ARSENATE-INDUCED TOXICITY: EFFECTS ON ANTIOXIDATIVE ENZYMES AND DNA DAMAGE IN *VICIA FABA*AIJUN LIN,^{†‡} XUHONG ZHANG,[§] YONG-GUAN ZHU,^{*‡||} and FANG-JIE ZHAO[#][†]Department of Environmental Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, People's Republic of China[‡]State Key Lab of Regional and Urban Ecology/Department of Soil Environmental Sciences, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, People's Republic of China[§]Beijing City University, Beijing 100083, People's Republic of China^{||}Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361003, People's Republic of China[#]Agriculture and the Environment Division, Rothamsted Research, Harpenden, Herts AL5 2JQ, United Kingdom

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Abstract—A glasshouse hydroponic experiment was conducted to investigate the toxicity of arsenate to broad bean (*Vicia faba*) plants when grown with external arsenate concentrations at 10 $\mu\text{mol/L}$. The treated plants showed no obvious symptoms of phytotoxicity, but shoot/root growth was inhibited. Lipid peroxidation in leaves and roots increased with the addition of arsenate, indicating oxidative stress. We investigated the responses to arsenate exposure in the activities of several representative antioxidant enzymes, including peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), in plant tissues, and the DNA damage in plant leaves and root tips induced by arsenate in this plant was detected for the first time. Arsenate addition increased POD activity in the leaves significantly but decreased its activity in the roots in the 10 $\mu\text{mol/L}$ treatment. Arsenate addition caused an induction of SOD and CAT activities in both leaves and roots, but not in roots at the arsenate concentration of 10 $\mu\text{mol/L}$. The DNA damage in *V. faba* was detected using Comet assay; in both leaves and roots, DNA damage increased with increasing arsenate concentrations, indicating genotoxicity of arsenate. These results indicate that arsenate toxicity causes oxidative stress in *V. faba*, which might be one of the mechanisms through which arsenic induces DNA damage.

Keywords—Arsenate   *Vicia faba*   Oxidative stress   DNA damage

INTRODUCTION

Arsenic is a metalloid frequently found in soils, water, and air, and its presence in the environment results from both natural and anthropogenic sources [1]. Major anthropogenic sources of As are metal processing, coal burning, and application of As-based pesticides or herbicides [2]. Inorganic arsenate and arsenite are the chemical forms most commonly detected in the environment, with arsenate and arsenite being the predominant form under anaerobic and aerobic conditions, respectively [3]. Some microorganisms can transform these compounds by oxidation/reduction or methylation/demethylation reactions [2,3]. Recently, public concern regarding this element has been increasing, because As may be toxic even at low concentrations [1]. Arsenic is highly toxic to plants and animals and may accumulate in plants, not only affecting plant growth but also entering the food chain, posing potential health risks to humans.

Arsenic is known to produce mutagenic and genotoxic effects on humans, causing, for example, lung, skin, and bladder cancers [4,5]. Kitchin [6] reviewed the mechanisms of As carcinogenesis and found evidence for three modes of action both in experimental systems (animal and human cells) and in human tissues: Chromosomal abnormalities, oxidative stress, and a continuum of altered growth factors leading to cell proliferation and promotion of carcinogenesis. Using the method of single-cell gel electrophoresis, or the Comet assay,

it was demonstrated that arsenite and dimethylarsinic acid induced DNA damage and DNA cross-linking in human cells [4]. Plants are one of the important components of natural and managed ecosystems; however, few studies have examined As-induced DNA damage and the underlying mechanisms in plants. In the past, many studies focused on the physiological effects, such as the inhibition of plant growth by As and the mechanisms of As resistance employed by plants [7]. It has been shown that As exposure leads to an overproduction of reactive oxygen species (ROS) or suppressed activities of enzymatic antioxidants in plant tissues, inducing oxidative stress [8–13]. Whether the oxidative stress induced by As exposure would lead to DNA damage in plants has not been investigated. Therefore, the aim of the present study was to evaluate the toxicity of arsenate, particularly oxidative stress and DNA damage in plant cells.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were of reagent grade and purchased from Sigma Chemical (Beijing, China) or Beijing Chemical (Beijing, China).

