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The protective role of selenium in *Vicia faba* L. subjected to cadmium stress

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Selenium (Se) is an essential micronutrient for many organisms, including plants, animals and humans. Selenium has also been shown to counteract various abiotic stresses induced in plants, but the associated mechanisms are rather complicated and still remain to be fully elucidated. Thus, this study was undertaken to evaluate the effect of selenium (as sodium selenite, Na_2SeO_3) at recommended dose (100 μM), a promising plant development regulatory substance in alleviating the deteriorative effect of cadmium (as cadmium chloride, CdCl_2) at 200 μM on faba bean plants. The results revealed that cadmium-stressed faba bean plants decreased significantly the growth characteristics (plant height, leaf area, dry weight and weight of 100 seed), photosynthetic pigments (chlorophyll *a+b* and carotenoids), photosynthetic activity ($^{14}\text{CO}_2$ -assimilation), antioxidant enzymes activity (superoxide dismutase, peroxidase and catalase), Ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBPCase) activity, glycinebetaine, total protein and macronutrient contents (N, P and K). Meanwhile, it increased significantly lipid peroxidation (malondialdehyde, MDA), H_2O_2 and free proline. Pretreatment of seeds with selenium (100 μM), for 12 h resulted in amelioration of the harmful effects of cadmium and increased finally the total yield

Keywords: *Vicia faba* L., Selenium, Photosynthetic activity ($^{14}\text{CO}_2$ -assimilation), Cadmium.

INTRODUCTION

Faba bean, *Vicia faba* L., is one of the world's most important legume crops. In Egypt it is used in the human diet, as animal feed, and for industrial purposes (Moussa, 2008).

Se is an essential micronutrient for animals and humans and appears to be a beneficial element for many plants (Feng et al, 2013). Se might act as an antioxidant, alleviating oxidative stress induced by environmental stressors, thus improving abiotic stress tolerance in plants (Feng et al, 2013). There is ample evidence for the ability of Se to improve plant resistance to cold, drought, salinity and heavy metals (Balakhnina and Nadezhkina, 2017). Selenium may regulate the reactive oxygen species (ROS) level in plants by stimulating spontaneous dismutation of $\text{O}_2^{\cdot-}$ to

H_2O_2 via direct interactions between ROS and Se-containing compounds and through the control of antioxidant enzyme activity (Feng et al., 2013). In addition, Kong et al. (2005) demonstrated that Se application maintained chloroplast and mitochondria ultrastructure, thereby improving photosynthesis and enhancing salt tolerance in sorrel seedlings. Furthermore, Se application significantly reversed the negative effects of salinity on the photochemical efficiency of photosystem II (PSII) in tomato seedlings (Diao et al, 2014).

The intensive use of high-phosphate fertilizers increased accumulation of metal ions, especially cadmium, in the soil (Taylor, 1997). Increased concentrations of cadmium in the environment have given rise to serious concern, because in the

form of Cd^{2+} cation it is highly mobile in soil and toxic to plants, animals and humans (Shukla et al, 2003). Cadmium is readily taken up by the cells of different plant species (Liu et al, 2007) and induces many morphological, physiological, biochemical and structural changes in plants, such as water imbalance, inhibition of seed germination, inhibition in photosynthesis, reduction of growth especially the root growth, disturbances in mineral nutrition, and sugar metabolism and therefore, strongly influences biomass production (Moussa, 2004) and finally can cause plant death (Kahle, 1993). Cadmium produces alterations in the membranes by inducing changes in their lipid composition (Ouariti et al, 1997) and affects the activities of enzymes associated with membranes, such as that of H^+ -ATPase (Fodor et al, 1995). Cadmium decreases photosynthetic rate due to reduced chlorophyll content and the enzymatic activity involved in CO_2 fixation (Greger and Ögren, 1991). In many plants Cd enhances the level of lipid peroxidation and alteration in antioxidant systems (Somashekaraiah et al, 1992). Harmful effects produced by Cd^{2+} might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins (Gouia et al, 2004).

The objectives of this study were to provide more information to better understand the mechanisms of the Se-induced enhancement of faba bean plant subjected to cadmium toxicity.

MATERIALS AND METHODS

Plant Materials and Growth Conditions.

