**RESEARCH ARTICLE** 



# Effects of silicon and titanium dioxide nanoparticles on arsenic accumulation, phytochelatin metabolism, and antioxidant system by rice under arsenic toxicity

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#### Abstract

Arsenic (As) is known to be one of the most toxic metalloids for humans and plants; however, little is known about the use of silicon (Si) and titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) in reducing As toxicity in rice (*Oryza sativa* L.). The experiment was conducted to examine the effects of Si-NPs (50 and 100 mg/L), TiO<sub>2</sub>-NPs (25 and 50 mg/L) and As (50  $\mu$ M) on growth, photosynthetic pigments, antioxidant defense system, glyoxalase system, expression of Si/As transporters, and genes involved in As sequestration in rice under hydroponic conditions. The results revealed that Si- and TiO<sub>2</sub>-NPs by upregulating the activity of antioxidant enzymes and glyoxalase cycle reduced hydrogen peroxide, methylglyoxal, malondialdehyde, and electrolyte leakage, and thus protected the photosynthetic apparatus and improved plant growth under As stress. By increasing the expression of *GSH1*, *PCS*, and *ABC1* genes, Si- and TiO<sub>2</sub>-NPs increased leaf and root accumulation of glutathione and phytochelatins and sequestered As in vacuoles, which protected plant cells from As toxicity. Si-NPs diminished As uptake and increased Si uptake in As-exposed rice plants by modulating the expression of Si/As transporters (*Lsi1*, *Lsi2*, and *Lsi6*). The results depicted that 100 mg/L Si-NPs treatment had the highest positive effect on plant growth and tolerance under As stress compared to other treatments. In general, Si- and TiO<sub>2</sub>-NPs augmented the growth of rice under As stress through different strategies, which can be used to design effective fertilizers to enhance the crop growth and yield in areas contaminated with toxic metals.

Keywords Arsenic · Nanoparticles · Si/As transporters · Phytochelatins · Oxidative stress · Rice

#### Introduction

Arsenic (As) is one of the most toxic elements in the environment, known as a non-threshold carcinogen. It has been revealed that irrigation with As-contaminated groundwater, industrial activities, and the application of As-based pesticides and fertilizers increase the contamination of paddy soils and, consequently, enhance the concentration of As in rice (Zhu et al. 2008; Zhao et al. 2009). As one of the most important cereals, rice (*Oryza sativa*) is the staple food of

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<sup>2</sup> Department of Biology, Science and Research Branch, Islamic Azad University, Teheran, Iran more than half of the people worldwide, which has high efficiency in accumulating As from the soil (Sun et al. 2012), which can result in reduced growth and yield of rice (Rahman et al. 2008). The entrance of As through the contaminated food into the human body could induce detrimental impacts including immune system disorders, cardiovascular disease, and various types of cancer (Shen et al. 2013; Kim et al. 2008). Therefore, an appropriate and efficient method to decrease the uptake and accumulation of As in rice is one of the major concerns of researchers.

Numerous emerging amendments have been considered with the purpose of diminishing the accumulation of toxic metals in plants and modifying soils contaminated with metals (Gerami et al. 2018; Rizwan et al. 2019; Ghorbani et al. 2020). Recently, nanotechnology has been widely used in various fields including medicine, industry, food production, and agriculture, which has unique achievements compared to other materials (Rafique et al. 2018; Bidi et al. 2021). The impacts of nanoparticles (NPs) on different plants under

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various environmental conditions were investigated and the response of plants varied according to the size and type of NPs, plant species, time exposure, and growth stage (Munir et al. 2018; Mohamed et al. 2017). Furthermore, the use of NPs in the remediation of soils contaminated by heavy metal has received attention worldwide (Hussain et al. 2018). Silicon (Si) and titanium dioxide (TiO<sub>2</sub>) NPs could be effective in the phytoremediation of areas polluted with heavy metals. Due to the low bioavailability of Si fertilizers, the application of Si-NPs may be the best choice for cereals including wheat (Hussain et al. 2019) and rice (Tripathi et al. 2016) as Si accumulator, in metal-contaminated soils. Chen et al. (2018) represented that the supply of Si-NPs lessened metal uptake and enhanced crop growth. Si-NPs have been shown to diminish As uptake more efficiently and thus increase the growth of maize under As stress, indicating a higher availability of Si-NPs than common silica fertilizer (Tripathi et al. 2016). Cai et al. (2017) indicated that  $TiO_2$ -NPs decreased the uptake and accumulation of lead and, as a result, promoted the growth of rice under lead stress. Rizwan et al. (2019) revealed that the foliar supply of TiO<sub>2</sub>-NPs and Si-NPs improved the antioxidant defense system and lessened the cadmium uptake in rice under cadmium toxicity, which was associated with increased growth and biomass of rice. However, Singh and Lee (2016) indicated that TiO<sub>2</sub>-NPs enhanced the accumulation of cadmium in soybean under cadmium stress. These results indicate that the impacts of NPs application depend on plant species, type of heavy metals, and NPs. Therefore, more research is required to accurately recognize the role of NPs in promoting the defense mechanism of plants under heavy metal toxicity.

Rice (*O. sativa*), as one of the most important cereal crops, is the staple food of more than half of the world's population. Rice is known to be the main source of toxic As entering the body of humans who consume grains at least once a day (Ghorbani et al. 2011; Zhang et al. 2020). In addition, the bioaccumulation factor of As in rice is higher than wheat and other cereals, which can be a threat to human health (Kong et al. 2018). Therefore, it is necessary to evaluate environmental efficient techniques to reduce the uptake and accumulation of As in rice plants.

