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SELECTION OF SHATTERING RESISTANCE MUTANTS AT THE MOLECULAR LEVEL IN OIL SEED RAPE

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ABSTRACT

Genetic variations in siliqua shattering resistance was observed selection of seven mutant population of canola. The aim of study was to determine and selection of shattering resistance mutant population in canola using molecular breeding techniques. The results showed that Mutant population 39 was the earliest in flowering date, (54.88days).By control, Mutant population 251 flowered after (104.83). Mutant population 251 gave the highest value of plant height (205.47cm), while mutant 36 gave the lowest value (131.1cm). Variation in number of racemes; was also obvious mutant population 251 gave the highest value (30.37cm); while mutant 36 gave the lowest value (6.75cm). For the trait of first raceme height, the exotic cultivar pactol was the tallest (35.7cm), while the mutant population (251) gave the shortest value (10.93cm). Mutant population 251 was the highest in fruiting zone length value (192.73cm), while mutant (36) gave the shortest value (117.84cm). Besides the first siliqua height was 121.47 cm for mutant population 251, while it was (42.63cm). Seed yield/plant of the local cultivar serw 4 was (54.13 g/plant), while the highest yield per plant among mutants was 51.60 g for mutant population 38. Mean 1000 seed weight (gm) was highest (3.93g/plant) for mutant 36, while mutant 251 gave the lowest 1000 seed weight (2.38g/plant). Mutant 37 gave the highest oil content (43.53%), and was while pactol gave the lowest oil content, (37.99%. The applied technique with respect to shattering resistance was very simple; entries were kept in the field without being harvested from 25- 28 days, after full maturity and harvesting was done manually by shaking.

Molecular analysis using twenty RAPD primers were used in the detection of polymorphism among the seven mutants and their control revealed a total of 175 polymorphic bands out of 235 reproducible products. This corresponds to a level of 74.47% polymorphism. The number of amplicons/primer ranged from 3 (OP-B05) to 18 (OP-O16), whereas the number of polymorphic bands per primer ranged from 2 (B05) to 16 (OP- O16). The total number of unique bands across the seven mutants and their control was 69 including 27 unique positive markers (UPM) and 42 unique negative markers (UNM).

Mutant 251 showed that different unique marker. Also primer OPC- 02 showed triple band. Unique markers.

Key words: ***B.napus, Shattering Resistance, Molecular analysis, RAPD.***

INTRODUCTION

Oil seed rape (*Brassica napus* L.) is one of the most important vegetable oil crops in the world. It is now the third important source of edible vegetable oils. It is a promising oil crop in Egypt that can help in

solving the local problem of oil production gap. Moreover; it can be grown in the new reclaimed lands as a winter crop. Improvement depends on the availability of genetic variability. Mutagenesis is a powerful tool to induce new genetic variability. The development of canola short ideotype, resistant to shattering and with high agronomical performance depends on the identification of relevant characteristics of the plant architecture prior to harvest. Losses occur due to adverse weather conditions during harvest, and due to the impact of the combiner machine. It is known that the structure and distribution of canopy components affect seed recovery as well as yield potential (Child and Evans 1989). In addition; canopy height and stem stiffness may affect the efficiency of seed recovery. Seed losses my also be affected by canopy characteristics and architecture which are determined by the plant morphology such as pod angles; length, thickness and width. Shatter susceptibility varies significantly in different genotypes (Child and Huttl, 1999). Recently; identification methods have focused on the application of DNA based markers. DNA based markers have number of advantages over other tests for cultivar discrimination in that DNA is unaffected by environmental factors or the developmental stage of the organism. The RAPD methodology has an advantage over other DNA fingerprinting methods in that it is fast and cheap. RAPD analysis using the genomic DNA from 40 or more rape seed plants is common (Mailer et al 1997).

The aims of this study were to find a marker assisted selection system for shattering resistance in seven rape seed mutants two exotic cultivars and one local cultivar, to determine the breeding and molecular markers genetic variability and to validate the genetic relationship among the tested mutants.

