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SELENIUM INCREASED THE EFFICIENCY OF ANTIOXIDANT SYSTEM IN ROOT CELLS OF TWO WHEAT CULTIVARS DIFFERING IN ALUMINIUM TOLERANCE

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Summary. The protective role of selenium (Se) against aluminum (Al) stress in two wheat cultivars (Benyswef 5 and Gemza 9) differing in their tolerance was investigated by evaluating the growth responses and the antioxidant properties of plants cultured hydroponically with Al (0 or 200 μM) and Se (0–10 μM Se).

Dry mass, cell death, Al-uptake, Se-uptake, extracellular generation of reactive oxygen species (ROS), $O_2^{\cdot -}$, H_2O_2 and $OH^{\cdot}$, lipid peroxidation and activities of the antioxidant enzymes ascorbate peroxidase (APX), guaiacol peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione-S-transferase (GST) in the roots were investigated. Our results indicated that Al reduced root dry mass of both cultivars and increased Al accumulation 5-fold and 3-fold compared with controls of cvrs. Gemza 9 and Benysef 5, respectively. Al induced generation of extracellular ROS, induction of lipid peroxidation and activation of peroxidase, superoxide dismutase, glutathione reductase and glutathione S-tranferase.

Se application up to 2 μM improved root growth and steadily decreased ROS and thiobarbituric acid reactive substances (TBARS) accumulation in plants treated with 0 and 200 μM Al. However, when applied at concentrations above 2 μM, Se induced stress in plants grown with or without Al. Significant changes in the activities of the antioxidant enzymes in the presence of Se were also found.

Our data provide evidence for the existence of an internal mechanism of tolerance involving increased antioxidant system activity in order to limit cellular damages and possibly linked to Al and Se interactions in both wheat cultivars studied, which was more apparent in cv. Gemza 9.

***Keywords*:** Aluminum, antioxidant enzymes, reactive oxygen species, selenium, wheat.

Abbreviations: Al – Aluminum; APX – Ascorbate peroxidase; DW – Dry weight; EDTA – Ethylendiamine tetraacetic acid; FW – Fresh weight; GR – Glutathione reductase; GSH-Px – Glutathione peroxidase; GST – Glutathione-S-transferase; H_2O_2 – Hydrogen peroxide; LPO – Lipid peroxidation; MDA – Malondialdehyde; NADH-PX – NADH-peroxidase; $O^{\cdot}_2$ – Superoxide ion; $OH^{\cdot}$ – Hydroxyl radical; POD – Peroxidase; PVP – Polyvinylpyrrolidone; ROS – Reactive oxygen species; Se – Selenium; SOD – Superoxidedismutase; TBA – Thiobarbituric acid; TBARS – Thiobarbituric acid reactive substances; TCA – Trichloroacetic acid.

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INTRODUCTION

Aluminum (Al) is the third most abundant chemical element in the earth crust. Solubilization of Al-containing minerals can be enhanced in acidic environments. Most soils are being acidified continuously during the process of agricultural modernization and in part due to acidic depositions from the atmosphere (Rengel, 2004). It has been estimated that about 50% of the cultivable area in the world is composed of acid soils (Panda et al., 2009). When the soil pH is lower (4.5–5.0), Al will be released mainly in phytotoxic form of Al^{3+}, solubilized in the soil water and absorbed by plant roots (Matsumoto, 2000).

Plants differ largely in their ability to tolerate Al and the mechanisms for Al tolerance or toxicity have not yet been fully elucidated. Although Al does not belong to the transition metals group that may induce oxidative stress, much of the current evidence suggest that Al can lead to the generation of reactive oxygen species (ROS) and cause oxidative damage to biomolecules. In fact, Yamamoto et al. (2002) have demonstrated that Al can alter mitochondrial functions, which lead to ROS production. The induction of cell death via a ROS-activated signal transduction pathway has been observed in barley (Pan et al., 2001).

Selenium is a non-metallic mineral that resembles sulfur (S) and occurs naturally as a trace element in most soils, rocks and waters. Se is an essential micronutrient and has important benefits for animal and human nutrition. However, it may be toxic to animals at high dosages. It is not clear whether Se is essential for plants or not (Terry et al., 2000). Se becomes toxic at higher levels due to the incorporation of Se into S-containing molecules, especially the non-specific replacement of cysteine (Cys) by Se-Cys in proteins (Brown and Shrift, 1981). At low concentrations, Se can counteract oxidative stress in plants through the reduction of lipid peroxidation and GSH-Px (Hartikainen et al., 2000). In contrast, at high concentrations, Se acts as a pro-oxidant and leads to a drastic reduction in yield. There is evidence that Se alleviates Cd-induced oxidative stress in broccoli plants (Pedrero et al., 2008). It has been suggested that the protective role of Se against Cd stress can be linked mainly to a reduction of active oxygen radicals in rape seedlings (Filek et al., 2008). Al produces oxidative damage in higher plants. An enhancement of the antioxidant properties in wheat cultivars as a consequence of Se supplementation under Al stress conditions is rather limited. In this work, the behavior of Al-tolerant wheat cultivar Gemza 9 and Al-sensitive cultivar (Benyswef 5) with regard to growth, cell death and antioxidant properties was investigated as well as the ability of Se to counteract Al-induced oxidative stress in wheat plants supplied with Se.

