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Full Length Research Paper

Efficiency of random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers for genotype fingerprinting and genetic diversity studies in canola (*Brassica napus*)

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Genetic diversity evaluations among 10 canola (*Brassica napus*) genotypes were determined using RAPD and ISSR markers. The RAPD and ISSR primers with the highest degree of polymorphism were selected. A total of 67 bands of polymorphic RAPD bands were detected out of 77 bands, with an average of 13.4 polymorphic fragments per primer. The number of amplified fragments with RAPD primers ranged from 8 to 21, with the size of amplicons ranging from 162 to 3154 bp. The polymorphism ranged from 68.4 to 100.0, with an average of 87%. The five ISSR primers produced 94 bands across 10 genotypes, of which 76 were polymorphic, with an average of 15.2 polymorphic fragments per primer. The number of amplified bands varied from 4 to 32, with size of amplicons ranging from 127 to 3011 bp. The percentage of polymorphism using ISSR primers ranged from 25 to 100.0 with an average of 78.8%. Dice similarity coefficient was calculated for all pairwise comparisons and was used to construct a UPGMA dendrogram. Clustering of genotypes within the groups was not similar when RAPD and ISSR derived dendrograms were compared, whereas, the pattern of clustering of the genotypes remained akin in ISSR and the combined data of RAPD and ISSR. The similarity coefficient ranged from 0.44 to 0.78, 0.41 to 0.72 and 0.47 to 0.73 with RAPD, ISSR and combined dendrogram, respectively. These results provided valuable information for fingerprinting that can be used to create wider genetic base for future canola breeding program.

Key words: *Brassica napus*, inter-simple sequence repeats (ISSR), random amplified polymorphic DNA (RAPD), genetic diversity.

INTRODUCTION

A great deal of research has been focused on oil crops of various plants, especially members of the mustard family (*Brassica*ceae) such as species of *Brassica*. Canola (rapeseed; *Brassica napus* L. genome AACC, 2 = 38) arises from spontaneous hybridization between turnip (*Brassica rapa*) (AA, 2n = 20) and cabbage (*Brassica oleracea*) (CC, 2n = 18). It is now the second largest oilseed crop over the world after soybean (*Glycine max*) providing 13% of the worlds supply (Abbas et al., 2009). Canola is primarily used for food and feed, but has recently gained an increasing interest as a source for bio-products (biodiesel). Besides that, the food and drug administration (FDA) approved canola oil with a qualified health claim (QHC) due to its ability to reduce the risk of coronary heart disease (Miller-Cebert et al., 2009).

Like any other crop species, to improve quality and quantity of *Brassica* spp., the presence of sufficient genetic diversity is very important. In the breeding process, significant improvement of quality and production was achieved, as well as utilization of rapeseed oil in human nutrition. However, genetic variability in this important crop is restricted with regard to many characters of value for breeding process (Marjanovic-jeromela et al., 2009). The success in breeding programs of a crop species largely relies on the presence of sufficient genetic diversity in the germplasm and knowledge about the characteristics of the genotypes and their genetic relationship. Various methods have been elaborated for this purpose. Pedigree analysis is the most widely used method for estimating the degree of similarity between varieties or populations, but the necessary information on ancestry is not always accurate or available. Application of morphological traits is hindered by their limited number and modifying effect of environmental factors in some cases. The spread of DNA markers has allowed the genome to be analyzed directly, thus, eliminating errors caused by environmental factors. By using these markers, the genome can be characterized with great accuracy. In addition to the estimation of degrees of relationship between different varieties, a further important use of these markers is to distinguish between genotypes. Numerous molecular markers have been used for variety identification in various plant species, which allow cultivar identification in early stages of plant development, being neutral to environmental effects (Mohammadi, 2002; Meszaros et al., 2007; Moghaddam et al., 2010). A variety of molecular markers including restriction fragment length polymorphism (RFLP) (Thormann et al., 1994), inter-simple sequence repeats (ISSR) (Carolyn et al., 2000; Rudolph et al., 2002; Chao-Zhi et al., 2003), amplified fragment length polymorphism (AFLP) (Sandip et al., 1999; Seyis et al., 2003; Jiang et al., 2007) and random amplified polymorphic DNA (RAPD) (Ashik Rabbani et al., 1998; Lazaro and Aguinagalde, 1998; Divaret et al., 1999), have been used to study the extent of genetic variation among the diverse group of important crop species in the genus *Brassica* (Afiah et al., 2007; Marjanovic-jeromela et al., 2009).

In this study, RAPD and ISSR markers based on the polymerase chain reaction (PCR) were applied. The value of RAPD analysis for efficient germplasm management in plants is already known (Young, 2000; Jaroslava et al., 2002). The technique is quick, easy and required less time. This detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence (Welsh and McClelland, 1990; Williams et al., 1990). ISSR or microsatellite are currently becoming the preferred technique for the molecular characterization of different plant species because of higher repeatability, codominant nature, specificity and having multiple alleles (Plieske and Struss, 2001; Halton et al., 2002). ISSRs are based on tandem repeats of short (2 to 6 bp) DNA fragments scattered throughout the genome that lie between conserved sequences. It permits detection of polymorphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats.

