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ORIGINAL ARTICLE

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, i.e., (SLM_{046}, Okapi, Licord, Fornax and Elite) were grown hydroponically to study effect of NaCl on root plasma membrane (PM) Lipid and protein alterations. The PM total sterols of Elite, Fornax and Licord were decreased while that of SLM_{046} and Okapi was increased in response to salt. Salt stress had no significant effect on PM total glycolipids and protein of both cultivars. The PM total phospholipids were increased in Elite, Licord and Fornax but it did not change significantly in SLM_{046} 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 (150 and 200 mM NaCl) PM of both cultivars was increased. The predominant fatty acid in (150 and 200 mM NaCl) of Elite was C17:0 and C18:1, respectively. However, in NaCl treated (150 and 200 mM) SLM_{046}, the predominant fatty acid was C20:0 and C17:0, respectively.

Key words: Plasma membrane lipids, Canola, Salinity stress

Introduction

Salinity is the 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 (Xu and Beardall, 1977). The plasma membrane (PM) might be the primary site of salinity injury[20,22]. Salt stress induces alteration in the PM permeability, which varying greatly between cultivars differing in salt tolerance[21,20]. PM permeability measurement probes alterations in PM lipid composition[41,35].

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[31,18]. In addition, some of the membrane lipids affect as lipid mediators, such as phosphatidic acid, free fatty acids and lysophospholipids, which are involved in signal transduction, vesicular trafficking and cytoskeletal reorganization in plant response to stresses[43,40,18]. Molecular changes in PM lipids of tolerant plants are supposed to enhance membrane integrity and this cellular functions under salinity[20,22,47]. Thus, investigating the response of PM lipids in various plants contrasting in their response to saline environment will help clearly to understand the PM correlation with plant salt tolerance mechanism. Alteration in PM proteins are reported in different plant cultivars under saline conditions[26,46,45]. Slight changes in PM proteins were found, however, in genotypes differing in salt sensitivity under saline conditions[13,14]. The objective of the present investigation was to characterize the lipid composition and polypeptide components of root PM

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of five canola cultivars in salinity conditions.

Materials and method

The canola cultivars (SLM_{046}, Okapi, Licord, Fornax and Elite) were obtained from the Seed and Improvement institute, Karaj, Iran. The seeds were kept at 4C were soaked in tap water for 1h 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[8]. The seeds of canola were placed in a dark incubator adjusted at 25C 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 treatment were applied 1/4- 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: 15/9 h photoperiod, 70-75% relative humidity, with day/night temperature of 18-22C, respectively.

Pm isolation:

Two- phase partitioning system Mansour *et al.* [21,22] 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) 250 mM sucrose, 5 mM EGTA, 5mM EDTA, 10 mM KF, 25 mM MOPS, and 1 mM PMSF. 2mM of DTT or 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 Mira cloth. A new homogenization medium was added to the tissue and was homogenized once again. The pooled solution was centrifuged at 10,000g for 20 min. The supernatant containing the PM and other membrane was then centrifuged at 50,000g for 1 h. The microsomal membrane pellet was resuspended in 5 mM potassium phosphate buffer (pH 7.8) containing 250 mM sucrose and 250 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 15000g for 5 min. The microsomal pellet was subjected to three successive phase partitioning steps. The upper phase, containing the PM fraction, centrifuged at 50,000g 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 0-4C. The purity of this PM preparation was based on Mansour *et al.*[23,24].

Pm total protein:

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

Electerphoresis:

SDS–PAGE was carried out using a discontinuous buffer system described by Laemmli [17] 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 85500.7 mm. Electrophoresis separation of the polypeptide's 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 destained in 50% methanol and 10% acetic acid. After destaining, 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[13]. 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 1,000g for 5 min. The upper waters 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.*[25] was used for 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 240C (7C min^{-1}), after which temperature was kept constant 240C 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 phospholipids were identified by co-chromatography with authentic standards. The area on TLC corresponding with each individual phospholipids was marked and assayed according to Ames[1].