Plant materials and treatments

Seeds of *Vicia faba*, purchased from Huazhong Normal University (Wuhan, Hubei Province, China), were sterilized in 70% (v/v) ethanol for 10 min. The seeds were washed with tap water and redistilled water, then sown in vermiculite in plastic pots. Fourteen days after germination, the seedlings were used in the experiments. The vermiculite was washed

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from the root system with tap water, and the roots were rinsed with redistilled water. Uniform seedlings were transferred to hydroponic pots, each containing 500 ml of nutrient solution. The modified Hoagland–Arnon nutrient solution [14,15] was used as the growth medium, and the composition of the nutrient solution was 1,000 $\mu\text{mol/L}$ of KNO_3 , 1,000 $\mu\text{mol/L}$ of $\text{Ca}(\text{NO}_3)_2$, 400 $\mu\text{mol/L}$ of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 150 $\mu\text{mol/L}$ of KH_2PO_4 , 2.5 $\mu\text{mol/L}$ of MnSO_4 , 0.5 $\mu\text{mol/L}$ of ZnSO_4 , 50 $\mu\text{mol/L}$ of Fe–ethylenediaminetetra-acetic acid (EDTA), 0.5 $\mu\text{mol/L}$ of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 5.0 $\mu\text{mol/L}$ of HBO_3 , 0.25 $\mu\text{mol/L}$ of Na_2MoO_4 , 0.1 $\mu\text{mol/L}$ of CoSO_4 , and 50 $\mu\text{mol/L}$ of NaCl . Plants were cultivated in a controlled-environment growth chamber at 25°C with a 14:8-h light:dark photoperiod with a light intensity of 350 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ and 70% relative humidity. After 28 d of growth in hydroponic culture, plants were treated with different concentrations of arsenate for 14 d. The arsenate concentrations tested were 0 (control), 5, and 10 $\mu\text{mol/L}$ supplied as Na_2HAsO_4 . Each treatment had four replicates. The nutrient solution was continuously aerated using air pumps and was replaced every 2 d.

Plant harvest and As analysis

After being treated for 14 d, the plants were harvested. Roots, stems, and leaves were separated and washed with redistilled water, and the fresh weights were determined. Roots and leaves were divided into three subsamples. Subsamples of leaves and root tips were used in the Comet assay and approximately 1.0 g (fresh wt) of roots or leaves in the enzymatic analyses; the remainders were dried (55°C for 72 h) to determine the dry weights and As concentrations. The dried plant tissues were ground in a stainless-steel mill and digested with concentrated HNO_3 . Temperature during digestion was controlled at a maximum of 120 to 130°C to avoid volatilization of As [16]. Digested samples were filtered and diluted with redistilled water to 25 ml. The As concentration was determined by inductively coupled plasma–optical emission spectroscopy (Optima 2000 DV; PerkinElmer, Shelton, CT, USA). Acid blanks were included to assess possible As contamination. A standard plant material (GBW07603) from the Department of Earth and Mine, Beijing, China, was used to verify the accuracy of As determination. The recovery rates for As was within $90 \pm 10\%$. The As concentration of the HNO_3 used was less than the detection limit of the inductively coupled plasma–optical emission spectroscopy (0.05 mg/L).

Estimation of lipid peroxidation

Lipid peroxidation was estimated following the method described by Verma and Dubey [17]. Fresh samples of roots or leaves (~200 mg) were ground in 0.25% thiobarbituric acid (TBA) in 10% trichloroacetic acid with a mortar and pestle. The mixtures were heated at 95°C for 30 min, cooled quickly in an ice bath, and centrifuged at 10,000 g for 10 min. The absorbance of the supernatant was recorded at 532 nm and corrected for unspecific absorbance by subtracting the absorbance at 600 nm. The solution of 0.25% TBA in 10% trichloroacetic acid served as a blank. Thiobarbituric acid–reactive substances (TBARS) were expressed as nmol/g fresh weight using an extinction coefficient of 155 mmol/L/cm.

