GENETIC RELATIONSHIPS AMONG SOME CANOLA CULTIVARS (*Brassica napus* L.) BASED ON ISSR AND RAPD ANALYSES

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Rape seed is one of the winter oil crops that grow well in Egypt's newly reclaimed lands. Its seeds contain more than 40% excellent edible semi dry oil. Consequently, rape-seed is considered a promising oil crop to decrease the gap between oil production and consumption in the country. Accordingly, looking for high yielding rape varieties adapted to the local conditions is considered an important objective (Sharaan, 1987). Gallab and Sharaan (2002) reported that the presence of double zero type of rape seed (oil contains less than 2% erucic acid and less than 30 µmol/g glucosinolate) encouraged its utility as a source of edible oil overall the world. Liu and Wang (2006) reported that polyploidization has been viewed as a highly dynamic process and a major force in the evolution of higher plants including many important crops. Based on the inter-simple sequence repeat (ISSR) analysis, the different degrees of A, B or C genomic modifications were observed in the three *Brassica* allopolyploids. ISSR data supported that a higher degree of ancestral genomic divergence gave rise to a greater frequency of genomic change of polyploidy.

Simple sequence repeats (SSR) are widespread in the genome and the average heterozygosity index of these markers has been reported to be higher than any other single locus approach (Powell *et al.*, 1996). Recently, inter-simple sequence repeat (ISSR) markers have emerged as an alternative system with reliability and advantages of microsatellites (SSR). The technique involves amplification of genomic segments flanked by inversely oriented and closely spaced microsatellite sequences by a single primer or a pair of primers based on SSRs anchored 5' or 3' ends with 1-4 purine or pyrimidine residues. The sequences of repeats and anchor nucleates are arbitrarily selected. Coupled with separation of the amplification products on a polyacrylamide or agarose gels, ISSR amplification can reveal a much larger number of fragments per primer than RAPD. It is concluded that ISSR technique provides a quick, reliable and highly informative system for DNA fingerprinting. ISSR markers are inherited in Mendelian mode and segregated as dominant markers. This technique has been widely used in the studies of cultivar identification, genetic mapping, gene tagging, genetic diversity, evolution and molecular ecology (Zietkiewicz *et al*., 1994; Leroy *et al*., 2000; Leroy *et al*., 2001; Wang, 2002; Bornet and

Branchard, 2004; Ripley and Roslinsky, 2005).

Comparison of ISSR and other PCR-based markers have shown their efficiency in plant breeding (Raman *et al*., 1999; Adams *et al*., 2003; Archak *et al*., 2003; Galvan *et al*., 2003; Mogg and Bond, 2003; Javidfar *et al*., 2006). As a result of these advantages and their universality and easiness of development ISSR markers are more and more requested. Gostimskii *et al.* (2005) reviewed briefly the organization and variation of plant genome using molecular markers with special emphasis on random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and other markers. They reported that markers have been demonstrated to be promising for identifying cultivars and determining the purity of genetic strains of pea. Genetic relationships between strains, cultivars, and mutants of pea have been studied. The possibility of using of molecular markers for studying somaclonal variation and detecting mutagenic factors in plants during long-term spaceflights is considered. The prospects of using DNA markers for understanding the organization and variability of higher plant genomes were discussed. Wolfe (2005) reported that inter-simple sequence repeat (ISSR) markers were originally devised for differentiating among closely related plant cultivars but have become extremely useful for studies of natural populations of plants, fungi, insects, and vertebrates. Liu *et al.* (2006) reported that the development of yellow-seeded varieties of *Brassica napus* for improving the oilseed quality, characteristics of lower fiber content and higher protein and oil content has been a major focus of breeding researches worldwide in recent years. A linkage map was constructed with 164 markers including 125 AFLP, 37 SSR, 1 RAPD and 1 SCAR markers distributed over 19 linkage groups.

This study was conducted to investigate oil productivity and some yield related traits in ten canola cultivars. Moreover, to study the genetic relationships among these cultivars based on the banding patterns of them using ISSR and RAPD-PCR techniques and to conduct the consensus tree of the ten cultivars under investigation.

