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Effect of NaCl and polyamines on plasma membrane lipids of wheat roots

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Abstract

Caryopses of a salt sensitive wheat cultivar (*Triticum aestivum* L. cv. Giza 163) were presoaked in 2.5 mM putrescine (Put), 5 mM spermidine (Spd) or 2.5 mM spermine (Spm) for 24 h and then subjected to 150 mM NaCl added to the growth medium for 15 d. Effects of NaCl and polyamines (PAs) on plasma membrane (PM) lipids, phospholipids, fatty acids, and free sterols were determined. NaCl treatment caused a decrease in total phospholipids, increase in saturated fatty acids and altered distribution of sterols and phospholipids. NaCl also induced increase in sterol/phospholipid ratio. PAs treatments (particularly Put and Spd) counterbalanced the NaCl deleterious effects on PM lipids.

Additional key words: fatty acids, phospholipids, plasma membrane, salinity, sterols, *Triticum aestivum*.

Introduction

Accumulating evidence points to a key role of polyamines (PAs) in several stress conditions: K^+ deficiency (Young and Galston 1984), increased soil acidity (Galston and Kaur-Sawhney 1987), chilling stress (Slocum and Galston 1985), osmotic and salinity stress (Krishnamurthy and Bhagwat 1989, Basu and Ghosh 1991, Reggiani *et al.* 1994, Mansour 2000). Mansour *et al.* (2001) demonstrated that presoaking of caryopses of salt sensitive wheat cultivar in PAs enhanced tolerance to NaCl due to altered ion uptake and transport under NaCl stress. Since ion transport is controlled by PM lipids (Russell 1989), one might expect that PAs may affect the PM lipid composition. It is believed that PM is involved in plant salt tolerance (Levitt 1980, Kuiper 1984, Cramer *et al.* 1985, Mansour 1997). Reports indicate that lipid composition of PM was changed under salinity (Kuiper 1984, Douglas 1985, Mansour *et al.* 1994, Wu *et al.* 1998). These changes in PM lipids may have a critical role in plant response to salinity stress because they may enable the PM to function properly under stress condition (Kuiper 1984, Mazliak 1989, Mansour *et al.* 1994). Therefore, this study was conducted to determine whether PAs enhanced plant performance under salinity correlates with alterations in PM lipid composition.

Materials and methods

Caryopses of salt sensitive *Triticum aestivum* L. (cv. Giza 163) were surface sterilized by 0.2 % $HgCl_2$ and then rinsed with distilled water. The caryopses were divided into four groups: three groups were presoaked for 24 h in different PAs (2.5 mM Put, 5 mM Spd, 2.5 mM Spm) and the fourth group, control, was presoaked in distilled water. PAs concentrations were chosen after a trial experiment and proven to be the most effective in alleviating the inhibitory effect of salinity on seedling growth (Mansour *et al.* 2001). The solutions were renewed every 4 h. Next, the caryopses were germinated on filter paper moistened with 10 cm^3 of ¼-strength modified Hoagland solution (MHS, pH 5.5 - 6.5, Epstein 1972) in the dark for 2 d. The seedlings were exposed

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Abbreviations: DPG - diphosphatidylglycerol; MHS - modified Hoagland solution; NT - nontreated plants; PA - phosphatidic acid; PAs - polyamines; PC - phosphatidylcholine; PE - phosphatidyethanolamine; PG - phosphatidyglycerol; PI - phosphatidylinositol; PS - phosphatidylserine; PM - plasma membrane; Put - putrescine; SC - salt control; Spd - spermidine; Spm - spermine.
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to 12-h photoperiod (irradiance of 440 μmol m^{-2} s^{-1}), light/dark temperature 25/22 °C and relative humidity 65 - 75 % in the growth chamber. The filter papers were changed daily with a new 10 cm^3 of MHS. After 5 d, the seedlings were transferred into containers with continuously aerated MHS and grown for 7 d. NaCl at 150 mM was added to MHS (except nontreated seedlings). The control seedlings (from seeds presoaked in distilled water) were further divided into two groups: salt control (SC), those received no PAs but only NaCl treatment, and non-treated plants (NT), those received neither PAs nor NaCl. The solutions were renewed weekly.