MATERIALS AND METHODS

Plant material

The field experiments in the present study were carried out during 2004/2005 and 2005/2006 seasons in Giza - Agric. Res .Station, ARC. Seven promising mutants population; (36, 37, 38, 39, 143 and 251) and their control French (Cresor) *Brassica napus* as well as the French cultivar pactol and the local commercial cultivar serw-4, were cultivated in field plots. The mutants population that were used in this study were developed in previous studies according to Sorour (1994 and 1995) using Gamma radiations. All entries were evaluated in a randomized complete block design with three replications in 2004/2005and 2005/2006. Each entry consisted of five rows 4m-long. Spacing between rows and plants within the row was kept at 60 cm and 15 cm, respectively. Thinning was done at one

plant/hill after 18 days of planting. Other agronomic practices were adopted according to the recommend methods.

Agronomic Traits

Data were recorded on a sample of ten individual plants/plot. The traits were days to 50% flowering, plant height (cm), number of racemes, height of first raceme, height of first siliques (cm), fruiting zone length (cm), 1000 seed weight, seed yield/plant and seed oil content. The obtained date were statistically analyzed and differences among entries were done using the Duncan multiple rang method. Steel and Torrie (1980). The background of the used entries is indicted in Table (1).

Table 1.Cultivars and Mutants used in the evaluation trial.

Mutant	Source
1- Cresor (34)	Local variety.
2- Mutant (251)	Cresor variety treated with 300 Gry.
3- Mutant (36)	Cresor variety treated with 200 Gry.
4- Mutant (37)	Cresor variety treated with 200 Gry.
5- Mutant (38)	Cresor variety treated with 200 Gry.
6- Mutant (39A)	Cresor variety treated with 200 Gry mutant in green.
7- Mutant (39B)	Cresor variety treated with 200 Gry (Chlorophyll mutant with albino leaves .
8- Mutant (143)	Cresor variety treated with 200 Gry.
9-Pactol	French
10-Serw-4	Local variety

Extraction and purification of genomic DNA

The DNA was isolated f seedling for 28 days from plants of the seven mutants population and their parent cultivar Cresor as well as two check cultivars Pactol and Serw-4 there control plants using a DNA easy Plant Mini Kit (Qiagen, Santa Clarita, CA). Fresh young leaves (0.5 mg) were ground in liquid nitrogen and used to extract DNA. The protocol was used as described in the manufacturer's instructions.

RAPD Analysis

A set of twenty random 10-mer primers was used in the detection of polymorphism among the seven mutants and their control. These primers were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems) at AGERI-Egypt. RAPD-PCR was carried out according to the procedure given by Williams *et al* (1990) with minor modifications. The

amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 2 mM dNTPs, 1 µM primer, 1 U *Taq* DNA polymerase and 25 ng template DNA. PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (*PE* Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min, and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts.

Data analysis

The banding patterns generated by RAPD-PCR marker analyses were compared to determine the genetic relatedness of the seven mutant population and their control. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal 1973). Dice formula: GSij = 2a/(2a+b+c) , where GSij is the measure of genetic similarity between individuals *i* and j, a is the number of bands shared by *i* and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in .

Results and Discussion

Table (1) revealed that Mutant population 39 was the earliest in flowering (54.88 days); Cresor 34 was moderate in flowering (77.33days) and Mutant population (251) was the latest (104.83days). Regarding plant height, Mutant (251)gave the highest value(205.47cm)followed by control Cresor(34) that gave the value (179.47cm) .The first and most promising local cultivar Serw- 4 was similar to the parental control of the mutant population (Cresor34). The exotic cultivar pactol gave the value (166.5 3 cm) on the other hand, one mutant population (36) .was to short and reached (131.1cm). Concerning No of racemes, Mutant 251 gave the highest number of racemes (30.37), Mutant (143) showed moderate No of racemes, while mutant 36 was the lowest and reached (6.75cm). As for the first of raceme height variety Pactol gave tallest hight value (35.7cm), Mutant 39 was moderate while Mutant 251 showed the shortest height value and reached (10.93cm). Also, regarding to fruiting zone, mutant 251 gave the highest value (192.73 cm). Mutant 39 reached (133.76 cm), while the shortest fruiting zone was for Mutant 36 (117.84cm). Regarding first siliqua height Mutant 251gave the highest value (121.47cm), while Mutant 143 was moderate in the height and the lowest value was Mutant 36 reached (42.63

