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RESEARCH PAPERS

Antioxidant Enzymes and Physiological Characteristics in Two Jerusalem Artichoke Cultivars under Salt Stress[1]

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Abstract—The effects of NaCl stress on the activity of antioxidant enzymes, lipid peroxidation, cell membrane stability, net photosynthetic rate, gas-exchange, and chlorophyll content were investigated in two Jerusalem artichoke cultivars, Dafeng (salt-tolerant) and Wuxi (salt-sensitive), grown under control (nutrient solution) or salt stress (nutrient solution containing 75, 150, and 225 mM NaCl) conditions for 7 days. In leaves of salt-tolerant cv. Dafeng, superoxide dismutase (EC 1.15.1.1), peroxidase (EC 1.11.1.7), and catalase (EC 1.11.1.6) activities significantly increased as compared to the controls, whereas no significant change was observed in cv. Wuxi. Lipid peroxidation and cell membrane injury were enhanced in both cultivars. Net photosynthesis and stomatal conductance decreased in response to salt stress, but cv. Dafeng showed a smaller reduction in photosynthesis than cv. Wuxi. The results indicated that stomatal aperture limited leaf photosynthetic capacity in the NaCl-treated plants of both cultivars. However, significant reduction in the leaf chlorophyll content due to NaCl stress was observed only in cv. Wuxi. These results suggested that salt-tolerant Jerusalem artichoke varieties may have a better protection against reactive oxygen species, at least in part, by increasing the activity of antioxidant enzymes under salt stress.

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Key words: Jerusalem artichoke - superoxide dismutase - peroxidase - catalase - photosynthesis - lipid peroxidation - salt stress

INTRODUCTION

Soil salinity is a major abiotic stress in plant agriculture strongly influencing plant productivity worldwide. Saline conditions reduce the ability of plants to absorb water, causing rapid reductions in the growth rate, and induce many metabolic changes similar to those caused by water stress. High salt concentrations in the external solution of plant cells cause several deleterious effects. Ionic imbalance is the first consequence of salt stress [1]. An increased concentration of Na^+ and Cl^- under salt (NaCl) stress is deleterious to several cellular systems [2]. It has been demonstrated that, under high salinity, not only the homeostasis of Na^+ and Cl^- but also of Ca^{2+} and K^+ ions are disturbed [3]. Secondly, high salt concentrations in the external solution impose a hyperosmotic shock due to the decrease in chemical activity of water and the loss of cell turgor. Third important effect of salt stress is reduced photosynthesis due to reduction in chloroplast stromal volume and/or generation of reactive oxygen species (ROS). Therefore, the elucidation of the plant biochemical and physiological mechanisms operating in response to these stresses are critical if we are to develop and introduce genetic or environmental improvement to salt stress tolerance.

Salt adaptation of plants has generally been studied, mainly focusing on the regulatory mechanisms of ionic and osmotic homeostasis [4]. In addition to ionic and osmotic imbalance, salt stress, like other abiotic stresses, also leads to oxidative stress through an increase in the cellular level of ROS, such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) [5, 6]. These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins, and nucleic acids [7]. During salt stress, ROS generation increases severalfold. Thus, it is imperative to assume that the regulation of antioxidant enzymes would be an important strategy for plants to resist salt stress. Salt-tolerant plants, besides being able to regulate the ion and water movements, should also have a better antioxidant system for effective ROS removal [8]. Some evidence suggests that resistance to oxidative stress may, at least in part, be involved in salt stress tolerance [9–11].

To mitigate the oxidative damage initiated by ROS, plants have developed the complex antioxidant systems. Antioxidant enzymes, such as superoxide dismutase (SOD), peroxidases (POD), and catalase (CAT)

[1] This text was submitted by the authors in English.

Abbreviations: CAT—catalase; MDA—malondialdehyde; NBT—nitroblue tetrazolium; POD—peroxidase; ROS—reactive oxygen species; SOD—superoxide dismutase.