Two phase partitioning system (Mansour *et al.* 1994) was used to isolate the root PM. The lipids were immediately extracted from the PM suspension as described by Mansour *et al.* (1994) and the lipid extracts were separated on two-dimensional thin layer chromatography according to Rouser *et al.* (1966). Solvent mixtures of chloroform:methanol:ammonia (65:25:5 v/v) in the first dimension and chloroform: aceton:methanol:acetic acid:water (30:40:10:10:5 v/v) in the second dimension were used.

For the analysis of fatty acids, lipids were saponified and fatty acids methyated with H_2SO_4 and methanol as described by Mansour *et al.* (1994) using H_2SO_4 instead of BF_3. Fatty acid methyl esters were determined by gas chromatography (*Hewlett Packard 5890*, Palo Alto, USA).

Free sterol classes were determined by gas liquid chromatography (*Vista 6000*, Palo Alto, USA) according to Ibrahim *et al.* (1994).

PM total lipids were determined according to March and Weinstein (1966) while PM total phospholipids were measured according to Connerty *et al.* (1964). PM total sterols were determined according to Zlatkis and Zak (1969).

The Student *t*-test was used to establish the significance of differences between means of control and treated plants.

Results and discussion

Despite precautions to reduce lipase activity (boiling isopropanol, high pH, EDTA, KF), 17.9 mol % of total phospholipids was obtained. About the same percentage was reported in other studies (Rochester *et al.* 1987, Norberg and Liljenberg 1991). Whether phosphatidic acid (PA) is a natural membrane constitutent is an open question.

Table 1. Total lipids, total phospholipids and total sterols of wheat root plasma membrane. Caryopses were presoaked in 2.5 mM Put, 5 mM Spd, or 2.5 mM Spm for 24 h and the seedlings were exposed to 150 mM NaCl for 15 d. Means ± SD of two duplicates (* - means significantly different from SC, $P = 0.05$).

Treatment	Lipids [mg g^{-1}(f.m.)]	Phospholipids [nmol g^{-1}(f.m.)]	Sterols [nmol g^{-1}(f.m.)]
NT	8.02 ± 0.87	83.56 ± 3.94*	3.32 ± 0.55
SC	7.39 ± 0.16	45.63 ± 5.23	2.66 ± 0.16
Put	8.27 ± 0.90	47.44 ± 4.07	2.72 ± 0.14
Spd	10.03 ± 0.95*	61.18 ± 6.41	2.97 ± 0.30
Spm	7.28 ± 1.14	47.94 ± 1.94	2.95 ± 0.18

NaCl at 150 mM had no significant effect on plasma membrane (PM) total lipids whereas it induced a decrease in PM total phospholipids (Table 1) and altered the relative distribution of phospholipids (Table 2) comparing with nontreated plants (NT). Phosphatidylcholine (PC) decreased from 25.4 to 11.4 %, phosphatidylglycerol (PG) decreased from 15.9 to 12.6 %, phosphatidylinositol (PI) increased from 6.8 to 8.4 %, phosphatidylserine (PS) increased from 8.8 to 21.7 %, phosphatidylethanolamine (PE) increased from 17.6 to 20.7 %, diphophatidylglycerol (DPG) increased from 7.1 to 11.6 %. These results are in agreement with findings of Mansour *et al.* (1994) and Wu *et al.* (1998) who reported changes in phospholipids under salt stress.

