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EFFECTS OF NaCl SALINITY LEVELS ON LIPIDS AND PROTEINS OF CANOLA (*BRASSICA NAPUS* L.) CULTIVARS

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ABSTRACT

Five canola (*Brassica napus* L.) cultivars (SLM046, Okapi, Licord, Fornax and Elite) were grown hydroponically to study the effect of NaCl on root plasma membrane (PM) lipid and protein alterations. The PM total sterols of Elite, Fornax and Licord were decreased while those of SLM046 and Okapi were increased in response to salt. Salt stress had no significant effect on PM total glycolipids and proteins of both cultivars. The PM total phospholipids were increased in Elite, Licord and Fornax but did not change significantly in SLM046 and Okapi after salinity stress. Molecular percentage of PM phospholipids and fatty acids of both cultivars was different in 0, 50 and 150 mM NaCl. The most abundant phospholipids in untreated Elite, Licord and Fornax PM were phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS), which changed into PG, PS, phosphatiylinositol (PI) and PC after salt treatment. Over 60% of the total fatty acids were saturated in PM of SLM046 and Okapi cultivars exposed to 150 and 200 mM NaCl. The predominant fatty acids in the cultivar Elite exposed to 150 and 200 mM NaCl were C 17:0 and C18:1, respectively. However, in the cultivar SLM046 treated with 150 and 200 mM NaCl, the predominant fatty acids were C20:0 and C17:0 respectively.

Key words: plasma membrane lipids, canola, salinity stress.

INTRODUCTION

Salinity is a major environmental factor limiting plant growth and productivity. Saline environments are generally correlated with changes in plant lipid metabolism (Bybordi et al., 2010; Ashraf and Haris, 2004). Fatty acids have been associated with damages provoked by a variety of environmental stresses. The plasma membrane (PM) might be the primary site of salinity injury (Mansour, 1997; Mansour and Salama, 2004). Salt stress induces alteration in the PM permeability, which varies greatly between cultivars differing in salt tolerance (Mansour and Stadelmann, 1994; Mansour, 1997). PM permeability measurement proves that alterations in PM lipid come from composition (Van Zoelen et al., 1978; Russell, 1989). The function of PM lipids in salt tolerance comes from the fact that membrane lipids have great influence on the membrane roles either by changing the membrane fluidity (Opekarova and Tanner, 2003; Lee 2003), or as lipid mediators, which are involved in signal transduction, vesicular trafficking and cytoskeletal reorganization in plant response to stresses (Wang, 2004; Testerink and Munnik 2005; Lin and Wu 1996). Molecular changes in PM lipids of tolerant plants are supposed to enhance membrane integrity and its cellular functions under salinity (Mansour, 1997; Mansour and Salama, 2004; Zongli et al., 1987). Thus, investigating the response of PM lipids in various plants contrasting in their response to saline environment will help understand the PM correlation with plant salt tolerance mechanism. Alterations in PM proteins are reported in different plant cultivars under saline conditions (Mansour et al., 2003; Yang et al., 2004; Wu et al., 2005). Slight changes in PM proteins were found, however, in genotypes differing in salt sensitivity under saline conditions (Hurkman et al., 1998; Kerkeb et al., 2001). The objective of the present investigation thus was to characterize the lipid composition and polypeptide components of root PM of five canola cultivars in salinity conditions.

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MATERIAL AND METHODS

Seeds of canola cultivars (SLM_{046}, Okapi, Licord, Fornax and Elite) were obtained from the Agricultural Research center, Karaj, Iran, and kept at 4°C. The seeds of canola cultivars were soaked in tap water for one hour and the water was renewed every 20 min. The seed of cultivars were then germinated in Petri dishes containing filter paper moistened with 15 ml of 1/4-strength modified Hoagland solution (MHS, Epstein, 1972), and placed in a dark incubator at 25°C for 5 days. In the sixth day of germination, uniform seedlings were transferred into 2 L black plastic pots containing 1/4-strength MHS for 7 days, which were continuously aerated. Five plants were fixed in a foam-disc supported at the top of each pot. For each cultivar, five treatments were applied ¼ - strength MHS plus 0, 50, 100, 150 and 200 mM NaCl. Each treatment was replicated two times. The period of salt exposure was 30 days. The solutions were renewed every 5 days during salt stress. The plants were left to grow in a controlled growth chamber under the following growth conditions: 15 hours photoperiod, 70-75% relative humidity, with day/night temperature of 18-22°C, respectively.

