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RESPONSE OF CANOLA LINES TO THREE EDAPHIC CONDITIONS IN RELATION TO BIOCHEMICAL GENETIC MARKERS

Ahmed, S. M. and S. A. Afiah*
Biological Sci. Dept., Fac. Educ., Ain Shams Univ., Heliopolis-Roxy, Egypt.
*Plant Gene. Res. Dept., Desert Research Center, El-Matariya, Cairo, Egypt.

Polymorphism among eight lines selected through Desert Research Center program from the progeny of two canola divergent ancestors (the German variety, sido and the erucic free Canadian one, canola 103). These lines with the Egyptian variety, serw 4 were sown under three environmental regimes and investigated by using the biochemical techniques (SDS-PAGE and isozyme electrophoresis). Among the eight genotypes tested, six had susceptibility index for seed yield/plant (S) value < 1, out of them L_1, L_5, L_6 and L_8 significantly surpassed the comparative variety Serw-4. 30 bands were recorded in seed protein patterns with polymorphism of 30%. Therefore, the lines 5 and 6 could be considered as the most salt tolerant genotypes, while lines 2 and 7 were the most sensitive ones. A slight variation in protein banding pattern and a considerable polymorphism (53%) in isozymes patterns were recorded. Three negative biochemical markers were detected in the sensitive genotype (line-2) at molecular weights of 42.01, 27.1 and 21.7 kDa in the protein banding pattern. Four biochemical markers for salinity were recognized in α and β–esterase banding patterns under Siwa conditions. The polymorphism ranged from 69% under Cairo conditions and 28% under Siwa conditions. But in case of β-esterase isozyme, the ratio was between 89% and 50%. The relations between the expression of specific genes and environmental conditions was discussed.

Keywords: Canola, SDS-PAGE, isozymes and biochemical genetic markers.

Development of salt tolerant field crops through breeding by utilizing the intervarietal variations is the need of the owner / end user. Canola crop is still outside the local cropping structure in spite of our acute shortage of edible oil. Such crop has advantages, i.e. high seed oil content (40-45%),

high protein in oil free meal (30-35%) and easy cultivation in newly reclaimed lands by the well adapted genotypes with relatively low costs (Sharaan *et al.*, 2006). Canola has many advantages in comparison with the other oil crops, e.g., sunflower, cotton, corn and peanuts. It is cultivated in winter in contrast with the other oil crops, which are grown in summer. So, it can cover the gap in edible oil production in Egypt (Afiah *et al.,* 2007 b).

Many authors used seed storage protein variability for the identification and characterization of species and cultivars or germplasm collections. Felix *et al.* (1996) used grain storage protein to characterize a set of the first filial generation (F_1) derived haploid lines of bread wheat. Haider (1998) used grains storage protein to discriminate among ten Egyptian maize cultivars. Simonsen and Heneen (1995) studied the genetic variation based on isozymes in 43 landraces and cultivars of *Brassica campestris* from China, 4 cultivars of *B. campestris from* Sweden and 1 from India, and 5 culivars of *B. olerasea* from Sweden and 1 from China (*B. alboglabra*). El-Shanshoury (2002) used seed protein by SDS-PAGE to study the genetic variability between 30 *Lathyrus sativus* samples collected from different countries. The resulted profiles showed different patterns indicating variability among accessions of different habitats. Electrophoretic techniques for protein and isozyme polymorphism have been used as identification method, which provide correlation between the altered expression of specific genes and changes in the environment. These changes in expression of genes would be involved in adaptation (Rashed *et al.*, 2004). Genetic markers can have many applications in different phases of selection programs, i.e. choice of parents, identification of favourable alleles and development of genotypes accumulating such alleles. Other applications remain for the most part in the domain of research. There is a scope for the impact of these methods depending on the results obtained as well as on the evolution of cost of the informative data. Thus, markers have already indisputably enriched the range of methods available to the breeder in manging and exploiting genetic variability (Young, 1999 and Dekkers and Hospital, 2002).

The aim of the present investigation is to use the biochemical techniques to determine the genetic variation among newly bred lines through the relations between the expression of specific environmental conditions.