cm). The Egyptian variety Serw-4 was the highest in seed yield /plant (54.13g/plant) .The most promising Mutant 38 had similar yielding ability is Serw- 4.The moderate Mutant 251 gave (40.73g), while the lowest yielding Mutant was 143, which reached (29.7g). Surprise irgly, the most productive plants of Serw-4 as average of two seasons showed a significantly less yielding ability than Mutant (143) in the second seamen. The reverse was true in the first season. Also, 1000 seed weight was the highest (3.9g) for Mutant 36, while Mutant 39 did not differ significantly from Mutant 36. Mutant 143 was moderate (3.5g), and Mutant 251 showed the lowest 1000 seed weight value the reached (2.4 g). .The results of oil content varied from (43.53) to (37.99 %) among the mutants. These results agree with those of Arnold (1997). Due to the high temperature prevailing for 25-28 after maturate during maturity period, large quantity of seed was lost and caused, a negative effect on the actual seed yield. They shattering or semi shattering is badly needed. A program for selecting, semi or shattering and non shattering was conducted. The applied technique of which is very simple Maturity. Harvesting was done manually by shaking. All the plants in all mutants which appeared to be shattering resistant or semi resistance shattering were selected according to previous Parameter. The entries showed variation in shattering resistance. Mutant 251 revealed highly resistance to shattering. While, Mutants 36, 37, 38 and 39 were semi resistance to shattering

Table (2) Showed that seed loss from siliqua in shattering is a major problem of canola production world wide and especially in Egypt. Narrow variation for shattering resistance is available in *Brassica napus*. However, considerable variation for shattering resistance is present in *B. napus*. Screening for shattering resistance in canola breeding programs is currently based on morphology and molecular markers. Also Mutant differed in their reaction from years to in other i.e. seed yield /plant was highest in variety Serw-4 followed by variety Pactol and the Mutants No .36,37 and 38 in 2004 -2005 ,season .But in 2005-2006 ,Mutant 38 had the highest seed yield /plant followed by Mutant 36 and Mutant 39. Mean of both season in dictated that both Serw-4 and Mutant 38 were significantly the higher yields compared to all other entries. Mean 1000 seed weight of both seasons was in flower of Mutant 36, Mutant 39, followed by Mutant 38. On the other hand average oil percentage of both seasons indicated superiority of Mutant 37 followed by Mutant 36 and Mutant 143. Some of there mutant, my therefore be recommended as new developed varieties after exposing to more yield trials on wider scale. The development of seven mutant and their control possessing high seed yield and high oil content have been the major objective of mutagenesis in breeding programs of rapeseed. Shattering resistance were found in mutants251, and semi shattering mutant 36, 37, 38, 39and 143. The seven entries were evaluated in seven mutant test majority

Table 2. Mean of flowering date, plant height, number of racemes /plant, first racemes height/plant, and fruiting zone in 2004 -2005 and 2005-2006 of the rape seed entries.

Entry	Flowering date		Mean	Plant height		Mean	No of racemes/plants		Mean	First Racemes height		Mean	Fruiting Zone (cm)		Mean
	2004-5	2005-6		2004-5	2005-6		2004-5	2005-6		2004-5	2005-6		2004-5	2005-6	
Cresor (34)	80,0 b	74.67 b	77.33 b	151.67 b	207.27 b	179.47 b	7.97 e	14.33 b	11.15 b	31.40 a	22.6 b	27.o b	120.93 b	185.0 b	152.97 b
Mutant 251	91.27 a	118.4 a	104.83 a	160.27 a	250.67 a	205.47 a	24.67 a	36.07 a	30.37 a	10.27 c	11.6 e	10.93 e	146.4 a	239.07 a	192.73 a
Mutant 36	63.4 g	48.47 e	55.93 e-f	138.27 d	123.93 g	131.1 f	8.33 d-e	5.17 d	6.75 d	31.67 a	16.05 c-d	23.86 b-c	127.8 b	107.88 g	117.84 e
Mutant 37	65.4 e	47.93 e-f	56.67 e	140.13 d	158.22 e	149.18 e	10.27 c	7.83 c	9.05 c	12.30 b-c	14.05 d	13.18 e	130.83 b	144.18 e	137.51 c
Mutant 38	63.8 f	47.0 e-f	55.40 f-e	142.93 c-d	146.88 f	144.91 e	9.73 c-d	7.87 c	8.80 c	11.6 b-c	10.87 e-f	11.23 e	131.33 b	136.02 f	133.68 c-d
Mutant 39	63,67 g-f	46.1 f- g	54.88 g	141.93 c-d	171.07 d	156.5 d	9.60 c-d-e	9.40 c	9.50 c	36.13 a	9.35 f	22.74 c	105.8 c	161.72 c-d	133.79 c-d
Mutant 143	69.53 c	55.0 d	62.27 c	141,13 d	177.63 d	159.38 d	8.93 c-d-e	12.93 b	10.93 b	14.0 b-c	9.63 e-f	11.82 e	127.47 b	168.0 c	147.73 b
Serw 4	67.47 d	44.7 3 g	56.1 e-f	150.37 b-c	198.67 c	174.52 b	12.6 b	9.8 c	11.2 b	18,0 b	17.33 c	17.67 d	87.73 d	181.33 b	134.53 c-d
Pactol	63.5 3 g-f	58.53 c	61.0 d	135.93 d	197.13 c	166.52 c	8.87 c-d-e	13.2 b	11.o3 b	33.4 a	38.00 a	35.70 a	102.53 c	159.13 d	130.83 d