PM isolation

Two-phase partitioning system (Mansour et al., 1994, 2004) was used to isolate the root PM of canola cultivars. The roots were washed in cold bi-distilled water and homogenized in a blender, with a homogenization medium (pH 7.5) containing 250 mM sucrose, 5 mM EGTA, 5 mM EDTA, 10 mM KF, 25 mM MOPS, and 1 mM PMSF. 2 mM of DTT was added as powder. The homogenization of the root tissue was carried out three times for 20 S each with a 10 S pause. The homogenate was filtered through miracloth. A new homogenization medium was added to the tissue and was homogenized once again. The pooled solution was centrifuged at 10,000 g for 20 min. The supernatant containing the PM and other membranes was then centrifuged at 50,000 g for one hour. The microsomal membrane pellet was re-suspended in 5 mM potassium phosphate buffer (pH 7.8) containing 250 mM sucrose and 50 mM KCl. The PM were prepared by partitioning of the microsomal suspension in 27g aqueous polymer two-phase system containing 6.5% dextrane T-500 (pharmacia), 6.5% polyethylene glycol 3350 in 250 mM sucrose, 5 mM potassium phosphate buffer (pH 7.8), 4 mM KCl and 25 mM DTT. Into the two-phase system, 108 µl of solution (333 mM DTE and 33.3 mM EDTA) was added, mixed thoroughly and centrifuged at 15,000 g for 5 min. The microsomal pellet was subjected to three successive phase partitioning steps. The upper phase, containing the PM fraction, was centrifuged at 50,000 g for 60 min. and the pellet resuspended in 1 ml of 5 mM MOPS - BTP (pH 7.5). All steps of this isolation procedure were carried out at 0-4°C. The purity of our PM preparation was based on Mansour et al. (1994, 2000).

PM total protein

The method of Henry (1974) was used to determine the PM total protein using bovine serum albumin as the standard.

Electrophoresis

SDS-PAGE was carried out using a discontinuous buffer system described by Laemmli (1970) to analyze the polypeptide from control and salinized fractions. After protein denaturation, the samples were applied to the top of SDS-PAGE that consisted of 5% (w/v) stacking gel and 7.5% (w/v) resolving gel with cross linking degree of 2.7% and plate dimensions of $85 \times 50 \times 0.7$ mm. Electrophoretic separation of the polypeptides was carried out in a Mini-Protein II cell (Bio-Rad) at 4 ◦ C for 90-120 min.

After electrophoresis, the gels were stained in 0.123% (w/v) Coomassie blue R-250 in 50% (v/v) methanol and 10% (v/v) acetic acid for 30-45 min and de-stained in 50% methanol and 10% acetic acid. After de-staining, the change in bands' staining intensity was measured by scanning with gel documentation device (Gel Doc.2000, Bio-Rad, version 1.1).

PM Lipid extraction and separation

Boiled isopropanol was immediately added to the PM suspension to inhibit the activity of lipases (Kates, 1972). Lipids were then extracted with 3.75 ml chloroform: isopropanol (2:1,v/v) and 2.25 ml of 0.1 M KCl was added to enhance the chloroform phase separation. Then the mixture was centrifuged in cold room at 1,000 g for 5 min. The upper water phase was re-extracted with 2 ml chloroform. The first and second chloroform phases (containing lipids) were collected and dried under CO_2 stream. The dried lipids were dissolved in 2.5 ml chloroform and stored at -80°C until analysis.

Determination of PM fatty acids

The method of Mansour et al. (2002) was used for the analysis of fatty acids. One milliliter of lipid extract, 6 ml of benzene and 15 ml of 10% alcoholic KOH were mixed. The tubes were refluxed for 4 h in boiling water bath and then the mixture was evaporated. Excess of diethyl ether was added and shaken well. The organic phase (upper phase) was pipetted and the aqueous phase (lower phase) was further washed three times with diethyl ether. The organic phase was used to determine the different classes of sterols and the aqueous phase was acidified to determine the different fatty acids. The aqueous phase containing fatty acids was acidified by 1 N H_2SO_4 and the pH was adjusted to two. The sample was methylated by the addition of 2.5 ml H_2SO_4 and 50 ml methanol and refluxed for 24 h. The mixture was evaporated; excess of diethyl ether was added and shaken well. The organic phase was evaporated to determine the different fatty acids.