Only a few papers comparing the results obtained by different molecular genetic methods have been published in the case of *B. napus* L. The capability of individual methods to differentiate the analyzed canola genotypes is described in this study. This study was therefore undertaken: (1) to determine the efficiency of RAPD and ISSR markers for estimating the genetic diversity; (2) to estimate the genetic diversity of canola genotypes based on molecular characterization. For this purpose, 10 canola (*B. napus* L.) genotypes were analyzed and the results of genetic distances estimated by ISSR and RAPD markers were compared.

MATERIALS AND METHODS

Plant materials

10 canola genotypes from Egypt, Canada, Germany and French were used in this study. The seeds were supplied by Desert Research Center. The genotypes included are shown in Table 1.

DNA extraction

Genomic DNA for each genotype was isolated using the protocol for medicinal and aromatic plants according to Anna et al. (2001). To remove RNA contamination, RNase A (10 mg/ml, Sigma, USA) was added to the DNA solution and incubated at 37°C for 30 min. Estimation of the DNA concentration in different samples was done by measuring optical density at 260 nm according to the following equation: Concentration (µg/ml) = OD_{260} x 50 x dilution factor. The quality of DNA was determined using agarose gel (0.8%) electrophoresis.

RAPD amplification

Amplification of RAPD fragments was performed according to Williams et al. (1990). A set of 25 random 10 mer primers (Operon Technology, USA) from groups A, B, C, D and E was used in detecting polymorphism among different canola genotypes but five primers only were successful in generating reproducible and reliable amplicons (Table 2). The amplification reaction was carried out in 25 µl reaction volume containing 1x PCR buffer, 4 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmole primer, 2 units Taq DNA polymerase and 25 ng template DNA.

PCR amplification was performed in a Perkin Elmer 2400 thermocycler (Germany), programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 37°C for 1 min and an extension step at 72°C for 2 min, followed by extension cycle for 7 min at 72°C in the final cycle.

ISSR amplification

15 primers for ISSR were used in the study but only five were successful in generating reproducible and reliable amplicons for different wild medicinal species. The names and sequences of the selected primers are shown in Table 3. PCR analysis was performed in 25 µl reaction as RAPD conditions and amplification was programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 40°C for 1 min and an extension step at 72°C for 2 min, followed by extension cycle for 7 min at 72°C in the final cycle.

Detection of PCR products

The products of both RAPD- and ISSR-based PCR analyses were detected using agarose gel electrophoresis (1.2% in 1X TBE buffer), stained with ethidium bromide (0.3 ug/ml) and then visually

Table 1. List of canola cultivars used in this study, their origin and pedigree information.

Number	Code	Origin	Pedigree and/selection history
1	Serw-4	NRV-ARC(Egypt)	Unknown
2	31/09	DRC, Mar NBL (Egypt)	T1x L5 Sel.31 Maryout 2005
3	27/09	DRC, Mar NBL (Egypt	T2x L5 Sel. Maryout 2005
4	Pactol	French	Unknown
5	Sedo	Germany	Unknown
6	14/09	DRC, Sudr NBL (Egypt)	C103/Sedo*2C103-14C-6Su-1Su-1Su-13Sw-2Sw-0Sw
7	Global	Canada	Unknown
8	5/09 (56/16)	DRC, Siwa NBL (Egypt)	Cresor/Duplo 18C-121Su-4Sw-15Sw-1Sw-0Sw
9	20/09	DRC, Mar NBL (Egypt)	T1x L1 Sel.20 Maryout 2005
10	10/09	DRC, Mar NBL (Egypt)	T1x Serw4 Sel.10 Maryout 2005

NRV-ARC, Newly released variety through agriculture research center-oil crops department; NBL#, newly bred lines selected through desert research center canola breeding program at Siwa, Ras-Sudr and Maryout experimental research stations.

Table 2. List of RAPD primers, the number of amplified products, the number of polymorphic and monomorphic bands and percentage of polymorphism obtained by analyzing 10 cultivars of canola.

S/N	Primer	Primer sequence (5´ 3’)	Mol. wt range (bp)	Total number of band	Number of polymorphic band	Number of monomorphic band	Polymorphism (%)
1	A12	5'-TCGGCGATAG-3'	167-3154	21	21	0	100.0
2	A16	5'-AGCCAGCGAA-3'	172-1790	19	13	6	68.4
3	A18	5'-AGGTGACCGT-3'	162-963	15	13	2	86.6
4	A09	5'-GGGTAACGCC-3'	164-1535	14	13	1	92.8
5	B11	5'-GTAGACCCGT-3'	239-688	8	7	1	87.5

Table 3. List of SSR primers, the number of amplified products, the number of polymorphic and monomorphic bands, and percentage of polymorphism obtained by analyzing 10 cultivars of canola.