Presoaking of caryopses in PAs increased PI and PC whereas it decreased PE and PS relative to SC plants (Table 2). The ratio of PC/PE was 0.55 in SC plants which and it increased to 2.0, 3.1 and 1.6 in PM of plants treated with Put, Spd and Spm, respectively. Stability of the membrane depends on bilayer/nonbilayer forming lipids: PC forms bilayer structure whereas PE form nonlamellar structure (Quinn 1983, Gange *et al.* 1985). Thus, the alteration in relative abundance of phospholipids by NaCl may affect PM stability and functions and hence plant sensitivity to NaCl (Kuiper 1984, Russell 1989, Lee 1991). On the other hand, the changes induced by PAs in PM phospholipids composition may occurr to restore the PM integrity and thus enhancing plant performance in saline environment. This suggestion is in agreement with the data reported by Basra *et al.* (1997) who indicated that exogenous application of PAs maintained membrane stability and enhanced recovery of growth during heat shock.

NaCl induced a decrease in the PM unsaturated fatty acids (16:1, 18:1, 20:1) and increased saturated fatty acids (16:0, 17:0, 18:0, 20:0) resulting in a lower unsaturated/saturated ratio (Table 3). Similar observations have been reported in PM of other plant species under salinity (Mansour *et al.* 1994, Surjus and Durand 1996, Wu *et al.* 1998). Alteration in fatty acid composition may change the bilayer thickness and

fluidity (Cogen *et al.* 1973, Rochester *et al.* 1987) which, in turn, affect membrane-bound enzymes and membrane properties. Put and Spd caused a decrease in PM saturated fatty acids and results in appearance of polyunsaturated fatty acid (18:3). Our results are consistent with those of Huang and Fu (1991) who found that treatment of ground nut seeds with Put or Spm alters the unsaturation of membrane fatty acids. The effect of PAs on fatty acid saturation may maintain the membrane fluidity in its physiological range so that the PM behaves normally under stress condition.

Table 2. Phospholipid composition [mol %] of root plasma membrane of plants grown in 150 mM NaCl for 15 d. Caryopses were presoaked in 2.5 mM Put, 5 mM Spd or 2.5 mM Spm for 24. Means ± SD of two duplicated (* and ** - means significantly different from salt control, $P = 0.05$ and $P = 0.01$, respectively).

Lipid class	NT	SC	Put	Spd	Spm
PA	17.9 ± 0.534**	12.7 ± 0.283	14.0 ± 0.424*	14.6 ± 0.636*	11.4 ± 0.212*
PI	6.8 ± 0.141*	8.4 ± 0.283	16.7 ± 0.778**	16.7 ± 0.007**	15.3 ± 0.212*
PS	8.8 ± 0.354**	21.7 ± 0.354	10.0 ± 0.424**	9.3 ± 0.424**	14.4 ± 1.414*
PC	25.4 ± 0.495**	11.4 ± 0.566	24.8 ± 0.778**	27.8 ± 0.282**	23.7 ± 0.845**
PE	17.6 ± 0.213*	20.7 ± 1.131	12.3 ± 1.344*	9.0 ± 0.919**	14.5 ± 0.778*
PG	15.9 ± 0.142*	12.6 ± 0.495	12.2 ± 0.007	12.7 ± 0.212	12.2 ± 0.212
DPG	7.1 ± 0.283*	11.6 ± 0.354	11.5 ± 2.051	11.0 ± 0.141	9.4 ± 1.202
PC/PE	1.40	0.55	2.00	3.09	1.64

Table 3. Fatty acid composition [mol %] of total phospholipids of root PM of plants grown in 150 mM NaCl for 15 d. Caryopses were presoaked in 2.5 mM Put, 5 mM Spd or 2.5 mM Spm for 24. Means ± SD of two duplicated (* and ** - means significantly different from SC, $P = 0.05$ and $P = 0.01$, respectively).