Uniform-sized faba bean (*Vicia faba* L.) cv. Giza 3 was purchased from the Crop Institute, Agriculture Research Center, and Giza, Egypt. The caryopsis was kept at 4°C . Seed were surface sterilized in 0.1 % (w/v) sodium dodecyl sulphate solution and thoroughly rinsed with sterile deionized water. The seeds were then soaked overnight (12 h) in either distilled water or $100\ \mu\text{M}$ freshly prepared selenium solution (as sodium selenite (Na_2SeO_3)) because it is more efficient than selenate, Hawrylak-Nowak (2015).

The seeds were germinated in pots (35cm high \times 30 cm diameter), each filled with 15 kg sandy loam soil. Ten seeds per treatment were sown in each pot at 3 cm depth. After emergence, the seedlings were thinned to four healthy seedlings per pot. Then, the stress treatments were imposed abruptly after 15 days by cadmium solution (200 mM), as cadmium chloride (CdCl_2). Plants were grown in a controlled environment

growth chamber with 15-h photoperiod; 65%–75% relative humidity; and day and night temperatures of 22 and 20°C . Photosynthetic photon flux density at maximum plant height was about $300\ \mu\text{M}\ \text{m}^{-2}\text{s}^{-1}$. Fertilization was by application of calcium super-phosphate (15.5 % P_2O_5) at a rate of 3 g/pot added during soil preparation. Potassium sulphate (48 % K_2O) was applied at a rate of 1.5 g/pot added in 2 equal doses during soil preparation and at 21 days after sowing. Ammonium sulphate (21.6 % N) was applied at a rate of 1 g/pot added in 3 applications at 21, 45 and 63 days after sowing. Cultural practices, such as weed control and irrigation, were performed as needed. The experimental design was randomized complete block design with three replicates. The treatments are, water (control), cadmium, CdCl_2 (200 μM), selenium (100 μM), and cadmium (200 μM) + selenium (100 μM).

Half of the seedlings were harvested 60 days after treatment with selenium (flowering stage) to record morphological and biochemical characters. Plant height was measured from the root collar to the tip of the highest leaf. Leaf area of green plus senesced leaves was determined with a leaf area meter (model LI 3100, LICOR, Lincoln, Neb.). For total dry matter production plant samples were dried in a convection oven at 70°C for 2 days and weighed.

From the other half of seedlings, weight of 100 seed were recorded at five months after treatment with selenium.

Chemical Analysis.

Chlorophyll *a*, *b* and total carotenoids were determined according to the method of according to Inskeep and Bloom (1985). Photosynthetic activity ($^{14}\text{CO}_2$ -fixation) was measured in the Radioisotope Department, Atomic Energy Authority, Cairo, Egypt, with the method published previously Moussa and Mohamed (2016). One pot from each treatment was placed under a bell jar and $^{14}\text{CO}_2$ was generated inside this chamber by a reaction between 10 % HCl and 50 μCi ($1.87 \times 10^6\text{Bq}$) $\text{NaH}^{14}\text{CO}_3 + 100\ \text{mg}\ \text{Na}_2\text{CO}_3$ as a carrier. Then the samples were illuminated with a tungsten lamp ($440\ \mu\text{mol}\ \text{m}^{-2}\text{s}^{-1}$). After 30 min exposure, the leaves were quickly detached from the stem, weighed and frozen for 5 min to stop the biochemical reactions, then subjected to extraction by 80 % hot ethanol. The ^{14}C was assayed in the ethanolic extracts using a Bray Cocktail (Bray, 1960) and a Liquid Scintillation Counter (LSC2-Scaler Ratemeter SR7, Nuclear Enterprises). Ribulose-1, 5-bisphosphate-

carboxylase/oxygenase (RuBPCase, EC 4.1.1.39) was determined following Warren et al. (2000). Malondialdehyde (MDA) contents were determined as described by Chen et al. (2013). The peroxidase activity (POD; EC: 1.11.1.7) was estimated using the method of Thomas et al. (1981). The activity of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) was determined as described by Chance and Maehly (1995). Hydrogen peroxide was measured according to methods of Patterson et al. (1994). Free proline content was estimated photometrically in acidic ninhydrin assay according to the method adopted by (Bates et al., 1973). Total protein was estimated spectrophotometrically by Bradford (1976). The amount of glycinebetaine was estimated according to the method of Grieve and Grattan (1983). The concentration of selected elements (N, P, and K⁺) was determined by the method of Goldstein et al. (2003).

