Changes in growth, osmotic potential and cell permeability of wheat cultivars under salt stress

M.M.F. MANSOUR

Department of Botany, Faculty of Science, Ain Shams University, Cairo 11566, Egypt

Abstract

10-d-old wheat seedlings were grown hydroponically in presence and absence of 100 mM NaCl for 7 d. Salt stress decreased growth of shoots and roots of both cultivars; fresh mass of sensitive cultivar being more affected. NaCl increased membrane permeability to urea, methylurea and ethylurea and decreased membrane partiality in root cortex cells of sensitive cultivar. Neither parameter changed by NaCl in resistant cultivar. NaCl treatment decreased water permeability and osmotic potential in both cultivars; sensitive cultivar was more affected. The results extends our previous data that cell membrane properties are different in salt sensitive and resistant genotypes and so cell permeability could be a potential trait indicating salt tolerance.

Introduction

Salinity stress has been reported to disturb the integrity of wheat and barley cell membrane resulting in increased membrane permeability and decreased lipid partiality (Mansour *et al.* 1993a). In previous work and other studies (Mansour 1990, Mansour *et al.* 1993b) genotypic differences in response to NaCl stress were reported. In the previous study subepidermal cells of leaf sheath and coleoptile of wheat and barley were used to test the effects of salinity stress on cytoplasmic factors. Studies on cellular level on a broader scale, therefore, would provide insights on the correlation of cytoplasmic factors with salt tolerance in higher plants. This approach could be achieved by exploring more different genotypes and tissues under salt stress. Differences in cytoplasmic viscosity and cell membrane permeability indicating differences in composition and/or structure of the cytoplasm and cell membranes which are expected to be reflected in plant growth. Thus salt resistant

---

Received 21 September 1993, *accepted* 7 January 1994.

Abbreviations: DM - dry mass; EU - ethylurea; FM - fresh mass; Ks - solute permeability constant; K_W - water permeability constant; MU - methylurea; RGR - relative growth rate; Pc - partition coefficient; U - urea; ψ_s - osmotic potential.

Acknowledgement: This work was supported by NUFFIC fellowship.

cells would be correlated with plant tolerance to salinity (Mansour 1990). Therefore, this contribution was conducted to study effects of NaCl stress on plant growth, osmotic potential, water and solute permeability in wheat cultivars differing in salt tolerance.

Materials and methods

Salt sensitive (Urban) and salt resistant (Sakha 8) cultivars of wheat (*Triticum aestivum* L.) were grown with and without 100 mM NaCl (for detail see Mansour *et al.* 1993c). Relative growth rate (RGR) was calculated by the formula used by Mansour (1990):

$$RGR = (\ln M_2 - \ln M_1)/(t_2 - t_1),$$

where M_1 and M_2 are the fresh mass or dry mass at time t_1 and t_2, respectively. For solute permeability (K_s), water permeability (K_w) and osmotic potential (ψ_s) measurements in root cortex cells, 1 cm long root sections (1 cm from the root tip) were plasmolyzed in 0.4, 0.6 (15 min each) and 0.8 M glucose for 30 - 45 min to reach osmotic equlibrium. The sections were then transferred in a droplet of the 0.8 M glucose into a perfusion chamber (closed by a cover glass sealed with vaseline) on the stage of a microscope. For K_s determination, the glucose solution was exchanged by an isoosmotic solution of U or MU and the length of deplasmolyzing protoplasts was measured at equal intervals. Because of higher EU permeability, the partial method was used (Stadelmann and Lee-Stadelmann 1989). K_w was measured in the same way as K_s but the 0.8 M glucose in the perfusion chamber was replaced with 0.5 M glucose and the course of deplasmolysis was recorded. K_S and K_W were calculated with the formulas of Stadelmann and Lee-Stadelmann (1989). When the root cortex cells reached osmotic equilibrium in 0.8 M glucose, protoplast length and cell dimensions were measured and O_g (osmotic groung value at turgor loss point was calculated, Lee-Stadelmann and Stadelmann 1989). ψ_S was calculated from O_g using Van't Hoff's equation.