Although few studies have shown that the application of Si- and TiO<sub>2</sub>-NPs can diminish the uptake and accumulation of As in As-stressed plants, a deeper understanding of the role of NPs in the mechanisms of reducing As accumulation in plants at the molecular and biochemical levels is needed. Apart from the positive role of Si- and TiO<sub>2</sub>-NPs on the plant defense systems, the role of Si- and TiO<sub>2</sub>-NPs on the transcription level of genes responsible in As uptake and translocation as well as the biosynthesis of phytochelatins (PCs) was assessed as a novel target for the use of Si- and TiO<sub>2</sub>-NPs in As-stressed plants. The results can provide long-term food security and safety by creating possible implications for marginal farming practices in areas polluted with As.

#### **Material and methods**

#### **Material and treatments**

Rice cultivar (Oryza sativa L. cv. IR64) seeds were obtained from the Rice Research Institute Amol-Iran. Cultivar IR64 was selected as the As-sensitive rice cultivar according to the results previously reported by Bidi et al. (2021). NaOCl (1%, w/v) and distilled water were employed for surface sterilization and washing of the seeds, respectively. The seeds were left to germinate and grow for 10 days in containers containing sterilized peat moss. Ten-day-old rice plants were moved to pots filled with 50% Hoagland medium (Hoagland and Arnon 1941). The pots were changed with fresh Hoagland (pH 6.0) every 4 days. The pots were held in controlled condition with 16-h light and 8-h dark (temperature, 25-22 °C; illumination intensity, 350–400  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, humidity: 60–70%). Rice seedlings were treated with As and NPs after 10 days (adaptation). As treatment was prepared using NaAsO<sub>2</sub> at concentrations of 0 and 50 µM and added to Hoagland solution. Both Si-NPs and TiO<sub>2</sub>-NPs were obtained from USA-Nano. Si-NPs (99% purity, 50 nm  $\geq$  size and 80–100 m<sup>2</sup>/g surface area) were prepared at concentrations of 50 and 100 mg/L and added to Hoagland. TiO<sub>2</sub>-NPs (99% purity, 200–220 m<sup>2</sup>/g surface area, and 20-30-nm size) with concentrations of 25 and 50 mg/L were added to Hoagland solutions. Seedlings were sampled 21 days after the start of treatments and after measuring the height of seedlings, they were transferred to - 80 °C. By incubating the seedlings at 68 °C for 48 h, total dry weight (TDW) was obtained (Ghorbani et al. 2009).

## Photosynthetic pigments and chlorophyll fluorescence

Fresh leaves were applied for homogenization with 3% acetone (v/v) and then centrifuged at  $10,000 \times g$  for 10 min. The supernatants were used for readings at 645, 663, and 470 nm and the amounts of Chl *a* and *b* and carotenoids were determined according to previous method by Sharma et al. (2012). Fv/Fm value was achieved by a PAM fluorometer (Walz; PAM 2500).

#### Root and shoot concentrations of As, Si, and Ti

After acidic digestion of dry root and shoot tissues in a mixture of  $HNO_3$ : $H_2O_2$  (4:1 ratio) on a hot plate, root and shoot concentrations of As, Si, and Ti were obtained by an ICP-MS (Agilent 7500 cx).

#### Root and leaf contents of glutathione (GSH) and PCs

Leaf and root contents of GSH were assessed through the procedure of Yu et al. (2003) and reading the change in absorbance of 420 nm following 2-nitro-5-thiobenzoic acid production from 5,5-dithio-bis(2-nitrobenzoic acid). By extracting non-protein thiols, leaf and root contents of PCs were measured through the method of De Vos et al. (1992).

#### Leaf contents of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and methylglyoxal (MG)

To determine  $H_2O_2$  content, fresh leaf tissues were extracted using 1% trichloroacetic acid (w/v). After centrifugation at 12,000×g for 10 min, the supernatants were mixed with KI (1 M) and 1 mM potassium phosphate buffer (pH 6.8), and read at 390 nm (Sinha et al. 2005).

Fresh leaves were used for extraction with 5% perchloric acid, and after centrifugation, the supernatants were neutralized with sodium carbonate and combined with monobasic sodium phosphate and N-acetylcysteine. After reading the mixture at 288 nm, the leaf MG level was achieved by Yu et al. (2003) method.

## Malondialdehyde (MDA) and electrolyte leakage (EL)

After homogenizing the fresh leaves in 0.1% trichloroacetic acid and centrifuging at  $10,000 \times g$  for 10 min, the supernatants were mixed with 0.5% thiobarbituric acid and incubated for 30 min at 95 °C. The supernatant was recorded at 532 nm and MDA level was obtained by Heath and Packer (1968) method.

Dionisio-Sese and Tobita (1998) method was employed to assess EL. After placing the leaf discs in tubes filled with distilled water, the tubes were kept at 32 °C for 2 h. After obtaining the electrical conductivity (EC1), the samples were transferred to 121 °C for 2 h and EC2 was recorded. Leaf EL was calculated using the following formula: (EC1/EC2) × 100.

#### **Enzyme extraction and assays**

Enzymatic extract of fresh leaf tissue was extracted using extraction buffer comprising potassium phosphate buffer (50 mM, pH 6.8), ascorbate (1 mM),  $\beta$ -mercaptoethanol (5 mM), glycerin (10%), and potassium chloride (100 mM) (Ghasemi-Omran et al. 2021). Then, after centrifuging the samples, the supernatant was employed to estimate enzyme activity. Five independent biological replications were used to determine the activity of enzymes, each of which was calculated from 3 to 5 technical replications.

