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The ameliorative effect of selenium on *Azolla caroliniana* grown under UV-B stress
L'effet bénéfique du sélénium sur la croissance d'*Azolla caroliniana* soumis à un stress de rayonnement UV-B

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Résumé de l'article

L'exposition de plants d'*Azolla* au rayonnement UV-B pendant 6 h a provoqué une diminution de la biomasse et du taux de croissance relative (TCR), coïncidant avec une augmentation du temps de doublement (TD) comparativement au témoin. Aussi, le contenu en protéines a diminué. Par contre, le peroxyde d'oxygène (H_2O_2) et le malondialdéhyde (MDA) se sont accumulés de façon significative dans les plants d'*Azolla* traités par rayonnement UV. À l'inverse, l'ajout de 1 ppm de sélénium (Se) a provoqué une augmentation importante de la biomasse et du contenu en protéines des plants d'*Azolla* exposés ou non au rayonnement UV, ainsi qu'une réduction importante du H_2O_2 et du MDA. De plus, l'ajout de Se a provoqué une augmentation importante du contenu total d'ascorbate et de glutathion chez les plants d'*Azolla* exposés ou non au rayonnement UV comparativement au témoin et aux plants d'*Azolla* exposés au rayonnement UV en présence de Se. Aussi, le potentiel d'oxydoréduction de glutathion (NrH) a augmenté significativement chez les plants d'*Azolla* exposés au rayonnement UV en présence de Se. Il y a également eu une augmentation importante (38 %) de l'activité d'ascorbate peroxydase (APX) chez les plants exposés au rayonnement UV comparativement au témoin. L'activité APX en présence de Se n'a pas changé de façon significative comparativement au témoin. L'activité de glutathion réductase (GR) a augmenté significativement chez l'*Azolla* exposé au rayonnement UV, mais ce n'était pas le cas pour l'activité de glutathion peroxydase (GSH-PX). Toutefois, l'activité de GSH-PX et l'activité de GR dans les plants d'*Azolla* traités ou non par rayonnement UV ont été rehaussées de façon significative par l'application de Se aux milieux nutritifs à une concentration de 1 ppm. Ainsi, nous pouvons conclure que le Se protège les plants d'*Azolla* du stress relié au rayonnement UV-B.

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Exposure of *Azolla* plants to UV-B radiation for 6 h resulted in a decrease in biomass and relative growth rate (RGR), which coincided with an increase in doubling time (DT) as compared with the control. Also, the protein content decreased. On the other hand, hydrogen peroxyde (H_2O_2) and malondialdehyde (MDA) accumulated significantly in UV-treated *Azolla* plants. Conversely, the addition of selenium (Se) at 1 ppm resulted in a significant increase in biomass and protein content of untreated and UV-treated *Azolla* plants, and a significant reduction in both H_2O_2 and MDA. Moreover, the addition of Se to UV-treated and untreated *Azolla* plants resulted in a significant increase in total ascorbate and total glutathione (GSH) contents compared with the control and UV-stressed *Azolla* plants. Also, glutathione redox potential (GSH/TG) increased significantly in UV-treated *Azolla* plants in the presence of Se. There also was a significant increase (38%) in ascorbate peroxidase (APX) activity in UV-treated plants compared with the control. APX activity in the presence of Se did not change significantly compared with the control. Glutathione reductase (GR) activity increased significantly in UV-treated *Azolla*, while glutathione peroxidase (GSH-PX) activity did not. On the other hand, both GSH-PX and GR activity in untreated and UV-treated *Azolla* plants were significantly enhanced by the application of Se to the nutrient media at a concentration of 1 ppm. Therefore, we can conclude that Se protects *Azolla* plants from UV-B stress.

Keywords: ascorbate, *Azolla caroliniana*, glutathione, glutathione peroxidase, selenium, UV-B radiation.