MATERIALS AND METHODS

Field experiments were conducted to study the response of eight newly bred lines of canola to different environmental conditions at three sites; Cairo (normal), Siwa (salt affected soil) and Mariyout (calcarious and slightly saline soil). The variations among newly bred lines descending from crossing between the canadian vareity (canola-103) and the german one (sido) were studied during 1996/1997 winter season (El-Hosary *et al.*,1999).

It is worthy to note that canola –103 free from erucic acid and sido < 2%. Seeds of the newly bred lines were obtained through pedigree selection under saline conditions of Desert Research Center (DRC) canola breeding program.The seeds of all newly bred lines and the check variety (Serw-4) were sown at the botanical garden of Fac. of Education, Ain Shams Univ., Siwa and Maryout Research Stations of DRC on the first week of November 2006. Some physical and chemical properties of the soil and irrigation water of the three experimental sites are shown in table (1). Samples of leaves were taken from all newly bred lines tested for isozymes experiments after 60 days from sowing. At harvest, seed yield/plant was recorded for plants of the experimental sites. The stress susceptibility index was computed according to Fischer and Mourer (1978).

Table (1). Some physical and chemical properties of the soil and irrigation water at the three experimental sites.

Location	Type	EC dSm^{-1}	pH	Cations (meq/l)				Anions (meq/l)			$CaCO_3$ %	Texture class
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}		
Free saline - soil type (Cairo)	Soil	1.82	7.35	5.18	3.3	9.2	0.49	0.19	10.4	7.58	3.04	Clay
Slightly saline (Maryout)	Soil	3.3	7.8	10.7	2.43	19.3	0.56	5.2	22.0	5.8	34	Clay loam
	Water	4.56	8.1	8.84	10.1	26.4	0.53	11.8	25.2	8.65	-	-
Salt affected soil (Siwa Oasis)	Soil	12.32	7.5	34.7	17.24	69.6	1.47	2.45	85.4	35.8	18.1	Sandy loam
	Water	4.01	7.3	8.69	9.08	21.5	0.48	10.3	20.5	8.74	-	-

SDS-polyacrylamide gel electrophoresis was performed in 10% acrylamide slab gels following the system of (Laemmli, 1970). Protein extraction was conducted by mixing 0.2 g of seeds with an equal weight of pure, clean, sterile fine sand. The seeds were then ground to fine powder using a mortar and pestle, and homogenized with 1.5 M Tris-HCl buffer, pH

8.8 in clean eppendorf tube and left in refrigerator over night, then centrifuged at 10,000 rpm for 10 min. The supernatant of each sample (contains protein extract) was kept in deep-freeze until use for electrophoretic analysis. A volume of 20 µl protein extract was added to an equal volume of treatment buffer. Then 20 µl of each sample was loaded in the gel.

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted to identify isozyme variation among the studied newly bred lines of canola using three isozymes systems. The utilized isozymes α, β-esterase and Aldehyde oxidase were separated in 10% polyacrylamide gel electrophoresis according to (Stegemann *et al.*, 1985). Isozymes extraction from canola samples was performed separately for each sample and location by homogenizing 0.5 g fresh leaf samples in 500 µl extraction buffer using a mortar and pestle. The extract was then transferred into clean eppendorf tubes and centrifuged at 10,000 rpm for five minutes. The supernatant was transferred to new clean eppendorf tubes and kept at –20°C until use for electrophoretic analysis. A volume of 50 µl extract of each sample was mixed with 25 µl of treatment buffer, then a volume of 50 µl from this mixture was applied to each well. After electrophoresis, the gels of α, β-esterase were soaked in 0.5 M borate buffer (pH 4.1) for 90 minutes at 4°C. This procedure lowers the pH of the gel from 8.8 to about 7 at which the reaction proceeds readily. The low temperature minimizes diffusion of the protein within the gel. The gel then was rinsed rapidly in two changes of double distilled water. The gel was stained for esterase activity by incubation at 37°C in a solution of 100 mg α-naphthyl acetate or β- naphthyl acetate (as a substrate) and 100 mg fast blue RR salt in 200 ml of 0.1 M phosphate buffer pH 6.5 (Scandalios, 1964). In case of Aldehyde oxidase (*Ao*), the gel was soaked in 100 ml of aldehyde oxidase staining buffer pH 8.6 containing 20 mg NBT, 10 mg EDTA, 25 mg NAD, 100 mg KCl, 10 ml benzaldehyde and 5 mg PMS (Jonathan and Wendel, 1990).

