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The alleviation of lead toxicity in *Vicia faba* L. by the application of selenium

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Abstract

Selenium (Se) is an essential element for humans but is not considered as essential for plants. However, its beneficial role in improving plant growth and stress tolerances is well established. In order to study the role of Se in lead (Pb) toxicity in Faba bean (*Vicia faba* L.).This experiment was carried out on plants grown for 2 weeks on Hoagland medium supplied with 50 µM Pb in the form of lead nitrate $Pb(NO_3)_2$ and/or Se concentrations of 1.5 and 6 µM in the form of sodium selenite Na_2SeO_3 with three replications. The result showed that Pb decreased chlorophyll "a" and chlorophyll "b". However, supplementation of Se restores the negative effect of Pb and increases pigment content. Osmolytes (soluble sugar and proline) were increased under Pb stress and further increase was observed with addition of Se. Pb also increased production of lipid peroxidation and the activities of antioxidant enzymes such as catalase and ascorbate peroxidase. Supplementation of Se decreased accumulation of lipid peroxidation and increased the activities of antioxidant enzymes to greater levels in roots and shoots.
The data suggest that Se might have an important protective effect in plants under Pb and may help to alleviate the adverse effect of Pb on the growth of *Vicia faba* L.

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Introduction

Lead (Pb), a heavy metal with characteristic toxic actions, has attracted considerable attention for its widespread distribution and potential risk to the environment. Plant processes such as the biosynthesis of nitrogenous compounds, carbohydrate metabolism and water absorption are adversely affected by increasing Pb levels insoil, even at every low concentration (John *et al.*, 2009; Hamid *et al.*, 2010). Furthermore, heavy metals also accumulate in different plant organs, and thereby enter the foodchain. However, the plant response to Pb contamination is a key research problem, and a special effort is underway to identify factors which can reduce Pb absorption or toxicity in plants. Selenium (Se) is an essential trace element for humans and animals, and some findings suggest that Se may be a beneficial element, which plays a novel role in plant biology for innovative crop production (Trumble *et al.*, 1998; Rayman, 2000; Terry *et al.*, 2000; Ellis and Salt, 2003; Hartikainen, 2005; Pilon-Smits *et al.*, 2009; Zhu *et al.*, 2009; Bañueloset *al.*, 2011; Hatfield *et al.*, 2014; Wu *et al.*, 2015). Selenium at low concentrations exerts positive effects for plants such as promoting growth, increasing antioxidative capacity, improving yield and quality, and delaying ripening and senescence (Hartikainen *et al.*, 2000; Xue*et al.*, 2001; Turakainen*etal.*, 2004; Broadley*et al.*, 2010; Pukacka *et al.*, 2011; Pezzarossa *et al.*, 2012). Applying Se fertilizer as base fertilizer or foliar spray has been used to increase the Se content in the edible portion of crops and to simultaneously counteract the detrimental effects of diverse environmental stresses, such as heavy metals (Feng *et al.*, 2013), drought (Ahmad *et al.*, 2016)and salt (Ardebili *et al.*, 2014) .Recently, the beneficial role of Se in alleviation of heavy metal- induced oxidative stress has been well established (Filek *et al.*, 2008; Han *et al.*, 2015). The vital role of Se in antioxidant protection from heavy metal stress has been observed in different biological systems (Yathavakilla and Caruso, 2007; Kumar *et al.*, 2012; Lin *et al.*, 2012; Malik *et al.*, 2012) including some crop plants (Lin *et al.*, 2012; Mroczek-Zdyrska and Wojcik, 2012; Mozafariyan *et al.*, 2014). Others have recognized that Se can detoxify many toxic heavy metals such as Pb, Cd, chromium (Cr), and arsenic (As) in plants (Shanker *et al.*, 1996a,b; Srivastava *et al.*, 1998; Feng *et al.*, 2013; Saidi *et al.*, 2014; Qing *et al.*, 2015). Though many studies have demonstrated that external Se may act as a chemical regulator to reduce heavy metal-induced oxidative stress, a better understanding of the mode of action of Se in prevention of heavy metal accumulation in plants should be achieved. Moreover, the possible mechanisms of the Se-enhanced tolerance and/or resistance of plants to Pb stresses remain unclarified. The present study was undertaken to investigate the effectiveness of Se (sodium selenite) application in the alleviation of Pb (lead nitrate) toxicity by ameliorating plant growth, the level of lipid peroxidation and enzymes activities.

