Silicon Nanoparticles More Efficiently Alleviate Arsenate Toxicity than Silicon in Maize Cultiver and Hybrid Differing in Arsenate Tolerance

Durgesh Kumar Tripathi [1], Swati Singh [2], Vijay Pratap Singh [3]*, Sheo Mohan Prasad [4]*, Devendra Kumar Chauhan [4]* and Nawal Kishore Dubey [1]*

[1] Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, India, [2] Department of Botany, Government Ramanuj Pratap Singhdev Post Graduate College, Sarguja University, Baikunthpur, India, [3] Ranjan Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Allahabad, Allahabad, India, [4] D.D. Pant Interdisciplinary Research Laboratory, Department of Botany, University of Allahabad, Allahabad, India

Alleviation of abiotic stress by silicon (Si) is well documented. But mitigation of abiotic stress by silicon nanoparticles (SiNPs) is not much known. Therefore, hydroponic experiments were conducted to investigate if SiNPs are more effective than Si in mitigation of arsenate (As^V; 25 and $50 \mu M$) toxicity in maize cultivar and hybrid differing in As^V tolerance. Under As^V stress, reduction in growth was accompanied by enhanced level of As and oxidative stress. As^V inhibited activities of antioxidant enzymes like ascorbate peroxidase, glutathione reductase, and dehydroascorbate reductase (except superoxide dismutase). The redox status of ascorbate and glutathione was disturbed by As^V as indicated by a steep decline in their reduced/oxidized ratios. However, addition of Si and SiNp ameliorates As^V toxicity in maize. Si and SiNp both could reduce As^V toxicity in maize cultivar and hybrid, which could be related with decreased accumulation of As and oxidative stress, and enhanced components of the ascorbate-glutathione cycle (AsA-GSH cycle). But lowering in the accumulation of As and oxidative stress markers, and enhancement in components of the AsA-GSH cycle was prominent in SiNp fed seedlings under As^V stress. The results also showed that SiNp are more effective in reducing As^V toxicity than Si, which is due to their greater availability to seedlings. Comparing responses of cultivar and hybrid, maize cultivar shows more resistance against As^V than hybrid.

Keywords: arsenate, maize, photosynthesis, silicon, SiNp

INTRODUCTION

In last two decades, millions of people are exposed to arsenic (As) poisoning due to drinking of As-contaminated water (Nordstrom, 2002; Patel et al., 2005; Zhao et al., 2009; Singh R. et al., 2015; Singh et al., 2015a). Arsenic is found in different oxidation states, with the most dominant forms being trivalent (As^{III}) and penta-valent (As^V), which are classified as potent carcinogens

Abbreviations: AsA, reduced ascorbate; APX, ascorbate peroxidase; DHA, dehydroascorbate; DHAR, dehydrateascorbate reductase; F_v/F_m, maximum photochemical efficiency of PS II; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; NPQ, non-photochemical quenching; qP, photochemical quenching; ROS, reactive oxygen species; Si, silicon; SiNp, silicon nanoparticle; SOD, superoxide dismutase; SOR, superoxide radical.

(Zhao et al., 2009). The potential sources of As in soil and water include both natural (e.g., weathering reactions, biological activity, volcanic emissions, etc.) and anthropogenic (mining, use of pesticides and wood preservatives, fossil fuels combustion, etc.; Patel et al., 2005; Zhao et al., 2009). Continuous increment in its concentration in the environment is one of the major public health problems all over the world. High As concentrations have been reported in different countries including, India, Bangladesh, China, Vietnam, Nicaragua, Brazil, France, USA, and several other countries (Patel et al., 2005; McClintock et al., 2012; Singh R. et al., 2015). In India, large area of water bodies and agricultural lands is highly polluted with As, being its concentrations between 50–300 mg L^{-1} and 32–105 mg kg^{-1}, respectively (Patel et al., 2005; Singh et al., 2015a,b). The level of As in drinking water is also higher (0.3–0.5 mg L^{-1}) than the prescribed limit (0.05 mg L^{-1}; Singh et al., 2015b). Studies have demonstrated that As adversely influences growth, development and metabolic process of plants, and consequently yield (Li et al., 2006; Ding et al., 2009; Singh et al., 2015b).

The transportation of As^V into the plant cell takes place through phosphate transporters and thus, affects homeostasis of main source of energy i.e., ATP, while As^{III} transportation occurs through aquaporins (nodulin26-like intrinsic protein family), and obstructs plant nutrient uptake, metabolism, and renovation of oxidative DNA damage (Kertulis-Tartar et al., 2009). There are increasing evidences, which show that As accelerates production of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet-}$) hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^\bullet OH$), causing major damage to the plant cell. Several studies demonstrated that As influences photosynthetic pigments, membrane system of chloroplasts, chlorophyll fluorescence, and RUBISCO enzyme (Li et al., 2006; Ding et al., 2009; Singh et al., 2015a,b). Thus, As causes adverse effects on plants from molecular to whole plant level.

Silicon (Si) is recognized as second most abundant element on the earth's crust and also one of the most prominent mineral constituents of some plants (Epstein, 1999). In the soil, it comprises more than 25% of the earth's crust (Currie and Perry, 2007) and is available to plants in the form of monosilicic acid [Si(OH)$_4$]. Since few decades, physiological role of Si in the regulation of abiotic and biotic stresses in plants has made huge interest among researchers (Savant et al., 1997; Currie and Perry, 2007). Several studies demonstrated that Si has numerous direct and indirect beneficial impacts on plant growth and development. For instance, it appreciably enhances resistance of plants to several toxic metals such as aluminum (Al), chromium (Cr), cadmium (Cd) iron (Fe), manganese (Mn), and zinc (Zn) (Horiguchi and Morita, 1987; Shi et al., 2005; Singh et al., 2011; Tripathi et al., 2012, 2015). There may be various strategies by which exogenous application of Si affects heavy metal resistance in plant species. It has been reported that Si supply could decrease the uptake of Mn (Horiguchi and Morita, 1987). However, Kidd et al. (2001) showed that Si stimulates maize root exudation of phenolics, which can chelate rhizosphere Al and thus, reduces its absorption. The valuable function of Si in resisting metal stress is well recognized in plants. However, an involvement of

Si-nanoparticles (SiNp) in the regulation of metal stress is not yet well-known.

In last decade, nanotechnology has emerged as a prominent tool for enhancing agricultural productivity. Nanotechnology includes processing, generating, manipulating and deploying nanomaterials (having one or more proportions in the order of 100 nm or less). It has been documented that nanomaterials show size dependent behavior, distinctive optical properties and high surface area, thus exhibit significant role in plant protection and nutrition (Liu et al., 2009; Nair et al., 2010). In agro-nanotechnological research, efforts are being made to identify remedies for various stresses including heavy metals. Despite the availability of ample literature on metal stress, a relationship between metal stress and nanoparticle interaction, and nanoparticle-mediated alleviation of metal stress are still not well-known.

In past few decades, silicon (Si) has emerged as one of the beneficial elements that involved in the regulation of various abiotic stresses in plants. However, little is known about involvement of silicon nanoparticles (SiNp) in the regulation of metal toxicity in plants. Therefore, in the present study our aims were to: (1) investigate effect of Si and SiNp on maize cultivar and hybrid (2) confirm whether SiNp or Si are more effective in alleviating As^V toxicity in maize cultivar and hybrid (3) investigate the comparative impact of Si and nano-Si on antioxidant response of maize cultivar and hybrid under As^V stress and (4) elucidate possible mechanisms by which nano-Si alleviates As^V toxicity in maize seedlings.

MATERIALS AND METHODS

Synthesis and Characterization of Silicon Nano-Particles (SiNp)

Silicon nano-particles (SiNp) were synthesized by sol-gel synthesis method using sodium silicate (Na$_2$SiO$_3$) according to the method of Liu et al. (2009) with some modifications. Briefly, Na$_2$SiO$_3$ was dissolved firstly in 475 mL distilled water (5 mM), and then the pH was adjusted to 3 with 1 mM HCl. After this, solution was stirred on water bath at 75°C for 2 h. The pH of the acidified Na$_2$SiO$_3$ was adjusted to 7.0 with NH$_4$OH at 75°C and solution mixed thoroughly for about 1.5 h on water bath. The obtained Si sol was further dialyzed with dialysis bag to remove impurities such as Na$^+$, NH$_4^+$, etc. and then evaporated to form the Si gel. The Si gel was further calcinated in furnace to obtain the SiNp powder. All solutions were freshly prepared at room temperature. The characterization of SiNp was performed by using scanning electron microscope (SEM), energy-dispersive X-ray spectroscopy (EDS), UV-Vis spectrophotometer, and X-ray diffraction (XRD) analysis.