MATERIAL AND METHODS

Plant growth and sample analysis

Two *Triticum aestivum* cultivars Gemza 9 (tolerant) and Benyswef 5 (sensitive) were used in this study. The seeds were obtained from Central Agricultural Research Institute, El DOKKY (Egypt). The seeds were rinsed in distilled water and surface-sterilized with 10% sodium hypochlorite solution for 10 min, washed and imbibed in

distilled water for 1 day. Then 15-20 seeds were planted onto plastic pots filled with sandy soil and grown in a growth chamber at 25°C with a 16 h/8 h light/dark photoperiod at a light intensity of 40 µmol m^{-2} s^{-1}. Ten days from germination, the seedlings were transferred to dark bottles filled with a continuously aerated basal nutrient solution proposed by Tolra et al. (2005). Two Al treatments (0 and 200 µM Al supplied as $AlCl_3$, MERCK reagent) were applied in combination with six Se levels (0, 1.0, 1.5, 2.0, 5.0 and 10.0 µM Se as $Na_2SeO_3.5H_2O$, MERCK reagent) in a completely randomized factorial design with replicates. pH of the nutrient solution was adjusted daily to 4.8 with diluted HCl or NaOH. Plants were harvested for growth and biochemical analysis 17 days after treatment.

Cell death

The loss of cell viability or cell death was evaluated using Evan's blue staining method as described by Baker and Mock (1994). The absorbance of Evan's blue released was measured at 600 nm.

Uptake of Al and Se

Subsamples of fresh material were dried at 65°C for 48 h to determine dry weight (DW), and the concentrations of Al and Se were then analyzed. Al was determined by flame atomic absorption spectrophotometry (FAAS), after plant samples were ashed at 500°C for 8 h and digested with hydrochloric acid (Azevedo et al., 2005).

For determination of Se concentration, the plant samples were digested in an acid mixture (16 ml 65% HNO_3, 2 ml 70% $HClO_4$ and 2 ml 95% H_2SO_4). Se was separated by an ammonium pyrrolidine dithiocarbamate-methyl isobutyl ketone (APDC-MIBK) extraction system and analyzed by using a coupled atomic absorption spectrophotometer graphite furnace (AAS-GF) at a wavelength of 196.1 nm (Kumpulainen et al., 1983).

$O^{\cdot}_2$, $OH^{\cdot}$ generation, H_2O_2 content and lipid peroxidation

Measurement of $O^{\cdot}_2$ was done according to the method of Kiba et al. (1997). The absorbance of the formed blue monoformazan was measured at 530 nm and its concentration was calculated using an extinction coefficient (ε) of 12.8 mM^{-1} cm^{-1}, which was an indirect measure of $O^{\cdot}_2$.

For estimation of extra-cellular OH, 10 excised root tips of equal length weighing 50 mg were incubated in 1ml of 10mM Na-phosphate buffer, pH 7.4 consisting of 15 mM 2-deoxy-D-ribose (SRL, Mumbai) at 37°C for 2 h (Halliwell et al., 1987). The absorbance of malondialdehyde (MDA) was measured at 532 nm and the concentration was calculated using an extinction coefficient (ε = 155 mM^{-1} cm^{-1}) and expressed in µmol g^{-1} FW.

Determination of H_2O_2 content was carried out according to Alexieva et al. (2001). The reaction was developed for 1 h in darkness and the absorbance was measured at 390 nm. The amount of H_2O_2 was calculated using a standard curve prepared with known concentrations of H_2O_2.

Lipid peroxidation was measured as the amount of MDA produced by the TBA reaction according to Dhindsa et al. (1981). The absorbance of the resulting supernatant was recorded at 532 and 600 nm. The non-specific absorbance at 600 nm was subtracted from that recorded at

532 nm. The concentration of MDA was calculated using an extinction coefficient (ε = 155 mM^{-1} cm^{-1}) and expressed in nmol g^{-1} FW.

Preparation of the enzyme extract and enzyme activity assay

Roots (0.5 g) were ground to a fine powder in liquid N_2 and then homogenized in 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 following the method of Gossett et al. (1994). The homogenate was centrifuged at 10,000 x *g* for 10 min at 4°C, the supernatants were collected and used for determination of the activities of ascorbate peroxidase, guaiacol peroxidase, glutathione reductase and glutathione S-transferase. Protein concentration in the enzyme extracts was determined by the method of Bradford (1976) using defatted BSA (Sigma, fraction V) as a standard.

Ascorbate peroxidase (APX) (EC 1.11.1.11) was assayed as described by Nakano and Asada (1981). The decrease in the absorbance at 290 nm for 1 min was recorded and the amount of the oxidized ascorbate was calculated using the extinction coefficient ε = 2.8 mM^{-1} cm^{-1}.

Guaiacol peroxidase (POD) (EC 1.11.1.7) activity was measured spectrophotometrically at 25°C following the method of Tatiana et al. (1999). The reaction started by the addition of the enzyme extract. The formation of tetraguaiacol was measured at 470 nm (ε = 26.6 mM^{-1} cm^{-1}).

Glutathione reductase (GR) (EC 1.6.4.2) activity was determined at 25°C by measuring the rate of NADPH oxidation as the decrease in absorbance at 340 nm (ε = 6.2 mM^{-1} cm^{-1}) according to the method of Halliwell and Foyer (1978). NADPH was added to start the reaction. For APX, POD, and GR, one unit of enzyme was defined as the amount of enzyme necessary to decompose 1 µmol of substrate per minute at 25°C.

