MOLECULAR ASSESSMENT OF GENETIC DIVERSITY IN SOME CANOLA HOMOZYGOUS LINES

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Canola (*Brassica napus* L.) is considered one of the most important oilseed (oil content 40 to 45%) and protein rich crops in the world. Its cultivation has increased tremendously in recent years and currently it is the second largest contributor to the world supply of vegetable oil (Ben Ghnaya *et al*., 2008).

Assessment of genetic diversity is one of the essential steps in every plant breeding programmes. Study of genetic relationships between different genotypes in any crop such as canola is necessary to develop favorable strategies for breeding, improvement and employment of germplasms or genetic resources (Paterson *et al.*, 1991). Therefore, the study of genetic diversity and relationships among plant germplasms using molecular markers is of great importance in crop improvement. Various types of molecular markers techniques based on the polymerase chain reaction (PCR) can be used to estimate genetic diversity and fingerprinting in crops such as random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSRs), simple sequence repeats (SSRs) (Leal *et al.*, 2010). The utility of RAPD or ISSR as molecular markers for investigating genetic relationships among plants has been clearly established. These techniques were proposed for fingerprinting and used to study population genetics, taxonomy and phylogeny of many plant species (Maugham *et al*., 1996; Wolf and Randle, 2001). RAPD and ISSR molecular marker techniques are suitable and powerful tools for studies and assessment of genetic diversity among *B. napus* species (Abdelmigid, 2012; Safari *et al.*, 2013). Three-primer based RAPD (Triple-RAPD) is a new strategy used to increase RAPD potential in genetic diversity by using three different primers combinations per reaction (Mansour *et al.*, 2008). The efficiency of each primer to differentiate between genotypes was assessed in any molecular marker techniques by the assessment of value known as resolving power (Rp) (Hasnaoui *et al*., 2010). This value is characteristic of a primer which reflects overall suitability of a marker technique for the purpose of identification, as it is related to the number of genotypes distinguished by that primer (Prevost and Wilkinson, 1999). Also, resolving power are very useful parameters for the molecular diagnosis efficiency of any molecular marker technique. Prevost and Wilkinson (1999) and Saini (2010) studied resolving power as tool for comparison the diagnostic efficiency of ISSR and RAPD techniques. On the other

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hand, Principal Coordinate (PCo) analysis was used for the purpose of estimating the genetic diversity and quantitative variation between genotypes in plant (Rahim *et al.*, 2008; Sesli and Yeğenoğlu, 2010).

The objective of this investigation was to compare the effectiveness of some DNA fingerprinting techniques in terms of the assessment of genetic diversity among several homozygous lines of Canola.

MATERIALS AND METHODS

Ten homozygous lines of canola (*Brassica napus* L.) from different locations or countries as shown in Table (1) were used in this study. In late 2014, the seeds of these lines were supplied from Genet. Dep. Fac. of Agric., Mansoura Univ.

Total DNA was extracted from seeds of the studied homozygous lines using EZ-10 Spin Column Plant DNA Mini-Preps Kit from Bio Basic Inc. Genomic DNA was used as a template for Polymerase Chain Reaction (PCR) amplification using 20 RAPD primers and 14 ISSR primers in detecting polymorphism among studied lines. Amplification reactions in RAPD technique were performed according to Williams *et al.* (1990) using 25 µl reaction mixture containing the following; 2.0 µl of template DNA (50 ng/µl), 2.5 µl of dNTPs (2.5 mM), 1.5 µl of $MgCl_2$ (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH_2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95°C for 5 minutes, followed by 35 cycles at 96°C for 30 seconds, 37°C for 30 seconds each, and 72°C for 30 seconds, then a final cycle of 72°C for 5 minutes. Also, the amplification reactions in ISSR technique were performed according to Wolfe *et al.* (1998) in 25 µl reaction volume containing the following reagents; 3.0 µl of template DNA (25 ng/ µl), 2.5 µl of dNTPs (2.5 mM), 2.5 µl $MgCl_2$ (2.5 mM), and 2.5 µl of 10 x buffer, 3.0 µl of primer (10 pmol), 1 µl of *Taq* polymerase (1 U/µl) and 12.5 µl of sterile dd H_2O. The PCRs were programmed for one cycle at 94°C for 4 min, followed by 45 cycles of 1 min at 94°C, 1 min at 57°C, and 2 min at 72°C the reaction was finally stored at 72°C for 10 min. The PCR products were separated on a 1.5% agarose gels and fragments sizes were estimated with 1.5 kbp ladder markers (1500,1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp).

To increase the potential of RAPD-PCR technique, two various combinations of three different primers (Triple-RAPD) were used together in the same PCR reaction according to Mansour *et al.* (2008).