XRD was performed on Rigaku D/max-2200 PC diffractometer operated at 40 kV/40 mA, using CuKα1 radiation with wavelength of 1.54 Å in the wide angle region from 20 to 80° on 2θ scale. SEM was performed on Jeol JXA 8100 EPMA at 15 Kv and the UV-Vis spectrum was recorded by systronic double beam spectrophotometer. EDS was also performed for the detection of elemental analysis.

Plant Materials and Growth Conditions

Seeds of maize cultivar (*Zea mays* L. cv. Nootan) and hybrid (*Z. mays* L. hyb. Shaktiman-4) were purchased from certified supplier of local market. Seeds were surface sterilized in 10% (v/v) sodium hypochlorite solution for 5 min and then washed thoroughly and soaked for 2–4 h in distilled water. After sterilization and soaking, uniform sized seeds were sown in plastic trays containing sterilized sand. Thereafter, trays were kept in the dark for seed germination at 25 ± 2°C. When germination reached maximum, seedlings were grown in a growth chamber (CDR model GRW-300 DGe, Athens) under photosynthetically active radiation (PAR) of 350 μmol photons m^{-2} s^{-1} and 86–90% relative humidity with 16:8 h day-night regime at 26 ± 1°C for 15 days. During seedlings' growth, seedlings were sprayed with water whenever required. Uniformed sized seedlings were used to analyze the impact of Si and SiNp on various physiological and biochemical parameters of maize cultivar and hybrid under As^V stress.

SiNp, Si, and As^V Treatments

Uniform sized seedlings gently up rooted from sand and their roots were washed in tap water. Thereafter, seedlings were acclimatized in half strength Hoagland's nutrient solution for 24 h. After this, As^V, Si, and SiNp treatments were given. Sodium arsenate (Na_2HAsO_4. $7H_2O$) was used as a source of As^V while sodium silicate (Na_2SiO_3) was used as a source of Si and its nanoparticles. The As^V concentrations (25 and 50 μM) which have been used in the present study are environmentally relevant while selection of Si and SiNp dose (10 μM) was based on screening experiments. The treatments include: control (no added As^V, Si, and SiNp), Si (10 μM), SiNp (10 μM), 25 μM As^V, 25 μM As^V+10 μM Si, 25 μM As^V+10 μM SiNp, 50 μM As^V, 50 μM As^V +10 μM Si, and 50 μM As^V+10 μM SiNp. In case of As^V+ Si and As^V + SiNp treatments, seedlings were pre-treated with Si and SiNp for 24 h and then they were given As^V treatments. Just after As^V and Si and SiNp treatments, seedlings were further grown in a growth chamber for 7 days under 350 μmol photons m^{-2} s^{-1} of PAR and 86–90% relative humidity with 16:8 h day-night regime at 26 ± 1°C. During this growth period, medium of various treatments was changed twice and aerated daily to avoid root anoxia. After 7 days of As^V, Si, and SiNp treatments, seedlings were harvested and various parameters were analyzed immediately.

Estimation of Growth and Photosynthetic Pigments

Growth was measured in terms of root and shoots length and plant fresh weight. Seedlings were selected randomly from control and treated samples, divided into root and shoot and then their fresh weight and length were determined.

For the estimation of photosynthetic pigments, 20 mg fresh leaves from control and treated seedlings were crushed in 5 ml of 80% acetone and pigments were extracted and centrifuged. The absorbance of pigment extract was read at 663, 646, and 470 nm. The amount of chlorophyll *a*, chlorophyll *b,* and carotenoids was calculated following the method of Lichtenthaler (1987).

Estimation of as Content

For the determination of As content, dried root and shoot samples (50 mg) from control and treated seedlings were digested in mixed acid (HNO_3:$HClO_4$; 85:15, v/v) until transparent solution was obtained. The volume of digested sample was maintained up to 30 mL with double distilled water. The content of As in digested samples was estimated by atomic absorption spectrometer (iCE3000 Series, model-3500 AAS, Thermo scientific, UK), fitted with specific lamp of particular metal using appropriate drift blank.

Chlorophyll a Fluorescence Measurements

For the assessment of photosynthetic performance, chlorophyll *a* fluorescence measurements were taken in the dark adapted (30 min) leaves of control and treated seedlings using hand held leaf fluorometer (FluorPen FP 100, Photon System Instrument, Czech Republic). The following fluorescence parameters: variable/maximum fluorescence ratio (F_v/F_m), photochemical quenching (qP) and non-photochemical quenching (NPQ) were measured. Measurements were taken in second leaf of six different plants of each treatment (Strasser et al., 2000).

Estimation of Superoxide Radical and Hydrogen Peroxide

Superoxide radicals (SOR; $O_2^{\bullet-}$) in control and treated seedlings were determined following the method of Elstner and Heupel (1976). This assay is based on formation of NO_2^- from hydroxylamine in the presence of $O_2^{\bullet-}$. Fresh leaves (50 mg) were crushed in 65 mM potassium phosphate buffer (pH 7.8) and centrifuged at 10,000 g for 10 min at 4°C. The reaction mixture consisted of 65 mM potassium phosphate buffer (pH 7.8), 10 mM hydroxylamine hydrochloride and leaf extract and then incubated for 20 min at 25°C. After this, 17 mM sulfanilamide and 7 mM naphthylethylene diamine dihydrochloride were mixed to the incubated reaction mixture. After 15 min of reaction, diethyl ether was mixed to the same reaction mixture gently and centrifuged at 2000 g for 5 min. The absorbance of the colored aqueous phase was recorded at 530 nm. A standard curve was prepared with $NaNO_2$ and used to calculate the production of $O_2^{\bullet-}$. The production of NO_2^- is equivalent to generation of $O_2^{\bullet-}$.

For the estimation of H_2O_2, fresh leaf samples (50 mg) from control and treated seedlings were crushed in 0.1% (w/v) trichloroacetic acid (Velikova et al., 2000). The reaction mixture (2 ml) contained tissue extract (0.5 ml), 10 mM potassium phosphate buffer (pH 7.0), and 1 M KI solution. Absorbance of reaction mixture was recorded at 390 nm to read increase in turbidity. Hydrogen peroxide concentration was calculated by using a standard curve prepared with H_2O_2.

Estimation of Lipid Peroxidation

Lipid peroxidation as malondialdehyde (MDA) content was estimated according to the method of Heath and Packer (1968). Fresh leaves (50 mg) from control and treated seedlings were crushed in 5% (w/v) TCA and centrifuged at 10,000 g for 10 min at 4°C. Reaction mixture contained thiobarbituric acid (0.5%)-TCA (20%) solution and tissue extract was boiled for 20 min in

water bath. After cooling, the absorbance of reaction mixture (TBA-MDA) was recorded at 532 and 600 nm. The values of non-specific absorption recorded at 600 nm were subtracted from the values recorded at 532 nm. The content of MDA was determined by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Estimation of SOD, APX, GR, and DHAR Activities

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured according to the method of Giannopolitis and Reis (1977). Fresh leaves (50 mg) from control and treated seedlings were homogenized in EDTA-phosphate buffer (50 mM; pH 7.8) and centrifuged at 15,000 g for 20 min at 4°C and supernatant was used as enzyme extract. Reaction mixture (3 ml) contained 50 mM potassium phosphate buffer (pH 7.8), 1.3 μM riboflavin, 0.1 mM EDTA, 13 mM methionine, 63 μM nitroblue tetrazolium (NBT), 0.05 M sodium carbonate (pH 10.2), and enzyme extract (0.1 ml). The reaction mixtures were illuminated for 20 min under white light intensity of 100 μmol photons m^{-2} s^{-1}. The photoreduction of NBT (formation of purple formazone) was recorded spectrophotometrically at 560 nm and compared with blank samples having no enzyme extract. One unit (U) of SOD activity is defined as the amount of enzyme required to cause 50% inhibition in reduction of NBT.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined according to the method of Nakano and Asada (1981). Fresh leaves (50 mg) from control and treated seedlings were homogenized in 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, centrifuged at 15,000 g for 20 min at 4°C and supernatant was used as enzyme. Reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H$_2$O$_2$, and enzyme extract. The decrease in absorbance due to H$_2$O$_2$ decomposition was measured at 290 nm. The enzyme activity was calculated by using an extinction coefficient of 2.8 mM^{-1}cm^{-1}. One unit (U) of enzyme activity is defined as 1 nmol ascorbate oxidized min^{-1}.

