SELENIUM INDUCES ANTIOXIDANT DEFENSIVE ENZYMES AND PROMOTES TOLERANCE AGAINST SALINITY STRESS IN CUCUMBER SEEDLINGS (*Cucumis sativus*)

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ABSTRACT

Selenium (Se) is not an essential nutrient for higher plants. Although, it is able to induce stress tolerance in many plant species. The present study was carried out to investigate the ability of Se to promote the tolerance of cucumber seedlings to salinity stress and to identify some biochemical parameter associated with Se–induced resistance in cucumber seedlings. Plants were irrigated with 2000 ppm NaCl after treatment with 1ppm Se for two weeks and samples collected after 7 days from started salt-treatment. The biochemical changes due to treatment with Se and /or NaCl were monitored by determination of lipid peroxidation (LPO), proline, phenolic compounds and percentage of electrolyte leakage (EL) from plant tissues. Moreover, the activities of peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and phenylalanine ammonialyase (PAL) were determined. The treatment of cucumber seedling with salinity (2000 ppm NaCl) in the presence or absence of selenium caused a remarkable elevation in free proline concentration and the level of phenolic compounds. Salinity stress caused a high level of lipid peroxidation which led to increase the percentage of electrolyte leakage from plant tissues. Se treatment caused a significant reduction in electrolyte leakage and malondialdehyde concentration of cucumber seedlings. The specific activities of antioxidant enzymes (POD, SOD, and APX) and PAL were significantly increased by the treatment with NaCl. Also, Se caused a significant elevation in the specific activities of POD, CAT, SOD, APX and PAL in cucumber seedlings grown under salt stress condition. On the other hand, Se caused a significant decrease in SOD activity in cucumber seedlings grown in non-salty condition. The isoenzymes pattern of POD demonstrated that peroxidase was upregulated by Se either in control or NaCl-treated seedlings. The results concluded that Se induce six POD isoenzymes in cucumber seedlings. Se reduces and overcomes the oxidative injury caused by salinity stress. Moreover, the protective effect of Se against salinity stress may be carried out through a mechanism included activation of PAL which is responsible for phenolic compounds biosynthesis. The accumulation of these phenolic compounds in Se-treated seedling leads to a high activities of antioxidant defensive enzymes and increase the tolerance level towards salinity stress.

1. INTRODUCTION

Soil salinity stresses plants in two ways. High concentration of salts in the soil makes it harder for roots to extract water and high concentration of salts within the plants can be toxic. Salts on the outside of roots have an immediate effect on cell growth and associated metabolism; toxic concentration of salts take time to accumulation inside plants before they affected plant function. Salinity is one of the major environmental factors that limiting global crop productivity. When plants subjected to salinity stress, the cretical balance between the formation of active oxygen species (AOS) and quenching activity of the antioxidant was disturbed. AOS generated due to salinity stress unbalances the cellular redox system in favor of oxidized forms resulting in oxidized damage to lipids, proteins and nucleic acids (**Halliwell and Gutteridge 1989**). Cucumber (*Cucumis sativus* L.) is one of the major vegetable crops grown in Egypt under the greenhouses conditions. Cucumber is a sensitive plant to oxidative stress (**Janoudi and Widders 1993**). So, we taken it in our study. Plant growth is limited by different unfavorable environmental conditions among which salt stress is considered to be one of the most important worldwide agricultural problems. High salinity can cause hyperosmotic stress and ion disequilibrium in plant cells, producing oxidative stress (**Hasegawa *et al* 2000; Zhu 2001**) and reducing plant growth. Also, many biotic and abiotic stresses induce an oxidative damage in plant cells. However, exposure of plants to salinity, drought, chilling or plant pathogens enhanced the production of reactive oxygen species (ROS) in plant cells (**Olmos *et al* 1997; Fieldes and Gerhardt 1998; Smirnoff 1993; Alscher *et al* 1997; Yordanov *et al* 2000; and Egert and Tevini 2002**). The enhanced production of reactive oxygen radicals is responsible for peroxidation of membrane lipids, photosynthetic pigments, protein and nucleic acids (**Smirnoff 1993; Alscher *et al* 1997; and Yordanov *et al* 2000**). The oxidative damage of the important molecules in plant cells affect most of the physiological process in plants and caused cell death. Many plants protect themselves by enhancement of the level of antioxidant such as carotenoids, ascorbic, α-tocopherol, flavonoids and phenolic compounds (**Egert and Tevini 2002**). Also, plants can tolerate the generation of ROS by enhancing antioxidative enzymes such as peroxidase, catalase and superoxide dismutase (**Egert and Tevini 2002; Siegel 1993 and Sancho *et al* 1996**). The hypothesis that selenium can protect plants against salinity stress was tested previously. Se-hyper accumulator plants such as indian mustard, cabbage and broccoli can tolerate the oxidative stress and have high level of resistance against wide range of biotic and abiotic stress (**Trelease and Trelease (1990) and Zhou 1990**). Moreover, the protective effect of Se on plants has been demonstrated as a decrease in lipid peroxidation and an increase in the activity of antioxidant enzymes in Cd-stressed in rape seedlings (**Filek *et al* 2007**). In addition Se may activate disproportion of superoxide radical and thus reduce the need of their scavenger superoxide dismutase (SOD) (**Hartikainen *et al* 2000**). Many reports suggested that Se is able to promote plant growth and induce stress tolerance against salt stress in sorrel seedlings (**Kong *et al* 2005**), UV-irradiation and senescence related stress in lettuce (**Hartikainen *et al* 1997; Hartikainen and Xue 1999; Xue and Hartikainen *et al* 2000 and Pennanen *et al* 2002**), UV-induce stress in reygrass (**Hartikainen *et al* 2000**) and photo oxidative stress in potato (**Seppänen *et al* 2003**). Phenylalanine ammonialyase (PAL) is considered to be a key enzyme of the phenylpropanoid biosynthesis (**Kacperska 1993**), catalyzing the transformation of L-Phenylalanine into trans-cinnamic acid, which is the prime intermediate in the biosynthesis of phenolic compounds (**Dixon *et al* 1992 and Levine *et al* 1994**). The activity of PAL was found to increase due to thermal stress (**Kacperska 1993; Levine *et al* 1994 and Leyva *et al* 1995**). PAL is considered to play a significant role in the mechanisms of stress acclimation in plant.

