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RESEARCH ARTICLE

Protective effect of 24-epibrassinolide against salt-induced destabilization of plasma membrane.

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Abstract

The study was undertaken to answer the question of whether 24-epibrassinolide has the ability to protect the plasma membrane against salt stress. Onion inner epidermal cells were exposed to 150 mMNaCl for 3h. Different protocols of 24-epibrassinolide, and three concentrations were studied (0, 1, 3 µM). NaCl increased plasma membrane permeability, number of cells with swollen protoplasm, and cell death. 24-epibrassinolide overcame Na^+-induced cellular alterations. 24-epibrassinolide prior to salt treatment had the greatest protective effect. 24-epibrassinolide at 3 µM was more effective than 1 µM. Plasma membrane protection under salinity stress was provided by 24-epibrassinolide, which may lead to improved salt tolerance.

Introduction:-

Salinity is one of the major abiotic factors limiting global agricultural productivity, rendering an estimated one-third of the world's irrigated land unsuitable for crops (Munns and Tester, 2008). High concentrations of salts in the soil lower the soil solution water potential and thus make it harder for roots to absorb water, i.e. the osmotic stress of salinity. Accumulation of salts (Na^+, Cl^-) within the plants causes specific ion toxicity and nutritional imbalance, i.e. the ionic stress of salinity (Munns and Tester, 2008). There is evidence indicating that the deleterious effect of salinity on plants is attributed to ionic impact rather than osmotic stress, either at whole plant or cellular level (Mansour et al., 1993a; Mansour 1995, 1997). Salt stress can also lead to oxidative stress through increasing reactive oxygen species, which cause cellular damage through oxidation of proteins, nucleic acids and membrane lipids (Gill and Tuteja, 2010). Glycophytes are believed to have cellular mechanisms for salt tolerance (Hasegawa et al., 2000; Mansour and Salama, 2004). Furthermore, plasma membrane (PM) stability has been recently reported as an effective selection criterion for salt tolerance in different crops (Farooq and Azam, 2006; Tiwari et al., 2010). It is therefore suggested PM as a primary site of salt injury (Cramer et al., 1985; Mansour, 1997; Mansour and Salama, 2004).

Brassinosteroids are natural substances wide spread in the plant kingdom and control the process of plant growth and development (Khirpach et al., 2000). Brassinosteroids have gained considerable interest because of their roles in affecting plant tolerance to stressful conditions such as high salinity, drought, chilling stress and nutrient deficiency (Khirpach et al., 2000; Bajguz and Hayat, 2009; Abbas et al, 2013). Exogenous application of 24-epibrassinolide (EB) ameliorates salt induced growth inhibition in different crop plants (Bajguz and Hayat, 2009; Abbas et al., 2013). Khirpach et al. (2000) demonstrated that one cellular physiological effect of brassinosteroids in plants is modulating fatty acid composition and hence membrane properties. Whether EB has a role in alleviating the salt-induced injurious effects on the PM is not yet elucidated and was addressed in this work.

Materials and methods:-

Innerepidermal cells of onion (*Allium cepa* cv. Kantartopu) were used as experimental material to study NaCl effects on the PM with or without EB treatments. Two sections (1 x 1 cm) of the inner epidermis of the third flashy scale were prepared. The sections were then peeled off from the parenchyma underneath and floated in distilled water.Four protocols of salt and EB were applied: (i) tissues were bathed in EB alone, to examine the effect of EB on the PM in absence of NaCl; (ii) EB was added to the NaCl solution, this treatment should indicate how much the damaging effect of NaCl can be attenuated by the presence of EB during exposure the PM to NaCl; (iii) EB was applied separately before NaCl imposition, prior treatment with EB is expected to test how such treatment can protect the membrane from the damaging effect of NaCl; and (iv) EB was applied after exposure to NaCl, subsequent treatment was intended to give information on the ability of EB to revert the NaCl damage. Three concentrations of EB (0, 1, 3 µM) are in the range usually found in plant tissue (Khirpach et al., 2000) were tested. Six treatments were applied. In the first two treatments, the tissue was exposed to distilled water or 150 mMNaCl for 3 h. In the third treatment, the tissue was exposed to EB alone (1 or 3 µM) for 1 h. In the fourth treatment, the tissue was exposed to a combined solution of 150 mMNaCl plus 1 or 3 µM EB for 3 h. In the remaining two treatments, the tissue was floated on 1 or 3 µM EB for 1h before or after exposure to 150 mMNaCl for 3h.