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed according to the method of Schaedle and Bassham (1977). The decrease in absorbance due to NADPH oxidation was read at 340 nm, and GR activity was calculated using an extinction coefficient of 6.2 mM^{-1} cm^{-1}. One unit (U) of enzyme activity is defined as 1 nmol NADPH oxidized min^{-1}.

Dehydroascorbate reductase (DHAR; EC 1.8.5.1) activity was assayed by the method of Nakano and Asada (1981). An increase in absorbance due reduced ascorbate formation was read at 265 nm, and DHAR activity was calculated using an extinction coefficient of 7.0 mM^{-1} cm^{-1}. One unit (U) of enzyme activity is defined as 1 nmol DHA reduced min^{-1}.

Determination of Ascorbate and Glutathione

Total ascorbate (AsA+DHA), reduced ascorbate (AsA), and dehydroascorbate (DHA) were determined by the method of Gossett et al. (1994). This assay is based on the reduction of Fe^{3+} to Fe^{2+} with ascorbic acid in acidic solution followed by formation of red chelate between Fe^{2+} and 2,2-bipyridyl. Ascorbate content was calculated using a standard curve prepared with L-ascorbic acid.

Total (GSH+GSSG), reduced (GSH) and oxidized glutathione (GSSG) contents were estimated by the enzyme recycling method of Brehe and Burch (1976). This method is based on sequential oxidation of GSH by 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) and reduction of GSSG in the presence of NADPH and glutathione reductase (GR; Type III from bakers' yeast; Sigma Chemical Company). The amount of glutathione was determined by using a standard curve prepared with GSH.

Statistical Analysis

Results were statistically analyzed by analysis of variance (ANOVA). Duncan's multiple range test was applied for mean separation for significant differences among treatments at $P <$ 0.05 significance level. The results presented are the means $\pm$ standard error of six replicates ($n = 6$).
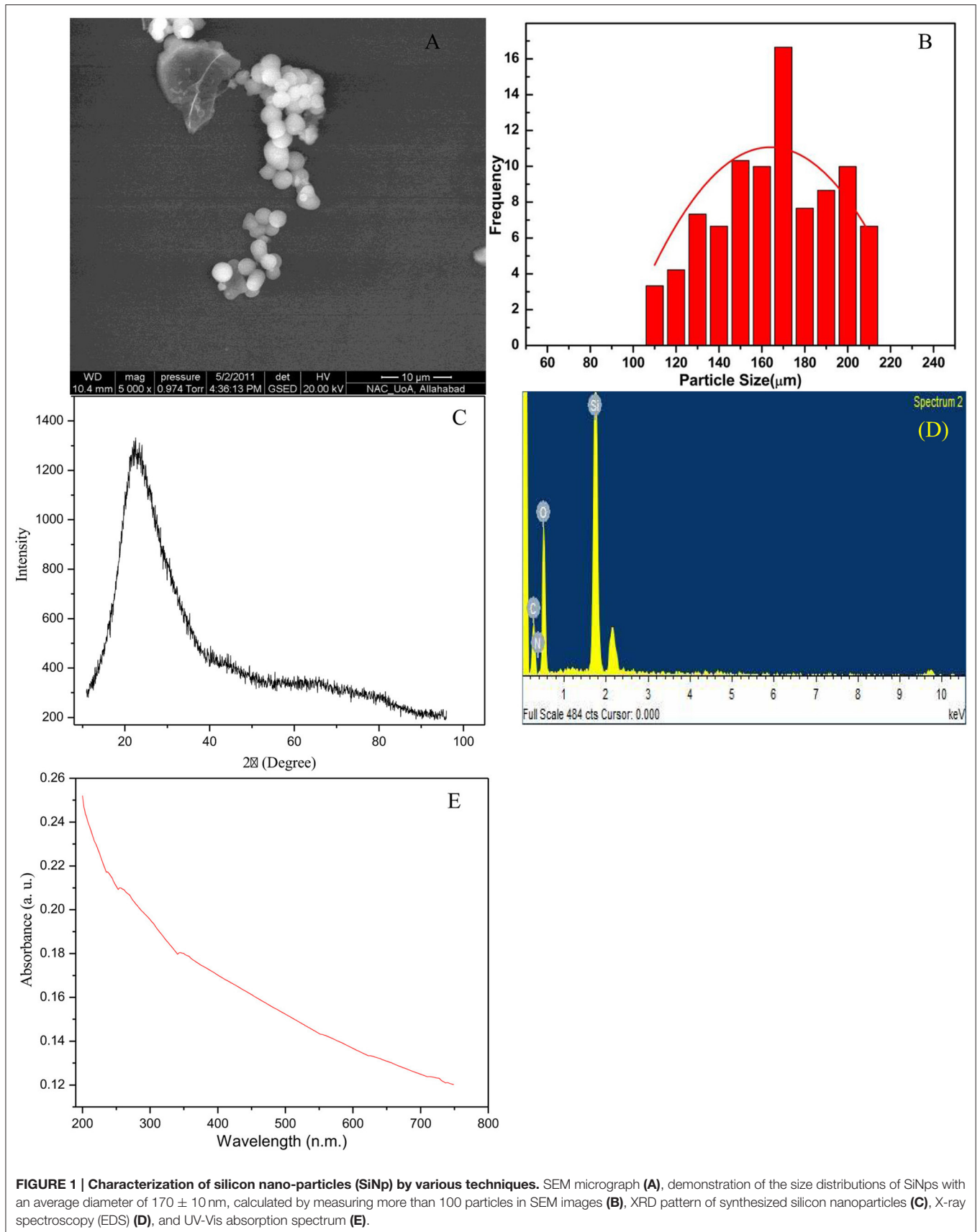
RESULTS

Characterization of SiNp

The morphology of synthesized SiNp was investigated by SEM, which shows almost spherical nanoparticles with size ranges from 150 to 200 nm (**Figure 1A**). The **Figure 1B** demonstrated the size distributions of SiNps with an average diameter of 170 $\pm$ 10 nm, calculated by measuring more than 100 particles in SEM images. The XRD pattern of the synthesized SiNp is shown in **Figure 1C**, which appears as broad band's with expression at $2'' =$ 22.8° representing that synthesized material is amorphous and composed of SiO$_2$ (Sooksaen et al., 2008). Elemental analysis of the sample using energy-dispersive energy-dispersive X-ray spectroscopy (EDS) is shown in **Figure 1D**. The analysis clearly indicates presence of silicon, carbon and oxygen in SiNPs. The UV-Vis spectroscopic analysis of synthesized SiNp was carried out in the range of 320–800 nm and shown in **Figure 1E**. The UV-Vis spectrum of SiNp clearly shows the absorption peak around ∼445 nm (Wilder et al., 1998).

Impact of Si and SiNp on Growth and As Accumulation under As^V Stress

Growth measured in terms of fresh weight was reduced significantly ($P < 0.05$) following As^V treatments in both maize cultivar and hybrid (**Figure 2**). Treatment with 25 and 50 μM of As^V reduced growth by 14 and 29% in maize cultivar while 19 and 34% in hybrid, respectively compared to the control. On the other hand, Si addition along with As^V treatments (25 and 50 μM) alleviated As^V-induced reduction in growth of maize cultivar and hybrid as percentage decline was only 7 and 19% and 8 and 22%, respectively. However, the addition of Si alone enhanced fresh mass of maize cultivar and hybrid by 6 and 7%, respectively over the value of control. Besides this, addition of SiNp along with 25 and 50 μM of As^V also significantly ($P < 0.05$) alleviated As^V-induced reduction in growth of maize cultivar and hybrid as reductions were only 4 and 12% and 5 and 16%, respectively. Thus, data indicate that SiNp were more effective in reducing ($P < 0.05$) As^V toxicity than Si. Furthermore, the addition of