[12] are the most important components in the system for ROS scavenging. SOD is one of the ubiquitous enzymes in aerobic organisms and plays a key role in cellular defense mechanisms against ROS. Its activity modulates the relative amounts of superoxide ($O_2^{\bullet -}$), and its enzymatic action results in the formation of H_2O_2 and $O_2 \cdot H_2O_2$ is still toxic and must be eliminated by conversion to H_2O in subsequent reactions. In tolerant plant species, a number of enzymes regulate H_2O_2 intracellular levels, CAT and POD being considered the most important. CAT, which is apparently absent from the chloroplasts, split H_2O_2 into water and molecular oxygen, whereas POD decomposes H_2O_2 by oxidation of co-substrates, such as phenolic compounds and/or antioxidants. They enable plants to protect themselves against the oxidative stress, whereas such activity was not observed in sensitive plants.

Malondialdehyde (MDA), an indicator of oxidative damage, showed a greater accumulation under salt stress. Cell membrane stability has widely been utilized to differentiate salt-tolerant and salt-sensitive cultivars [13, 14], and in some cases a higher membrane stability could be correlated with abiotic stress tolerance.

Jerusalem artichoke (*Helianthus tuberosus* L.) is a C_3 warm-season plant that could be cultivated at a relatively low cost with zero irrigation [15]. During recent years, Jerusalem artichoke has been recognized as a good source of fructose and inuline [16], and therefore, it has a potential application in several industries. Although Dafeng and Wuxi are two Jerusalem artichoke cultivars widespread in semi-arid regions of north and south of China [17], studies related to Jerusalem artichoke tolerance towards salt stress conditions are scarce. Therefore, the aim of the present study was to study the effect of NaCl salinity on the activity of key antioxidant enzymes, membrane lipid peroxidation, cell membrane stability, photosynthesis, the stomatal conductance, and total chlorophyll content in order to better understand the salt stress tolerance of Jerusalem artichoke.

MATERIALS AND METHODS

Seeds of two Jerusalem artichoke (*Helianthus tuberosus* L.) cultivars, Dafeng and Wuxi, obtained from the Shandong medium examination base of Nanjing Agricultural University of China, were sown in 20-mesh quartz sand. All the experiments were conducted in a greenhouse. The maximum and minimum temperatures were 31 and 22°C, respectively. After germination, the seedlings were transferred to sand-filled plastic pots with diameter and height of 15 cm. Each pot contained a single plant and was watered with half-strength Hoagland's nutrient solution at every alternate day. Seedlings growing uniformly (in 48 pots) were selected after 20 days, randomly divided into 8 sets with 6 pots per set. Each pot was considered as one replicate.

Experimental plants were exposed to salinity by adding NaCl to the growth medium (75 mM every 12 h) until the final concentrations of 75, 150, and 225 mM. All the treatment solutions were prepared in the half-strength Hoagland's solution. The amount of evaporated water was determined by weighing the pots, and the weight loss was replenished daily by adding distilled water. Plants were harvested after 7 days of treatments for biochemical analyses and for the study of physiological indices.

The net photosynthetic rate (P_n) and stomatal conductance (g_s) were measured with a portable Photosynthesis System (Li-6400, LI-COR, United States). Temperature in the leaf chamber was adjusted to 25°C, the photon flux density was 1000 μmol/(m^2 s), and the ambient CO_2 concentration was 350 μmol/mol. All measurements were carried out between 8 : 00 and 11 : 00 a.m.

Total chlorophyll in leaves was extracted with 80% acetone, and its content was determined according to Wellburn [18].

Leaves detached from plants were used to determine NaCl-induced oxidative damage. Stress-induced oxidative damage was determined by measuring the thiobarbituric acid-reactive materials, mainly MDA. Sample preparation and MDA determination were performed after Zhao and Tan [19]. The MDA content was calculated using an extinction coefficient of 155/(mM cm).

Membrane permeability was evaluated from electrolyte leakage from the cells. Leaves receiving various treatments were washed twice with distilled water, then put in autoclave (120°C) for 20 min, and the conductivity of external medium was measured using a Conductivity Meter (EC214, HANNA Instruments, Italy), following the method described by Lutts et al. [20].

