

Effect of salt on malondialdehyde and antioxidant enzymes in seedling roots of Jerusalem artichoke (*Helianthus tuberosus* L.)

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Abstract Two cultivars of Jerusalem artichoke (*Helianthus tuberosus* L.) differing in genotype, Red skin (cv. R., salt-tolerant but low-yield) and White skin (cv. W., salt-sensitive but high-yield), were used to investigate malondialdehyde (MDA) content and antioxidant enzyme activity changes in their roots under a hydroponic culture system with 250 mM NaCl. The results showed that MDA contents in roots of the two genotypes increased, but MDA content of cv. R. was higher than that of cv. W. Changes in all antioxidant enzymes in roots of both varieties exhibited a similar trend, namely increased initially and then decreased. However, there were still some differences existing between the two cultivars. In other words, activities of the other two antioxidant enzymes except catalase (CAT) and peroxidase (POD) in roots of cv. R. were less

than controls at 48 h, while all others except ascorbate peroxidase (APX) in roots of cv. W. were greater than controls. The peak of superoxide dismutase (SOD) activity of cv. W. was observed to appear earlier than that of cv. R. CAT activity of cv. W. was significantly greater than the value of cv. R. and the latter showed a moderate trend. POD activity of cv. R. obtained the maximum at 6 h, whereas the peak of cv. W. displayed at 24 h. APX activity of cv. R. declined more than that of cv. W. These results suggested that there was a lower efficiency of scavenging reactive oxygen species (ROS) in cv. R. roots. Concomitantly, salt stress caused more severe damage to roots of cv. R. Antioxidant enzymes in roots were inadequate to elucidate salt-tolerance mechanisms of the whole plant.

Keywords *Helianthus tuberosus* · Reactive oxygen species · Roots · Salt-tolerant

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Abbreviations

APX	Ascorbate peroxidase
AsA	Ascorbate
CAT	Catalase
H ₂ O ₂	Hydrogen peroxide
MDA	Malondialdehyde
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase

Introduction

Salinity is a major abiotic stress affecting approximately 7% of the world's total land area resulting in billion dollar

losses in crop production around the globe (Shabala and Cuin 2007). With the use of chemical fertilizers and development of irrigation agriculture, salinity land areas have increased year by year. Salt stress causes multifarious adverse effects in plants. Among them, production of reactive oxygen species (ROS) is one of the deleterious effects. When molecular O₂ undergoes reduction, it gives rise to ROS such as superoxide (O₂[−]), hydrogen peroxide (H₂O₂) and the hydroxyl radical (·OH). Singlet oxygen (¹O₂), which may arise due to the reaction of O₂ with excited chlorophyll, is also considered as one of the potential ROS (Ashraf 2009). These ROS are highly reactive because they can interact with a number of cellular molecules and metabolites thereby leading to a number of destructive processes causing cellular damage. Plants possess antioxidant metabolites, enzymes and non-enzymes to a variable extent, which have the ability to detoxify ROS (Ashraf 2009). Antioxidants have been touted as beneficial for enhancing plant stand and mitigating the effects of biotic and abiotic stresses (Singh et al. 2010). Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (POD) (Xue and Liu 2008; Ashraf 2009). Among them, SOD catalyzes the dismutation of O₂[−] to molecular oxygen and H₂O₂ (Giannopolitis and Ries 1977). CAT catalyzes a redox reaction in which dismutation of H₂O₂ gives rise to water and oxygen (Ashraf 2009). Tetrاغuaiacol is produced by the reaction of guaiacol with H₂O₂ in the presence of POD (Chen and Wang 2006). APX catalyzes the oxidation of ascorbate (AsA) by H₂O₂ and gives rise to monodehydroascorbate radical (Ashraf 2009). Malondialdehyde (MDA) is one of the final decomposition products in membrane lipid peroxidation, described as an indicator of the damage extent of membrane lipid peroxidation. It has widely been utilized to differentiate salt-tolerant and salt-sensitive cultivars (Xue and Liu 2008).

Jerusalem artichoke (*Helianthus tuberosus* L.) originates in North America and belongs to Asteraceae *Helianthus* genus. According to the color of tuber, it can be divided into red and white varieties. Jerusalem artichoke is tolerant to drought, cold and has a strong adaptability (Liu et al. 2003; Shi 2008). Thus, in the present study, Red skin (cv. R., salt-tolerant but low-yield) and White skin (cv. W., salt-sensitive but high-yield) were compared for their salt responses. Although parts of works have been done to screen salt-tolerant Jerusalem artichoke cultivars, most of works were performed on leaves (Xue and Liu 2008; Long et al. 2009). The immediate feedback of Jerusalem artichoke roots in a short and successive time course, especially different salt-tolerant genotypes, has not been investigated in detail. Given the above facts, we carried out this work at root levels. The paper aimed at revealing the salt-tolerant differences of the two genotypes with a short-

onset salt stress and providing a scientific theory for breeding a salt-tolerant and high-yield cultivar in future.