DNA banding patterns generated from RAPD, ISSR and Triple-RAPD techniques were analyzed by GelAnalyzer 3 program. These data scoring amplicons (bands) as present (1) or absent (0) for each primer and entered in the form of a binary data matrix. From this matrix, the molecular distances MD were estimated

using Nei & Li coefficients (Nei and Li, 1979) by computational package MVSP 3.1. Also, depending on this matrix, Cluster analysis and Principal Coordinate (PCo) analysis were performed using the same program.

Resolving power (Rp) of the primers was calculated according to Prevost and Wilkinson (1999) using the formula:

$$Rp = \sum I_a = \sum 1 - [2 \times (0.5 - p)]$$

Where, I_a (amplicon informativeness) was calculated for each amplicon scored individually by the primer, p being the ratio of studied lines containing the I amplicon.

RESULTS AND DISCUSSION

From Figs (1, 2 and 3), it appeared that all the three molecular marker techniques confirmed to be valid in discriminating between the ten homozygous lines of canola, these techniques revealed reproducible polymorphic patterns.

In these molecular marker techniques, 20 RAPD and 14 ISSR primers were then used to analyze the genetic diversity of ten homozygous lines of canola. Only eight RAPD and five ISSR primers as well as two various combinations of Triple-RAPD were successful in generating reproducible and reliable amplicons as shown in Table (2). A total of 115 amplicons, ranging from 109 to 1476 bp, were amplified using all the molecular marker techniques applied in this study, 59 (51.3%) of them were polymorphic; of which were 21 (18.3%) unique markers.

RAPD analysis

In the RAPD analysis, eight primers were successfully produced multiple band profiles with a number of amplicons varied from 2 (for OP-Q18) to 10 (for OP-B07 and OP-C19) with 144 (for OP-C19) to 1090 bp size range (for OP-C09) and polymorphism % between 00.0 (for OP-Q18) to 62.5% (for OP-A10). The primer OP-C19 showed the highest Rp (17.4) while, the lowest Rp (4.0) values was exhibited by the primer OP-Q18. Moreover, the primer OP-A09 produced the highest number of unique markers (4 positive and negative).

Triple-RAPD analysis

Data obtained from Triple-RAPD technique using two various combinations of three different primers presented in Table (2), showed that all combinations were successfully produced multiple band profiles. These combinations were able to produce a number of amplicons 7 and 10 as well as, polymorphism% were 85.7 and 40.0% while, the Rp value were 10.2 and 15.8 for triple a and b, respectively with 109 to 506 bp size range (for triple a). On the other hand, the triple-b only produced three unique markers.

Comparison between RAPD and Triple-RAPD techniques

In comparison between data obtained from the two Triple-RAPD combinations and their primers separately in RAPD technique (Figs 1, 2 and Table 2), it can be noted that the combination of

three primers resulted in the appearance of new amplicons that were not obtained when each used primer separately. This result was in agreement with those obtained by Mansour *et al.* (2008). The total number of amplicons resulting from two Triple-RAPD combinations (10.0 and 7.0) were higher than the average of amplicons for the three primers were used in each combination (8.7 and 3.7). Also, the Rp values recorded for two Triple-RAPD combinations (15.8 and 10.2) were higher than the average of Rp values for the three primers were used in each combination (15.1 and 6.0). The results of these genetic diversity parameters may reveal that reproducible banding patterns for Triple-RAPD combinations surpassed that from single primer RAPD. This reproducibility might improve results using of further primer combinations.

ISSR analysis

Only five ISSR primers were successfully to produced multiple band profiles with a number of amplicons (Fig. 3 and Table 2) varied from 4 (for 14A primer) to 12 (for HB-10 primer) with 164 to1476 bp size range for the same previous primer (HB-10), which showed that the highest polymorphism% and RP were 66.67% and 16.4, respectively. Also, the Rp values for HB-08 and HB-12 primers were equal to that of HB-10 primer. On the other hand, all ISSR primers except HB-15 produced unique markers ranged from one (for 14A) to three (for HB-08 and HB-12).

The primers HB-08 and HB-10 that were based on the repetitive motif GA produced more amplicon (9 and 12 amplicon, respectively) than the primers based on any other repetitive motifs used in this study. This result was in harmony with what was obtained by Abdelmigid (2012) and Safari *et al.* (2013). Abdelmigid (2012) who indicated that the same repetitive motif $(GA)_n$ was more frequent than the repetitive motif targeted by the other ISSR primers successfully in generating reliable amplicons in canola. Also, from the results obtained by Safari *et al.* (2013) observed that among 110 amplicons, 72 amplicons were generated by primers that contain the same repetitive motif (GA or AG). From all of these observations, it can be indicated that the microsatellites content of repetitive motif $(GA)_n$ are more frequent than the repetitive motif targeted by the other ISSR primers successfully in generating reliable amplicons in canola.

