Genetic markers associated with salt tolerance in canola *(Brassica napus* L.)

(Received: 01.09.2006; Accepted: 17.09.2006)

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ABSTRACT

This study aimed at elucidating the genetic variation between five selected canola cultivars grown under Egyptian environment for salt tolerance. Seedlings from three local and two German cultivars were subjected to salt stress for two weeks. Plant growth, leaf osmotic adjustment, peroxidase isozyme, protein banding patterns and RAPD analyses were performed. Salt stress decreased leaf osmotic potential in all cultivars. The results showed that the cultivar Masrri-L16 can maintain higher osmotic potential of the cells than the other cultivars, leading to enhancement of the ability to tolerate salt stress. Salt stress induces a new peroxidase bands and increases the band intensity, indicating the protective role of peroxidase enzyme. The genetic polymorphism between the cultivars was detected by protein and RAPD analyses. In total three (21.4%) and 78 (52%) polymorphic bands were detected for protein and RAPD, respectively. The comparison between the two protocols revealed that the latter gave more markers and more conclusive results. These molecular markers were sufficient to distinguish among five canola genotypes. The genotype-specific markers represent 12.3% of the total markers detected by both analyses, 94.7 % of them were RAPD markers. Thirteen RAPD markers may be considered as markers for salt tolerance in the cultivar Masrri-L11 and five markers for the cultivar Masrri-L16. These markers can be verified as being RAPD markers associated with salt tolerance in the two canola genotypes that help in marker-assisted selection breeding programs.

Keywords: *Osmotic adjustment, peroxidase, protein markers, RAPD markers, salt tolerance, canola genotypes*

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INTRODUCTION

Canola is considered as one of the most important sources of vegetable oil and protein-rich meal worldwide. Salinity is one of the major causes of loosing agricultural land and reducting crop productivity worldwide. The progressive salinization of soil was estimated to be around 20% of irrigated land (Ghassemi *et al.*, 1995). Since high salt conditions seriously affect growth, developments of salinity tolerant cultivars have been a major objective of most breeding programs (Bohnert and Jensen, 1996).

Genetic variability within a species offers a valuable tool for studying mechanisms of salt tolerance. The analysis of genetic variation and relatedness in germplasm are of great value for genetic resources conservation

and plant breeding programs, to determine the best crosses between different genotypes. Over the years, the methods for assessing genetic diversity have ranged from classical strategies such as morphological analysis to biochemical and molecular techniques (Demissie *et al.*, 1998). Several molecular approaches have been used to identify, diagnose, delimit species and assess phylogenetic relationships between different cultivars. Three molecular methods random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and more recently DNA sequencing, have been the most extensively applied.

The random nature of RAPDs means that they complement isozyme variation, which only reflects differences in protein-coding genes (Williams *et al.*, 1990). Although less reliable than allozymes for the estimation of genetic parameters in populations of out-crossing diploids (Liu and Furnier, 1993), they can detect more polymorphism. Usefulness of RAPDs in *Brassica oleracea* has been demonstrated for variety identification (Hu and Quiros, 1991), gene bank management (Kresovich *et al.*, 1992), taxonomic studies (Demeke *et al.*, 1992) and gene diversity evaluation (Margale *et al.*, 1995). Liu and Furnier (1993) demonstrated that RAPD markers are very useful for discriminating individual genotypes. The utility of these molecular markers for the establishment of genetic relationships among *B. oleracea* and its wild relatives has been demonstrated (Làzaro and Auginagaldc, 1998), and among barely cultivars (Hussein *et al.*, 2005). Hussein *et al.* (1998 and 2000) completed the molecular markers for genome mapping in *B. napus* L.

In the present work, three local and two German canola cultivars were subjected to salt stress. Plant water status and plant growth and molecular genetic markers related to salt tolerance in canola cultivars at the protein, isozyme and randomly amplified polymorphic DNA (RAPD) levels were determined.