The main objective of the present investigation is to evaluate the protective effect of Selenium against salinity stress in cucumber seedlings and try to correlates the Se–induced salinity tolerance in cucumber seedlings with the activities of antioxidant defensive enzymes. The ability of Se to increase the phenolic compound biosynthesis through the activation of phenylalanine ammonialyase (PAL) was also studied.

2. MATERIALS AND METHODS

2.1. Plant material and growth condition

Cucumber seeds (*Cucumis sativus, L. cv* Zena) were cultivated in pots with sandy soil in a greenhouse for ten days (day by day). After germination, the seedlings (32-old days) were divided into two groups; the first group was irrigated with Hoagland's nutrient solution for two weeks, while, the second group was irrigated with the same nutrient

solution containing 1 ppm Se in form of sodium selenite for 2 weeks. Each group was subdivided into two subgroups. One of them was irrigated with NaCl solution (2000 ppm) and the other irrigated with distilled water as control. After 7 days of salinity treatment, samples from all groups were collected and used for determination of fresh weight, dry weight and electrolyte leakage. Samples also were frozen at -20°C and analysis was carried out.

2.2. Moisture content

Moisture content of cucumber seedlings was determined as recommended by **A.O.A.C. (1975)**. A known weight of cucumber seedling was dried in a ventilation oven at 70°C for 24 hours, then at 105°C for three hours. The results were calculated per grams for each 100 g fresh sample.

2.3. Measurement of electrolyte leakage

The electrolyte leakage was measured according to the method described by (**Kong et al 2005**). Twenty leaf discs from each subgroup were excised and rinsed thoroughly with double distilled water to remove contamination caused by sampling. Samples were then transferred to tubes with 20 ml double distilled water. The electrical conductivity (E_0) of the solution was immediately measured using an electrical conductivity meter (DDSJ-308A, Shanghai) at 25°C. The tubes were incubated at 30ºC for 30 min, and the electrical conductivity (E_1) measured again. Subsequently, the tubes were placed in boiling water bath for 20 min and the electrical conductivity (E_2) read after the tubes had cooled to 25ºC. The electrolyte leakage percentage (EL %) of leaf cells was calculated in accordance to the following equation:

$$EL\ (\%) = (E1 - E_0) / (E2 - E_0) \times 100$$

2.4. Lipid peroxidation

The level of lipid peroxidation was measured by determination of malondialdhyde (MDA) in fresh seedling tissues as described by (**Heath and Packer 1968**). One gram of fresh seedling tissue was homogenized in 5 ml of 0.1 % (w/v) TCA. The homogenate was centrifuged at 10000x g for 5 min, and then 4 ml of thiobarbuteric acid (0.5% in TCA 20%) was added to 1 ml of the supernatant. The mixture was heated to 95°C for 30 min and then quickly cooled in ice bath. The contents were centrifuged at 10000x g for 15 min and the absorbance of supernatent was measured at 532 nm. The MDA content was calculated using a extinction

coefficient of 155 mM^{-1} cm^{-1}. MDA content expressed as µmol. g^{-1} fresh weight (FW).

2.5. Determination of phenolic compound

Phenolic compound were extracted from fresh seedling samples with ethanol 80%. Total phenol content was determined in **Shahidi and Naczk (1995)**. Using Folin-ciocalteus reagent at 725 nm. Adding 1 ml of each extract, 0.5 ml Folin reagent and 7.5 ml distilled water then shaking for 3 minutes then 1 ml Na_2CO_3 was added, the blue color developed was determined after 1h at 725 nm against blank. The phenolic compound concentrations were determined by using the standard curve of catichol (100 ppm). The concentrations of phenolic compound were expressed as µg. g^{-1} dry weight (DW).