FIGURE 1 | Characterization of silicon nano-particles (SiNp) by various techniques. SEM micrograph **(A)**, demonstration of the size distributions of SiNps with an average diameter of 170 ± 10 nm, calculated by measuring more than 100 particles in SEM images **(B)**, XRD pattern of synthesized silicon nanoparticles **(C)**, X-ray spectroscopy (EDS) **(D)**, and UV-Vis absorption spectrum **(E)**.
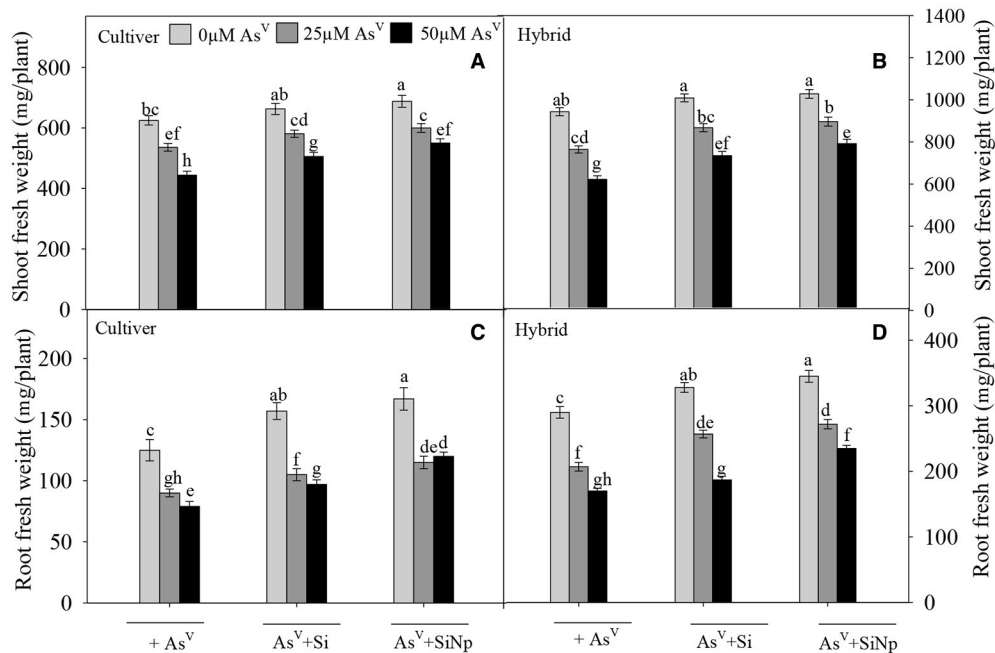
FIGURE 2 | Effects of Si and silicon nanoparticles (SiNp) on growth (fresh weight of roots and shoots), of maize cultivar (A,C) and hybrid exposed (B,D) to As^V stress. Data are means ± standard error of three independent experiments with two replicates in each experiment ($n = 6$). Bars followed by different letter(s) show significant difference at $P < 0.05$ significance level according to the Duncan's multiple range test.

SiNp alone significantly increased fresh mass of maize cultivar and hybrid by 10 and 9%, respectively over the respective value of control (**Figure 2**).

Similar to the As^V-induced reduction in growth, the accumulation of As was significantly ($P < 0.05$) higher in As^V-stressed maize cultivar and hybrid (**Figure 3**). Maize cultivar and hybrid seedlings grown under 25 and 50 μM of As^V treatments accumulated about 124 and 272 μg As g^{-1} dry weight and 156 and 312 μg As g^{-1} dry weight, respectively. However, with the addition of Si (25 μM As^V + Si and 50 μM As^V + Si) metal accumulation significantly reduced and it was only 110 and 226 μg As g^{-1} dry weight and 136 and 246 μg As g^{-1} dry weight in maize cultivar and hybrid seedlings, respectively (**Figure 3**). Similarly, the addition of SiNp with 25 and 50 μM of As^V also significantly ($P < 0.05$) reduced the accumulation of As in maize cultivar and hybrid as accumulation was only 104 and 212 μg As g^{-1} dry weight and 126 and 234 μg As g^{-1} dry weight, respectively.

Impact of Si and SiNp on Photosynthetic Pigments Under As^v Stress

Data pertaining to the photosynthetic pigments i.e., total chlorophyll, carotenoids, and protein are presented in **Table 1**. The results reveal that As^V (25 and 50 μM) treatments significantly ($P < 0.05$) declined chlorophyll contents of maize cultivar by 8 and 16% while in hybrid it was declined by 11 and 19%, respectively. Similar to the results of chlorophyll content, carotenoids, and total protein were also significantly decreased in maize cultivar and hybrid. Under 25 and 50 μM of As^V treatments, carotenoids and protein were decreased by 6 and

13% and 16 and 24% in cultivar and by 9 and 16% and 18 and 27%, respectively in hybrid (**Table 1**). On the other hand, the addition of Si and SiNp in nutrient medium alleviated ($P < 0.05$) As^V-induced decline in total chlorophyll, carotenoids and total protein in comparison to the As^V treatments alone (**Table 1**).

Impact of Si and SiNp on Photosynthetic Performance under As^v Stress

The results pertaining to changes in chlorophyll a fluorescence parameters such as F_v/F_m, qP, and NPQ are shown in **Figures 4A–F**. The results reveal that As^V (25 and 50 μM) exposure decreased F_v/F_m and qP by 11 and 19% and 14 and 21% in maize cultivar while by 14 and 22% and 16 and 24% in hybrid, respectively as compared to the control (**Figures 4A–D**). In contrast to this, treatment of plants with As^V at 25 and 50 μM increased NPQ by 18 and 25% in maize cultivar and by 21 and 28%, respectively in hybrid (**Figures 4E,F**). On the other hand, Si and SiNP treatments alone did not bring significant ($P < 0.05$) changes in chlorophyll a fluorescence parameters. Furthermore, the addition of Si and SiNp with As^V significantly ($P < 0.05$) ameliorated As^V-induced negative impacts on F_v/F_m, qP, and NPQ in comparison to the As^V treatments alone (**Figure 4**).

Impact of Si and SiNp on Reactive Oxygen Species and Lipid Peroxidation under As^v Stress

It has been reported in earlier studies that As^V stress was associated with the production of ROS, which ultimately cause
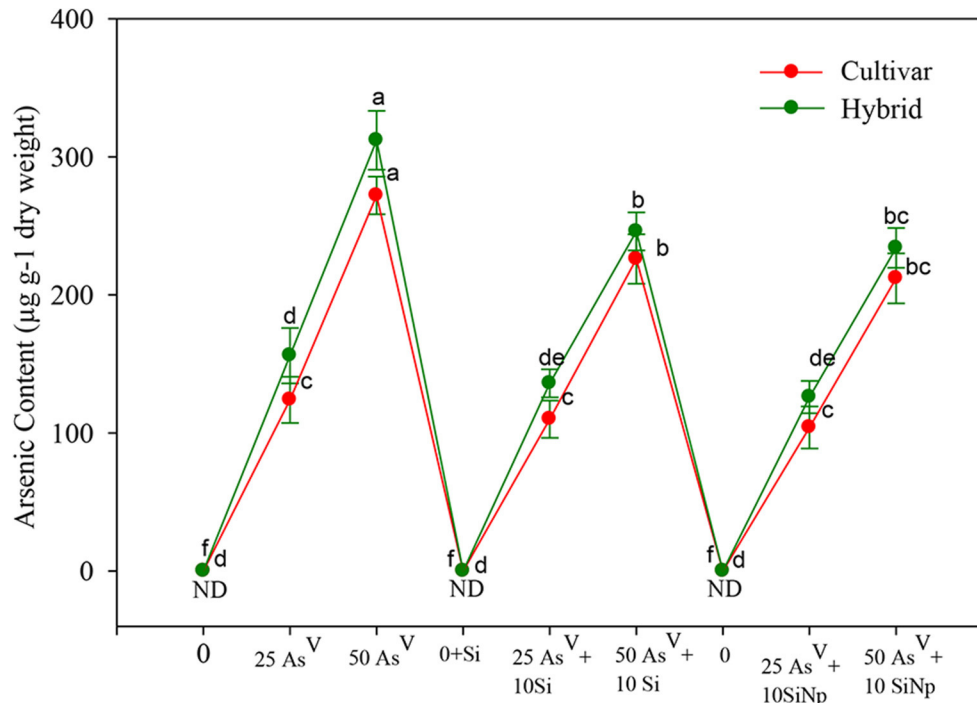
FIGURE 3 | Effects of Si and silicon nanoparticles (SiNp) on the accumulation of As in maize cultivar and hybrid exposed to As^V stress. Data are means ± standard error of three independent experiments with two replicates in each experiment ($n = 6$). Bars followed by different letter(s) show significant difference at $P < 0.05$ significance level according to the Duncan's multiple range test.

damage to macromolecules. Therefore, in order to investigate oxidative stress in both maize cultivar and hybrid, we examined levels of SOR and H_2O_2 in plants whose growth was suppressed substantially by As^V (25 and 50 μM). The results show that 25 and 50 μM of As^V enhanced SOR and H_2O_2 content by 18 and 27% and 24 and 32% in maize cultivar and by 23 and 32% and 28 and 37%, respectively in hybrid (**Figures 5A–D**). Contrary to As^V, Si, and SiNp treatments alone did not significantly ($P < 0.05$) change SOR and H_2O_2 contents in maize cultivar and hybrid (**Figures 5A–D**). Further, when Si and SiNp was added jointly with As^V, they significantly ($P < 0.05$) reduced As^V-mediated enhancement in SOR and H_2O_2. Under 25 μM As^V +Si, 50 μM As^V +Si, 25 μM As^V +SiNp, and 50 μM As^V +SiNp combinations, SOR declined up to 11 and 17%, 6 and 10% in cultivar and 15 and 20% and 7 and 11% in hybrid while H_2O_2 decreased up to 14 and 21% and 6 and 11% in cultivar and 18 and 24% and 9 and 14%, respectively in hybrid (**Figures 5A–D**).