The fatty acid methyl esters were determined by gas chromatography on a HP-5890 (Hewlett Packard, Little Falls, DE) equipped with a flame ionization detector. A HP-FFAP (free fatty acid phase) column (25 m, 0.3 mm diameter) was used with helium (35 cm s^{-1}) as a carrier gas. The injector temperature was 250°C, detector temperature was 260°C, and the temperature program during the analysis went from 50 to 240°C (7°C min^{-1}), after which temperature was kept constant 240°C for 30 min.

Determination of PM Phospholipid classes

Phospholipids (polar lipids) were assayed according to Deinstrop and Weinheim (2000). The lipid extract was spotted along a glass thin layer chromatography plate. Phospholipid classes were separated by two-dimensional TLC with solvent mixtures of chloroform: methanol: deionized bidistilled water (75:25:2.5, v/v) in the first direction. After allowing sufficient time for drying, the plate was developed at right angles to the first development in chloroform:methanol:acetic acid:water (80:9:12:2, v/v).

After phospholipid separation, spots were located by exposure to I2 vapor. Individual phospho-lipids were identified by co-chromatography with authentic standards. The area on TLC corresponding with each individual phospholipids was marked and assayed according to Ames (1966).

Determination of PM total glycolipids

The total glycolipids were determined by the anthrone reaction using glucose as the standard according to Mansour et al. (1994). To dried lipid extract, methanol, distilled water and anthrone reagent was added, waterbathed and measured spectroscopy at 620 nm.

Determination of PM total sterols

Total sterols were determined according to Naudet and Hautfenne (1985) with cholestrol as the standard. Glacial acetic acid and sulfuric acid were added to the lipid extract, left in the dark for 10 min and measured by spectroscopy at 415 nm.

Statistics

Analysis of variance was performed using statistical analysis system version 9.1 (SAS). The mean contents at each time were compared by t tests using the least significant

difference method, considering $P<0.05$ as statistically different.

RESULTS

NaCl significantly decreased the total sterols of Elite, Fornax and Licord cultivars, whereas it increased the total sterols of SLM046 and Okapi (Table 1). The PM total protein and PM glycolipids showed a non-significant decrease in both cultivars (Table 1). Total phospholipids of PM in canola roots were significantly increased in Elite, Licord and Fornax in response to NaCl stress. Salt stress decreased the ratio of glycolipid/ phospholipid of PM in Elite, Licord and Fornax cultivars, whereas this ratio was increased in SLM046 and Okapi. The PM sterol/phospholipid ratio was decreased in Elite and Licord, but it was increased in SLM046 and Okapi. The most abundant phospholipids in the Elite, Fornax and Licord PM of control plants were phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS), respectively, which when combined made up 59% of the total phospholipids.

Table 1. Effect of salinity levels on the PM total protein, total glycolipids, total sterols, total phospholipids, sterol/phospholipid ratio and glycolipid/phospholipid ratio of canola cultivars