Determination of pm total glycolipid:

The total glycolipids were determined by the enthrone reaction using glucose as the standard according to Mansour *et al.*[21]. To dried lipid extract, methanol, distilled water and enthrone reagent was added, water bathed and measured spectroscopy at 620 nm.

Determination of pm total sterols:

Total sterols were determined according to Zhang *et al.* (1969), with cholesterol as the standard. Glacial acetic acid and sulfuric acid were added to the lipid extract, left in the dark for 10 min and measured spectroscopy at 415 nm.

Statistics:

Analysis of variance were done by statistical analysis system version 9.1 (SAS). The mean contents at each time were compared by t tests using the least significant difference method, where $P<0.05$ deemed statistically different.

Results and Discussion

NaCl decreased significantly total sterols of Elite, Fornax and Licord cultivars whereas it increased the total sterols of SLM_{046} and Okapi (Table1). The PM total protein and PM glycolipids showed non-significantly decrease in both cultivars (Table 1). Total phospholipids of PM of canola roots were significantly increased in Elite, Licord and Fornax in response to NaCl stress (Table 1). Salt stress decreased the ratio of glycolipid/phospholipid of PM in Elite, Licord and Fornax cultivars whereas this ratio increased in SLM_{046} and Okapi (Table 1). The PM sterol/phospholipid ratio was decreased in Elite and Licord but it was increased in SLM_{046} and Okapi (Table 1). The most abundant phospholipids in the Elite, Fornax and Licord PM in control plants were phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylserine (PS), respectively, which when combined, made up 59% of the total phospholipids. 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). The most dominant phospholipid species in the control PM of SLM_{046} 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 (Table 2). In SLM_{046} and Okapi, PG increased while other phospholipid species (PS, PI, PC and PE) were decreased on 150 and 200 mM NaCl (Table 2). Except PG/PE ratio in Elite and Okapi increased and PG/PE ratios were 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% (SLM_{046}) 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 both cultivars. The predominant fatty acid in salinity levels (150 and 200 mM NaCl) root PM of Elite was C17:0 and C18:1.

However, the PM abundant fatty acid of SLM_{046} in salinity levels (150 and 200 mM NaCl) was C17:0 and C20:0, respectively (Table 3).

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

Lipid/Protein	SLM_{046}				
	0 mM	50 mM	100mM	150 mM	200 mM
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