Leaf activity of catalase (CAT) enzyme was achieved by Aebi (1984) method and reading the difference in the absorbance of the mixture solution contained enzyme aliquot, 2% H<sub>2</sub>O<sub>2</sub>, and 50 mM potassium phosphate buffer (pH 6.8) at 240 nm for 2 min.

Leaf activity of superoxide dismutase (SOD) was achieved through the procedure given by Giannopolitis and Reis (1977) and showing the difference in absorbance of the reaction mixture consisting of enzyme aliquot, potassium phosphate buffer (50 mM, pH 7.8), Na<sub>2</sub>CO<sub>3</sub> (0.05 M, pH 10.2), nitro blue tetrazolium (63  $\mu$ M), EDTA (0.1 mM), methionine (13 mM), and riboflavin (1.3  $\mu$ M) at 560 nm.

Leaf activity of ascorbate peroxidase (APX) was obtained through the procedure of Nakano and Asada (1981) and recording reduction of absorbance in the reaction solution including enzyme aliquot, 50 mM potassium phosphate buffer (pH 7.0), ascorbate (0.5 mM),  $H_2O_2$  (0.1 mM), and EDTA (0.1 mM) at 290 nm.

Glutathione reductase (GR) activity was determined according to Schaedle and Bassham (1977) and showing absorbance changes in the reaction solution (0.1 M potassium phosphate buffer (pH 7.0), EDTA (1 mM), oxidized GSH (1 mM), NADPH (0.2 mM), and enzyme aliquot) at 340 nm.

Leaf activity of glyoxalase (Gly) I was obtained through the procedure of Hossain et al. (2010) and reading absorbance changes in the solution contained 100 mM K phosphate buffer (pH 7.0),  $MgSO_4$  (15 mM), MG (3.5 mM), GSH (1.7 mM), and enzyme extract at 240 nm.

Leaf activity of Gly II was achieved through the procedure of Principato et al. (1987) and reading absorbance changes in the reaction solution contained 5,5-dithio-bis(2nitrobenzoic acid) (0.2 mM), S-lactoylglutathione (1 mM), 100 mM Tris–HCl buffer (pH 7.2), and enzyme aliquot at 412 nm.

The leaf contents of the proteins were estimated by the procedure previously described by Bradford (1976) using bovine serum albumin as standard.

#### Expression of genes in roots and leaves

TRIzol reagent (Invitrogen, USA) and RevertAid<sup>TM</sup> Reverse Transcriptase kit (Fermentas, Germany) were used for total RNA extraction and first-strand cDNA synthesis, respectively. RT-PCR was done by Thermo Scientific Maxima SYBR Green qPCR Master Mix. *Actin* gene was used as an internal control for normalization. The expression of target genes was analyzed through the procedure of Livak and Schmittgen (2001). The primers of the target genes and *Actin* gene are listed in Table 1, which were designed using Primer3 program.

Table 1 The sequences of primers used in qPCR reactions

Gene name	5'-primer-3'	Accession no
Lsil	F: GTTGCTCAGGCTTCTCAACC R: AGTTGTTGCTGGCCATTTCT	XM_015770687
Lsi2	F: CTCGCTGCTCGTCTTCTTCT R: GGTACGTTTGATGCGAGGTT	XM_015776731
Lsi6	F: GTCCGTTGATTCGTTGTCCT R: TCACGAACACAAGCAGGAAC	XM_015788648
GSH1	F: ATCTACGCTTTGTCCCCATTC R: ATATTCCCAGAGGTTCGGTG	NM_001203879
PCS	F: TCGCTTCAAATACCCTCCTC R: TTTACTTGGGCTGGATCCTC	LC192429
ABC1	F: CCATGGCTAGGGCTGTTTAT R: GTTCTCCCTTGATGCACCTT	NM_104680
Actin	F: TCCTCCGTGGAGAAGAGCTA R: GCAATGCCAGGGAACATAGT	XM_015774830

#### **Statistical analysis**

The results were analyzed by SAS 9.1.3 software and the least significant difference (LSD) test was performed to achieve a significant difference between the means at the confidence level of P < 0.05. The results of morphological and biochemical traits are the mean  $\pm$  standard deviation (SD) of five replications (transcription level was obtained from three biological replications).

#### Results

#### Plant height and biomass

Arsenic (50  $\mu$ M) treatment significantly lessened the height and TDW by 35.8 and 40.8%, respectively, in comparison with non-treated plants. The addition of Si- and TiO<sub>2</sub>-NPs did not have a significant impact on plant height. However, 100 mg/L Si-NPs significantly raised TDW by 12.2% relative to control plants. In As-exposed plants, the supply of Si- and TiO<sub>2</sub>-NPs significantly restored the height and TDW, with 100 mg/L Si-NPs having the greatest effect (Table 2).

### Photosynthetic pigments and chlorophyll fluorescence

Under As stress, the contents of Chl *a* and *b* lowered significantly by 55.8 and 64.2% over the control ones. However, the application of Si- and TiO<sub>2</sub>-NPs enhanced Chl *a* and *b* contents in As-treated plants. In As-stressed plants, 100 mg/L Si-NPs most effective at improving Chl *a* and *b* contents (Table 2). As stress declined the carotenoid content by 53% over untreated plants. In As-treated rice, the supply of 50 and 100 mg/L Si-NPs enhanced the carotenoid content by 34.5 and 75%; however, 25 and 50 mg/L TiO<sub>2</sub>-NPs raised carotenoids by 16.9 and 47.3%, respectively, in comparison with plants treated with only As (Table 2). Arsenic toxicity decreased Fv/Fm; however, the addition of Si- and TiO<sub>2</sub>-NPs significantly increased Fv/Fm value in As-exposed rice. In As-stressed plants, the highest Fv/Fm value was recorded under 100 mg/L Si-NPs application (Table 2).