Enzyme extraction and assay was performed after Chen et al. [21] with minor modifications. 0.5 g of mature leaves, collected from plants grown under various treatments, were ground with a prechilled mortar and pestle with 5 ml of ice-cold sodium phosphate buffer (62.5 mM, pH 7.8) containing 1.0% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 12000 *g* for 20 min at 4°C. The pellet was discarded, and the supernatant was stored at 4°C until the assays of enzymatic activities and protein content.

SOD activity was assayed by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT). The reaction mixture, with a final volume of 3.0 ml, contained 1.5 ml of sodium phosphate buffer (62.5 mM), 0.3 ml of riboflavin (20 μM), 0.3 ml of methionine (130 mM), 0.3 ml of EDTA (100 μM), 0.3 ml of NBT (750 μM), 0.05 ml of enzyme extract, and 0.25 ml of deionized water. The reaction was carried out under light flux of 75 μmol/(m^2 s) for 20 min, after which, the light was switched off. The absorbance was measured at 560 nm in a UV-120-02 spectrophotometer (Shimadzu, Japan). One unit of SOD activity

was defined as the amount of enzyme required to cause a 50% inhibition of the NBT photoreduction rate.

For the measurement of POD activity, 2.9 ml of 100 mM sodium phosphate buffer (pH 7.0) containing 20 mM guaiacol and 2% H_2O_2 was mixed with 0.1 ml of enzyme extract. POD activity was evaluated from guaiacol oxidation (extinction coefficient 28.5 (mM cm). The absorbance at 470 nm was measured at 1-min intervals for 5 min. An increase in the absorbance (0.01 unit/min) was equated to one unit of POD activity.

CAT activity was assayed by monitoring a disappearance of H_2O_2 (extinction coefficient of 39.8/(mM cm) by measuring a decrease in absorbance at 240 nm. The 3.0 ml of the reaction mixture contained 0.2 M sodium phosphate buffer (pH 7.8), 0.1 M H_2O_2, 0.2 ml of the enzyme extract and deionized water, as described in Ghazi et al. [22] with minor modifications.

The activities of SOD, POD, and CAT were expressed as units per milligram of protein. Protein was determined according to Bradford [23] by using BSA as a standard.

Data in the text and figures are expressed as means of six replicates and their standard errors. All data were subjected to one-way ANOVA tests, and means of six replicates were compared by the Student-Newman-Keul's multiple-range test. Comparisons with $P < 0.05$ were considered significantly different.

RESULTS

SOD activity in cv. Dafeng exhibited a significant increase with the increasing severity of NaCl stress, whereas salt treatment had no significant impact on SOD activity of cv. Wuxi (Fig. 1a). In cv. Dafeng, SOD activity increased in the 150 and 225 mM NaCl treatments by 47 and 84%, respectively, as compared to the control plants.

POD and CAT activities significantly increased with increasing NaCl levels only in cv. Dafeng at all stress levels (Figs. 1b, 1c). However, in cv. Wuxi, no significant changes in POD and CAT activities were observed at all NaCl levels. In cv. Dafeng, 150 and 225 mM NaCl caused, respectively, 48 and 93% increase in POD and 71 and 84% increase in CAT activity in comparison to the control plants.

NaCl stress caused a significant increase in the levels of MDA in both Jerusalem artichoke cultivars (Fig. 1d). However, the degree of MDA accumulation in cv. Wuxi was higher than that in cv. Dafeng, indicating a higher rate of lipid peroxidation due to salt stress. The MDA accumulation, averaged 61% in cv. Wuxi, increased with NaCl treatments. In cv. Dafeng, a slight decrease in the MDA content was observed under 75 mM NaCl. But 150 and 225 mM NaCl treatments caused a 15 and 30% increase in MDA content, respectively.