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

Phospholipid	Elite					Fornax				
	0 mM	50 mM	100mM	150 mM	200 mM	0 mM	50 mM	100 mM	150 mM	200 mM
PA	5.5±1.52	5.2±1.38	5.1±1.28	4.9±1.22	4.8±1.20	5.21.18	5.0±1.09	4.9±1.02	4.8±1.04	4.6±1.02
PS	12.5±3.85	14.5±3.66	14.8±3.69	15.9±3.66	16.2±3.65	12.23.60	13.8±3.66	14.4±3.70	15.8±3.66	16.6±3.52
PI	11.2±3.36	13.8±3.35	14.8±3.38	16.2±3.33	17.7±3.31	11.02.88	12.9±3.51	16.4±3.96	18.5±4.12	20.8±4.42
PG	20.2±4.42	19.1±4.38	18.8±4.33	18.5±4.30	18.1±4.28	20.14.38	19.2±4.12	18.1±3.98	17.2±4.11	16.2±3.88
PC	19.5±3.77	18.4±3.71	18.2±3.70	18.1±3.60	17.9±3.59	19.23.71	18.1±3.66	17.2±3.59	15.9±3.88	15.8±3.76
PE	12.2±3.12	10.6±2.98	10.1±2.88	9.9±2.80	9.6±2.76	12.13.68	11.1±3.21	10.2±2.88	9.9±2.77	9.22.70
DPG	18.9±3.46	18.4±3.42	18.2±3.39	16.5±3.32	15.7±3.30	20.23.77	19.8±3.33	18.8±3.12	17.6±3.02	16.8±2.89
PC/PE	1.60	1.73	1.80	1.82	1.86	1.58	1.61	1.68	1.60	1.71
PG/PE	1.65	1.80	1.86	1.86	1.87	1.66	1.71	1.77	1.73	1.76
Phospholipid	Licord					Okapi				
	0 mM	50 mM	100mM	150 mM	200 mM	0 mM	50 mM	100 mM	150 mM	200 mM
PA	4.9±1.01	4.7±1.02	4.5±1.08	4.2±1.02	3.8	6.6±1.18	5.9±1.15	5.5±1.12	5.2±1.11	5.1±1.09
PS	11.9±2.88	12.8±2.89	13.6±2.99	14.2±3.13	15.8	16.6±3.18	15.4±3.02	15.1±3.12	14.6±3.10	12.1±2.87
PI	10.8±2.72	13.5±3.13	16.5±3.66	18.9±3.66	20.2±3.88	13.8±3.13	10.8±2.98	9.9±1.99	8.4±1.90	8.1±1.87
PG	20.2±4.32	18.6±3.86	17.6±3.59	17.2±3.46	16.1±3.77	24.9±3.88	29.6±4.41	39.6±4.98	46.6±5.28	51.9±5.66
PC	18.2±3.41	17.6±3.66	16.9±3.60	15.8±3.28	14.9±3.18	22.6±3.66	17.6±3.38	12.4±3.28	9.6±2.96	8.2±2.88
PE	11.2±3.19	10.6±3.60	10.1±3.11	9.9±1.21	9.4±1.20	16.6±3.12	14.2±3.41	13.1±3.32	12.1±2.98	11.4±2.66
DPG	22.5±3.88	22.2±3.80	20.7±3.86	19.9±3.38	19.7±3.66	8.9	6.5	4.4	3.5	5.9
PC/PE	1.54	1.66	1.68	1.6	1.58	1.36	1.23	0.79	0.78	0.71
PG/PE	1.80	1.75	1.74	1.73	1.71	1.5	2.08	3.02	3.85	4.55

Phospholipid	SLM_{046}				
	0 mM	50 mM	100mM	150 mM	200 mM
PA	7.6±2.22	7.2±2.18	6.6±2.11	6.2±2.02	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

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

Fatty acid	Elite					Fornax				
	0 mM	50 mM	100mM	150 mM	200 mM	0 mM	50 mM	100 mM	150 mM	200 mM
$C_{16:0}$	19.5±0.33	12.4±0.22	11.2±0.20	10.2±0.18	9.1±0.15	19.1±2.36	13.6±1.96	12.4±1.77	11.2±1.55	10.8±1.41
$C_{17:0}$	5.9±0.18	19.2±2.28	25.1±2.31	27.8±0.47	30.3±2.66	7.1±0.33	16.0±2.12	23.4±2.66	24.3±2.77	27.2±2.90
$C_{18:0}$	21.5±0.38	18.2±1.96	12.1±1.18	10.2±1.08	8.2±1.02	21.8±0.41	18.9±2.28	12.6±1.99	12.4±1.80	8.9±1.10
$C_{18:1}$	27.5±0.41	25.9±2.96	25.2±2.90	25.1±2.89	25.0±2.79	27.6±2.99	26.6±2.89	26.2±2.88	26.1±2.80	25.9±2.70
$C_{18:2}$	7.2±0.19	6.1±0.16	5.9±0.15	5.8±0.14	5.5±0.13	6.6±0.22	6.4	6.3	6.2	6.0±0.22
$C_{20:0}$	18.4±0.29	18.2±0.28	20.5±0.38	20.9±0.38	21.9±0.45	17.8±0.39	18.5±0.45	19.1±0.49	20.8±0.60	21.8±0.71
Unsaturated/saturated	0.55	0.52	0.50	0.48	0.46	0.54	0.51	0.48	0.46	0.45
Fatty acid	Licord					Okapi				
	0 mM	50 mM	100mM	150 mM	200 mM	0 mM	50 mM	100 mM	150 mM	200 mM
$C_{16:0}$	19.5±1.48	14.8±1.28	13.6±1.33	12.6±1.26	11.4±1.20	7.2±0.22	8.8±0.28	9.1±0.30	9.9±0.33	10.8±0.34