Lipid peroxidation is a marker to measure the effect of abiotic stresses such as As^V stress on lipids. Accordingly, 25 and 50 μM of As^V also enhanced lipid peroxidation (MDA content) by 28 and 34% in maize cultivar and by 32 and 38%, respectively in hybrid (**Figures 5E,F**). On the other hand, the addition of Si and SiNp alone did not influence the production of MDA in maize cultivar and hybrid. Besides this, they significantly ($P < 0.05$) lowered As^V-induced lipid peroxidation in maize cultivar and hybrid as MDA content declined up

to 18 and 10% in cultivar and 21 and 15% in hybrid under 25 μM As^V +Si and 25 μM As^V +SiNp combinations and 24 and 14% in cultivar and 26 and 18% in hybrid, respectively, under 50 μM As^V +Si and 50 μM As^V + SiNp combination (**Figures 5E,F**).

Impact of Si and SiNp on the Activities of Enzymatic Antioxidants Under As^V Stress

In order to investigate the role of Si and SiNp in the regulation of antioxidant enzymes and the relationship between ROS and the cellular defense system, the activities of SOD, APX, GR, and DHAR were examined in As^V-stressed maize cultivar and hybrid with and without Si and SiNp supplementation. The results related to the activities of enzymatic antioxidants are shown in **Table 2**. The results show that As^V treatments (25 and 50 μM) significantly ($P < 0.05$) increased SOD activity in maize cultivar and hybrid. In contrast to SOD activity, 25 and 50 μM of As^V significantly ($P < 0.05$) inhibited the activities of APX (24 and 31% and 31 and 38%), GR (28 and 34% and 32 and 39%), and DHAR (34 and 39% and 38 and 44%) in maize cultivar and hybrid, respectively as compared to the value of control (**Table 2**). In contrast to this, Si and SiNp alone significantly ($P < 0.05$) increased the activity of all enzymes studied in maize cultivar and hybrid. Moreover, the addition of Si and SiNp with As^V significantly ($P < 0.05$) alleviated As^V-mediated inhibitions in APX, GR, and DHAR (**Table 2**).

TABLE 1 | Effects of Si and silicon nanoparticles (SiNp) on total chlorophyll (total Chl), carotenoids (Car) and protein content of maize cultivar and hybrid exposed to As^V stress.

	Total Chl (μg g^{-1} fresh weight)		Car (μg g^{-1} fresh weight)		Protein (mg g^{-1} fresh weight)	
	Cultiver	Hybrid	Cultiver	Hybrid	Cultiver	Hybrid
As^V (μM)						
0	1370 ± 23.8ab	1480 ± 24.1c	446 ± 7.1ab	481 ± 7.3b	12.4 ± 0.20c	12.7 ± 0.20ab
25	1260 ± 19.3d	1317 ± 23.2ef	419 ± 5.7bc	438 ± 5.1d	10.4 ± 0.10fg	10.4 ± 0.13g
50	1151 ± 17.9e	1199 ± 20.3g	288 ± 4.6e	404 ± 4.8e	9.4 ± 0.10h	9.3 ± 0.14h
As^V (μM) + Si						
0	1439 ± 26.9a	1569 ± 25.7ab	464 ± 9.1a	505 ± 7.3a	12.8 ± 0.12ab	13.2 ± 0.16a
25	1329 ± 22.1bc	1406 ± 26.1cd	432 ± 8.0ab	457 ± 5.7bc	11.4 ± 0.18e	11.4 ± 0.13de
50	1247 ± 20.2d	1332 ± 18.9e	410 ± 6.1cd	338 ± 4.2f	10.5 ± 0.18f	10.7 ± 0.15f
As^V (μM) + SiNp						
0	1466 ± 27.3a	1613 ± 28.7a	473 ± 5.3a	515 ± 7.1a	13.0 ± 0.17ab	13.3 ± 0.22a
25	1356 ± 18.7ab	1450 ± 23.3c	437 ± 4.3ab	471 ± 5.6b	12.0 ± 0.11d	12.1 ± 0.14c
50	1315 ± 12.5bc	1406 ± 21.6cd	424 ± 5.3bc	462 ± 4.8bc	13.6 ± 0.13a	11.7 ± 0.12cd

Data are means ± standard error of three independent experiments with two replicates in each experiment (n = 6). Different letter(s) within same column show significant difference at P < 0.05 significance level according to the Duncan's multiple range test.

TABLE 2 | Effects of Si and silicon nanoparticles (SiNp) on the activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) in maize cultivar and hybrid exposed to As^V stress.
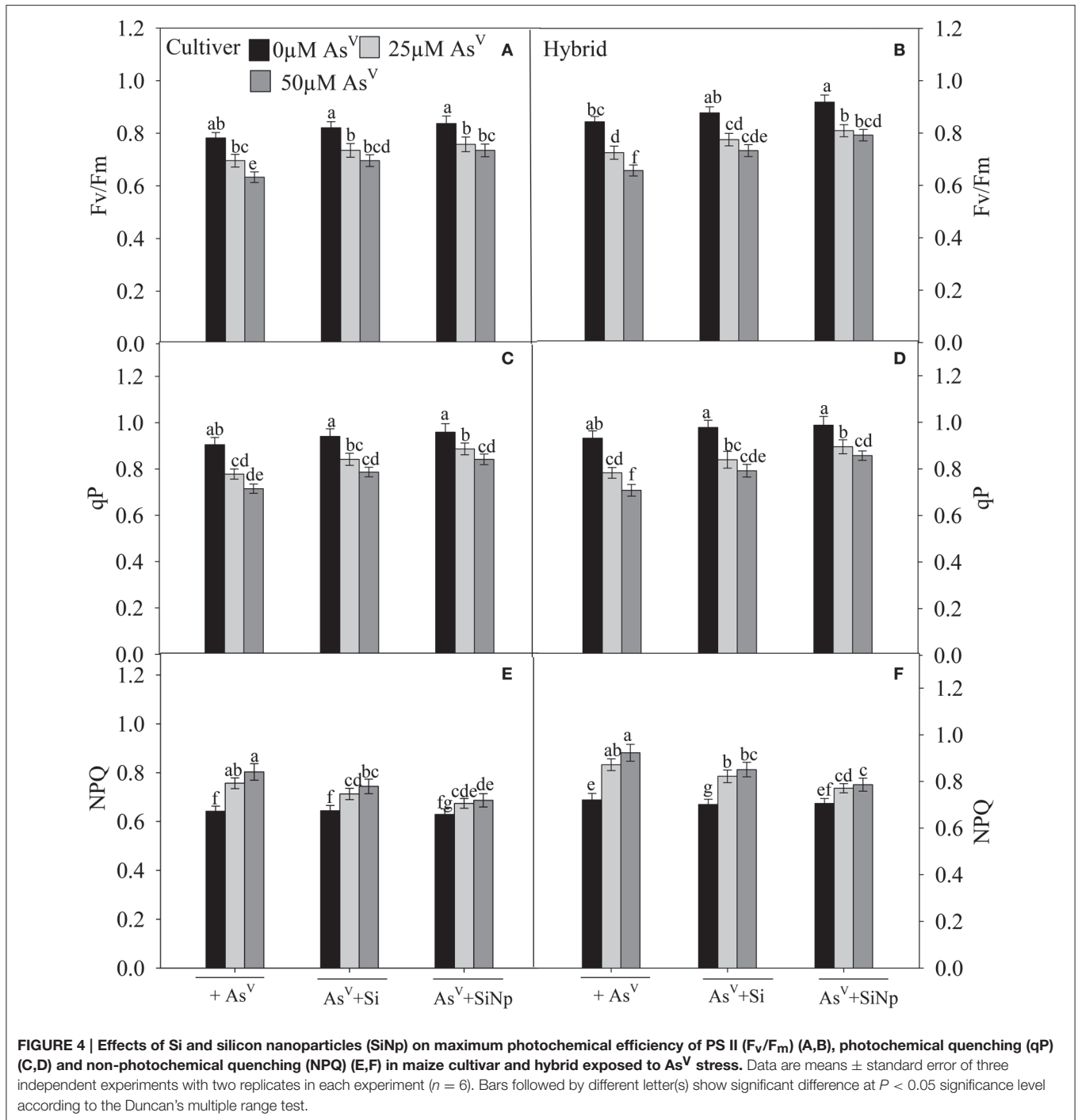
Treatments	SOD Units mg^{-1} protein		APX Units mg^{-1} protein		GR Units mg^{-1} protein		DHAR Units mg^{-1} protein	
	Cultiver	Hybrid	Cultiver	Hybrid	Cultiver	Hybrid	Cultiver	Hybrid
As^V (μM)								
0	44.5 ± 1.8g	32.7 ± 1.7gh	24,342 ± 197.6c	26,572 ± 166c	86.7 ± 4.3c	67.8 ± 1.4c	887.6 ± 10.4c	643.7 ± 10.6ab
25	58.7 ± 1.3b	46.4 ± 1.1b	18,500 ± 184gh	18,334 ± 191h	62.4 ± 2.5gh	46.1 ± 1.3gh	585.8 ± 11.6h	399.1 ± 8.9fg
50	83.3 ± 2.2a	51.0 ± 1.6a	16,796 ± 175i	16,474 ± 183i	57.2 ± 1.6i	41.3 ± 1.2i	541.4 ± 9.3i	360.5 ± 5.4h
As^V (μM) + Si								
0	46.5 ± 1.3ef	33.7 ± 1.7g	26,046 ± 247ab	28,963 ± 249b	91.9 ± 2.6ab	73.2 ± 1.3ab	976.4 ± 7.3ab	682.3 ± 9.5a
25	54.3 ± 1.6cd	43.2 ± 1.5cd	20,447 ± 239f	21,257 ± 242f	69.4 ± 1.7f	51.5 ± 1.4f	692.3 ± 12.5ef	463.5 ± 6.8de
50	58.7 ± 1.4bc	45.1 ± 1.1bc	18,743 ± 233g	19,132 ± 2 28g	63.3 ± 1.3g	47.5 ± 1.1g	630.2 ± 9.7g	418.4 ± 5.7f
As^V (μM) + SiNp								
0	45.4 ± 1.9ef	33.1 ± 1.7g	27,019 ± 191a	30,026 ± 233a	94.5 ± 2.4a	75.9 ± 1.2a	1011.9 ± 14.8a	695.2 ± 8.9a
25	51.6 ± 1.6de	39.2 ± 1.2ef	22,394 ± 198d	23,383 ± 242d	77.2 ± 3.2d	56.9 ± 1.3d	763.3 ± 9.9d	527.8 ± 7.4c
50	53.4 ± 1.9cde	41.5 ± 1.9e	21,421 ± 189e	22,055 ± 237e	72.8 ± 2.8de	54.2 ± 1.6de	718.9 ± 8.7e	482.8 ± 4.4d