MATERIALS AND METHODS

Plant material and culture conditions

The experiment reported here was conducted in the Genetic Engineering Research Center, Faculty of Agriculture- Cairo University, Egypt. Three local canola cultivars namely (Serw-4, Masrri-L11, and Masrri-L16) and two German cultivars namely (Semu-304 and Semu-248) were used. Two seeds were planted in plastic pots (3 L) each containing a mixture of sandy soil and peat moss (1:1 v:v). Seedlings were irrigated daily with 400 ml of Hoagland solution and the soil water tension was maintained ≤ 60 k P_a. Thirty days after planting, the seedlings were subjected to salt stress by the addition of 0, 100, 200 and 300 mM NaCl to the Hoagland solution for 15 days. The temperature was 25°C and the photosynthetically active radiation was 2743 μ mole m^{-2} s^{-1} (photosynthetic active radiation PAR). There were five replications per NaCl treatment and the control (no treatment with NaCl).

Determination of leaf water relations

Leaf samples were frozen in liquid nitrogen, and stored at -20°C. Tissues were thawed and centrifuged at 1,200 xg for 25 min at 4 °C to extract the cell sap. Osmotic potential (ψ_s) of the cell sap was measured using a vapor pressure osmometer (model 5,500, Wescor, Logan, UT, USA). Osmotic adjustment (OA) was calculated as the differences in (ψ_s) between salinized and control plants.

Measurement of plant dry weight

Plants were harvested two weeks after initiation of the salt treatment. The harvested plants were dried at 80°C in an air-forced

draught oven for more than three days, and weighed.

SDS-protein electrophoresis

Protein extraction was performed using two -week- old seedlings. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for total storage proteins according to the method described by Laemmli, (1970).

Isozyme analysis

Isozyme extraction was performed using two -week- old seedlings as well as leaf tissues from NaCl treated plants. Tissue (400 mg) was ground in 2 ml extraction buffer (0.1% (w/v) Tris-citric acid, pH 7.5; 1% (w/v) polyvinyl pyrolidone (PVP); 0.1% (w/v) ascorbic acid and 0.1% (w/v) cysteine) and centrifuged at 5333 xg (JS - 5.2 rotor), at 4 °C for 5 min. Twenty µl of extracted samples were used for electrophoresis on polyacrylamide gel (SDS-PAGE) according to the method of Stegmann *et al.* (1983), using Pharmacia electrophoresis apparatus (GE-4).

Peroxidase detection

Peroxidase was detected by incubating the gel in darkness for one hour at 37°C in a mixture of 15 ml of 10% benzidine (in 95% ethanol); 85 ml of 1mM potassium acetate and 1 ml of 1% H_2O_2 (pH 4.7). After the incubation period, the gel was rinsed in distilled water and fixed in 50% glycerol for one hour. Rf value of each band was calculated as follow:

$$\mathbf{Rf} = \frac{\textbf{Distance traveled by the band from the top of the running gel}}{\textbf{Distance traveled by the tracking dye}}$$

DNA extraction and RAPD analysis

Total genomic DNA was isolated using the method descried in Rogers and Bendich (1985). PCR reactions were conducted using arbitrary 10-mer primers (Operon Technology, Inc., Alameda, CA, USA). The names and sequences of the primers that gave clear bands were as fellows:

Primer	Sequence
OPA-07	5`-.GAAACGGGTG-.3`
OPA-18	5`-.AGGTGACCGT- 3`
OPF-04	5`- GGTGATCAGG- 3`
OPG-12	5`- CAGCTCACGA- 3`
OPK-02	5`-.GTCTCCGCAA- 3`
OPK-04	5`- CCGCCCAAAC- 3`
OPK-10	5`- GTGCAACGTG- 3`
OPK-14	5`- CCCGCTACAC- 3`
OPM-13	5`- GGTGGTCAAG- 3`
OPN-13	5`-.AGCGTCACTC- 3`
OPP-15	5`- GGAAGCCAAC-3`
OPQ-12	5`- AGTAGGGCAC-3`

The reaction mixture (20 µl) contained 10 ng DNA, 200 µM dNTPs, 1 µM primer, 0.5 units of Red Hot Taq polymerase (AB-gene Housse, UK) and 10-X Taq polymerase buffer (AB-gene Housse, UK). Samples were heated to 94°C for 5 min and then subjected to 35 cycles of 1 min at 94°C; 1 min at 35°C and 1 min at 72°C. The amplification products were separated in 1% (w/v) agarose gel in 1 x TBE buffer and visualized by staining with ethidium bromide. Reproducibility of DNA profiles was determined by replicating all

RAPD reactions at least three times. Variations among canola genotypes across the primers were evaluated from pairwise comparisons for the proportion of shared bands amplified (Nei, 1978). The similarity coefficient was calculated by using statistical software package STATISTICA-_SPSS (Stat Soft Inc.).