Materials and methods

Plant Material and Growth Conditions

Vicia faba L. minor (Aguadulce) plants were grown hydroponically in a laboratory condition. Seeds were germinated on moist filter paper in plastic containers containing half-strength Hoagland's nutrient solution (with $NH_4H_2PO_4$ to protect the plants from the short term fluctuations in P supply) in the dark at 25 °C for 7 days. Then, the seedlings were transplanted into 3 L plastic containers (ten plants per one container) with modified full-strength Hoagland's medium (Hoagland and Arnon 1959) .containing the following mineral components: KNO_3 (6 mM),$Ca(NO3)_2.4H_2O$ (4 mM), $MgSO_4.7H_2O$ (2 mM), iron citrate (0.85 µM), H_3BO_3 (46 µM), $MnCl_2.4H_2O$ (9 µM),$ZnSO_4.7H_2O$ (0.76 µM), $CuSO_4.5H_2O$ (0.32 µM), and $H_2MoO_4.2H_2O$ (0.11 µM). $NH_4H_2PO_4$ was removed from Hoagland's medium to prevent precipitation of lead phosphate (Wierzbicka and Potocka, 2002).

To determine the effect of Pb and Se, the growth medium was supplemented with 50 µMPb in the form of Pb $(NO_3)_2$ (Sigma) and/or 1.5 µM or 6 µM Se in the form of Na_2SeO_3 (Sigma). The Pb and Se concentrations were chosen on the basis of preliminary experiments and literature data. Two-week cultivation was performed at 25/20°C (day/

night) with 16 h/8 h (day/night) photoperiod at photosynthetic active radiation 150 µmol m^{-2} s^{-1}. During the experiment, the nutrient solution was continuously aerated and its losses were supplemented daily with dH_2O. The medium was changed once a week and its pH was kept at 5.0-5.2.Analyses were performed on fresh material 14 days after Pb and/or Se addition, and the measurements were performed in triplicate.

Analytical methods

Determination of Chlorophyll, Proline and Soluble sugars

The method used for extraction of chlorophyll is the traditional method established by Holden (Holden M, 1975) which is a maceration of the plant in acetone. For the determination of proline, the technique used is that of Monneveux and Nemmar (Monneveux and Nemmar, 1986). Soluble sugars were determined by the method of Schields and Bunnet (Schields, Burnett ,1960) using anthrone in sulfuric acid.

Determination of Lipid Peroxidation

The level of lipid peroxidation was measured by estimating malondialdehyde (MDA, a product of lipid peroxidation) following the method of Heath and Packer (1968) (Heath and Packer , 1968) . Leaf samples (0.5 g) were homogenized in 3mL 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at 10000 ×g for 10min. Supernatant (1mL) was mixed with 4mL thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA), heated in a water bath at 95°C for 30min, and then quickly cooled by transferring to an ice bath. MDA content was measured by observing the difference in absorbance at 532nm using an extinction coefficient of 155mM−1 cm−1 and expressed as nmol of MDAg^{-1} FW.