[L'effet bénéfique du sélénium sur la croissance d'*Azolla caroliniana* soumis à un stress de rayonnement UV-B]

L'exposition de plants d'*Azolla* au rayonnement UV-B pendant 6 h a provoqué une diminution de la biomasse et du taux de croissance relative (TCR), coïncidant avec une augmentation du temps de doublement (TD) comparativement au témoin. Aussi, le contenu en protéines a diminué. Par contre, le peroxyde d'oxygène (H_2O_2) et le malondialdéhyde (MDA) se sont accumulés de façon significative dans les plants d'*Azolla* traités par rayonnement UV. À l'inverse, l'ajout de 1 ppm de sélénium (Se) a provoqué une augmentation importante de la biomasse et du contenu en protéines des plants d'*Azolla* exposés ou non au rayonnement UV, ainsi qu'une réduction importante du H_2O_2 et du MDA. De plus, l'ajout de Se a provoqué une augmentation importante du contenu total d'ascorbate et de glutathion chez les plants d'*Azolla* exposés ou non au rayonnement UV comparativement au témoin et aux plants d'*Azolla* exposés au rayonnement UV en présence de Se. Aussi, le potentiel d'oxydoréduction de glutathion (NrH) a augmenté significativement chez les plants d'*Azolla* exposés au rayonnement UV en présence de Se. Il y a également eu une augmentation importante (38 %) de l'activité d'ascorbate peroxydase (APX) chez les plants exposés au rayonnement UV comparativement au témoin. L'activité APX en présence de Se n'a pas changé de façon significative comparativement au témoin. L'activité de glutathion réductase (GR) a augmenté significativement chez l'*Azolla* exposé au rayonnement UV, mais ce n'était pas le cas pour l'activité de glutathion peroxydase (GSH-PX). Toutefois, l'activité de GSH-PX et l'activité de GR dans les plants d'*Azolla* traités ou non par rayonnement UV ont été rehaussées de façon significative par l'application de Se aux milieux nutritifs à une concentration de 1 ppm. Ainsi, nous pouvons conclure que le Se protège les plants d'*Azolla* du stress relié au rayonnement UV-B.

Mots clés : ascorbate, *Azolla caroliniana*, glutathion, glutathion peroxydase, rayonnement UV-B, sélénium.

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INTRODUCTION

Selenium (Se) is an important element for human and animal nutrition, due to its role in a series of biochemical reactions enhancing antioxidant activity (Rayman 2002). In contrast, Se is not considered essential for plants (Terry *et al.* 2000; Kápolna *et al.* 2009). Nevertheless, several studies have shown that at low concentrations, Se exerts a beneficial effect on the growth and stress tolerance of plants by enhancing their antioxidative capacity (Xue and Hartikainen 2000; Xue *et al.* 2001; Djanaguiraman *et al.* 2005; Kong *et al.* 2005; Lyons *et al.* 2009).

Many studies have shown deleterious UV effects, such as reduced photosynthesis and protein contents, impaired chloroplast function, and DNA damage (Agrawal 1992; Lesser *et al.* 1994). Ultraviolet radiation also produces oxidative stress that arises from the deleterious effects of active oxygen species (Costa *et al.* 2002; Ibrahim and Mostafa 2007) that react with lipids, pigments, proteins and nucleic acid (Dai *et al.* 1997; Nasibi and M-Kalantari 2005).

The addition of Se at low concentrations alleviated the oxidative stress caused by UV irradiation in lettuce (*Lactuca sativa* L.) and ryegrass (*Lolium perenne* L.) (Hartikainen and Xue 1999; Hartikainen *et al.* 2000), and in strawberry (*Fragaria* x *ananassa* Duch.) (Valkama *et al.* 2003). Pennanen *et al.* (2002) reported that Se, in addition to increasing the growth of plants, delayed the death of plants subjected to severe UV stress. Furthermore, at an optimal level, Se was able to increase the antioxidative capacity of senescing plants and delay senescence in lettuce, ryegrass and soybean (*Glycine max* L.) (Xue *et al.* 2001; Djanaguiraman *et al.* 2005). Selenium also improved the recovery of potato plants from light and chilling stress (Seppänen *et al.* 2003), and enhanced salt resistance in sorrel (*Rumex patientia* L.) seedlings (Kong *et al.* 2005).

