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Glutathione-triggered Mitigation in Salt-induced Alterations in Plasmalemma of Onion Epidermal Cells

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ABSTRACT

Plasma membrane permeability, protoplasm swelling and cell viability were used to investigate the influence of exogenous glutathione (0.5 mM) in alleviating NaCl-induced cellular alterations of onion bulb inner epidermis. Glutathione was added simultaneously with 150 mM NaCl or before salt treatment. Plasma membrane permeability to urea, the numbers of swollen protoplasm and cell mortality were increased under 150 mM NaCl salt stress. Glutathione ameliorated NaCl-induced plasma membrane changes and maintained its permeability and cell viability. The ameliorative effect of glutathione was more pronounced when added together with salt. The alleviating effect of glutathione might be through scavenging active oxygen species and hence lipid peroxidation produced under salt stress. It is therefore, proposed that glutathione may ameliorate NaCl-indcued alterations on the plasma membrane, which may enhance salt tolerance in plants.

Key Words: Salinity; Plasma membrane; Glutathione; Permeability; Cell mortality; Protoplasm swelling

INTRODUCTION

Salinity has a major impact on plant growth and productivity. About one billion ha of land are affected by salinity (Szaboles, 1994). The majorty of crop plants are relatively salt-sensitive and are unable to tolerate low level of salinity (Levitt, 1980). Salt imposes several kinds of stresses upon plant. It causes drastic changes in the water potential, ion toxicity, ion imbalance and oxidative stress (Levitt, 1980; Cramer *et al*., 1985; Hasegawa *et al*., 2000; Zhu, 2001).

Tolerance to salt stress is achieved at the cellular level (Levitt, 1980; Hasegawa *et al*., 2000). Understanding the mechanism of salt tolerance can, therefore be expected from studying salt responses at the cellular level of plants differing in their sensitivity to salinity stress. Mansour *et al*. (2005) and Mansour and Salama (2004) report differences in salt tolerance of various crop plant at the cellular level.

Previous studies reported that the plasma membrane may be the primary site of salt injury (Levitt, 1980; Mansour, 1997; Mansour & Salama, 2004; Salama *et al*., 2007). Several reports investigated the response of the plasma membrane to salinity in genotypes differing in their salt tolerance (Mansour *et al*., 2005; Salama *et al*., 2007). Changes in the composition/structure of plant cell membrane have been found in saline environment (Kuiper, 1984; Mansour *et al*., 2002; Salama *et al*., 2007). The previous studies indicate that plasma membrane lipid alteration induced by saline conditions may have a critical role in plant response to salinity. The changes in the PM lipid by salt may result in membrane properties that enable the cell membrane to physiologically function under salt stress (Kuiper, 1984; Quinn, 1989; Mansour *et al*., 2002; Salama *et al*., 2007). Passive permeability is directly affected by a change in the membrane lipid matrix (Mansour & Salama, 2004; Mansour *et al*., 2005). Cell membrane permeability has been used to detect alterations in the physical and chemical status of the plasma membrane, thus indicating membrane damage under various conditions (Chen *et al*., 1991; Mansour *et al*., 1993a; Mansour *et al*., 2005).

Salt stress induces an oxidative stress via cellular accumulation of damaging active oxygen species (AOS) including superoxide radicals, hydrogen peroxide and hydroxyl radicals (Noctor & Foyer, 1998; Khan & Panda, 2002). AOS cause oxidative damage to different cellular components including membrane lipid, protein and nucleic acid (Halliwell & Gutteridge, 1986; Mittler, 2002). Plant cope with this via producing enzymatic and non-enzymatic antioxidants (Wang *et al*., 2003; Blokhina *et al*., 2003). Among non-enzymatic antioxidants, glutathione has been indicated to scavenge oxygen species and improve seed germination and seedling growth under salt stress (Roxas *et al*., 1997; Millar *et al*., 2003; Hussain *et al*., 2008). Glutathione is a tripeptide (α-glutamyle cysteinylglycine), which has been detected virtually in all cell compartments (Millar *et al*., 2003).

This investigation was carried out to study the effect of NaCl on plasma membrane in absence and presence of

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glutathione. Different approaches of glutathione treatment have been used. Plasma membrane permeability, protoplasm intactness and cell viability were used to detect the alteration in membrane structure/composition and the impact of glutathione to ameliorate the salt-induced changes in plasma membrane were analyzed.

