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Selenium Alleviates the Arsenic Toxicity in Sunflower Seedling

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Authors' contributions

This work was carried out in collaboration between all authors. Author SI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NN and CY managed the analyses of the study. Author SI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Hydroponic experiments were performed to investigate physiological mechanisms of selenium (Se) mitigation of As toxicity in sunflower. The exposure of plants to 10 µM arsenic (As) inhibited biomass production and intensively increased accumulation of As in both roots and leaves. As also enhanced hydrogen peroxides (H_2O_2) content and lipid peroxidation as indicated by malondialdehyde (MDA) accumulation. Presoaking seeds with Se (5, 10 and 20 µM) markedly alleviated the negative effect of As on plan growth and led to a decrease in oxidative damages as evidenced by the lowered H_2O_2 and MDA content. Se particularly enhanced the activities of catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX), but lowered that of superoxide dismutase (SOD). As important antioxidants, ascorbate (AsA) and glutathione (GSH) contents in sunflower leaves exposed to As were significantly decreased by Se treatment. These

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results reveal the potentiating effect of selenium in regulating Arsenic induced oxidative stress in sunflower seedling.

Keywords: Antioxidants; arsenic; selenium; Helianthus annuus; oxidative stress.

1. INTRODUCTION

Arsenic (As) is ubiquitous in nature appearing in various chemical forms, mainly due to differences in soil redox potential, pH and biological activity. Inorganic As species are considered more toxic than organic ones [1]. Arsenic, like other metals and metalloids can either be taken up by plants or washed down into the groundwater, and present a risk to human health. However, the most important factor determining whether arsenic in the soil gets into the plant-based food crops we eat every day, is the genetic makeup of the plant itself [2].

Arsenic affects the growth and development of plants, and causes toxicity resulting in various biochemical and physiological disorders [3]. At the morphological level, an excessive amount of As causes stunted growth [4], leaf chlorosis and necroses [5], reduction in leaf area and photosynthesis activities [6]. At cell level, Arsenic induces oxidative stress as evidenced by enhanced lipid peroxidation, hydrogen peroxide (H_2O_2) production and ion leakage [3].

Despite the fact that arsenic is a nonessential element for plants, it is easily taken up by roots and transported to other parts of the plant, being toxic to living cells at very low concentrations [3]. This feature makes arsenic a serious problem since the As-enriched plants can be transferred to the food chain. Therefore, this toxic element could be incorporated into the human diet through edible plants, causing toxicity [7].

Selenium, in contrast, is an essential micronutrient necessary for antioxidative reactions and hormone balance in human and animal cells. The antioxidant and anticarcinogenic properties attributed to some seleno compounds [8] justify the increasing interest in growing selenium-enriched vegetables, which represent an important source of this element in the human diet [9]. According to current thinking, higher plants do not require Se, and the question of whether Se is an essential element to plants remains controversial. Recent studies suggest that selenium may be beneficial to biological functions in plants [10,11] due to its antioxidative action. However, Se often exerts a dual effect on plant growth. At lower concentration, Se can protect plants against the damage induced by heavy metals, drought [12], UV-B [13], salt [14], water [15] and high temperature [16]. Whereas at higher dose, Se acts as a pro-oxidant and cause damage to plants [10].

It is of considerable interest to know whether external Se could act as a regulator or capable of initiating antioxidant intervention strategy to respond to oxidative stress brought about by As. In fact, accumulating evidence suggested that Se exerts positive effects, such as, growth improvement, increase of antioxidative capacity, reduction of reactive oxygen species (ROS) and lipid peroxidation product [17]. Consequently, the main objective of this study was to investigate the potential role of external Se in modulating As-induced oxidative stress and to provide a basis for developing strategies to reduce risks associated with As toxicity.