Several studies have shown that the protective role of Se against oxidative stress in higher plants coincided with enhanced glutathione peroxidase (GSH-PX) activity and decreased lipid peroxidation (Hartikainen *et al.* 2000; Xue *et al.* 2001; Djanaguiraman *et al.* 2005). Cartes *et al.* (2005) demonstrated that selenite was more efficient than selenate at promoting GSH-PX activity in ryegrass plants. In addition, Se affected the activity of catalase, superoxide dismutase, and glutathione S-transferase (Xue and Hartikainen 2000).

The addition of Se can influence activity and antioxidant levels in stressed plants and thus regulate reactive oxygen species (ROS) levels. In plants, GSH-PX is a powerful scavenger of H_2O_2 and lipid peroxide with the help of glutathione. GSH-PX is believed to be a key enzyme that can be widely and robustly activated by Se in various plants exposed to diverse environmental stresses (Feng *et al.* 2013).

GSH-PX and glutathione reductase (GR) appear to be a complementary pair of enzymes. While GSH-PX reduces toxic hydroperoxides that are formed as a result of oxidative stress, GR converts oxidized glutathione to its reduced form.

The role of Se in mitigating environmental stresses has been extensively investigated in animals and humans and, to a lesser extent, in plants. The aim of this study is to investigate the role of Se in mitigating UV-B radiation effect on biomass and on the antioxidant system in *Azolla caroliniana* Willd. plant.

MATERIALS AND METHODS

Plant material and growth condition

Azolla caroliniana (known as water velvet) was provided in 1982 by Prof. Weam El-Aggan from the Catholic University of Louvain (Louvain, Belgium); it was identified by Prof. G.A. Peters of Kettering Laboratory (Yellow Springs, OH, USA). The plants were acclimated in the greenhouse of the Faculty of Science of Alexandria University (Alexandria, Egypt), in 2500 mL polyethylene vessels that were filled with a nitrogen-free, modified Hoagland solution (2/5 concentration, pH 5.1) in which KNO_3 and $Ca(NO_3)_2$ were replaced by KCl and $CaCl_2$, respectively. About 5 g (fresh mass) of *Azolla* from the stock material were inoculated in each vessel to make a new subculture, and so on. The plants were freed from epiphytic microorganisms by thorough washing with distilled water. The cultures were grown in a growth chamber under a 16-h photoperiod at irradiance of 1200 µmol m^{-2} s^{-1} (cool white fluorescent tubes) and light/dark temperatures of 28-30/20-25°C (stock culture). Before being used, the plants were surface-sterilized with 0.2% Clorox (El-Aggan 1982), then thoroughly washed with distilled water.

Selenium treatment and growth estimation

5 g of *Azolla* plants were transferred to 250 mL vessels containing 2/5 modified Hoagland solution and treated with 1 ppm Se in the form of sodium selenite (Hassan and Mostafa 2015) for 7 d, and were then exposed to UV-B radiation supplied by three UV-emitting tubes (TL12/100W/01, Philips, Holland) positioned 50 cm above leaf level. Rack height, lamp spacing and lamp power were adjusted as described by Ibrahim and Mostafa (2007) to maintain a total daily flux of biologically effective UV-B radiation doses of 5.75 KJ $m^{-2}d^{-1}$. Plants were rotated under the lamp banks to minimize potential effects resulting from microenvironment variations for 6 h. Samples were taken for chemical analyses. The number of generations and doubling time (DT) were determined from the fresh mass and duration of experiment using the equation given in Peters *et al.* (1979): n (final mass) = n° × 2G, where G = number of generations, n° = initial mass of *Azolla* plants (mass of inoculum); DT = duration of experiment per generation. Relative growth rate (RGR) was calculated by using the formula of Subudhi and Watanabe (1981): RGR [kg $kg^{-1}d^{-1}$] = 0.693 DT^{-1}.

Estimation of hydrogen peroxide (H_2O_2)

Hydrogen peroxide content was determined according to Velikova *et al.* (2000). Plant tissues (50 mg) were homogenized in an ice bath with 5.0 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 rpm for 15 min. The supernatant was used in the assay for H_2O_2. The reaction mixture contained 0.5 mL of plant extract, 0.5 mL of 10 mM potassium phosphate buffer (pH), and 0.1 mL 1 M KI. The content of H_2O_2 was calculated by comparing it

with a standard calibration curve using different concentrations of H_2O_2, and the results were expressed as µmol H_2O_2 g^{-1} f.m.