Cell membrane stability as expressed in terms of membrane injury is represented in the table. The extent of membrane damage was assessed by the measurement of electrolyte leakage in tolerant and sensitive cultivars. The ion leakage was correlated with increasing NaCl concentration. The increase was stronger in the leaves of cv. Wuxi than in cv. Dafeng, which means that cv. Dafeng had better cell membrane stability.

NaCl stress caused a significant decrease in the net photosynthetic rate and stomatal conductance with an increase in the NaCl concentration in both Jerusalem artichoke cultivars (table). In cv. Wuxi, the average decreases in the net photosynthetic rate and stomatal conductance were about 40 and 43%, respectively. In cv. Dafeng, however, the net photosynthetic rate was decreased by 12, 26, and 36% and stomatal conductance was reduced by 17, 35, and 50% when plants were subjected to 75, 150, and 225 mM NaCl, respectively.

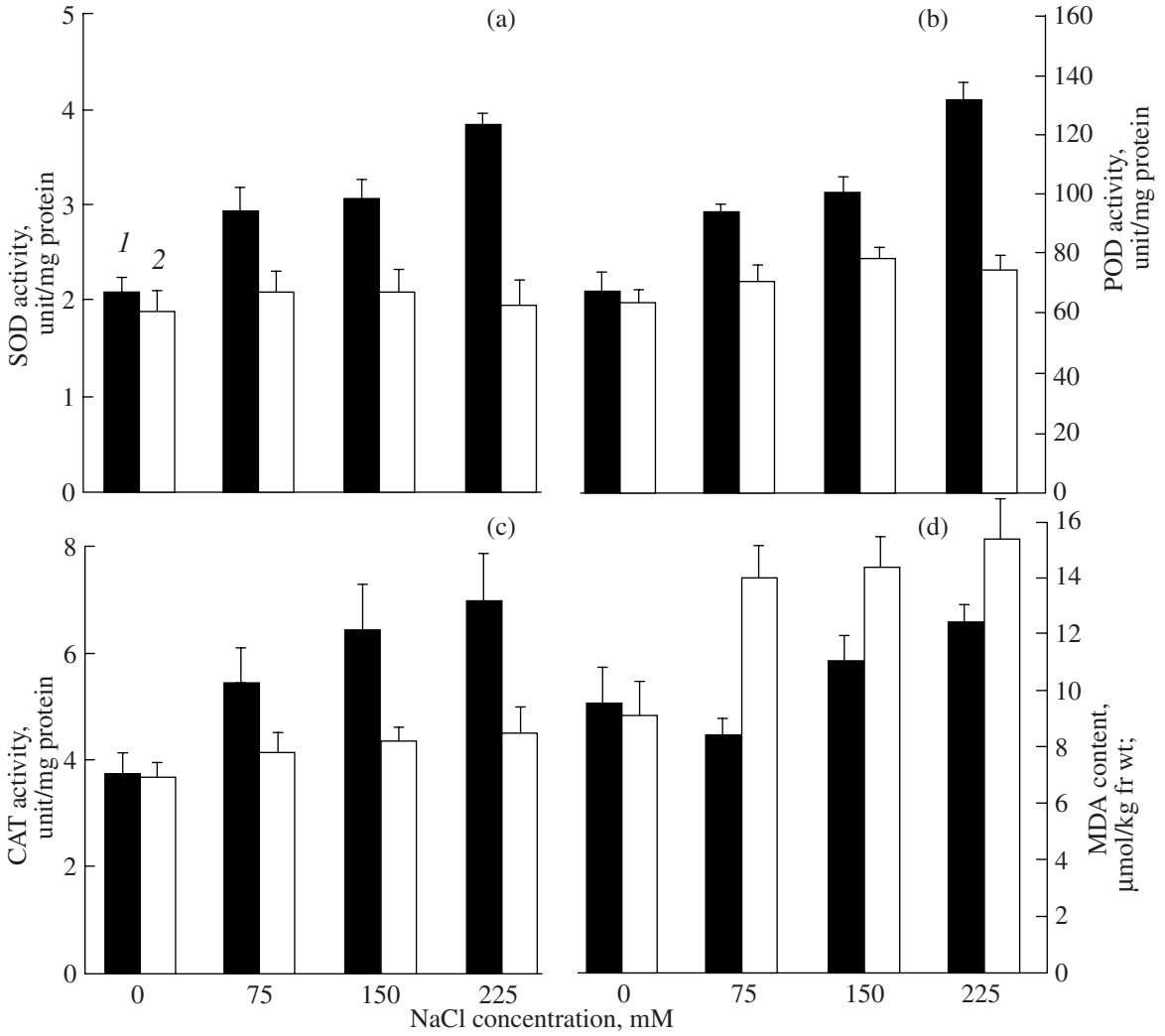
In cv. Wuxi, all NaCl treatments led to a 27% reduction in the content of total chlorophyll. In cv. Dafeng, the 150 and 225 mM NaCl treatments caused, respectively, a 12 and 23% decrease in the total chlorophyll in comparison with the control plants (table).