S/N	Primer	Primer sequence (5´ 3’)	Mol. wt range (bp)	Total number of band	Number of polymorphic band	Number of monomorphic band	Polymorphism (%)
1	HB10	(GA)6CC	194	32	32	0	100.0
2	HB11	(GT)6CC	127	15	11	4	73.3
3	HB12	(CAC)3GC	265	4	1	3	25.0
4	HB14	(CTC)3GC	154	24	23	1	95.8
5	HB0 8	(GA)6GG	284	19	19	0	100.0

examined with UV transilluminator and photographed using a CCD camera (UVP, UK).

Data analysis

The RAPD and ISSR reproducible bands were scored as present (1) or absent (0), each of which was treated as independent locus regardless of its intensity. By comparing the banding patterns of genotypes for a specific primer, genotype -specific bands were identified. Faint or unclear bands were not considered. The binary data generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Band size was estimated by comparing with 1 kb ladder

(Invitrogen, USA) using Totallab, TL120 1D v2009 (nonlinear Dynamics Ltd, USA). Data generated by RAPD and ISSR primers were used to compile a binary matrix for cluster analysis (NTSYSpc Ver. 2.1). Genetic similarity among accessions was calculated according to Dice similarity coefficient and used to construct a dendrogram using unweighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (SHAN) routine (Sneath and Sokal, 1973).

RESULTS

Polymorphisms detected by RAPD and ISSR markers

High level of polymorphism was observed among the studied *B. napus* genotypes (Tables 2 and 3). A total of 15 ten (10) mer arbitrary oligonucleotide primers were initially used to establish RAPD-PCR fingerprints of the 10 samples belonging to canola genotypes. Only five primers successfully generated reproducible polymorphic products (Figure 1). The five primers used for RAPD analysis detected a total of 77 fragments, with an average of 15.4 fragments per primer. The percentage of polymorphism ranged from 68.4% (A16) to 100% (A12) with an average of 87%. Of the 77 amplified bands, 67 were polymorphic, with an average of 13.4 polymorphic bands per primer. A total of 22 unique bands were identified out of the polymorphic ones. The number of bands detected by each primer depended on primer, sequence and the extent of variation in specific genotype. The number of amplified fragments varied from eight (B11) to 21 (A12) and the amplicon size varied from 162 bp (A18) to 3154 bp (A12).

ISSR primers produced different numbers of DNA fragments, depending on their simple sequence repeat motifs (Figure 2). The ISSR primers produced 94 bands across the 10 genotypes, of which 76 were polymorphic. A total of 28 unique bands were identified out of the polymorphic ones. The number of bands ranged from four (HB12) to 32 (HB10) and the amplicon size varied from 127 to 3011 bp. Average number of bands and polymorphic bands per primer were 18.8 and 15.2, respectively. The percentage of polymorphism ranged between 25% (HB12) and 100% (HB10, HB8) with an average of 78.8%. Both RAPD and ISSR bands were scored for presence (1) or absence (0) among the genotypes and used for UPGMA analysis.

Genetic similarities based on RAPD and ISSR markers

Genetic similarity was calculated from the dice similarity index value for all the 10 accessions of *B. napus* considering ISSR and RAPD approaches individually as well as together. Based on RAPD markers alone, the maximum genetic similarity was 0.78 between Egyptian cultivars 14/09 (Sudr NBL) and 20/09 (Mar NBL), while the lowest genetic similarity of 0.44 was between Egyptian genotypes Serw-4 (NRV-ARC) and 10/09 (Mar NBL) (Table 5). Based on ISSR markers alone, the maximum genetic similarity was 0.72 between Egyptian genotypes (5/09, 20/09 and 10/09), Canadian (Global), French (Pactol) and Dutch (Sedo) genotypes, while the lowest genetic similarity of 0.41 was observed between Egyptian (Serw-4, 31/09), Canadian (Global) and French (Pactol) genotypes (Table 6). Based on both the marker systems together, the maximum genetic similarity was 0.73 between Canadian (Global) and Egyptian (5/09), while the French cultivar (Pactol) exhibited the lowest genetic similarity of 0.47 with both of Egyptian cultivars (Serw-4, 31/09) (Table 7).

Phylogenetic analysis based on RAPD and ISSR

The phylogenetic relationships among 10 accessions of *B. napus* were analyzed by UPGMA method (Figure 3). The cluster result indicated that all the genotypes could be distinguished by RAPD and ISSR markers, respectively. However, the relationship among the accessions revealed by RAPD analysis were somewhat different from that revealed by the analysis of ISSR. A dendrogram based on UPGMA analysis grouped the 10 accessions into one main cluster and single genotype of the Egyptian genotype Serw-4 (NRV) formed a separate OUT in cluster showing less similarity coefficient (0.57) with the other genotypes (Figure 3a). Genotypes within main cluster II were further divided into three subclusters (IIa, IIb and IIc). Subcluster IIa comprised Egyptian genotypes (31/09, 14/09, 20/09 and 10/09) with Canadian and Dutch (Sedo and Global, respectively). Subcluster IIb comprised both Egyptian (5/09 and 10/09), while Egyptian (27/09) and French (Pactol) genotypes formed subcluster IIc. Within cluster IIc, 27/09 and Pactol appeared to be closer to each other with similarity coefficient of 0.70.