Estimation of lipid peroxidat

Estimation of lipid peroxidat was measured as described by Zhang *et al.* (2007). About 1.5 mL of plant extract was homogenized in 2.5 mL of 5% thiobarbituric acid (TBA) dissolved in 5% TCA. The mixture was heated at 95°C for 15 min, then quickly cooled on ice and centrifuged at 5,000 rpm for 10 min. The absorbance of the supernatant was measured at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value measured at 600 nm. The concentration of malondialdehyde (MDA) was calculated in µmol g^{-1} f.m.

Estimation of ascorbate and glutathione levels

Reduced glutathione was determined using the method described in Moron *et al.* (1979). A homogenate was prepared with 0.5 g of plant extract with 5 mL of 5.0% TCA, and it was then centrifuged at 1,000 rpm for 10 min. The supernatant was used for the estimation of GSH with 2 mL of freshly prepared 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) solution to 0.1 mL of plant extract and 0.9 mL sodium phosphate buffer (pH 8). The intensity of the resulting yellow colour was measured using a spectrophotometer at 412 nm after 10 min. The values are expressed as nmol GSH g^{-1}. GSH estimation was done using the method of Griffith (1980) by adding 0.1 mL of neutralized supernatant to a standard solution mixture consisting of 0.5 mL of 0.1 M sodium phosphate buffer (pH 7.5) containing ethylenediaminetetraacetic acid (EDTA), 0.2 mL of 6 mM DTNB, 0.1 mL of 2 mM nicotinamide adenine dinucleotide phosphate (NADPH), and 0.1 mL of 1 U yeast GR. Changes in absorbance at 412 nm were monitored at 25 ± 2°C until the absorbance reached 5 U.

Extraction and estimation of ascorbate were done using the method described by Oser (1979). The reaction mixture consisted of 2% sodium molybdate, 0.2 mL 0.15 N H_2SO_4, 0.1 mL 1.5 mM Na_2HPO_4, and 1 mL of tissue extract. The mixture was incubated at 60°C in a water bath for 40 min. After centrifugation at 3,000 g for 10 min, absorbance was read at 660 nm.

Preparation of enzyme extract and assay of enzyme activity

Leaf tissues (0.5 g) were ground to a fine powder in liquid N_2 and then homogenized in 0.2 mL of 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM D-isoascorbic acid, 2% (w/v) polyvinylpyrrolidone (PVP) and 0.05% (w/v) Triton X-100 using a chilled pestle and mortar, following the method of Gossett *et al.* (1994). The homogenate was centrifuged at 10,000 x g for 10 min at 4°C, and the supernatant was collected and used for assays of ascorbate peroxidase (APX), GSH-PX and GR. Protein concentrations in the enzyme extract and plant extract were determined according to the method of Bradford (1976) using defatted bovine serum albumin (BSA) (Sigma, fraction V) as a standard.

Ascorbate peroxidase (EC 1.11.1.11)

APX was assayed as described by Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 0.2 mM EDTA, 0.5 mM ASA and 0.25 mM H_2O_2. The reaction was started at 25°C by adding H_2O_2 after having added the enzyme extract. The decrease in absorbance at 290 nm was recorded for 1 min and the amount of ascorbate oxidized was calculated from the extinction coefficient 2.8 mM^{-1} cm^{-1}. Enzyme activity was expressed in terms of µmol ascorbate oxidized mg^{-1} protein min^{-1}.

Glutathione peroxidase (EC 1.11.1.9)

GSH-PX activity was modified from Flohé and Günzler (1984). For the enzyme reaction, 0.2 mL of the supernatant was mixed with 0.4 mL GSH (Sigma product), and the mixture was put in an ice bath for 30 min. The mixture was then centrifuged for 10 min at 3,000 rpm; 0.48 mL of the supernatant was placed in a cuvette, and 2.2 mL of 0.32 M $NaHPO_4$ and 0.32 mL of 1.0 mM 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB, Sigma) were added for colour development. The absorbance at 412 nm was measured with a spectrophotometer after 5 min. Enzyme activity was calculated as a decrease in GSH with reaction time compared with that of the non-enzyme reaction.