Glutathione S-transferase (GST) (EC 2.5.1.13) activity was measured according to Mannervik and Guthenberg (1981) by following the changes in the absorbance at 340 nm.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was measured in fresh roots (1g) after homogenization in 8 cm^3 potassium phosphate buffer (50 mM, pH 7.8) containing 0.1 mM Na_2EDTA and 1% insoluble PVP. The homogenate was centrifuged at 20,000 x *g* for 20 min. The supernatant was collected and used for the assay of SOD following the method of Beyer and Fridovich (1987).

Statistical analysis

Data were subjected to statistical analysis, and the results were expressed as means ± SE of three independent replicates for every data set. Furthermore, for each Al level, the effect of Se on the response variables was investigated by polynomial regression analysis ($P \leq 0.05$).

RESULTS

Effect of Al and Se treatments on plant growth, cell death and contents of Al and Se

Our results showed that wheat was significantly affected by the addition of Al and Se to the growth medium (Fig. 1A). The Al concentration of 200 µM reduced root DW of both wheat cultivars by about 55% and 40% in cvrs. Benyswef 5 and

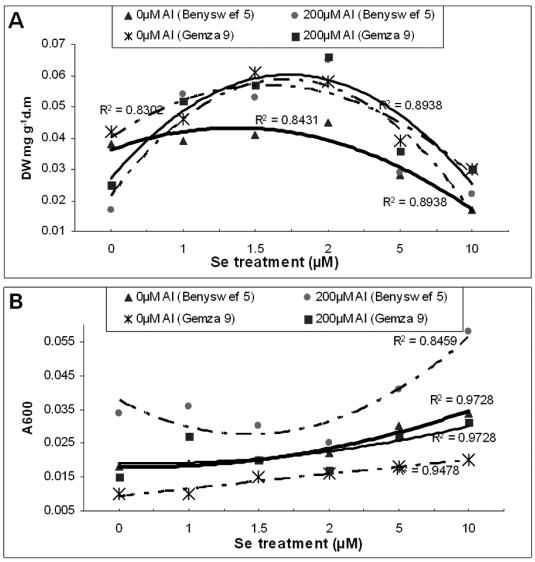


Figure 1. Dry weight (A) and cell death (B) of roots of two wheat cultivars (Benyswef 5 and Gemza 9) cultured hydroponically at different Al and Se supply levels. Data are means of three replicates. Solid lines represent a significant polynomial regression equation for the estimated effect of Se on the dry mass and cell death in Al-treated and untreated plants at $p \leq 0.05$.

Gemza 9, respectively. The application of Se up to 2 μM significantly promoted the root growth of plants treated with either 0 or 200μM Al. Thus, in cv. Gemza 9 grown without Al, the dry matter yield increased by 9.5%, 45.6% and 38% in plants treated with 1, 1.5 and 2 μM Se, respectively. The corresponding values of DW in cv. Benysewf 5 were 2.6%, 8.7% and 18% at the same Se levels, respectively. In Al-treated plants the addition of 2 μM Se into the culture raised the yield by 71% and 57% in cvrs. Benyswef 5 and Gemza 9, respectively. On the other hand, the addition of 5 or 10 μM Se significantly decreased the DW, and this effect was more pronounced when wheat plants were supplied with 200μM Al. The percent reduction in DW at 10 μM Se in the presence of 200 μM Al was 42% and 29% in cvrs. Benyswef 5 and Gemza 9, respectively. Accordingly, cv. Benyswef 5 was more sensitive to the high dose of Se than cv. Gemza 9.

Likewise, a dose response for cell death was evident for treatments with different concentrations of Se. Increasing concentration of Se affected considerably cell death. Cell death correlated better with the uptake of 200μM Al alone (R=0.94) rather than with treatment of Se in both wheat cultivars (R=0.893), but the cell death was markedly higher in cv. Benyswef 5 compared with cv. Gemza 9. The interaction between Al and Se up to 2 μM Se reduced cell death, however, higher concentrations of Se (5 and 10 μM) increased cell death (Fig. 1B).

More or less equal contents of Al were found in the roots of both cultivars at all Se concentrations, however, the addition of Se at 2 μM led to insignificant changes in Al content (Fig. 2A). Se application enhanced the accumulation of Al. After a 7-day exposure to 200 μM Al the contents of Al in the roots was almost from 2.7 times (cv. Gemza 9, tolerant) to 5 times (cv. Benyswef, 5 sensitive) those in the corresponding control (without Al). Seedlings without Al treatment contained some Al, which may be related to the purity of the growing nutrient salts. Application of Se up to 2 μM greatly reduced the content of Al in cv. Benyswef 5, but increased this content at higher concentrations of Se as compared with Al treated seedlings.

It was found that the Se content was significantly increased due to Se application. At 10 μM Se, Se content was more than 2.5-fold higher in cv. Benyswef 5 than in cv. Gemza 9. Plants

supplied simultaneously with Se and Al accumulated more Se than those grown with Se alone, and this effect was more noticeable in cv. Benyswef 5 than in cv. Gemza 9 at all Se concentrations applied to the culture media (Fig. 2B).

