The effect of NaCl on antioxidant enzyme activities in potato seedlings

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Abstract

The effect of NaCl on the growth and activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) were investigated in the seedlings of four potato cultivars (Agria, Kennebec; relatively salt tolerant, Diamant and Ajax; relatively salt sensitive). The shoot fresh mass of Agria and Kennebec did not changed at 50 mM NaCl, whereas in Diamant and Ajax it decreased to 50 % of that in the controls. In Agria and Kennebec, SOD activity increased at 50 mM NaCl, but no significant changes observed in Diamant and Ajax. At higher NaCl concentration, SOD activity reduced in all cultivars. CAT and POD activities increased in all cultivars under salt stress. Unlike the other cultivars, in Ajax seedlings, APX activity increased in response to NaCl stress. We also observed new POD and SOD isoenzyme activities and changes in isoenzyme compositions under salt stress. These results suggest that salt-tolerant potato cultivars may have a better protection against reactive oxygen species (ROS) by increasing the activity of antioxidant enzymes (especially SOD) under salt stress.

Additional key words: ascorbate peroxidase, catalase, peroxidase, salt stress, *Solanum tuberosum*, superoxide dismutase.

Introduction

Salinity is one of the major abiotic stresses affecting plant growth, development and productivity. Plants exposed to salt stress, undergo changes in their metabolism in order to cope with the changes taking place in their environment (Gueta-Dahan *et al.* 1997). One of the biochemical changes occurring when plants are subjected to biotic or abiotic stresses is the production of reactive oxygen species (ROS). The main sites of ROS production in the plant cell during abiotic stress are chloroplasts, mitochondria and microbodies (Breusegem 2001). ROS are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage lipids, proteins and nucleic acids (Halliwell and Gutteridge 1985, Fridovich 1986, Davies 1987, Meloni *et al.* 2003). Plants posses a number of antioxidant systems that protect them from these potential cytotoxic effects. Antioxidant enzymes are the most important components in the scavenging system of ROS (Meloni *et al*. 2003, Núñez *et al*. 2003). Superoxide dismutase (SOD) is a major scavenger of $^{\cdot}O_2^-$ and its enzymatic action results in the formation of H_2O_2. Catalase (CAT), ascorbate peroxidase (APX) (Chen and Asada 1989) and a variety of general peroxidases (POD) (Chang *et al*. 1984) catalyze the breakdown of H_2O_2. Therefore, this enzyme system eliminates the damaging effects of toxic oxygen species.

In many countries, potatoes are cultivated in arid and semi arid regions, where shortage or poor water quality are major factors limiting plant growth and yield (Ahmed and Abdullah 1979). Although, potato (*Solanum tuberosum* L.) is classified as a moderately salt sensitive, variation in salt sensitivity has been observed among different cultivars (Ahmed and Abdullah 1979, Heuer and Nadler 1998). Agria, Kennebec (relatively salt tolerant cultivars), Diamant and Ajax (relatively salt sensitive cultivars) were considered in this work.

The objective of the present study was to determine the effect of salinity on the growth and antioxidant enzymes (SOD, CAT, POD and APX) in relatively salt sensitive and tolerant potato cultivars.

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Abbreviations: APX - ascorbate peroxidase (EC 1.11.1.11); CAT - catalase (EC 1.11.1.6); NBT - nitroblue tetrazolium; SOD - superoxide dismutase (EC 1.15.1.1); POD - peroxidase (EC 1.11.1.7); PVP - polyvinylpyrolidone; GA_3 - gibberellic acid; NAA - naphthalenacetic acid; STS - silver thiosulfate; EDTA - ethylenediaminetetraacetic acid

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Materials and methods

Plants and treatments: Potato (*Solanum tuberosum* L.) cultivars, namely Agria, Kennebec (relatively salt tolerant), Diamant and Ajax (relatively salt sensitive) were used in this experiment. The plants were maintained by subculture of nodal cuttings on sterile medium consisting of MS (Murashige and Skoog 1962) salts and vitamins, 2 mg dm^{-3} Ca-pantothenate, 2 mg dm^{-3} gibberrelic acid (GA_3), 0.01 mg dm^{-3} naphthalene acetic acid (NAA), 1 mg dm^{-3} silver thiosulfate (STS), 30 g dm^{-3} sucrose and 7 g dm^{-3} agar. The shoot cultures were grown in 500 cm^3 glass jars capped with translucent closures. The culture conditions were 16-h photoperiod with irradiance of 150 µmol m^{-2} s^{-1} and temperature of 25 °C.