MATERIALS AND METHODS

Plant material and tissue preparation. Onion bulbs (*Allium cepa* L., Giza 6) were obtained from the National Agricultural Research Center. Dokki, Giza, Egypt. The bulbs were stored at room temperature (20°). Epidermal cells of onion scale were used as experimental material to study NaCl effects on the cell membrane with or without glutathione. Two sections (0.5 × 0.5 cm each) of the middle portion of the inner epidermis of third fleshy scale counting from outside, were prepared with a sharp razor. Next the epidermis sections were peeled off from the parenchyma and floated in balanced water (25 mM KCl + 3.7 mM $CaCl_2$) for 1 min.

Treatments. The sections were divided into four groups: group I, received neither NaCl nor glutathione (non-treated sections); group II, was floated on 150 mM NaCl only for 3 h; group III, was floated on 150 mM NaCl + 0.5 mM reduced glutathione for 3 h and group IV, was treated with 0.5 mM reduced glutathione for 2 h and then exposed to 150 mM NaCl for 3 h. After the previous treatments were applied the sections were subjected for cell membrane permeability and cell viability measurements.

Plasma membrane permeability measurement. The plasmolytic method (Stadelmann & Lee-Stadelmann, 1989) was used to study the permeation of non-electrolyte solute, urea, through the plasma membrane of individual intact cells. Plasmolytic techniques were used as a sensitive probe for *in vivo* detection of the changes in the membrane structure/composition under various conditions (Fennell & Li, 1985; Zhao *et al*., 1987; Chen *et al*., 1991; Mansour *et al*., 1993a; Mansour, 1995). The cells were plasmolyzed by placing the section in 0.4 M glucose for 10 min. Then the section was transferred to 0.6 M glucose for another 10 min and finally to 0.8 M glucose for 45 min to reach osmotic equilibrium. The section was then transferred to a perfusion chamber mounted on a microscope stage. The glucose solution for the final concentration (0.8 M) in the perfusion chamber was replaced by an isotonic solution of urea. As the protoplasts deplasmolyzed by urea permeation the change in the protoplast length was measured at timed intervals using an eye piece micrometer. Permeability coefficients K_s (cm s^{-1}) were calculated by the formula of Stadelmann and Lee-Stadelmann (1989) using a computer software.

$$K_s = b/4 \times \frac{(L_2 - L_1) - b/3 \times \ln (L_2/L_1)}{(L_0 - b/3) \times (t_2 - t_1)} \times F$$

Where K_s is the permeability coefficient in cm s^{-1}, b is the inner width of the cell, L_0 is protoplast length in the final concentration of the osmoticum (0.8 M glucose), L_1 and L_2 are the protoplast length at times t_1 and t_2 during deplasmolysis, F is the conversion factor in cm/eye piece micrometer unit. The permeability coefficients of 25-30 cells were calculated for each treatment.

Cell viability. Plasmolysis and non-plasmolysis (Lee-Stadelmann & Stadelmann, 1989) of the cells were used as an indicator for cell viability. In addition number of dead cells (not plasmolyzed) and swollen protoplasm were counted in five sections per treatment. Five fields of vision were chosen at random per section and examine for viable cells (plasmolyzed), dead cells (non-plasmolyzed) and swollen protoplasm.

RESULTS AND DISCUSSION

Treatment of onion bulb scale sections with 150 mM NaCl for 3 h significantly increased the permeability coefficient (k_s) for urea compared with non-treated onion bulb scale sections (Table I). These results were agreed with the previous published reports, where salt stress significantly increased the permeability constant in the sensitive plant species (Mansour, 1995; Mansour, 1997; Mansour, 1998; Mansour *et al*., 2005). The increase in membrane permeability may results from salt-induced plasma membrane lipid composition alteration and/or peroxidation (Mansour, 1997; Shalata & Neumann, 2001; Mansour & Salama, 2004). The increase in membrane permeability was parallel with the increase in the number of swollen protoplasm and the number of dead cells (Table II), where salt increased both parameters relative to the non-treated sections. Our results were consistent with the finding of other studies (Mansour, 1995; Mansour & Salama, 2004; Salama, 2009). High salinity increased lipid peroxidation, which was associated with an increase in the electrolyte leakage, indicative of membrane damage (Bandeoglu *et al*., 2004). Increased cell mortality was brought about by damage cell membrane induced by salt stress.