Plasmolysis and cytoplasmic streaming were used as evidence of cell viability (Lee-Stadelmann and Stadelmann, 1989; Mansour 1995). The cells were plasmolyzed in stepwise increasing glucose solution Non plasmolysis of the cells was used as an indicator for cell death, which was confirmed by the absence of cytoplasmic streaming. The number of dead cells, cells with swollen protoplasm and total number of cells (viable cells, dead cells, cells with swollen protoplasm) was determined in 5-7 sections per treatment. One field of vision in the middle of each section was chosen and evaluated.

Plasmolytic techniques (Stadelmann and Lee-Stadelmann, 1989; Mansour et al., 1993a; Mansour, 1997, 2013) were used to measure the permeation of urea and methylurea through the PM. After plasmolysis of the cells, the sections were transferred in a droplet of the same solution into a perfusion chamber (Mansour, 1997, 2013), and an isotonic solution of urea or methylurea was introduced. As the cells deplasmolyzed, the protoplast length was measured at time intervals. Three to five cells were measured from one section. Five to seven sections were measured per treatment. Protoplast length was plotted vs. time for each cell individually. The permeability coefficient (K_s, in cm s^{-1}) was calculated from the plot by the formula of Stadelmann and Lee-Stadelmann (1989) using a computer program.

Statistics:-

One tailed Student's t-test was applied to compare the mean values of control and treated tissues.

Results and discussion:-

Treating onion epidermis with 150 mMNaCl for 3 h significantly increased the permeability coefficient (K_s) for urea and methylurea (Table 1). EB administered in different protocols reduced the K_s values even below the values of control cells (Table 1). The protective effect of EB was greater with 3 µM EB than 1 µM, and was more pronounced when EB was applied prior to NaCl treatment (Table 1). Perturbation of the PM and hence increased its permeability in response to high salinity has been documented in several previous reports, and was attributed to oxidative stress and ionic impact rather than osmotic stress of salinity (Leopold and Willing, 1984; Ding et al. 2012; Mansou, 2013). It is also reported that salt stress altered PM lipid composition/structure (Kerkeb et al., 2001; Mansour and Salama, 2004; Wu et al., 2005; Salama et al., 2007; Mansour, 2013; Mansour et al., 2015) and increased membrane lipid peroxidation (Ding et al., 2012; Wang et al., 2013; Mansour, 2013), which subsequently change membrane properties and functions. Nonelectrolytes permeate membranes through its lipid portion and protein transporters (Mansour, 1997, 2013; Kojima et al., 2006; Wang et al., 2008). It can be, therefore, inferred that NaCl-induced permeability changes reported here brought about by alterations in the composition of PM lipids and proteins (Mansour et al., 2015). Lipid peroxidation and oxidative damage of proteins of PM may also participate in increased PM permeability induced by NaCl (Mansour, 2013). Our conclusion is based on the fact that changes in lipid metabolism and lipid peroxiation occur in as shortly as 1 h: Gapinska et al. (2008) indicated that lipid peroxidation increased after 1 h of tomato roots salt treatment, and PM lipid alteration occurred after 3 h of choline treatment, a precursor of phosphatiylcholine biosynthesis (Mansour et al., 1993b). EB significantly prevented alteration of PM permeability suggesting that EB had a protective effect on PM integrity which enables the PM to remain physically intact and physiologically functional under salt imposition. The significant permeability decrease after 1 h of EB

application alone indicates that EB was taken up by the cells and incorporated into the PM already during this relatively short time interval.

NaCl imposition considerably increased the number of dead cells as well as the number of cells with swollen protoplasm (see Mansour, 1995) compared with untreated controls (Table 2). EB significantly reduced the cells mortality and the number of cells showing swollen protoplasm (Table 2) compared with their controls. EB applied alone reduced considerably the cell mortality and protoplasmic swelling (Table 2). Treatments with EB before exposure to NaCl were more effective than simultaneous or subsequent treatments in reducing the number of dead cells and those with swollen protoplasm (Table 2). Application of EB in 3 µM was more efficient than 1 µM in mitigating the NaCl-induced cellular alterations. NaCl-induced perturbation of PM integrity resulted in increased Na^+ passive influx into the cytosol which leads to cellular metabolism disruption and increased water uptake into the cytoplasm resulting in protoplasmic swelling (Mansour, 1995, 2013), events that eventually lead to cell death. These deleterious effects of Na^+ on the PM were shown in increased cell mortality and number of cells with swollen protoplasm (Table 2). The previous cellular alterations were remarkably reduced by EB, more so with 3 µM EB prior treatment. This again is related to the ability of EB to stabilize the PM under salinity stress and thus may confer salt tolerance (Ding et al., 2012). What supports our contention is the notion that PM is thought to be the primary site of salt injury and hence maintaining its integrity will enhance plant performance in saline conditions (Leopold and Willing, 1984; Cramer et al., 1985; Mansour, 1997, 2013; Mansour and Salama, 2004; Mansour et al., 2015).