Data are means ± standard error of three independent experiments with two replicates in each experiment (n = 6). Different letter(s) within same column show significant difference at P < 0.05 significance level according to the Duncan's multiple range test.

Impact of Si and SiNp on Ascorbate and Glutathione Levels under As^V Stress

The results related to amounts of ascorbate and glutathione and their redox status are shown in **Tables 3, 4**. Under As^V (25 and 50 μM) treatments, though total ascorbate increased by 14 and 25% in cultivar and 13 and 21% in hybrid and total glutathione content enhanced by 17 and 29% in cultivar and 12 and 24%, respectively in hybrid, however, their oxidized forms increased ($p < 0.05$) by 154 and 263% in cultivar and 168 and 199% in hybrid and 166 and 371% in cultivar and 175 and 350%, respectively in hybrid (**Tables 3, 4**). This results into a steep decline in reduced ascorbate/dehydroascorbate
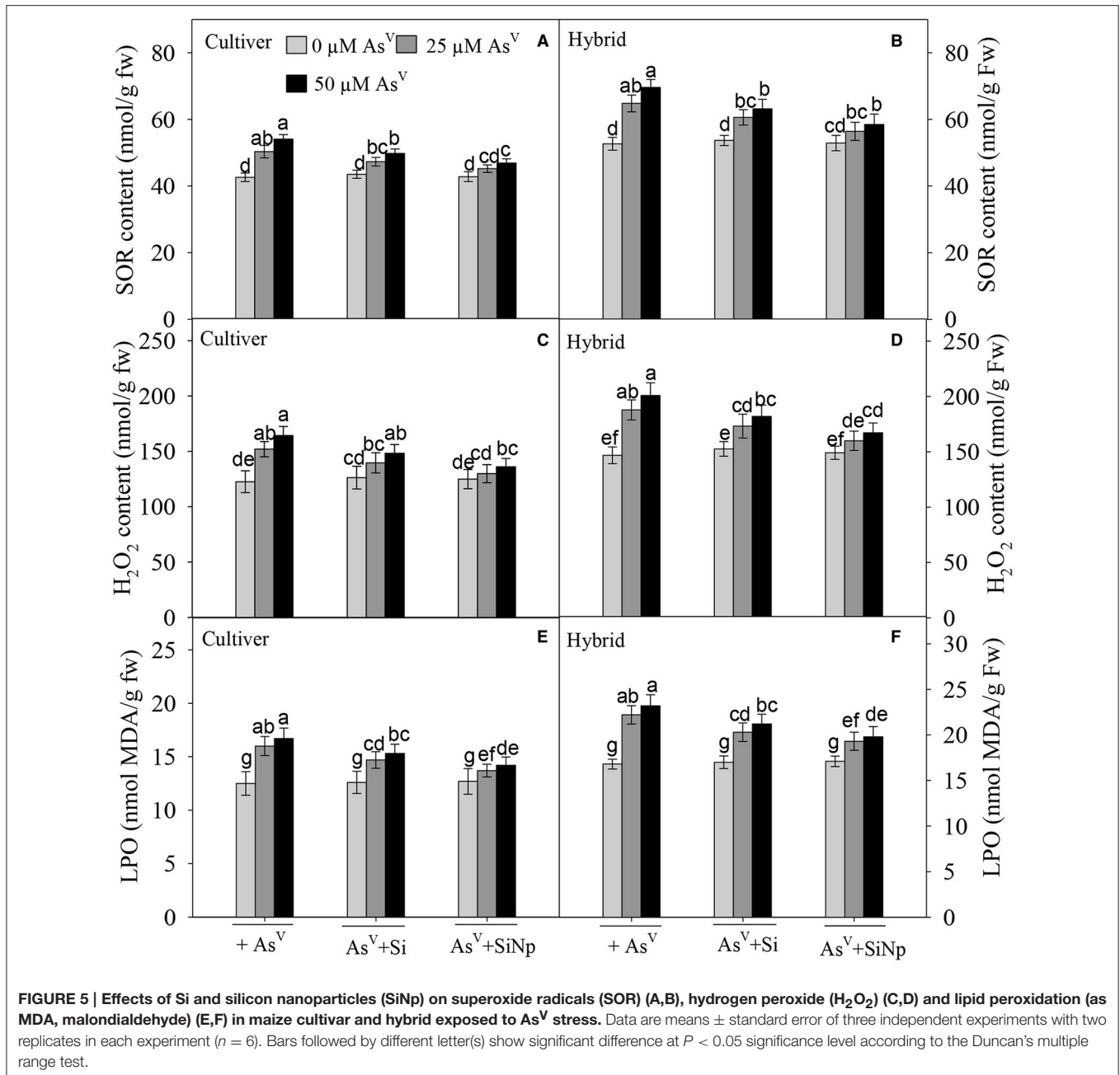
and reduced glutathione/oxidized glutathione ratios as they were only 6.8 and 4.2 in cultivar and 4.6 and 3.8 in hybrid and 5.4 and 3.4 in cultivar and 4.6 and 2.8 in hybrid, respectively following As^V (25 and 50 μM) exposure (**Tables 3, 4**). On the other hand, Si and SiNp alone enhanced levels of reduced ascorbate and glutathione as well as their reduced/oxidized ratios in maize cultivar and hybrid (**Tables 3, 4**). Furthermore, application of Si and SiNp together with As^V (25 and 50 μM) attenuated As^V-induced diminish in levels of reduced ascorbate and glutathione as well as their reduced/oxidized ratios in both maize cultivar and hybrid seedlings (**Tables 3, 4**).