#### Root and shoot concentrations of As, Si, and Ti

Arsenic accumulated in the roots and shoots of rice under 50  $\mu$ M As treatment. However, Si- and TiO<sub>2</sub>-NPs application declined the root and shoot accumulation of As in Asexposed rice, with 100 mg/L Si-NPs having the stronger effect (Table 3). Arsenic treatment lessened the root and shoot concentrations of Si by 54.5 and 62.1%, respectively, compared to non-treated plants. In non-stressed (control)

**Table 2** Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) and titanium dioxide nanoparticles (TiO<sub>2</sub>, 25 and 50 mg/L) (NPs) on growth, photosynthetic pigments, and chlorophyll fluorescence (Fv/Fm) of rice seedlings under arsenic (As, 0 and 50  $\mu$ M) toxicity

Treatments	Height (cm)	Total dry weight (g)	Chlorophyll a (mg g <sup>-1</sup> fw)	Chlorophyll b	Carotenoids	Fv/Fm
Control	35.19±1.55a	$3.77 \pm 0.19b$	2.67±0.18a	2.04±0.16b	$0.315 \pm 0.022a$	$0.685 \pm 0.019$ bc
50 mg/L Si-NPs	$37.02 \pm 1.08a$	$4.03 \pm 0.21$ ab	$2.69 \pm 0.23a$	$2.03 \pm 0.19b$	$0.309 \pm 0.022a$	$0.712\pm0.018\mathrm{ab}$
100 mg/L Si-NPs	$37.09 \pm 1.17a$	$4.23 \pm 0.34a$	$2.68 \pm 0.21$ a	$2.35 \pm 0.11a$	$0.337 \pm 0.016a$	$0.716 \pm 0.010a$
25 mg/L TiO <sub>2</sub> -NPs	$35.60 \pm 1.37a$	$3.82 \pm 0.18b$	$2.66 \pm 0.19a$	$2.21 \pm 0.16$ ab	$0.319 \pm 0.023a$	$0.698 \pm 0.015$ abc
50 mg/L TiO <sub>2</sub> -NPs	$35.85 \pm 1.33a$	$3.83 \pm 0.18b$	$2.71 \pm 0.14a$	$2.03 \pm 0.11b$	$0.314 \pm 0.015a$	$0.676 \pm 0.017c$
50 µM As	$22.59 \pm 1.69e$	$2.23 \pm 0.18e$	$1.18 \pm 0.17$ d	$0.73 \pm 0.11e$	$0.148 \pm 0.015e$	0.397±0.011 g
50 µM As+50 mg/L Si-NPs	$28.94 \pm 1.16 \mathrm{bc}$	$2.74 \pm 0.13$ cd	$1.99 \pm 0.15b$	$1.10 \pm 0.12d$	$0.199 \pm 0.014$ cd	$0.529 \pm 0.016 \mathrm{e}$
50 µM As + 100 mg/L Si-NPs	$30.93 \pm 1.39b$	$2.87 \pm 0.12c$	$2.47 \pm 0.15a$	$1.56 \pm 0.08c$	$0.259 \pm 0.017b$	$0.624\pm0.020\mathrm{d}$
$50 \mu\text{M As} + 25 \text{mg/L TiO}_2$ -NPs	$25.75 \pm 1.35d$	$2.38 \pm 0.16e$	$1.49 \pm 0.17c$	$1.02 \pm 0.13$ d	$0.173 \pm 0.015$ de	$0.491 \pm 0.018 \mathrm{f}$
$50 \mu M As + 50 mg/L TiO_2-NPs$	$27.64 \pm 1.72$ cd	$2.45 \pm 0.16$ de	$1.95 \pm 0.14$ b	$1.34 \pm 0.13c$	$0.218 \pm 0.015c$	$0.540 \pm 0.017e$

The same letters above the bars (means  $\pm$  SD, n = 5) indicate no significant difference (LSD test; P < 0.05)

Table 3	Effect of silicon nanoparticles (Si-NPs, 5	50 and 100 mg/L) and titanium	dioxide nanoparticles	(TiO2, 25 and 50 mg/L	) (NPs) on the root
and sho	ot concentrations of arsenic (As), Si and 7	Γi in rice seedlings under As (0	and 50 µM) toxicity		