Table 2 Cont.

Entry	First siliques		Mean	Seed yield \plant(g)		Mean	1000 seed weight(g)		Mean	% of oil content		Mean
	2004-5	2005-6		2004-5	2005-6		2004-5	2005-6		2004-5	2005-6	
Cresor (34)	87.27 a	147.0 b	117.13 b	37.85 d	27.2 e	32.52 e	2.78 d-e	3.02 c	2.90 e	40.71b-c	40.03 d	39.89b
Mutant 251	58.33 d	148.6 a	121.47 a	39.07 d	42.4 c	40.73 d	2.35 f	2.42 d	2.38 f	39.94 c	39.34 d	39.52c-d
Mutant 36	31.67 f	53.6 f	42.63 f	45.33 b-c	49.27 b	47.3 b	3.95 a	3.90 a	3.93 a	42.84 a	42.46a-b	42.84a-b
Mutant 37	31.53 f	71.52 d	51.53 e	45.33 b-c	44.27 c	44.8 b-c	3.58 b-c	3.63 a-b	3.61 c-d	42.76 a	43.11 a	43.53a
Mutant 38	38.87 e	62.62 c	50.74 e	43.87 c	59.33 a	51.6 a	3.69 a- b	3.74 a-b	3.72 b-c	41.58 b	41.66b-c	41.58b-c
Mutant 39	40.73 e	73.1 d	56.92 d	32.87 e	53.o7 b	42.97c-d	3.84 a- b	3.89 a	3.86 a-b	40.27c	40.57c-d	40.27e-d
Mutant 143	68.27 c	86.13 c	77.20 c	23.47 f	35.93 d	29.7 f	3.37 c	3.55 b	3.46 d	41.6b	41.94a-b	41.60a-b
Serw 4	75.6 b	26.59	51.07 e	67.47 a	40.20 c	54.13 a	2.92 d	2.97 c	2.95 e	40.27c	38.11 e	40.40c-e
Pactol	77.5b	24.47g	51.00 e	47.80 b	42.40 c	45.16b-c	2.51 e-f	2.46 d	2.49 f	37.82d	40.39 d	37.99 d

of seven mutant shattering at harvest time; especially in late harvesting (late May). All mutant that appeared to be resistant to shattering and semi shattering were selected and sown in 2004/2005 and 2005/2006.

Nowadays, the molecular genetic. The fluctuation of molecular markers represents the major difficulty for improving breeding program of rape seed. For that reason we try to go further step to study breeding at the molecular level rather than the morphological one in order to have more accurate discrimination among tested mutants and checks.