MATERIALS AND METHODS

Ten canola cultivars namely, Pactol (French cultivar), Serw 4 (Egyptian cultivar) [obtained from Oil Crops Department, Field Crops Research Institute, ARC, Giza, Egypt], Arabella, Idol, Anja, Aurora, Bingo, Caramba, Ediga and Star (German cultivars) [obtained from Plant Genetics and Crop Plant Research Institute (IPK), Gatersleben, Germany] were used in this study. The ten cultivars were grown up to the seed maturity stage in a randomized complete block design with four replications during the two successive seasons 2004-2005 and 2005-2006. Traits measured were plant height (cm), number of branches/plant, number of pods/plant, seed weight/plant (g), oil yield (g)/plant and seed oil percentage.

Oil was extracted from seeds using diethyl ether and measured according to A.O.A.C (1990). Oil percentage calculated as the ratio of oil content/plant to seed weight/plant x 100.

The data obtained from the two successive seasons were statistically analyzed using Mstat C program. Correlations were calculated and analyzed for the oil productivity and some yield related traits using Costat program.

DNA was extracted according to Junghans and Metzlatt (1990) from the ten cultivars. The extracted DNA was used to perform RAPD-polymerase chain reaction (PCR) according to Williams *et al*. (1990) using 15 arbitrary 10-mer primers (Operon Technologies, Inc.) as presented in Table (1) and ISSR-PCR according to Wang (2002) using 12 primers as shown in Table (2). PCR products were analyzed on 1.2% agarose gels and visualized using ethidium bromide and UV transilluminator. The consensus tree was developed based on the banding patterns of the ten cultivars using SPSS statistical analysis program to study the genetic relationships among the ten cultivars at the molecular level.

RESULTS AND DISCUSSION

Results of the six yield-related traits (Table 3) showed significant differences among cultivars in the first and the second seasons as well as the combined of both seasons. Cultivar Idol recorded the highest number of pods/plant; seed weight and oil yield/plant as well as seed oil percentage, while cultivar Ediga was the lowest for these traits. Plant height ranged from 123.63 cm (Arabella) to 243.63 cm (Bingo), indicating a wide range of variation in this trait among canola cultivars and in turn it is possible to improve this trait through selection. Regarding number of branches/plant, Arabella cultivar produced the highest number and averaged 14.38 branches/plant, while cultivar Bingo was the lowest with an average of 5 branches/plant. Concerning number of pods/plant the average of the two seasons ranged from 235.44 to 515.38 pods/plant for Ediga and Idol cultivars, respectively. This wide range of variation confirms the possibility of improving this trait through selection. Cultivar Idol yielded the highest seed weight/plant with an average of 26.67 g., while cultivars Pactol and Ediga averaged 14.73 and 15g being the lowest, respectively.

Oil yield/plant ranged from 5.78 to 13.43 g in cultivars Ediga and Idol, respectively. The last investigated trait was oil percentage which ranged from 37.88% to 50.28% in cultivars Ediga and Idol, respectively. The results over all traits indicated that cultivar Idol recorded the highest oil yield/plant and the other related traits, while cultivar Ediga was the lowest.

The results of correlation between all studied traits revealed that there are significant positive correlation between the plant pod number and both of seed

weight/plant, oil percentage and oil yield/plant (0.728, 0.446 and 0.714, respectively), as well as, between seed weight/plant and both of oil percentage and oil yield/plant (0.540 and 0.972, respectively) as shown in Table (4). On the other hand, the correlation between number of branches and oil% was positively significant (0.329). Soomro *et al*. (2005) investigated correlation of grain yield and its components in 8 commercial *Brassica* sp. cultivars and they reported that grain yield showed significantly positive correlation with plant height, seed index, number of branches/plant and pods/plant.

RAPD results in Table (5) produced scorable banding patterns with seven primers out of the 15 used ones. Figure (1) represents some RAPD primers banding patterns. A total of 46 DNA fragments were amplified with different lengths overall the ten cultivars with the seven primers. The results also showed that 19 DNA amplified fragments were monomorphic in the ten cultivars and 27 were polymorphic. Five DNA amplified fragments were considered as cultivar specific markers where primer OP-O06 produced the first unique band with length of 2850 bp in cultivar Idol only. Another cultivar-specific marker was produced with length of 2550 bp in cultivar Caramba using primer OP-Z13. In addition the third cultivar-specific marker was produced with length of 280 bp in cultivar Serw 4 using primer OP-O15. Finally two cultivar specific markers were produced with lengths of 850 and 610 bp in cultivar Bingo using primer OP-O07. The cultivar-specific marker using primer OP-O06 with length of 2850 bp in cultivar Idol may be related to the high oil content where cultivar Idol was the highest one in oil content.