2.6. Determination of free proline

Free proline content of fresh seedling tissues was determined according to the method described by (**Petters et al 1997**). The proline content was expressed as µg. g^{-1}(FW).

2.7. Preparation of crude enzyme extract

Plant seedling (500 mg) was homogenized in 1ml of chilled Potassium phosphate buffer (100 mM, pH7.0) containing 0.1mM EDTA and 1% polyvinyl pyrrolidone (PVP) (w/v) at 4º C. Homogenate was squeezed through four layers of cheese cloth and extract thus obtained was centrifuged at 15,000× g for 15 min at 4º C. Supernatant was used to measure the activities of POD, CAT, SOD, APX and PAL.

The protein content in the enzyme crude extracts was measured according to **Lowry et al 1951**.

2.8. Assay of Peroxidase activity

Peroxidase, POD (E.C 1.11.1.7) activity in enzyme crude extract was determined as described by (**Hammer Schmidt et al 1982**). The reaction mixture (3 ml) consisted of 0.25% (V/V) Guaiacol in 10 mM sodium phosphate buffer (pH= 6.0 containing 10 mM H_2O_2). 25 µl of the crude enzyme extract was added to initiate the reaction. The absorption changes at 470 nm were followed by using spectrophotometrically (UV-visible-160A, Shimadzu). The activity was calculated by measuring the absorbance changes at 470 nm per min. A unit of peroxidase activity is defined as that amount of enzyme which cause 0.01 ΔOD. min^{-1}. The specific activity expressed as (IU. mg^{-1} protein).

2.9. Assay of SOD activity

Superoxide dismutase, SOD (E.C 1.15.1.1) activity was assayed by the method of (**Beauchamp and Fridovich 1971**) by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture (3ml) contained 40mM phosphate buffer (pH 7.8), 13mM methionine, 75µM NBT, 2µM riboflavin, 0.1mM EDTA and 100µl of enzyme crude extract. The test tubes were shaken and placed 30cm below light source consisting of 15 W fluorescent lamp. The absorbance was taken at 560nm. The activity of SOD was expressed as unit mg^{-1} protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light and enzyme activity was expressed as unit's mg protein (IU. mg^{-1}protein).

2.10. Assay of Catalase activity

Catalase, CAT (E.C 1.11.1.6) activity was determined using a modified method developed by (**Aeby 1984**). The reaction mixture (3 ml) contained 10 mM H_2O_2 and 100 µl of enzyme extract in 50 Mm phosphate buffer (pH 7.0). Enzyme activity was assayed by monitoring the decrease in absorbance at 240 nm as a consequence of H_2O_2 consumption. A unit of CAT activity (U) is defined as the decomposition of 1 µmol H_2O_2 per min. CAT activity was expressed as unit's mg protein (IU. mg^{-1}protein).

2.11. Assay of ascorbate peroxidase activity

Ascorbate peroxidase, APX (E.C 1.11.1.11) activity was measured as a decrease in absorbance at 290 nm for 1 min using L-ascorbate as standard (**Nakano and Asada 1981**). The assay mixture 3 ml consisted of 0.5 mM Ascorbic acid, 0.1 mM H_2O_2, 0.1 mM EDTA, 50 mM sodium phosphate buffer (pH 7.0), and 100 µL enzyme extract. A unit of APX activity is defined as the decreased of 1µmol of L-ascorbate per min and evaluated by comparison with a standard curve (0.5µmol to 20µmol L-ascorbate). APX activity was expressed as unit's mg protein (IU. mg^{-1}protein).

2.12. Assay of phenylalanine ammonia-lyase activity

The activity of phenylalanine ammonia-lyase, PAL (E.C 4.3.1.5) was assayed by the method described by (**He *et al* 2001**). The PAL assay reaction consisted of 100 µL crude enzyme extract and 900 µL of 6 µmol phenylalanine in 500 mM Tris-

HCl buffer (pH 8·5). The mixture was incubated at 37°C for 1 h and measured spectrophotometrically at 290 nm. Absorbance at 290 nm was measured and the amount of trans-cinnamic acid formed was evaluated by comparison with a standard curve (0.067 µmol 0.1ml^{-1} trans-cinnamic acid). PAL activity was expressed as unit's mg protein (µmol h^{-1} mg^{-1} protein).

2.13. Native PAGE & Peroxidase isoenzyme

The isozyme separation of POD was carried out in accordance to the method described by (**Rao *et al* 1997**). Equal amounts of protein (200 µg) from each sample extract were mixed with loading buffer and subjected to continuous poly acrylamide gel electrophoresis (PAGE) under non-denaturing and non-reducing conditions. Samples were loaded in sets into gel after separation. The gels were stained with benzidine (0.25 mg /100 ml distilled water containing 0.5 ml of glacial acetic acid) then added ten drops of 1 % freshly hydrogen peroxide to POD isozyme pattern.