Materials and methods

Plant material and stress treatments

Tubers of cv. R. and cv. W. were independently collected from Xiuyan of Liaoning Province and Yulin of Shanxi Province, China. Tubers of the two genotypes were cut into small square pieces with a single bud, and about 1 g weight, sterilized with 75% ethanol (v/v) for 30 min, and washed thoroughly with distilled water. Subsequently, tuber slices were immersed into 2.9 × 10^{−5} M Gibberellic acid (GA3) for 5 min in order to uniformly maintain the future seedlings. Thereafter, they were rinsed drastically with distilled water, and germinated on moist sand in an incubator at 25°C. The relative uniformly germinated slices with buds were picked out and cultured with half-strength Hoagland's nutrient solution in the tissue culture house. The nutrient solution was renewed once in every 2 days.

The seedlings were subjected to 150 mM sodium chloride (NaCl) treatment by supplying salt to the nutrient solution after 21 days. Next day, it was replaced with a new half-strength Hoagland's nutrient solution that contained 250 mM NaCl. Subsequently, cv. R. and cv. W. were investigated for their responses to NaCl stress. Compared with the controls, analyses were done at 3, 6, 12, 24, 48 h after treatment. A part of mixed roots (5-cm section from root tip) were treated and used for MDA determination and the others were frozen in liquid nitrogen and kept at −80°C for further antioxidant enzymes analysis.

MDA contents assay

MDA contents in roots of cv. R. and cv. W. suggested the severity of their lipid peroxidation. MDA content was measured on the basis of thiobarbituric acid reaction protocol (Heath and Packer 1968).

Antioxidant enzymes extraction and assay

Antioxidant enzymes extraction was carried out according to Zhang et al. (2009) with minor modifications. Briefly, roots were ground into fine powder in liquid nitrogen with a pre-cooled mortar and pestle. Exactly 0.5 g of each sample was extensively homogenized in 2 ml of homogenate buffer containing 50 mM sodium phosphate dibasic and sodium phosphate monobasic (Na₂HPO₄–NaH₂PO₄, pH 7.8), 0.2 mM ethylenediaminetetraacetic acid (EDTA) and 1% w/v polyvinylpyrrolidone (PVPP) in an icy

bath. The homogenate was transferred into an Eppendorf tube and centrifuged at $12,000 \times g$ for 20 min at 4°C . The supernatant was transferred to a new tube for determination of enzyme activities. All extraction procedures were performed at 4°C . The absorption was determined on a Shimadzu UV-1800 spectrophotometer.

SOD activity assay was carried out according to Zhang et al. (2009) as follows: the 3 ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM nitro-blue tetrazolium (NBT), 2 mM riboflavin, 10 mM EDTA and 0.1 ml enzyme extract. One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of NBT reduction measured at 560 nm. SOD activity was expressed as U mg^{-1} protein.

CAT activity was determined by measuring the decrease in the absorbance of H_2O_2 at 240 nm with time according to Chen and Wang (2006). A 0.01 decrease in absorbance per minute at 240 nm was defined as one unit of the enzyme activity. The 3 ml reaction mixture consisted of 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 ml enzyme extract. CAT activity was expressed as U mg^{-1} protein min^{-1} .

POD activity was estimated according to Chen and Wang (2006) as described below. Activity was detected by measuring the increase in absorbance at 470 nm due to guaiacol oxidation. A 0.01 increase in absorbance per minute at 470 nm was defined as one unit of the enzyme activity. The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol, 1.0 mM H_2O_2 and 0.1 ml enzyme extract. The activity was expressed as U mg^{-1} protein min^{-1} .

APX activity was measured based on Zhao et al. (2002) by monitoring the rate of AsA oxidation at 290 nm. The assay mixture contained 0.25 mM AsA, 1.0 mM H_2O_2 , 0.1 mM EDTA, and 0.2 ml enzyme extract in 25 mM phosphate buffer (pH 7.0). APX was detected according to the reduction value of the absorbance at 290 nm per unit time. The extinction coefficient of AsA was $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ at 290 nm. The activity of APX was calculated in the light of $\mu\text{mol AsA mg}^{-1} \text{ protein h}^{-1}$.