ISSR-results as shown in Table (6) using eight primers out of twelve ones produced scorable banding patterns. A total number of 92 DNA fragments were amplified with different lengths overall the ten cultivars under investigation. The results showed that 33 DNA amplified fragments were monomorphic in the ten cultivars and 59 amplified fragments were polymorphic. Figure (2) represents some ISSR banding patterns. Eight DNA amplified fragments considered as cultivar-specific markers. In Anja, primer 844A produced a cultivar-specific marker with length of 1350 bp and two cultivar-specific markers with lengths of 460 and 420 bp were produced in cultivar Star using the same primer. It also produced a cultivar-specific marker for cultivar Aurora and Ediga with length of 900 bp and 670 bp, respectively. Cultivar-specific markers with lengths of 910 and 570 bp in were produced in cultivar Caramba using primers HB1 and HB9, respectively. Primer HB1 produced one more cultivar-specific marker with lengths of 900 bp in cultivar Star.

According to RAPD results the most two closely related cultivars were Ediga and Star (Table 7) with the highest similarity index (0.923), also cultivars Arabella and Anja were closely related

with high similarity index (0.918). On the other hand, the results indicated that the two most distantly related cultivar were Bingo and Ediga with low similarity index (0.698), these cultivars located very far from each other in the consensus tree (Fig. 3). Results indicated also that the two contrasting cultivars in oil content (Idol and Ediga) were distantly related cultivars to some extent, where they located in two different sub-clusters in the consensus tree with moderate similarity index (0.807).

The results of RAPD dependent consensus tree and similarity indices shown in Table (7) and Fig. (3) indicated that cultivars Aurora, Caramba and Bingo located in one of the two main clusters in the consensus tree. While, cultivar Serw 4 is located in a sub-cluster of the second main cluster and cultivars Idol, Anja, Arabella, Pactol, Star and Ediga are located in the other sub-cluster of the second main cluster of the consensus tree.

Similarity indices and consensus tree were developed on the base of the banding patterns of the ten cultivars using 12 ISSR primers as shown in Table (8) and Fig (4). According to ISSR results (Table 8), the most two closely related cultivars were Anja and Ediga with the highest similarity index (0.883). On the other hand the most two distantly related cultivars were Bingo and Ediga with low similarity index (0.723), the two cultivars located very far. The results indicated also that the two contrasting cultivars in oil content (Idol and Ediga) were moderately related cultivars where they located in two different sub-clusters in the consensus tree with moderate similarity index (0.835).

The results indicated that the consensus tree was divided the cultivars into two main clusters, the first included cultivars Anja, Ediga, Caramba, Idol and Star. The second main cluster included cultivars Aurora, Serw 4, Arabella, Bingo and Pactol (Fig. 4).

Cultivars distribution on the consensus tree according to the banding patterns of ISSR differed than that based on RAPD banding patterns which may be due to that each technique amplified different parts of the genome, so it is better to use the combination of the banding patterns of the two technique to use more segments of the genome that will increase the validity of the consensus tree. Results of the combined data as shown in Fig. (5) and Table (9) exhibited that the most two closely related cultivars were Idol and Anja with the highest similarity index (0.874) and cultivars Star and Ediga with similarity index (0.862). On the other hand, the most two distantly related cultivars were Ediga and Bingo with low similarity index (0.714).

The consensus trees of the ten cultivars were developed based on their banding patterns with the 12 used ISSR primers, 15 RAPD primers and the combination of both techniques (Fig. 5) showed that the consensus tree was divided into two main clusters, the first

included cultivars Idol, Anja, Star, Ediga, Pactol and Serw 4 and the second main cluster included cultivars Caramba, Arabella, Aurora and Bingo. The results indicated that the two contrasting cultivars in oil content (Idol and Ediga) were moderately related cultivars where they located in two different sub-clusters in the consensus tree with moderate similarity index (0.826).

The present findings agreed with those of Azzam and Abbas (2005), who recorded significant differences between Pactol and Serw 4 cultivars for yield and yield components traits. Results are also in accordance with the findings of Reddy *et al*. (2002), who reported that inter simple sequence repeat ISSR is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic DNA (RAPD). They also reported that ISSR markers are highly polymorphic and are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology.

The aforementioned results corroborated that RAPD and inter simple sequence repeat (ISSR) sequences as molecular markers can lead to the detection of polymorphism and identifying cultivars which confirmed the findings of Bornet *et al.* (2002) and Gostimskii *et al.* (2005).