RESULTS AND DISCUSSION

Saline susceptibility index of seed yeild/plant (S) under salt affected soil at Siwa Oasis relative to adequate free saline conditions at Cairo experimental site was calculated. Among the nine genotypes tested, six had (S) value < 1, out of them L_1, L_5, L_6 and L_8 significantly surpassed the comparative variety Serw-4 by relative percentages of superiority 30.6%, 33.9%, 36.2% and 23.2%, respectively (Table 2). Therefore, the lines 5 and 6 could be considered as the most salt tolerant genotypes, while lines 2 and 7 were the most sensitive ones. If these stable newly bred lines show biochemical genetic markers for salt tolerance, it would be beneficial to exploit the genetic make-up and could be released as a new tolerant varieties.

These findings are more or less in harmony with those obtained by El-Saied and Afiah (1998) and Afiah *et al.* (2007 a).

SDS-Protein Analysis

Figure (1) demonstrated the SDS- protein profiles of canola seeds samples, while table (3) revealed their densitometrical analysis and represented the dominant occurrence of bands because polymorphism is detected as presence (+) or absence as (-) criteria. 30 bands were recorded in seed protein patterns with polymorphism of 30%. From them, 21 monomorphic bands were recognized, while the remaining 9 bands were considered as polymorphic ones, (Table 3). The low level of protein polymorphism could be attributed to the conservative nature of the seed protein. This conclusion was in accordance with Bonfitto *et al.* (1999). Low level of protein polymorphism was also reported in early ripening peach of Sinai (Mansour *et al.*, 1998) and in nine mung bean cultivars (Hassan, 2001). The detected intraspecific polymorphism in the resulted protein profiles could be attributed to some environmental stresses (Sammour *et al.*, 1993). Although, the protein polymorphism was slight, three negative biochemical markers were detected in the sensitive genotype (line-2) at molecular weights of 42.01, 27.1 and 21.7 kDa in the protein banding pattern (Table 4).

Table (2). Seed yeild/plant as combined over the three experimental sites and saline susceptibility index (S).

Genotype	Seed yeild/plant	S	RS%
Serw-4	15.85 ^{f}	2.61	-
L-1	20.70 ^{b}	1.45	30.6
L-2	15.48 ^{f}	0.66	2.33
L-3	18.17 ^{d}	0.87	14.6
L-4	16.31 ^{e}	1.71	2.90
L-5	21.23 ^{ab}	0.21	33.9
L-6	21.60 ^{a}	0.93	36.2
L-7	15.53 ^{f}	0.18	2.01
L-8	19.54 ^{c}	0.38	23.2

Values followed by the same letter(s) are not significantly different according to Duncan's multiple range test

RS%: Relative Superiority percentage over the check variety (Serw-4)

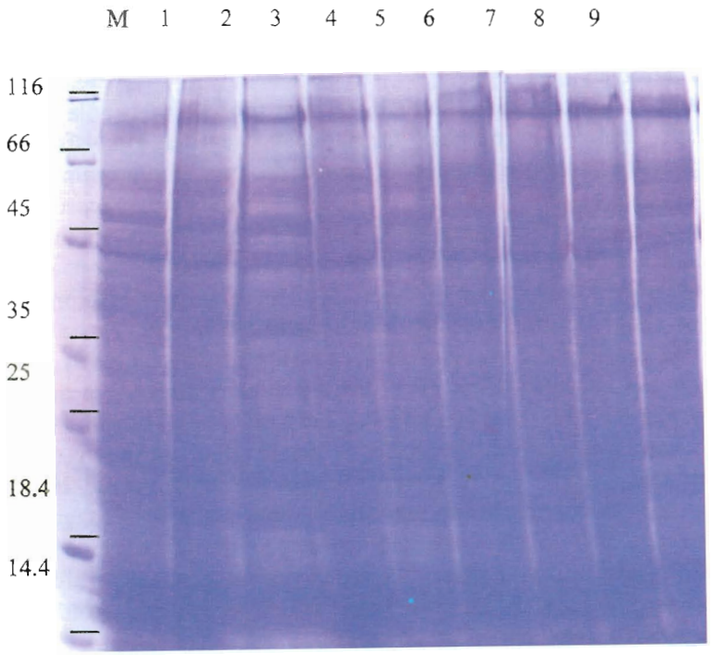

Fig. (1). SDS-Protein banding pattern of eight newly bred lines and the check variety (Serw-4) of canola *(Brassica napus* L).