Lipid/Protein	Salinity levels, mM				
	0	50	100	150	200
			Elite cultivar		
Proteins (mg dl^{-2})	3.35 ± 0.88	2.28 ± 0.89	2.18 ± 0.70	1.98 ± 0.50	1.90 ± 0.4
Sterols (nmol g^{-1})	38.5 ± 7.77	30.2 ± 7.12	20.10 ± 6.26	19.2 ± 6.11	19.1 ± 5.96
Glycolipids (nmol g^{-1})	84.3 ± 3.33	65.5 ± 3.12	60.4 ± 2.96	58.3 ± 2.88	57.2 ± 2.80
Phospholipids (nmol g^{-1})	81.5 ± 8.10	80.5 ± 18.9	200.4 ± 2.22	209.4 ± 23.2	209.8 ± 23.8
Sterol/phospholipids	0.48	0.17	0.10	0.09	0.09
Glycolipids/phospholipids	1.03	0.36	0.30	0.27	0.27
			Formax cultivar		
Proteins (mg dl^{-2})	4.18 ± 2.22	3.82 ± 2.18	3.12 ± 1.96	2.86 ± 1.88	2.22 ± 1.77
Sterols (nmol g^{-1})	40.8 ± 4.46	48.9 ± 4.55	52.8 ± 5.12	53.4 ± 5.22	54.6 ± 5.38
Glycolipids (nmol g^{-1})	100.6 ± 8.18	84.6 ± 7.60	82.5 ± 7.18	81.1 ± 7.08	76.4 ± 7.96
Phospholipids (nmol g^{-1})	130.5 ± 11.12	140.6 ± 12.3	150.2 ± 13.18	155.8 ± 14.18	159.6 ± 14.18
Sterol/phospholipids	0.32	0.34	0.35	0.34	0.34
Glycolipids/phospholipids	0.77	0.60	0.54	0.52	0.47
			Licord cultivar		
Proteins (mg dl^{-2})	5.8 ± 2.22	3.89 ± 1.88	2.99 ± 1.77	2.22 ± 1.66	1.98 ± 1.22
Sterols (nmol g^{-1})	39.8 ± 4.48	32.6 ± 4.38	28.2 ± 3.96	25.4 ± 3.77	20.8 ± 3.32
Glycolipids (nmol g^{-1})	85.8 ± 7.76	65.4 ± 7.18	62.4 ± 6.96	60.4 ± 6.61	58.9 ± 6.14
Phospholipids (nmol g^{-1})	82.3 ± 7.66	160.4 ± 18.2	180.9 ± 18.22	199.6 ± 19.22	202.8 ± 19.36
Sterol/phospholipids	0.48	0.20	0.16	0.13	0.10
Glycolipids/phospholipids	1.04	0.40	0.34	0.30	0.29
			Okapi cultivar		
Proteins (mg dl^{-2})	5.9 ± 1.88	4.8 ± 1.70	4.4 ± 1.66	4.1 ± 1.60	3.2 ± 1.52
Sterols (nmol g^{-1})	137.2 ± 17.70	146.2 ± 17.22	150.2 ± 18.2	152.4 ± 18.90	155.5 ± 18.99
Glycolipids (nmol g^{-1})	122.5 ± 14.20	85.4 ± 8.22	84.4 ± 8.12	82.2 ± 8.22	80.3 ± 8.10
Phospholipids (nmol g^{-1})	141.2 ± 18.20	92.4 ± 14.40	91.2 ± 13.88	89.2 ± 13.77	87.4 ± 13.28
Sterol/phospholipids	097	1.58	1.64	1.77	0.96
Glycolipids/phospholipids	0.87	0.92	0.93	0.93	
			SLM_{046} cultivar		
Proteins (mg dl^{-2})	6.6 ± 2.22	5.9 ± 1.89	4.9 ± 1.88	4.4 ± 1.76	4.1 ± 1.66
Sterols (nmol g^{-1})	140.2 ± 16.22	149.6 ± 17.22	152.6 ± 17.70	157.2 ± 18.8	158.4 ± 18.9
Glycolipids (nmol g^{-1})	128.4 ± 14.18	88.6 ± 9.87	86.7 ± 8.87	81.4 ± 8.21	79.2 ± 7.96
Phospholipids (nmol g^{-1})	145.4 ± 18.2	99.4 ± 9.99	90.2 ± 9.17	87.3 ± 8.99	86.2 ± 8.81
Sterol/phospholipids	0.96	1.50	1.69	1.77	1.77
Glycolipids/phospholipids	0.88	0.89	0.96	1.80	1.83

Each value is the mean ± SD of two replications.

After salt stress, the dominant phospholipids became PG, PS, phosphatidylinositol (PI), and PC, respectively, which when combined made up 65% of the total phospholipids (Table 2).

Table 2. Effect of salinity levels on the PM Phospholipid composition (nmol%), PC/PE ratio and PG/PE ratio of canola cultivars