Comparison of RAPD, Triple-RAPD and ISSR data

From results presented in Tables (3 and 4), six homozygous lines of canola were characterized by 21 unique markers (four positive and 17 negative). These marker loci were nine unique markers (one positive and eight negative) by RAPD technique, three unique markers (one positive and two negative) by Triple-RAPD combinations and nine unique markers (two positive and seven negative) by ISSRs technique. Out of these nine makers, five unique markers were gener-

ated by primers that contain the repetitive motif (GA) suggesting that this microsatellite is more successful in generating reliable markers in canola. Among the ten homozygous lines of canola, six showed unique markers; DH3 line had the highest number of negative markers using all techniques, while Pactol was had the highest number of positive markers (three positive markers). These results, indicates that all techniques applied in this study were succeeded in showing different molecular markers which can be relied upon in distinguishing between studied homozygous canola lines. Although, ISSR technique was the best in terms of the average resolving power Rp (13.6) and number of marker amplicon/PCR reaction (1.8), all techniques still considered suitable tools for sufferable fingerprinting diagnostic markers for all studied homozygous lines in canola. These findings were in harmony with that illustrated previously by many studies, such as Fernández *et al.* (2002) in Barley, Hussein *et al.* (2005) in Date Palm, Abd El-Hady *et al.* (2010) in Vigna and Abdelmigid (2012) in Canola.

Molecular distances

Based on RAPD, Triple-RAPD, ISSRs, and combined data presented in Tables (5 to 8) the highest molecular distance (MD) according to Triple-RAPD data (Table 6) was among DH3 with both serw4 and serw8 (0.33), while the lowest MD according to the same data was between serw4 and serw8 (0.00). According to RAPD, ISSR, and combined data the MD ranged from 0.02 to 0.20, 0.01 to 0.24 and 0.05 to 0.21, respectively.

Correlation coeffecient RAPD, Triple-RAPD and ISSR dissimilarity matrices

From MD data for all molecular marker techniques applied in this study, the compatibility and degree of correlations among MD revealed by RAPD, Triple-RAPD, ISSRs and combined data were calculated (Table 9). Highly significant positive correlations were found among all of these molecular marker techniques and combined data. This result was in agreement with Sharaf *et al.* (2009) who found highly significant correlation coefficients among RAPD, ISSR, AFLP and their combined data in seven cotton genotypes. The correlation between ISSRs and combined data was the highest (0.887) followed by RAPD technique with combined data (0.835), then Triple-RAPD with combined data (0.742). On the other hand, the lowest correlation was recorded among RAPD and Triple-RAPD data (0.535).

These positive correlations between all molecular techniques and combined data, indicate the reliability of the combined data for molecular distances in accurate assessment for genetic diversity, as well as in identifying the genetic relationships between all the canola lines. This result was in agreement with those obtained by Abd El-Hady *et al.* (2010) who indicated that combined RAPD and ISSR analyses provided the possibility of

identifying the investigated Vigna genotypes.

Cluster analysis and principal coordinate based on combined data

Accordingly, Cluster analysis and Principal Coordinate (PCo) analysis for ten homozygous lines of canola were performed based on the relative genetic distances from combined data (Figs 4 and 5).

The PCo analysis determines the consistency of the differentiation among the cultivars defined by the cluster analysis (Adhikari *et al*., 2015). Cluster analysis indicated that the genetic distances between all the canola homozygous lines were very low, indicating that degree of similarity were high between these lines. Nevertheless, PCo analysis has managed to divide these lines into four groups. The first eigenvector (PCo axis1) accounted for 27.65% of the combined data variation among groups. This value indicated that the PCo analysis was succeeded in assessment of genetic diversity and description of heterogeneity within studied lines (Sonja *et al*., 2008).

DNA profile

Finally, it became clear that these molecular techniques were efficient in terms of assessing genetic diversity among the canola homozygous lines, suggesting the possibility of using results of these techniques in signing genetic fingerprinting for these lines. Therefore, these genetic fingerprints for all homozygous lines of canola were performed as DNA-profile diagram (Fig. 6) based on 115 amplicons obtained using RAPD, Triple RAPD and ISSR techniques. This profile showed that the amplicons per line were variously ranged from 77 (for DH3) to 107 (for Pactol) with average number of 93.3. In addition, six homozygous Lines showed 21 marker loci, these markers were spread over these homozygous lines variously differentiate each line for the other. These results indicated that DNA-profiling diagram also is a useful tool for molecular identification for these studied homozygous lines. So, it was deduced that RAPD and ISSR primers used in this investigation were with high degree of confidence for the molecular identification. This was in agreement with those results obtained by Archak *et al*. (2003). Based on that, these techniques have the potential to identify specific markers for homozygous lines of canola, which indicates the possibility of using these markers as resources for the breeding and management of canola. Therefore, it is proposed to use a largest possible number of primers for these techniques or apply other additional techniques to have better assessment for genetic variability in germplasm of canola. And thus gaining more efficiently exploitation of available variations to improve this important economically crop (Abdelmigid, 2012).