Table (3). Number and type of the bands produced by SDS-PAGE and the percentage of the polymorphism detected in nine newly bred lines and the check variety (Serw-4) of canola (*Brassica napus* L).

Monomorphic bands	Polymorphic bands		Polymorphism%
	unique	shared	
21	1	8	30%

Isozymes Analysis

In the present investigation, three isozymes, which are α and β–esterases and aldehyde oxidase, were used to discriminate newly bred lines under study as shown in Fig.(2) and tables (5-8). Electrophretic α-naphthyl esterase patterns of fresh leaf samples for canola newly bred lines, were shown in Fig (2) and table (5). The polymorphism ranged from 69% under Cairo conditions and 28% under Siwa conditions. But in case of β-esterase isozyme the ratio was between 89% and 50% as appeared in Fig (2) and table (6). The highest polymorphism was 89% in β-esterase isozyme under

Cairo conditions, while the aldehyde oxidase isozyme did not show any polymorphism under the three regions. Among 57 bands recorded, twenty-seven bands were recognized as monomorphic and 30 ones were polymorphic with polymorphism ratio 53%. It was worthy to notice that, the number and position of monomorphic bands of samples varied in the three locations, also the five unique bands of samples No.'s (8), (7) and (9) in α, β–esterase patterns which appeared under Cairo conditions, disappeared in the other two locations.

Table (4). The molecular weights (Mol. wt) of seed-protein bands of eight newly bred lines and the check variety (Serw-4) of canola *(Brassica napus* L).

Mol.wt (K.Da)	Serw-4	L1	L2	L3	L4	L5	L6	L7	L8
126.3	+	-	-	-	+	+	-	+	+
99.88	+	+	+	+	+	+	+	+	+
61.96	+	+	+	+	+	+	+	+	+
58.78	+	+	+	+	+	+	+	+	+
51.36	+	+	+	+	+	+	+	+	+
49.24	+	+	+	+	+	+	+	+	+
45.53	-	-	+	-	-	-	+	+	+
44.29	+	+	+	+	+	+	+	+	+
43.59	+	+	+	+	+	+	+	+	+
42.01	+	+	-	+	+	+	+	+	+
40.61	+	+	+	+	+	+	+	+	+
40.08	+	-	-	-	-	-	-	-	+
38.68	+	-	-	-	-	-	-	-	+
37.63	+	+	+	+	+	+	+	+	+
36.22	+	-	-	-	-	-	-	-	-
35.00	+	+	+	+	+	+	+	+	+
32.89	+	+	+	+	+	+	+	+	+
31.05	-	+	+	+	+	+	+	+	+
27.10	+	+	-	-	+	+	+	+	+
23.96	+	+	+	+	+	+	+	+	+
23.04	+	+	+	+	+	+	+	+	+
21.7	+	+	-	-	+	+	+	+	+
20.87	+	+	+	+	+	+	+	+	+
20.25	+	+	+	+	+	+	+	+	+
19.32	+	+	+	+	+	+	+	+	+
16.97	+	+	+	+	+	+	+	+	+
16.53	+	+	+	+	+	+	+	+	+
15.46	+	+	+	+	+	+	+	+	+
14.93	+	+	+	+	+	+	+	+	+
14.13	+	+	+	+	+	+	+	+	+