Treatments	As in root	As in shoot	Si in root	Si in shoot	Ti in root	Ti in shoot
	mg/kg DW		g/kg DW		mg/kg DW	
Control	_	_	$3.98 \pm 0.14d$	4.56±0.17d	$9.43 \pm 0.88d$	5.29±0.81d
50 mg/L Si-NPs	_	_	$6.12 \pm 0.20b$	$6.38 \pm 0.23b$	$8.43 \pm 1.37d$	$5.49 \pm 1.00d$
100 mg/L Si-NPs	-	-	8.97±0.18a	$7.77 \pm 0.18a$	$9.50 \pm 1.10d$	$4.30 \pm 1.21$ d
25 mg/L TiO <sub>2</sub> -NPs	-	-	$3.81 \pm 0.19d$	$4.41 \pm 0.26d$	$21.84 \pm 1.99 \mathrm{b}$	$14.35 \pm 1.12c$
50 mg/L TiO <sub>2</sub> -NPs	-	_	$3.94 \pm 0.17$ d	$4.34 \pm 0.29$ d	28.01 ± 1.41a	23.64 ± 1.48a
50 µM As	508.7 <u>+</u> 18.6a	90.17±7.67a	$1.81 \pm 0.18 \mathrm{f}$	$1.73 \pm 0.14 \mathrm{f}$	$5.34 \pm 0.59e$	$4.14 \pm 0.79$ d
50 µM As + 50 mg/L Si-NPs	379.3±18.5c	$46.57 \pm 4.82d$	$3.37 \pm 0.19e$	$3.55 \pm 0.19e$	$4.89 \pm 0.68e$	$4.16 \pm 1.03$ d
50 µM As+100 mg/L Si-NPs	273.0±19.7d	$36.00 \pm 5.48e$	$4.94 \pm 0.17c$	$5.13 \pm 0.23c$	$4.49 \pm 1.21e$	$4.11 \pm 1.20d$
$50 \mu\text{M As} + 25 \text{mg/L TiO}_2$ -NPs	476.0±15.5b	$77.92 \pm 5.66b$	$1.95 \pm 0.23 f$	$1.99 \pm 0.15 f$	$17.98 \pm 1.41c$	13.99±1.45c
$50 \mu M As + 50 mg/L TiO_2-NPs$	$463.0 \pm 21.9b$	$69.90 \pm 5.65c$	$2.08\pm0.20\mathrm{f}$	$2.06\pm0.17\mathrm{f}$	$23.98 \pm 1.64$ b	$19.73 \pm 1.41b$

The same letters above the bars (means  $\pm$  SD, n = 5) indicate no significant difference (LSD test; P < 0.05)

and As-stressed plants,  $TiO_2$ -NPs application did not have a significant impact on the shoot and root concentrations of Si. However, Si-NPs significantly enhanced the shoot and root accumulation of Si in both non-stressed and As-stressed plants, with 100 mg/L Si-NPs having a better effect (Table 3). The addition of As to the hydroponic medium had no significant impact on the shoot and root amount of Ti. In control and As-stressed plants, Si-NPs supply did not have a significant impact on the shoot and root concentrations of Ti. However, the exogenous application of TiO<sub>2</sub>-NPs enhanced the shoot and root accumulation of Ti and the highest level of Ti accumulation was observed at 50 mg/L TiO<sub>2</sub>-NPs (Table 3).

## Hydrogen peroxide ( $H_2O_2$ ), methylglyoxal (MG), malondialdehyde (MDA), and electrolyte leakage (EL)

The addition of As significantly enhanced the leaf accumulation of  $H_2O_2$  over normal conditions. However, the application of Si- and TiO<sub>2</sub>-NPs declined the leaf accumulation of  $H_2O_2$  in As-stressed rice, with 100 mg/L Si-NPs having the most beneficial effect (Fig. 1A). The leaf content of MG increased 2.8-fold under 50  $\mu$ M As stress over control ones. In As-treated rice, the supply of 50 and 100 mg/L Si-NPs diminished MG content by 29.3 and 43%, respectively, while the supply of 25 and 50 mg/L TiO<sub>2</sub>-NPs lessened MG level by 24 and 32.5%, respectively, over plants treated

Fig. 1 Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) and titanium dioxide nanoparticles (TiO<sub>2</sub>, 25 and 50 mg/L) (NPs) on hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, A), methylglyoxal (MG, B), malondialdehyde (MDA, C), and electrolyte leakage (EL, D) of rice seedlings under arsenic (As, 0 and 50  $\mu$ M) toxicity. The same letters above the bars (means ± SD, *n* = 5) are not significantly different (LSD test; *P* < 0.05)



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with only As (Fig. 1B). Leaf MDA content showed a significant increase in plants treated with As over the control plants. However, in As-treated rice, the supply of 50 and 100 mg/L Si-NPs and 25 and 50 mg/L TiO<sub>2</sub>-NPs diminished leaf MDA level by 23.1, 34.8, 18.1, and 26.2%, respectively, compared to plants treated with only As (Fig. 1C). A significant increase in EL levels was observed in the leaves of Asexposed plants over control plants. However, the application of Si- and TiO<sub>2</sub>-NPs significantly reduced EL level in Asstressed plants, with 100 mg/L Si-NPs having the stronger effect (Fig. 1D).

#### Antioxidant enzymes and glyoxylate system

A significant increase in leaf activity of CAT, SOD, APX, and GR in As-treated plants was recorded by 28.9, 53.3, 36.8, and 69.9%, respectively, compared to non-treated plants. However, the exogenous application of Si- and  $TiO_2$ -NPs further raised the leaf activity of CAT, SOD, APX, and GR compared to plants treated with As alone. In As-stressed plants, the highest activity of antioxidant enzymes was recorded under 50 mg/L TiO<sub>2</sub>-NPs (Fig. 2A, B, C, D).

The addition of 50  $\mu$ M As enhanced the leaf activity of Gly I by 34.8% over non-treated plants. The supply of 50 and 100 mg/L Si-NPs and 25 and 50 mg/L TiO<sub>2</sub>-NPs further raised Gly I activity by 32.8, 59.4, 23.9, and 77.6%, respectively, in As-exposed plants than plants treated with only As (Fig. 2E). Arsenic treatment induced the activity of Gly II enzyme compared to control samples. However, the use of Si- and TiO<sub>2</sub>-NPs further enhanced the activity of Gly II in As-treated rice than plants treated with As alone, with 50 mg/L TiO<sub>2</sub>-NPs having the stronger effect (Fig. 2F).