Our results showed different mutant performance in flowering date, plant height (cm), No. of racemes , first raceme height (cm), fruiting zone (cm), first silique (cm), seed yield /plant, 1000 seed weight (g) and % of oil content. The parameters were good to select the potential mutant, which has good traits. These results agree with those by Williams, (1989). The variety Serw 4 gave the highest seed yield/ plant, while the mutant 37 gave the highest percentage of oil content. These results suggest that the comparison between mutants, controls and two check varieties could be made in order to determine the best performing mutants and their control. Also mutant 39 earliest in flowering. While mutant 251 was the latest in flowering onset. The mutant 251 gave green growth in longer time, but duration flowering was short it Rangel from 4-5 the mutant 251 in vary high in shattering resistances, while the branches in all stems, the flower color white and creamy in 2 days, and convert ion to yellow color.

Table 3. Phenotypic correlation coefficient between different traits

Character	Plant height (cm)	No. of racemes	First racemes (cm)	Fruiting zone (cm)	First siliques (cm)	Seed yield (gm)	Weight 1000 seed (gm)	%of oil
Flowering date	**0.46	**0.84	-0.07	**0.39	**0.64	-0.27	**-0.60	0.05
Plant height (cm)		**0.72	-0.11	**0.91	**0.0.62	-0.22	**-0.50	**0.88
No. of raceme			-0.23	**0.68	**0.64	-0.13	**-0.60	**0.82
First raceme s				*0.32	-0.21	-0.17	-0.20	0.30
Fruiting zone					**0.56	*-0.30	*-0.29	0.60
First siliques						.-0.16	.**0.36	0.12
Seed yield /plant (g)							0.10	0.31*
Weight 1000 seed (g)								0.35*

* indicate significance at 0.05 and 0.01 probability levels ,respectively.

Phenotypic Correlation coefficients etween different traits are presented in Table (3). The results revealed that flowering date had positive and highly significant correlation with each of plant height (cm), No. of racemes, fruiting zone, first silique and weight of 1000 seed. While the correlation between the first racemes, seed yield and % of oil were negative.

Plant height had, positive and highly significant correlation with each of No. of racemes, fruiting zone, first silique, and weight of 1000 seed while the correlation between first racemes, seed yield and % of oil was negative.

Regarding to No. of racemes, there was a positive and significant coefficient correlation with fruiting zone first silique and weight of 1000 seed while the correlation between first racemes, seed yield and %of oil was negative.

Also first racemes had positive and highly significant correlations with each of fruiting zone, while the correlation between first siliques seed yield /plant, weight 1000 seed and %of oil were negative.

Fruiting zone had positive and highly significant correlation with first silique, seed yield /plant and weight 1000 seed , while the correlation between % of oil were negative.

Also first silique had positive and highly significant correlations with weight of 1000 seed, while the correlation between seed yield /plant, and %of oil were negative.

Besides seed yield/plant had positive and highly significant correlation with each of % of oil, while the correlation between weight of 1000 seed was negative. Regarding to weight of 1000 seed it had positive and highly significant correlation of %oil.

RAPD analysis

A total of 20 decamer primers (Table 4) were screened against DNA of seven mutants and their control (Fig. 1). The amplification profiles of the seven mutants and their control revealed a total of 175 polymorphic bands out of 235 reproducible products. This corresponds to a level of 74.47% polymorphism. The number of amplicons /primer ranged from 3 (OP-B05) to 18 (OP-O16), whereas the number of polymorphic bands per primer ranged from 2 (B05) to 16 (OP- O16). (Table 5).

Genetic relationships among the seven mutants and the control

The genetic relationships among the seven mutants and their control examined using the Dice coefficient to compute the similarity matrices. These similarity matrices were employed to generate dendrogram using the UPGMA method. Based on the RAPD data, the genetic similarities ranged from 60.6% to 89.5% (Fig. 2). In this respect, Ali *et al* (2007) evaluated the similarity coefficient among thirty local and exotic *Brassica* genotypes using RAPD markers and found that the pairs of similarity coefficients ranged from 21.54 to 59.36%.