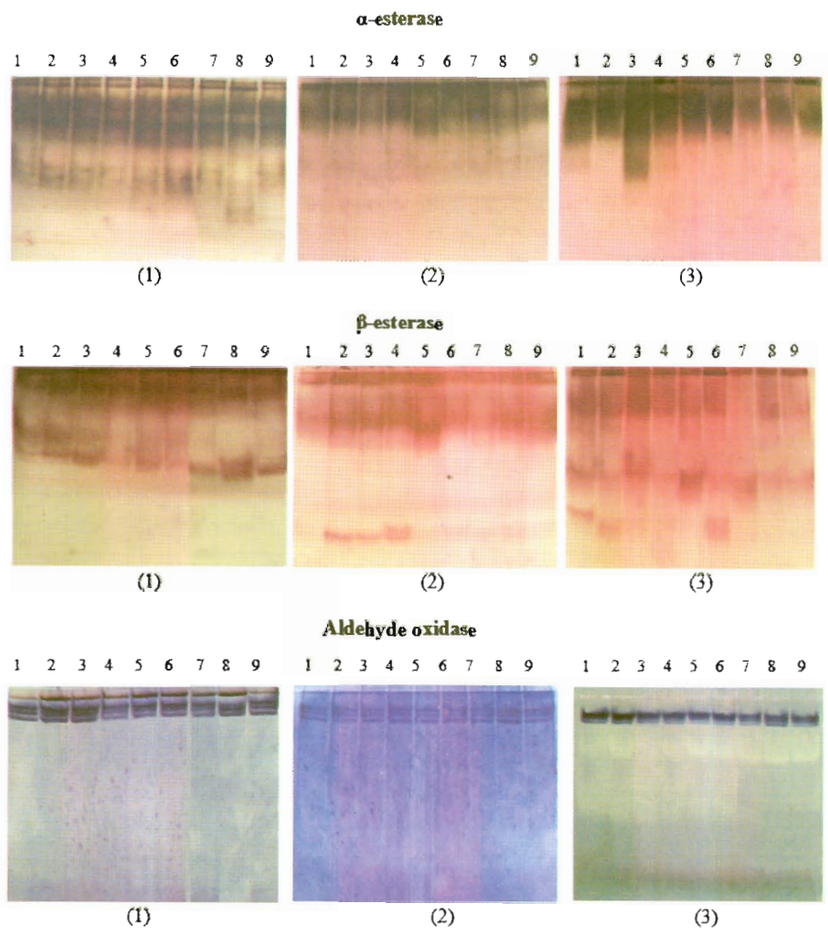

Fig. (2). Electrophoritic banding patterns of α, β–esterase and aldehyde oxidase isozymes for eight newly bred lines as well as the check variety (No.9) of canola (*Brassica napus* L).

1 under Cairo conditions
2 under Siwa conditions
3 under Mariuot conditions

Table (5). The presence (+) and absence (-) of bands in α-esterase isozyme profiles of eight newly bred lines and the check variety (Serw-4) of canola (*Brassica napus* L) in three locations.

Location	Band No.	Serw-4	L1	L2	L3	L4	L5	L6	L7	L8
Cairo	1	+	+	+	+	-	-	+	-	+
	2	-	-	-	-	+	+	-	-	-
	3	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+
	7	+	+	+	-	+	+	+	-	+
	8	-	-	-	-	-	-	-	+	-
	9	+	+	+	+	+	+	+	-	+
	10	+	+	+	-	+	+	+	+	+
	11	+	+	+	-	+	+	-	+	-
	12	-	-	-	-	-	-	-	+	-
	13	-	-	-	-	-	-	+	+	+
Siwa Oasis	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+
	4	-	-	+	+	+	-	+	-	-
	5	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+
	7	+	+	+	+	+	-	+	+	+
Mariyout	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	-	+	-	-	-	-	-	-
	4	-	-	+	+	-	-	-	-	-
	5	+	-	+	+	-	-	-	-	-

Location	Band No.	Serw-4	L1	L2	L3	L4	L5	L6	L7	L8
Cairo	1	+	+	+	+	-	-	+	-	+
	2	-	-	-	-	+	+	-	-	-
	3	+	+	+	+	+	+	+	+	+
	4	+	+	+	+	+	+	+	+	+
	5	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+
	7	+	+	+	-	+	+	+	-	+
	8	-	-	-	-	-	-	-	+	-
	9	+	+	+	+	+	+	+	-	+
	10	+	+	+	-	+	+	+	+	+
	11	+	+	+	-	+	+	-	+	-
	12	-	-	-	-	-	-	-	+	-
	13	-	-	-	-	-	-	+	+	+
Siwa Oasis	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+
	4	-	-	+	+	+	-	+	-	-
	5	+	+	+	+	+	+	+	+	+
	6	+	+	+	+	+	+	+	+	+
	7	+	+	+	+	+	-	+	+	+
Mariyout	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	-	+	-	-	-	-	-	-
	4	-	-	+	+	-	-	-	-	-
	5	+	-	+	+	-	-	-	-	-

Table (6). The presence (+) and absence (-) of bands in β-estrase isozyme profiles of eight newly bred lines and the check variety (Serw-4) of canola (*Brassica napus* L.) in three locations.