Effect of Al and Se treatments on extracellular generation of ROS

Our results on the extra-cellular generation of $O^{\cdot}_2$ following treatment with different concentrations of Se alone, Al or Al in combination with Se clearly indicated a dose-dependent increase of $O^{\cdot}_2$ due to Se in both wheat cultivars. The extra-cellular generation of $O^{\cdot}_2$ correlated with the severity of Se treatment, and it was always significantly higher in cv. Benyswef 5 than in cv. Gemza 9. Insignificant changes were recorded up to 2 µM Se. It was found that the generation of the superoxide ion increased in Al treated plants by 55% and 69% compared with the corresponding controls in cvrs. Benyswef 5 and Gemza 9, respectively. When the combined effects of Se and Al were analyzed, it was found that lower extra-cellular generation of $O^{\cdot}_2$ was provoked by different Se concentrations, especially at lower concentrations up to 2 µM Se in both wheat cultivars (Fig. 3A).

The generation of $OH^{\cdot}$ induced by Se was also increased following a dose-response at 5 and 10µM Se and insignificant changes were recorded at low concentrations up to 2 µM Se in both wheat cultivars (Fig. 3B). In the absence of Se, 200 µM Al increased $OH^{\cdot}$ about 6-fold and 8-fold in cvrs. Benyswef 5 and Gemza 9, respectively. Se applied up to 1.5 µM greatly decreased the $OH^{\cdot}$ generation as compared with Al-treated roots only while at higher concentrations of Se the $OH^{\cdot}$ generation was increased in both wheat cultivars.

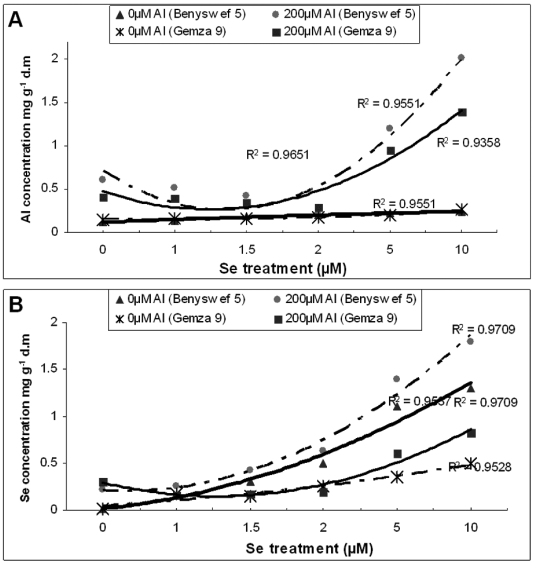


Figure 2. Concentrations of Al (A) and Se (B) in the roots of two wheat cultivars (Benyswef 5 and Gemza 9) cultured hydroponically at different Al and Se supply levels. Data are means of three replicates. Solid lines represent a significant polynomial regression equation for the estimated effect of Se on Al or Se concentrations in Al-treated and untreated plants at $p \leq 0.05$.

Al increased hydrogen peroxide contents after 7 days of exposure. The increase was 47% in the roots of the sensitive cv. Benyswef 5, but reached only 24% in the roots of the tolerant cv. Gemza 9 compared with the corresponding control. At the same time, in the presence of 1.5 µM Se, Al induced a decrease in hydrogen peroxide content in the roots of the sensitive cv. Benyswef 5 to attain the value of the control while in the tolerant cv. Gemza 9, a concentration of 2 µM Se was required to attain the value of the control (Fig. 3C).