Table 4. Primer code, number of monomorphic amplicons, number of polymorphic amplicons, total number of amplicons and percentage of polymorphism as revealed by RAPD markers of seven mutants and their control

Primer	Monomorphism	Polymorphism	Total	Percentage
OP-D16	6	6	12	50.00%
OP-A11	5	8	13	61.54%
OP-S03	7	6	13	46.15%
OP-B06	2	8	10	80.00%
OP-B01	3	13	16	81.25%
OP-C20	4	6	10	60.00%
OP-D19	1	6	7	85.71%
OP-S01	5	7	12	58.33%
OP-B13	1	9	10	90.00%
OP-C02	6	7	13	53.85%
OP-B09	2	7	9	77.78%
OP-B05	1	2	3	66.67%
OP-B17	4	8	12	66.67%
OP-S02	0	8	8	100.00%
OP-B03	2	12	14	85.71%
OP-O16	2	16	18	88.89%
OP-C03	5	10	15	66.67%
OP-O14	1	14	15	93.33%
OP-P01	1	8	9	88.89%
OP-A09	2	14	16	87.50%
Total	60	175	235	74.47%

Table 5. The seven mutants and the control as characterized by unique positive and/or negative RAPD markers

Primer	Unique Negative Marker	Unique Positive Marker
OP-D16	---	220, 315, 450
OP-A11	310, 390	180, 230, 1350
OP-S03	400, 600, 800, 1100	720
OP-B06	610, 660, 1070	450, 500, 1800
OP-B01	80, 270, 310, 620	1500
OP-C20	700	900
OP-D19	570, 860	---
OP-S01	1700	---
OP-B13	900, 1000, 1070	---
OP-C02	1200, 1500	900, 1050, 1400
OP-B09	1070, 1350	---
OP-B05	700	---
OP-B17	450, 460	220, 870
OP-S02	550, 600	560
OP-B03	600	240
OP-O16	510, 530	300, 500, 570, 600
OP-C03	75, 130, 450	190
OP-O14	420, 530, 800, 900	220, 490
OP-P01	500	---
OP-A09	800	380
Total	42	27

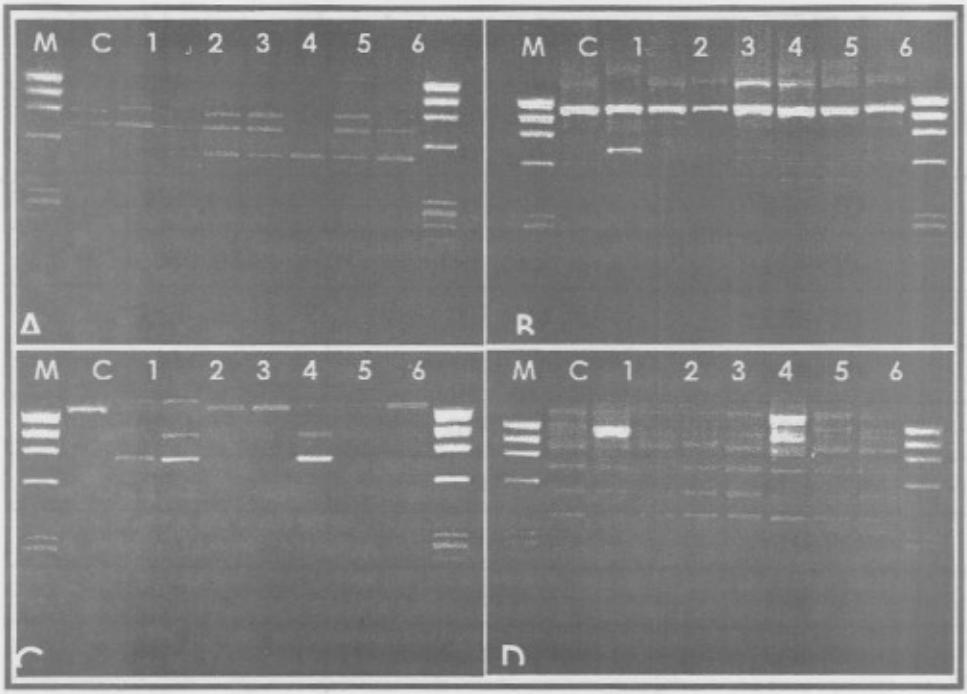

Fig. 1. Profiles of the seven mutants and the control as revealed by different RAPD primers (A: OPD-19, B: OPS-01, C: OPB-09 and D: OPC-02). M: phi x 174 DNA marker, C: control, 1-7: mutants.