RESULTS

Salt stress reduces plant biomass production, Fig. (1) indicates that plant dry weight was decreased with increasing NaCl concentration. The cultivar Masrri-L16 showed a relatively higher dry weight followed by cultivar Masrri-L11, while the growth of the cultivars Serw-4 and Semu-249 were significantly affected by salt stress (Fig. 1).

The osmotic potential (ψ_s) of the salt treated plants decreased with increasing NaCl concentrations and the decrease was more pronounced in Masrri-L16. Osmotic adjustment (O.A.) increased with increasing NaCl concentration, and was greater in Masrri-L16 followed by Masrri-L11 and the lowest was in Semu-249 (Table 1).

Fig. (2) and Table (2) show the peroxidase profiles and the RF values of the control and salt treated canola plants. A polymorphic band with the Rf value of about 0.28 could be detected which can distinguish the cultivar Serw-4 from other cultivars used. Salinity stress induced three bands with the Rf values of about 0.28, 0.33 and 0.37 respectively (Table 2). Band intensity of the salt treated plants was higher than the control plants in all cultivars except the salt sensitive cultivar Semu-248 (Lane 5, Fig. 2).

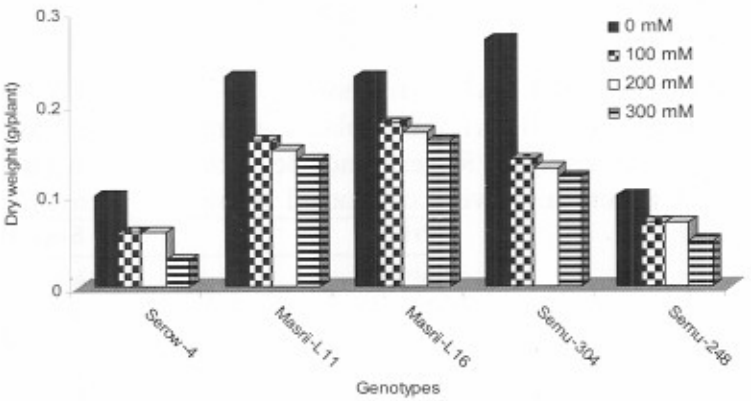

Fig. (1): The effect of salt stress on dry weight in the five canola cultivars.

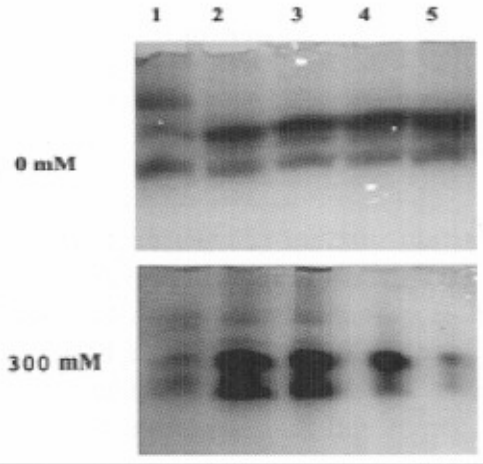

Fig. (2): Peroxidase isozyme profile of the control and salt treated canola plants. Lanes 1-5 are the canola cultivars Serw-4, Masrri-L11, Masrri-L16, Semu-304 and Semu-248, respectively.

Table (1): Osmotic potential (ψ_s) and osmotic adjustment (O.A.) in the five canola cultivars under salinity stress.