Statistical Analysis.

All obtained data were subjected to the statistical analysis and means were compared according to LSD at 5% level test described by Gomez and Gomez (1992).

RESULTS AND DISCUSSION

Growth characteristics.

Data presented in Table (1) revealed that Cd treated plants (200 µM) showed significant decrease in growth characteristics (plant height, leaf area, dry weight and weight of 100 seed) and these results are in agreement with that of (Moussa and Sabah, 2010). The growth inhibition produced by Cd could be at least partially due to the effect of this heavy metal on the photosynthesis rate (Metwally et al, 2003). Meanwhile, plants pretreated with selenium (100 µM) showed a significant increase in this parameter (Moussa and Ahmed, 2010).

Photosynthetic pigments, photosynthetic activity (¹⁴CO₂-assimilation) and Rubisco.

Data presented in Table (2) revealed that Cd treated plants (200 µM) showed significant decrease in photosynthetic pigments (chlorophyll *a+b* and carotenoids), photosynthetic activity

(¹⁴CO₂-assimilation) and RuBPCase activity as compared to the control plants (Moussa and Sabah, 2010). Meanwhile, plant treatment with selenium (100 µM) showed a significant increase in this parameter (Moussa and Ahmed, 2010). Feng et al. (2013) reported that, the restoration of photosynthesis in stressed plants after Se application may be closely related to the decreased ROS levels, reactivation of antioxidants, restored structure of the damaged chloroplasts and enhanced production of other vital metabolites (such as GSH and SH-like substances). On the other hand, Se enhancement effect was attributed to its effect in stimulation of chlorophyll formation and protection of photosynthetic apparatus and consequently decreased the damage caused by water stress (Pennanen et al, 2002). Also, Se treatments increased photosynthetic activity (Djanaguiraman et al, 2010 and Wang et al, 2012)

Antioxidant enzyme activities (catalase, peroxidase and superoxide dismutase), glycinebetaine, lipid peroxidation levels (malondialdehyde), free proline, H₂O₂ and total protein.

The results presented in Table (3) revealed that Cd treated plants showed a significant decrease in antioxidant enzyme activities (superoxide dismutase, peroxidase and catalase), glycinebetaine and total protein as compared to the control plants (Moussa and Sabah, 2010). Plants pre-treated with selenium increased significantly this parameter (Moussa and Ahmed, 2010). Meanwhile, Cd treated plants showed a significant increase in lipid peroxidation levels (malondialdehyde), free proline and H₂O₂ as compared to the control plants. These results were confirmed by the results of Maria et al. (2006). Also, Se treatments decreased the lipid peroxidation levels (malondialdehyde), free proline and H₂O₂ (Hawrylak-Nowak, 2009, Moussa and Ahmed, 2010 and Azadeh et al, 2012). Se reduced the lipid peroxidation and exerted positive effects on the cell membrane stability. Accumulation of GB represents a major biochemical adaptation in several bacteria, plants and animals (Rhodes and Hanson, 1993).

Table 1. Effect of cadmium in faba bean plants pretreated with selenium on growth characteristics (plant height, leaf area, dry weight and weight of 100 seed).

| Treatments | Plant height (cm) | Leaf area (cm ² /plant) | Dry weight (g/plant) | Weight of 100 seed(g) |
|-------------------------------------|-------------------|------------------------------------|----------------------|-----------------------|
| Control | 38 ^c | 91 ^c | 10.4 ^c | 60.8 ^c |
| Cadmium (200 µM) | 26 ^d | 68 ^d | 6.2 ^d | 41.5 ^d |
| Selenium (100 µM) | 46 ^a | 123 ^a | 15.3 ^a | 95.2 ^a |
| Cadmium (200µM) + Selenium (100 µM) | 40 ^b | 102 ^b | 11.1 ^b | 64.1 ^b |

^aValues in a column followed by the same letter are not significantly different; $P \leq 0.05$, Duncan's multiple range test. Data are means of three replicates.