A dendrogram based on UPGMA analysis with ISSR data is shown in Figure 3b. The 10 genotypes were grouped into two main clusters. Cluster I comprised Egyptian genotypes Serw-4 (NRV) and 31/09 with similarity coefficient of 0.54. Genotypes within cluster II were further divided into three subclusters. The first subcluster (IIa) comprised of Egyptian (27/09), French (Pactol) and Dutch (Sedo) genotypes, the second subcluster (IIb) comprised of both Egyptian genotypes (14/09 and 5/09) and the Canadian (Global) one. Global and 5/09 genotypes appeared to be closer to each other with similarity coefficient of 0.72. The remaining two Egyptian genotypes (20/09 and 10/09) were grouped within the third subcluster (IIc) with similarity coefficient of 0.72.

The ISSR and RAPD data were combined for UPGMA cluster analysis. The UPGMA dendrogram thus obtained from the cluster analysis of ISSR and RAPD data is shown in Figure 3c. The clustering pattern of the genotypes in the combined analysis remained similar to the ISSR dendrogram, while the RAPD dendrogram showed

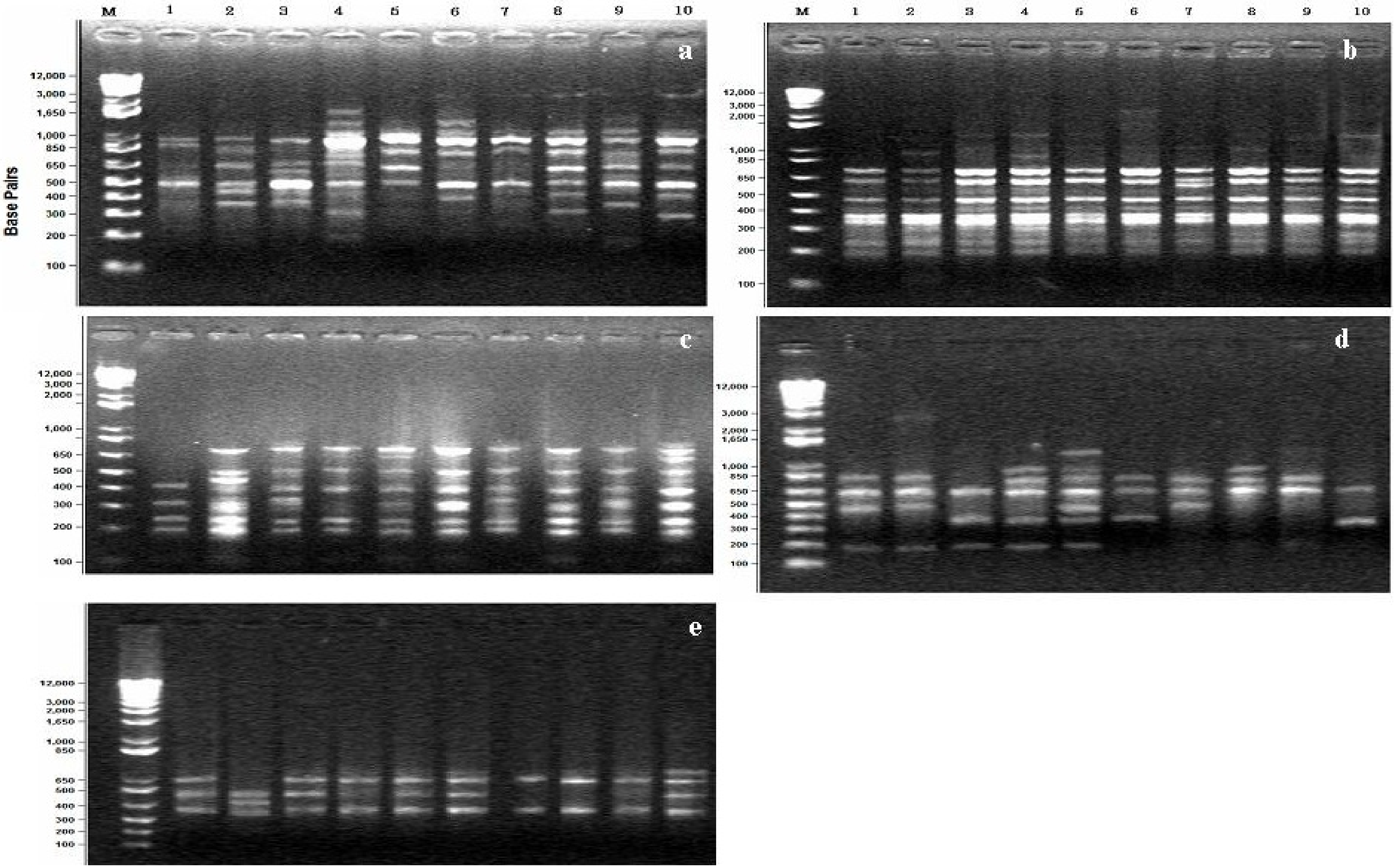


Figure 1. RAPD-PCR amplification products of 10 accessions of canola produced by (a) primers A12; (b) primer A16; (c) primer A18; (d) primer A9; (e) primer B11. Lane M is 1 kb ladder and lanes 1 to 10 represent different canola accessions as listed in Table 1.

