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Beneficial Effects of Selenium for Alleviating Cadmium Toxicity in *Pisum sativum* L.

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Abstract: Selenium (Se) has been proved to be an essential element for humans and animals. Selenium has also been found to be beneficial to plants. In Egypt, selenium deficiency in the diet is a common problem. To counteract this problem, Se compounds have been used to increase the Se content in the edible parts of crops, through foliar sprays or base application of fertilizers. Selenium has also been shown to counteract various abiotic stresses induced in plants by cold, drought, high light, water, salinity and heavy metals, but the associated mechanisms are rather complicated and still remain to be fully elucidated. This paper reports the effects of selenium application on some physiological characteristics of pea (*Pisum sativum* L.) exposed to cadmium (Cd) stress. Thus, this study was undertaken to evaluate the effect of selenium (as sodium selenate, Na_2SeO_4) at recommended dose (50 µM), a promising plant development regulatory substance in alleviating the deteriorative effect of cadmium (as cadmium chloride, $CdCl_2$) at 100 µM on pea plants.

The results revealed that cadmium-stressed pea plants treated with selenium had increased levels of photosynthetic pigments (chlorophyll a+b and carotenoids) as compared with cadmium-stressed plants. The level of antioxidant system components (superoxide dismutase, peroxidase, catalase, and glutathione peroxidase) increased in response to selenium treatment as compared to cadmium-stressed plants. Enhanced antioxidant activities helped to decrease oxidative damage from Cd and develop tolerance against cadmium stress in selenium-treated pea plants. An increase in the degree of cadmium tolerance induced by selenium was indicated by the improvement of photosynthetic pigments and consequently the photosynthetic activity. Cadmium treatments decreased the relative water content, as compared to the control plant. Meanwhile, relative water content increased in response to selenium treatment as compared to cadmium-stressed plants. Cadmium treatments decreased the macronutrient level (N, P and K) as compared to the control plants. Meanwhile cadmium-stressed pea plants pretreated with selenium had an increased level of these element contents. Selenium tended to counterbalance the Cd-induced changes in nutrients, it also reduced the lipid peroxidation and exerted positive effects on the cell membrane stability.

The data provided evidence that selenium treatment reduced the adverse effects of cadmium stress on pea plants, and might play a key role in providing stress tolerance by stimulation of the antioxidant system as a stress protection mechanism. Eventually, the results of the present investigation clearly manifested that the addition of the selenium (50 µM), to pea plants grown in soil contaminated by cadmium up to 100 µM, boosted plants to overcome or even reduce cadmium toxicity and thus obtained relatively better growth, better quality and yield, as well as better chemical composition.

Key Words: *Pisum sativum*, selenium, cadmium, antioxidant enzymes, relative water content.

1. Introduction

Selenium (Se) is considered to be an essential trace element for human, animals, and some species of microorganisms. Although, Se is not confirmed to be required by higher plants [1]. Several studies demonstrate that at low concentrations it plays an important role in antioxidative reactions and hormone balance in plant cells such as enhancing the activity of glutathione peroxidase [2,3]. For instance, [4] reported that low level of exogenous Se (5 and 10 µM) generally stimulates growth as well as photosynthetic pigments accumulation in NaCl treated cucumber seedlings.

Several studies demonstrate that selenium supply may exert diverse beneficial effects, including growth promoting activities [5,6]. Moreover, some plant species grown in Se-enriched media have shown enhanced resistance to certain abiotic stresses, e.g. drought [7], salinity [8] and heavy metals [2] stresses. Selenium exerts beneficial effects on growth and stress tolerance of plants by enhancing their antioxidative capacity [9]. It was shown that Se has the ability to regulate the water status of plants under drought conditions [10].

Recent researches have demonstrated that Se is not only able to promote growth and development of plants, but also increases resistance and antioxidant capacity of plants subjected to various stresses [6]. Moreover, a stimulatory effect of foliar application of Se on nitrogen assimilation has been reported for barley [22].