The effect of Se treatment on LPO was determined by evaluating the tissue

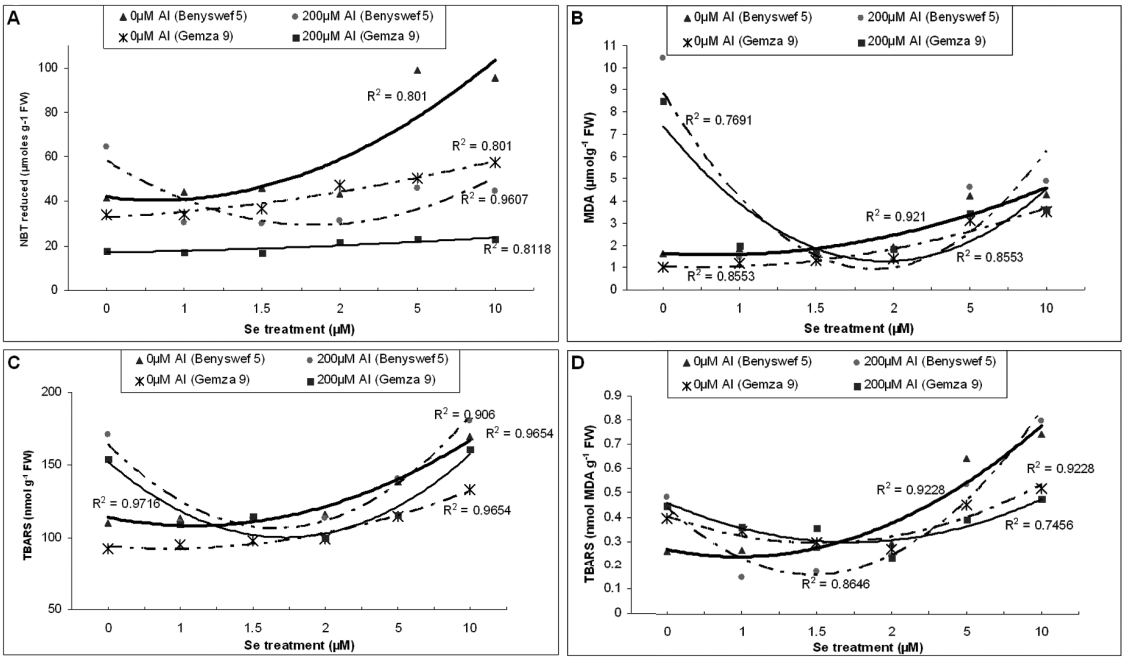


Figure 3. Extra-cellular generation of superoxide ion (A), hydroxyl radicle (B), hydrogen peroxide (C) and lipid peroxidation (D) in the roots of two wheat cultivars (Benyswef 5 and Gemza 9) cultured hydroponically at different Al and Se supply levels. Data are means of three replicates. Solid lines represent a significant polynomial regression analysis equation for the estimated effect of Se on reactive oxygen species accumulated in Al-treated and untreated plants at $p \leq 0.05$.

contents of TBARS. Se toxicity caused a significant increase in TBARS contents of the root tissue in a dose-dependent manner (Fig. 3D). TBARS accumulation was statistically significant above 2 µM Se level. There were 3-fold and 1.3-fold- increases at 10 µM Se level in cvrs. Benyswef 5 and Gemza 9, respectively. Insignificant changes in the TBARS accumulation was observed in response to lower concentrations of Se (Fig. 3D). Nevertheless, at a concentration of Se up to 2 µM, plants supplied with Al accumulated less TBARS in the roots of both wheat cultivars compared with controls and those cultivated with Se alone, but above this level TBARS steadily increased.

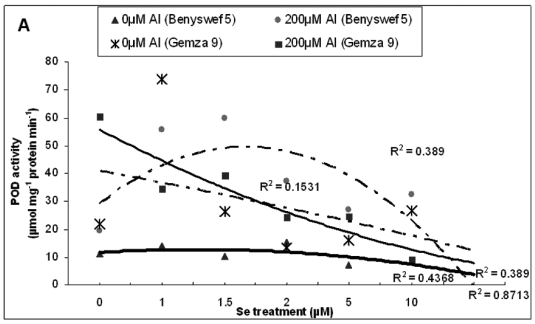
Effect of Al and Se treatments on antioxidant enzymes

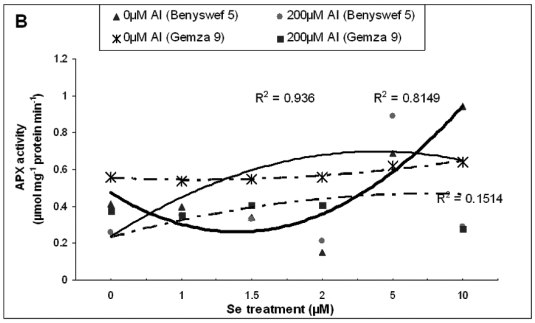
The activity of peroxidase was highest in plants supplied with Al than in those without Al (Fig. 4A). The application of Al alone enhanced the enzyme activity in the roots by about 1.9-fold and 2.6-fold in cvrs. Benysef 5 and Gemza 9, respectively compared with their corresponding controls. In the absence of Al, the application of Se up to 2 µM promoted POD activity, whereas higher Se concentrations steadily decreased its activity, but the activity was higher in the presence of Al. On the other hand, Se lowered the root APX activity in plants cultured without Al, and the greatest

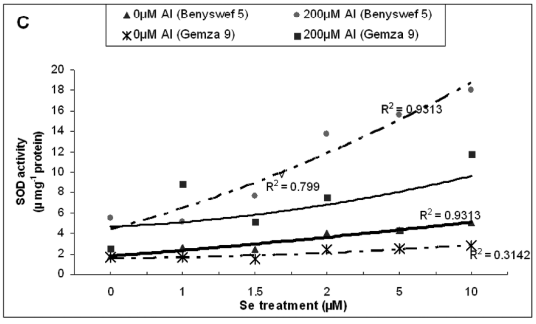
inhibition was recorded at 2 µM Se in cv. Benyswef 5 whereas an insignificant change was recorded in cv. Gemza 9. In Al-treated plants, APX activity was inhibited at Se concentrations up to 1.5 µM. Nevertheless, an activation of APX occurred at 5 µM Se while at 10 µM Se the enzyme activity was inhibited (Fig. 4B).

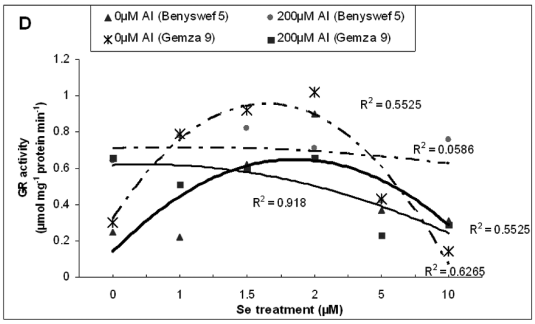
There was a slight increase of SOD enzymes after Se treatment. A significant increase was not detected at all Se levels in cv. Gemza 9 whereas in cv. Benyswef 5 a significant increase was recorded. SOD activity increased almost 1.5-fold and 2.7-fold due to treatment with 200 µM Al in cvrs. Gemza 9 and Benyswef 5, respectively (Fig. 4C). However, in plants supplied with Al, Se application up to 2 µM insignificantly diminished SOD activity. In contrast, at 5 or 10 µM Se, SOD activity of Al-stressed plants significantly increased in cv. Benyswef 5. In cv. Gemza 9, SOD activity of Al-stressed plants increased at all Se concentrations applied.