2. MATERIALS AND METHODS

2.1 Plant Material and Growth Conditions

Seeds of sunflower (*Helianthus annuus*) were sterilized and divided into two groups. One half of the seeds were soaked in Se (5, 10 and 20 µM) as selenate solution (Na_2SeO_4 10 H_2O) for 24 h, the other half of the seeds was soaked in water (control), and then the both groups were allowed to germinate on moist filter paper in the dark. Four-day-old, dark grown seedlings, were transferred to plastic beakers (6 L capacity, 6 plants per beaker) filled with nutrient solution containing: 1.0 mM $MgSO_4$, 2.5 mM $Ca(NO_3)_2$, 1.0 mM KH_2PO_4, 2.0 mM KNO_3, 2.0 mM NH_4Cl, 50 µM EDTA–Fe–K, 30 µM H_3BO_3, 10 µM $MnSO_4$, 1.0 µM $ZnSO_4$, 1.0 µM $CuSO_4$ and 30 µM $(NH4)_6Mo_7O_{24}$. After an initial growth period of 5 days, treatments were performed by adding 10 µM Na_2HAsO_4 to the nutrient solution. As dose used in this work are chosen appropriately to expose the plants to moderate levels of As. Plants were grown in a growth chamber with a 16-h-day (25ºC)/8-h-night (20ºC) cycle, an irradiance of 150 µmol m^{-2} s^{-1}, and 65-75% relative humidity. The nutrient solution was buffered to pH 5.5 with HCl/KOH, aerated, and

changed twice per week. After 4 days of As-treatment, roots of the harvested plantlets were soaked in 20 mM EDTA for 15 min to remove adsorbed metals and washed carefully using distilled water to eliminate any contamination. Primary leaves were harvested and immediately stored in liquid nitrogen. Three independent culture experiments were performed in order to check reproducibility. For biomass production, As determination and biochemical analyzes, five plantlets from each replication of all treatments were selected.

2.2 Determination of Total Arsenic Concentrations

Dry plant material was powdered and wet-digested in acid mixture (HNO_3:$HClO_4$, 3:1, v/v) at 100°C. As concentrations were determined by atomic absorption spectrophotometer (Perkin-Elmer, Analyst 300) using an air–acetylene flame.

2.3 Determination of Lipid Peroxidation, Hydrogen Peroxide Production, Glutathione (GSH) and Ascorbate (AsA) Pool

The level of lipid peroxidation in plant leaves was determined by estimation of the thiobarbituric acid reactive substances (TBARS) which was expressed as the malondialdehyde (MDA) concentration based on the method of Hodges et al. [18]. Briefly, fresh leaf sample (0.2 g) was ground in 0.1% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 10.000 *g* for 5 min. To 1 mL supernatant, 4 mL (TBA) [5% TBA (w/v) in 20% TCA (w/v)] was added. The mixture was heated at 100ºC for 30 min and then cooled in an ice bath. After centrifugation at 10.000 *g* for 10 min, the absorbance of the supernatant was measured at 532 nm. The value was corrected for the non specific absorption at 600 nm. Lipid peroxidation level was expressed as nmol MDA formed using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Hydrogen peroxide (H_2O_2) levels were determined according to Sergiev et al. [19]. Leaf tissue (0.5 g) was homogenized in an ice bath with 5 mL TCA (0.1%, w/v). The homogenate was centrifuged at 12.000 *g* for 15 min and 0.5 mL of the supernatant was added to 0.5 mL potassium phosphate buffer (10 mM, pH 7.0) and 1 mL potassium iodide (KI) (1 M). The absorbance of the supernatant was measured at 390 nm and the concentration of H_2O_2 was obtained using a standard curve. Results were expressed as nmol g^{-1}DW.

Glutathione (GSH) concentration was determined by the method of Ellman [20] based on the development of a yellow colour when 5,5-dithiobis-2-nitrobenzoic acid (DTNB) was added to compounds containing sulfhydryl groups. 500 µL tissue homogenate in phosphate buffer were added to 3 mL 4% (v/v) sulfosalicylic acid. The mixture was centrifuged at 3.000 *g* for 15 min. Then, 500 µl supernatant were taken and added to Ellman's reagent. The absorbance was measured at 412 nm after 10 min of reaction. Total GSH concentration was expressed as µmol g^{-1}FW.