Enzyme extractions

Fresh root and leaf samples (~500 mg) were ground in liquid nitrogen using a mortar and pestle. The ground samples were homogenized on ice in 10 ml of solution containing 50

mmol/L of potassium phosphate buffer and 1% (w/v) polyvinylpyrrolidone (pH 7.8) and kept at 4°C for 10 min. The homogenates were filtered and centrifuged at 4,000 g for 15 min at 4°C. The supernatants were used for further analyses.

Peroxidase assay

The peroxidase (POD) activity was determined at 25°C with guaiacol [18]. In the presence of H_2O_2 , POD catalyzes the transformation of guaiacol to tetraguaiacol (a brown product). The increase in absorbance was measured at 470 nm at 30-s intervals for up to 2 min using a spectrophotometer (Ultraviolet-3010; Shimadzu, Tokyo, Japan). The reaction mixture contained 100 mmol/L of potassium phosphate buffer (pH 6.0), 33 mmol/L of guaiacol, and 0.3 mmol/L of H_2O_2 .

Superoxide dismutase assay

The activities of superoxide dismutase (SOD) were determined using the published method for photoreduction of nitroblue tetrazolium chloride (NBT) with some modifications [9,19]. The assay mixture at a total volume of 3 ml in a 5-ml cuvette contained 13 mmol/L of methionine, 10 $\mu\text{mol/L}$ of riboflavin, 75 $\mu\text{mol/L}$ of NBT, 10 $\mu\text{mol/L}$ of EDTA, 0.05 mol/L of phosphate buffer (pH 7.8), and enzyme extracts. The blue color developed after illumination of the mixtures, and after 30 min of reaction, the absorbance at 560 nm was recorded in a spectrophotometer (Ultraviolet-3010). Reduction of NBT under illumination was measured with or without the addition of the enzyme extract, and the difference between the two measurements was used to calculate the activity of SOD. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light.

Catalase assay

The catalase (CAT) activities in leaf and root tissues were determined according to the method described by Aravind and Prasad [9] with some modifications. The reaction medium consisted of 50 mmol/L of potassium phosphate buffer (pH 7.0) and 19 mmol/L of H_2O_2 in a final volume of 3 ml. The decrease in the absorbance of H_2O_2 was recorded at 240 nm for 2 min using a double-beam spectrophotometer (Ultraviolet-3010), and 50 mmol/L of potassium phosphate buffer (pH 7.0) was used as the blank.

DNA damage in leaves and root tips

The Comet assay was used to detect DNA damage in the leaves and root tips of *V. faba* induced by arsenate in the experiment. After harvest, leaves and root tips were washed three times with double-distilled water, then blotted dry with filter paper (Waterman, Maidston, UK). All operations were conducted under dim or yellow light to avoid DNA damage by light. Plant material was placed in a 60-mm Petri dish on ice and covered with 250 μl of cold, $1\times$ phosphate buffer solution (130 mmol/L of NaCl , 7 mmol/L of Na_2HPO_4 , 3 mmol/L of NaH_2PO_4 , and 50 mmol/L of EDTA; pH 7.5). Each leaf or root tip was cut gently into pieces with a new razor blade. The pieces were washed in the buffer by repeated pipetting using a micropipette. The nuclei were released and collected in the buffer.

The nuclei suspension was used in the alkaline Comet assay, and the slides were prepared as described by Navarrete et al. [20] and Gichner et al. [21] with some modifications [22]. After preparation, the slides were put in freshly prepared, cold alkaline buffer (300 mmol/L of NaOH and 1 mmol/L of

Fig. 1. *Vicia faba* plant biomass in dry weight (g) for three different arsenate treatments. Data represent the mean $\pm$ standard error ($n = 4$). □ = As, 0 $\mu\text{mol/L}$ (control); ▨ = As, 5 $\mu\text{mol/L}$ treatment; ■ = As, 10 $\mu\text{mol/L}$ treatment.