The treatments consisted of four salinity levels (0, 50, 75 and 100 mM NaCl). NaCl was added to the medium and five single nodes were cultured in any jar with 10 replicates for each treatment. The culture conditions were the same as above. After six weeks, the shoot fresh mass was recorded for each treatment and the plant materials were stored at -20 °C for subsequent analysis.

Protein and enzyme extraction: One gram of frozen shoots was homogenized in 1 cm^3 of an ice cold solution containing 100 mM Tris-HCl (pH 7), 20 % glycerol, 1 % PVP (Clulow *et al.* 1993). The homogenate was then centrifuged for 30 min at 18 000 *g*. A portion of the eluent was analyzed immediately for catalase activity, and the remainder was stored at -20 °C for subsequent analysis of SOD, POD and APX. Protein content was determined by the method of Bradford (1976).

Enzyme activity determination: Catalase activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 0.3 cm^3 of 3 % H_2O_2, 2.5 cm^3 of 0.05 M phosphate buffer (pH 7), and 0.2 cm^3 extract (Aebi 1983). APX activity was assayed by monitoring the ascorbic acid-dependent reduction of H_2O_2 at 265 nm in a reaction mixture containing 2 cm^3 of 0.05 M phosphate buffer (pH 6.5), 0.2 cm^3 of 3 % H_2O_2, 0.2 cm^3 of 50 µM ascorbate. Reaction was started by adding 0.1 cm^3 extracted protein (Arrigoni *et al.* 1992). POD activity was measured by the H_2O_2-dependent oxidation of benzidine at 530 nm, in a reaction mixture containing 2 cm^3 of 0.2 M acetate buffer (pH 4.8), 0.2 cm^3 of 3 % H_2O_2, 0.2 cm^3 of 0.04 M benzidine and 0.1 cm^3 extracted protein (Abeles and Biles 1991). SOD activity was measured by monitoring the inhibition of nitroblue tetrazolioum (NBT) reduction at 560 nm (Giannopolitis and Ries 1977). The reaction mixture contained 50 mM phosphate buffer (pH 7), 0.1 mM Na-EDTA, 75 µM riboflavin, 13 mM methionine and 0.01 - 0.02 cm^3 enzyme extract. Reaction was carried out in test tubes at 25 °C under fluorescent lamp (40 W) with irradiance of 75 µmol m^{-2} s^{-1}. The reaction was allowed to run for 8 min and stopped by switching the light off. Blanks and controls were run in the same manner but without irradiation and enzyme, respectively. Under the experimental condition, the initial rate of reaction, as measured by the difference in increase of absorbance at 560 nm in the presence and absence of extract, was proportional to the amount of enzyme. The unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photoreduction by 50 %. SOD activity values are given in units per mg of protein (Martinez *et al.* 2001). For other enzymes (CAT, POD, APX), the activity was defined as the changes in absorbance per minute for 1 mg protein.

Electrophoresis and isoenzyme visualization: Non-denaturating polyacrylamid gel electrophoresis (PAGE) was carried out using 10 % resolving gel at 4 °C and 65 mA (Hames and Rickwood 1990). POD isoenzymes were visualized by incubating of the gels in a solution consisting of 80 cm^3 of 0.2 M acetate buffer (pH 5), 8 cm^3 of 3 % H_2O_2, 4 cm^3 of 0.04 M benzidine. POD isoenzymes were appeared with brown bands after 30 - 60 min at 4 °C (Van Loon 1971). SOD isoenzymes were visualized by incubating the gels for 30 - 45 min in the dark in a solution consisting of 100 cm^3 of 0.2 M Tris-HCl (pH 8), 4 mg riboflavin, 4 mg Na-EDTA, 20 mg NBT and illuminating of the gels until the bands became apparent (Wendel and Weeden 1990).