A considerable increase in GR activity was observed in a dose-dependent manner (Fig. 4D). The highest specific activity was observed at 2µM Se. GR activity increased 2.5-fold due to treatment with 200 µM Al in cvrs. Gemza 9 and Benyswef 5, respectively. However, in plants supplied with Al, Se application









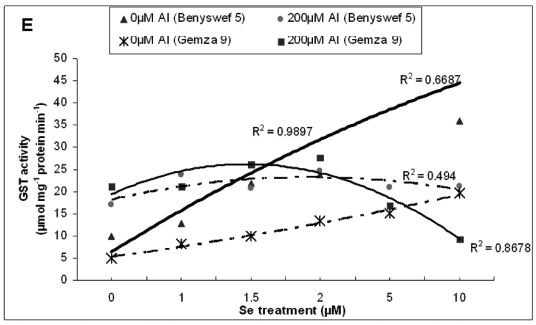


Figure 4. Changes in the activities of POD (A), APX (B), SOD (C), GR (D) and GST (E) in the roots of two wheat cultivars (Benyswef 5 and Gemza 9) cultured hydroponically at different Al and Se supply levels. Data are means of three replicates. Solid lines represent a significant polynomial regression analysis equation for the estimated effect of Se on reactive oxygen species accumulated in Al-treated and untreated plants at $p \leq 0.05$.

up to 2 µM stimulated GR activity in both cultivars. In contrast, at 5 and 10 µM Se, GR activity in Al-stressed plants significantly decreased in both cultivars.

GST activity increased at all Se levels in a dose-dependent manner. The highest GST activity was observed at 10 µM Se. There were about 4-fold differences between control and 10 µM Se treatment in cv. Benyswef 5 (Fig. 4E). In cv. Gemza 9 GST decreased up to 5 µM Se concentration followed by an increase at 10 µM Se. Treatment with 200 µM Al resulted in an increase in GST activity in both cultivars. Se application up to 2 µM increased GST activity. In contrast, at 5 or 10 µM Se, GST activity in Al-stressed plants decreased in cv. Gemza 9 while in cv. Benyswef 5 it increased up to 5 µM Se followed by a decrease.

DISCUSSION

Plants have a remarkable but differential ability to take up and accumulate various essential and non-essential elements including heavy metals from their external environment. In the present investigation, the exposure of wheat cultivars to 200 µM Al decreased root DW by about 55 and 40% in cvrs. Benyswef 5 and Gemza 9, respectively. These results indicated that the primary target of Al injury was the root system as a result of an inhibition of elongation and cell division in the root meristematic zone (Doncheva et al., 2005). The present investigation showed that Se applied at 2 µM promoted yield whereas growth was greatly inhibited when 5 and 10 µM Se were added (Fig. 1A). Furthermore, in the absence of Se, root DW of Al-treated plants was significantly improved at low Se supply in both wheat cultivars, indicating the role of Se to mitigate the toxic effect of Al. Several reports provided evidence that at low Se levels the yield of non accumulator plants like ryegrass was increased (Xue et al., 2001).

Plant growth responses were closely related to Al and Se content in wheat root tissues. A stronger reduction of root DW was detected in cv. Benyswef 5 compared with cv. Gemza 9. This may be due to the fact that cv. Benyswef 5 accumulated more Al than cv. Gemza 9. Even though, an obvious DW reduction was observed after the addition of 200 µM Al together with 5 or 10 µM Se (Fig. 1A). Plants simultaneously supplied with Al and Se also accumulated more Se in their root tissues compared with those supplemented with Se alone at all Se concentrations tested. Cv. Benyswef 5 was more sensitive to high levels of Se than cv. Gemza 9. The content of Se in cv. Benyswef 5 was more than 2.5-fold higher compared with cv. Gemza 9. The difference in Se content among the two wheat cultivars resulted in pronounced differences in dry matter and this could be used as a suitable trait for Se tolerance. In the present investigation, the apparent increase in Al or Se uptake in Al-stressed plants could be due to either alterations of extracellular and intracellular structures due to Al-toxicity (Kochian et al., 2005) or alterations in protein structure at high Se levels as Se can replace sulphur in amino acids (Terry et al., 2000). Recently, it has been shown that Se has the ability to regulate the water status of plants under drought conditions (Kuznetsov et al., 2003). In contrast, at low Se levels, the increased Se uptake by Al-Se-supplied wheat plants compared with plants treated with Se alone may

suggest the role of Se to counteract the toxic effects induced by Al in both wheat cultivars.