Enzyme extraction and activity determination

Fresh leaves were homogenized in 50 Mm potassium phosphate buffer pH 7.6, the homogenized samples were centrifuged at 12000 ×g for 20 min and the supernatant was used as crude enzyme extract in CAT and APX (Loggini *et al*., 1999).Catalase (CAT) activity was determined as a decrease in absorbance at 240 nm for 3 min following decomposition of H_2O_2(Cakmak and Horst, 1991).The reaction mixture 3 ml contained 50mM phosphate buffer pH 7.2, 15 mM $H_2O_2$and 100 µl of crude enzyme extract. The activity was calculated using the extinction coefficient 39400 $M^{-1}cm^{-1}$. Ascorbate peroxidase (APX) activity was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 3 min in 3 ml of a reaction mixture containing 50 Mm potassium phosphate buffer pH 7.2, 0.5 Mm ascorbic acid, H_2O_2 and 100 µl of crude enzyme extract (Nakano and Asada, 1981). The activity was calculated using the extinction coefficient 2800$M^{-1}cm^{-1}$.

Statistical Analysis

The results were statistically analyzed using one way Analysis of Variance (ANOVA) to determine the degree of significance for the obtained variations by the used treatments.

Results

Effect of Pb stress and Se on biochemical constituents of Vicia faba L.

Photosynthetic pigments

The (Fig.1) illustrates the effect of Pb stress, on the chlorophyll content of faba bean. The chlorophyll a and b content of Pb-treated plants was 33% and 25% lower, respectively, when compared with the control.The application of Se alone increased photosynthetic pigments (chla andchl b) compared to control. In combination with Pb treatment, all the concentrations of Se (1.5 and 6µM) significantly increased photosynthetic pigments as compared with untreated plants grown under Pb stress.

Proline content

The proline responses of *Vicia faba* plant to Pb and Se is summarized in the (Fig. 2).Pb stress increased proline content by 40 and 55% in shoot and root respectively, than control. Se supplementation to Pb stressed plants increased the proline content by 16 and 35% in 50 µM Pb+1.5 µM Se and 36 and 27% in 50 µM Pb+6 µM Se in shoot and root, respectively, than corresponding Pb alone exposed plants.

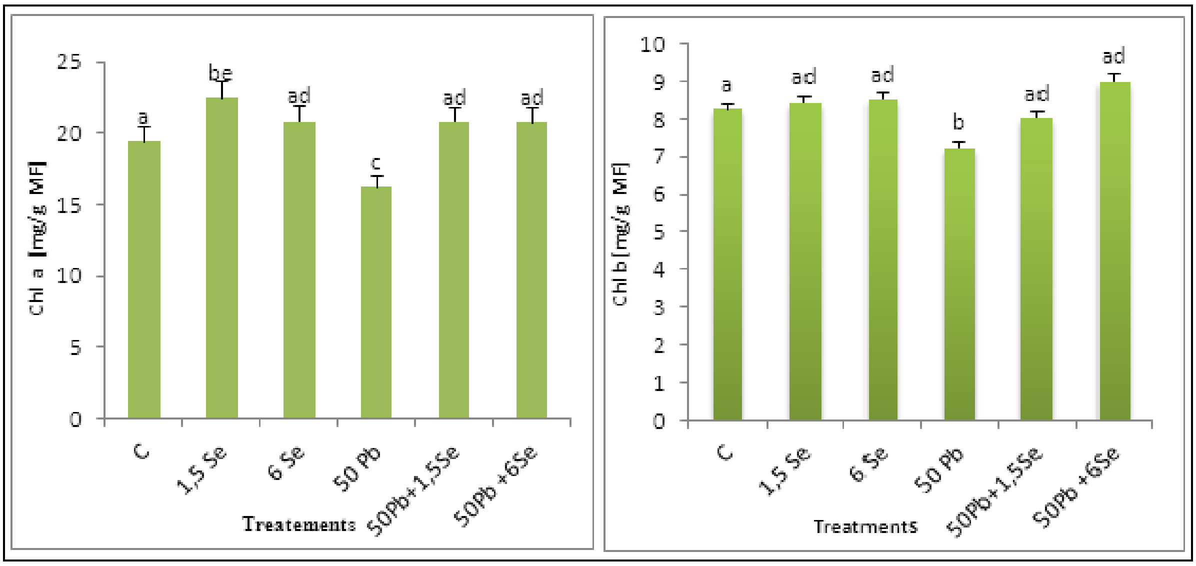

Fig. 1. Effect of Se and/or Pb application on chlorophyll a and b concentrations in shoots of *Viciafaba* L submitted to 50µM Pb for 14 days. Values with different letters differ significantly from each other at p<0.05.

