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NaCl enhances thylakoid-bound SOD activity in the leaves of C_3 halophyte *Suaeda salsa* L.

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Abstract

The halophyte *Suaeda salsa* L., exposed to different NaCl concentrations, was used to study effect of NaCl salinity on superoxide dismutase (SOD) activities. The fresh weight of plants was markedly enhanced and leaf net photosynthesis rate was not reduced by high NaCl treatment indicating that *S. salsa* possesses an effective antioxidative response system avoiding oxidative damage. The SOD activity and the proportion of chloroplast SOD increased with the increase of salt concentration or with time. Activity of thylakoid-bound SOD constituted the major part of total chloroplast SOD activity. Results in this study suggest that SOD in chloroplasts of *S. salsa*, especially the thylakoid-bound SOD which scavenges $O_2^{\bullet -}$ radicals in situ, plays an important role in the resistance to oxidative stress induced by high-salinity.

Keywords: *Suaeda salsa* L.; SOD; Chloroplasts; NaCl stress

1. Introduction

During normal metabolism, plants generate reactive oxygen species (ROS), including superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and singlet oxygen (O_2^1). ROS are overproduced in plants under stress, including drought and desiccation, salt stress, chilling, heat shock, heavy metals, ultraviolet radiation, air pollutants such as ozone and SO_2, mechanical stress, nutrient deprivation, pathogen attack and high light stress [1-5]. Injury caused by ROS is known as oxidative stress, which is the major cause of damage in plants exposed to different stressors [2]. There is increasing evidence that NaCl salinity is one factor leading to oxidative stress in plant cells [6–8]. High NaCl concentrations seem to impair electron transport and lead to the formation of ROS in chloroplasts [9–11].

Plants have well-developed defense systems against ROS, involving both enzymatic and non-enzymatic mechanisms. Plants respond to a rise in ROS that the defense system is unable to remove with increased enzymatic or non-enzymatic antioxidant processes [12], but the mechanisms underlying these processes are not well understood [13]. SOD has a central role in the antioxidant defense network. SOD is the key enzyme to diminish the concentrations of superoxide [14]. SOD catalyzes the disproportion of superoxide radicals ($O_2^{\bullet -}$) to yield molecular oxygen and hydrogen peroxide (H_2O_2). The control of the steady-state $O_2^{\bullet -}$ levels by SOD is an important protective mechanism against cellular oxidative damage, since $O_2^{\bullet -}$ acts as a precursor of more cytotoxic or highly reactive oxygen derivatives, such as peroxynitrite or $HO^{\bullet}$ [15]. Therefore, SOD is usually considered the first line of defense against oxidative stress [16]. Increased SOD activity was correlated with increased protection from damage associated with oxidative stress [10].

The question arises whether an effective antioxidative response system (ARS) is really responsible for an increased level in salt tolerance. In general, with respect to salt tolerance, plants can be divided into two groups, i.e., halophytes and non-halophytes. Halophytes are able to tolerate high levels of salinity and to finish their life cycle in native saline soil. Moreover, their growth is enhanced by certain concentrations of salt, in general ranging between

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100 and 200 mmol/L NaCl, depending on plant species, developmental stage and environmental conditions [17,18]. In contrast, growth of non-halophytes is markedly reduced even by low concentrations of NaCl and they are not able to finish their life cycle in native saline soil [19]. There are several examples of NaCl-dependent up-regulation of ARS components in non-halophytes. For instance, Mittova et al. [20] found that the selective up-regulation of a set of antioxidative enzymes effectively alleviated the salt-dependent oxidative stress and damage in the salt-tolerant tomato species Lpa. On the other hand, little is known about NaCl-dependent changes of ARS components in halophytes.

The Chenopodiaceae *Suaeda salsa* L., a C_3 plant, is native to saline soil and adapted to the high-salinity region in the north of China [21]. Our recent studies have shown that high-salinity (100–400 mmol/L NaCl) led to a slight increase of CO_2 assimilation rate and did not affect photosystem II photochemistry [22,23], suggesting that *S. salsa* may have an effective antioxidative response system to protect oxidative stress induced by salt stress.