This study indicates that RAPD, Triple-RAPD and ISSR molecular marker techniques, as well as the analyses based on these techniques such as cluster analysis, principal coordinate analysis and DNA profile are suitable tools for as-

sessing genetic diversity, signing genetic fingerprinting and also potential to identify specific markers for the canola homozygous lines. Moreover, highly significant positive correlations were found among all of these molecular marker techniques and combined data, this indicate that the possibility of relying the combined data for molecular distances in accurate assessment for genetic diversity. All of these lead to consider these techniques as important tools for breeding and improvement of canola.

SUMMARY

In order to assess genetic diversity among ten homozygous lines of Canola, eight RAPD and five ISSR primers as well as two various combinations of Triple-RAPD were successful in generating reproducible and reliable amplicons. A total of 115 amplicons were amplified using all molecular marker techniques applied in this study, 59 (51.3%) of them were polymorphic of which 21 (18.3%) were unique markers. ISSR technique was the best in terms of the average resolving power Rp (13.6) and number of marker amplicons/PCR reaction (1.8). From all of the targeted microsatellites by ISSRs, the repetitive motif (GA)n was more frequent from all repetitive motifs targeted by the other ISSR primers. Highly significant positive correlations were found for molecular distance among all of these molecular marker techniques and combined data, which indicates the reliability of the combined data for molecular distances in accurate assessment for genetic diversity and identifying the genetic relationships between all studied homozygous lines in canola. Accordingly, Cluster analysis and Principal Coordinate (PCo) analysis based on combined data were used for indicating degree of similarity which was high between all the studied homozygous lines. Moreover, PCo analysis have managed to divide these lines into four groups indicating that PCo analysis was succeeded in assessment of genetic diversity and description of heterogeneity within studied lines. On the other hand, the genetic fingerprint for all homozygous lines of canola was performed as DNA-Profile diagram, showing that the amplicons per homozygous line were differentiated for each other with average of 93.3 amplicons and with an appropriate number of diverse molecular markers, indicating that DNA-Profile also is a useful tool for molecular identification. Based on that, these techniques have the potential to identify specific markers for homozygous lines of canola, which indicates the possibility of using these markers as reliable resources for breeding and improvement of canola.

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Table (1): Name and origin of different canola homozygous lines used in this study.

No.	Name	Country of origin	Pedigree
1	Fido	England	Exotic homozygous line
2	Drakkar	France	Exotic homozygous line
3	Pactol	France	Exotic homozygous line
4	Serw4	Egypt	Homozygous line obtained *via* anther culture
5	Serw6	Egypt	Homozygous double haploid line obtained from natural haploid plant
6	Serw8	Egypt	Homozygous line
7	DH1	Egypt	Homozygous double haploid line obtained from anther culture in Serw4
8	DH3	Egypt	Homozygous double haploid line obtained from anther culture for hybrid between Drakkar X Serw6
9	DH4	Egypt	Homozygous double haploid lines obtained from anther culture for hybrid between Serw6 X Fido
10	DH5	Egypt	

Table (2): List of primers for the three molecular marker techniques, number of amplicons types, total number of amplicons, percentage of polymorphism and resolving power obtained by analyzing different canola homozygous lines.

Molecular marker technique	Symbol	Primer Name	Primer sequence (5´→ 3´)	Molecular size range (bp)of amplicons	Number of amplicon types			Total number of amplicos	Polymorphism (%)	Resolving power Rp)
					Monomorphic	Polymorphic without unique	Unique (+ or -)			
RAPD	a	OP- A01	5´CAGGCCCTTC3`	288 :709	3	3	1	7	57.14	12.4
	b	OP- A09	5´GGGTAACGCC3`	241:808	5	-	4	9	44.44	15.6
	c	OP- A10	5´GTGATCGCAG3`	167:644	3	3	2	8	62.50	13.8
	d	OP-B07	5´GGTGACGCAG3`	158:719	7	3	-	10	30.00	17.2
	e	OP- C09	5`AGGCTGGGTG3`	470:1090	2	3	-	5	60.00	7.2
	f	OP- C19	5´GTTGCCAGCC3`	144:769	6	3	1	10	40.00	17.4
	g	OP-E15	5´ACGCACAACC3`	380:799	2	1	1	4	50.00	6.8
	h	OP-Q18	5´AGGCTGGGTG3`	393:569	2	-	-	2	00.00	4.0
Triple RAPD	Triple (a)	OP-A01 OP-A09 OP-C19	5´CAGGCCCTTC3` 5´GGGTAACGCC3` 5´GTTGCCAGCC3`	109:506	6	4	-	10	40.00	15.8
	Triple (b)	OP- C09 OP-E15 OP-Q18	5`AGGCTGGGTG3` 5´ACGCACAACC3` 5´AGGCTGGGTG3`	136:430	1	3	3	7	85.71	10.2
ISSR	a	14A	5 $(CT)_8$TG3`	256:768	2	1	1	4	50.00	6.2
	b	HB-08	5 $(GA)_6$GG3`	275:1418	4	2	3	9	55.56	16.4
	c	HB -10	5`$(GA)_6$CC3`	164:1476	4	6	2	12	66.67	16.4
	d	HB -12	5`$(CAC)_3$GC3`	335:1408	4	3	3	10	60.00	16.4
	e	HB -15	5`$(GTG)_3$GC3`	252:859	5	3	-	8	37.50	12.8
Total					56	38	21	115		

