (2019) in rice, Tripathi et al. (2016) in maize, and Khan *et al.* (2020) in wheat. Plants possess antioxidant defense mechanisms to combat abiotic stresses-induced oxidative stress, which can improve plant tolerance and adaptability. The results indicated that the application of Si-NPs boosted the activities of MDHAR, DHAR, APX, and POX enzymes in rice seedlings during the As stress. Rizwan *et al.* (2019) reported similar improvements in antioxidant enzyme activity by using Si-NPs in rice under cadmium toxicity. According to Khan *et al.* (2020), Si-NPs boosted the activity of POX, APX, and superoxide dismutase (SOD) and lowered the contents of H_2O_2 and superoxide anion, declined oxidative stress, and hence improved wheat growth and biomass under heavy metal toxicity. Khan *et al.* (2020) showed that Si-NPs addition by raising the activities of APX, POX, and SOD and declining the contents of H_2O_2 and superoxide anion, lessened the oxidative stress and hence improved wheat growth and biomass during heavy metal toxicity. In another study, Ghorbani *et al.* (2020) revealed that raising the activity of the antioxidant enzymes and glyoxalase system can boost the tolerance of plants during As toxicity. Thus, our results demonstrate that Si-NPs upregulated the activities of antioxidant enzymes, which might help plants boost their defense systems in response to As toxicity.

Preservation of chlorophyll content and accumulation of osmolites compounds such as proline can play an important role in improving plant tolerance under heavy metal stress (Ghorbani *et al*. 2022). Our results showed that the application of Si-NPs by modulating enzymes involved in chlorophyll and proline metabolism (chlorophyllase, aminolevulinic acid dehydratase, proline dehydrogenase, pyrroline-5-carboxylate synthase), increased chlorophyll and proline contents in the As-stressed rice seedlings, which will play an important role in boosting plant adaptation under the As toxicity. Ghorbani *et al.* (2021) previously reported that modulating the metabolism of proline and chlorophyll under the As stress could improve plant growth and biomass. Improving proline and chlorophyll metabolism can be due to the positive effects of Si-NPs on ameliorating oxidative stress and maintaining ionic homeostasis under the As stress. Thus, Si-NPs by modulating the metabolism of proline and chlorophyll, and consequently, increasing the content of chlorophyll and proline, strengthen the defence system and increase the tolerance of rice plants under As stress.

The results showed that the addition of As enhanced the relative expression of *Lsi1*, *Lsi2*, and *Lsi6* genes, which is compatible with the raised As concentration in the shoot and root. Similarly, Mousavi *et al.* (2020) found that As treatment increased the expression of the *Lsi1*, *Lsi2*, and *Lsi6* genes. As toxicity diminished the uptake of Si and mineral nutrients (N, P, Ca, Mg, and K). Since As and Si are absorbed and translocated by similar transporters in rice plants, the decline in Si absorption during As addition might be attributable to the rhizosphere's competitive influence (Ma *et al.* 2007; Yamaji *et al.* 2008). As a result, As toxicity reduced Si uptake and accumulation in rice plants, which was compensated for by upregulation

of Si/As transporter expression; due to the high concentration of As in the rhizosphere, it enhanced As absorption and concentration in rice plants. Si-NPs diminished As accumulation in the root and shoot and increased Si accumulation in the root and shoot during the As toxicity, suggesting that the presence of Si in the rhizosphere effectively decreases the As absorption by the roots, which is compatible with the findings of Khan and Gupta (2018) and Tripathi *et al.* (2013). The application of Si-NPs also effectively improved the leaf concentrations of nutrient minerals (N, P, Ca, K, Mg) in the As-stressed plants, which could play an important role in maintaining ionic homeostasis and, consequently, improving plant tolerance under the As toxicity. The positive effect of Si-NPs on nutrient accumulation can be due to the role of Si-NPs in modulating the expression and performance of transporters involved in nutrient uptake as well as maintaining membrane potential (Koleva *et al.* 2022). The use of Si-NPs reduced the relative expression of Si/As transporters, which is compatible with a reduction in the demand for Si absorption due to enhanced Si accumulation. Therefore, Si-NPs can play a crucial role in improving plant growth and biomass under the As toxicity by reducing the As uptake and increasing Si and mineral nutrients.