In the present study, the uptake of Se and Al were in good correlation with generation of ROS in the order of superoxide ion> hydroxyl radical > hydrogen peroxide. It was found that the generation of the superoxide ion increased upon Al treatment by 53% and 69% in cvrs. Benyswef 5 and Gemza 9, respectively compared with the corresponding controls. The extra-cellular generation of $O^{\cdot}_2$ correlated with the severity of Se treatment, and it was always significantly higher in cv. Benyswef 5 (in untreated plants) than in cv. Gemza 9 (in control and Se-treated roots). An insignificant change was recorded up to 2 µM Se. Jones et al. (2006) suggested that in maize roots Al exposure may lead to an uncontrolled production of ROS possibly by the formation of excessive amounts of Al superoxide semi reduced radical ions. This may be due to the pro-oxidant activity of Al in biological systems owing to the formation of the aluminum superoxide semi-reduced radical ion that may contribute to the foregone Al-induced oxidative stress (Exley, 2004). It has been shown that Al^{3+} rapidly binds to membrane lipids, replacing the Ca^{2+} bridges between the phospholipid head groups and inducing a rigidification of the lipid bilayer (Jones et al., 2006). Therefore, ROS accumulation could occur in wheat cultivars because of lack of detoxification capacity or an inhibition of ROS detoxification enzymes.

Peroxidase can also catalyse a third type of reaction that results in the production of $OH^{\cdot}$ from H_2O_2 in the presence of $O^{\cdot}_2$ (Liszkay et al., 2003). NADH-PX has a primary role in contributing to oxidative burst that results in generation of $O^{\cdot}_2$, H_2O_2 and $OH^{\cdot}$ on the cell surface. Thus, while the two cultivars upregulated ROS at low and moderate Se, their responses varied at a severe Se level. Cv. Gemza 9 controlled to some extent the up-regulation, whereas cv. Benysewf 5 failed to up-regulate ROS at higher Se levels alone or in combination with Al.

In the present study, H_2O_2 and lipid peroxidation content increased upon Al treatment in both wheat cultivars (Fig. 3C, D), because H_2O_2 is not compartmentalized in the cell and may inactivate enzymes by oxidizing their thiol groups. On the other hand, lipid peroxidation was the first type of oxidative damage to be occurred. Its overall effects include a decrease of membrane fluidity, an increase in the leakiness of the membrane, and damage to membrane proteins, enzymes, and ion channels (Garg and Manchanda 2009). Our result showing that Al can induce lipid peroxidation confirmed earlier reports on the effect of Al in different plants (Panda et al., 2003; Meriga et al., 2004). According to our result the ability of 2 µM Se to decrease root TBARS levels by almost 50% in both cultivars after treatment with 200µM Al demonstrated that plants increased the uptake of Se as a consequence of root Al injury. Furthermore, this result also suggested that at low Se levels an extra amount of Se was taken up by plants to alleviate the Al-induced oxidative stress and improve the antioxidant system ability (Mora et al., 2008). In contrast, the increase in TBARS contents at high Se levels (5 or 10 µM Se) indicated that Se may act as a pro-oxidant in wheat and this mechanism can, in addition to the metabolic disturbance, contribute to

Se toxicity (Hartikainen et al.,2000). The results of the present study indicated that the exposure of both wheat cultivars to 200 µM Al increased cell death to a higher extent than the exposure to Se alone (Fig. 1B). However, in plants supplied with Al, Se application up to 2 µM scarcely diminished cell death. In contrast, 5 or 10 µM Se significantly increased cell death in both wheat cultivars which attained 3-fold the control value at 10 µM Se. Tamas et al. (2004) reported that barley peroxidase was activated 48 h after the onset of Al treatment and concluded that H_2O_2 produced in barley roots during the early phase of Al stress might play an active role in the induction of cell death. It is known that after a longer exposure to Al stress or at higher Al concentrations, H_2O_2-induced necrosis can occur (Simonovicova et al., 2004; Tamas et al., 2005). Pan et al. (2001) observed also that low concentrations of Al induced an oxidative burst and PCD in barley root tips, while higher concentrations caused necrosis. Recent genetic evidence suggests that ROS do not trigger PCD or senescence by causing physicochemical damage to the cell, but rather these metabolites act as signals that activate genetically programmed pathways of gene expression that lead to regulated cell suicide events (Foyer and Noctor, 2005). Application of 2 µM Se in the presence of Al controlling ROS production might therefore be a promising avenue of genetic engineering to enhance the tolerance of plants to Al stress in both wheat cultivars.

The fluctuations in the level of lipid peroxidation due to the addition of Se were accompanied by significant changes in the activity of the antioxidant enzymes in the wheat plants cultured with or without Al. Low Se addition levels activated POD in the roots of plants growing with or without Al, but at 5 or 10 µM Se root POD activity was inhibited in Al-treated plants (Fig. 4A). Furthermore, compared with no Se addition, a great increase of POD activity in the roots of Al-stressed plants occurred, which seemed to reflect an increased hydrogen peroxide (H_2O_2) production at low Se supply levels.

APX exhibited the opposite behavior with respect to POD at low Se levels. At low concentration, Se decreased APX activity in roots irrespective of added Al (Fig. 4B). At 5 µM Se, APX was activated in the roots of Al-treated plants, but 10 µM Se inhibited the enzyme activity in both wheat cultivars although the activity was originally higher in cv. Gemza 9 both in control and Se-treated plants.