DISCUSSION

Many biotic and abiotic stresses, including salt stress, disrupt the cellular homeostasis of the cells, and further enhance the production of ROS in the plants cells [24].The production of ROS during stresses results from photorespiration, from the photosynthetic apparatus, and from mitochondrial respiration [5]. The increased rate of ROS production in chloroplasts under salt stress is well known [25]. The increase in the activity of antioxidant enzymes under salt and water stresses could indicate the increased ROS production and the build-up of a protective mechanism to reduce oxidative damage triggered by stress in plants.

Because salt stress is related to increased ROS generation [5, 6], salt stress resistance may depend, at least in part, on the regulation of the antioxidant defense system, which includes antioxidant compounds and several antioxidant enzymes. In this work, salinity led to a significant increase in SOD activity in cv. Dafeng but not in cv. Wuxi, suggesting that the salt-tolerant genotype has a better $O_2^{\bullet-}$ · radical scavenging ability. It has been shown that salinity increases SOD activity in salt-tolerant cultivars and decreases it in salt-sensitive pea cultivars [9]. Similar to the present study, there are some reports [22] showing an increased SOD activity under salt-stress conditions [26].

In the present study, the POD activity was significantly increased in cv. Dafeng but was not significantly changed in cv. Wuxi. Increased SOD activity, without an accompanying increase in the ability to scavenge H_2O_2, can result in enhanced cytotoxicity by the even more destructive hydroxyl radical generated from H_2O_2 in a metal-catalyzed Haber–Weiss reaction. In the present study, the enhancement of POD activity under



The effects of NaCl on (a) SOD, (b) POD, and (c) CAT activities and (d) MDA content in leaves of two Jerusalem artichoke cultivars. (*1*) Cv. Dafeng; (*2*) cv. Wuxi.
The seedlings were exposed to various treatments for 7 days. Each data point represents mean of six replicates and its standard error.

salt stress in cv. Dafeng indicated that it had a higher capacity to decompose H_2O_2 generated by SOD.

Meanwhile, salt stress led to a significant increase in CAT activity in cv. Dafeng, whereas no significant increase in CAT activity was observed in cv. Wuxi. Therefore, it could be hypothesized that CAT is the most important among the H_2O_2 scavenging enzymes in leaves. Our results are in agreement with those of Rout and Shaw [8], who suggested that CAT was the most important H_2O_2-scavenging enzyme leading to salt tolerance in aquatic macrophytes. In addition, various responses of CAT have been found in plants under salt stress: from the large increase in activity to no changes [27], for example in mulberry [28], cotton [14], and barley cultivars [29]. Thus, our results suggest that POD and CAT activities coordinated with SOD activity play a central protective role in the $O_2^{\bullet-}$ and H_2O_2 scavenging process [10, 29] and the active involvement of these enzymes is related, at least in part, to salt-induced oxidative stress tolerance in Jerusalem artichoke.