Total soluble sugar contents

Pb stress increased soluble sugars content by 39 and 33 % in shoot and root respectively, than control. Se supplementation to Pb stressed plants did not influence the total soluble sugar than corresponding Pb alone exposed plants.

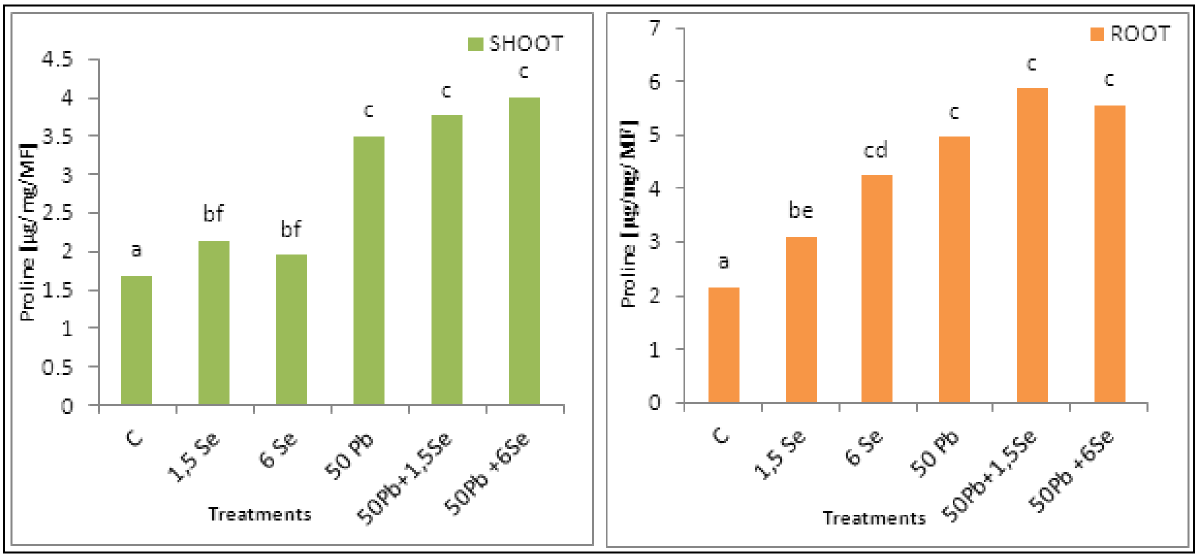

Fig. 2. Effect of Se and/or Pb application on proline content in shoots of *Viciafaba* L submitted to 50µM Pb for 14 days. Values with different letters differ significantly from each other at p<0.05.

Lipid peroxidation MDA

The Lipid peroxidation (MDA) of the *Vicia faba* L. shoots and roots (Fig.4) was significantly affected by Pb and Se. The MDA content in shoots part was significantly increased with the increasing of Se doses by 18% and 19% respectively for 1.5 µM and 6µM Se under non Pb stress conditions. Pb treatment induces an increasing of 38 % relative to the control and the combination of Pb and Se, the lower (1.5 lM) Se concentration did not exert an effect on the MDA level , but the higher (6 µM) Se concentration decreased the MDA level by17 % in comparison to the plants treated with Pb . At the roots level, there was also an increase in MDA contents with Se application and withPb stress. However, MDA content was not affected by the application of Se under Pb conditions.