Protein concentration was detected according to the Bradford (Bradford, 1976) method.

Statistical analysis

Physiological and biochemical data were presented as mean \pm standard deviation (SD) of three biological replicates. Student's *t* test ($P < 0.05$) was selected for data statistics between the cultivars at the same time point and asterisks indicated significant differences using a *t* test. Duncan's new multiple range test ($P < 0.05$) was used for data statistics of each cultivar at different time points and

lower case letters *a*, *b*, *c*, *d*, and *e* indicated statistical significance.

Results

Effect of NaCl on MDA contents

MDA levels of cv. R. and cv. W. expressed an upward trend and cv. R. was significantly higher than cv. W. in the process of salt treatment (Fig. 1). MDA content of cv. R. increased significantly at the onset of salt treatment, while dropping provisionally at 6 h but still greater than controls. Later, MDA content rose progressively, up to the maximum at 48 h, and it was 1.31-fold higher than the control. MDA content of cv. W. showed a subdued trend in the whole process of salt treatment. At 48 h the peak appeared and it was 1.2-fold higher than the control, nevertheless, compared with cv. R., it was significantly lower. MDA content of cv. R. was 1.7-fold higher than cv. W. at 48 h.

Effect of NaCl on antioxidant enzymes activities

Activities of antioxidant enzymes (SOD, CAT, POD and APX) in roots of two varieties exhibited a similar change. That is to say, increased first and then decreased, but obvious differences existed (Fig. 2). More specifically, peaks of activities of antioxidant enzymes appeared at different times and the activities of antioxidant enzymes were also different in the last stage of salt stress.

Activities of SOD in the two cultivars showed a similar trend, but the activity of cv. W. was significantly greater than that of cv. R. The peak of SOD of cv. W. was

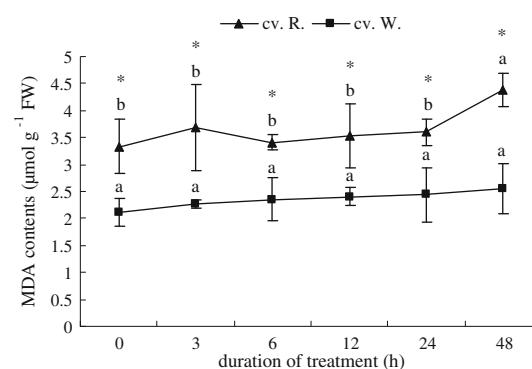


Fig. 1 Effect of NaCl on MDA contents in roots of *Helianthus tuberosus* L. Values are presented as mean \pm standard deviation (SD) of three biological replicates. Student's *t* test ($P < 0.05$) was selected for data statistics between both cultivars at the same time point and asterisks indicated significant differences using a *t* test. Duncan's new multiple range test ($P < 0.05$) was used for data statistics of each cultivar at different time points and lower case letters *a* and *b* indicated statistical significance