Cultivars	NaCl (mM)	Ψs	OA
Serw-4	0	-2.97	
	100	-4.46	1.49
	200	-6.45	3.48
	300	-8.68	5.71
Masrri-L11	0	-3.06	
	100	-4.96	1.9
	200	-6.53	3.47
	300	-8.42	5.36
Masrri-L16	0	-3.11	
	100	-4.21	1.1
	200	-6.21	3.1
	300	-9.54	6.43
Semu-304	0	-3.79	
	100	-5.21	1.42
	200	-6.45	2.66
	300	-8.94	5.15
Semu-248	0	-3.79	
	100	-4.54	0.75
	200	-5.79	2.00
	300	-6.66	2.87

Table (2): RF values for peroxidase isozyme of the control (0 mM NaCl) and salt treated canola plants. Lanes 1-5 are the canola cultivars Serw-4, Masrri-L11, Masrri-L16, Semu-304 and Semu-248, respectively.

Rf	0 mM NaCl					*Rf*	300 mM NaCl				
	1	2	3	4	5		1	2	3	4	5
0.28	+	-	-	-	-	0.28	+	+	+	+	-
0.41	+	+	+	+	+	0.33	+	-	-	-	-
0.62	+	+	+	+	+	0.37	-	+	+	-	-
						0.41	+	+	+	+	+
						0.62	+	+	+	+	+

Protein banding patterns of the five canola genotypes are presented in Fig. (3) and the data scored are in Table (3). SDS-protein patterns exhibited a maximum number of 14 bands, which are not necessarily present in all samples; three of them were polymorphic (21.4%). The protein band with the molecular size of 20.13 KDa is considered as a genotype-specific band for the cultivar Semu-248 (Table 3).

Table (3): SDS-protein patterns in five canola genotypes where (+) means presence and (-) means absence. Broad-range protein marker was used to detect M.W. of the protein bands.

MW (KDa)	Cultivars				
	Serw-4	Masrri-L11	Masrri-L16	Semu-304	Semu-248
106.11	-	+	+	+	+
100.19	+	+	+	+	+
90.42	+	+	+	+	+
56.73	+	+	+	+	+
48.42	+	+	+	+	+
42.81	+	+	+	+	+
37.09	+	+	+	+	+
32.00	-	+	+	+	+
29.90	+	+	+	+	+
26.55	+	+	+	+	+
23.00	+	+	+	+	+
20.13	-	-	-	-	+
17.15	+	+	+	+	+
14.4	+	+	+	+	+

Table (4): Primers used in RAPD analysis and their number of bands and size range.

Primer	Number of scorable bands	Size range of scorable bands (b.p)
OPA-07	10	180-1324
OPA-18	9	200-1881
OPF-04	13	180-1435
OPG-12	17	180-2180
OPK-02	13	130-1658
OPK-04	12	288-1396
OPK-10	15	261-1668
OPK-14	12	244-1848
OPM-13	12	292-1570
OPN-13	13	221-1470
OPP-15	11	185-1762
OPQ-12	11	251-1234
Total	**148**	

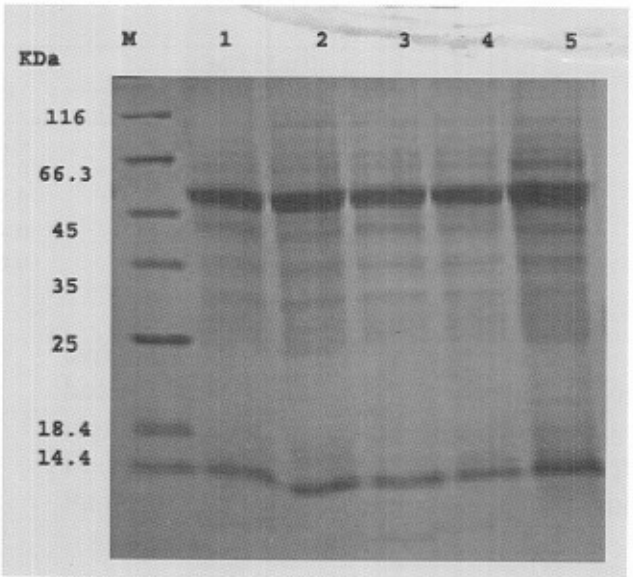

Fig. (3): The SDS-PAGE of total protein extracted from the leaves of five canola genotypes (Lane 1: Protein marker, lanes 2-6 are the canola cultivars Serw-4, Masrri-L11, Masrri-L16, Semu-304 and Semu-248, respectively.