Fig. 2. Thiobarbituric acid-reactive substances (TBARS) in roots and leaves of *Vicia faba* under different arsenate addition. Data represent the mean $\pm$ standard error ($n = 4$). □ = As, 0 $\mu\text{mol/L}$ (control); ▨ = As, 5 $\mu\text{mol/L}$ treatment; ■ = As, 10 $\mu\text{mol/L}$ treatment.

Na_2EDTA ; pH > 13) at 4°C to allow the DNA to denature. Electrophoresis was then conducted at 4°C for 15 min at 300 mA. After being neutralized with 0.4 mol/L of Tris-HCl (pH 7.5), each slide was stained with 50 μL of 13 mg/L of ethidium bromide solution and viewed with a Zeiss Axioskop fluorescence microscope (Axioskop 2 Mot Plus; Zeiss, Jena, Germany) with an excitation filter of 510 to 560 nm and a barrier of 590 nm. The Comet images were captured, and the image-analysis system (CASP) was employed to measure various Comet parameters, including the tail length, tail DNA, tail moment (TM), and Olive tail moment (OTM) [23]. The tail length, as a rough estimate of the DNA migration, was recorded in arbitrary units. Tail DNA refers to the percentage of DNA in the Comet tail. Tail moment is the integrated value of DNA density in the tail multiplied by the migration distance. Olive tail moment is the product of the distance (in x direction) between the center of gravity of the head and the center of gravity of the tail and the percentage of tail DNA. For each slide, 50 randomly chosen cells were analyzed for the values of tail length, tail DNA, TM, and OTM.

Data analysis

Means of four replicates and standard errors are presented. Analysis of variance was performed using the software Genstat® for Windows® [24].

Table 1. As concentrations in plant tissue at different arsenate treatments^a

As concn. ($\mu\text{mol/L}$)	Root (mg/kg dry wt)	Stem (mg/kg dry wt)	Leaf (mg/kg dry wt)
0	—	—	—
5	33.17 $\pm$ 2.94	6.59 $\pm$ 0.33	6.27 $\pm$ 1.39
10	94.24 $\pm$ 6.61	7.98 $\pm$ 0.46	7.81 $\pm$ 0.72

^a Values are presented as the mean $\pm$ standard error. — = below the detection limit of the inductively coupled plasma-optical emission spectroscopy (2.5 mg/L of As).

RESULTS

Effects of arsenate on growth of *V. faba*

Compared with the control, the growth of *V. faba* was significantly ($p < 0.05$) inhibited by arsenate addition, but no significant difference was found between the two added arsenate concentrations (Fig. 1). The root elongation was inhibited, and the arsenate-treated roots, especially those in the 10 $\mu\text{mol/L}$ treatment, were shorter and thicker than those in the control.

Fig. 3. Activities of peroxidase (POD) in roots and leaves of *Vicia faba* under different arsenate addition. Data represent the mean $\pm$ standard error ($n = 4$). Different letters above columns indicate significant differences among different arsenate treatments as determined by least significant difference at the 5% level. The changes in POD activities were represented by the changes in optical density at 470 nm (OD470). □ = As, 0 $\mu\text{mol/L}$ (control); ▨ = As, 5 $\mu\text{mol/L}$ treatment; ■ = As, 10 $\mu\text{mol/L}$ treatment.

Fig. 4. Activities of superoxide dismutase (SOD) in *Vicia faba* plants treated with different concentrations of arsenate. Data represent the mean $\pm$ standard error ($n = 4$). Different letters above columns indicate significant differences among different arsenate treatments as determined by least significant difference at the 5% level. One unit (U) of activity is the amount of protein required to inhibit 50% initial reduction of nitroblue tetrazolium chloride under light. $\square$ = As, 0 $\mu\text{mol/L}$ (control); $\square$ with diagonal lines = As, 5 $\mu\text{mol/L}$ treatment; $\blacksquare$ = As, 10 $\mu\text{mol/L}$ treatment.