#### Root and shoot contents of PCs and GSH

The application of As caused a significant rise in the root and leaf contents of PCs by 52.8 and 104%, respectively, over the

Fig. 2 Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) and titanium dioxide nanoparticles (TiO<sub>2</sub>, 25 and 50 mg/L) (NPs) on the leaf activities of catalase (CAT, A), superoxide dismutase (SOD, B), ascorbate peroxidase (APX, C) and glutathione reductase (GR, D), glyoxalase I (Gly I, E), and glyoxalase II (Gly II, F) enzymes of rice seedlings under arsenic (As, 0 and 50 µM) toxicity. The same letters above the bars (means  $\pm$  SD, n = 5) are not significantly different (LSD test; P < 0.05)



normal conditions. The addition of Si- and TiO<sub>2</sub>-NPs significantly raised the root and leaf contents of PCs in As-treated rice. The supply of 50 and 100 mg/L Si-NPs enhanced the root content of PCs by 20.1 and 24.4% and the leaf content of PCs by 21.2 and 36.7%, respectively; however, 25 and 50 mg/L TiO<sub>2</sub>-NPs enhanced the root accumulation of PCs by 54.2 and 65.7% and the leaf accumulation of PCs by 53.7 and 63.1%, respectively, in comparison to plants exposed to only As (Fig. 3A, B). A significant increase in the root and leaf contents of GSH was obtained in plants exposed to 50 µM As over non-treated rice. In control plants, NPs supply had no significant impact on the root and leaf accumulation of GSH; however, in As-exposed plants, Si- and TiO2-NPs enhanced the root and leaf contents of GSH than plants treated with As alone, and TiO2-NPs had the most beneficial impacts on GSH accumulation than Si-NPs (Fig. 2C, D).

#### Expression of As transporter, PCs, and GSH1 genes

The addition of As in hydroponic medium enhanced mRNA level of *Lsi1* in the roots and leaves of rice by 6.6- and 2.1-fold, respectively, than control plants. In the roots, Si- and TiO<sub>2</sub>-NPs lowered *Lsi1* expression in As-exposed rice over plants treated with only As, which recorded the greatest decrease in 100 mg/L Si-NPs treatment. In the leaves of As-treated rice, the supply of Si-NPs reduced the expression level of *Lsi1*, while TiO<sub>2</sub>-NPs had no significant impact on mRNA level of *Lsi1* (Fig. 4A).

The treatment of 50  $\mu$ M As enhanced the transcription levels of *Lsi2* and *Lsi6* in roots and leaves over control. In As-stressed rice, the addition of Si-NPs downregulated the expression of *Lsi2* and *Lsi6* in both root and leaf tissues, while TiO<sub>2</sub>-NPs supply had no significant impact on the expression of *Lsi2* and *Lsi6* in comparison to plants exposed to only As (Fig. 4B, C).

The application of 50 µM As significantly enhanced the level of GSH1 expression in roots and leaves. The application of Si- and TiO<sub>2</sub>-NPs further upregulated the expression level of GSH1 in the root and leaf of As-treated rice, which was the highest increase in 50 mg/L TiO<sub>2</sub>-NPs treatment (Fig. 5A). Enhancements of 4.5- and 3.2-fold in PCS expression level were recorded in the leaf and root of rice seedlings, respectively, over control ones. However, the supply of Si- and TiO<sub>2</sub>-NPs further upregulated the mRNA level of PCS in the root and leaf of As-treated rice, with 50 mg/L TiO<sub>2</sub>-NPs having the stronger effect (Fig. 5B). The transcription level of ABC1 in root and leaf showed 3.3- and 2.1-fold upregulation under As stress, respectively, compared to those under normal conditions. However, Si- and TiO<sub>2</sub>-NPs significantly enhanced ABC1 gene expression in the root and leaf of As-exposed rice. In the roots of As-stressed plants, the highest expression level of ABC1 gene was obtained under the treatments of 100 mg/L Si-NPs and 50 mg/L TiO<sub>2</sub>-NPs, while in the leaves, the highest expression of ABC1 was recorded in the plants treated with 50 mg/L TiO<sub>2</sub>-NPs (Fig. 5C).

**Fig. 3** Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) and titanium dioxide nanoparticles (TiO<sub>2</sub>, 25 and 50 mg/L) (NPs) on the root and leaf contents of phytochelatins (PCs, A and B) and glutathione (GSH, C and D) of rice seedlings under arsenic (As, 0 and 50  $\mu$ M) toxicity. The same letters above the bars (means ± SD, n = 5) are not significantly different (LSD test; P < 0.05)



**Fig. 4** Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) and titanium dioxide nanoparticles (TiO<sub>2</sub>, 25 and 50 mg/L) (NPs) on the expression of *Lsi1* (A), *Lsi2* (B), and *Lsi6* (C) genes of rice seedlings under arsenic (As, 0 and 50  $\mu$ M) toxicity. The same letters above the bars (means ± SD, *n*=3) are not significantly different (LSD test: *P* < 0.05)