7	100.0						
8	89.5	100.0					
4	86.7	84.5	100.0				
1	84.0	83.9	83.0	100.0			
5	83.0	83.3	87.8	83.3	100.0		
3	81.0	81.9	87.1	79.5	80.7	100.0	
6	78.8	79.7	80.9	76.8	76.8	76.0	100.0
2	69.1	69.9	67.6	71.7	68.3	61.2	60.6	100.0

Fig. 2. The genetic similarity matrices among the seven mutants and the control as computed according to Dice coefficient from RAPD data.

Ali *et al* (2007) studied the level of polymorphism among 30 local and exotic *Brassica* genotypes using four random primers and found that level of genetic polymorphism was in the range of 21.54 to 59.36%. On the other hand, Ananga *et al* (2008) evaluated thirty accessions representing two diploid *Brassica* species (*Brassica rapa* and *Brassica oleracea* var. *virids)* and fifteen tetraploid cultivars *(Brassica napus)* using 13 sets of RAPD primers which revealed 126 highly polymorphic bands with an average of 10 per primer. Also, the results of UPGMA dendrogram showed that *B. rapa* was highly diverse and was supported from three different basal branches, while *B. napus* accessions were generally monophyletic.

The RAPD based dendrogram separated the Mutant (251) from all the other mutants and their control. Besides there were clusters, one containing all the other mutants and their control, except the Mutant (39A), which was clustered only in the second group (Fig. 3). The separation of the Mutant (251) from all the other mutants and their control my be due to the exposure to high level of gamma radiation (300 Gry). Also mutant 251 showed a unique marker of up to 4380 bP, less than 6557bP in primer PS.o1, while primer 0PB-09, showed unique markers up to 23130 bP, also primer OPC-02, showed triple band between molecular markers 941 bP to 23130 bP. Also Mutants 36, 37, 38, with similar shattering, while Mutant 39A and 39B at the same shattering but Mutant (39 B) white found that primer OPD-19 is unique markers in 23130Bp also found unique markers approximately 222bP. In addition primer OPb-09 Mutant 39A (green) found that unique markers up to 23130 Bp, and 9416 Bp. Also primer opC-o2 overlapped in three bands starting with molecular weight 9557bp up to 23130 bP.

Mutants identification by unique markers

T he twenty decamer primers detected unique positive and/or negative specific markers identifying six out of the seven mutants. Each of these primers revealed unique markers for one or more mutants. The total number of unique bands across the seven mutants and their control was 69 including 27 unique positive markers (UPM) and 42 unique negative markers (UNM). The number of both UPM and UNM ranged from zero to 4 in the different mutants. The mutant (251) was characterized by the highest number of UPM and UNM, (18 and 22, respectively). Table (5) shows the number of both UPM and UNM as reveald by 20 decamer RAPD primers.

CONCLUSION

The selection of shattering resistance in breeding programs is very important for canola .The calculated similarity matrix showed considerable genetic variability in the studied seven mutants and their control showing variation in shattering resistance. The Mutant 251 revealed very high resistance to shattering, while Mutant 36, 37, 38 and 39 were semi resistance shattering. The Mutant 251 showed a unique marker from all the other mutants. Other entries were clustered into two clusters one clustered containing all the other mutant and their control and the other Mutant 251.

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انتخاب الطفرات المقاومة للانفراط على المستوى الجزيئى فى نباتات الكانولا

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يهدف هذا البحث الى استخدام طرق التربية الجزيئية لتحديد الطفرات المقاومة للإنفراط فى الكانولا وباستخدام طرق التربية التقليدية فى الكشف عن الطفرات المقاومة للإنفراط بترك النباتات بعد النضج الفسيولوجى من ٢٥ : ٢٨ يوم ويتم هز النباتات للكشف عن الطفرات المقاومة للإنفراط وعدم المقاومة للإنفراط وقد وجدت إختلافات بين الطفرات حيث كانت الطفرة ٢٥١ شديدة المقاومة للإنفراط

أما الطفرات ٣٦ و ٣٧ و ٣٨ و ٣٩ كانت نصف مقاومة للإنفراط بينما الطفرة ١٤٣ كانت نصف مقاومة للإنفراط ومتأخرة فى النضج ١٥ يوم .

ولقد أظهرت النتائج تباين في الصفات حيث اذهرت الطفرة ٣٩ بعد ٥٤,٨٨ يوم بينما الطفرة ٢٥١ كانت متأخرة جدا عن باقي الطفرات.