SUMMARY

Oil productivity and some yield related traits were investigated in ten cultivars of canola (*Brassica napus* L.) These traits are plant height, branch number, seed weight/plant, number of pods/plant, oil yield/plant and seed oil percentage. Significant variations were detected among the ten cultivars under investigation for all traits. Moreover, results indicated that there are significant positive correlations between number of pods/plant and both of seed weight/plant, oil% and oil yield/plant, as well as, between seed weight/plant and both of oil% and oil yield/plant. The correlation between number of branches and oil% was positively significant.

RAPD-results produced scorable banding patterns with 7 primers out of 15. A total number of 46 DNA fragments were amplified with different lengths overall the ten cultivars with the seven primers. The results showed that 19 DNA amplified fragments were monomorphic in the ten cultivars and 27 were polymorphic. Eight DNA amplified fragments were considered as cultivar specific markers. ISSR-results using 8 primers out of twelve produced scorable banding patterns. A total number of 92 DNA fragments were amplified with different lengths overall the ten cultivars. The results showed that 33 DNA amplified fragments were monomorphic in the ten cultivars and 59 amplified fragments were polymorphic. Eight DNA amplified fragments were considered as cultivar-specific markers. The similarity indices and the consensus trees of the ten cultivars were developed based on the banding patterns of 12 ISSR primers, 15

RAPD and the combination of both techniques indicated that the two contrasting cultivars in oil content (Idol and Ediga) were distantly related cultivars.

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Table (1): RAPD primer names and their sequences.

Name	Sequence	Name	Sequence	Name	Sequence
OP-O01	GGCACGTAAG	OP-O15	TGGCGTCCTT	OP-Z10	CCGACAAACC
OP-O06	CCACGGGAAG	OP-O18	CTCGCTATCC	OP-Z13	GACTAAGCCC
OP-O07	CAGCACTGAC	OP-O20	ACACACGCTG	OP-Z15	CAGGGCTTTC
OP-O09	TCCCACGCAA	OP-Z03	CAGCACCGCA	OP-Z16	TCCCCATCAC
OP-O11	GACAGGAGGT	OP-Z06	GTGCCGTTCA	OP-Z17	CCTTCCCACT

Table (2): ISSR primer names and their sequences.

Name	**Sequence**	**Name**	**Sequence**	**Name**	**Sequence**
HB1	$(CT)_8TG$	HB12	$(CAG)_3GC$	17898A	$(CA)_6AC$
HB8	$(GA)_6GG$	HB15	$(GTG)_3GC$	17898B	$(CA)_6GT$
HB9	$(GT)_6GG$	844A	$(CT)_8AC$	17899A	$(CA)_6AG$
HB10	$(GA)_6CC$	844B	$(CT)_8GC$	17899B	$(CA)_6GG$

Table (3): Oil productivity and some related traits of ten canola cultivars in 2004/2005, 2005/2006 and the combined of the two seasons.

Cultivar	Season	Plant height (cm)	No. of branches/ plant	No. of pods /plant	Seed weight /plant (g)	Oil %	Oil yield /plant (g)
Aurora	2004-2005	135.75	8.25	271.25	17.36	42.30	7.35
	2005-2006	122.00	6.00	240.00	16.61	44.50	7.39
	Average	128.88	7.13	255.63	16.99	43.40	7.37
Caramba	2004-2005	139.50	12.25	260.25	15.12	42.79	6.47
	2005-2006	148.00	12.25	179.25	14.85	45.64	6.77
	Average	143.75	12.25	219.75	14.99	44.22	6.62
Bingo	2004-2005	242.25	5.50	211.25	16.15	41.23	6.67
	2005-2006	245.00	6.50	272.50	16.68	43.44	7.24
	Average	243.63	6.00	241.88	16.42	42.34	6.96
Idol	2004-2005	131.00	6.75	540.75	25.07	48.37	12.12
	2005-2006	120.50	5.75	490.00	28.26	52.18	14.74
	Average	125.75	6.25	515.38	26.67	50.28	13.43
Arabella	2004-2005	128.25	14.00	277.50	17.96	47.94	8.61
	2005-2006	119.00	14.75	343.75	22.67	52.14	11.82
	Average	123.63	14.38	310.63	20.32	50.04	10.22
Anja	2004-2005	129.23	8.40	219.67	12.22	41.95	5.12
	2005-2006	160.31	9.83	546.83	22.27	45.85	10.21
	Average	144.77	9.12	383.25	17.25	43.90	7.67
Pactol	2004-2005	129.26	8.02	171.47	14.46	45.76	6.62
	2005-2006	166.06	9.39	450.97	15.00	45.64	6.85
	Average	147.66	8.71	311.22	14.73	45.70	6.74
Serw 4	2004-2005	141.50	6.87	164.03	15.40	44.72	6.89
	2005-2006	180.97	11.14	491.64	18.56	49.25	9.12
	Average	161.24	9.01	327.84	16.98	46.99	8.01
Star	2004-2005	132.59	6.87	246.46	16.16	41.41	6.70
	2005-2006	160.00	7.58	329.00	19.17	42.06	8.06
	Average	146.30	7.23	287.73	17.67	41.74	7.38
Ediga	2004-2005	137.44	6.86	138.48	13.30	39.37	5.24
	2005-2006	174.50	7.07	332.40	16.70	37.87	6.32
	Average	155.97	6.97	235.44	15.00	38.62	5.78
$LSD_{0.05}$ (1)		11.68	3.41	112.05	3.44	2.13	1.56
$LSD_{0.05}$ (2)		13.80	2.56	145.69	5.67	0.79	2.64
$LSD_{0.05}$ (Combined)		9.50	1.98	94.65	3.38	1.14	1.54