Ascorbate (AsA) concentration was determined spectrophotometrically by using dinitrophenyl-hydrazine according to Mukherjee and Choudhuri [21]. The assay is based on the reduction of Fe^{3+} to Fe^{2+} by AsA. Briefly, leaf samples were powdered in liquid nitrogen and extracted in 6% (w/v) trichloroacetic acid (TCA), 2% (w/v) dinitrophenyl-hydrazine in 50% H_2SO_4 and 10% (v/v) thiourea in 70% ethanol. The homogenate was boiled in a water bath for 15 min, cooled at room temperature, and centrifuged at 1.000 *g* for 10 min at 4ºC. The resulting pellet was dissolved with 80% H_2SO_4. The absorbance was measured at 530 nm. A calibration curve was prepared using ascorbic acid as standard and utilised for calculations. Results were expressed as µmol g^{-1} FW.

2.4 Determination of Antioxidative Enzyme Activities

Frozen leaf tissue (0.4 g) was homogenized in 4 mL ice-cold extraction buffer (50 mM potassium phosphate, pH 7.0, 0.4% PVPP) using a pre-chilled mortar and pestle. The homogenate was squeezed through a nylon mesh and centrifuged for 30 min at 14.000 g at 4ºC. The supernatant was used for assays of the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX). All spectrophotometric analyses were conducted at 25ºC by using a Perkin Elmer's LAMBDA 25/35/45 UV/Vis spectrophotometer.

SOD (EC1.15.1.1) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Beauchamp and Fridovich [22]. The reaction mixture (1 mL) included 50 mM

phosphate buffer (pH 7.4), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA, 2 µM riboflavin and 100 µL enzyme extract. The reaction was allowed to proceed for 15 min illuminated with fluorescent tubes. Absorbance of the reaction mixture was read at 560 nm. SOD activity was expressed as U mg^{-1} protein. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of photochemical reduction of NBT.

CAT (EC1.11.1.6) activity was assayed by the decomposition of hydrogen peroxide according to Aebi [23]. The reaction mixture (1 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.1% H_2O_2 and 100 µL enzyme extract. The decrease of H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (ε=39.4 mM^{-1} cm^{-1}). CAT activity was expressed as U mg^{-1} protein.

APX (EC1.11.1.1) activity was determined by the method of Nakano and Asada [24]. The reaction mixture (2 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.3 mM ascorbate, 0.1 mM H_2O_2 and 100 µL enzyme extract. The reaction was initiated by addition of H_2O_2 and the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm. Activity of APX was calculated by using the molar extinction coefficient for ascorbate (ε=2.8 mM^{-1} cm^{-1}). APX activity was expressed as U mg^{-1} protein.

GPX (EC 1.11.1.9) activity was measured by a spectrophotometric method according to Drotar et al. [25]. The reaction mixture (250 µL) contained 2 mM glutathione, 1 mM NADPH, 1 mM EDTA, 2 mM t-butyl hydroperoxide and 0.5 µg of glutathione reductase in 100 mM sodium phosphate buffer (pH 7.0) and 10 µg of extracted proteins. The rate of NADPH oxidation was measured at 340 nm. GPX activity was expressed as U mg^{-1} protein.

2.5 Determination of Soluble Protein Concentration

Soluble protein concentration was measured according to Bradford [26] using the bovine serum albumin (BSA) protein assay reagent (Pierce, BSA Protein Assay Kit, USA) with BSA as the standard protein.

2.6 Statistical Analysis

All statistical analyses were carried out with GraphPad Prism 4.02 for Windows (GraphPad Software, San Diego, CA). Significant differences between treatment effects were determined by 1-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons with statistical significance of $P<0.05$. Number of replications (n) in tables/figures denotes individual plants measured for each parameter among 13-day-old plants. Results were expressed as mean ± standard error of the mean (mean ± SEM).