Phospholipid	Salinity levels, mM				
	0	50	100	150	200
Elite cultivar					
PA	5.5 ± 1.52	5.2 ± 1.38	5.1 ± 1.28	4.9 ± 1.22	4.8 ± 1.20
PS	12.5 ± 3.85	14.5 ± 3.66	14.8 ± 3.69	15.9 ± 3.66	16.2 ± 3.65
PI	11.2 ± 3.36	13.8 ± 3.35	14.8 ± 3.38	16.2 ± 3.33	17.7 ± 3.31
PG	20.2 ± 4.42	19.1 ± 4.38	18.8 ± 4.33	18.5 ± 4.30	18.1 ± 4.28
PC	19.5 ± 3.77	18.4 ± 3.71	18.2 ± 3.70	18.1 ± 3.60	17.9 ± 3.59
PE	12.2 ± 3.12	10.6 ± 2.98	10.1 ± 2.88	9.9 ± 2.80	9.6 ± 2.76
DPG	18.9 ± 3.46	18.4 ± 3.42	18.2 ± 3.39	16.5 ± 3.32	15.7 ± 3.30
PC/PE	1.60	1.73	1.80	1.82	1.86
PG/PE	1.65	1.80	1.86	1.86	1.87
Formax cultivar					
PA	5.2 ± 1.18	5.0 ± 1.09	4.9 ± 1.02	4.8 ± 1.04	4.6 ± 1.02
PS	12.2 ± 3.60	13.8 ± 3.66	14.4 ± 3.70	15.8 ± 3.66	16.6 ± 3.52
PI	11.0 ± 2.88	12.9 ± 3.51	16.4 ± 3.96	18.5 ± 4.12	20.8 ± 4.42
PG	20.1 ± 4.38	19.2 ± 4.12	18.1 ± 3.98	17.2 ± 4.11	16.2 ± 3.88
PC	19.2 ± 3.71	18.1 ± 3.66	17.2 ± 3.59	15.9 ± 3.88	15.8 ± 3.76
PE	12.1 ± 3.68	11.1 ± 3.21	10.2 ± 2.88	9.9 ± 2.77	9.2 ± 2.70
DPG	20.2 ± 3.77	19.8 ± 3.33	18.8 ± 3.12	17.6 ± 3.02	16.8 ± 2.89
PC/PE	1.58	1.61	1.68	1.60	1.71
PG/PE	1.66	1.71	1.77	1.73	1.76
Licord cultivar					
PA	4.9 ± 1.01	4.7 ± 1.02	4.5 ± 1.08	4.2 ± 1.02	3.8
PS	11.9 ± 2.88	12.8 ± 2.89	13.6 ± 2.99	14.2 ± 3.13	15.8
PI	10.8 ± 2.72	13.5 ± 3.13	16.5 ± 3.66	18.9 ± 3.66	20.2 ± 3.88
PG	20.2 ± 4.32	18.6 ± 3.86	17.6 ± 3.59	17.2 ± 3.46	16.1 ± 3.77
PC	18.2 ± 3.41	17.6 ± 3.66	16.9 ± 3.60	15.8 ± 3.28	14.9 ± 3.18
PE	11.2 ± 3.19	10.6 ± 3.60	10.1 ± 3.11	9.9 ± 1.21	9.4 ± 1.20
DPG	22.5 ± 3.88	22.2 ± 3.80	20.7 ± 3.86	19.9 ± 3.38	19.7 ± 3.66
PC/PE	1.54	1.66	1.68	1.6	1.58
PG/PE	1.80	1.75	1.74	1.73	1.71
Okapi cultivar					
PA	6.6 ± 1.18	5.9 ± 1.15	5.5 ± 1.12	5.2 ± 1.11	5.1 ± 1.09
PS	16.6 ± 3.18	15.4 ± 3.02	15.1 ± 3.12	14.6 ± 3.10	12.1 ± 2.87
PI	13.8 ± 3.13	10.8 ± 2.98	9.9 ± 1.99	8.4 ± 1.90	8.1 ± 1.87
PG	24.9 ± 3.88	29.6 ± 4.41	39.6 ± 4.98	46.6 ± 5.28	51.9 ± 5.66
PC	22.6 ± 3.66	17.6 ± 3.38	12.4 ± 3.28	9.6 ± 2.96	8.2 ± 2.88
PE	16.6 ± 3.12	14.2 ± 3.41	13.1 ± 3.32	12.1 ± 2.98	11.4 ± 2.66
DPG	8.9	6.5	4.4	3.5	5.9
PC/PE	1.36	1.23	0.79	0.78	0.71
PG/PE	1.5	2.08	3.02	3.85	4.55
SLM_{046} cultivar					
PA	7.6 ± 2.22	7.2 ± 2.18	6.6 ± 2.11	6.22.02	5.6 ± 1.77
PS	17.2 ± 3.13	16.2 ± 2.99	15.5 ± 2.72	14.8 ± 2.70	12.9 ± 2.46
PI	14.1 ± 2.77	11.2 ± 2.18	10.6 ± 2.10	9.2 ± 2.09	8.9 ± 1.96
PG	25.2 ± 3.87	26.1 ± 3.89	36.2 ± 4.48	41.9 ± 4.96	4.7 ± 5.18
PC	22.8 ± 3.26	19.9 ± 3.14	14.4 ± 3.03	12.4 ± 2.87	10.1 ± 1.77
PE	17.2 ± 3.11	15.9 ± 2.89	14.2 ± 2.77	14.1 ± 2.76	12.2 ± 1.66
DPG	5.9	3.5	2.5	2.2	1.9
PC/PE	1.32	1.25	1.01	0.93	0.82
PG/PE	4.3	1.64	2.54	2.97	3.85