Table (4): Correlation between the oil productivity and some of its related traits in canola (overall the ten cultivars and the two years).

Traits	Plant height (cm)	No. of branches / plant	No. of pods / plant	Seed weight /plant (g.)	Oil%
No. of branches/ plant	-0.158				
No. of pods/ plant	0.016	0.143			
Seed weight /plant (g.)	-0.128	0.102	0.728***		
Oil %	-0.289**	0.329**	0.446***	0.540***	
Oil content /plant (g.)	-0.199	0.157	0.714***	0.972***	0.714***

Significant at the level of 5% * Significant at the level of 1%

Table (5): Number of amplified fragments and specific markers of the ten canola cultivars using seven RAPD primers.

RAPD primers	TAF	Aurora		Caramba		Bingo		Idol		Arabella		Anja		Pactol		Serw 4		Star		Ediga		TSM
		AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
O06	5	3	0	2	0	4	0	5	1	3	0	3	0	3	0	3	0	3	0	1	0	1
O07	6	4	0	3	0	6	2	2	0	2	0	2	0	1	0	3	0	2	0	2	0	2
O15	4	2	0	2	0	3	0	3	0	3	0	3	0	3	0	3	1	3	0	3	0	1
O20	9	6	0	6	0	5	0	6	0	7	0	8	0	9	0	5	0	7	0	6	0	0
Z03	7	6	0	7	0	7	0	5	0	6	0	6	0	5	0	5	0	5	0	5	0	0
Z10	6	5	0	4	0	6	0	5	0	4	0	4	0	5	0	6	0	5	0	4	0	0
Z13	9	5	0	9	1	8	0	7	0	6	0	4	0	4	0	4	0	3	0	3	0	1
Total	46	31	0	33	1	39	2	33	1	31	0	30	0	30	0	29	1	28	0	24	0	5

TAF= Total amplified fragments, AF= Amplified fragments, TSM= Total specific markers, SM= Specific markers

Table (6): Number of amplified fragments and specific markers of the ten canola cultivars using eight ISSR primers.

ISSR primers	TAF	Aurora		Caramba		Bingo		Idol		Arabella		Anja		Pactol		Serw 4		Star		Ediga		TSM
		AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
844A	15	11	1	6	0	5	0	8	0	10	0	5	1	6	0	9	0	5	2	5	1	5
844B	14	7	0	8	0	6	0	11	0	3	0	8	0	7	0	5	0	11	0	8	0	0
17889B	9	3	0	8	0	5	0	9	0	3	0	9	0	5	0	8	0	8	0	8	0	0
HB1	9	6	0	5	1	6	0	1	0	1	0	1	0	1	0	3	0	5	1	3	0	2
HB9	11	10	0	10	1	9	0	10	0	8	0	9	0	7	0	10	0	10	0	8	0	1
HB12	11	11	0	11	0	11	0	11	0	11	0	11	0	9	0	11	0	11	0	11	0	0
HB15	16	16	0	16	0	11	0	12	0	16	0	14	0	15	0	16	0	15	0	14	0	0
17899A	7	4	0	5	0	6	0	5	0	5	0	3	0	6	0	5	0	4	0	3	0	0
Total	92	68	1	69	2	59	0	67	0	57	0	60	1	56	0	67	0	69	3	60	1	8

TAF= Total amplified fragments, AF= Amplified fragments, TSM= Total specific markers, SM= Specific markers

Table (7): Similarity indices for the ten canola cultivars on the base of their banding patterns with RAPD primers.