Glutathione reductase (EC 1.6.4.2)

GR activity was determined at 25°C by measuring the rate of NADPH oxidation as the decrease in absorbance at 340 nm (extinction coefficient = 6.2 $mM^{-1}cm^{-1}$) according to the method of Halliwell and Foyer (1978). The reaction mixture (0.1 mL) consisted of 100 mM Tris-HCl (pH 7.8), 21 mM EDTA, 0.005 mM NADPH, 0.5 mM oxidized glutathione (GSSG), and the enzyme. NADPH was added to start the reaction. For APX, GPX and GR, enzyme activity was expressed in µmol mg^{-1} protein min^{-1}.

Statistical analysis

A statistical analysis was done using the Statistical Package for Social Sciences (SPSS Statistics 20.0, IBM Corporation, Armonk, NY, USA) software. Arithmetic mean, standard deviation, and ANOVA for more than two groups were used, followed by Duncan's multiple range test (MRT) to detect significant differences between the two groups. The level of significance was set at 0.05.

RESULTS AND DISCUSSION

The exposure of *Azolla* plants to UV-B radiation for 6 h resulted in a decrease in biomass and RGR, which coincided with an increase in DT as compared with the control. Also, the protein content decreased. Compared with the control, the reduction in biomass and protein content was 13% and 22%, respectively. Similarly, several studies have reported that exposure of plants to UV radiation resulted in a marked decrease in fresh mass and protein content (Agrawal 1992; Nasibi and M-Kalantari 2005). It has been reported that a reduction in fresh mass under UV radiation might be related to the enhancement of ROS generation (Mahdavian *et al.* 2008) and hence to the destruction of plasma membranes (Dai *et al.* 1997; Selvakumar 2008). Thus, the reduction in biomass in UV-treated *Azolla* plants might be related to a decrease in water absorption resulting from the destruction of plasma membranes and proteins due

to generation of ROS. Nasibi and M-Kalantari (2005) reported that ROS destroy macromolecules such as proteins, polysaccharides, lipids and DNA.

Conversely, the addition of Se at a concentration 1 ppm resulted in a significant increase in biomass and protein content of untreated and UV-treated *Azolla* plants (Table 1). With Se, UV-treated *Azolla* plants' biomass and protein content were 1.8 to 1.6-fold those of UV-treated plants without Se, respectively (Table 1). Similarly, some studies have demonstrated the benefits of adding a small amount of Se, including increased tuber yield and starch concentration in young potato leaves (Turakaine *et al.* 2004), and increased growth in ryegrass and lettuce exposed to UV-B radiation (Hartikainen and Xue 1999; Pennanen *et al.* 2002).

The positive synergistic effect of Se and UV radiation was found to be partly associated with the antioxidative role of Se in increasing GSH-PX activity (Xue and Hartikainen 2000). In this investigation, the addition of Se at the optimum concentration (1 ppm) to UV-treated *Azolla* growth media coincided with the promotion of plant growth and protein content, thus indicating that Se may have particular biological functions in the alteration of the antioxidant defense system.

Data in Table 2 show a significant accumulation of H_2O_2 and MDA in UV-treated *Azolla* plants. H_2O_2 and MDA contents were 2.6 and 1.9-fold those of the control, respectively. It has been shown that the induction of ROS generation was an early effect of UV radiation in plants (Kakani *et al.* 2003). In this study, the exposure of *Azolla* plants to UV radiation enhanced the accumulation of H_2O_2 and MDA contents. In accordance with these observations, Wang *et al.* (2010) reported that the production of O_2^- and H_2O_2 in *Zea* plants increased with UV-B radiation and that their continuous accumulation resulted in lipid peroxidation. Also, Nasibi and M-Kalantari (2005) suggested that peroxy radicals could abstract hydrogen from other unsaturated fatty acids, thus leading to a chain reaction of peroxidation and breakdown of the structure and function of membrane lipids of rapeseed (*Brassica napus* L.) exposed to UV-B radiation.