Results and discussion

NaCl treatment significantly reduced FM, DM as well as RGR (on FM and DM bases) of shoots and roots of cv. Urban and cv. Sakha 8 (Table 1). On the percentage basis, the reduction in the shoot and root FM and RGR (FM basis) due to salt exposure was greater than the reduction in DM and RGR (DM basis) in both cultivars. However, no visual symptoms of wilting were observed in both cultivars upon salt exposure. This might be due to increased stomatal resistance (Downton *et al.* 1985) and decreased cell osmotic potential (Greenway and Munns 1980). Cv. Urban showed higher reduction in shoot and root FM and RGR (FM basis) by NaCl as compared with cv. Sakha 8. This may be attributed to the higher reduction in water permeability induced by NaCl in root cortex cells of cv. Urban (to 60 % of the salt exposure the reduction in DM and RGR (DM basis) was more or less comparable in both cultivars suggesting that parameters other than growth should be considered to differentiate between salt sensitive and resistant wheat cultivars. This is also because the data on growth in saline environment is rather contradictory: while Kuiper *et al.* (1988) considered reduced growth as a trait for salt tolerance, Weimberg and Shannon (1988) used vigorous growth as a criterion for wheatgrass selection.

Table 1. Fresh mass (FM), dry mass (DM) and relative growth rate (RGR) of wheat shoots and roots exposed to 100 mM NaCl for 7 d. Each value is the mean ± SD of 30 - 42 measurements of 3 replications.

	FM [g plant^{-1}]		RGR_{FM} [d^{-1}]		DM [g plant^{-1}]		RGR_{DM} [d^{-1}]	
	shoot	root	shoot	root	shoot	root	shoot	root
cv. Urban								
Control	0.75 ± 0.006	0.37 ± 0.05	0.18 ± 0.01	0.15 ± 0.02	0.09 ± 0.01	0.03 ± 0.003	0.20 ± 0.02	0.15 ± 0.01
NaCl	0.45 ± 0.003 a	0.22 ± 0.01 a	0.10 ± 0.001 a	0.07 ± 0.01 a	0.07 ± 0.001 b	0.02 ± 0.002	0.16 ± 0.002 b	0.12 ± 0.01 b
cv. Sakha 8								
Control	0.89 ± 0.02	0.41 ± 0.02	0.26 ± 0.004	0.23 ± 0.01	0.10 ± 0.002	0.04 ± 0.004	0.26 ± 0.003	0.22 ± 0.01
NaCl	0.65 ± 0.02 a	0.31 ± 0.01 a	0.19 ± 0.01 a	0.16 ± 0.004 a	0.08 ± 0.01 b	0.03 ± 0.001 b	0.22 ± 0.01 a	0.18 ± 0.01 b

a,b indicate significant difference from control at 1 and 5 % level, respectively.

NaCl increased U, MU and EU permeability in cv. Urban while it had no effect on K_s values of cv. Sakha 8 suggesting differences in lipid matrix of membranes of both cultivars (Mansour *et al.* 1993a). The differences in permeability constant to urea family permeators between the controls of sensitive and resistant cultivars also pointed out to these dissimilarties in the membrane lipid portion of both cultivars. These results suggest that salt resistant cultivar may have inherent or inducible membrane properties which maintain membrane integrity under salt stress. The previous proposal is supported by the fact that NaCl induced various changes in the plasma membrane lipid composition of sensitive wheat cultivar (Mansour *et al.* 1993c), while resistant cultivars always have such lipid characteristics that correlate with salt tolerance (Kuiper 1984). The slope of the regression line ($1/K_s$ *vs* 1/Pc) of cv. Urban was decreased by NaCl stress (0.011 *vs* 0.029 for control value) suggesting decreased membrane partiality by salt exposure (Fig. 1). This suggests change in the fatty acid residues of the membrane core by NaCl; probably altered packing density of lipid molecules or different relative proportion of lipid species induced by NaCl (Mansour *et al.* 1993a, Chen *et al.* 1991). NaCl effect on lipid partiality of cv. Urban was not found with cv. Sakha 8 (slope value of 0.0081 *vs* 0.0079 for control value) which again confirms different membrane composition/structure in both cultivars.