Salt stress is known to result in an extensive lipid peroxidation, which has often been used as an indicator of salt-induced oxidative damage to membranes [13]. It has been demonstrated that both osmotic and ionic effects are involved in NaCl salinity effects and limit the photosynthesis and respiration, leading to an increase in ROS generation. The increased rate of ROS generation and their decreased scavenging are responsible for secondary oxidative damages like peroxidation of membrane lipids and the loss of membrane semipermeability. Parallel to these observations, we observed that the degree of MDA accumulation was higher in cv. Wuxi than in cv. Dafeng. The lower level of lipid peroxidation suggests, therefore, that salt-toler-

The effects of NaCl on cell membrane injury, net photosynthetic rate (P_n), stomatal conductance (g_s), and chlorophyll (Chl) content in leaves of two Jerusalem artichoke cultivars

Cultivar	NaCl concentration, mM	Cell membrane injury, %	P_n, μmol CO_2/(m^2 s)	g_s, mmol/(m^2 s)	Chl, g/kg fr wt
Dafeng	0	23.1[f]	18.2[a]	0.596[a]	1.46[a]
	75	26.7[e]	16.0[b]	0.495[b]	1.41[b]
	150	29.4[c]	13.5[c]	0.385[c]	1.29[c]
	225	32.9[b]	11.6[d]	0.298[d]	1.13[d]
Wuxi	0	21.9[f]	19.6[a]	0.553[a]	1.61[a]
	75	28.1[d]	11.8[d]	0.371[c]	1.18[d]
	150	32.2[b]	11.4[d]	0.268[e]	1.17[d]
	225	36.3[a]	11.5[d]	0.262[f]	1.16[d]

Notes: Values marked with the same letter are not significantly different according to Student-Newman-Keul's multiple-range test. The seedlings were exposed to various treatments for 7 days. Each data point represents mean of six replicates.

ant plants are better protected against oxidative damage under salt stress. Similar results demonstrating the correlation between lipid peroxidation and the antioxidant system activity were also reported by other researchers [13]. These authors suggested that the reduction of MDA content was due to increased antioxidant enzyme activities, which reduced H_2O_2 levels and membrane damage.

Cell membrane stability has been used to assess tolerance of various plant species [28]. In the present study, a smaller percent of membrane injury was observed in cv. Dafeng compared to cv. Wuxi, which further supports the tolerance of cv. Dafeng. Less electrolyte leakage is correlated with the greater membrane integrity under stressful conditions, and such genotypes are characterized as salt stress-tolerant ones.

In glycophytes, the inhibition of photosynthesis under salinity stress may be due, at least in part, to stomatal closure [30], although direct salt effects on several biochemical and photochemical processes have been also reported [31]. In both Jerusalem artichoke cultivars, the photosynthesis was reduced probably because there was a reduction in the stomatal conductance (table). Parallel decreases in stomatal conductance and net photosynthesis under NaCl salinity have been reported for cotton [14]. Our results suggested that the stomatal closure limited leaf photosynthetic capacity in the NaCl-treated plants of both cultivars. Significant declines in the leaf chlorophyll content due to NaCl stress, however, were observed only in cv. Wuxi. Delfine et al. [32] reported no changes in the chlorophyll content spinach (*Spinacia oleracea* L.) plants salt stressed for 20 days. Here, also, no significant change in the chlorophyll content was found in cv. Dafeng during 7-days salt stress. In NaCl treatment, the reduction in stomatal conductance accompanied by decreased leaf chlorophyll content could contribute to the higher reduction of the leaf photosynthetic rate in cv. Wuxi as compared with that in cv. Dafeng.

Cv. Dafeng, a Jerusalem artichoke cultivar, which exhibited a higher salt tolerance, had also the higher P_n than cv. Wuxi under salt stress, the higher activity of antioxidant enzymes scavenging the production of ROS immediately, maintaining cell membrane stability, and increasing chlorophyll content. Although the obtained results showed that the difference in the antioxidant enzymes activity and physiological indices in the two Jerusalem artichoke cultivars may, at least in part, explain the mechanisms underlying oxidative stress injury and subsequent tolerance to salinity, the mechanisms involved in the process are complicated and are largely unknown and need to be elucidated.

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