Statistical analysis

Results are presented as means values ± standard deviasion of five replicates. Data were analsed by one way analysis of variance (ANOVA) using Student's t-test to test the different significance. Comparisons with *P*- values of *p* < 0.05 were considered significantly different.

RESULTS

Selenium induces growth of cucumber seedlings grown under salinity stress

Data in **(Fig.1.A)** indicated that salt treatment of cucumber seedlings for 7 days caused a significant reduction in the fresh weight. Moreover, the fresh weight of salt–stressed seedlings was decreased by about 36 % of control fresh weight. While, Se-treatment caused a significant increase in the fresh weight of cucumber seedlings grown in the presence or absence of salinity stress by about 17 % and 15 % respectively.

Also, (Fig.1.B) indicated that salt treatment of cucumber seedlings for 7 days caused a slight decrease in dry weight. Moreover, the dry weight of salt–stressed seedlings were decreased by about 14 % of control dry weight. Under 2000 ppm NaCl stress condition, cucumber seedlings treated with 1 ppm Se in form of selenite grew better than control. The results indicated that selenium at concentration of 1 ppm significantly promote the growth of salt-stressed and non salt-stressed cucumber

seedlings. Where, the seedling dry weight of sele-
nium treated cucumber seedlings grown in the
presence or absence of salt stress condition exhib-
ited the maximum dry weight by about 73 % and
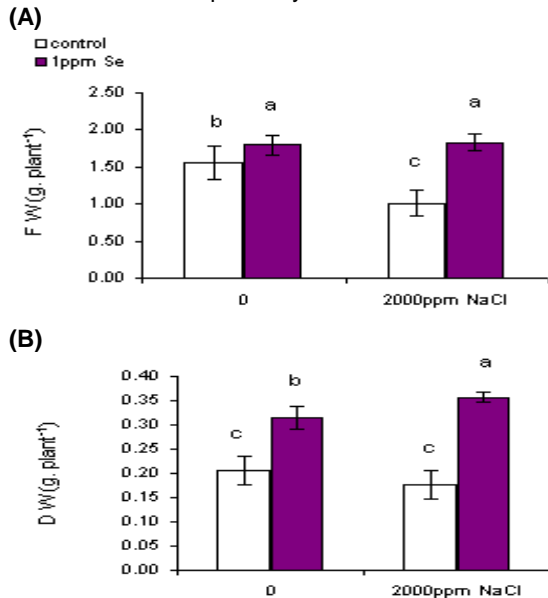53 % of control respectively.

(A)

(B)

**Fig. 1. Effects of Se on the growth of cucumber
seedlings grown in the presence or ab-
sence of 2000 ppm NaCl. Biomass pro-
duction of the plant as (FW g. plant^{-1}) (A)
and as (DW g. plant^{-1}) (B). Data present-
ed as means of 5 replicates ± SD. Bars
treatments with different letters are sig-
nificant different ($P < 0.05$)**

**Selenium improves cucumber seedlings re-
sistance against membrane damage under sa-
linity stress**

    MDA was taken as a marker for the degree of
lipid peroxidation as MDA produced by peroxida-
tion of unsaturated fatty acids in plant cell mem-
brane. (**Fig. 2.A**) indicates that salinity treatment
led to a significant increase in MDA. Whereas, lipid
peroxidation of plant tissues increased by salinity
treatment about one fold. Treatment of NaCl-
treated cucumber seedlings with 1ppm Se highly
reduced the level of lipid peroxidation expressed
as MDA content. Moreover, Se treatment reduced
content of MDA to the level closed to of untreated
cucumber seedling (control). The treatment of Se
only caused a slight increase in MDA content
about 25 % of control.

    To determine the effect of lipid peroxidation on
the performance of plant cell, the extend of mem-
brane damage was assessed indirectly by deter-
mining the amount of solute leakage from leaf
cells. The conductivity measurements presented in
(**Fig. 2.B**) showed that treatment of cucumber
seedlings with 2000 ppm NaCl led to a highly sig-
nificant increase in the electrolyte leakage. Moreo-
ver, the treatment with NaCl increased the level of
EL by about 300%. While, treatment of NaCl–
stressed cucumber seedling with Se caused a high
reduction in the electrolyte leakage by about 38%.
Moreover, the treatment of Se only caused in-
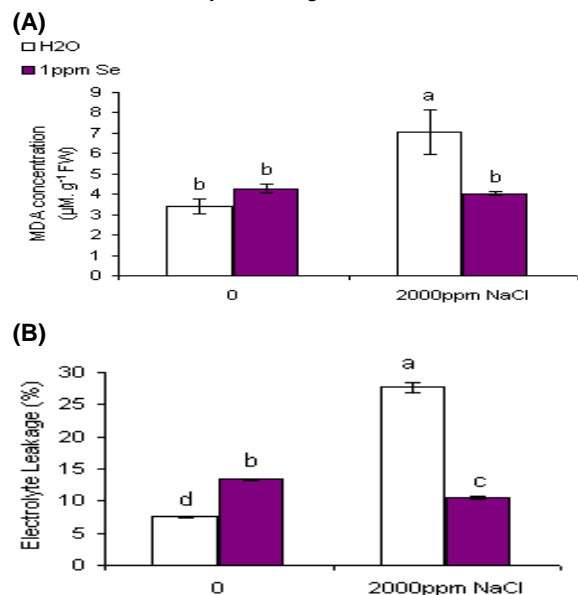creased in electrolyte leakage about 76%.