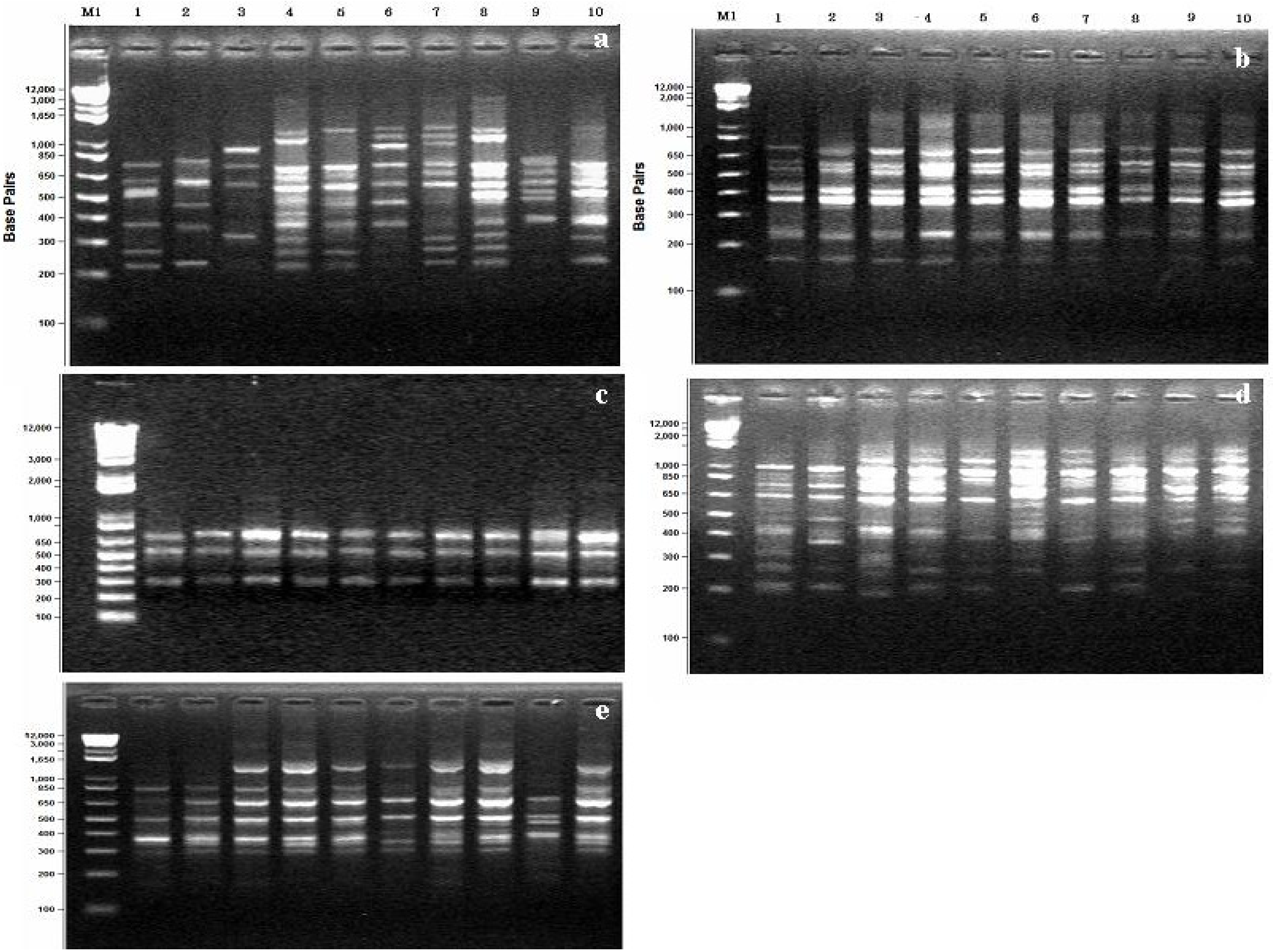


Figure 2. ISSR-PCR products of 10 accessions of canola produced with (a) primer HB10; (b) primer HB11; (c) primer HB12; (d) HB14; (e) HB8. Lane M is 1 kb ladder and lanes 1 to 10 represent different canola accessions as listed in Table 1.

Table 4. Comparison of DNA marker systems in *B. napus*.