#### Discussion

The results of the present study showed that As toxicity reduced photosynthetic pigments and Fv/Fm value and, as a result, inhibited the growth of rice (Table 2), which is consistent with previously reported results on rice (Ghorbani et al. 2020; Bidi et al. 2021) and maize (Tripathi et al. 2016). As stress has been reported to lessen plant growth and yield by inducing oxidative stress, diminishing chlorophyll

content, reducing the absorption of iron and its translocation to the leaves, and inhibiting stomatal conductance (Mousavi et al. 2020). However, the application of both NPs improved photosynthetic pigments and, consequently, restored growth and biomass under As phytotoxicity. The positive impacts of NPs on enhancing plant growth and yield under normal and stressful conditions have been previously documented (Gohari et al. 2020; Bidi et al. 2021; Tripathi et al. 2016). Rizwan et al. (2019) indicated that Si- and TiO<sub>2</sub>-NPs by **Fig. 5** Effect of silicon nanoparticles (Si-NPs, 50 and 100 mg/L) and titanium dioxide nanoparticles (TiO<sub>2</sub>, 25 and 50 mg/L) (NPs) on the expression of *GSH1* (A), *PCS* (B), and *ABC1* (C) genes of rice seedlings under arsenic (As, 0 and 50  $\mu$ M) toxicity. The same letters in the columns (means  $\pm$  SD, n = 3) are not significantly different (LSD test; P < 0.05)



diminishing the accumulation of cadmium and improving the activity of antioxidant enzymes enhanced rice growth under cadmium phytotoxicity. Numerous studies have shown that the supply of NPs improved plant biomass under toxic metal stress by improving the antioxidant defense system and enhancing the accumulation of mineral nutrients (Hussain et al. 2018; Gohari et al. 2020; Bidi et al. 2021). Improvements in photosynthetic pigments induced by Si- and TiO<sub>2</sub>-NPs in rice (Zhang et al. 2020; Rizwan et al. 2019) and maize (Tripathi et al. 2016) plants under heavy metal stress have already been documented. The application of Si- and TiO<sub>2</sub>-NPs has been shown to protect the thylakoid membranes and photosynthetic apparatus from heavy metal stress by improving the activity of antioxidant enzymes and decreasing the level of toxic radicals (Khan et al. 2020; Rizwan et al. 2019). Asgari et al. (2018) noted that Si-NPs

improve photosynthetic pigments by facilitating the uptake of nutrients through xylems. Therefore, the application of nanoparticles increased rice growth under As toxicity by restoring chlorophyll contents and improving the performance of the photosynthetic apparatus.

Stressful conditions enhance the level of compounds such as H<sub>2</sub>O<sub>2</sub> and MG, which induce oxidative stress and damage bio-membranes, resulting in elevated EL (Bidi et al. 2021; Ghorbani et al. 2018a, 2021). Here, As toxicity enhanced leaf levels of H<sub>2</sub>O<sub>2</sub> and MG, which was accompanied by increased levels of MDA and EL (Fig. 1), indicating the induction of oxidative stress. Increased accumulation of proline, sugar, and malondialdehyde compounds and induction of oxidative stress in rice (Ghorbani et al. 2020; Bidi et al. 2021), soybean (Ahmad et al., 2020), and mustard (Ahmad et al., 2021) have already been described. However, the supply of Si- and TiO<sub>2</sub>-NPs effectively decreased the accumulation of H<sub>2</sub>O<sub>2</sub> and MG and thus protected membrane lipids and reduced MDA level and EL, indicating the positive effects of these NPs in alleviating As-induced oxidative stress. The reduction in H<sub>2</sub>O<sub>2</sub>, MG, and MDA levels, and thus the alleviation of EL and oxidative stress induced by the supply of Si- and TiO<sub>2</sub>-NPs, has previously been documented by Khan et al. (2020) in wheat, Tripathi et al. (2016) in maize, and Rizwan et al. (2019) in rice. To counteract environmental stresses-induced oxidative stress, plants have defense mechanisms including antioxidant defense system and glyoxylate cycle in which strengthening this mechanism can increase plant adaptation and tolerance (Ghorbani et al. 2018b, 2019). The results revealed that the supply of both Si- and TiO<sub>2</sub>-NPs upregulated the leaf activities of SOD, CAT, GR, APX, Gly I, and Gly II enzymes under As toxicity (Fig. 2). Enhancing the leaf activities of antioxidant enzymes and the glyoxylate system can play a major role in improving the rice growth under As phytotoxicity (Ramezani et al. 2021). Similar results of improving the activity of antioxidant enzymes by the application of Si- and TiO<sub>2</sub>-NPs in rice under cadmium toxicity have already been documented by Rizwan et al. (2019). Gohari et al. (2020) revealed that TiO<sub>2</sub>-NPs protected bio-membranes under oxidative stress induced by salinity stress by increasing the activity of guaiacol peroxidase (GP), CAT, APX, and SOD enzymes and decreasing the level of  $H_2O_2$ . Khan et al. (2020) indicated that the supply of Si-NPs by increasing the activity of SOD and peroxidase (POD) and reducing the levels of H<sub>2</sub>O<sub>2</sub> and MDA, diminished oxidative stress and reduced EL and thus enhanced the growth of wheat under heavy metal phytotoxicity. Ghorbani et al. (2020) showed that augmenting the activity of the glyoxalase system and antioxidant enzymes can effectively promote the tolerance of plants under As phytotoxicity. Therefore, our findings confirm that Si- and TiO<sub>2</sub>-NPs increased the activity of antioxidant enzymes and the glyoxalase system, which can play an effective role in strengthening the immune system of plants in response to As phytotoxicity. However,  $TiO_2$ -NPs further increased the activity of the glyoxalase system and antioxidant enzymes than Si-NPs, which show that the effectiveness effects of NPs on plant tolerance under stressful conditions vary with the type of NPs (Rizwan et al. 2019).