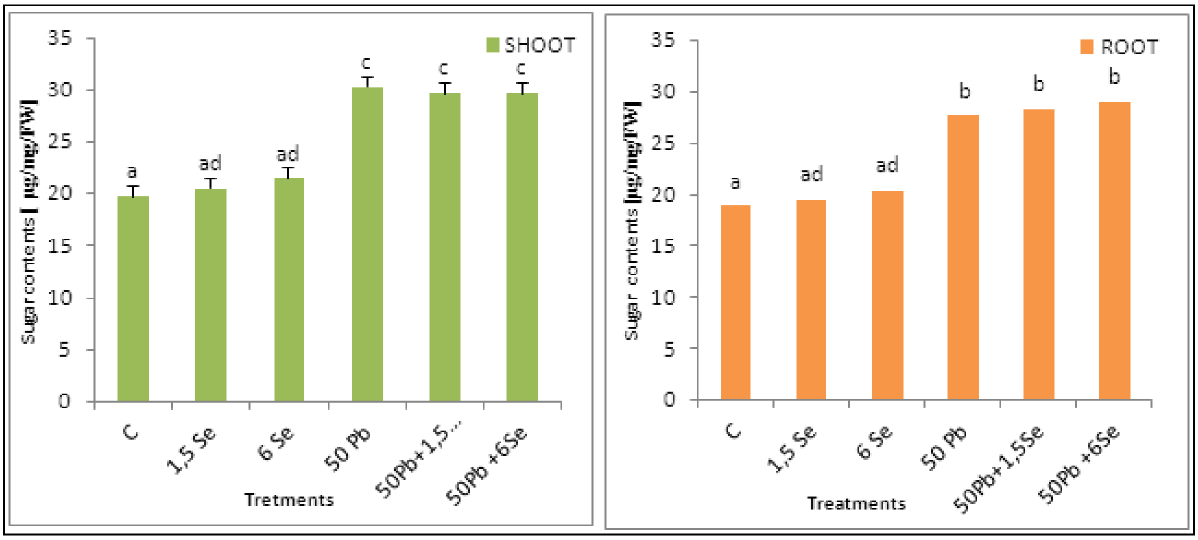

Fig. 3. Effect of Se and/or Pb application on total soluble sugars in shoots of *Vicia faba* L. submitted to 50µM Pb for 14 days. Values with different letters differ significantly from each other at $p<0.05$.

Effect of Pb stress and Se on antioxidant system of Viciafaba L.

APX activity

The ascorbate peroxidase activities (APX) were significantly affected by Pb (Fig .5) in both compartments (shoots and roots). In the shoots exposed to Pb or 1.5 or 6 µM Se, an increase in the APx activity by 53, 39, and 46 %, respectively, was noted in comparison to the control. The addition of 1.5 µM Se to the growth medium with Pb maintained the APx activity at the control level, which was 50 % lower than in the shoots exposed to the Pb alone. However, addition of 6 µM Se to the growth medium with Pb increased the APx activity by 63 %.