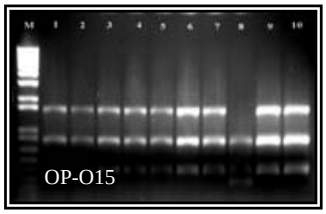
Cultivars	Aurora	Caramba	Bingo	Idol	Arabella	Anja	Pactol	Serw 4	Star
Caramba	0.875								
Bingo	0.857	0.861							
Idol	0.844	0.848	0.861						
Arabella	0.839	0.875	0.829	0.906					
Anja	0.820	0.825	0.783	0.857	0.918				
Pactol	0.787	0.762	0.754	0.825	0.852	0.900			
Serw 4	0.833	0.742	0.794	0.839	0.800	0.814	0.847		
Star	0.847	0.787	0.776	0.885	0.847	0.862	0.897	0.877	
Ediga	0.764	0.772	0.698	0.807	0.836	0.852	0.852	0.830	0.923

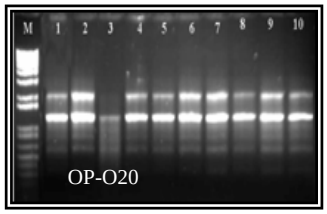
Table (8): Similarity indices for the ten canola cultivars on the base of their banding patterns with ISSR primers.

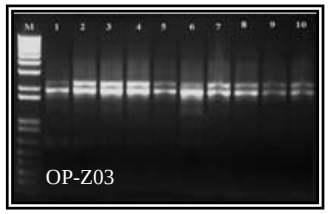
Cultivars	Aurora	Caramba	Bingo	Idol	Arabella	Anja	Pactol	Serw 4	Star
Caramba	0.803								
Bingo	0.835	0.813							
Idol	0.756	0.868	0.778						
Arabella	0.825	0.850	0.786	0.768					
Anja	0.750	0.837	0.739	0.882	0.729				
Pactol	0.790	0.800	0.730	0.748	0.772	0.759			
Serw 4	0.844	0.838	0.794	0.806	0.832	0.803	0.829		
Star	0.774	0.783	0.766	0.809	0.809	0.837	0.752	0.724	
Ediga	0.750	0.837	0.723	0.835	0.763	0.883	0.759	0.819	0.837

Table (9): Similarity indices for the ten canola cultivars on the base of combination of the banding patterns with RAPD and ISSR.

Cultivars	Aurora	Caramba	Bingo	Idol	Arabella	Anja	Pactol	Serw 4	Star
Caramba	0.826								
Bingo	0.843	0.830							
Idol	0.784	0.861	0.808						
Arabella	0.830	0.859	0.802	0.814					
Anja	0.772	0.833	0.755	0.874	0.793				
Pactol	0.789	0.787	0.739	0.774	0.800	0.807			
Serw 4	0.841	0.808	0.794	0.816	0.822	0.806	0.835		
Star	0.796	0.784	0.769	0.832	0.753	0.845	0.798	0.839	
Ediga	0.754	0.817	0.714	0.826	0.786	0.874	0.788	0.822	0.862







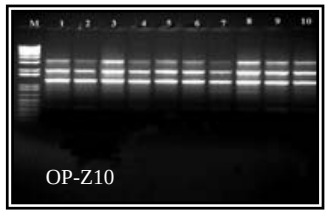
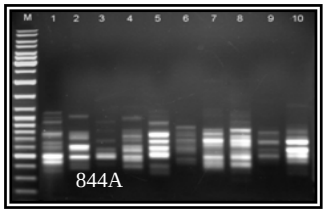
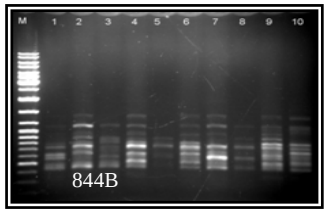
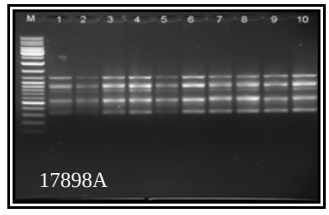