Statistics: All data were subjected to a two-way analysis of variance and significance was determined at the 95 % confidence ($P \leq 0.05$) limits.

Results and discussion

The shoot fresh mass of Diamant and Ajax at 50 mM NaCl decreased approximately to 50 % of that in the controls, but no significant reduction of Agria shoot fresh mass was observed. The shoot fresh mass reduction on Kennebec was very low at this NaCl concentration (Fig. 1). Then, based on the reduction of shoot fresh mass, Agria and Kennebec were more salt tolerant than Diamant and Ajax at low NaCl concentration. At higher NaCl concentration, the growth of all cultivars significantly reduced.

Superoxide dismutase that catalyses the conversion of the superoxide anion to H_2O_2, performs the first step in

the removal of ROS (Breusegem *et al.* 2001, Rout and Show 2001). In the present study we observed a differential response in SOD activity in relatively salt sensitive (Diamant, Ajax) and tolerant (Agria, Kennebec) cultivars (Fig. 2*A*). Whereas, Agria and Kennebec exhibited an 87 and 27 % increase, respectively, in SOD activity at 50 mM NaCl, salt treatment had no significant impact on SOD activity of Diamant and Ajax. SOD activity reduced slightly in all of the cultivars at 75 mM NaCl. Similar increases in the activity of SOD have been reported by Hernandez *et al.* (1993) in the salt tolerant pea cultivar when exposed to NaCl. Also, in cotton, high SOD activity was associated with salt tolerance (Gossett *et al.* 1994, Meloni *et al.* 2003). Rout and Shaw (2001) observed similar changes in aquatic macrophytes. In contrast, Benavides *et al.* (2000) reported that, SOD activity increased significantly in the salt-sensitive clone of potato but remained unchanged in salt-tolerant ones when exposed to NaCl. Therefore, our results are opposite to that of Benavides *et al.* (2000). In Agria and Kennebec, increased SOD activities probably coped with injuring effects of $O_2^{\bullet -}$, but at higher NaCl level (75 mM) the protective role was inefficient and resulted to damaging effects on the plant growth. H_2O_2 scavenging enzyme activities significantly changed in response to NaCl stress. In the present study, POD and CAT activities increased in all cultivars under salt stress (Fig. 2*B*,*C*). CAT activities in Agria, Diamant, Kennebec and Ajax increased 393, 52, 33 and 32 % above the control levels, respectively. On the other hand, when plants exposed to 50 mM NaCl, POD activities in Agria, Diamant, Kennebec and Ajax were 485, 116, 62 and 77 % higher than in the controls, respectively. APX exhibited different manners. While, APX activity in Ajax at 75 mM NaCl increased 64 % above the level of that in 0 mM NaCl, in other cultivars the activity showed a decreasing pattern (Fig. 1*D*).

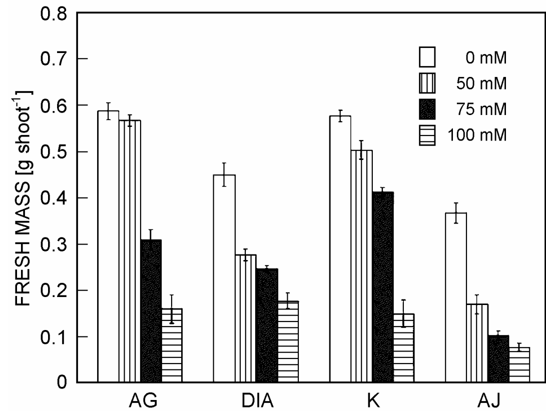

Fig. 1. The shoot fresh masses of potato seedlings under NaCl stress. *Bars* indicate SE. AG - Agria, DIA - Diamant, K - Kennebec, AJ - Ajax.