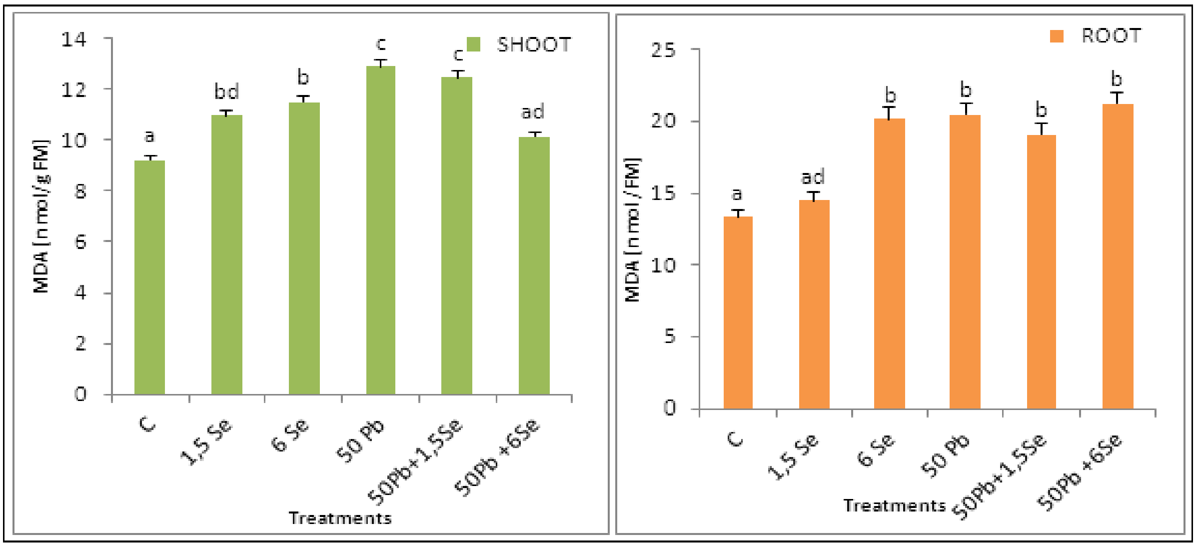

Fig. 4. Effect of Se and/or Pb application on MDA in shoots and roots, of *Viciafaba*L submitted to 50µM Pb for 14days. Values with different letters differ significantly from each other at $p<0.05$.

In root,Pb induced a 42% increase in APx activity in comparison to the control, addition of Se, both 1.5 µM and 6 µM, to the Pb-treated plants increased APx activity by 10% and 15%, respectively, in comparison to plants supplemented with Pb alone.

Catalase activity

The (Fig. 2) shows the effect of different levels of se and Pb treatments on CAT activity in shoots and roots of *Viciafaba* L. In the absence of Pb, Se at the concentrations of 1.5 and 6 1µM increased CAT activity in the shoots by 44 and53 %, respectively. The simultaneous treatment with Pb and 6 µM Se increased CAT activity (64 %) in comparison to the control. In the groups exposed to both Pb and Se, CAT activity was significantly lower in comparison to the 50 µM Pb-treated plants, that is, 54 and 23 % at 1.5 µM Se and 6µM Se, respectively. In roots, Treatments with 6 µM Se as well as 50 µMPb and 50 µM Pb+1.5 µM Se or Pb+6 µM Se did not change CAT activity.