Fig (1): Banding patterns for 10 Canola cultivars with some RAPD primers. (M- Marker 1- Aurora 2- Caramba 3- Bingo 4- Idol 5- Arabella 6- Anja 7- Pactol 8- Serw 4 9- Star 10- Ediga)







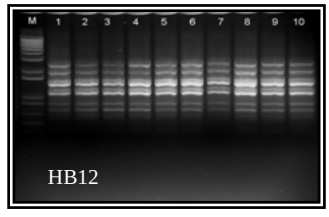


Fig (2): Banding patterns for 10 Canola cultivars with some ISSR primers. (M- Marker 1- Aurora 2- Caramba 3- Bingo 4- Idol 5- Arabella 6- Anja 7- Pactol 8- Serw 4 9- Star 10- Ediga)

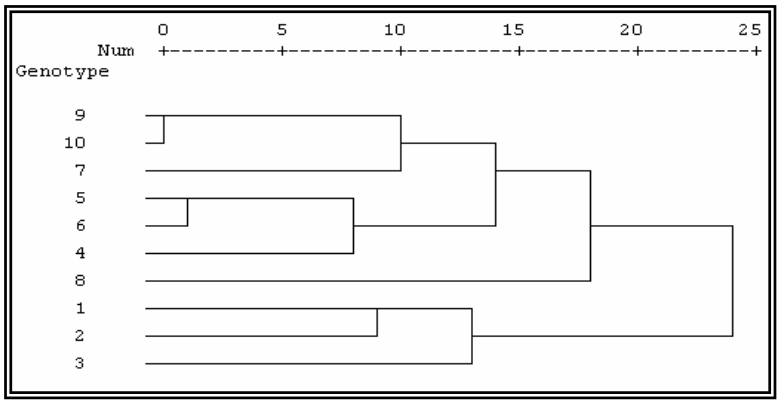


Fig. (3): Consensus tree for ten canola cultivars developed on the base of their banding patterns with RAPD primers.

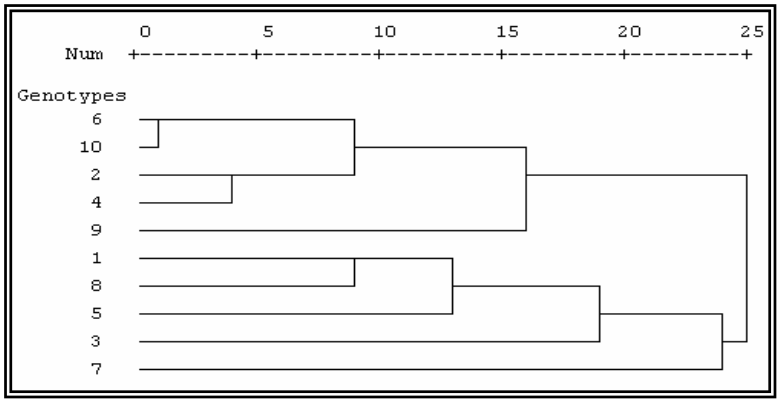


Fig. (4): Consensus tree for ten canola cultivars developed on the base of their banding patterns with ISSR primers.

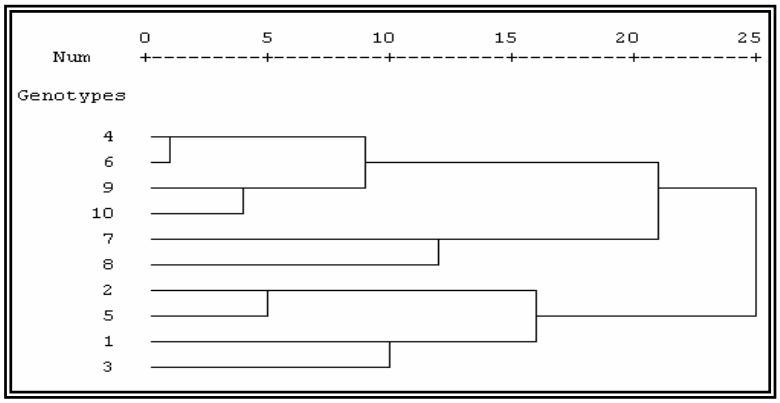


Fig. (5): Consensus tree for ten canola cultivars developed on the base of their banding patterns with combination of RAPD and ISSR.