Arsenic uptake

The As concentrations in *V. faba* roots, stems, and leaves increased significantly ($p < 0.05$) with As addition in the culture solution (Table 1). Tissue As concentrations followed the trend roots > stems and leaves, with most As being retained in the roots, especially in the treatment with high arsenate. In the leaves and stems, arsenate addition led to significant ($p < 0.05$) increases in As concentrations compared to the control, but no significant difference was found between the two arsenate treatments.

Effect of arsenate on lipid peroxidation

Lipid peroxidation in roots and leaves of *V. faba*, measured as TBARS, increased with increasing arsenate concentrations in the culture solution (Fig. 2). Arsenate addition led to significant increases in the TBARS level in leaves compared to the control ($p < 0.05$), but no significant difference was found between the treatments with 5 and 10 $\mu\text{mol/L}$ of arsenate. Compared with the control, root TBARS level was significantly ($p < 0.05$) increased by the 10 $\mu\text{mol/L}$ of arsenate treatment but not by the 5 $\mu\text{mol/L}$ of arsenate treatment.

Fig. 5. Activities of catalase (CAT) in *Vicia faba* plants treated with different concentrations of arsenate. Data represent the mean $\pm$ standard error ($n = 4$). Different letters above columns indicate significant differences among different arsenate treatments by least significant difference at the 5% level. The changes in CAT activities were represented by the changes in optical density at 290 nm (OD290). $\square$ = As, 0 $\mu\text{mol/L}$ (control); $\square$ with diagonal lines = As, 5 $\mu\text{mol/L}$ treatment; $\blacksquare$ = As, 10 $\mu\text{mol/L}$ treatment.

Effects of arsenate on activities of antioxidative enzymes

POD activity. The activities of POD in the leaves and roots of *V. faba* are shown in Figure 3. In the leaves, the arsenate addition at 5 $\mu\text{mol/L}$ increased the POD activity significantly ($p < 0.05$) compared with the control, but no significant difference was observed between the 10 $\mu\text{mol/L}$ of arsenate treatment and the control. In the roots, no significant difference was found in POD activities between the control and the 5 $\mu\text{mol/L}$ of arsenate treatment, but arsenate addition at 10 $\mu\text{mol/L}$ led to a significant ($p < 0.05$) decrease in POD activity compared with the control.

SOD and CAT activities. The activities of SOD and CAT were affected by the arsenate treatments (Figs. 4 and 5). The activities of SOD and CAT in the leaves increased significantly ($p < 0.05$) with increasing arsenate concentration. In the roots, arsenate addition at 5 $\mu\text{mol/L}$ increased SOD and CAT activities significantly ($p < 0.05$) compared with the control, but the 10 $\mu\text{mol/L}$ of arsenate treatment led to a significant ($p < 0.05$) decrease in SOD and CAT activities compared with the control.

Table 2. DNA damage in leaves of *Vicia faba* under different arsenate treatments^a

As concentration ($\mu\text{mol/L}$)	Parameters of DNA damage			
	Tail length ^b	% Tail DNA	TM	OTM
0	41.6 $\pm$ 9.6	43.6 $\pm$ 2.7	22.8 $\pm$ 5.6	16.7 $\pm$ 3.0
5	101.5 $\pm$ 9.2	75.6 $\pm$ 1.8	78.3 $\pm$ 7.2	46.4 $\pm$ 4.8
10	120.8 $\pm$ 4.4	80.8 $\pm$ 0.3	99.3 $\pm$ 2.3	52.1 $\pm$ 4.8

^a Values are presented as the mean $\pm$ standard error. TM = tail moment; OTM = olive tail moment.

^b Relative length in arbitrary units.

Fig. 6. Comet images of plant nuclei in the leaves of *Vicia faba* under arsenate treatments of 0, 5, and 10 $\mu\text{mol/L}$.

