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Amelioration of NaCl-induced Alterations on the Plasma Membrane of *Allium Cepa* L. by Ascorbic Acid

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Abstract: The influence of exogenous ascorbic acid (0.5 mM) in counteracting NaCl-induced cellular alteration of onion bulb inner epidermis was investigated. Plasma membrane permeability, protoplasm swelling and cell viability were measured under the effect of 150 mM NaCl in presence and absence of ascorbic acid. Ascorbic acid was added simultaneously with salt or before salt treatment. Salt stress at 150 mM NaCl increased PM permeability to urea, the numbers of swollen protoplasm and cell mortality. Ascorbic acid protected NaCl-induced PM alterations and hence maintain its permeability and cell viability. The protective effect of ascorbic acid was more pronounced when added simultaneously with NaCl. The mitigative effect of AA might be via direct interaction with PM components and/or inhibition of lipid peroxidation induced by AOS formed under salinity. It is possible that ascorbic acid counteract the deleterious effect of NaCl on the cell membrane which may lead to increased salt tolerance in plants.

Key words: salinity, plasma membrane, ascorbic acid.

INTRODUCTION

Salinity represents one of the environmental stresses that threat plant growth and productivity. Salt stress exhibits its adverse effects on plants through decreasing the water potential of the root medium, ion imbalance, ion toxicity due to excessive Na^+ or Cl^- uptakes and cellular accumulation of damaging active oxygen species (Shalata and Neumann, 2001).

The plasma membrane may be the primary site of response to salinity and may correlate with salt tolerance (Cramer *et al*., 1985; Mansour, 1997). Plasma membrane response to salinity differs in salt sensitive and resistant plant species. Salt stress drastically changes the PM permeability of salt sensitive species, in the marginal effect on tolerant one (Mansour, 1997; Mansour and Salama, 2004; Mansour *et al*., 2005). In addition, PM lipid alterations in saline conditions are always in unfavorable direction that affects PM properties and functions in salt sensitive cultivars (Mansour, 1997; Mansour and Salama, 2004; Salama, *et al*., 2007). Several reports indicate that the changes in the membrane lipid may have a critical role in plant response to salinity stress. This is because lipid alterations induced by salt may result in membrane properties that enable the cell membrane to physiologically function under salt stress (Kuiper, 1984; Mazliak, 1989; Quinn, 1989; Mansour *et.al*., 2002). Therefore, the importance of studying the PM responses to salinity comes from its crucial role in plant salt tolerance mechanism (Levitt,1980; Mansour,1997; Mansour *et al*., 2003; Mansour and Salama, 2004). Furthermore, cell membrane stability has been used to screen for salt tolerant wheat varieties (Farooq and Azam, 2006). Permeability of the PM is a reliable indicator to prob the change in the lipid matrix and may correlate with plant salt tolerance (Levitt, 1980; Stadelmann and Lee-Stadelmann, 1989; Mansour, 1997; Mansour, 1998; Mansour and Salama, 2004).

Oxidative stress is a secondary stress results from salt imposition, which leads to active oxygen species formation (Khan and Panda, 2002). AOS, can damage essential membrane lipids as well as proteins and nucleic acid (Inzė and Van Montaque, 1995; Noctor and Foyer, 1998). Levels of AOS in plant cells are normally controlled by protective antioxidant activity. The anti oxidative system includes ascorbate, glutathione, α-tocopherols and enzymes (Hernandez *et al*., 2000; Jose *et al*., 2002; Khan and Panda, 2002). Various associations between saline environments and endogenous levels of water-soluble anti-oxidants and for anti-oxidative enzymes have been reported (Gossett *et al*., 1994; Shalata and Tal, 1998; Tsugane *et al*., 1999; Shalata and Neumann, 2001). Salt tolerance capacity of salt tolerant species is therefore, closely related to the balance between the formation of AOS and its removal by anti oxidative system. This paper investigated the

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effect of NaCl on plasma membrane permeability, protoplasm intactness and cell viability used as indicators to study alteration in membrane structure / composition in absence and presence of 0.5 mM ascorbic acid. This aims to test the mitigative effect of ascorbic acid on PM protection under salinity.