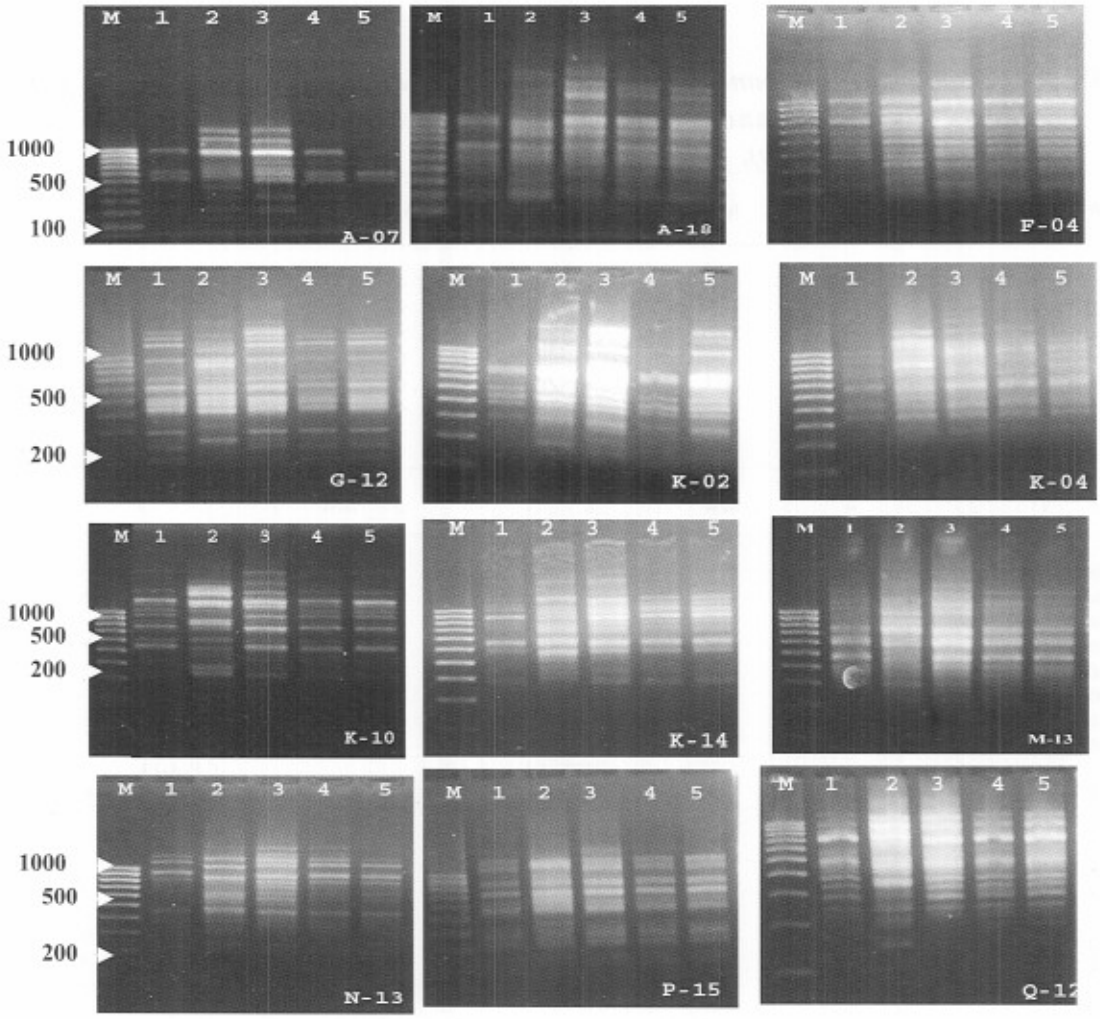

Fig. (4): RAPD banding patterns of the five canola genotypes using 12 selected random primers, M: 1 kbp plus DNA ladder, 1-5: the canola cultivars Serw-4, Masrri-L11, Masrri-L16, Semu-304 and Semu-248.