(A)

(B)

**Fig. 2. Effects of Se on lipid peroxidation ex-
pressed as malondialdehyde (MDA)
concentration (µMol. g^{-1} FW) (A) and
electrolyte leakage percentage (EL %)
(B) in cucumber seedlings grown in the
presence or absence of 2000 ppm
NaCl. Data presented as means of 5
replicates ± SD. Bars treatments with
different letters are significant different
($P < 0.05$)**

**Selenium increases the concentration of pro-
line and phenolic compounds in cucumber
seedlings grown under salinity stress**

    Proline and phenols contents were estimated in
Se-treated and control cucumber seedlings grown
in the presence or absence of salinuty stress. Pro-
line and phenols were increased by 165 % and
118% respectively by treatment with 2000 ppm
NaCl as compared with control. (**Fig.3. A,B**).

The treatment of cucumber seedlings grown under salinity condition with Se caused a significant increase in proline and phenolic compounds contents. Where, Proline content in cucumber seedlings grown under salinity stress increased by about 53 % as a result of treatment with 1 ppm Se. On the other hand, phenolic compounds were increased by about 245 % in salt-stressed cucumber seedling due to Se treatment. In salt-untreated cucumber seedlings (control) Se treatment did not cause significant change in Proline content. While, Phenolic compounds were significantly increased by Se-treatment as compared with control of cucumber seedlings. **(Fig. 3.B)**.
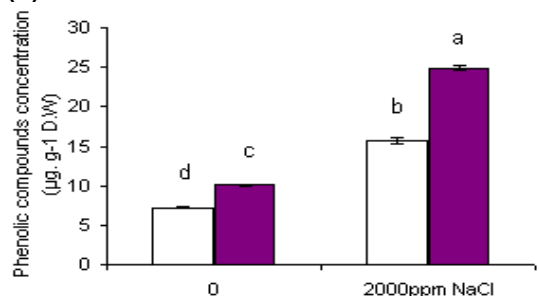
(A)
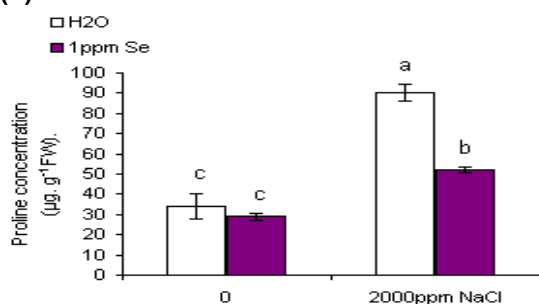
(B)

Fig. 3. Effects of Se on Proline concentration (μg. g^{-1} FW) (A) and Phenolic compounds concentration as (μg. catichol g^{-1} DW) (B) in cucumber seedlings grown in the presence or absence of 2000 ppm NaCl. Data presented as means of 5 replicates ± SD. Bars treatments with different letters are significant different ($P < 0.05$)

Se induces the activities of antioxidant enzymes in cucumber seedlings grown under salinity stress

Data in **(Fig. 4.A),** Showed that salinity stress significantly increased in the specific activity of POD about 110 % of control. Se-treatment caused a significant elevation in specific activity of POD in the presence or absence of 2000ppm NaCl. More-

over, Se treatment increased specific activity of POD about 280 % and 120 % of control respectively. CAT activity was decreased in cucumber seedlings irrigated with 2000 ppm NaCl by about 30 % of control. Se treatment caused a significant increase in the specific activity of catalase in the presence or absence of salinity stress. Moreover, Se treatment increased the specific activity of catalase about 311 % and 220 % of control respectively **(Fig. 4.B)**. Data of SOD activity indicated that salinity stress caused a significant increase in the specific activity of SOD about 20 % of control in cucumber seedlings. Also, Se-treatment caused a significant increase in the specific activity of SOD about 80 % of control in cucumber seedlings irrigated with 2000 ppm NaCl. While, Se treatment of cucumber seedlings grown in non salty medium caused a significant decrease in SOD activity about 15 % of control. But, The irrigation with 2000 ppm NaCl in the presence of Se led to a significant elevation in SOD activity **(Fig. 4.C)**. APX activity was significantly increased in cucumber seedlings irrigated with 2000 ppm NaCl by about 19 % of control. Se treatment caused a significant increase in APX activity in cucumber seedlings grown in the presence or absence of salinity stress. Moreover, Se treatment increased the specific activity of APX (65 % and 30 %) in the presence or absence of 2000 ppm NaCl respectively **(Fig.4.D).**

Se induces the activity of phenyalanine ammonia-lyase in cucumber seedlings grown under salinity stress

(Fig. 5). Showed that PAL activity was significantly increased in cucumber seedlings which irrigated with 2000 ppm NaCl by about 50 % of control. Se treatment caused a significant increase in PAL activity in cucumber seedlings grown in the presence or absence of salinity stress. Moreover, Se treatment increased the specific activity of PAL (150 % and 90 %) respectively.