FIGURE 4 | Effects of Si and silicon nanoparticles (SiNp) on maximum photochemical efficiency of PS II (F_v/F_m) (A,B), photochemical quenching (qP) (C,D) and non-photochemical quenching (NPQ) (E,F) in maize cultivar and hybrid exposed to As^V stress. Data are means ± standard error of three independent experiments with two replicates in each experiment ($n = 6$). Bars followed by different letter(s) show significant difference at $P < 0.05$ significance level according to the Duncan's multiple range test.

DISCUSSION

Similar to previous studies, the results of the present study revealed that As^V significantly ($P < 0.05$) reduced growth of maize cultivar and hybrid seedlings, which has occurred due to the enhancement in the accumulation of As in seedlings (**Figures 2**, **3**). This kind of As^V toxicity response is well-documented (Li et al., 2006; Singh R. et al., 2015; Singh et al., 2015a,b). Additionally, the results revealed that As^V negatively affects pigments, total protein, photosynthesis, and the status of antioxidants (SOD, APX, GR, and DHAR, reduced ascorbate and glutathione), and significantly increased level of oxidative stress markers like SOR, H_2O_2, and damage to lipids (**Figures 4**, **5**; **Tables 1–4**). To explain As^V-induced toxicity in plants, numerous hypotheses, and mechanisms have been proposed but in the present investigation As^V-mediated toxicity

FIGURE 5 | Effects of Si and silicon nanoparticles (SiNp) on superoxide radicals (SOR) (A,B), hydrogen peroxide (H$_2$O$_2$) (C,D) and lipid peroxidation (as MDA, malondialdehyde) (E,F) in maize cultivar and hybrid exposed to As^V stress. Data are means ± standard error of three independent experiments with two replicates in each experiment ($n = 6$). Bars followed by different letter(s) show significant difference at $P < 0.05$ significance level according to the Duncan's multiple range test.

to maize cultivar and hybrid seedlings may be associated with its harmful impacts on pigments, photosynthesis and macromolecules such as lipids due to higher accumulation of As.

On contrary, the addition of Si and SiNp significantly ($P < 0.05$) reduced toxicity level of As^V in maize cultivar and hybrid by ameliorating As^V-induced negative effects on growth, pigments, photosynthetic process and protein, which was accompanied by a decreased level of As accumulation (**Figure 3**; **Table 1**). Si-mediated alleviation of As^V toxicity complies with mechanisms, which were reported in earlier studies (Horiguchi and Morita, 1987; Tripathi et al., 2015; Wang et al., 2015). Regarding SiNp-mediated alleviation of As^V toxicity, somewhat similar

mechanisms have been observed as reported for Si. For instance, lowering in the accumulation of As and oxidative damage and enhancement in antioxidant defense system (**Figures 3**, **5**; **Tables 2–4**). In recent years, nanotechnology has been established as a powerful technology and shown a greater potential as an environment cleaner technology including alleviation of toxicities of various heavy metals (Dickinson and Scott, 2010). Further various studies also demonstrated metal nanoparticles mediated alleviation of metal toxicity in plants. (Dubchak et al., 2010; Wang et al., 2012). However, the role of nanoparticles and their mechanisms of action in plant growth and development is not yet well known. But their high surface

TABLE 3 | Effects of Si and silicon nanoparticles (SiNp) on total ascorbate (AsA+DHA), reduced ascorbate (AsA), dehydroascorbate (DHA), and reduced ascorbate/dehydroascorbate ratio (AsA/DHA) in maize cultivar and hybrid exposed to As^V stress.

Treatments	Total ascorbate (nmol g^{-1} fresh weight)							
	Cultiver				Hybrid			
As^V (μM)	AsA + DHA	AsA	DHA	AsA/DHA	AsA + DHA	AsA	DHA	AsA/DHA
0	644.5 ± 12.9g	602.1 ± 13.6f	42.4 ± 1.4f	14.2 ± 0.090c	567.7 ± 17.6g	525.0 ± 6.9gh	42.7 ± 2.7gh	12.3 ± 0.10c
25	734.7 ± 14.6de	640.5 ± 16.3cd	94.2 ± 2.7b	6.8 ± 0.70h	641.5 ± 12.9de	526.9 ± 9.5gh	114.6 ± 3.7b	4.6 ± 0.080f
50	805.6 ± 15.7bc	650.7 ± 12.7de	154.9 ± 3.9a	4.2 ± 0.60i	686.9 ± 13.4bc	543.8 ± 8.3g	143.1 ± 2.1a	3.8 ± 0.070g
As^V (μM) + Si								
0	696.1 ± 11.8f	654.2 ± 18.1cd	41.9 ± 1.7fg	15.6 ± 0.10ab	601.8 ± 11.3f	562.2 ± 7.7f	39.6 ± 1.3hi	14.2 ± 0.090b
25	766.9 ± 19.4cd	683.5 ± 17.3bc	83.4 ± 2.2cd	8.2 ± 0.060f	664.2 ± 8.7cd	584.2 ± 9.3cd	80.0 ± 2.1d	7.3 ± 0.080e
50	818.5 ± 20.1ab	724.4 ± 14.6ab	94.1 ± 3.3c	7.7 ± 0.080fg	681.2 ± 11.2bc	586.6 ± 13.1cd	94.6 ± 3.2c	6.2 ± 0.067g
As^V μM) + SiNp								
0	741.2 ± 15.4cd	697.8 ± 15.7bc	43.4 ± 1.8f	16.1 ± 0.10a	755.0 ± 10.1bc	707.2 ± 6.9a	47.8 ± 1.7g	14.8 ± 0.091a
25	799.2 ± 14.6bc	722.3 ± 16.9ab	76.9 ± 2.4de	9.4 ± 0.080d	686.9 ± 7.6b	613.8 ± 9.8bc	73.1 ± 1.6f	8.4 ± 0.051d
50	850.7 ± 13.9a	762.1 ± 13.7a	88.6 ± 2.7cd	8.6 ± 0.070e	720.9 ± 9.8a	635.1 ± 11b	85.8 ± 2.7de	7.4 ± 0.042e

Data are means ± standard error of three independent experiments with two replicates in each experiment (n = 6). Different letter(s) within same column show significant difference at P < 0.05 significance level according to the Duncan's multiple range test.

TABLE 4 | Effects of Si and silicon nanoparticles (SiNp) on total glutathione (GSH+GSSG), reduced glutathione (GSH), oxidized glutathione (GSSG) and reduced glutathione/oxidized glutathione ratio (GSH/GSSG) in maize cultivar and hybrid exposed to As^V stress.

Treatments	Total glutathione (nmol g^{-1} fresh weight)							
	Cultiver				Hybrid			
As^V (μM)	GSH + GSSG	GSH	GSSG	GSH/GSSG	GSH + GSSG	GSH	GSSG	GSH/GSSG
0	843.7 ± 19.2h	785.5 ± 8.2e	58.2 ± 6.1gh	13.5 ± 0.18bc	785.4 ± 12.7g	728.5 ± 14.8d	56.9 ± 5.1fg	12.8 ± 0.10bc
25	987.1 ± 17.9ef	832.1 ± 7.7e	155.0 ± 11.9c	5.4 ± 0.10g	879.6 ± 16.4ef	722.5 ± 17.5d	157.1 ± 7.7bc	4.6 ± 0.11gh
50	1088.4 ± 23.4bc	841.0 ± 9.3f	274.4 ± 14.3a	3.4 ± 0.11i	973.9 ± 25.3bc	717.6 ± 17.2de	256.3 ± 8.6a	2.8 ± 0.9i
As^V (μM) + Si								
0	936.5 ± 11.9g	873.6 ± 8.9cd	62.9 ± 7.7g	13.9 ± 0.17ab	856.1 ± 17.3ef	796.2 ± 16.2bc	59.9 ± 5.6f	13.3 ± 0.18ab
25	1046.2 ± 18.4de	910.3 ± 9.1cd	135.9 ± 16.7de	6.7 ± 0.13ef	926.8 ± 18.7cd	796.3 ± 17.8bc	130.5 ± 7.6d	6.1 ± 0.14e
50	1113.7 ± 24.3ab	934.1 ± 7.9bc	179.6 ± 12.6b	5.2 ± 0.10gh	997.4 ± 23.4ab	822.4 ± 20.1ab	175.0 ± 9.2b	4.7 ± 0.12g
As^V(μM)+ SiNp								
0	995.6 ± 19.8de	930.9 ± 8.6bc	64.7 ± 7.9g	14.4 ± 0.20a	895.3 ± 16.5g	834.8 ± 16.7ab	60.5 ± 3.1f	13.8 ± 10a
25	1079.9 ± 23.3bc	957.2 ± 9.7b	122.7 ± 9.8ef	7.8 ± 0.11d	950.3 ± 22.6ef	828.5 ± 18.3ab	121.8 ± 8.1de	6.8 ± 0.11d
50	1155.9 ± 27.2a	1007.7 ± 12.3a	148.2 ± 11.2d	6.8 ± 0.10e	1028.9 ± 29.3bc	865.6 ± 19.1a	163.3 ± 7.8bc	5.3 ± 0.09f

Data are means ± standard error of three independent experiments with two replicates in each experiment (n = 6). Different letter(s) within same column show significant difference at P < 0.05 significance level according to the Duncan's multiple range test.

to volume ratio that increases their reactivity and possible biochemical activity of nanoparticles might play a role in their action (Adrees et al., 2015). Considering greater potential of SiNp in alleviating AsV stress than Si, it is reported that SiNp have greater availability to seedlings than Si (unpublished data), thus exerting greater ameliorative effect in maize cultivar and hybrid.