RAPD analysis indicated that all primers used resulted in the appearance of PCR products with a variable number of bands. The data show that 148 DNA markers were detected among the five canola cultivars, of which 78 bands were polymorphic (52%) (Fig.4 and Table 4). The highest number of RAPD bands was detected for primer OPG-12 (17 bands), while the lowest was scored for OPA-18 (9 bands). Table (5) shows the polymorphic bands generated from each primer.

Similarity indices for any pair of cultivars used in the present study are shown in Table (6). The data indicate a close relationship as a whole, between the examined canola cultivars, although differences in their salt-tolerance were very close. The specific RAPD markers for the different canola cultivars are listed in Table (7). Seventeen out of the 78 generated RAPD markers were found to be genotype- specific (21.7%). The largest numbers of RAPD specific markers were scored for Masrri-L11 (13 markers) while Masrri-L16 scored five markers.

Table (5): RAPD polymorphic bands generated by twelve different primers in the five canola cultivars (1-5: the canola cultivars Serw-4, Masrri-L11, Masrri-L16, Semu-304, Semu-248, respectively).

	OPA-07						OPA-18						OPF-04				
MW	1	2	3	4	5	MW	1	2	3	4	5	MW	1	2	3	4	5
1324	-	+	+	-	-	1881	-	+	+	-	-	1435	-	+	+	+	+
1072	-	+	+	-	-	1606	-	+	+	+	+	1111	-	-	+	-	+
841	+	+	+	+	-	1371	-	++	+	+	+	352	-	+	+	+	+
770	-	-	+	+	-	627	-	+	+	+	+	300	-	-	+	-	-
466	-	+	-	-	-	411	-	+	-	-	-	260	+	+	+	-	+
315	-	+	+	-	-	289	-	-	-	-	-	180	-	+	+	+	+
225	-	+	+	-	-												
180	-	+	-	-	-												
	OPG-12						OPK-02						OPK-04				
MW	1	2	3	4	5	MW	1	2	3	4	5	MW	1	2	3	4	5
2180	-	+	+	-	-	1658	-	+	+	-	+	1396	-	-	+	-	-
1766	+	-	+	+	+	1400	-	+	+	-	+	1150	-	+	+	+	-
1200	-	+	+	+	+	1043	-	+	+	+	+	1000	-	+	+	+	+
886	-	+	-	-	-	938	-	+	-	-	-	861	+	+	-	+	+
346	-	+	-	-	-	755	-	-	+	-	-	770	-	+	+	-	+
300	+	-	+	+	+	185	-	+	+	-	-	689	-	+	-	-	-
276	-	+	+	-	-	130	-	-	+	-	-	288	-	+	+	-	+
251	+	-	+	+	+												
	OPK-10						OPK-14						OPM-13				
MW	1	2	3	4	5	MW	1	2	3	4	5	MW	1	2	3	4	5
1371	-	+	+	-	-	1848	-	+	+	-	-	1570					
1180	-	+	-	-	-	1191	+	-	-	+	+	1400	-	-	+	+	-
774	+	-	+	+	+	741	-	+	+	+	+	900	+	-	+	+	+
751	+	+	-	-	-	631	-	+	+	+	+	478	-	+	+	-	-
430	+	-	+	+	+	421	-	+	+	-	-	388	+	-	+	+	+
328	-	+	-	-	-	305	-	+	+	+	+	355	-	+	+	+	+
309	-	+	+	+	+							292	-	-	+	-	+
261	-	+	+	-	-								-	+	+	-	-
	OPN-13						OPP-15						OPQ-12				
MW	1	2	3	4	5	MW	1	2	3	4	5	MW	1	2	3	4	5
1470	-	-	+	+	-	1762	-	-	+	-	-	589	-	+	-	-	
856	+	-	-	-	-	565	-	+	+	-	-	430	-	-	+	+	
809	-	+	+	+	+							418	-	+	-	-	
706	-	+	+	-	-							383	+	-	+	+	
583	-	+	+	+	+							295	-	+	-	-	
319	-	+	+	-	-							251	-	+	-	-	
221	-	+	+	-	-												

Table (6): Similarity index (%) of each pair of the examined canola cultivars.