Se up-regulates peroxidase iso-enzymes

Peroxidase isoforms were analyzed by Native PAGE. POD exist as multiple isoform **(Fig. 6)**. In the profile of POD isozymes of control cucumber seedlings Four bands (I,II,IV and V) were detected (lane A). Salinity-treatment and Se-treatment induced the appearance of new band (VI). Also, Se-treatment induced the appearance of two new bands (III and VI) were detected (laneD) in cucumber seedlings grown under salinity stress. Whereas, Se caused a quite strong staining intensity in new synthezied bands in salt-stressed cucumber seedlings. Se has a high ability to up-regulate POD isoenzymes (I,III,IV,V and VI) under salty conditions.
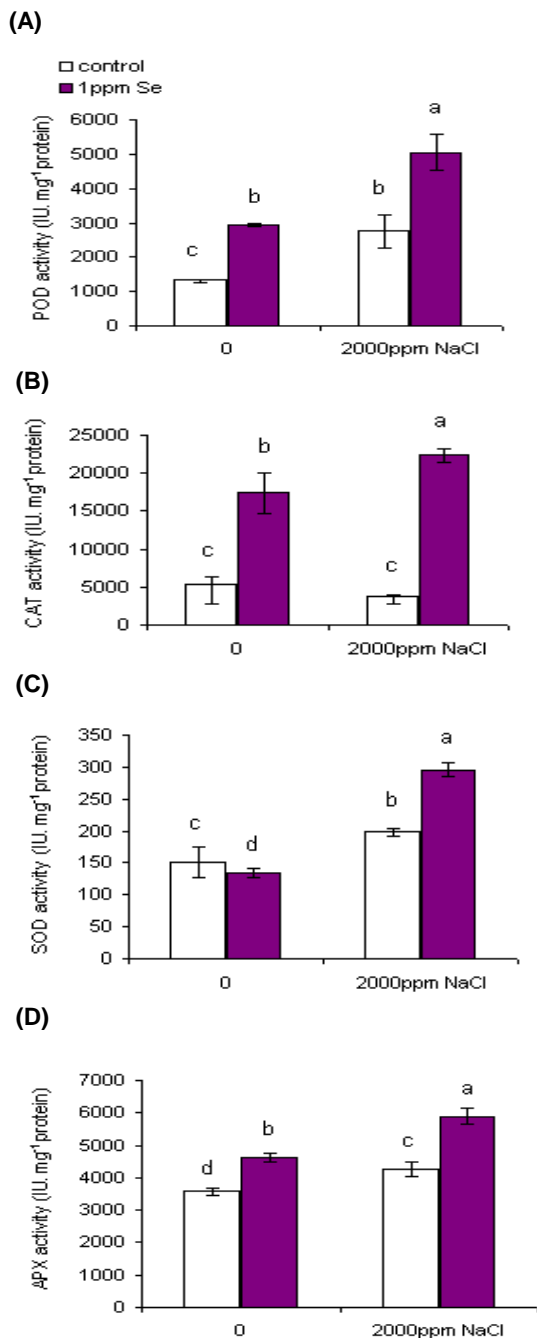
(A)

(B)

(C)

(D)

Fig. 4. Effect of selenium on the activities of POD (IU. mg^{-1} protein) (A), CAT (IU. mg^{-1} protein) (B), SOD (IU. mg^{-1} protein) (C) and APX (IU. mg^{-1} protein) (D) in cucumber seedling grown under salt stress (2000 ppm NaCl). Data presented as means of 5 replicates ± SD. Bars treatments with different letters are significant different (*P < 0.05*)

Fig. 5. Effect of selenium on the activity of PAL (µmol. Cinnamic acid h^{-1} mg^{-1} protein) in cucumber seedling grown under salt stress (2000 ppm NaCl). Data presented as means of 5 replicates ± SD. Bars treatments with different letters are significant different (*P < 0.05*)
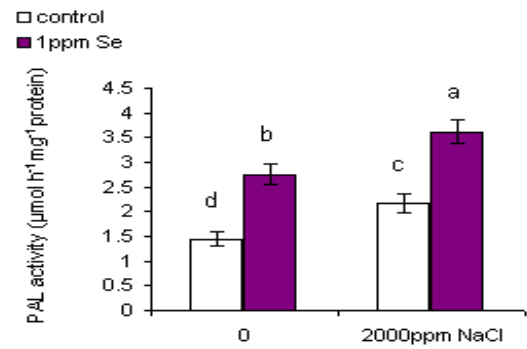