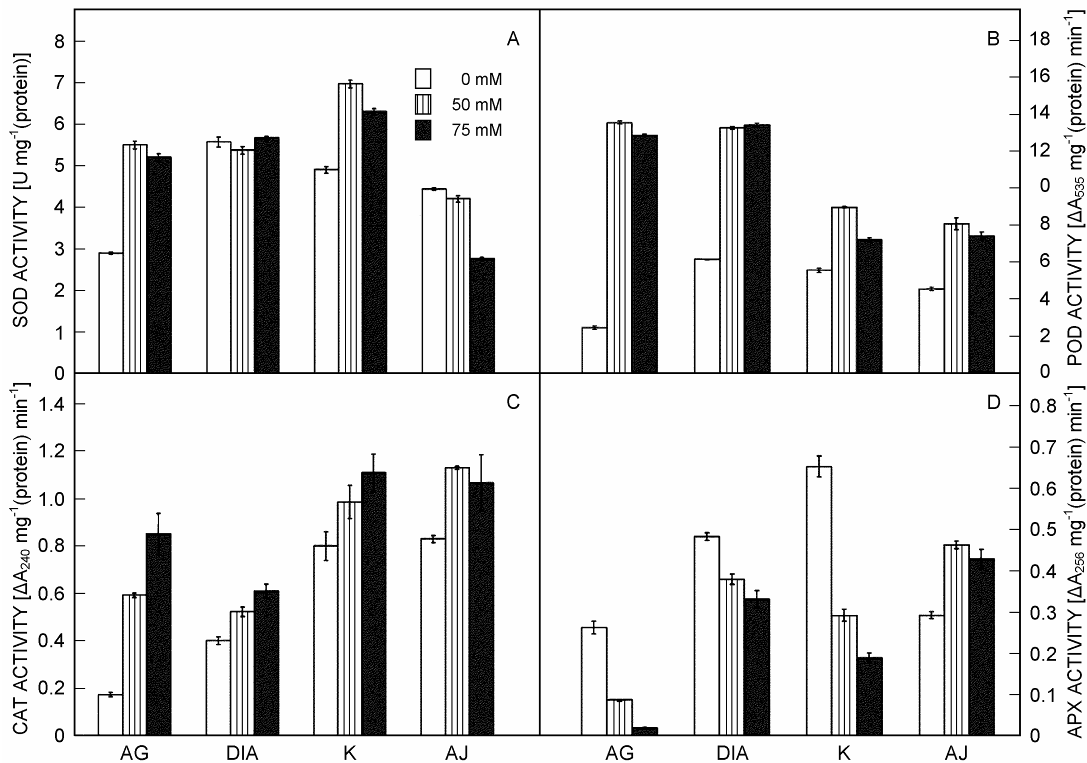

Fig. 2. Antioxidant enzyme responses to NaCl treatments in the potato cultivars (AG - Agria, DIA - Diamant, K - Kennebec, AJ - Ajax). *A* - SOD, *B* - POD, *C* - CAT, *D* - APX. *Bars* indicate SE.

There are different reports on the responses of H_2O_2 scavenging enzymes to salt treatments. In cotton, it has been reported that, under salt stress, H_2O_2 scavenging enzyme activities increased in salt tolerant cultivars and reduced or remained unchanged in non-tolerant ones (Gossett *et al*. 1994, 1996, Meloni *et al*. 2003). Rout and Shaw (2001) observed that the activity of CAT and POD increased significantly in salt-tolerant aquatic macrophytes in response to the salt treatment, while APX activity significantly reduced, but in salt-sensitive plants the activity of APX increased. Benavides *et al*. (2000) concluded that APX was probably more important than CAT in the H_2O_2 detoxification in potato exposed to NaCl.

According to the present results, POD and CAT probably had more important role in H_2O_2 detoxification than APX, although in Ajax, APX together with CAT and POD plays detoxifying role in the plant. Then, we suggest that the importance of the enzymes in H_2O_2 detoxification depends on the cultivar. On the other hand, the elevated H_2O_2 detoxifying enzyme activities without an accompanying increase in the ability to scavenge superoxide radical result in damaging effects in plants.