The most dominant phospholipid species in the control PM of SLM046 and Okapi were PC, PE, PS and PG, whereas those in 150 and 200 mM NaCl PM were PG, PE, PS and diphosphatidylglycerol (DPG). Except PI, increasing salinity levels had no significant effect on the content of the other phospholipid classes of Elite, Licord and Fornax PM. In SLM046 and Okapi, PG was increased while other phospholipid species (PS, PI, PC and PE) were decreased on 150 and 200 mM NaCl. PG/PE ratio in Elite and Okapi increased and decreased in Licord.

The saturated fatty acids with chain length 16, 17, 18 and 20 accounted for 70% (Elite), 68.7% (Fornax), 69% (Licord), 77.7% (Okapi) and 80.5% (SLM046), whereas the unsaturated fatty acids with one (18:1) and two (18:2) double bonds contributed to 30.5% (Elite), 31% (Fornax), 22.3% (Licord), 19.5% (Okapi) and 38% (SLM_{046}) in 200 mM NaCl level. Increasing salinity levels changed the distribution of fatty acids and thus decreased the ratio of the unsaturated/ saturated in all cultivars. The predominant fatty acids in root PM of Elite at salinity levels of 150 and 200 mM NaCl were C17:0 and C18:1. However, the abundant fatty acids of the PM in SLM_{046} at salinity levels of 150 and 200 mM NaCl were C17:0 and C20:0 respectively (Table 3).

DISCUSSION

Since PM is the primary cell structure that encounters surrounding salinity, determining how salinity affects its architecture/ composition will give insight into its implication on salt tolerance mechanism in plants. It has been shown that PM lipid composition was correlated with salt tolerance in non-halophytes and halophytes (Blits and Gallagher, 1990; Wu et al., 2005; Kerkeb et al., 2001; Goncalo et al., 2003). We found that addition of more than 150 mM NaCl to the growth medium for 15 days affected differently the PM lipid and protein content and composition of the five canola cultivars contrasting in their response to salinity. Reports indicate that Cl-toxicity is the main cause for salt sensitivity (Cramer et al., 1994; Mansour et al., 2005). Salt-induced PM lipid changes most likely correlated with Cl- accumulation in sensitive cultivars (Kuiper, 1984; Racagni et al., 2003).

The increase of total sterols in the PM of SLM_{046} and Okapi cultivars as a result of increasing salinity might have a significant value for salt tolerance of these cultivars, as it was suggested in other species (Blits and Gallagher, 1990; Wu et al., 1998; Kerkeb et al., 2001). Free sterols are of great importance during salt stress because they can regulate membrane enzyme activities (Sandstorm and Cleland, 1989; Ros et al., 1990), membrane permeability and fluidity (Cramer et al., 1985; Simon, 1974) and hence affect membrane ion absorption (Kuiper, 1984; Russel, 1989). However, the putative role of free sterols in salt adaptation is argued (Mansour and Salama, 2004). The effect of increasing salinity levels on the total PM phospholipids observed in this study was reported in previous studies (Wu et al., 2005; Mansour et al., 2002).