DNA damage

The DNA migration in leaf and root tip samples increased with the addition of arsenate (Figs. 6 and 7). In both leaves and roots, the values of tail length, tail DNA, TM, and OTM were all significantly ($p < 0.05$) increased in both arsenate treatments compared with the control (Tables 2 and 3). In roots, the values of all Comet assay parameters were significantly higher at 10 $\mu\text{mol/L}$ of arsenate compared with those at 5 $\mu\text{mol/L}$ of arsenate. In leaves, the values of tail length and TM were significantly higher at 10 $\mu\text{mol/L}$ of arsenate compared with those at 5 $\mu\text{mol/L}$ of arsenate.

DISCUSSION

Arsenic is phytotoxic and is expected to negatively affect plant growth [7]. Under the present experimental conditions, *V. faba* exposed to two concentrations of arsenate showed biomass reduction (Fig. 1) and inhibition of root elongation. The toxicity threshold for As differs between plants but usually is within the range of 5 to 100 mg/kg in shoots for nonhyperaccumulating plants [25]. The concentrations of As in leaves and stems of *V. faba* in the present experiment were only slightly greater than 5 mg/kg (Table 1), suggesting that the plants were stressed with only a mild degree of As toxicity, and more severe symptoms of phytotoxicity, such as necrosis, were not observed in the present experiment.

Heavy metal and metalloid toxicity is considered to induce the overproduction of ROS (e.g., O_2^- and H_2O_2) and may result in significant oxidative damage to cellular constituents, such as DNA, proteins, and lipids [13]. Membrane lipids are especially prone to attack by free radicals, and lipid peroxidation is one of the main manifestations of oxidative damage [26]. In the present study, an increase in the level of TBARS in the leaves and roots of *V. faba* with arsenate addition indicated that the lipid peroxidation occurred in the tissues of *V. faba* in response to arsenate, which is consistent with a previous study using *Holcus lanatus* L. [8]. It is suggested that ROS may be generated through the conversion of arsenate to arsenite [8,27].

High levels of lipid peroxidation might be expected to correspond with high levels of SOD activity [8]. Plants are protected against the oxidative stress by the operation of intricate antioxidative systems, comprising both enzymatic systems, such as SOD, ascorbate peroxidase, guaiacol peroxidase, POD, and CAT, and nonenzymic systems that act as free radical scavengers, such as ascorbic acid, thiols, and cysteine [28]. Superoxide dismutase converts superoxide radicals (O_2^-) into O_2 and H_2O_2 and acts in the first line of defense against oxidative stress [13]. In the present study, the activities of SOD in the leaves of *V. faba* were increased with increasing arsenate concentration, indicating that exposure to arsenate induced the formation of O_2^- , which stimulated the biosynthesis of SOD. The situation is different, however, in the roots: In the 5 $\mu\text{mol/L}$ of arsenate treatment, the SOD activity of the roots was the highest, whereas in the 10 $\mu\text{mol/L}$ of arsenate treatment, the SOD activity of the roots was significantly inhibited, possibly as a result of a more severe degree of toxicity, as shown by the aberrant coralloid root morphology. The decrease in SOD activity also could be a result of lipid peroxidation, because in the 10 $\mu\text{mol/L}$ of arsenate treatment, the level of TBARS was significantly increased compared with the 5 $\mu\text{mol/L}$ of arsenate treatment, indicating severe membrane damage and inhibited cell processes, which could have led to the inhibition of SOD synthesis. This observation is in agreement with a previous study [8], which showed that the SOD activity of *H. lanatus* increased in response to low-concentration arsenate exposure but was inhibited in response to at high-concentration arsenate exposure. Catalase, which catalyzes the removal of H_2O_2 formed during the reaction catalyzed by SOD, is another component in the antioxidant enzymatic system in plant cells to control ROS [9,29]. In our experiments the patterns of CAT and SOD induction by arsenate were very similar, which underlined the complementary functions of these two enzymes.