$C_{17:0}$	6.8±0.48	15.4±1.66	22.3±3.33	22.7±3.28	28.3±4.11	26.7±3.33	20.2±2.96	19.1±2.89	18.6±2.80	17.2±2.77
$C_{18:0}$	22.4±3.36	19.2±3.33	13.2±2.88	12.2±2.77	7.4±1.31	5.2±0.18	3.2±0.14	3.1±0.13	2.9±0.12	2.6±0.11
$C_{18:1}$	27.3±3.96	26.1±3.99	25.9±3.88	25.8±3.79	25.5±3.66	23.5±3.38	14.1±2.18	13.8±2.10	13.6±1.99	13.5±1.89
$C_{18:2}$	6.2±0.19	5.9±0.22	5.8±0.23	5.8±0.28	5.5±0.22	14.2±2.88	10.2±2.14	9.9±2.10	9.2±1.89	8.8±1.66
$C_{20:0}$	17.6±0.66	18.6±0.60	19.2±0.89	20.9±0.90	21.9±0.91	23.2±3.96	43.5±6.66	45±6.60	45.8±6.56	47.1±6.46
Unsaturated/saturated	0.54	0.52	0.50	0.46	0.44	0.60	0.48	0.36	0.30	0.28

Table 3: Continue

Fatty acid	SLM_{046}				
	0 mM	50 mM	100mM	150 mM	200 mM
$C_{16:0}$	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.

Discussion:

Since PM is the primary cell structure that encounter 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[3.45,14,11]. We found that addition 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[6,27]. Salt induced PM lipid changes most likely correlated with Cl^- accumulation in sensitive cultivars[15,32]. Increasing total sterols of the PM of SLM_{046} and Okapi cultivars by increasing salinity might have a significant value for salt tolerance of this cultivars, as it was suggested in other species[3,44,14]. Free sterols are of great importance during salt stress because they can regulate membrane enzyme activities[36,34] membrane permeability and fluidity[5,37] and hence affecting membrane ion absorption[15,35]. However, the putative role of free sterols in salt adaptation is argued[22]. Increasing salinity levels effect on the total PM phospholipids observed in this study has been reported in previous studies[45,25].

No significant change in the PM total glycolipids and proteins was found in both cultivars. Mansour *et al*.[23] found that salt stress did not affect the level of glycolipids in the root PM of wheat. The salt-induced increase in sterol/phospholipid ratio of SLM_{046} and Okapi PM is consistent with the finding of Kerkeb *et al*.[14]. Increase in the sterol/phospholipid ratio of PM has been interpreted to correlate with plant salt adaptation[16]. Increase in SLM_{046} and Okapi PM glycolipid/phospholipid ratio was previously found in cowpea and related to its haloadaptation, as the greater this ratio is the lower the membrane ion permeability[42]. The relative compositional change in SLM_{046} 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 [28] whereas others (e.g., PG, PC) are lamellar formers[10]. PG/PE ratio was decreased in Licord cultivar whereas it was increased in Okapi by increasing salinity levels. Which is in agreement with other reports in oat (Norberg and lijenberg, 1991) and wheat[25]. Although PC tends to form bilayer configuration, it was decreased in the PM of SLM_{046} 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. This interpretation is supported by the of Racagni *et al*.[32] who found an increase in PI in sensitive tomato in saline condition. The molar percentage of saturated (C16, C17, C18, C20) and unsaturated (C18.1, C18.2) fatty acids were different in salinity levels. This may suggest that both 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*.[21], WU *et al*. [44], Mansour and salama[22] and Wu *et al*.[45].

It has been reported that chain length and saturation of fatty acids affect bilayer thickness and fluidity, which regulate membrane different functions [15,33,35,29]. Increasing salinity levels reduced the PM fluidity of the halophyte *Spartina patens*[45] 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 [4,22,9].

In conclusion, PM composition was already significant 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 special importance in the adaptation of SLM_{046} and Okapi to salinity. Changes in PM proteins may have a role in SLM_{046} and Okapi adaptation to salt.

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