Table 2. Effect of cadmium in faba bean plants pretreated with selenium on photosynthetic pigments (chlorophyll *a+b* and carotenoids, mg g⁻¹FW), photosynthetic activity (10³ Becquerel mg⁻¹FW) and RuBPCase (mg.g⁻¹FW).

| Treatments | Chlorophyll (a+ b) | Carotenoids | Photosynthetic Activity | RuBPCase |
|-------------------------------------|--------------------|-------------------|-------------------------|-------------------|
| Control | 5.03 ^c | 2.13 ^c | 25.7 ^c | 22.6 ^b |
| Cadmium (200 µM) | 3.01 ^d | 0.93 ^d | 14.6 ^d | 14.7 ^d |
| Selenium (100 µM) | 7.25 ^a | 3.72 ^a | 41.4 ^a | 35.2 ^a |
| Cadmium (200µM) + Selenium (100 µM) | 5.26 ^b | 2.33 ^b | 27.1 ^b | 21.9 ^c |

^aValues in a column followed by the same letter are not significantly different, $P \leq 0.05$, Duncan's multiple range test. Data are means of three replicates.

Table 3. Effect of cadmium in faba bean plants pretreated with selenium on SOD (units mg⁻¹protein), POD (units mg⁻¹protein), CAT (µMH₂O₂/min.gFW), glycinebetaine (µM g⁻¹DW), MDA (µM g⁻¹FW), free proline (µmol g⁻¹FW), H₂O₂ (µM g⁻¹FW) and total protein (mg g⁻¹FW).

| Treatments | SOD | POD | CAT | Glycine-betaine | MDA | Free proline | H ₂ O ₂ | Total protein |
|-------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------------------|-------------------------------|--------------------|
| Control | 19.2 ^b | 33.7 ^c | 16.3 ^c | 18.7 ^c | 24.7 ^b | 523 ^c | 33.2 ^c | 117.2 ^c |
| Cadmium (200µM) | 10.1 ^d | 19.4 ^d | 7.8 ^d | 9.4 ^d | 41.8 ^a | 810 ^a | 62.8 | 63.8 ^d |
| Selenium (100 µM) | 34.3 ^a | 49.6 ^a | 26.1 ^a | 32.5 ^a | 25.4 ^b | 518 ^d | 34.2 ^c | 144.8 ^a |
| Cadmium (200µM) + Selenium (100 µM) | 18.2 ^b | 35.2 ^b | 17.4 ^b | 22.1 ^b | 28.2 ^c | 543 ^b | 38.5 ^b | 128.6 ^b |

^aValues in a column followed by the same letter are not significantly different, $P \leq 0.05$, Duncan's multiple range test. Data are means of three replicates.

Table 4. Effect of cadmium in faba bean plants pretreated with selenium on the macronutrient contents of N, P and K (mg g⁻¹DW).

| Treatments | N | P | K |
|---|--------------------|--------------------|--------------------|
| Control | 102.7 ^b | 99.4 ^a | 88.7 ^a |
| Cadmium (200 µM) | 72.6 ^d | 52.1 ^c | 62.8 ^c |
| Selenium (100 µM) | 126.8 ^a | 112.8 ^a | 123.8 ^a |
| Cadmium (200µM) + Selenium (100 µM) | 101.3 ^c | 88.2 ^b | 90.4 ^b |

^aValues in a column followed by the same letter are not significantly different, $P \leq 0.05$, Duncan's multiple range test. Data are means of three replicates.

Inorganic macronutrient contents (nitrogen, phosphorus and potassium).

It is evident from Tables (4) that the cadmium treatments decreased significantly the content of most major nutrient elements (N, P and K) and these results are in agreement with that of Keck (1978) who reported that Cd-induced inhibition in K uptake by oat (*Hordeum vulgare*) roots concluded that one of the first sites of Cd action is the plasmalemma K⁺ carrier (ATPase). Meanwhile, selenium treatments tended to counterbalance the Cd-induced changes in nutrients, and increased significantly total contents of N, P and K (Maria et al, 2010)

CONCLUSION

Pretreatment of faba bean seeds with selenium (100 µM), for 12 h resulted in amelioration of the harmful effects of cadmium and increased finally the total yield.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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