No significant change in the PM total glycolipids and proteins was found in studied cultivars. Mansour et al. (1994) found that salt stress did not affect the level of glycolipids of the root PM in wheat. The salt-induced increase in sterol/phospholipid ratio in the PM of SLM_{046} and Okapi cultivars is consistent with the finding of Kerkeb et al. (2001). The increase in the sterol/phospholipid ratio of the PM has been interpreted to correlate with plant salt adaptation (Kuiper, 1985). The increase in the PM glycolipid/phospholipid ratio, as found by us in cultivars SLM_{046} and Okapi, was previously found in cowpea and related to its halo-adaptation, as the greater this ratio is, the lower the membrane ion permeability (Vazquez-Duhalt et al., 1991). The relative compositional change in SLM046 and Okapi cultivars PM phospholipids might maintain the PM integrity and functions in saline conditions. This could be explained by the fact that some classes of phospholipids (e.g. PE, PA) are non-lamellar formers (Melchoir, 1982) whereas others (e.g., PG, PC) are lamellar formers (Gange et al., 1985). PG/PE ratio was decreased in Licord cultivar whereas it was increased in Okapi by

increasing salinity levels. This is in agreement with other reports in oat (Norberg and Liljenberg, 1991) and wheat (Mansour et al., 2002). Although PC tends to from bilayer configuration, it was decreased in the PM of SLM046 and Okapi cultivars by increasing salinity levels.

It appears that effect of decreased PC on the PM stability was overcome by the increased PG obtained in tolerant cultivars under salinity. PI was increased significantly in Elite, Licord and Fornax by increasing salinity levels, which might correlate with salt sensitivity.

Table 3. Effect of salinity levels on the PM fatty acid composition (mol%) and unsaturated/saturated ratio of canola cultivars

Fatty acid	Salinity levels, mM				
	0	50	100	150	200
			Elite cultivar		
$C_{16:0}$	19.5 ± 0.33	12.4 ± 0.22	11.2 ± 0.20	10.2 ± 0.18	9.1 ± 0.15
$C_{17:0}$	5.9 ± 0.18	19.2 ± 2.28	25.1 ± 2.31	27.8 ± 0.47	30.3 ± 2.66
$C_{18:0}$	21.5 ± 0.38	18.2 ± 1.96	12.1 ± 1.18	10.2 ± 1.08	8.2 ± 1.02
$C_{18:1}$	27.5 ± 0.41	25.9 ± 2.96	25.2 ± 2.90	25.1 ± 2.89	25.0 ± 2.79
$C_{18:2}$	7.2 ± 0.19	6.1 ± 0.16	5.9 ± 0.15	5.8 ± 0.14	5.5 ± 0.13
$C_{20:0}$	18.4 ± 0.29	18.2 ± 0.28	20.5 ± 0.38	20.9 ± 0.38	21.9 ± 0.45
Unsaturated/saturated	0.55	0.52	0.50	0.48	0.46
			Formax cultivar		
$C_{16:0}$	19.1 ± 2.36	13.6 ± 1.96	12.4 ± 1.77	11.2 ± 1.55	10.8 ± 1.41
$C_{17:0}$	7.1 ± 0.33	16.0 ± 2.12	23.4 ± 2.66	24.3 ± 2.77	27.2 ± 2.90
$C_{18:0}$	21.8 ± 0.41	18.9 ± 2.28	12.6 ± 1.99	12.4 ± 1.80	8.9 ± 1.10
$C_{18:1}$	27.6 ± 2.99	26.6 ± 2.89	26.2 ± 2.88	26.1 ± 2.80	25.9 ± 2.70
$C_{18:2}$	6.6 ± 0.22	6.4	6.3	6.2	6.0 ± 0.22
$C_{20:0}$	17.8 ± 0.39	18.5 ± 0.45	19.1 ± 0.49	20.8 ± 0.60	21.8 ± 0.71
Unsaturated/saturated	0.54	0.51	0.48	0.46	0.45
			Licord cultivar		
$C_{16:0}$	19.5 ± 1.48	14.8 ± 1.28	13.6 ± 1.33	12.6 ± 1.26	11.4 ± 1.20
$C_{17:0}$	6.8 ± 0.48	15.4 ± 1.66	22.3 ± 3.33	22.7 ± 3.28	28.3 ± 4.11
$C_{18:0}$	22.4 ± 3.36	19.2 ± 3.33	13.2 ± 2.88	12.2 ± 2.77	7.4 ± 1.31
$C_{18:1}$	27.3 ± 3.96	26.1 ± 3.99	25.9 ± 3.88	25.8 ± 3.79	25.5 ± 3.66
$C_{18:2}$	6.2 ± 0.19	5.9 ± 0.22	5.8 ± 0.23	5.8 ± 0.28	5.5 ± 0.22
$C_{20:0}$	17.6 ± 0.66	18.6 ± 0.60	19.2 ± 0.89	20.9 ± 0.90	21.9 ± 0.91
Unsaturated/saturated	0.54	0.52	0.50	0.46	0.44
			Okapi cultivar		
$C_{16:0}$	7.2 ± 0.22	8.8 ± 0.28	9.1 ± 0.30	9.9 ± 0.33	10.8 ± 0.34
$C_{17:0}$	26.7 ± 3.33	20.2 ± 2.96	19.1 ± 2.89	18.6 ± 2.80	17.2 ± 2.77
$C_{18:0}$	5.2 ± 0.18	3.2 ± 0.14	3.1 ± 0.13	2.9 ± 0.12	2.6 ± 0.11
$C_{18:1}$	23.5 ± 3.38	14.1 ± 2.18	13.8 ± 2.10	13.6 ± 1.99	13.5 ± 1.89
$C_{18:2}$	14.2 ± 2.88	10.2 ± 2.14	9.9 ± 2.10	9.2 ± 1.89	8.8 ± 1.66
$C_{20:0}$	23.2 ± 3.96	43.5 ± 6.66	45 ± 6.60	45.8 ± 6.56	47.1 ± 6.46
Unsaturated/saturated	0.60	0.48	0.36	0.30	0.28
			SLM_{046} cultivar		
$C_{16:0}$	7.7 ± 0.22	8.9 ± 0.28	10.0 ± 0.42	11.2 ± 0.39	13.1 ± 0.40
$C_{17:0}$	26.2 ± 3.22	21.1 ± 2.96	20.0 ± 2.88	18.2 ± 2.39	19.2 ± 2.46
$C_{18:0}$	5.5 ± 0.12	3.8 ± 0.10	3.3 ± 0.11	3.1 ± 0.12	2.3 ± 0.11
$C_{18:1}$	23.8 ± 3.28	14.2 ± 6.66	13.3 ± 5.66	12.1 ± 5.41	11.1 ± 5.39
$C_{18:2}$	14.5 ± 1.96	10.5 ± 1.14	9.6 ± 1.11	9.5 ± 1.09	8.4 ± 0.96
$C_{20:0}$	22.3 ± 2.28	41.5 ± 8.82	43.8 ± 8.49	45.9 ± 8.66	45.9 ± 8.55
Unsaturated/saturated	0.65	0.40	0.28	0.25	0.22