Marker system	Number of primer	Polymorphism (%)	Average number of ands/primer	Average number of polymorphic band/primer
RAPD	5	87.0	15.4	13
SSR	5	78.8	18.8	15.2
RAPD+SSR	10	83.6	17.1	14.3

Table 5. Dice similarity coefficient of 10 genotypes based on RAPD data analysis.

Genotype	Serw-4	31/09	27/09	Pactol	Sedo	14/09	Global	5/09	20/09	10/09
Serw-4	1.00									
31/09	0.63	1.00								
27/09	0.55	0.60	1.00							
Pactol	0.48	0.54	0.70	1.00						
Sedo	0.64	0.68	0.74	0.68	1.00					
14/09	0.62	0.66	0.66	0.63	0.76	1.00				
Global	0.64	0.72	0.72	0.64	0.73	0.74	1.00			
5/09	0.50	0.66	0.59	0.66	0.73	0.71	0.74	1.00		
20/09	0.64	0.68	0.64	0.64	0.69	0.77	0.78	0.70	1.00	
10/09	0.44	0.56	0.69	0.60	0.67	0.71	0.60	0.74	0.60	1.00

Table 6. Dice similarity coefficient of 10 genotypes based on SSR data analysis.

Genotype	Serw-4	31/09	27/09	Pactol	Sedo	14/09	Global	5/09	20/09	10/09
Serw-4	1.00									
31/09	0.54	1.00								
27/09	0.55	0.55	1.00							
Pactol	0.47	0.41	0.64	1.00						
Sedo	0.54	0.51	0.62	0.72	1.00					
14/09	0.52	0.43	0.67	0.61	0.56	1.00				
Global	0.41	0.44	0.58	0.59	0.64	0.68	1.00			
5/09	0.58	0.52	0.63	0.63	0.62	0.69	0.72	1.00		
20/09	0.50	0.50	0.62	0.56	0.51	0.59	0.54	0.62	1.00	
10/09	0.52	0.49	0.63	0.64	0.59	0.67	0.68	0.69	0.72	1.00

some variation in the clustering of canola genotypes. Cluster analysis performed from combining data of both markers generated a dendrogram that separated the genotypes into two distinct clusters. The first cluster (I) comprised Egyptian genotypes Serw-4 (NRV) and 31/09with similarity coefficient 0.58. The second cluster (II) was further divided into two subclusters. French and Dutch genotypes (Pactol and Sedo) were grouped into subcluster IIa and a single Egyptian genotype (27/ 09) formed a separate OUT in cluster showing less similarity coefficient (0.675) with other genotypes. Subcluster IIb comprised four Egyptian genotypes (14/09, 5/09, 20/09 and 10/09) with the Canadian one (Global). As in ISSR-based dendrogram, Global and 5/09 appeared to be closer to each other with similarity coefficient 0.70. In the same subcluster IIb, each of the Egyptian genotypes 20/09 and 10/09 diverged into separate outgroup.

Comparison of RAPD and ISSR markers

It is important to understand that different markers have different properties and will reflect different aspects of genetic diversity. RAPD (Williams et al., 1990) and ISSR (Zietkiewicz et al., 1994) markers have advantages and disadvantages for assessing genetic diversity. In this study, the RAPD and ISSR surveys among the 10 accessions of *B. napus* revealed 87 and 78.8% of polymorphic bands, respectively (Table 4).

Table 7. Dice similarity coefficient of 10 genotypes based on RAPD+SSR data analysis.

Genotype	Serw-4	31/09	27/09	Pactol	Sedo	14/09	Global	5/09	20/09	10/09
Serw-4	1.00									
31/09	0.58	1.00								
27/09	0.55	0.58	1.00							
Pactol	0.47	0.47	0.67	1.00						
Sedo	0.59	0.60	0.68	0.70	1.00					
14/09	0.57	0.54	0.66	0.62	0.66	1.00				
Global	0.50	0.57	0.64	0.61	0.68	0.70	1.00			
5/09	0.55	0.59	0.61	0.64	0.67	0.70	0.73	1.00		
20/09	0.56	0.59	0.63	0.60	0.60	0.68	0.64	0.66	1.00	
10/09	0.48	0.53	0.66	0.62	0.63	0.69	0.64	0.71	0.66	1.00

DISCUSSION

Canola crop has advantages of high seed oil content (40 to 45%), high protein in oil free meal (30 to 35%) and easy cultivation in newly reclaimed lands by the well adapted genotypes with relatively low costs (Afiah et al., 2007; Sharaan et al., 2006). The two marker systems, ISSR and RAPD used in this study have also been used as effective tools to evaluate genetic diversity and to throw light on the phylogenetic relationships in *Brassica* (Marjanovic-jeromela et al., 2009), *Ricinus* (Gajera et al., 2010) and *Trigonella* (Dangi et al., 2004). These studies have given important clues in understanding species relationship, which may further assist in developing and planning breeding strategies.