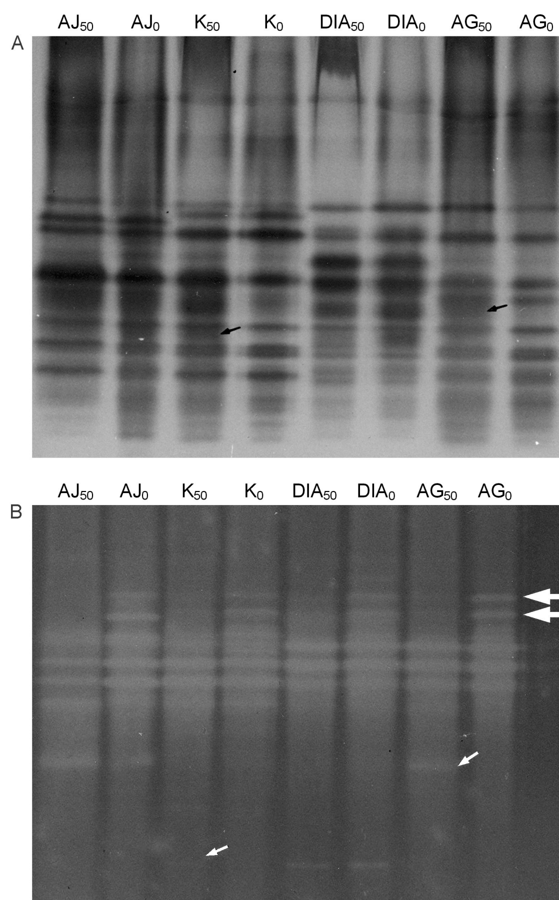

Fig. 3. Effects of 50 mM NaCl on POD (*A*) and SOD (*B*) isoenzymes in potato cultivars (AG - Agria, DIA - Diamant, K - Kennebec, AJ - Ajax). *Large arrows* indicate decreased isoenzyme content and *small arrows* indicate new isoenzyme bands.

The isoenzyme compositions of POD and SOD at 50 mM NaCl were studied using 10 % PAGE. The electrophoretic profils of POD and SOD isoenzymes were affected by NaCl treatment (Fig. 3). This alteration in POD isoenzymes profil was expressed as increased intensity of some isoenzyme bands and the appearance of new isoenzyme bands under salt stress (Fig. 3*A*). For example, isoenzymes with relative mobility (Rm) 0.536 in Agria or with Rm 0.587 in Kennebec were appeared in 50 mM NaCl.

In all the cultivars, the electrophoretic profil of SOD isoenzymes was also altered at 50 mM NaCl (Fig. 3*B*). But unlike POD, the alteration in SOD isoenzyme profils was expressed mostly as a reduction in the isoenzyme activities (bands with Rm 0.39 and 0.42) in all the cultivares. In Ajax these isoenzymes were eliminated. New SOD isoenzymes with Rm 0.7 and 0.08 were appeared at 50 mM NaCl in Agria and Kennebec, respectively. Then these new SOD and POD isoenzyme bands may be associated with salt tolerance character of Agria and Kennebec.

There are many reports on the changes in the activity of various SOD isoenzymes and corresponding mRNA under osmotic stresses (Mittler and Zilinskas 1993, Zhu and Scandalios 1994). Therefore with respect to expression of new SOD and POD isoenzymes and changes in the constitutive enzyme pools, we suggested that plant response to salinity could be expressed at the transcriptional and post-transcriptional levels.

In conclusion, this study showed that the difference of SOD, POD, CAT and APX activities in the relatively salt sensitive and tolerant potato cultivars could be ascribed to the difference in mechanisms underlying oxidative stress injury and subsequent tolerance to salinity. On the other hand, the importance of the enzymes in H_2O_2 detoxification depends on the cultivar. And that, the elevated H_2O_2 detoxifying enzyme activities without an accompanying increase in the ability to scavenge superoxide radical result in damaging effects in plants. Finally, these results suggest that salt-tolerant potato cultivars may have a better protection against reactive oxygen species (ROS) by increasing the activity of antioxidant enzymes (especially SOD) under salt stress.

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