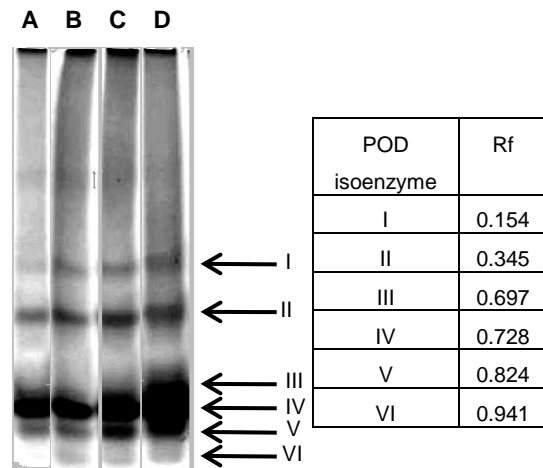| POD isoenzyme	Rf
I	0.154
II	0.345
III	0.697
IV	0.728
V	0.824
VI	0.941

Fig. 6. Effect of Se on peroxidases isoenzymes detected in soluble protein extract from untreated cucumber seedlings (control) (A), Se-treated cucumber seedlings (B), Salt-stressed cucumber seedlings (C) and Se- treated cucumber seedlings grown under salty stress condition (D). Equal amounts of protein (200 µg) were loaded from each sample

DISCUSSION

The production of reactive oxygen species (ROS) is the most important cause of plant cell damage when plants subjected to salt stress, thus leading to growth suppression (**Dionisio-Sese and Tobita 1998; Zhu 2001**). The observed reduction in the cucumber seedling fresh and dry weight as a result of salinity stress could explaind by the ac-

cumulation of H_2O_2 in plant cells (**Fig.1. A,B**). Howevere, the accumulation of H_2O_2 may lead to increased mechanical strength and lower the extensibility of plant cell walls, which can rapidly reduce seedling growth (**Schuiitzendiibel and Polle 2002**). Se-treatment caused a significant increase in the fresh and dry weights of cucumber seedlings grown in the presence or absence of salinity stress (**Fig.1.A,B**). Se at concentration of 1 ppm was able to stimulate the growth of salt-stressed cucumber seedling by increased the antioxidant enzymes activities that reduce the level of accumulation of ROS. Our results are in agreement with previous reports in sorrel seedlings (**Kong et al 2005**), UV-irradiation and senescence related stress in lettuce (**Hartikainen and Xue 1999; Xue and Hartikainen 2000 and Pennanen et al 2002**), UV-induce stress in reygrass (**Hartikainen et al 2000**) and photo oxidative stress in potato (**Seppänen et al 2003**).

The enhanced production of reactive oxygen species in salt stressed seedlings, led to high level of lipid peroxidation and caused a remarkable accumulation of MDA in plant tissues as shown in (**Fig. 2.A**). The level of MDA, one of the major TBA reactive metabolites, increased in NaCl–treated cucumber seedlings by about two times (**Fig. 2.A**). The results, we obtained confirmed that Se is able to reduce the lipid peroxidation in salt stressed seedlings. This protective effect of Se could be attributed to its ability to reduce oxygen radicals that are produced in the presence of salinity stress. Electrolyte leakage is important index of the physiological functions of the cell. Adversities such as drought, salinity and high and low temperatures initially damage the structure of the cell membrane leading to an increase in membrane permeability and resulting in leakage of intracellular contents (**Jia et al 2002**). Electrolyte leakage is an effective marker of plant cell membrane damage. The observed elevation of lipid peroxidation in salt-stressed cucumber seedlings led to a highly significant increased in the level of electrolyte leakage by about 300% (**Fig.2.B**). Whereas, Se treatment under salinity stress caused a significant reduction in both electrolyte leakage and MDA concentration. This reduction by Se treatment could be attributed to a high activities of some antioxidant enzymes such as POD and SOD which were highly consistent with the finding of (**Gueta-Dahan et al 1997**) in citrus and (**Dionisio-Sese and Tobita 1998**) in rice seedlings.