On the other hand, in this study, the addition of Se at a concentration of 1 ppm to the nutrient medium of UV-treated or untreated *Azolla* plants significantly reduced H_2O_2 and MDA contents, thus revealing that the addition of Se results in a marked reduction in ROS generation, which is possibly due to the reactivation of antioxidants, especially the H_2O_2 quencher (e.g. GSH-PX) in *Azolla* plants. Other studies have shown that an optimal Se concentration inhibits lipid peroxidation in many stressed plants (Cartes *et al.* 2005; Yao *et al.* 2010; Kumar et *al.* 2012).

In the present study, the addition of Se to UV-treated and untreated *Azolla* plants resulted in a significant increase in total ascorbate and GSH contents compared with the control and UV-stressed *Azolla* plants (Table 3). Also, glutathione redox potential (GSH/TG) increased significantly with the addition of Se to UV-treated *Azolla* plants. These observations could reflect the enhancement of GR activity to allow the reducing agent (GSH) to eliminate H_2O_2 through APX or GSH-PX. Reduced glutathione plays an important role in the antioxidant defense system of *Azolla* since it participates in the regeneration of ascorbate through dehydroascobate reductase, and it can also react with singlet oxygen and OH radical to protect protein thiol groups (Asada 1994). The enhancement of ascorbate and glutathione levels by Se compared with the control and UV-stressed *Azolla* plants reflects the capacity of GSH to synthesize organic Se. This conclusion is supported by Hasanuzzaman and Fujita (2011) who found that ASA and GSH contents were enhanced by Se in both salt- and drought-stressed rapeseed seedlings.

Table 1. Growth characterized by biomass, doubling time (DT), relative growth rate (RGR) and total protein of *Azolla* plants exposed to UV-B radiation for 6 h with or without Se (1 ppm) after 7 d. Values are mean ± SD (n = 3).

Treatment	Biomass [g culture^{-1}]	DT [d]	RGR [Kg Kg^{-1}d^{-1}]	Protein [mg g^{-1} d.m.]
Control	10.17 ± 1.03	6.88	0.10	30.27 ± 3.01
Se	12.80 ± 1.17*	5.47	0.13	55.6 ± 5.65*
UV	8.82 ± 0.98	7.90	0.09	25.6 ± 2.65*
Se + UV	15.57 ± 1.56*	4.50	0.15	41.3 ± 4.001*
p	0.013*	_	_	0.013*

*Difference is significant at $p \leq 0.05$.

Table 2. Changes in H_2O_2 content and lipid peroxidation (µmol g^{-1} f.m.) in *Azolla* plants exposed to UV-B radiation for 6 h with or without Se (1 ppm) after 7 d. Values are mean ± SD (n = 3).

Treatment	H_2O_2	MDA
Control	0.36 ± 0.036	3.40 ± 0.021
Se	0.08 ± 0.0112*	1.35 ± 0.0738*
UV	0.92 ± 0.085*	6.37 ± 0.062*
Se + UV	0.18 ± 0.0012*	2.24 ± 0.204*
p	0.025*	0.016*

* Difference is significant at $p \leq 0.05$.

There was a significant (38%) increase in APX activity in UV-treated plants compared with the control. APX activity did not change significantly in the presence of Se compared with the control. GR activity increased significantly, while GSH-PX activity did not change significantly in UV-treated *Azolla* plants compared with the untreated control. On the other hand, both GSH-PX and GR activities were significantly enhanced by the application of Se at a concentration of 1 ppm to the nutrient media of untreated and UV-treated *Azolla* plants. There was a 2.7-fold increase in GSH-PX activity with Se application in UV-treated plants compared with the control (Table 4).