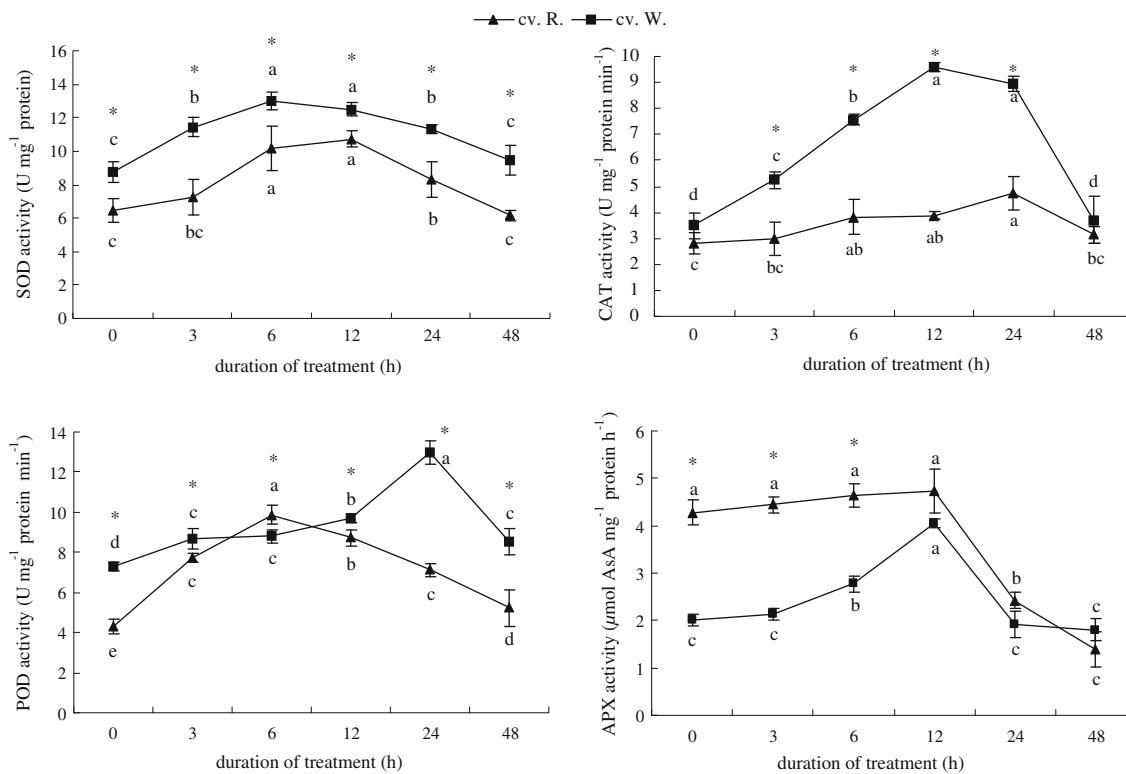


Fig. 2 Effect of NaCl on antioxidant enzymes activities in roots of *Helianthus tuberosus* L. Values are presented as mean \pm standard deviation (SD) of three biological replicates. Student's *t* test ($P < 0.05$) was selected for data statistics between both cultivars at

the same time point and asterisks indicated significant differences using a *t* test. Duncan's new multiple range test ($P < 0.05$) was used for data statistics of each cultivar at different time points and lower case letters *a*, *b*, *c*, *d*, and *e* indicated statistical significance

observed at 6 h, whereas the maximum of cv. R. appeared at 12 h, up to 12.98 and 10.71 U mg⁻¹ protein, respectively. But the height of SOD activity of cv. W. arose earlier. Finally SOD activities were 107 and 96% as compared to their controls, respectively. However, although there was no significant difference between 0 and 48 h, SOD activity of cv. W. still presented stronger than that of cv. R., and it was 1.53-fold of cv. R. at 48 h.

Activities of CAT exhibited a sharp difference. CAT activity of cv. W. was significantly higher than cv. R. and the summit value of the former was 2.47-fold of the latter at 12 h. However, the latter showed a moderate trend, its maximum appeared at 24 h and it turned up later than the former. The summits of two cultivars were 2.76- and 1.69-fold greater compared with their controls, respectively. At the last stage of NaCl treatment, activity of cv. W. was 3.69 U mg⁻¹ protein min⁻¹ and the value of cv. R. was 3.14 U mg⁻¹ protein min⁻¹, and there was no significant difference existing between the two cultivars.

Compared with cv. W., the peak of POD activity in cv. R. displayed earlier. However, peaks of SOD and CAT activities in cv. R. appeared later than those of cv. W. POD activity of cv. R. obtained the height at 6 h, whereas the peak of cv. W. displayed at 24 h, 2.29-fold and 1.78-fold

greater than controls, respectively. POD activities in two cultivars were still greater than their respective controls at 48 h.

At the early stage of salt treatment, APX activity of cv. R. was significantly greater than cv. W. APX activities of two cultivars simultaneously received the maximums at 12 h and APX activity of cv. R. was higher than cv. W., but there was no significant difference. Subsequently APX activities of both cultivars decreased and they were 32.74 and 90.34% of their controls at 48 h, independently. APX activity of cv. R. declined more than that of cv. W.

Discussion

Most crops are glycophytes; thus they are not capable of growing in high concentrations of salt in the soil (Shabala and Cuin 2007). Membrane lipid peroxidation of plant tissues often occurs in abiotic and senescence stresses. Mandhania et al. (2006) found that as compared to a salt-tolerant line wheat cultivar (KRL-19), a salt-sensitive line wheat cultivar (WH-542) suffers greater damage to cellular membranes due to lipid peroxidation as indicated by higher accumulation of MDA. Xue and Liu (2008) reported that

NaCl stress causes a significant increase in MDA levels in leaves of both Jerusalem artichoke cultivars and the degree of MDA accumulation in cv. Wuxi (salt-sensitive) is higher than that in cv. Dafeng (salt-tolerant), indicating a higher rate of lipid peroxidation due to salt stress. Our results indicated that root cells of salt-tolerant cv. R. made more obvious victims in response to salt stress. With the extent of salt treatment time, more and more victims were emphasized in cv. R. root cells to cope with salt stress.