The intensive use of high-phosphate fertilizers increased accumulation of metal ions, especially cadmium, in the soil [12]. 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 [13,14].

Cadmium is readily taken up by the cells of different plant species [15] 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 [16,17] and finally can cause plant death [18]. Cadmium produces alterations in the membranes by inducing changes in their lipid composition [19] and affects the activities of enzymes associated with membranes, such as that of H^+-ATPase [20]. Cadmium decreases photosynthetic rate due to reduced chlorophyll content and the enzymatic activity involved in CO_2 fixation [21]. In many plants Cd enhances the level of lipid peroxidation and alteration in antioxidant systems [22]. Harmful effects produced by Cd^{2+} might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins [23].

2. Materials and Methods

Uniform-sized pea seeds (*Pisum sativum* L.*)* cv. Master was purchased from the Crop Institute, Agriculture Research Center, Giza, Egypt. Grains were sterilized in 70 % ethanol and 3.1% NaOCl and then thoroughly rinsed with sterile deionized water. The seeds were then soaked overnight (12 h) in either distilled water or 50 µM freshly prepared selenium solution (as sodium selenate, Na_2SeO_4).

The seeds were germinated in pots (40 cm high × 35 cm diameter), each filled with 15 kg sandy loam soil with 2.5% organic matter and available N, P and K concentrations of 170, 80 and 200 mg kg^{-1}. 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. 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 440 µM $m^{-2}s^{-1}$. 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, $CdCl_2$ (100 µM), Selenium (50 µM), and Cadmium (100 µM) + Selenium (50 µM).

Plants were harvested 25 days after starting the treatments for chemical analyses. Fresh samples or deep-frozen samples were used for the biochemical assays.

Chemical Analysis.

Chlorophyll *a,b* and total carotenoids were determined according to [24]. Photosynthetic activity ($^{14}CO_2$-fixation)

was measured in the Radioisotope Department, Atomic Energy Authority, Cairo, Egypt, with the method published previously [25]. One pot from each treatment was placed under a bell jar and $^{14}CO_2$ was generated inside this chamber by a reaction between 10 % HCl and 50 µCi ($1.87x10^6$ Bq) $NaH^{14}CO_3$+100 mg Na_2CO_3 as a carrier. Then the samples were illuminated with a tungsten lamp (440 µmol $m^{-2}s^{-1}$). 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 [26] and a Liquid Scintillation Counter (*LSC2-Scaler Ratemeter SR7, Nuclear Enterprises*). Relative water content (RWC) was measured and calculated according to [27]. Fresh samples were ground in the presence of liquid nitrogen and measurements were undertaken using spectrophotometer (Specord 200, Analytical Jena, Germany). The activity of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) was determined as described by [28]. Peroxidase (POD, EC 1.11.1.7) activity was determined using the guaiacol test at 470 nm [29]. The glutathione peroxidase (GPX, EC 1.11.1.9) activity was determined as the decrease in absorbance at 340 nm due to the oxidation of NADPH [30]. Lipid peroxidation was measured in terms of malondialdehyde (MDA) content using the thiobarbituric acid reaction as described by [31]. H_2O_2 concentration was determined by the potassium titanium oxalate method [32]. Free proline content was estimated photometrically in acidic ninhydrin assay according to the method adopted by [33]. Total protein was estimated spectrophotometrically by the [34].

Statistical Analyses.

Data were analyzed using a sigma stat (3.5) with Tukey test ($P < 0.05$). Correlation analysis using Spearman Rank Order Correlation in sigma stat (3.5) were conducted to determine the relationship between measurement parameters.

3. Results and Discussion

Photosynthetic pigments, photosynthetic activity ($^{14}CO_2$-assimilation) and relative water content.