Location	Band No.	Serw-4	L1	L2	L3	L4	L5	L6	L7	L8
Cairo	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	-	-	-
	3	-	-	-	-	-	-	-	+	-
	4	-	-	-	-	-	-	+	-	-
	5	+	+	+	-	+	+	-	-	-
	6	-	-	-	-	-	-	-	-	+
	7	+	+	+	-	-	-	-	+	-
	8	+	+	+	+	+	+	+	-	+
	9	-	-	-	+	-	-	-	+	-
Siwa Oasis	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	-	+	-	+	+	-	+	-	-
	4	+	+	+	+	+	+	-	+	+
	5	-	-	-	-	-	-	-	+	-
	6	+	+	+	+	+	+	+	+	+
	7	+	+	-	+	+	+	+	+	+
	8	+	+	+	+	+	-	+	+	+
Mariyout	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	-	-	+	+	+	+	-	-
	4	+	+	+	+	+	+	+	+	+
	5	-	-	-	-	-	-	+	+	+
	6	+	-	-	-	-	-	-	-	-
	7	-	+	+	+	+	+	+	+	+

Location	Band No.	Serw-4	L1	L2	L3	L4	L5	L6	L7	L8
Cairo	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	-	-	-
	3	-	-	-	-	-	-	-	+	-
	4	-	-	-	-	-	-	+	-	-
	5	+	+	+	-	+	+	-	-	-
	6	-	-	-	-	-	-	-	-	+
	7	+	+	+	-	-	-	-	+	-
	8	+	+	+	+	+	+	+	-	+
	9	-	-	-	+	-	-	-	+	-
Siwa Oasis	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	-	+	+	+	+	-	+	-	-
	4	+	+	+	+	+	+	-	+	+
	5	-	-	-	-	-	-	-	+	-
	6	+	+	+	+	+	+	+	+	+
	7	+	+	-	+	+	+	+	+	+
	8	+	+	+	+	+	+	+	+	+
Mariyout	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	+	-	+	+	+	+	+	-
	4	+	+	+	+	+	+	+	+	+
	5	-	-	-	-	-	-	+	+	+
	6	+	-	-	-	-	-	-	-	-
	7	-	+	+	+	+	+	+	+	+

Table (7). The presence (+) and absence (-) of bands in aldehyde oxidase isozyme profiles of eight newly bred lines and the check variety (Serw-4) of canola (*Brassica napus* L.) in three locations.

location		Serw-4	L1	L2	L3	L4	L5	L6	L7	L8
Cairo	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+
Siwa Oasis	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+
Mariyout	1	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+	+

These observations could be explained on the bases of adaptations to the local environmental conditions associated with selection processes through generations. Our results agreed with El-Saied and Afiah (2004) in Lentil under rainfed conditions and Hassan *et al.* (2002), who revealed extensive polymorphism in esterase isozyme among ten accessions representing five *Populus* species from different locations and recorded also high variations among samples of the same species. The same finding was obtained by Ahmed (2003), who recorded intraspecific polymorphism in isozyme patterns within the *Brassica* and *Raphanus* species that were collected from different geographical locations.

Four biochemical markers for salinity were recognized in α and β–esterase banding patterns under Siwa conditions (Tables 5 and 6). One negative marker (band No. 7) was scored in the tolerant genotype (line-6) in α–esterase pattern. On the other hand, two negative markers (bands No.'s 4 and 7) were recorded in the tolerant genotype (line-6) and the sensitive one (line-2), while the band No. 5 was considered as a positive marker in the sensitive genotype (line-7) in β–esterase pattern. These results were similar to Afiah *et al.* (2007) who detected only one biochemical genetic marker which was identified among 140 polymorphic bands detected from SDS-PAGE and isozyme banding profiles in three hybrids of canola parental genotypes and their F_1 and F_2 populations.