Fatty acids	NT	SC	Put	Spd	Spm
16:0	29.9 ± 0.14	42.4 ± 0.92	5.1 ± 1.06**	7.3 ± 0.35**	3.6 ± 0.07**
16:1	11.9 ± 0.28	4.5 ± 0.57	5.8 ± 1.13	15.3 ± 0.43**	8.2 ± 0.21*
17:0	-	4.7 ± 0.14	38.6 ± 0.85**	10.3 ± 0.28**	21.5 ± 0.71**
18:0	13.7 ± 0.28	25.3 ± 0.64	3.5 ± 0.71**	15.6 ± 0.92**	25.6 ± 0.57
18:1	10.1 ± 0.14	2.6 ± 0.57	25.0 ± 1.49**	16.9 ± 0.92**	5.1 ± 0.35*
18:2	-	3.0 ± 0.14	1.4 ± 0.28*	5.0 ± 0.14**	7.7 ± 0.42**
18:3	-	-	2.2 ± 0.21	7.4 ± 0.64	4.7 ± 0.21
20:0	19.0 ± 0.06	14.5 ± 1.20	7.3 ± 1.06*	9.3 ± 1.27*	14.4 ± 2.19
20:1	15.8 ± 0.28	3.2 ± 0.35	11.1 ± 0.28**	13.0 ± 0.28**	9.4 ± 0.28**
Unsat./sat.	0.594	0.153	0.837	1.355	0.539

Table 4. Composition of free sterols [mol %] of root PM of plants grown in 150 mM NaCl for 15 d. Caryopses were presoaked in 2.5 mM Put, 5 mM Spd or 2.5 mM Spm for 24. Means ± SD of two duplicated (* and ** - means significantly different from SC, $P = 0.05$ and $P = 0.01$, respectively).

Sterols	NT	SC	Put	Spd	Spm
Cholesterol	7.8 ± 0.354**	12.1 ± 0.495	5.8 ± 1.061**	5.9 ± 0.071**	8.2 ± 3.182
Campasterol	62.0 ± 0.071**	51.0 ± 4.101	55.4 ± 6.152	57.0 ± 0.424	43.3 ± 10.395
Stigmasterol	24.3 ± 2.546*	35.4 ± 5.233	37.0 ± 4.667	35.2 ± 0.035	45.5 ± 5.728
β-Sitosterol	6.0 ± 2.121	1.6 ± 0.566	1.9 ± 0.425	2.0 ± 0.495	3.2 ± 1.485
Sterol/phospholipids	0.04	0.06	0.06		0.06

Although total free sterols did not change under salt stress (Table 1), the relative proportions of cholesterol and stigmasterol was increased and that of campesterol and β-sitosterol was decreased (Table 4). Alterations in PM sterols upon salt exposure have been reported in various plant species (Douglas 1985, Mansour *et al.* 1994, Brown and DuPont 1989, Wu *et al.* 1998). NaCl increased sterol/phospholipids ratio (Table 4) which is

consistent with finding of Navari-Izzo *et al.* (1988) in maize. High ratio of sterol/phospholipids is considered as important characteristic of PM deterioration (Lees and Thompson 1980, Stelleart and Genus 1994). In this study sterol/phospholipids ratio was decreased by PAs treatment, but it still was elevated compared with NT controls. Sterols are important class of the membrane lipids because they can regulate membrane enzyme activities (Sandstrom and Cleland 1989, Larsson *et al.* 1990), membrane permeability (de Kruijff 1973), membrane ion absorption (Kuiper 1984, Russell 1989) and membrane fluidity (Cogen *et al.* 1973). Therefore, NaCl-induced decrease in the ratio of more planner sterols (cholesterol, campesterol) to less plannar sterols (stigmasterol, β-sitosterol), and increase in the sterol/ phospholipid ratio may affect PM functions and properties (Mansour *et al.* 1994, Surjus and Durand 1996). PAs-induced alterations in PM sterols, however, may mitigate those promoted by NaCl.

It seems that NaCl-induced changes in PM lipids were in the direction to deteriorate the PM while PAs-induced alterations were in a favorable direction to maintain PM stability and functions, which was reflected in their effect on the growth enhancement under salt stress (Mansour *et al.* 2001).

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