Table (3): Six canola homozygous lines characterized by unique positive and/or negative markers, marker loci size and total number of markers identifying each line.

Molecular marker technique	Homozygous lines	Unique positive markers			Unique negative markers			Total markers
		Size of marker loci (bp)	Primer	Total positive markers/ Line	Size of marker loci (bp)	Primer	Total negative markers/ Line	
RAPD	DH3				241 372 424	OP- A09	3	5
					223	OP- C19	1	
					252	OP- E15	1	
	Drakkar				326 370	OP- A10	2	2
	Pactol	598	OP- A09	1	709	OP- A01	1	2
Triple RAPD	DH3				219 430	Triple (b)	2	2
	Pactol	350	Triple (b)	1				1
ISSR	DH1				399	HB -08	1	3
					324	HB -10	1	
					404	HB -12	1	
	DH3				275 4476	HB -08	2	3
					1040	HB -12	1	
	DH5				768	14 A	1	1
	Serw8	335	HB -12	1				1
	Pactol	1476	HB -10	1				1

Table (4): Comparison of DNA marker techniques in different canola homozygous lines.

Molecular marker techniques	Number of PCR reaction	Gel Polymorphism					Total number of Amplicons	Average number of polymorphic amplicons/ PCR reaction	Average of polymorphism (%)	Average number of marker amplicons / PCR reaction	Average resolving power (Rp)
		Polymorphic amplicons	Total markers			Total number of Polymorphic amplicon					
			Unique (+)	Unique (-)	Total						
RAPD	8	16	1	8	9	25	55	3.13	45.45	1.125	11.80
Triple RAPD	2	7	1	2	3	10	17	5.00	58.82	1.500	13.0
ISSR	5	15	2	7	9	24	43	4.80	55.81	1.800	13.6
Total	15	38	4	17	21	115					

Table (5): Molecular distances (MD) between the ten canola homozygous lines based on Dice dissimilarity index for RAPD technique.

	DH1	DH3	DH4	DH5	Fido	Drakkar	Serw4	Serw6	Serw8
DH3	0.15								
DH4	0.09	0.13							
DH5	0.09	0.17	0.06						
Fido	0.13	0.14	0.05	0.07					
Drakkar	0.16	**0.20**	0.06	0.06	0.05				
Serw4	0.06	0.15	0.05	0.07	0.08	0.10			
Serw6	0.08	0.12	0.05	0.05	0.06	0.10	0.08		
Serw8	0.10	0.13	**0.02**	0.08	0.07	0.09	0.07	0.07	
Pactol	0.13	0.15	0.06	0.08	0.07	0.08	0.11	0.09	0.08

Table (6): Molecular (MD) between the ten canola homozygous lines based on Dice dissimilarity index for Triple-RAPD technique.

	DH1	DH3	DH4	DH5	Fido	Drakkar	Serw4	Serw6	Serw8
DH3	0.24								
DH4	0.04	0.30							
DH5	0.12	0.27	0.08						
Fido	0.08	0.22	0.12	0.04					
Drakkar	0.11	0.25	0.15	0.07	0.03				
Serw4	0.11	**0.33**	0.15	0.14	0.10	0.07			
Serw6	0.18	0.26	0.14	0.13	0.17	0.20	0.20		
Serw8	0.11	**0.33**	0.15	0.14	0.10	0.07	**0.00**	0.20	
Pactol	0.21	0.28	0.26	0.17	0.13	0.10	0.10	0.23	0.10

Table (7): Molecular distances (MD) between the ten canola homozygous lines based on Dice dissimilarity index for ISSR technique.