Peroxidase is present in both prokaryotes and eukaryotes and is considered to play a role in antioxidant defense, which uses guaiacol or glutathione, pyridine nucleotides, and ascor-

Fig. 7. Comet images of plant nuclei in the root of *Vicia faba* under arsenate treatments of 0, 5, and 10 $\mu\text{mol/L}$.

Table 3. DNA damage in roots of *Vicia faba* under different arsenate treatments^a

As concentration ($\mu\text{mol/L}$)	Parameters of DNA damage			
	Tail length ^b	% tail DNA	TM	OTM
0	72.1 $\pm$ 6.4	53.9 $\pm$ 6.4	40.7 $\pm$ 7.5	25.0 $\pm$ 3.9
5	85.9 $\pm$ 8.6	73.9 $\pm$ 4.9	65.5 $\pm$ 10.8	38.1 $\pm$ 6.0
10	144.3 $\pm$ 4.6	91.0 $\pm$ 1.0	131.6 $\pm$ 2.6	68.9 $\pm$ 0.1

^a Values are presented as the mean $\pm$ standard error. TM = tail moment; OTM = olive tail moment.

^b Relative length in arbitrary units.

bate as the electron donors in the scavenging of H_2O_2 in the cell [30]. Peroxidase could play a role in alleviating oxidative stress induced by heavy metals or metalloids. In the present study, the POD activities of roots and leaves were increased under the 5 $\mu\text{mol/L}$ of arsenate treatment, indicating that the accumulation of H_2O_2 in plant tissues may have stimulated the biosynthesis of POD. In the 10 $\mu\text{mol/L}$ of treatment, however, POD, like SOD, was not increased in roots (see above).

Prolonged or high levels of exposure to heavy metals or metalloids can lead to a buildup of ROS, which in turn can damage cells through membrane peroxidation, loss of ions, protein cleavage, and most notably, DNA damage, leading to mutagenesis or cell death [13,31]. The results suggested that arsenate toxicity induced oxidative stress in the leaves and roots of *V. faba*. The Comet assay, which is widely used to evaluate the genotoxicity of chemicals in the environment [32], was employed to assess DNA damage induced by arsenate in *V. faba*. Both in leaves and in roots, the DNA damage increased with increasing arsenate concentration, indicating that arsenate-induced DNA damage in *V. faba* and the levels of DNA damage imposed by arsenate could be quantified by Comet assay.

In the leaves, the pattern of DNA damage was consistent with the increase in antioxidative enzyme capacities. In the roots, the enzyme capacities increased in the 5 $\mu\text{mol/L}$ of arsenate treatment but decreased at the 10 $\mu\text{mol/L}$ of arsenate treatment, which could have contributed to oxidative stress. The latter could relate to the strongly altered coralloid root structure at this treatment. Using Comet assay, Mancini et al. [33] showed that the hydrogen peroxide treatment caused DNA damage in tobacco plants, particularly the transgenic lines with deregulated levels of CAT and MnSOD. Another possible explanation for the low activity levels of antioxidative enzymes in the roots of the 10 $\mu\text{mol/L}$ of arsenate treatment was the timing of the measurements. The present study analyzed plant materials exposed to arsenate for 14 d. At this time, arsenate toxicity could already have inhibited the biosynthesis of the antioxidative enzymes, resulting in more accumulation of ROS. Hu et al. [34] reported that one of the mechanisms of As genotoxicity to human cells was to inhibit DNA repair in human cells by inducing ROS accumulation. In the present study, arsenate was found to induce oxidative stress in *V. faba*, which could be one of the mechanisms of DNA damage. Using the Comet assay of the human lung fibroblast cell line MRC-5, it was shown that As could induce DNA damage at the concentration, similar to the findings in the present study [4], indicating that the Comet assay using plant cells also could be used to assess the genotoxicity of chemicals.

In conclusion, our results suggest that arsenate toxicity causes oxidative stress and DNA damage in *V. faba* and that the DNA damage may be one of the results of the oxidative stress.

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