GSH-PX, with the help of GSH, is a powerful scavenger of H_2O_2 and lipid hydroperoxides. GSH-PX is believed to be a key enzyme that can be widely and robustly activated by Se in various plants exposed to diverse environmental stresses (Feng *et al.* 2011). In this study, under UV-stress and in the absence of Se, H_2O_2 in *Azolla* plants may be quenched by APX, while in the presence of Se, H_2O_2 is primarily scavenged by GSH-PX instead of APX. Similar results were obtained with *Chlamydomonas* cells (Takeda *et al.* 1997). The increased activity of the GSH-PX enzyme in response to Se suggests a unique role for this enzyme in counteracting oxidative stress in plants. Se-pretreated rapeseed seedling exhibited increased ASA levels and significantly enhanced APX, dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), GSH-PX and catalase activity compared with drought-stressed plants without Se (Hasanuzzaman and Fujita 2011). The positive synergistic effect of Se and UV radiation was found to be associated with the antioxidative role of Se through increased GSH-PX and catalase activity, whereas APX responded negatively (Xue and Hartikainen 2000).

The insignificant changes in ascorbate content of UV-treated *Azolla* plants in the absence of Se may be due to its participation in reducing H_2O_2, which is catalyzed by a high level of APX activity. Ascorbate is the principal electron donor and it is oxidized to dehydroascorbate in the reaction of APX with H_2O_2, then reduced back to ascorbate in the presence of GR (Zhang and Kirkham 1996). Hence, the levels of ascorbate and GSH play an important role in oxidative defense. These insignificant changes in ascorbate level might be correlated with the increased activity of APX and GR or could be due to its participation in the ascorbate-glutathione oxidative pathway. It was shown that there was a decrease in ascorbate level in *Bruguiera parviflora* (Roxb.) Wight & Arn., which is likely due to its role in reducing H_2O_2 to H_2O (Parida *et al.* 2004).

CONCLUSION

Several studies reported that the addition of Se enhanced the biosynthesis of glutathione fractions and activity of peroxidases (Hasanuzzaman and Fujita 2011; Feng and Wei 2012; Malik *et al.* 2012) as well as GR activity (Mittler 2002). As shown in this study, the addition of Se caused a significant increase in GSH-PX, GSH/TG as well as GR activity. These observations were accompanied with a significant decrease in H_2O_2 and MDA contents. These results might indicate that the application of Se can influence the activity of GSH-PX for scavenging ROS through the ascorbate-glutathione cycle. Therefore, Se might protect *Azolla* plants from UV stress.

Table 3. Changes in ascorbate, reduced glutathione, oxidized glutathione (nmol g^{-1} f.m.), total glutathione (TG) and GSH/TG ratio in *Azolla* plants exposed to UV-B stress for 6 h with or without Se (1 ppm) after 7 d. Values are mean ± SD (n = 3).

Treatment	Ascorbate	GSSG	GSH	TG	GSH/TG
Control	3.39 ± 0.32	0.95 ± 0.058	9.70 ± 0.68	10.65 ± 1.1	0.91
Se	4.22 ± 0.41*	4.68 ± 0.51*	13.04 ± 1.1*	17.72 ± 2.65*	0.74
UV	3.72 ± 0.32	4.77 ± 0.36*	2.73 ± 0.29*	7.50 ± 1.07*	0.36
Se + UV	4.96 ±0.43*	4.75 ± 0.48*	9.56 ± 1.02*	14.31 ± 1.33*	0.67
p	0.021*	0.001*	0.001*	0.013*	_

* Difference is significant at $p \leq 0.05$.

Table 4. Changes in ascorbate peroxidase, glutathione peroxidase and glutathione reductase specific activity (µmol mg^{-1} protein min^{-1}) in *Azolla* plants exposed to UV-B stress for 6 h with or without Se (1 ppm) after 7 d. Values are mean ± SD (n = 3).

Treatment	APX	GSH-PX	GR
Control	8.68 ± 0.98	7.66 ± 0.78	2.07 ± 0.365
Se	8.87 ± 0.89	9.42 ± 0.95*	3.04 ± 0.365*
UV	11.95 ± 1.23*	8.27 ± 0.86	3.53 ± 0.541*
Se + UV	7.43 ± 0.85	20.74 ± 1.23*	7.72 ± 0.98*
p	0.035*	0.01*	0.001*

* Difference is significant at $p \leq 0.05$.

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