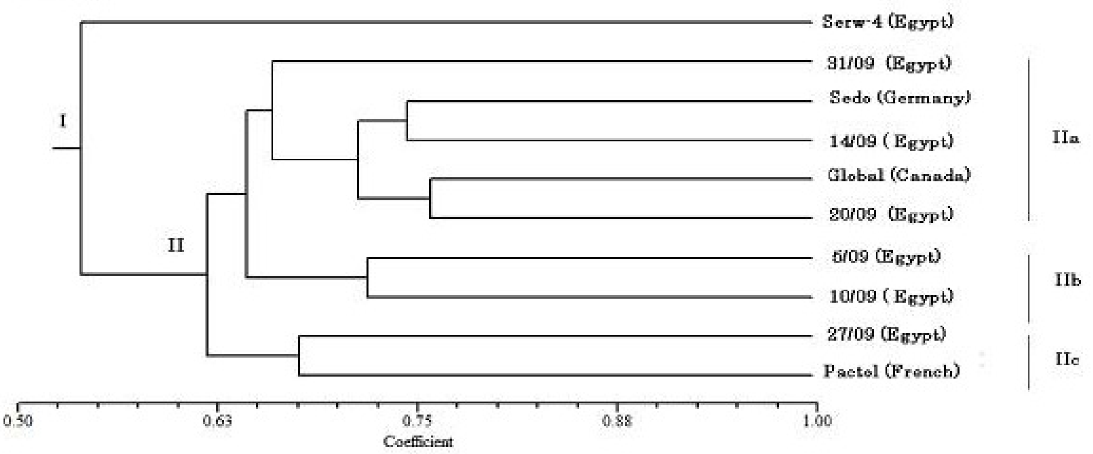
Variations in DNA sequences lead to polymorphism which is indicative of genetic diversity. The results indicated the presence of wide genetic variability as a result of the high polymorphism among the studied genotypes of *B. napus*. Based on RAPD and ISSR markers data, the percentage of the polymorphic fragments were 87 and 78.8%, respectively. This high level of polymorphism could be attributed to the location of those genotypes in different regions and/or their pedigree information. Also, higher numbers of bands for each primer indicates the existence of larger genetic diversity among the genotypes under investigation (Agrama and Tuinstra, 2003). Primers with higher polymorphic bands are more efficient in studying genetic diversity and discrimination of the genotypes (Roman et al., 2004; Moghaddam et al., 2009). In this investigation, the RAPD and ISSR primers produced 77 and 94 bands respectively, with average of 15.4 and 18.8 per primer and polymorphism percentage of 87 and 78.8%, respectively. In other studies, percentage of polymorphic primers in rapeseed reported was 76% (Hollden et al., 1994), 14% (Roman et al., 2004) and 6% (Mailer et al., 1997). Average number of bands reported in the literature were 3 (Ishida et al., 2000), 3.36 (Roman et al., 2004), 5.54 (Shiran et al., 2004), 8.6 (Kimura et al., 2000) and 9.6 (Sandip et al., 1999).

Both RAPD and ISSR molecular markers have been used successfully in population genetic studies (Parsons et al., 1997; Li and Ge, 2001). Both markers are proved to be efficient and inexpensive way to provide molecular data. The ISSR method has been reported to be more reproducible (Goulao and Oliveira, 2001) and produces more complex marker patterns than the RAPD approach (Chowdhury et al., 2002), which is advantageous when differentiating closely related cultivars. Nevertheless, on the basis of higher percent of polymorphism (RAPD = 87%; ISSR = 78.8%) and similarity matrix, the RAPD markers were marginally more informative than ISSR in the assessment of genetic diversity in the studied canola.

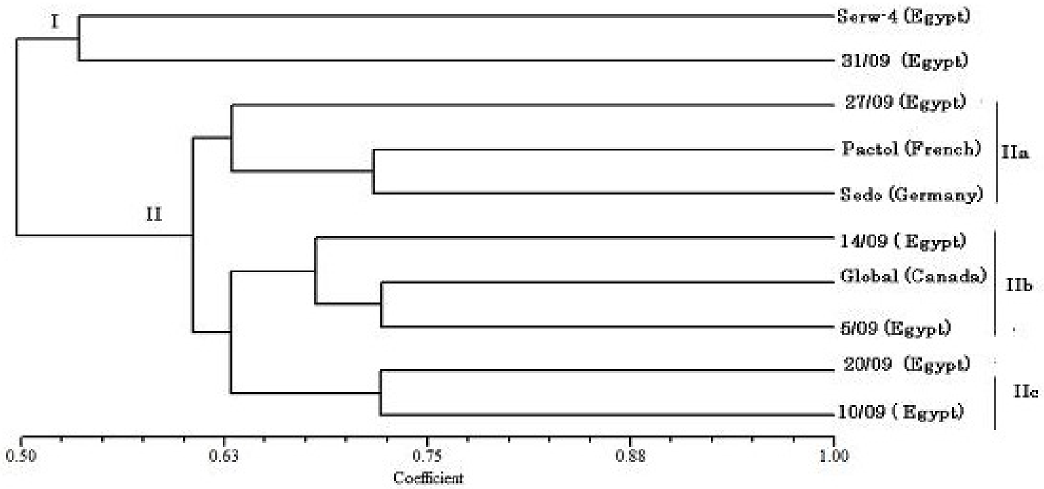
Similar results were reported in *Cladesia grandis* (Chen et al., 2006) and castor (Gajera et al., 2010). However, both of the markers worked effectively in the assessment of genetic diversity in canola as they exhibited more than 60% polymorphism.