3. RESULTS

3.1 Effects of Se Pretreatment on Growth Response and As Distribution

In the present study, we have examined the effect of As on developing sunflower seedling after 4 days treatment of hydroponic culture, and the subsequent effect of Se in conferring tolerance to As toxicity. Exposure to As (10 µM) decreased both root and leaf fresh weights by about 55% and 40%, respectively (Table.1). Under As stress conditions, pre-soaking with Se significantly promoted plant growth and markedly alleviated As-induced growth inhibition (Table.1). The best-growing seedlings were consistently apparent with lower Se (5 µM) dose (increase by about 27% and 32% in root and leaf fresh weights, respectively, compared to As-treated plant). Whereas, at higher Se doses (20 µM), no significantly difference was observed as compared to As-treated plants (Table 1).

The effects of Se supplementation on As contents in sunflower leaves are summarized in Table 2. As addition to the nutrient solution resulted in a high accumulation of this metal within plant organs reaching 4.201 mg g^{-1} DW in roots and a value of 0.0641 mg g^{-1} DW in leaves (Table 2). Pretreatment with Se significantly decreased As concentration in both roots and leaves. The most prominent effect was at 5 µM Se, the concentration that significantly decreased As accumulation by about 21% and 39%, in roots and leaves, respectively. Contrarily, at highest Se dose, no significantly difference was observed as compared to As-treated plant (Table 2).

3.2 Effects of Se Pretreatment on MDA, H_2O_2, Glutathione (GSH) and Ascorbate (AsA) Pool in Leaves of Sunflower Seedlings under As Stress

Lipid peroxidation (MDA) increased upon As exposure by nearly 2 times as compared to the control (Table 3). Pretreatment with Se before As

application decreased the level of MDA by about 40% and 28% at 5 and 10 µM, respectively. By contrast, no major changes were observed at higher Se dose (20 µM) or in the presence of Se alone (Table 3).

Table 1. Effects of Se pretreatment on biomass accumulations in sunflower seedling submitted during 4 days to As stress. Data are means ± SEM (n=5). Values within rows followed by the same letter(s) are not significantly different according to Tukey's test, (P<0.05)

Se (µM)	As in nutrient solution	
	0 (µM)	10(µM)
Root FW (g plant^{-1})		
0	1.650±0.07b	0.750±0.09e
5	1.890±0.09a	1.030±0.09c
10	1.850±0.08a	0.900±0.07d
20	1.620±0.09b	0.735±0.08e
Leaf FW (g plant^{-1})		
0	2.220±0.08b	1.32±0.04e
5	2.780±0.09a	1.95±0.02c
10	2.750±0.08a	1.50±0.01d
20	2.450±0.06b	1.25±0.02e

Table 2. Effects of Se pretreatment on (As) concentrations (µg g^{-1}DW) in roots and leaves of sunflower seedling submitted during 4 days to As stress. Data are means ± SEM (n=5). Values within rows followed by the same letter(s) are not significantly different according to Tukey's test, (P<0.05)

Se (µM)	As concentration (mg g^{-1} DW)	
	Roots	Leaves
0	4.201±0.145a	0.0641±0.097a
5	3.320±0.110b	0.0390±0.064b
10	3.692±0.122b	0.0445±0.056b
20	4.090±0.134ac	0.0580±0.058ac

The effects of Se and As on H_2O_2 production are summarized in Table 3. Compared to control, treatment of plant with 10 µM As increased H_2O_2 content by nearly 3 times. Se alone did not significantly affect H_2O_2 production. By contrast, presoaking with 5 µM of Se decreased leaf H_2O_2 content by 41% relative to As-stressed plants grown without Se application.

GSH concentration increased under As stress conditions by about 47%. Such an increase was obviously less pronounced in Se-pretreated plants before As application (Table 3). AsA concentration significantly increased (+47%) under As stress conditions as compared to control (Table 3). This increase was reduced by Se pretreatment by about 31.6% and 27.75%, respectively, at 5 and 10 µM. In plants pretreated with Se and non-subjected to As stress, no significant difference with the control was detected in leaf MDA, H_2O_2, GSH, and AsA concentrations.