	Serw-4	Masrri-L11	Masrri-L16	Semu-304
Masrri-L11	56.7			
Masrri-L16	65.5	76.3		
Semu-304	83.1	66.9	81	
Semu-248	83.1	69.6	79.7	93.9

Table (7): Genotype-specific RAPD markers of three local and two German canola cultivars.

Genotype	Genotype-specific marker	Number of Marker
Serw-4	OPN13-865	1
Masrri-L11	OPA 07-466, OPA 07-180, OPA 18-411, OPA 18-289, OPG 12-346, OPG 12-886, OPK 02-938, OPK 04-689, OPK 10-1180, OPK 10-328, OPQ 12-589, OPQ 12-418, OPQ 12-251.	13
Masrri-L16	OPF 04-300, OPK 02-755, OPK 02-130, OPK 04-1396, OPP 15-1762.	5

DISCUSSION

The detrimental effects of high salinity on plants can be observed at the whole-plant level as the death of plants and/or decreases in productivity (Allakhverdiev *et al.*, 2000). In the present work salt stress was found to reduce plant biomass production. The cultivar Masrri-L16 showed a relatively high dry weight followed by cultivar Masrri-L11, while plant growth of the cultivars Serw-4 and Semu-248 were remarkably affected.

It is well known that osmotic adjustment involves the net accumulation of solutes in a cell in response to salinity, and consequently, the osmotic potential decreases, which in turn attracts water into the cells and enables the turgor to be maintained (Neumann *et al.*, 1988). The osmotic potential (ψ_s) of the salt treated plants was decreased with increasing NaCl concentration and the decrease was more pronounced in Masrii-L16 and Masrii-L11. These results suggest that Masrii-L16 is able to maintain a higher osmotic potential in the cells due to the increase in the osmoticum concentration, leading to the maintenance of plant growth and to enhancement of the ability to tolerate salt stress. Alarcon *et al.* (1994) observed a direct relationship between the degree of the saline stress applied and the decrease in water stress as evidenced by the decrease of leaf turgor pressure in tomato plants. The cultivars used differed for their ability to tolerate salt stress and the cultivars Masrri-L16 and Masrri-L11 are salt tolerant, while Serw-4 and Semu-248 are the most salt sensitive cultivars.

Isozyme loci have been used as markers in a number of genetic studies, such as genetic diversity in *Brassica juncea* (Kumar and Gupta, 1985); genetic diversity in *B. rapa* (Persson *et al.*, 2001), testing genome construction of different species of *Brassica* (Chen *et al.*, 1989); isozyme loci and their linkage in *B. campestris* (Nozaki *et al.*, 1995) and isozyme markers as seed coat color (Rahman, 2001).

Peroxidases are enzymes related to polymer synthesis in cell wall (Bowles, 1990), as well as it plays an important role in the prevention of oxidative damage caused by environmental stress to the membrane lipids (Kalir *et al.*, 1984). Salt stress increased peroxidase bands intensity and induced some new bands. Salt tolerant cultivars Masrri-L16 and Masrri-L11 showed higher band intensity compared with the other cultivars. These results are in agreement with those of Gaspar *et al.* (1985) who reported an increase in peroxidase activity in cultivars sensitive to salt,

which could be responsible for the ability of such cultivars to adapt to external stimulus.

Genetic diversity in 14 taxa of the *Brassica oleracea* L. group and wild relatives ($2n$ =18) has been studied by two different approaches: isozymes and RAPD markers (Làzaro and Aguinagalde, 1996). In the present paper, five canola genotypes were studied using protein and RAPD markers. The SDS-protein patterns exhibited a maximum number of 14 bands; three of them were polymorphic (21.4%). A disadvantage of these biochemical markers seems to be their relatively low level of polymorphism, probably as a result of the genetic similarity of modern cultivars. They are suitable for the differentiation of *B. napus* from other Brassicas (*B. oleracea*, *B. rapa* etc.), but for the identification of individual oilseed rape cultivars, it is necessary to use additional marker systems for precise cultivar description (Rahman *et al.*, 2004).