The mechanism by which EB act on the PM structure and function is most likely that EB interacts with the membrane components in such a way as to stabilize its structure and thus counterbalances the salt effects on PM (Talaat and Shawky, 2012), and hence its integrity is maintained under salinity stress. In addition, EB might protect PM from oxidative damage via antioxidant enzyme induction reported in several studies (Bajguz and Hayat, 2009; Talaat and Shawky, 2012, Ding et al., 2012; Wang et al., 2013) and in turn the PM integrity is retained in saline environment. Both mechanisms might ensure retaining the functional properties of the PM at high salinity and thus may enhance salt tolerance.

Table 1: Mean values of permeability coefficient (K_s) for urea and methylurea of onion bulb scale inner epidermal cells subjected to different treatments of NaCl and 24-epibrassinolide (EB). Each value is the mean ± SD (number of cells in parentheses from 5-7 sections). Arrows indicate transfer to the second treatment (time in parentheses). All treatments are significantly different at P › 0.01 from cells receiving only 150 mMNaCl.

Treatment	Permeability coefficient (cm s^{-1} X 10^8)	
	Urea	Methylurea
0 mMNaCl (3h)	4.22±0.46 (22)	16.55±1.91 (18)
150 mMNaCl (3h)	8.86±1.13 (21)	21.89±2.30 (20)
1 µM EB (1h)	2.64±0.33 (27)	14.34±0.99 (20)
3 µM EB (1h)	2.11±0.41 (29)	12.08±1.07 (19)
1 µM EB (1h) → 150 mMNaCl (3h)	2.07±0.22 (20)	14.07±2.09 (13)
3 µM EB (1h) → 150 mMNaCl (3h)	1.64±0.17 (25)	13.11±0.87 (21)
150 mMNaCl (3h) → 1 µM EB (1h)	7.09±0.77 (24)	19.01±1.17 (17)
150 mMNaCl (3h) → 3 µM EB (1h)	6.55±0.89 (31)	18.23±2.04 (16)
1 µM EB + 150 mMNaCl (3h)	4.11±0.84 (26)	16.17±1.49 (20)
3 µM EB + 150 mMNaCl (3h)	3.23±0.61 (30)	15.50±0.76 (22)

Table 2: Mean of the number of dead cells and cells with swollen protoplasm of onion bulb scale inner epidermis cells subjected to different treatments of NaCl and 24-epibrassinolide (EB). Each value is the mean ± SD of evaluated cells in 5-7 sections. Arrows indicate transfer to the second treatment (time in parentheses). All treatments are significantly different at P › 0.01 from cells receiving only 150 mMNaCl

Treatment	Dead cells		Cells with swollen protoplasm	
	number	%[a]	number	%[a]
0 mMNaCl (3h)	0.6±0.08	0.47	0.0±0.000.0	
150 mMNaCl (3h)	15.07±1.19	19.0	28.31±2.58	29.0
1 µM EB (1h)	0.00±0.00	0.0	0.03±0.002	0.02
3 µM EB (1h)	0.00±0.00	0.0	0.00±0.00	0.0
1 µM EB (1h) → 150 mMNaCl (3h)	1.30±0.07	1.2	2.80±0.31	2.9
3 µM EB (1h) → 150 mMNaCl (3h)	0.00±0.00	0.0	1.61±0.20	1.6
150 mMNaCl (3h) → 1 µM EB (1h)	4.80±0.37	6.1	4.11±0.51	4.7
150 mMNaCl (3h) → 3 µM EB (1h)	2.97±0.13	3.7	3.12±0.64	3.2
1 µM EB + 150 mMNaCl (3h)	1.11±0.06	1.7	2.27±0.19	2.3
3 µM EB + 150 mMNaCl (3h)	0.80±0.05	1.0	1.90±0.33	2.0

[a] indicates percentages of the mean of the total number of cells in 5-7 sections (67-93).

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