3.3 Effects of Se Pretreatment on Antioxidant Enzyme Activities in As-treated Plant Leaves

Arsenic application increased SOD activity by about 46% as compared to the control (Fig. 1A). By contrast, CAT, APX and GPX activities were decreased in As treated leaves by about 41, 45 and 34% (Fig. 1B-D). Se application decreased SOD activity upon As exposure and alleviated the inhibitory effect of As on CAT, APX and GPX activities. The most obvious effect was at 5 µM Se, the concentration that induced an increase of 28.5%, 30% and 21% in CAT, APX and GPX activities, respectively (Figs. 1B-D). In plants pretreated with Se and non-subjected to As stress, no significant difference with the control was detected in leaf antioxidant enzyme activities.

4. DISCUSSION

The present study was performed to investigate the pronounced role of Se in protecting plant from As induced oxidative stress in sunflower seedling. In our experiment, the exposure of plants to As treatment decreased both root and leaf fresh weights and increased As accumulation within plant organs. In agreement, mungbean exposed to As showed depleted shoot and root dry biomass [27]. Upon translocation to the shoot, As can severely inhibit plant growth by slowing or arresting expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility and fruit production [28].

The underlying mechanisms of As tolerance in plants are not completely explained. Understanding the biochemical and molecular responses to As stress is essential for the holistic perception of plant resistance mechanisms under As stress. The effects of various environmental stresses on plants are known to be mediated, at least partially, by an enhanced production of reactive oxygen species (ROS) [29,30]. ROS and MDA are the well known markers for determining the extent of oxidative stress and considered to

be the most important contributors to growth inhibition. Our results indicated that As exposed plants had enhanced levels of H_2O_2 and MDA which together affected the cell membrane functionality and integrity. These were in turn interfering with the biosynthesis of photosynthetic machinery and impaired the subsequent growth. A drastic increase in H_2O_2 may have as consequence a lower extensibility of plant cell walls, which can rapidly terminate growth [29]. This could explain the decrease in fresh mass observed in sunflower leaves treated with 10 µM of As.

Increase of H_2O_2 content may also inactivate enzymes by oxidizing their thiol groups. However, in As-stressed plants pretreated with Se, H_2O_2 level and lipid peroxidation were much

Table 3. Effects of Se pretreatment on MDA, H2O2, GSH and AsA concentration in sunflower leaves submitted during 4 days to As stress. Means of n=5±SEM from three independent experiments. Different letters mean significance of difference between the treatments (P<0.05, Tukey's test)

Treatment		MDA	H_2O_2	GSH	AsA
Se (µM)	As (µM)	(nmol g^{-1}FW)	(nmol g^{-1}FW)	(µmol g^{-1} FW)	(µmolg^{-1} FW)
0	0	37.03±0.610c	50.21±0.110d	13.65±0.381d	6.67±0.260c
5	0	38.17±0.418c	48.56±0.319d	13.50±0.318d	5.89±0.140c
10	0	37.29±0.501c	49.57±0.415d	12.87±0.388d	6.20±0.282c
20	0	40.35±0.411c	51.09±0.354d	13.36±0.424d	6.45±0.158c
0	10	72.50±0.625a	148.05±0.311a	26.08±0.579a	11.75±0.393a
5	10	43.58±0.555b	87.32±0.358c	17.30±0.450b	8.04±0.170b
10	10	52.26±0.544b	96.27±0.408c	20.10±0.301c	8.49±0.167b
20	10	68.75±0.412a	131.35±0.532ab	24.18±0.419a	10.59±0.220a

