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Protective Role of Ca Against NaCl Toxicity in Jerusalem Artichoke by Up-Regulation of Antioxidant Enzymes*[1]

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ABSTRACT

The ameliorative effect of external Ca^{2+} on Jerusalem artichoke (*Helianthus tuberosus* L.) under salt stress was studied through biochemical and physiological analyses of Jerusalem artichoke seedlings treated with or without 10 mol L^{-1} $CaCl_2$, 150 mmol L^{-1} NaCl, and/or 5 mmol L^{-1} ethylene-bis(oxyethylenenitrilo)-tetraacetic acid (EGTA) for five days. Exposure to NaCl (150 mmol L^{-1}) decreased growth, leaf chlorophyll content, and photosynthetic rate of Jerusalem artichoke seedlings. NaCl treatment showed 59% and 37% higher lipid peroxidation and electrolyte leakage, respectively, than the control. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were decreased by NaCl, indicating an impeded antioxidant defense mechanism of Jerusalem artichoke grown under salt stress. Addition of 10 mmol L^{-1} $CaCl_2$ to the salt solutions significantly decreased the damaging effect of NaCl on growth and chlorophyll content and simultaneously restored the rate of photosynthesis almost to the level of the control. Ca^{2+} addition decreased the leaf malondialdehyde (MDA) content and electrolyte leakage from NaCl-treated seedlings by 47% and 24%, respectively, and significantly improved the activities of SOD, POD, and CAT in NaCl-treated plants. Addition of EGTA, a specific chelator of Ca^{2+}, decreased the growth, chlorophyll content, and photosynthesis, and increased level of MDA and electrolyte leakage from NaCl-treated plants and from the control plants. EGTA addition to the growth medium also repressed the activities of SOD, POD, and CAT in NaCl-treated and control seedlings. External Ca^{2+} might protect Jerusalem artichoke against NaCl stress by up-regulating the activities of antioxidant enzymes and thereby decreasing the oxidative stress.

Key Words: antioxidant enzymes, calcium, Jerusalem artichoke, lipid peroxidation, salt stress

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Soil salinity is one among the major abiotic stresses that influence plant productivity (Bohnert *et al.*, 1995). Saline conditions reduce the water absorption ability of plants, cause rapid reductions in growth, and induce many metabolic changes similar to those caused by water stress. High salt concentrations in soil adversely affect several metabolic processes in plants, most often leading to death. Cellular ionic imbalance in plant cells is the first consequence of salt stress (Niu *et al.*, 1995; Zhu *et al.*, 1997). Increased concentrations of Na^+ and Cl^- in cells exert numerous deleterious impacts on vital cellular processes and functions (Serrano *et al.*, 1999). It has been shown that under high salinity not only the homeostasis of Na^+ and Cl^- but also the distribution of Ca^{2+} and K^+ is disturbed (Rodriguez-Navarro, 2000). Higher concentrations of salt in the external environment of roots impose a hyperosmotic shock in plants because of a decrease in the chemical activity of water and loss of cell turgor. Because of the reduction in chloroplast stromal volume and/or increased generation of reactive oxygen species (ROS) (Price and Hendry, 1991) under salt stress, the rate of photosynthesis is also decreased.

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Salt adaptation in plants is mainly dependent on the regulatory mechanisms of ionic and osmotic homeostasis (Niu *et al.*, 1995; Hare *et al.*, 1998; Yeo, 1998; Zhu, 2003). In addition to ionic and osmotic imbalance, salt stress also causes oxidative stress through an increase in cellular level of ROS, such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals (Alscher *et al.*, 1997; Mittler, 2002; Neill *et al.*, 2002). These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to lipids, proteins, and nucleic acids (Alscher *et al.*, 1997; Imlay, 2003). During salt stress, ROS generation in plant cells increases by several folds (Smirnoff, 1993). Thus, it is imperative to assume that regulation of antioxidant enzymes could be an important strategy of plants in fighting against salt stress. Some earlier studies have also shown that resistance to oxidative stress, at least in part, is involved in salt stress tolerance of plants (Gosset *et al.*, 1994; Gueta-Dahan *et al.*, 1997; Hernández *et al.*, 2000; Mittova *et al.*, 2002; Badawi *et al.*, 2004; de Azevedi Neto *et al.*, 2006).

It is well-known that calcium plays an important role in the adaptation of plants to diverse kinds of environmental stresses including salt stress (Bowler and Fluhr, 2000). Ameliorating effect of Ca^{2+} on Na^{+}-induced growth inhibition of root has been shown (Cramer *et al.*, 1988); Ca^{2+} prevents the salinity-induced shortening of growth zones of root (Bernstein *et al.*, 1993). Volume and length of cell are generally reduced in salinity conditions; however, Ca^{2+} prevents salinity-induced decrease in cell volume and length (Azaizeh *et al.*, 1992). Similarly, it has previously been shown that Ca^{2+} restores the photosynthesis under NaCl stress through increasing stomatal conductance (Perera *et al.*, 1995). It was also shown that Na^{+}/Ca^{2+} ratio largely affects the transport of water in the root and to the leaf growing zones (Cramer, 1992). There exists sufficient evidence showing that salinity alters the ionic content and transport in plants. Calcium has several regulatory functions over membrane property and ionic transport in halophytes and glycophytes. For example, increasing the external Ca^{2+} concentration reduces the transport of Na^{+} through ion channels, thereby reducing Na^{+} influx in root cells (Tyerman *et al.*, 1997). Although a general protective role of Ca^{2+} against NaCl toxicity in plants is well-known (Hyder and Greenway, 1965), the role of Ca^{2+} in salt stress-induced oxidative stress tolerance of plants has not been confirmed.

Jerusalem artichoke (*Helianthus tuberosus* L.) is a C_3 warm-season plant that can be cultivated at relatively low cost with zero irrigation (Monti *et al.*, 2005). Jerusalem artichoke has been recognized recently as a good source of fructose and inuline (Baldini *et al.*, 2004; Saengthongpinit and Sajjaanantakul, 2005), and consequently has a potential application in several industries. Some evidences suggest that Jerusalem artichoke possesses a certain degree of salt tolerance, and can be grown in unused saline soils of costal areas (Liu *et al.*, 2004; Long *et al.*, 2005). To the best knowledge of the authors, no attempt has previously been made to study the antioxidant system of Jerusalem artichoke under salt stress. The aim of the present study was to investigate the effect of hyper salinity (NaCl) on the activity of some key antioxidant enzymes of plants and the effect of Ca^{2+} enrichment on NaCl-induced salt stress adaptation and antioxidant system of Jerusalem artichoke.

MATERIALS AND METHODS

Plants and treatments

Seeds of Jerusalem artichoke (*Helianthus tuberosus* L.) were sown in 20-mesh quartz sand. The maximum and minimum temperatures were 31 and 22 °C, respectively. Seedlings were transferred to sand-filled plastic pots having diameter and height as 15 cm. Each pot had a single plant, and was watered on alternate days with half-strength Hoagland nutrient solution (Zhang *et al.*, 2005). All pots were placed in a greenhouse to avoid rainfall. When the seedlings were 21 days old, they were subjected to various treatments as follows: control (a); 10 mmol L^{-1} $CaCl_2$ (b); 150 mmol L^{-1} NaCl (c); 150 mmol L^{-1} NaCl + 10 mmol L^{-1} $CaCl_2$ (d); 150 mmol L^{-1} NaCl + 5 mmol L^{-1} ethylene-bis(oxyethylenenitrilo)-tetraacetic acid (EGTA) (e).

Uniform seedlings in a total of 30 pots were selected, randomly divided into 5 sets with 6 pots per