The mechanisms of salt tolerance in plants have been discussed several times in different plants by researchers. For example, tomato (Shalata and Tal 1998), pea (Hernandez et al. 1993, 2001), maize (Neto et al. 2006), canola (Ashraf and Ali 2008) and so on. Unfortunately, there has not been a definite interpretation so far. The role of different antioxidants as potential selection criteria for improving plant salt tolerance has been critically discussed. With the advances in molecular biology and availability of advanced genetic tools considerable progress has been made in the past two decades in improving salt-induced oxidative stress tolerance in plants by developing transgenic lines with altered levels of antioxidants of different crops (Ashraf 2009). Antioxidant enzymes (SOD, CAT, POD and APX) and non-enzymatic metabolites, which are associated with salt tolerance, have been the focus of studies and lots of works have been done on antioxidants. For examples, Neto et al. (2006) reported that SOD and APX increase in the maize leaves of salt-stressed plants in salt-tolerant and salt-sensitive cultivars, and the increase in the activities of antioxidant enzymes is more marked in the salt-tolerant cultivar than in the salt-sensitive one. Additionally, in the roots of the salt-tolerant maize cultivar, the activities of SOD and CAT decrease with salt treatment, whereas those of APX remain unaltered in comparison with the control. Similarly, SOD activity in potato increases at lower salt level in salt-tolerant cultivars. However, at higher salt levels, SOD activity is reduced in all cultivars. In contrast, the activities of CAT and POD increase in all cultivars, but no difference is discerned in cultivars differing in salt tolerance (Rahnama and Ebrahimzadeh 2005). Mandhania et al. (2006) found that CAT, POD and APX increase in both wheat cultivars. Khan and Panda (2008) found that a positive association of some important antioxidant enzymes such as SOD, POD and CAT in two rice cultivars exists. Similarly, the same results of *Brassica napus* are also discovered (Ashraf and Ali 2008).

In our study, the peak of SOD activity of cv. R. appeared later than that of cv. W. and the latter activity was greater. It indicated that active oxygen scavenging system in cv. W. was activated efficiently and removed ROS. In most of the time of salt stress, the activities of both the cultivars were greater than their respective controls. Only at the last phase of salt treatment SOD activity reduced and was lower in cv.

R. as compared to its controls. It showed that SOD activities of Jerusalem artichoke roots in both salt-tolerant and salt-sensitive cultivars increased at a higher salt level. Afterward, SOD activities decreased and it was perhaps related to the damage caused by excessive ROS accumulation in root cells. Although our results were not absolutely identical with the previous conclusions, our results performed a similar aspect to Rahnama and Ebrahimzadeh (2005) and the maize leaves of Neto et al. (2006). In addition, the results that were different from the previous persons' conclusions could be caused by species specificity. CAT activity in cv. W. increased more than that of cv. R. However, activities of SOD and CAT in roots were not adequate to demonstrate that salt tolerance in cv. W. was greater than cv. R. Hernandez et al. (2001) suggested that salt stress can cause oxidative stress and enhance the antioxidant capacity in the leaf apoplast of pea cultivars. However, this anti-oxidative response does not seem to be sufficient to remove the harmful effects of high salinity particularly in the salt-sensitive cultivar that results in necrotic leaf lesions. In the process of salt stress, many necrotic leaf lesions appeared in leaves of cv. W., which was similar to Hernandez et al. (2001). It was bound to result in damages to plant photosynthesis and affect other plant functions. Finally, more damages were caused to the whole plant. This was perhaps the reason why cv. W. was sensitive to salt. POD activities in two cultivars increased, and the peak of cv. W. came out greatly. However, Cavalcanti et al. (2004) found that the survival of cowpea plants under salt stress is not mediated by POD. In contrast, APX activities in two cultivars decreased. Although APX activity in cv. W. remained higher and showed variable expression during two time points, Katsuhara et al. (2005) reported that the removal of ROS is not sufficient to influence the overall plant salt tolerance. Thus, although the roots of cv. W. suffered less salt damages, it could not decide the whole plant destiny.

Antioxidant changes and the mechanism of salt tolerance differed between different species and cultivars of a single species. It is known that the mechanism of salinity tolerance is a complex character. No single elucidation can explain the mechanism of plant salt tolerance. Here we only discussed salt tolerance from the levels of MDA and antioxidant enzymes. But those seemed to be inadequate to interpret the mechanism of salt tolerance. Thus, specific mechanisms including sodium, potassium, chloride ions, ROS and antioxidant enzyme activity changes in the aerial parts and in roots are still further studied to find out the traits that are related to salt tolerance.

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