Each value is the mean ± SD of two replications.

This interpretation is supported by the results of Racagni et al. (2003) who found an increase of PI in sensitive tomato in saline conditions. The molar percentage of saturated (C16, C17, C18, C20) and unsaturated (C18.1, C18.2) fatty acids were different at different salinity levels. This may suggest that cultivars already have different abundance of PM fatty acids, which further changed differently in saline conditions. In addition, relative compositional changes in fatty acids induced by NaCl resulted in decreased unsaturated/ saturated ratio, more so in tolerant cultivar. Similar reduction in this ratio has been reported by Mansour et al. (1994) Wu et al. (1998, 2005) and Mansour and Salama (2004).

It has been reported that chain length and saturation of fatty acids affect bilayer thickness and fluidity, which regulate membrane different functions (Kuiper, 1984; Rochester et al., 1987; Russell, 1989; Neffati and Marzouk, 2008). Increasing salinity levels reduced the PM fluidity of the halophyte Spartina patens (Wu et al., 2005). Greater reduction in the PM unsaturation of SLM_{046} and Okapi may be, therefore, related to salt adaptation, as suggested by several reports that a less fluid membrane may reduce Na^+ and CL^- permeability (Mansour and Salama, 2004; Francois and Kleiman, 1990).

In conclusion, PM composition was already significantly different among the salt tolerant and sensitive cultivars in absence of salt exposure. In addition, the same salt level resulted in different response of PM lipids and protein patterns in the five cultivars with different degrees of salt tolerance. The changes in lipid composition under NaCl stress appear to be in the favorable direction to maintain membrane stability, which may be of special importance in the adaptation of cultivars SLM_{046} and Okapi to salinity. Changes in PM proteins may also have a role in the SLM_{046} and Okapi adaptation to salt.

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