Conclusions

Our results indicated that Si-NPs improved the plant height and total dry weight of rice under the As stress. The application of Si-NPs declined the As uptake and improved the Si uptake in rice via modulating the mRNA levels of Si/As transporters (*Lsi1*, *Lsi2*, *Lsi6*). Si-NPs addition also raised the activities of antioxidant enzymes and reduced the contents of H_2O_2 and superoxide anion, therefore, diminishing oxidative stress and enhancing the biomass and growth of rice plants in the As-toxicity conditions. Si-NPs by modulating the metabolism of proline and chlorophyll, improved the content of proline and chlorophyll in the As-stressed plants. In general, our results expedited a more reasonable insight into how Si-NPs reduce the As absorption and translocation, enhance plant defense processes, and eventually improve plant adaptation in the As-stress conditions, which helps in developing practical fertilizers to diminish the As stress in the As-polluted regions in rice.

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Conflict of interest

The authors declare that there is not any conflict of interest regarding the publication of this manuscript.

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تاثیر نانوذرات سیلیس بر کاهش سمیت آرسنیک در برنج (*Oryza sativa* L.)

طاهره کیانی[1]، لیلا پیشکار[1]، نسرین سرتیپ‌نیا[1]، علیرضا ایران بخش[2] و گیتی برزین[1]

۱- گروه زیست شناسی، واحد اسلامشهر، دانشگاه آزاد اسلامی، اسلامشهر
۲- گروه زیست شناسی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران
*مسئول مکاتبه؛ Email: Pishkar@iiau.ac.ir

چکیده

آرسنیک یکی از سمی‌ترین متالوئیدها برای گیاهان شناخته شده است، ولی اطلاعات کمی در مورد استفاده از نانوذرات سیلیس در کاهش سمیت آرسنیک در برنج وجود دارد. این تحقیق به منظور بررسی اثرات غلظت‌های نانوذرات سیلیس (۵۰ و ۱۰ میلی‌گرم بر لیتر) و آرسنیک (۵۰ میکرومولار) بر رشد گیاه، متابولیسم کلروفیل و پرولین، سیستم دفاعی آنتی اکسیدانی، همئوستازی یونی و بیان ناقل‌های سیلیس/آرسنیک در برنج در شرایط هیدروپونیک در سال ۱۴۰۰ در دانشگاه آزاد اسلامی واحد اسلامشهر انجام شد. نتایج نشان داد که افزودن نانوذرات سیلیس به محیط کشت هیدروپونیک با تنظیم فعالیت آنزیم‌های آنتی اکسیدانی، سطح پراکسید هیدروژن و سوپراکسید آنیون را کاهش داد و از دستگاه فتوسنتزی محافظت کرده و باعث بهبود رشد گیاه تحت تنش آرسنیک شد. نانوذرات سیلیس با تعدیل بیان ناقلین سیلیس/آرسنیک (*Lsi1*، *Lsi2*، *Lsi6*)، باعث کاهش جذب آرسنیک و افزایش جذب سیلیس در گیاهان برنج تحت تنش آرسنیک شد. کاربرد نانوذرات سیلیس با افزایش جذب عناصر مغذی، باعث حفظ همئوستازی یونی در گیاه برنج تحت تنش آرسنیک شد. به طور کلی، نانوذرات سیلیس باعث افزایش رشد برنج تحت تنش آرسنیک شد، که می‌تواند برای طراحی کودهای موثر برای افزایش رشد و عملکرد محصول در مناطق آلوده به آرسنیک استفاده شود.

واژه‌های کلیدی: آرسنیک؛ برنج؛ تنش اکسیداتیو؛ ناقلین سیلیس/آرسنیک؛ نانوذرات