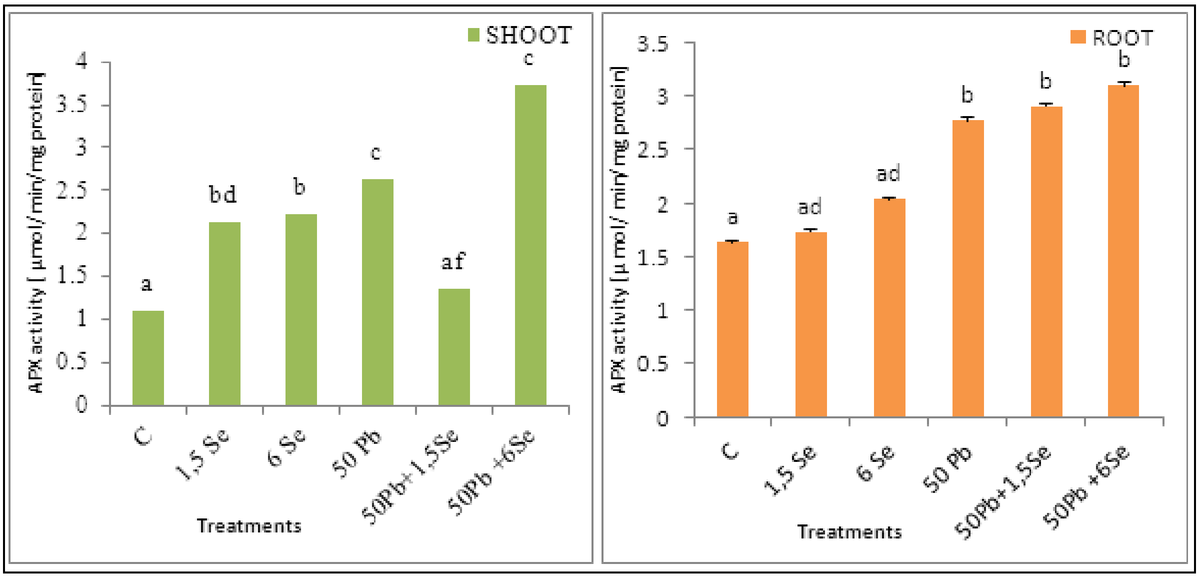

Fig. 5. Effect of Se and/or Pb application on APX activities in shoots and roots, of *Vicia faba* L submitted to 50µM Pb for 14days. Values with different letters differ significantly from each other at $p<0.05$.

Discussion

Selenium is an essential element for growth maintenance of plants and is also very much beneficial in reducing the adverse effects of stresses. Present experiment is designed to investigate the protective role of Se during Pb stress.

The photosynthetic pigments are important macromolecules that are produced by plants, and play a vital role in photosynthesis, which is responsible for plant growth and dry matter production.

The reduction in the rates of the pigments in the present study can be the result of an inhibition of the synthesis of chlorophyls by blocking of the ions Mg, Mn and Fe (Liu *et al.*, 2008; Chatterjee, 2004;Gopal andRizvi,2008).Or by increasing the chlorophyllase activity (Liu *et al.*, 2008). Or, the destruction of the chloroplasts by the Species Reactivates of Oxygen (ERO) induced by the presence of lead (Sharma and Dubey, 2005). Murkowski and Skórska (2008) on *Cucumis sativa* and *Scenedesmus quadricauda* showed that the reduction in the photosynthetic activity is the resultant of the inhibition of the transport of the electrons in the photosystem II (PSII).

Meanwhile, plant treatment with selenium showed a significant increase in this parameter. Fenget *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 Pb stress (Pennanen *et al.*, 2002). Also, Se treatments increased photosynthetic activity (Djanaguiraman *et al.*, 2010; Wang *et al.*, 2012).