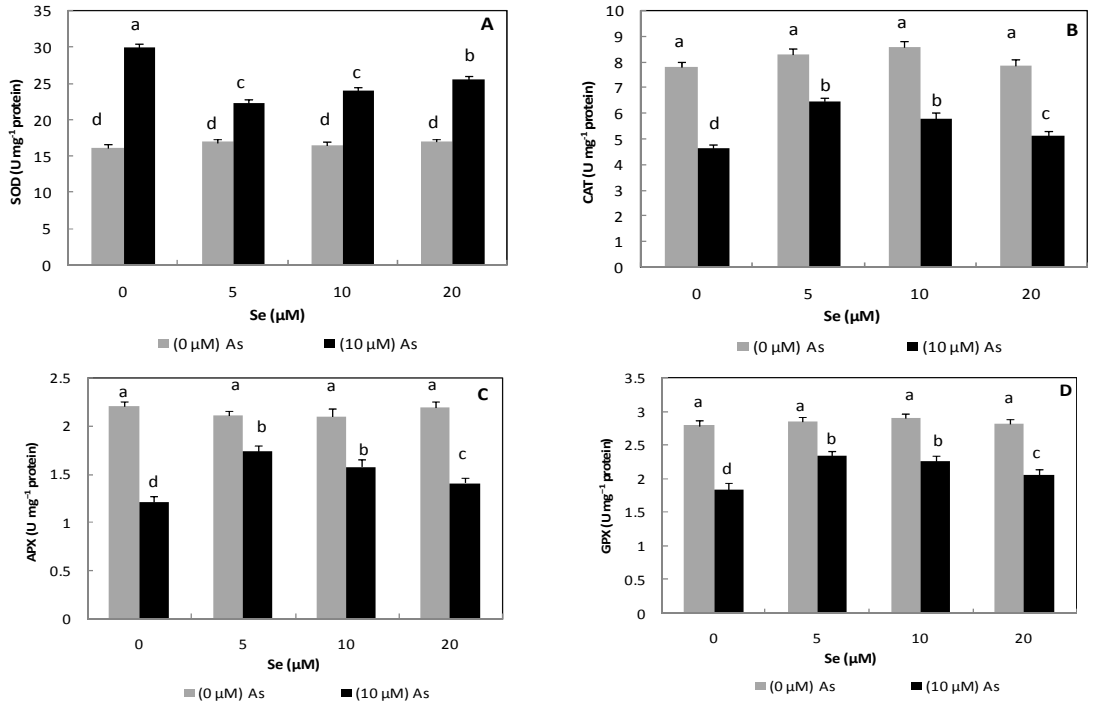

Fig. 1. Effects of Se pretreatment on SOD (A), CAT (C), APX (D) and GPX (E) activities in sunflower seedling submitted during 4 days to As stress. Data are means ± SEM (n=5). Values within rows followed by the same letter(s) are not significantly different according to Tukey's test, (P<0.05)

lower than in plants treated with As only. Se subdued H_2O_2 and MDA formation is supported by several recent reports [16,27,30], which indicated the protective effect of Se in lowering H_2O_2 and lipid peroxidation rate under As stress conditions.

Recent studies have shown that, at low concentrations, Se can protect plants from various abiotic stresses by enhancing the antioxidant capacity [11,13,14,30]. The present study was performed to analyze the mechanisms of the beneficial effect of Se on sunflower plants exposed to toxic As dose. Se pretreatment enhanced plant growth under As application and the most prominent effect was observed at lower Se dose. Whereas, at high dose Se acts as a pro-oxidant and cause damage to plants [10]. Ameliorative impact of lower Se dose on sunflower seedling can be attributed to the role of Se in nutrient uptake [31], stomatal regulation [32] and photosynthetic capacity [27]. Se is believed to possess a strong ability to combine with heavy metals, such as Cd, Hg, Ag and Pb, to form nontoxic Se-metal complexes [31,33]. However, in plants, nontoxic Se-metal complexes have not yet been detected.