أما صفة ارتفاع النبات فأظهرت الطفرة ٢٥١ كان اطول النباتات بينما الطفرة ٣٦ أعطت أقل قيمة لهذه الصفة. أما صفة عدد الأفرع وجد أن الطفرة ٢٥١ أعطت أعلى قيمة بينما الطفرة ٣٦ أعطت أقل قيمة لهذه الصفة. أما صفة إرتفاع أول فرع ثمري وجد أن الصنف باكتول أعطى أعلى قيمة وجد أن الطفرة ٢٥١ أعطت اول فرع ثمري على ارتفاع منخفض .

بالنسبة للمنطقة الثمرية وجد أن الطفرة ٢٥١ أعطت أعلى قيمة بينما الطفرة ٣٦ أعطت أقل قيمة. أما صفة إرتفاع أول قرن وجد أن الطفرة ٢٥١ أعطت أعلى قيمة بينما الطفرة ٣٦ أعطت أقل قيمة. أما بالنسبة إلى محصول النبات وجد أن الصنف سرو٤ أعطى أعلى قيمة وكذلك وجد أن الطفرة ٣٨ كانت عالية في صفة محصول النبات تتعادل مع سرو٤ بينما وجد أن الطفرة (١٤٣) أعطت أقل قيمة. أما صفة وزن ال ١٠٠٠ بذرة فقد وجد أن الطفرة ٣٦ أعطت أعلى محصول . وكذلك أن صفة محصول الزيت وجد أن الطفرة ٣٧ أعطت أعلى قيمة بالنسبة المئوية للزيت بينما وجد أن الصنف باكتول أعطى أقل قيمة في النسبة المئوية للزيت.

وقد أظهر التحليل الجزيئي لكل من الطفرات والكنترول باستخدام عشرون من بادئات التكبير العشوائي لجزيئيات الحامض النووي RAPD – DNA وعددها ٢٣٥ شظية من DNA من بينهم ١٧٥ شظية متباينة تمثل نسبة تباين 74.47% وتراوح عدد شظايا / بادئ ما بين ٣ شظايا (بادئ B05) إلى ١٨ شظية (بادئ O16) بينما تراوحت عدد الشظايا المتباينة / بادئ ما بين ٢ شظايا (بادئ B05) إلى ١٦ شظية (بادئ O16) وكانت إجمالي عدد الشظايا المتباينة الفريدة في الطفرات ٦٩ شظية فريدة من بينها ٢٧ شظية فريدة موجبة و ٤٢ شظية فريدة سالبة.

ولقد أظهر التحليل على المستوى الجزيئى أن الطفرة ٢٥١ قد أنفصلت فى مجموعه واحدة وبها مجموعه من ى المعلمات الفريدة وكانت إحدى المعلمات أعلى من ٤٣٨٠ قاعدة وأقل من ٦٥٥٧ قاعدة مع البادئ

OPB-O9وكما أظهرت معلمات فريدة ٢٣١٣٠ قاعدة مع البادئ OPC-O2 كما أظهرت مجموعه من المعلمات الفريدة متداخلة مع بعضها البعض .

كما أظهرت الطفرة ٣٩ أ الخضراء العادية بمقارنتها بالطفرة ٣٩ البيضاء إختلافات على المستوى الجزيئى أى أن الطفرة البيضاء مع البادئ OPD19معلمات فريدة أعلى من ٢٣١٣٠ قاعدة ايضا وجد معلمات فريدة عند ٢٢٢ قاعدة مع البادئ OPB –O9 مع الطفرة ٣٩ الخضراء معلمات فريدة عند ٢٣١٣٠ قاعدة و ١٠٥٨ قاعدة.

ويظهر ان أنتخاب طفرات مقاومة للإنفراط مهم جدا فى برامج التربية حيث تم إنتخاب طفرات مقاومة للإنفراط بالطرق التقليدية وطرق التربية الجزيئية حيث كان الطفرة ٢٥١ شديدة المقاومة للإنفراط عن باقى الطفرات وكانت الطفرات ٣٦ و٣٧ و ٣٨ و٣٩ نصف مقاومة للإنفراط الطفرة ١٤٣ كانت متأخرة فى النضج ١٥ يوم .