Table (8). Number and type of the bands produced by the three isozymes and the percentage of the polymorphism detected by each isozyme in eight newly bred lines as well as the check variety (Serw-4) of canola (*Brassica napus* L).

Isozyme system	Location	Monomorphic band no.	Polymorphic band No.		Polymorphism%
			unique	shared	
α-esterase	Cairo	4	2	7	69%
	Siwa Oasis	5	0	2	28%
	Mariyout	2	0	3	60%
β-esterase	Cairo	1	3	5	89%
	Siwa Oasis	4	0	4	50%
	Mariyout	2	1	3	67%
Aldehyde oxidase	Cairo	3	0	0	00%
	Siwa Oasis	3	0	0	00%
	Mariyout	3	0	0	00%
Total No. of bands		27	6	24	53%

CONCLUSION

In summating SDS-PAGE, isozymes techniques are useful in the establishment of biochemical genetic markers for salt tolerance in canola crop for screening the most tolerant genetic resources rapidly and safely. The results of this investigation provide some markers associated either positively or negatively with salt tolerance which could be used to enhance breeding programs aimed at improvement of the canola genotypes for salt tolerance by pyramiding genes controlling this polygenic character with the aid of marker-assisted selection.

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استجابة سلالات من الكانولا لثلاثة ظروف ارضية مختلفة وعلاقة ذلك بالكاشفات الوراثية البيوكيميائية

شوكت محمود أحمد ، سامى عبد العزيز عافية*
قسم العلوم البيولوجية- كلية التربية - جامعة عين شمس- القاهرة- مصر
* قسم الاصول الوراثية النباتية - مركز بحوث الصحراء - المطرية - القاهرة- مصر

هذا البحث استخدم التقنيات البيوكيميائية المتمثلة فى التفريد الكهربى لكل من بروتينات البذور الجافة والمشابهات الانزيمية لدراسة التباين الوراثى بين سلالات جديدة ناتجة من برنامج تربية الكانولا بمركز بحوث الصحراء بالإضافة لصنف مقارن وهو سرو-٤، وكذلك دراسة التعبير الجينى تحت الظروف البيئية المتباينة فى القاهرة ومريوط وواحة سيوة. وقد تمثلت المادة الوراثية فى ثمانية سلالات ناتجة عن الانتخاب المنسب خلال الأجيال الإنعزالية لنواتج التهجين بين الصنف الالمانى Sedo والصنف الكندى الخالى من الحمض الدهنى إيروسك canola103

أظهرت النتائج أن أربعة سلالات هى:L1-L5-L6-L8 قد سجلت نسب تفوق اعلى من الصنف المقارن سرو-٤، وتم اعتبار ان السلالتين L5-L6 هما الأكثر تحملاً للملوحة فى حين كانت السلالتان L2-L7 هما الأكثر حساسية. وعلى الرغم من تسجيل نسبة منخفضة من التباين ٣٠% فى طرز البروتين، إلا أنه تم الحصول على ثلاثة كاشفات سالبة فى السلالة L2 عند الأوزان الجزيئية ٤٢,٠١-٢٧,١-٢١,٧ كيلو دالتون. على الجانب الآخر تم تسجيل نسبة أعلى من التباين فى طرز المشابهات الإنزيمية هى ٥٣% . لم يظهر إنزيم الدهيد اوكسيديز أى تباين فى المناطق الثلاث وقد تفاوتت نسبة التباين فى إنزيم بيتا استيريز بين ٨٩% تحت ظروف القاهرة و٥٠% تحت ظروف واحة سيوة، فى حين كان التفاوت ما بين ٦٩% و٢٨% فى إنزيم الفا استيريز، وهذا يعكس اختلاف الفعل الجينى تحت الظروف البيئية المختلفة. هذا وقد سجلت طرز المشابهات الإنزيمية أربعة كاشفات بيوكيميائية (ثلاثة منها سالبة والرابعة موجبة) تحت الظروف الملحية لمنطقة سيوة، مما يعكس نفعية تلك التقنيات فى محاولة إعتماد تلك السلالات كأصناف لها القدرة على الإنتاجية فى الأراضى حديثة الإستصلاح وذلك بعد دراسة العلاقة بين التعبير الجينى والظروف البيئية المختبرة.