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 materials to study NaCl effects on the cell membrane with or without ascorbic acid. 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.

Treatments

The sections were divided into four groups: group I received neither NaCl nor ascorbic acid (non treated sections), group II was soaked in 150 mM NaCl only for 3h, group III was soaked in 150 mM NaCl + 0.5 mM ascorbic acid for 3h, and the fourth one was treated with 0.5 mM ascorbic acid for 2h and then exposed to 150mM NaCl for 3h. 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 and Lee Stadelmann, 1989) was used to study the permeation of nonelectrolyte 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 and Li, 1985; Zhao *et al*., 1987; Chen *et al*., 1991; Mansour *et al*., 1993; Mansour, 1995). The cells were plasmolyzed by placing the section in 0.4 M glucose for 10 min then 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 (o.8 M) in the perfusion chamber was replaced with 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).

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

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 by using a computer program.

Cell Viability:

Plasmolysis and non plasmolysis (Lee-Stadelmann and 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.

RESULTS AND DISCUSSION

Salt treatment (150 mM NaCl) extended for three hours significantly increased the permeability coefficient (K_s) of urea compared to untreated onion bulb scale sections (Table I). These results are consistent with previously published reports where salinity significantly increased membrane permeability in sensitive plant species (Mansour, 1995; Mansour, 1997; Mansour, 1998; Mansour *et al*., 2005). Changes in PM permeability were attributed to NaCl-induced PM lipid structure/composition alterations (Mansour, 1997; Mansour and Salama, 2004). Similarly, NaCl treatment increased the number of swollen protoplasm and dead cells (Table II). Our results agreed with the finding of other studies (Mansour, 1995; Mansour and Salama, 2004). Increased

cells mortality was brought about by damaged cell membrane induced by NaCl stress. Impaired plasma membrane structure by salt exposure caused passive Na^+ influx into the cytoplasm followed by water and subsequent protoplasm swelling.

It is interesting to mention that the increase in membrane permeability was correlated with the increase in the numbers of swellen protoplasm and the numbers of dead cells. Salinity alters the membrane lipid matrix via regeneration of active oxygen species (AOS) (Inzė and Van Montaque, 1995; Noctor and Foyer, 1998) and changed lipid metabolism (Mansour, 1997, Mansour and Salama, 2004). Blokhina *et. al*., (2003) reported that one of the main cellular components susceptible to damage by free radicals is lipids (peroxidation of unsaturated fatty acids of membranes) which consequently change membrane properties and functions. It is reported that salt treatment of tomato seedlings for 6h induced AOS formation and consequently membrane lipid peroxidation (Shalata and Neumann, 2001). NaCl alterations of PM lipidcomposition have been reported in several studies (Mansour *et al*., 1994; Wu *et al*., 2005; Salama *et al*., 2007).

Treatment with 0.5 mM ascorbic acid with or before salt treatment significantly reduced the permeability coefficient (K_s) compared with salt treated cells (Table I). Reduction in permeability constant was more pronounced when ascorbic acid was added simultaneously with salt (Table I). The numbers of swollen protoplast and dead cells were significantly decreased with ascorbic acid treatment compared with salt treated sections (Table II). The alleviating effect of ascorbic acid on cell mortality and swollen protoplasm was more pronounced when NaCl and ascorbic acid used together (Table I), consistent with permeability measurements. The mitigative effect of ascorbic on PM reported in this paper is in agreement with the study of Shalata and Neumann (2001), who found that exogenous ascorbic acid increases tomato seedling salt resistance through lipid peroxidation.