Data presented in Table (1) revealed that Cd treated plants showed significant decrease in photosynthetic pigments (chlorophyll a+b and carotenoids), photosynthetic activity ($^{14}CO_2$-assimilation) and relative water content by 39.9, 46.8, 43.6 and 30.7 %, respectively as compared to the control plants. These results were in correspondence with those of [27,37]. Meanwhile, plant treatment with selenium (50 µM) + cadmium (100 µM) showed a significant decrease in the photosynthetic pigments (chlorophyll a+b and carotenoids), photosynthetic activity ($^{14}CO_2$-assimilation) and relative water content by 9.5, 7.4, 7.3 and 4.9 %, respectively as compared to the control plants. These results were consistent with those of [38]. The net photosynthetic rate and stomatal conductance of higher plants leaves are known to decrease as relative water content decrease [39]. In addition, the antioxidative effect of Se particularly on the chloroplasts can delay senescence [40]. 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 [41].

Elemental Analysis.

Determination of K^+ in pea plants was done by flam photometer (Jenway, PFP-7). The method of [35] was applied for phosphorus estimation. Total nitrogen was determined using the Kjeldahal method according to [36].

It was observed that the chlorophyll content increased significantly with Se application in the present study, which is in agreement with the positive effects of Se treatment in delaying the loss of chlorophyll in senescing *Vicia faba* plants [38] and drought-stressed wheat plants [7].

Table 1. Effect of cadmium (100 µM) in pea plants pre-treated with selenium (50 µM) on chlorophyll *a+b* (mg g^{-1}FW), carotenoids (mg g^{-1}FW), photosynthetic activity (10^3 Becquerel mg^{-1}FW) and relative water content (%).

Treatments	Chlorophyll *(a+ b)*	Carotenoids	Photosynthetic Activity	Relative Water Content
Control	4.63 [b]	1.75 [a]	15.320 [a]	74.6 [a]
Cadmium (100 µM)	2.78 [d]	0.93 [c]	8.632 [c]	51.7 [c]
Selenium (50 µM)	4.95 [a]	1.63 [b]	15.021 [a]	75.2 [a]
Cadmium (100 µM) + Selenium (50 µM)	4.19 [c]	1.62 [b]	14.197 [b]	70.9 [b]

Data are means of three replicates. Duncan's test: within each column, same letter indicates no significant difference between treatments ($P<0.01$).

The increase in chlorophyll a and chlorophyll b contents of pea leaves may be attributed to Se effect over protection of chloroplast enzymes and thus increasing the biosynthesis of photosynthetic pigments [41]. When plants are subjected to environmental stress, their chloroplasts are damaged, leading to disrupted photosynthesis. However, the addition of appropriate levels of Se can somewhat reduce the damage to the chloroplasts and increase the chlorophyll contents [42]. Through proteomic analysis, [43] revealed that in rice (*Oryza sativa* L.) seedlings, low doses of Se enhanced photosynthesis. In addition, in sorghum, Se application significantly increased the photosynthetic rate, stomatal conductance and transpiration rate [44]. 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), Renwei et al. [42].

Antioxidant enzyme activities (catalase, peroxidase and superoxide dismutase), glutathione peroxidase, lipid peroxidation levels (malondialdehyde), free proline, H_2O_2 and total protein.

The results presented in Table (2) revealed that Cd treated plants showed a significant decrease in antioxidant enzyme activities (superoxide dismutase, peroxidase and catalase), glutathione peroxidase and total protein by 38.9, 39.7, 46.4, 32.5 and 44.7 %, respectively as compared to the control plants. Meanwhile, Cd treated plants showed a significant increase in lipid peroxidation levels (malondialdehyde), free proline and H_2O_2 by 44.2, 31.3, and 34.7 %, respectively as compared to the control plants. These results were confirmed by the results of [45]. Plants pre-treated with selenium (50 µM) + cadmium (100 µM) showed a significant decrease in the antioxidant enzyme activities (superoxide dismutase, peroxidase and catalase), glutathione peroxidase and total protein by 6.9, 3.3, 10.2 and 8.5 %, respectively as compared to the control plants. Also, decreased the lipid peroxidation levels (malondialdehyde), free proline and H_2O_2 by 11.0, 10.8, and 12.9 %, respectively as compared to the control plants. These results are in agreement with that of [38,46].