The present work has provided strong evidence of the protective role of Se against oxidative stress resulting from As stress. It is possible that in the presence of Se, a portion of the oxidative radicals is removed in a non-enzymatic way [34]. Data obtained in this paper indicate that sunflower seedling tried to cope with the As-induced oxidative stress by strengthening their antioxidant capabilities, since the AsA and GSH levels were significantly increased upon As exposure. Furthermore, our data suggest that when Se-pretreated plants must cope with As-induced oxidative damages, their AsA utilization is higher than that occurring under As stress only. This is substantiated by the behavior of APX, the activity of which was more enhanced in Se-pretreated plants subjected to As than in those subjected to As only. Under As stress condition, the reduction in leaf AsA content induced by Se could be due to a less efficient recycling of the AsA oxidized forms.

The data suggest that Se plays an important antioxidant role in protecting plants from oxidative stress. In Se-pretreated plants, the initially decreased activities of SOD and increased activities of CAT, APX and GPX cooperatively controlled the As-induced H_2O_2 at high homeostatic levels contrarily to the mode during plant–pathogen interactions [35]. It seems to suggest that Se-reduced H_2O_2 permit sunflower seedling to respond more effectively to As-induced oxidative damage. It is well established that CAT has a high reaction rate but a low affinity for H_2O_2, whereas APX has a high affinity for H_2O_2 and is able to detoxify low concentrations of H_2O_2 [36]. Therefore, it is possible that stimulation of CAT and APX activities by Se decreases the level of H_2O_2 in sunflower leaves, which may be a possible mechanism in plant defense strategy against As-induced oxidative stress. The possible mechanisms of the Se-enhanced resistance and/or tolerance of plants to As stresses have not been fully clarified.

5. CONCLUSION

Accumulating evidence suggested that Se activates protective mechanisms that can alleviate oxidative stress in both enzymatic and non-enzymatic ways. Therefore, based on these findings, Se might be able to down regulate As-induced oxidative damages through the inhibition of ROS production and indirectly by regulation of antioxidative system including GSH biosynthesis. The regulation of ROS levels by Se may be a key mechanism for counteracting Arsenic toxicity in sunflower plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cullen WR, Reimer KJ. Arsenic speciation in the environment. Chem Rev. 1989;89:713-764.
2. Meharg AA, Hartley WJ. Arsenic uptake and metabolism in arsenic resistant and non resistant plant species. New Phytologist. 2002;154:29–43.
3. Li WX, Chen TB, Huang ZC, Lei M, Liao XY. Effect of arsenic on chloroplast ultra structure and calcium distribution in arsenic hyperaccumulator *Pteris vittata* L. Chemosphere. 2006;62:803-809.
4. Tang T, Miller DM. Growth and tissue composition of rice grown in soil treated with inorganic copper, nickel, and arsenic. Commun Soil Sci Plant Anal. 1991; 22:2037-2045.
5. Frans R, Horton D, Burdette L. Influence of MSMA on straighthead, arsenic uptake

and growth response in rice (*Oryza sativa* L.). Arkansas AES Rep Ser. 1988;302:1-12.