One of the major ways in which higher plants transmit information concerning changes in the changing environment is via the production of bursts of superoxide at the plasma membrane (Barnes & Mayfield, 2003; Terman & Brunk, 2006). Furthermore, salinity induced production of AOS, which cause oxidative damage to different cellular components including membrane lipid (Noctor & Foyer, 1998; Mittler, 2002). We propose that salt exposure for 3 h may induce AOS and thus affect the plasma membrane. Membrane lipid peroxidation was induced in tomato seedling after 6 h salt treatment via the production of AOS (Shalata & Neumann, 2001). In addition, Mansour *et al*. (1993b) reported that choline chloride treatment for 2 h changes cell membrane permeability, which indicates choline chloride uptake and incorporation and in turn lipid changes. Several reports indicated that salt treatment

Table I. Cell membrane permeability coefficient (K_s, cm s^{-1}) of onion epidermal cells after exposure to 150 mM NaCl in presence and absence of 0.5 mM glutathione, each value is the mean ± S.D of 25-30 cells. Values with letters a, b are significantly different from the salt treated sections at $P< 0.01$, Salt treated cells are significantly different from cells received neither NaCl nor glutathione

Treatment	Permeability coefficient (cm $s^{-1} \times 10^{8}$)
0 m M NaCl	5.91 ± 0.40
150 mM NaCl (3 h)	8.76 ± 0.60
150 mM NaCl + 0.5 mM glutathione (3 h)	6.87 ± 0.50[a]
0.5 mM glutathione (2h) → 150 mM NaCl (3 h)	7.76 ± 0.47[b]

Table II. Number of dead and swollen cells of onion inner epidermis after exposure to 150 mM NaCl in presence and absence of 0.5 mM glutathione, each value is the mean ± S.D of 25-30 cells, Values with letters a, b are significantly different from the salt treated sections at $P<0.01$, Salt treated cells are significantly different from cells received neither NaCl nor glutathione

Treatment	Dead cells	Swollen cell
0 mM NaCl	00.6 ± 0.50	00.0 ± 0.0
150 mM NaCl (3 h)	07.8 ± 4.16	00.9 ± 1.6
150 mM NaCl + 0. 5 mM glutathione (3 h)	00.6 ± 0.90[a]	00.2 ± 0.5[a]
0.5 mM glutathione (2h) →150 mM NaCl (3 h)	01.6 ± 1.50[b]	02.0 ± 1.6[b]

induced alterations in the plasma membrane lipid structure/composition (Mansour *et al*., 1994; Mansour *et al*., 2002; Wu *et al*., 2005).

The increase in the plasma membrane permeability caused by salt stress was significantly reduced by the exogenous application of 0.5 M glutathione, simultaneously with or before salt treatment (Table I). The numbers of swollen protoplast and dead cells were significantly decreased by glutathione treatment compared with salt treated sections (Table II). The mitigative effect of glutathione on cell permeability, cell mortality and swollen protoplasm was more pronounced when glutathione and salt were used together (Table I & II). The results might indicate that glutathione ameliorated the injurious effect of salt on the plasma membrane. These results were consistent with the previous studies, which reported that ascorbic acid (as antioxidant) mitigated the increase in the plasma membrane permeability and cell mortality under salt stress in onion bulb scales (Salama, 2009). In addition, Shalata and Nemann (2001) found that exogenous ascorbic acid increases tomato seedling salt tolerance through lipid peroxidation mitigation. Exogenous glutathione increased phenol peroxidase activity with the reduction of damage effect of salt on (*Brassica napus* L.) as well as it reacts with H_2O_2 and maintain the membrane integrity (Kattab, 2007) and hence enhance canola salt tolerance. Similarly, exogenous application with Hydrogen peroxide partially alleviates the salt effect on wheat seedlings via the production of antioxidant defense system including glutathione (Devi *et al*., 2008).

In summary, glutathione may mitigate the deleterious effect of salt via protection of PM. This may occur through lipid peroxidation prevention and maintenance of cellular redox balance (Foyer & Noctor, 2005). Stabilization of PM by glutathione may reduce passive Na^+ influx and thus enhance plant salt tolerance.

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