Chloroplasts are a major source of ROS in the leaf cells [11,24]. As such, specific ROS detoxification systems, located in both the thylakoid and the stromas have evolved [24,25]. Little information is available about the effects of salt stress on the chloroplastic antioxidant system [20].

The objective of this study was to investigate the effect of NaCl stress on SOD activity in leaves of halophyte *S. salsa*. For this purpose, we examined (1) the growth; (2) radical damage (MDA content); (3) leaf total SOD activity and chloroplastic SOD activity (thylakoid-bound and stromal SOD). We try to clarify the role of SOD in relation to salt tolerance in halophyte *S. salsa*.

2. Materials and methods

2.1. Plant material and NaCl treatments

Seeds of *S. salsa* L. were soaked in tap water for 3 h, sown in sand and kept in the dark at 25 °C for 3 d. After germination the seedlings were transferred to a greenhouse and cultivated under a photoperiod of 15 h d^{-1} (light intensity 600 μmol m^{-2} s^{-1}; relative humidity of 70–80%; day temperature: 30 ± 2 °C; night temperature: 23 ± 2 °C). Three-week-old seedlings were transplanted into plastic pots (20 cm in diameter and 22 cm in height, seven plants per pot) containing sand and irrigated with half-strength Hoagland nutrient solution. After reaching about 15 cm in height, the seedlings were subjected to NaCl treatment. NaCl concentrations were stepped up in 50 mmol/L every 12−h increments until final concentrations (0, 100, 200, and 400 mmol/L) were achieved. NaCl was dissolved in Hoagland nutrient solution and plants were watered daily. Physiological parameters were performed after 7-day treatment except time-course experiments.

2.2. Measurements of net photosynthetic rate

The net photosynthetic rate was measured with a portable Photosynthesis System (Li-6400, LI-COR, Inc., USA).

2.3. Determination of malondialdehyde content

Malondialdehyde (MDA) is a final decomposition product of lipid peroxidation induced by oxidative stress, and has been used as an indicator of lipid peroxidation. MDA content was determined by the thiobarbituric acid (TBA) reaction, as described by Lin et al. [26] and Buege and Aust [27].

2.4. Preparation of crude SOD extracts

One gram of plant material was homogenized at 4 °C in 2 mL of medium: 100 mmol/L K-phosphate buffer (pH 7.8) containing 3 mmol/L $MgSO_4$, 3 mmol/L EDTA and 2% (W/V) polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 17900 × *g* (Eppendorf Centrifuge 5417R) for 5 min at 4 °C. The supernatant was used for determination of SOD and protein content.

2.5. SOD activity and protein assay

In the presence of SOD the photochemical reduction of nitro blue tetrazolium (NBT) is inhibited and the level of inhibition was used to quantify the enzyme. SOD was assayed according to Giannopolitis and Ries [28] with some modifications. The reaction medium was composed of 50 mmol/L K-phosphate buffer (pH 7.8), 0.1 mmol/L EDTA, 13 mmol/L methionine, 60 μmol/L riboflavin, 2.25 mmol/L NBT and an appropriate aliquot of extract in a final volume of 4 mL. The reaction mixture was illuminated with light intensity of 72 μmol m^{-2} s^{-1} for 15 min and turning the lights off stopped the reaction. A control reaction was always performed wherein all the steps and components were exactly the same as described above, except that crude enzyme was replaced with an equal volume of phosphate buffer (pH 7.8) [29]. Assays were always carried out at 25 °C. The reaction was measured at 560 nm. The photochemical procedure was chosen because it is more reliable in crude extracts than other methods. One unit of enzyme activity was defined as the quantity of enzyme that reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.

The protein content was determined according to Bradford [30] using bovine serum albumin as standard.

2.6. Isolation of chloroplasts

Chloroplasts were isolated from 50 g leaves at the start of the light period according to the method of Sgherri et al. [31] with some modifications. The leaves were placed at −15 °C for 1 h, and then washed with cold deionized water and