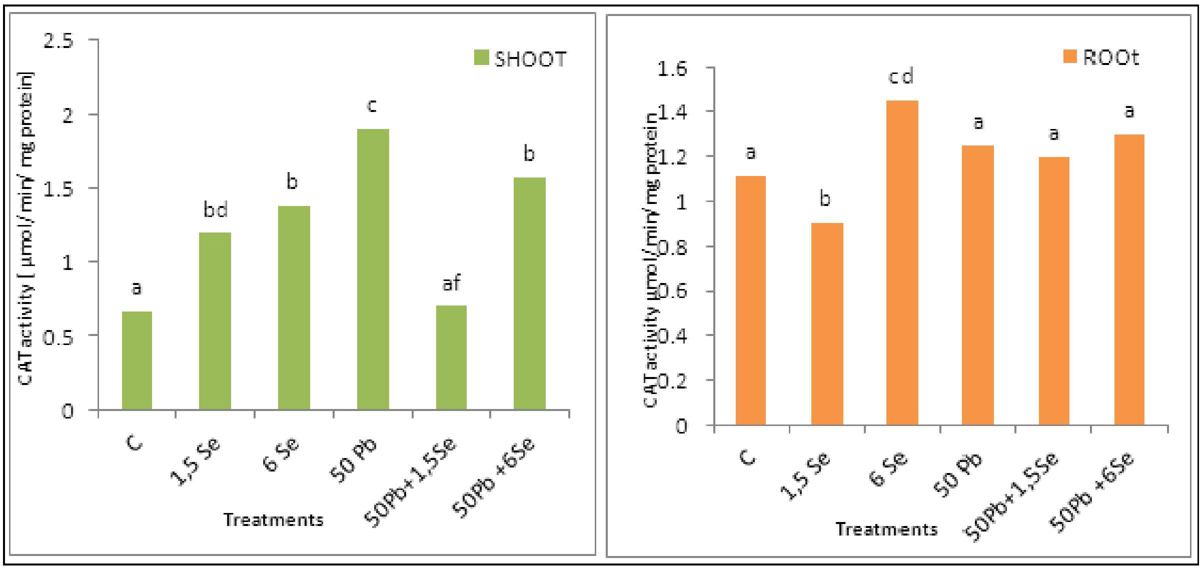

Fig. 6. Effect of Se and/or Pb application on Catalase activity in shoots and roots, of *Vicia faba* L submitted to 50µM Pb for 14days. Values with different letters differ significantly from each other at $p<0.05$.

Accumulation of various compatible solutes (prolineand sugars compounds) under abiotic stresses is a key adaptive strategy to counteract stress-induced osmotic damage (Majumder *et al.* 2010). In the current investigation, Pb treated plants showed higher accumulations of osmolytes, i.e. proline and total soluble sugars in shoot and roots. Increased concentration of osmolytes in Pb plants indicates Pb-induced osmotic shock in plant tissues. But, application of Se improved the osmotic-adjustment capacity of Pb plants by further enhancing the level of compatible solutes in plant tissues. Improvement in osmotic-adjustment capacity in the form of elevated level of osmolytes in Se-treated plants may also be the possible reason of augmentation in photosynthetic activities under Pb stress. Hawrylak-Nowak (2009) and Nawaz *et al* (2013) worked on cucumber and wheat, respectively, and also reported that exogenous application of Se is responsible for elevation in the level of various osmolytes in plants grown under abiotic stresses.

Pb stress can stimulate the production reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen (Lee *et al.*, 2001). One effect of free oxygen radicals accumulation in plant cells under stress is lipid peroxidation via oxidation of unsaturated fatty acids leading to membrane deterioration (Marschner, 1995). MDA is one of the final products of the peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Sharma *et al.*, 2011). The MDA level was reduced in the presence of the higher Se concentrations introduced to the growth medium with Pb. This indicates that the Se effect on the MDA level depends on the concentration applied, its content in the specific tissue, and the Se form. Consistent with our results, several researchers have also shown inhibition of lipid peroxide accumulation after Se supplementation in the form of selenite (Hawrylak- Nowak *et al.,* 2014). These results can be attributed to the antioxidative properties of Se, especially in lower doses (Hartikainen *et al.*, 2000). However, for selenate, the lower Se concentrations decreased lipid peroxidation, whereas the higher Se concentration intensified it (Cartes *et al.*,2005).

Plants have a defensive system of antioxidants to protect against ROS. This system comprised various enzymes, i.e. CAT , APX ,POD, GR and GPX . The combined action of all these antioxidant enzymes is vital to neutralize ROS.

APX is believed to function as a signaling substance for the fine modulation of ROS, whereas CAT (mainly localized in peroxisomes) might be responsible for the removal of excess ROS (Mittler, 2002). In stressed plants, Se was observed to significantly reactivate these two enzymes, particularly CAT (Yao *et al.*, 2009a,b; Djanaguiraman *et al.*, 2010; Yao *et al.*, 2010a,b; Hasanuzzaman *et al.*, 2011; Yao *et al.*, 2011; Malik *et al.*, 2012). Thus, it is reasonable to speculate that the increased activities of these enzymes may indicate excess ROS (H_2O_2) in these studies.

Conclusion

In conclusion, the present study illustrates the antioxidant properties of Se to avoid Pb toxicity in Faba bean seedling. Therefore, based on these findings, Se might be able to down regulate Pb-induced oxidative damages through the inhibition of ROS production and indirectly by regulation of antioxidative system. The regulation of ROS levels by Se may be a key mechanism for counteracting environmental stress in plants.

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