It is well known that the extent of oxidative stress is determined by the capability of the plant antioxidative defense system to suppress the toxic levels of ROS at the cellular level (Gratao et al., 2005). POD and APX function concurrently to detoxify H_2O_2 to water in higher plants (Apel and Hirt, 2004). Thus, at low Se levels, the APX inhibition can be attributed to the increased POD activity. On the other hand, the inhibition of POD and APX at higher Se supply levels (5 and 10 µM Se) was not efficient enough to protect the wheat cells from the Al-induced toxic damage and coincided with the increase of oxidative damage to cell membranes. These results also suggest that the pro-oxidant nature of Se when applied at higher concentrations might be due to the induction of ROS in the cell, but not through antioxidant enzymes. Rıos et al. (2009) have shown that when applied at high concentrations, Se diminished

APX activity, triggered H_2O_2 production and induced lipid peroxidation in lettuce plants as a consequence of Se toxicity. These facts give reason to suppose that ROS production observed in our study far exceeded the ROS scavenging capacity when 5 or 10 μM Se was applied, thus leading to the inactivation of defense enzymes.

Our results demonstrated that Se-mediated changes in the activities of the antioxidant enzymes were dose-dependent. We did not observe any significant changes in total SOD activity in the roots of cv. Gemza 9 in the wheat seedlings upon treatment with Se up to 2 μM, yet significantly higher activities were observed in cv. Benyswef 5, which may be related to the superoxide ion produced in the cells. Al increased SOD activity by about 2.6-fold and 1.6-fold in cvrs. Benyswef 5 and Gemza 9, respectively. The interaction of Al and Se greatly enhanced SOD activity in both wheat cultivars. This increase coincided with the reduction of cell damage of root membranes suggesting that Se was able to diminish the level of superoxide ion in root tissue of Al-stressed plants at a low concentration. Se with charges of positive four and positive six reacts directly with cysteine clusters in the catalytic subunits of enzymes, such as protein kinase, oxidizing the sulfhydryl groups to disulfide linkages, and thereby inactivating the enzyme (Spallholz 1994). Since oxidation of sulfhydryl groups is also associated with the production of both superoxide and peroxide, cell damage induced by Se may be triggered by either enzyme inactivation or ROS production.

According to the results obtained in this study, GST and GR activities were significantly increased in the roots of both wheat cultivars exposed to Se and Al (Fig. 4D, E). Stimulation of GSH synthesis by oxidative stress was reported by May and Leaver (1993). On the contrary, Se may interfere with the synthesis of GSH. The addition of Se strongly decreased sulfate-induced GSH accumulation in spinach leaf disks (De Kok, and Kuiper, 1986). In addition, incubation of spruce needles with Se caused a considerable reduction in GSH content (Bosma et al., 1991). Thus, interference of GSH synthesis in plants by Se or other Se compounds may diminish plant defense against hydroxyl radicals and oxidative stress. As a result of the substantial decrease in GSH synthesis, GR activity might be induced to compensate GSH through a reduction of the existing GSSG in the cell. Similar to our study, GR activity was significantly increased when coffee cell cultures were exposed to sodium selenite (Gomes-Junior, 2007).

Plants contain several GSH-dependent detoxifying enzymes, markedly GSTs, which collectively constitute 1% of the soluble protein in maize leaves. In this study, GST activity was also significantly increased by Al and Se treatments. Nevertheless, the activity was higher in cv. Gemza 9 (tolerant cultivar) than in cv. Benyswef 5 (sensitive one). The interaction between Se and Al increases GST, which has been considered as a marker for Al-stress in wheat. It is postulated that GSTs have additional functions other than catalyzing the formation of GSH conjugates. It was also demonstrated that several stress-inducible GSTs protected plants from oxidative damage by functioning as GSH-Px (Roxas et al.,1997). Similarly, higher GSH-Px activities have been reported

in ryegrass when exposed to elevated concentrations of sodium selenate or sodium selenite (Cartes et al., 2005). Se is also important for the antioxidant response in plant organisms. Se is an antioxidant at low concentrations. As a component of glutathione peroxidase, a widely recognized direct or indirect function of Se is the removal of reactive oxygen species (Drake, 2006). Thus, the ability of Se to offset the absorption of Al and consequently reduce its toxicity in both wheat cultivars is the most interesting result in the current study.

CONCLUSIONS

The present investigation strongly suggests that Se alleviates the toxic effect of Al in tolerant and sensitive wheat cultivars after 7-d exposure. Higher selenium level caused oxidative stress in the cultivars causing membrane damage through production of ROS. Consequently, it is more likely that antioxidant enzyme activities may not be part of the mechanism/s involved in Se stress tolerance. Nevertheless, Se-induced oxidative stress occurred at high concentrations exhibiting its role as a pro-oxidant, which was supported by previous reports (Cartes et al., 2005). These results suggest that there must be additional ways in which Se tolerant plants can alleviate the effects of Al stress. It is also suggested that detection and analysis of induced proteins might help to increase our understanding on the mechanisms of Se tolerance under Al stress in plants. It is well known that ROS-scavenging enzymes, APX, POD, SOD, GR and GST comprise numerous isoenzymes with different properties and ubiquity at the cellular level. Therefore, further studies are needed to determine if Se affects one particular or several isoforms of the antioxidant enzymes under Al stress conditions. Such studies will help to elucidate the potential contribution of Se to specific plant physiological processes.

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