	DH1	DH3	DH4	DH5	Fido	Drakkar	Serw4	Serw6	Serw8
DH3	0.26								
DH4	0.12	0.16							
DH5	0.16	**0.24**	0.14						
Fido	0.20	0.18	0.15	0.10					
Drakkar	0.15	0.19	0.13	0.09	0.04				
Serw-4	0.17	0.18	0.12	0.07	0.03	**0.01**			
Serw-6	0.16	0.20	0.11	0.06	0.04	0.03	0.01		
Serw-8	0.22	0.23	0.17	0.10	0.05	0.06	0.05	0.07	
Pactol	0.22	0.23	0.17	0.12	0.08	0.09	0.08	0.09	0.05

Table (8): Molecular distances (MD) between the ten canola homozygous lines based on Dice dissimilarity index for combined data.

	DH1	DH3	DH4	DH5	Fido	Drakkar	Serw4	Serw6	Serw8
DH3	0.19								
DH4	0.10	0.16							
DH5	0.12	**0.21**	0.09						
Fido	0.15	0.17	0.10	0.08					
Drakkar	0.15	**0.21**	0.10	0.07	**0.05**				
Serw-4	0.11	0.18	0.09	0.08	0.07	0.06			
Serw-6	0.12	0.16	0.08	0.06	0.07	0.08	0.07		
Serw-8	0.14	0.20	0.09	0.10	0.07	0.07	0.05	0.09	
Pactol	0.17	0.20	0.13	0.11	0.08	0.09	0.10	0.11	0.07

Table (9): Correlation between all molecular techniques based on molecular distances MD between ten homozygous lines.

Molecular marker technique	RAPD	T-RAPD	ISSR
T-RAPD	0.536**		
ISSR	0.552**	0.543**	
Combined data	0.835**	0.742**	0.887**