As PCR techniques have been developed over the last 15 years, a wealth of new DNA marker technologies has arisen enabling the generation of high-density molecular maps for all the major Brassica crop species. Molecular markers have also been extensively used in analysis of genetic diversity in Brassica crops. Based on the data obtained by RAPD analysis, it was possible to discriminate between the five canola genotypes used in the present study. The genotype-specific markers indicate that 13 markers distinguish the cultivar Masrri-L11 and five markers distinguish the cultivar Masrri-L16. These markers can be verified as being RAPD markers associated with salt tolerance in the two canola genotypes. Subsequent experiments need to be achieved to determine the linkage between these RAPD markers and gene(s) for salt tolerance in canola cultivars used in the present study.

ACKNOWLEDGMENTS

This research is fully supported by the National Strategy for Biotechnology and Genetic Engineering, Science and Technology Center at the Academy of Scientific Research and Technology, Ministry of Scientific Research. All the technical approaches of the research activities are carried out at the Genetic Engineering Research Center (GERC), Faculty of Agriculture, Cairo University, Giza-Egypt.

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الملخص العربي

تحديد الواسمات الوراثية المتعلقة بصفة تحمل الملوحة فى الكانولا

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كان الهدف من هذه الدراسة هو تحديد الاستجابات الوراثية لخمسة من أصناف الكانولا المنزرعة تحت الظروف المصرية لصفة تحمل الملوحة. تم تعريض بادرات ثلاثة من الاصناف المحلية علاوة على إثنين من الاصناف الالمانية لتركيزات متدرجة من الملح (NaCl) و تم أخذ بيانات على كل من الصفات التالية: الوزن الجاف، المحتوى المائى لخلايا الاوراق، و كذلك تم دراسة التغير الناتج من تأثير معاملات الملوحة على أنماط شرائط البروتين و المشابهات الانزيمية. أدت الملوحة الزائدة الى خفض الضغط الاسموزى لخلايا كل الاصناف تحت الدراسة واوضحت النتائج أن الصنف مصرى لـ ١٦ (Masrri-L16) استطاعت خلاياه الحفاظ على ضغطها الاسموزى عند الحد الذى يسمح لها بامتصاص المياه تحت ظروف الملوحة مقارنة بباقى الاصناف تحت الدراسة.أدت الملوحة أيضا الى استحداث شريط جديد من شرائط البيروكسيديز فى صنف سرو-٤ (Rf = 0.33)، كذلك أدت الى زيادة كثافة شرائط البيروكسيديز فى كل الاصناف و هذا يعكس الدور الذى يلعبه البيروكسيديز فى حماية الخلايا من الاضرار الناتجة من تاثير الملح. تم تحديد التباينات الوراثية بين الاصناف الخمسة على مستوى أنماط شرائط البروتين و كذلك شرائط الـ RAPD. أظهرت شرائط البروتين تباينا فيما بينها بنسبة ٢١,٤ % بينما أظهرت شرائط الـ RAPD ٥٢ %. يمكن القول أن الواسمات الوراثية المتحصل عليها كافية للتمييز بين الاصناف الخمسة تحت الدراسة. أوضحت النتائج أن الواسمات الوراثية المتخصصة و المميزة للأصناف تمثل ١٢,٣ % من اجمالي الواسمات المتحصل عليها لكل من البروتين و الـ RAPD : ٩٤,٧ من الواسمات كانت خاصة بالـ RAPD، ١٣ واسم من واسمات الـ RAPD يمكن اعتبارها واسمات متعلقة بصفة التحمل للملوحة و مميزة للصنف مصرى لـ ١١ و خمسة واسمات تميز الصنف مصرى لـ ١٦، و هذه الواسمات يمكن استخدامها كواسمات لصفة تحمل الملوحة فى الكانولا و يمكن تطبيقها فى برامج التربية للحصول على اصناف كانولا متحملة للملوحة.