At the present, several mechanisms are known by which Si alleviates abiotic stresses such as heavy metal toxicities, salinity, drought, chilling and freezing stresses. However, several other routes of Si action in mitigation of metal toxicity remain to be investigated. Some mechanisms, which are frequently discussed in the literature regarding Si-mediated alleviation of heavy metal stresses comprise: (1) stimulation of antioxidant systems, (2)

complexation or co-precipitation of toxic metal ions with Si, (3) immobilization of toxic metal ions in growth media, (4) uptake processes, (5) compartmentation of metal ions within plants, and (6) Si may modify the cation binding capacity of the cell wall (Kopittke et al., 2012). The results of the present study revealed that the addition of SiNp reduced As accumulation in maize cultivar and hybrid seedlings, which resulted into better photosynthetic performance, decreased levels of oxidative stress markers (ROS, H$_2$O$_2$, and MDA) and improved antioxidant defense system (**Figures 2–5**; **Tables 1–4**). The reduction in As accumulation may be described on the basis of formation of barrier in root endodermis for As^V as reported in other studies (Feng et al., 2009; Singh et al., 2013). Moreover, the addition of SiNp reduced membrane lipid

peroxidation caused by excess metal and significantly improved cell wall breadth in root epidermis (data not shown), and thus declining As absorption and accumulation. It is reported that nano silica is easily absorbed by plants than inorganic silica (Suriyaprabha et al., 2012), and thus justifying greater protective impact of SiNp than Si under As^V stress in maize seedlings.

Application of Si and SiNp lowered the level of oxidative stress. It is further proven by increased activity of superoxide dismutase (SOD). SOD comprises the first line of defense system against ROS which catalyzes dismutation of superoxide anion into H_2O_2 and O_2 in chloroplast. Significant enhancement of SOD has been reported in an earlier study (Kuniak and Skodowska, 2001). In the present study, the activity of SOD in cultivar and hybrid maize treated with As^V and Si and SiNp together showed enhanced activity, which suggested the important role of SOD in scavenging ROS. However, application of Si and SiNp decreased SOD activity in cultivar and hybrid maize (**Table 2**). These results imply that it might be due to lower accumulation of superoxide anion in cultivar and hybrid maize seedlings. It has been well documented that the ascorbate-glutathione (AsA-GSH cycle) cycle is the main mechanism which scavenges ROS successfully in plants under abiotic and biotic stresses (Schmidt et al., 1999; Liang et al., 2003; Asada, 2006). The activity of APX eliminates H_2O_2. Moreover, GR and DHAR both are principally accountable for providing substrate (reduced glutathione and ascorbate, respectively) for APX during scavenging of H_2O_2. The present study indicated that APX, DHAR, and GR were inhibited by excess As^V, and (**Table 2**) in accordance with the change of their activities in barley and bentgrass (Liang et al., 2003; Mittler et al., 2004; Foyer and Noctor, 2011). However, application of Si and SiNp enhanced their activities under As^V stress demonstrating that rising AsA-GSH cycle components is one of key mechanisms to counteract As^V stress in maize cultivar and hybrid as evidenced from data of oxidative stress (**Table 2**).

Furthermore, ascorbate and glutathione are recognized as the heart of the redox hub. Both antioxidants are abundant and stable with proper redox potentials that act together in several pathways and maintained in a commonly reduced condition (Foyer and Noctor, 2011). Mittler et al. (2004) demonstrated that both antioxidants are the element of an extremely complex and complicated plant antioxidative system. In the present study, oxidative stress caused by As^V in maize seedlings and defense provided by both Si and SiNp may be linked with high levels of reduced ascorbate and glutathione, which are crucial non-enzymatic antioxidant having low molecular weight and act in coordinative way in AsA-GSH cycle for the elimination of H_2O_2 (**Tables 3, 4**). Oxidative stress induced by As^V (25 and $50\,\mu M$) in maize cultivar and hybrid were further correlated with the redox status of ascorbate (AsA+DHA, AsA, and DHA) and glutathione (GSH+GSSG, GSH, and GSSG). It has been shown that ascorbate (AsA) plays an important role in scavenging ROS directly and indirectly (Foyer and Noctor, 2011; Singh et al., 2015a). Furthermore, the protective role of ascorbate (AsA) against oxidative stress has been demonstrated in *Arabidopsis* ascorbate-deficient (*vtc1*) mutant (Gao and Zhang,

2008). Another antioxidant, GSH, plays an important role in the synthesis of phytochelatins and in the removal of ROS (Kalinowska and Pawlik-Skowrónska, 2010) and also acts as a substrate in the detoxification of peroxidation products of lipids and proteins *via* the activity of glutathione-*S*-transferase (Li et al., 2006; Gajewska and Skłodowska, 2010). Chen et al. (2010) have reported that exogenous application of GSH alleviates Cd-induced accumulation of $O_2^{\bullet-}$, H_2O_2, and MDA in barley seedlings. The results of the present study showed that As^V (25 and $50\,\mu M$) severely alters the redox status of AsA and GSH (**Tables 3, 4**). Though AsA+DHA and GSH+GSSG increased by As^V (25 and $50\,\mu M$) exposures, however, DHA and GSSG were greatly increased, which result into a steep decline in AsA/DHA and GSH/GSSG ratios (**Tables 3, 4**). These results clearly reveal that As^V (25 and $50\,\mu M$) stress causes alteration in the redox status of the cell by interfering with AsA and GSH pools. However, addition of Si and SiNp restores the redox status of AsA and GSH pools as indicated by high ratios of AsA/DHA and GSH/GSSG and thus maintaining the redox buffering in maize cultivar and hybrid seedling (**Tables 3, 4**). The results of enhanced AsA and GSH contents correspond with the enhanced activities of the AsA-GSH cycle enzymes i.e., GR and DHAR and thus, suggesting a role of Si and SiNp in triggering up-regulation of antioxidant defense system. Further, these results show that application of Si and SiNp under As^V (25 and $50\,\mu M$) stress re-establishes the redox status of the cell and thus mitigates As^V toxicity.

CONCLUSION

The results showed that As^V (25 and $50\,\mu M$) caused decreases in growth of maize cultivar and hybrid seedlings by interfering with the photosynthesis, contents of photosynthetic pigments, SOD, APX, GR, DHAR, AsA, and GSH by enhancing oxidative damage through the accumulation of As. However, application of Si and SiNp protects maize cultivar and hybrid seedlings against As^V by decreasing the accumulation of As and ROS, and enhancing levels of SOD, APX, GR, DHAR, AsA, and GSH, which re-establish the redox status of the cell and thus mitigate As^V toxicity. Comparing results of Si and SiNp, it was observed that reductions in the accumulation of As and oxidative stress markers were higher under SiNp and As^V combinations than Si, which is due to greater availability of SiNp to seedlings than Si. Further, results show that levels of components of the AsA-GSH cycle were higher in SiNp and As^V combinations than Si, which subsequently counterbalance ROS mediated damage to macromolecules more efficiently. Thus, the present study provides evidence that SiNp were more effective than Si in reducing As^V stress in maize cultivar and hybrid. Further, the results of the present study may be interesting from agronomical point of view in crop breeding.

AUTHOR CONTRIBUTIONS

DKT, VPS, SMP, DKC, and NKD designed experiments. DKT and SS performed experiments. DKT, SS and VPS, analyzed data and wrote the manuscript.

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