Table 1: Cell membrane permeability constant (K_s, cm s^{-1}) of onion scale leaf after exposure to 150 mM NaCl in presence and absence of 0.5 mM ascorbic acid. 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 least *P* 0.01. Salt treated cells are significantly different from cells received neither NaCl nor ascorbic acid.

Treatment	Permeability coefficient (cm $s^{-1} \times 10^{8}$)
0 mM NaCl	7.35 ± 0.30
150 mM NaCl (3h)	9. 52 ± 0.34
150 mM NaCl + 0.5 mM ascorbic acid (3h)	7.22 ± 0.32[a]
0.5 mM ascorbic acid (2h) → 150 mM NaCl (3h)	7.51 ± 0.34[b]

Table II: Number of dead and swollen cells of onion scale leaf after exposure to 150 mM NaCl in presence and absence of 0.5 mM ascorbic acid. 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 least *P* 0.01. Salt treated cells are significantly different from cells received neither NaCl nor ascorbic acid.

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

Ascorbic acid may alleviate the membrane damage induced by salt effects via scavenging AOS, and thus maintain membrane permeability in its proper range. On the other hand, ascorbic acid may directly interact with PM components in such a way that protects membrane structure and properties. Therefore, it could be proposed that ascorbic acid inhibition of salt-induced increase in lipid peroxidation by AOS, or its binding to the membrane lipids may stabilize the PM structure, and so maintain its properties and functions. This may reduce passive NaCl influx into the plant and hence maintain growth at high salinity.

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REFERENCES

Blokhin, O., E. Virolainen and K.V. Fagerstedt, 2003. Antioxidants, oxidative damage and oxygen deprivation stress: a Review. Ann. Bot., 91: 179-194.

Chen, J., E. Sucoff and E. Stadelmann, 1991. Aluminum and temperature alteration of cell membrane permeability of *Quercus rubra*. Plant Physiol., 96: 644-649.

Cramer, RC., A. Luchli and V.S. Polito, 1985. Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cell. A primary response to salt stress? Plant Physiol., 79: 207-211.

Farooq, S. and F. Azam, 2006. The use of cell membrane stability technique to screen for salt tolerant wheat varieties. J. Plant Physiol., 163: 629-637.

Fennell, A. and P.H. Li, 1985. Temperature response of plasma membranes in tuber-bearing *Solanum* species. Plant Physiol., 80: 470-472.

Gossett, D., E. Millhollon and M. Cran-Lucas, 1994. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci., 34: 706-714.

Hernandez, J.A., J. Jinenez, P. Mullineaux and F. Sevilla, 2000. Tolerance of pea (*Pisum sativum* L.) to long term stress is associated with induction of antioxidant defences. Plant Cell Environ., 23: 853-862.

Inzè, D. and M. Van Montague, 1995. Oxidative stress in plants. Curr. Opin. Biotech., 6: 153-158.

Jose, A., J.A. Hernandez, S. Maria and B. Almansa, 2002. Short term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plant., 115: 251-257.

Khan, M.H. and S.K. Panda, 2002. Induction of oxidative stress in roots of *Oryza sativa* L. in response to salt stress. Biol. Plant., 45: 525-627.

Kuiper, P.J.C., 1984. Function of plant cell membrane under saline conditions: Membrane lipid composition and ATPase. In: Salinity tolerance in plants. R.C. Staples and G. H. Toenniessen eds., Wiley, New York, 77-91.

Lee-Stadelmann, O.Y. and E.J. Stadelmann, 1989. Plasmolysis dnd deplasmolysis. In: Methods in Enzymology. S. P. Coldwich and N. O. Kaplan. Eds. Vol, 174, Biological transport (Biomembranes, Part U). Academic Press, New York, 225-246.

Levitt, J., 1980. Responses of plants to environmental stresses, II. Water, radiation, salt and other stresses. Academic, New York.

Mansour, M.M.F., 1995. NaCl alterantion of plasma membrane of *Allium cepa* epidermal cells. Alleviation by calcium. J. Plant Physiol., 145: 726-730.