Table 2. Effect of cadmium (100 µM) in pea plants pre-treated with selenium (50 µM) on SOD (units mg^{-1} protein), POD (units mg^{-1} protein), CAT (µMH_2O_2/min.gFW), GPX (µMNADPH/min.gFW), MDA (µM g^{-1}FW), free proline (µmol g^{-1} FW), H_2O_2 (µM g^{-1}FW) and total protein (mg g^{-1} FW).

Treatments	SOD	POD	CAT	GPX	MDA	Free Proline	H_2O_2	Total Protein
Control	13.1 [b]	21.4 [b]	12.7 [a]	25.8 [a]	17.8 [c]	419 [c]	28.4 [c]	128.9 [b]
Cadmium (100 µM)	8.0 [d]	12.9 [d]	6.8 [c]	17.4 [c]	31.9 [a]	610 [a]	43.5 [a]	71.3 [d]
Selenium (50 µM)	14.7 [a]	23.5 [a]	12.2 [a]	26.8 [a]	16.5 [c]	407 [c]	27.0 [c]	136.8 [a]
Cadmium (100 µM) + Selenium (50 µM)	12.2 [c]	20.7 [c]	11.4 [b]	22.7 [b]	20.0 [b]	470 [b]	32.6 [b]	117.9 [c]

Data are means of three replicates. Duncan's test: within each column, same letter indicates no significant difference between treatments (P<0.01).

Several studies have shown that a protective role of Se against the oxidative stress in higher plants coincided with enhanced GPX activity and decreased lipid peroxidation [2]. The finding of the present study was in conformity with previous studies which state that Se can alter antioxidant levels in plants and detoxify superoxide radicals, thus preventing oxidative damage and protecting the membranes and enzymes [28].

Hawrylak-Nowak [4] reported that, Se treatment at concentration of 5 and 10 µM increased proline content. The activation of antioxidant enzymes including SOD, CAT, APX and POD by Se supplementation has been reported in some plant species [28]. The plants treated with selenate induce higher increases in enzymes that detoxify H_2O_2, especially ascorbate peroxidase and glutathione peroxidase, thereby improving stress resistance [9,47].

Inorganic macronutrient contents (nitrogen, phosphorus and potassium).

It is evident from Tables (3) that the content of most major nutrient elements estimated in this study (N, P and K) decreased in Cd (100 µM) treated plants by 23.5, 33.8, 43.7 %, respectively as compared to the control plants. Meanwhile, plants treated with selenium (50 µM) + cadmium (100 µM) showed a significant deccrease in the above parameters by 5.1, 11.6 and 13.8 %, respectively as compared to the control plants. These results were confirmed by the results of [48].

Table 3. Effect of cadmium (100 µM) in pea plants pre-treated with selenium (50 µM) on the macronutrient contents of N, P and K (mg g^{-1}DW).

Treatments	N	P	K
Control	78.3 [b]	88.5 [a]	105.4 [a]
Cadmium (100 µM)	57.4 [d]	48.3 [c]	81.3 [c]
Selenium (50 µM)	82.3 [a]	86.4 [a]	107.6 [a]
Cadmium (100 µM) + Selenium (50 µM)	71.9 [c]	79.9 [b]	98.5 [b]

Data are means of three replicates. Duncan's test: within each column, same letter indicates no significant difference between treatments (P<0.01).

Positive effects of selenium in the form of selenate on potassium accumulation were also observed by [49]. The positive effect of selenium on K^+ and Ca^{+2} accumulations was also observed by [50] in spinach roots. Keck [51] 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). As for wheat, Trivedi and Erdei [52] found a similar decrease in the K concentration in response to Cd. Reports have shown that proper doses of Se can protect plants against the damage caused by heavy metals, including As, mercury (Hg), Pb, Cd, Zn, Cu, chromium (Cr) and Sb [42].

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