The results indicated that As treatment upregulated the transcription levels of genes associated with As uptake and translocation (Lsi1, Lsi2, and Lsi6) (Fig. 4), which is consistent with enhanced As concentration in the root and shoot. Enhanced mRNA levels of Lsi1, Lsi2, and Lsi6 genes under As treatment have also been recorded by Mousavi et al. (2020). As stress also lowered the uptake of Si and its accumulation in the shoots. Because Si and As are absorbed and distributed in rice plants through similar transporters (Ma et al. 2004, 2007; Yamaji et al. 2008), the reduction in Si uptake under As stress may be due in part to the competitive effect of Si and As in the rhizosphere. Thus, As stress decreased the uptake and accumulation of Si in rice plants, which to compensate for this decrease, the expression level of Si/As transporters upregulated. Due to the high concentration of As in the growth medium, it increased the uptake and concentration of As in rice plants. Si-NPs reduced the root and shoot accumulation of As and increased the root and shoot accumulation of Si under As stress, indicating that the presence of Si in the rhizosphere effectively reduces the uptake of As by the root, which is consistent with the results of Tripathi et al. (2013) and Khan and Gupta (2018). Application of S-NPs downregulated the expression level of Si/As transporters, which is consistent with a decrease in the need for Si uptake due to the increased accumulation of Si. The reduction in As uptake in the presence of Si may be due to the high affinity of Si/As transporters (Lsi1, Lsi2, Lsi6) towards Si than As (Khan and Gupta 2018). Si has also been reported to block the apoplasmic transports and restrict the entry of metals by binding to cell wall components and inducing structural modifications (Lukačová et al. 2013). Therefore, by modulating the expression of Si/As transporters, Si-NPs increased Si uptake and decreased As uptake under As phytotoxicity, which can effectively improve the tolerance of rice plants under As stress. TiO2-NPs did not have a significant impact on the expression of As transporters and, as a result, the root and shoot accumulation of As, which indicates that these NPs improve plant tolerance under As toxicity through a different mechanism from that of Si-NPs.

GSH is one of the predominant non-protein thiols in plants, which plays an outstanding role in enhancing plant tolerance under stressful conditions. GSH, as an important precursor to PCs, one of the most important chelating agents for toxic metals, plays a crucial role in the detoxification of heavy metals. Si- and TiO<sub>2</sub>-NPs upregulated the root and leaf expression of *GSH1*, *PCS*, and *ABC1* genes (Fig. 5),

which is consistent with the increase in root and leaf concentrations of GSH and PCs (Fig. 3). Overexpression of GSH1 gene has been reported to increase the leaf accumulation of GSH and thus protect the photosynthetic apparatus under heavy metal stress, indicating the importance of glutathione in reducing heavy metal toxicity (Ivanova et al. 2011). Guo et al. (2008) revealed that the simultaneous overexpression of GSH1 and PCS1 genes increased the accumulation of PCs and thus improved the tolerance of Arabidopsis thaliana to As and cadmium toxicity. Thus, our findings indicated that the supply of Si- and TiO<sub>2</sub>-NPs upregulated the expression of GSH1, PCS, and ABC1 genes and increased the production of GSH and PCs, resulting in As sequestration in vacuoles and protection of plant cells against arsenic toxicity. However, TiO<sub>2</sub>-NPs upregulated the expression of PCS, GSH1, and ABC1 more than Si-NPs, which is consistent with the higher accumulation of As in TiO<sub>2</sub>-NPs-treated plants and the higher need for PCs and GSH.

#### Conclusions

The ability of plants to detoxify, reduce the uptake, or sequester toxic pollutants can play a crucial role in improving the growth and yield of crops in areas contaminated with toxic metals. Our findings revealed that Si- and TiO2-NPs supply through different strategies enhanced the biomass and growth of rice under As phytotoxicity. By modulating the expression of Si/As transporters (Lsi1, Lsi2, and Lsi6), Si-NPs enhanced Si uptake and lowered As uptake in rice, while TiO<sub>2</sub>-NPs had no significant impacts on the expression of Si/As transporter and, consequently, As and Si accumulation. Both Si- and TiO<sub>2</sub>-NPs increased the leaf activity of the glyoxalase system and antioxidant enzymes and decreased levels of H<sub>2</sub>O<sub>2</sub>, MG, MDA, and EL, thereby reducing oxidative stress and improving plant growth under As stress. However, TiO<sub>2</sub>-NPs increased the activity of antioxidant enzymes and the glyoxalase system more effectively than Si-NPs. By increasing the transcription level of GSH1, PCS, and ABC1, Si- and TiO<sub>2</sub>-NPs increased the root and leaf accumulations of GSH and PCs, resulting in As sequestration in vacuoles and protection of plant cells against As toxicity. However, GSH and PCs levels were higher in TiO2-NPs-treated plants than those in Si-NPs-treated plants. The results revealed that although Si- and TiO<sub>2</sub>-NPs improved the tolerance of rice plants under As toxicity through different mechanisms, Si-NPs, especially 100 mg/L Si-NPs treatment, had the highest induction effect on improving the growth and tolerance of rice plants compared to other treatments. In general, the results expedite a better understanding of how Si- and TiO<sub>2</sub>-NPs decline As uptake and transport, improve plant defense mechanisms, and ultimately enhance

plant adaptation under As toxicity, which could produce a novel approach for designing effective fertilizers to reduce As toxicity in As-contaminated areas in rice.

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**Data availability** All data used or analyzed during this study are available from the corresponding author on reasonable request.

#### Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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