6. Knauer K, Behra R, Hemond H. Toxicity of inorganic and methylated arsenic to algal communities from lakes along an arsenic contamination gradient. Aquatic Toxicol. 1999;46:221-230.
7. Smedley PL, Edmunds WM, Pelig-Ba KB. Mobility of arsenic in groundwater in the Obuasi gold-mining area Ghana: Some implications for human health. In: Appleton, J.D., Fuge, R., McCall, G.J.H. (Eds.), Environmental Geochemistry and Health. Geological Society Special Publication, London. 1996;113:163-181
8. Spallholz JE, Shriver BJ, Reid TW. Dimethyldiselenide and methylseleninic acid generate superoxide in an *In vitro* chemiluminiscence array in the presence of glutathione: Implications for the anticarcinogenic activity of L-selenomethionine and L-seleno methylselenocysteine. Nutr. Cancer. 2001; 40:34–41.
9. Fishbein L. In metals and their compounds in the environment; Merian, E., Ed.; VCH: Weinheim. 1991;1153-1189.
10. Hartikainen H, Xue T, Pironen V. Selenium as an anti-oxidant and pro-oxidant in ryegrass. Plant Soil. 2000;225:193-200.
11. Cartes P, Jara AA, Pinilla L, Rosas A, Mora ML. Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. Ann Appl Biol. 2010;156:297–307.
12. Hasanuzzaman M, Fujita M. Selenium pretreatment upregulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biol Trace Elem Res. 2011;143:1758–76.
13. Yao XQ, Chu JZ, Ba CJ. Antioxidant responses of wheat seedlings to exogenous selenium supply under enhanced ultraviolet-B. Biol Trace Elem Res. 2010;136:96–105.
14. Hasanuzzaman M, Hossain MA, Fujita M. Selenium-induced up-regulation of the antioxidant defense and methylglyoxal detoxification system reduces salinity-induced damage in rapeseed seedlings. Biol Trace Elem Res. 2011;143:1704–21.
15. Wang CQ. Water-stress mitigation by selenium in *Trifolium repens* L. J Plant Nut Soil Sci. 2011;174:276–82.
16. Djanaguiraman M, Devi DD, Shanker AK, Sheeba JA, Bangarusamy U. Selenium an antioxidative protectant in soybean during senescence. Plant Soil. 2005;272:77–86.
17. Turakainen M, Hartikainen H, Seppanen MM. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J Agr Food Chem. 2004;52:5378–82.
18. Hodges DM, DeLong JM, Forney CF, Prange RK. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 1999;207: 604-11.
19. Sergiev I, Alexieva V, Karanov E. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. CR Acad Bulg Sci. 1997;51:121–24.
20. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophy. 1959;82:70-77.
21. Mukherjee SP, Choudhuri MA. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Physiol Plant. 1983;58:166–70.
22. Beauchamp C and I. Fridovich. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 1971;44:276–87.
23. Aebi H. Catalase *In vitro*. Method Enzymol. 1984;105:121–126.
24. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 1981;22:867–80.
25. Drotar A, Phelps P, Fall R. Evidence for glutathione peroxidase activities in cultured plant cells. Plant. Sci. 1985;42:35-40.
26. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976; 72:248-54.
27. Malik JA, Goel S, Kaur N, Sharma S, Singh I, Nayyar H. Selenium antagonises the toxic effects of arsenic on mungbean (*Phaseolus aureus* Roxb.) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms. Environ Exp Bot. 2012;77: 242–248.

28. Garg N, Singla P. Arsenic toxicity in crop plants: Physiological effects and tolerance mechanisms. Environ Chem Lett. 2011;9: 303–321.
29. Schützendübel A, Polle A. Plant responses to abiotic stresses: Heavy metal induced oxidative stress and protection by mycorrhization. J Exp Botany. 2002;53: 1351-1365.
30. Feng R, Weic C, Tu S. The roles of selenium in protecting plants against abiotic stresses. Environ Exp Bot. 2013; 87:58-68.
31. Shanker K, Mishra S, Srivastava S, Srivastava R, Daas S, Prakash S. Study of mercury-selenium (Hg–Se) interactions and their impact on Hg uptake by the radish (*Raphanus sativus*). Plant Food Chem Toxicol. 1996;34:883-86.
32. Djanaguiraman M, Prasad PVV, Seppänen M. Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol Biochem. 2010;48: 999–1007.
33. Belzile N, Wu GJ, Chen YM, Appanna VD. Detoxification of selenite and mercury by reduction and mutual protection in the assimilation of both elements by *Pseudomonas fluorescens*. Sci Total Environ. 2006;367:704–14.
34. Terry N, Zayed AM, De Souza MP, Tarun AS. Selenium in higher plants. Annu Rev Plant Physiol Plant Mol Biol. 2000;51:401–32.
35. Chen Z, Silva H, Klessig D.F. Active oxygen species in the induction of plant systematic acquired resistance by salicylic acid. Science. 1993;262:1883–86.
36. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 2002;7:405–41.

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