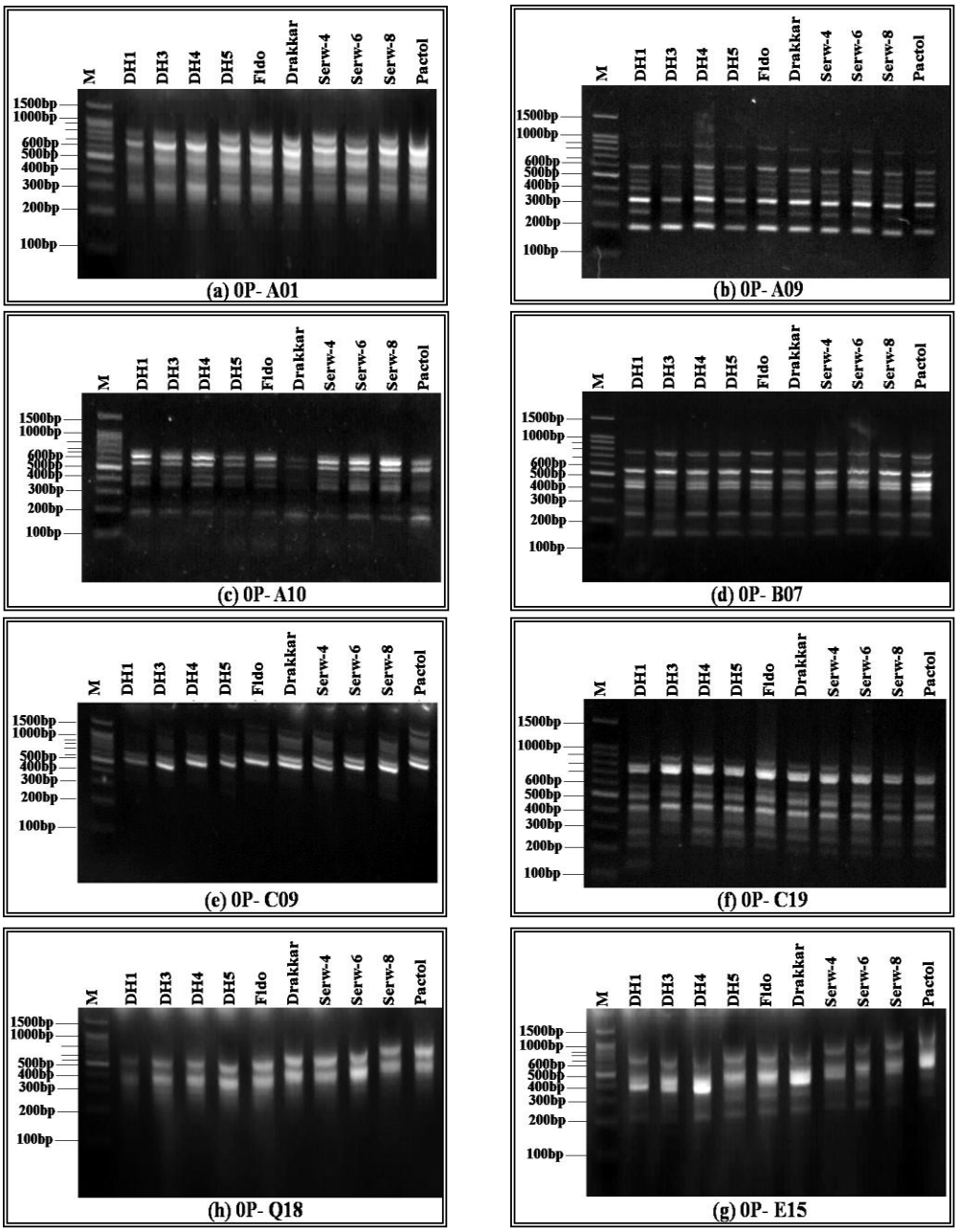


Fig. (1): Banding patterns of RAPD-PCR products for homozygous lines of canola produced with eight primers. M: 1.5 kb ladder and lanes 2 to 11 represent the ten homozygous lines.

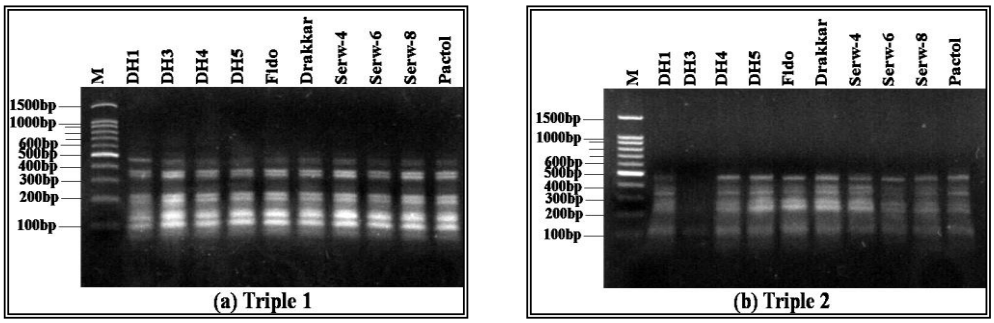


Fig. (2): Banding patterns of Triple-RAPD PCR products for homozygous lines of canola produced with eight primers. M: 1.5 kb ladder and lanes 2 to 11 represent the ten homozygous lines.

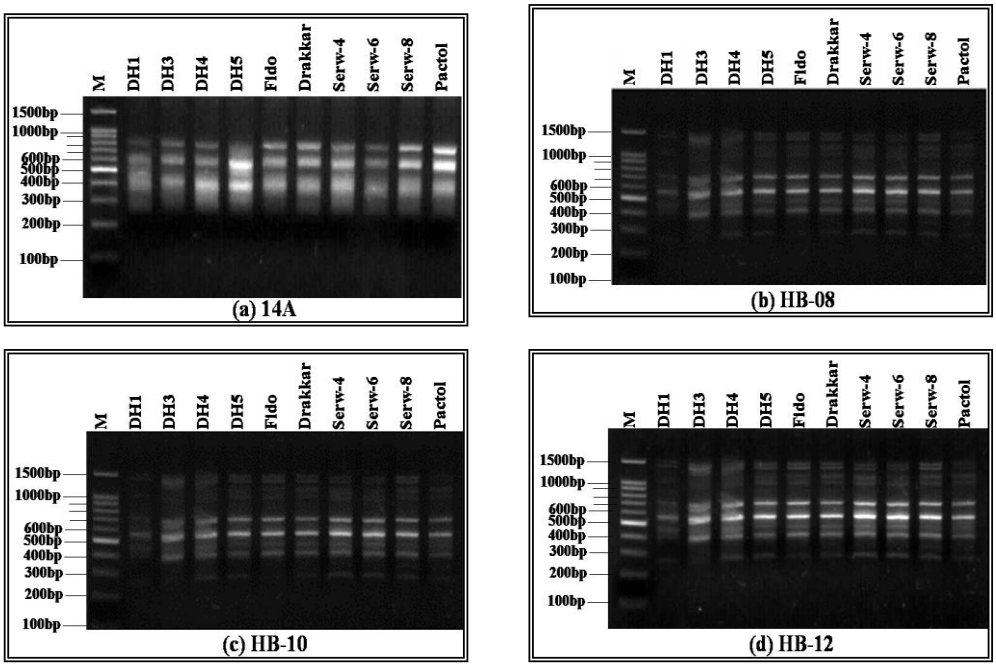
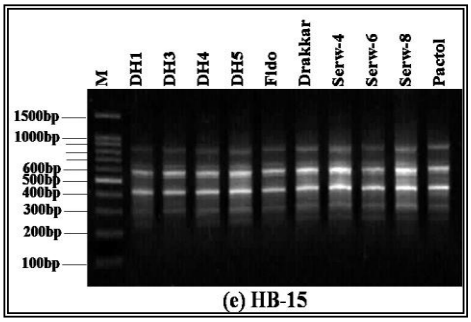


Fig. (3): Banding patterns of ISSR -PCR products for homozygous lines of canola produced with five primers. M: 1.5 kb ladder and lanes 2 to 11 represent the ten homozygous lines.