Table 2. Effects of 100 mM NaCl on permeability constants (K_s) for permeation of urea (U), methylurea (MU) and ethylurea (EU), water permeability constant (K_w) and osmotic potential of root cortex cells of wheat cultivars. Each value is the mean ± SD of the cell number given in parentheses.

	K_s (U) [× 10^{-7} cm s^{-1}]	K_s (MU) [× 10^{-7} cm s^{-1}]	K_s (EU) [× 10^{-7} cm s^{-1}]	K_w [× 10^{-4} cm s^{-1}]	ψ_s [MPa]
cv. Urban					
Control	3.03±0.32(13)	29.48±2.02(11)	47.41±3.76(11)	3.31±0.10(13)	-0.64±0.17(19)
NaCl	6.51±0.39(12)[a]	36.30±2.77(15)[a]	55.99±5.09(12)[a]	1.99±0.21(14)[a]	-0.98±0.10(16)[a]
cv. Sakha 8					
Control	9.58±0.99(11)	48.37±2.99(14)	77.11±4.39(11)	3.92±0.34(14)	-0.74±0.10(19)
NaCl	8.83±0.87(13)	50.01±5.52(13)	76.73±3.77(13)	3.60±0.41(12)[b]	-0.98±0.12(22)[a]

a,b indicate significant difference from control at 1 and 5 % level, respectively.

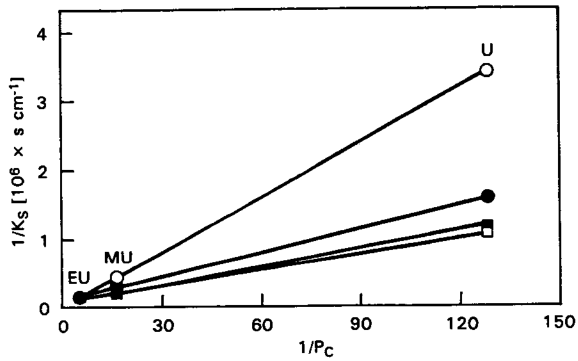

Fig. 1. Permeation resistance ($1/K_s$) of U, MU and EU as a function of 1/Pc, where Pc is the partition coefficient of U, MU or EU in octanol/water at 20 °C (*closed symbols* - NaCl treated plants, *open symbols* - control plants; *circles* - cv. Urban, *squares* - cv. Sakha 8). A steep slope means that permeation resistance across the membrane changes greatly with the lipid solubility of the permeator. A flat slope indicates that the membrane has lower lipid partiality, and permeation resistance increase only slightly with the lipid solubility of the permeator.

NaCl stress significantly decreased, with different degrees, permeability to water in both cultivars (Table 2). Alterations or differences in membrane structure or composition is believed to be causes for changes in water permeability (Finkelstein 1987). Zekri and Parsons (1989) report that hydraulic conductivity decreased more in sensitive citrus rootstocks compared with tolerant ones in response to salinity stress which was attributed to a higher decrease in membrane permeability to water of sensitive rootstocks. Our results are in agreement with their findings. NaCl decreased water permeability while increased U, MU and EU permeability (Table 2) which suggests that water and urea series permeators do not cross the cell membranes in the same way (Chen *et al.* 1991).