Many reports indicated that proline accumulated during many biotic and abiotic stress (**Aspinall and Paleg 1981; Yoshiba et al 1997; Hare et al 1999 and Chen et al 2001**). This response is typical with observed accumulation of proline in salt-stressed plant tissues (**Fig. 3.A**). Treatment of cucumber seedlings grown under salinity condition with Se caused a significant increase in proline content by about 53 % of control. The accumulation of proline in cucumber seedling by salt stress could be explained by increasing biosynthesis or inhibition of proline degradation. Proline biosythensis from glutamate is composed of two enzyme reaction involving Δ^1-pyrroline-5-carboxylate synthetase (P5CS) and Δ^1-pyrroline-5-carboxylate reductase (P5CR) (**Hayashi et al 2000**). An increase of P5CR activity by salinity stress was observed in leaves of wheat and P5CS activity did not show a specific response to salinity stress (**Zhi-Qiang et al 2007**). Thus, it's obvious that P5CR activity is in tight relation to free proline content. Also, Proline accumulation in plant cells exposed to salt is often considered to be involved in stress resistance mechanisms (**Aspinall and Paleg 1981; Yoshiba, et al 1997; Hare et al 1999; Chen et al 2001**). Also, Ornithine-δ-aminotransferase (OAT) catalyzes the first step in the pathway of conversion of ornithine to proline (**Hare et al 1999**). OAT activity was found to increase significantly in detached rice leaves exposed to excess Cu (**Chen et al 2001**). The increase in OAT activity may have, to some extent, contributed to the elevated content of proline. Increase in OAT activity along with an increase in the content of proline has also reported in wheat under cold stress (**Charest and Phan 1990**), and in *Brassica juncea* under salt stress (**Vogel and Kopac 1960**). The enzymes proline dehydrogenase (PDH) and Δ^1-pyrroline-5-carboxylate dehydrogenase (P5CDH) are reported to catalyze proline oxidation (**Yoshiba et al 1997 and Hare et al 1999**). Activities of thses two enzymes have no effect of CuSO4 stressed in detached rice leaves (**Chen et al 2001**). These results suggest that proline oxidation (or degradation) contribute little to proline accumulation in detached rice leaves under CuSO4 stress condition. Therefore, the accumulation of proline in cucumber seedling by salt stress could be explained by increasing the activities of (P5CR, P5CS or OAT) or inhibition of proline degradation enzymes (PDH and P5CDH). The effect of Se on the proline level could be due to its effect on one of proline metabolism enzymes and need furthur investigations.

The high phenols content in cucumber seedling treated with Se in the presence or absence of salinity stress is highly corelated to observed high PAL activities in these seedlings. So, Se enhanced

the biosynthesis of phenolic compounds by induction the activity of PAL which catalyzed the formation of trans–cinnamic acid by deamination of L–Phenylalanine. The production of trans–Cinnamic acid acts as the primer intermediary step in the biosynthesis of phenolic compounds (**Kacperska 1993; Dixon** *et al* **1992; Rosler** *et al* **1997 and Rivero** *et al* **2001**). Oxidation of phenolic compounds by polyphenol oxidase and peroxidase in leaves leads to the production of quinones (**Thypyapong** *et al* **1995**) which responsible for the production of active oxygen species under oxidative stress (**Pillinger** *et al* **1994**).

ROS generated due to salinity stress unbalances the cellular redox system in favor of oxidized forms resulting in oxidized damage to lipids, proteins and nucleic acids. So, plants adapted different protective mechanism to scavenge the free radicals produced under oxidative stress. In the present study, salinity stress resulted in a significant increase in the specific activities of (POD, SOD, APX and PAL) and a significant decrease in CAT activity in cucumber seedlings. This response indicates that enhanced production of free radicals under salinity sressed condition. Similar results have been found in NaCl-stressed roots of *chrysanthemum* (**Hossain** *et al* **2004**) and Cd-stressed in Rape seedlings (**Filek** *et al* **2007; Rout and Shaw 2001**). Se treatment caused a significant elevation in the specific activities of antioxidant enzymes (POD, CAT, SOD and APX) and PAL either NaCl–treated cucumber seedlings which indicate that Se can increase the tolerance of plants against oxidative stress (**Fig.4,5**). This protective effect of Se has been demonstrated as a known action of its antioxidant effect because it forms selenocysteine, part of the active center of antioxidant enzymes (**Levander and Burk 1994**) and as an increase in the activity of antioxidative enzymes (**Xue** *et al* **2001 and Sappänen** *et al* **2003**). Also. the present study showed that the specific activity of SOD was slightly reduced by Se treatment of control seedlings which indicate that Se may enhance the spontaneous disproportion of superoxide radicals and thus reduce the need of their scavenger SOD (**Hartikainen** *et al* **2000**). The induction of individual isozymes to salinity stres is interpreted as a response to augmented ROS generation. Both of salt treatment and Se-treatment exert differential effects on the expression of the multiple forms of POD (**Fig.6**). The salt-treated plants were capable of synthesizing new isoform of POD, which could be considered as a response to salinity-induced oxidative damage.

The results suggested that enzymatic removal of H_2O_2 by POD is the domainant pathway during salinity stress. Also, new synthezied isoforms of POD were detected in plants treated with Se in the presence of salinity stress. Whereas, Se enhanced POD activity because it forms selenocysteine, part of the active center of peroxidase (**Levander and Burk 1994**). The increased in peroxidase denisty and intensity in both of Se treatments could be a result of increasing the expression of POD genes.

The protective role of Se on the salt stress in cucumber seedlings could be due to : (1) reduction of oxygen radicals, (2) osmotic regulation by synthesis of osmoregulatory compound such as proline, (3) increasing the biosynthesis of non-enzymatic antioxidants such as phenols by increasing the activity of PAL and (4) induction the activities of some antioxidant enzymes and biosynthesis of new peroxidase isozymes.

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