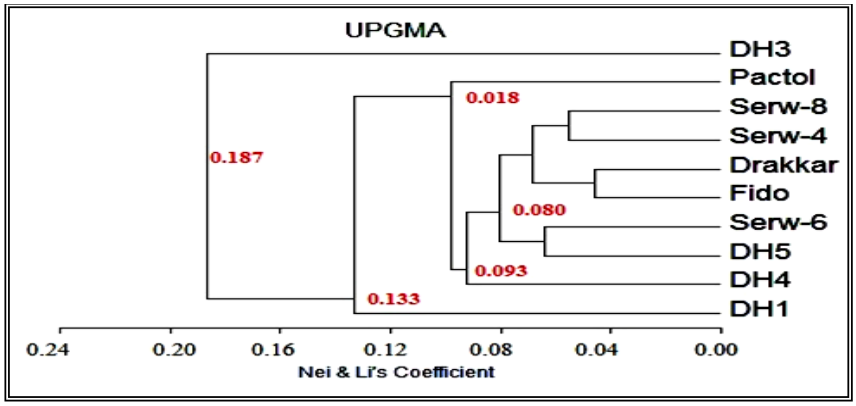


Fig. (4): UPGMA dendrogram indicating the genetic relationships among ten homozygous lines based on dice dissimilarity index for combined data.

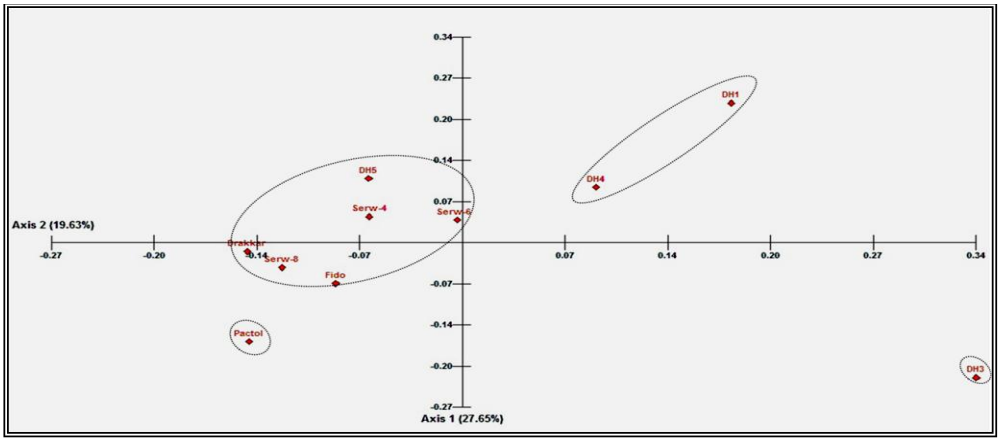


Fig. (5): Principal Coordinate (PCo) analysis for ten homozygous lines based on dice dissimilarity index for combined data.

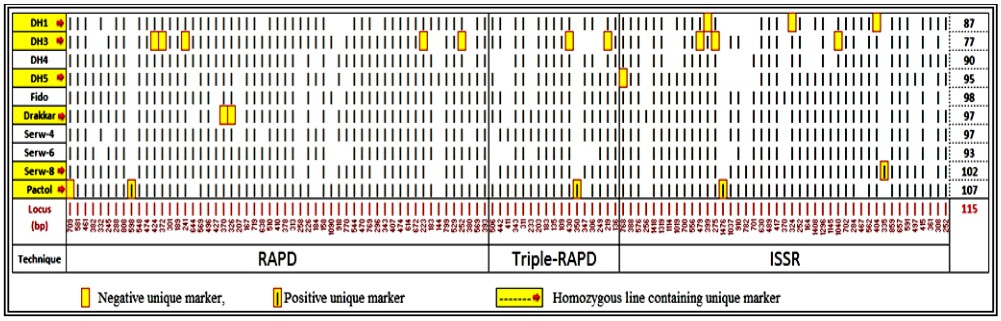


Fig. (6): DNA-profile representation of RAPD, Triple RAPD and ISSR fingerprints of Canola homozygous lines based on 115 amplicons 21of them were marker loci according to Adhikari *et al.* (2015).