Mansour, M.M.F., 1997. Cell permeability under salt stress. In: Strategies for improving salt tolerance in higher plants.P.K. Jaiwal, R.P. Singh and A. Gulati. eds. Oxford and IBH, New Delhi, 87-110.

Mansour, M.M.F., 1998. Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. Plant Physiol. Bichem., 36: 767-772.

Mansour, M.M.F. and K.H.A. Salama, 2004. Cellular basis of salinity tolerance in plants. Enveiron. Exp. Bot., 52: 113-122.

Mansour, M.M.F., O.Y. Lee–Stadelmann and E.J. Stadelmann, 1993. Salinity stress and cytoplasmic factors. A comparison of cell permeability and lipid partiality in salt sensitive and salt resistant cultivars and lines of *Triticum aestivum* and *Hordium vulgare*. Physiol. Plant., 88: 141-148.

Mansour, M.M.F., K.H.A. Salama, M.M. Al-Mutawa and A.F. Abou Hadid, 2002. Effect of NaCl and polyamines on plasma membrane lipids of wheat roots. Biologia Plant., 45(2): 235-239.

Mansour, M.M.F., K.H.A. Salama, F.Z.M. Ali and A.F. Abou Hadid, 2005. Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. Gen. Appl. Plant Physiol., 31: 29-41.

Mansour, M.M.F., K.H.A. Salama, M.M. Al-Mutawa, 2003. Transport proteins and salt tolerance in plants. Plant Sci., 164: 891-900.

Mansour, M.M.F., P.R. Van hasselt and P.J. Kuiper, 1994. Plasma membrane lipid alterations induced by NaCl in winter wheat roots. Physiol. Plant., 92: 473-478.

Mazliak, P., 1989. Membrane response to environmental stress: The lipids viewpoint-introductory overview. In: Biological role of plant lipids. P. A. Blacs, K. Gruiz, T. Krammer eds. Budapest and Plenum Publich, New York, 505-509.

Noctor, G. and C.H. Foyer, 1998. Ascorbate and glutathione: Keeping active oxygen under control. Ann. Rev. Plant Physiol. Plant Mol. Biol., 49: 249-279.

Quinn, P.J., 1989. Membrane stability under thermal stress. In: Biological role of plant lipids. P. A. Blacs, K. Gruiz, T. Krammer eds. Budapest and Plenum Public, New York, 511-515.

Salama, K.H.A., M.M.F. Mansour, F.Z.M. Ali and A.F. Abou Hadid, 2007. NaCl-induced changes in plasma membrane lipids and proteins of *Zea mays* L. cultivars differing in their response to salinity. Acta Physiol. Plant., 29: 351-359.

Shalata, A. and P.M. Neumann, 2001. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J. Exp Bot., 52(364): 2207-2211.

Shalata, A. and M. Tal, 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicum pennellii*. Physiol. Plant., 104: 169-174.

Stadelmann, E.J. and O.Y. Lee-Stadelmann, 1989. Passive permeability. In: Methods in Enzymology. S. P. Colowich and N. O. Kaplan, eds. Vol. 174 Biological transport (Biomembranes, Part U). Academic Press, New York, 246-266.

Tsugane, K., K. Koboyashi, Y. Niwa, K. Wada and H. Koboyashi, 1999. A reactive *Arabidopsis* mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification. The Plant Cell., 11: 1195-1206.

Wu, J., D.M Seliskar and J. Gallagher, 2005. The response of plasma membrane lipid composition in callus of the halophytes, *Spartina patens*, to salinity stress. Am J Bot., 92: 852-858.

Zhao, X.J., E. Sucoff and E.J. Stadelmann, 1987. Al^{3+} and Ca^{2+} alteration of membrane permeability of *Quercus rubra*root cortex cells. Plant Physiol., 83: 157-162.