Salt treatment significantly decreased ψ_S of the root cortex cells of cv. Urban and cv. Sakha 8 (Table 2). However, in cv. Sakha 8 the decrease in ψ_S corresponded only to the depression of ψ_S in the external medium by NaCl (about 0.25 MPa). On the other hand, for cv. Urban the decrease in ψ_S exceeded the lowering of external ψ_S due to NaCl which is presumably due to more accumulation of salt by roots (Munns 1993). This result is consistent with our previous finding in subepidermal cells of leaf sheath and coleoptile of wheat and barley under salt stress (Mansour *et al.* 1993b). Organic osmotics (betaine/proline) and ions might be also the contributors to the decrease in ψ_S (Greenway and Munns 1980).

In conclusion, growth of cv. Urban and cv. Sakha 8 did not markedly differ under salt stress. Higher reduction in cv. Urban FM in relation to that of cv. Sakha 8 may be attributable to NaCl effect on water permeability of root cortex cells in cv. Urban. Though ψ_S decreased in both cultivars under salt stress, the decrease in cv. Urban was higher than that of cv. Sakha 8. Varying degree of NaCl effects on K_w, K_S and membrane partiality of cv. Urban and cv. Sakha 8 clearly indicate that cell membrane composition/structure is not the same for both cultivars. The effect of salinity stress on the cytoplasmic parameters tested in this investigation and in our previous studies using different genotypes and tissues was always consistent and reproducible suggesting more reliable traits to differentiate between wheat sensitive and resistant cultivars.

References

Chen, J., Sucoff, E., Stadelmann, E.: Aluminum and temperature alteration of cell membrane permeability of *Quercus rubra*. - Plant Physiol. **96**: 446-449, 1991.

Downton, W., Grant, W., Robinson, S.: Photosynthetic and stomatal responses of spinach leaves to salt stress. - Plant Physiol. **77**: 85-88, 1985.

Finkelstein, A.: The unmodified membrane. - In: Finkelstein, A.: Water Movement through Lipid Bilayers, Pores, Plasmalemma. Theory and Reality. Pp. 39-106. Wiley, New York 1987.

Greenway, H., Munns, R.: Mechanisms of salt tolerance in nonhalophytes. - Annu. Rev. Plant Physiol. **31**: 149-190, 1980.

Kuiper, P.J.: Functioning of plant cell membranes under saline stress conditions: membrane lipid composition and ATPases. - In: Staples, R., Toenniessen, D. (ed.): Salinity Tolerance in Plants. Pp. 77-91. Wiley, New York 1984.

Lee-Stadelmann, O., Stadelmann, E.: Plasmolysis and deplasmolysis. - In: Colowick, S.P., Kaplan, N. (ed.): Methods of Enzymology. Vol. 174. Pp. 225-246. Academic Press, New York 1989.

Mansour, M.: Studies on the Protoplasm of Salt Sensitive and Salt Tolerant Plants. - Ph. D. Thesis, University of Minnesota, Minnesota 1990.

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

Mansour, M., Lee-Stadelmann, O., Stadelmann, E.: Osmotic potential and cytoplasmic viscosity in *Triticum aestivum* and *Hordeum vulgare* under salt stress. A comparison of sensitive and resistant lines and cultivars. - J. Plant Physiol. **142**: 623-628, 1993b.

Mansour, M., Van Hasselt, P., Kuiper, P.J.: Plasma membrane lipid alterations induced by NaCl in winter wheat roots. - Physiol. Plant., in press.

Munns, R.: Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. - Plant Cell Environ. **16**: 15-24, 1993.

Stadelmann, E., Lee-Stadelmann, O.: Passive permeability. - In: Colowick, S.P., Kaplan, N. (ed.): Methods of Enzymology. Vol. 174. Pp. 246-266. Academic Press, New York 1989.

Weimberg, R., Shannon, M.: Vigor and salt tolerance in 3 lines of tall wheatgrass. - Physiol. Plant. **73**: 232-237, 1988.

Zekri, M., Parsons, L.: Growth, and root hydraulic conductivity of several citrus rootstocks under salt and polyethylene glycol stress. - Physiol. Plant. **77**: 99-106, 1989.