Knowledge of genetic similarity (distance) between genotypes and among individuals or populations is useful in a breeding program because it permits organization of germplasm and provides more efficient sampling of genotypes to cross for the development of populations (Afiah et al., 2007). In this study, the UPGMA analysis dendrograms and similarity coefficients revealed good relationships between some cultivars (Tables 5 and 7; Figure 3). However, the results obtained from cluster analysis based on RAPD and ISSR data sets were also different. In general, the UPGMA analysis dendrograms did not show any clear pattern of clustering according to the locations from where accessions were collected. It is clear from the cluster analysis that accessions from one geographical region grouped together in some cases while they were placed in different clusters in certain cases (Figure 3). Cluster analysis based on ISSR data assigned the genotypes in two main groups (Figure 3b). In this grouping, two Egyptian genotypes (Serw-4 and 31/09) were located in a single cluster and separated from other Egyptian genotypes. On the other hand, European genotypes (Pactol and Sedo) were located close to each other. Grouping based on RAPD data showed no distinct separation of Egyptian genotypes

a) RAPD



b)ISSR



c) RAPD+ISSR

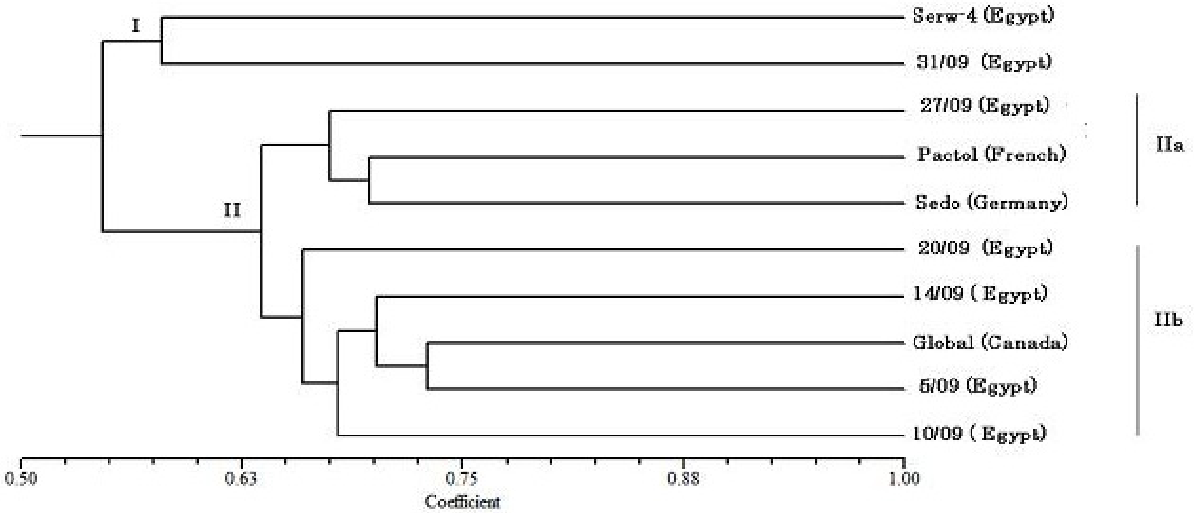


Figure 3. Dendrograms obtained from 10 accessions of canola with UPGMA based on Dice coefficient. (a) RAPD data-based dendrogram; (b) ISSR data-based dendrogram; (c) combined (ISSR and RAPD) data based dendrogram.

from European and Canadian types (Figure 3a). Clustering of canola genotypes based on the combined RAPD and ISSR data revealed similar results with those of ISSR alone and could differentiate some of Egyptian cultivars from the rest of the studied canola genotypes. In similar studies, the superiority of the ISSR marker system in comparison with the RAPD marker system was observed (Goulao and Oliveira, 2001; Moghaddam et al., 2009).

Thus, there was no clear clustering pattern of geographically closer genotypes in this study indicating that the association between genetic similarity and geographical distance was less significant. Therefore, it is necessary to use more number of accessions from each location to confirm the available pattern. Similar results were reported in castor (Gajera et al., 2010), *Melocanna* (Lalhruaitluanga and Prasad, 2009), rapeseed (Marjanovic-Jeromela et al., 2009) and in *Trigonella* (Dangi et al., 2004).

A possible explanation for the difference in resolution of RAPDs and ISSRs is that the two marker techniques target different portions of the genome which are subjected to different mechanisms generating genetic variation (Lalhruaitluanga and Prasad, 2009). Polymorphic bands of a given RAPD primers may bind to many parts of the genome, so each primer may give information on the polymorphism of several chromosome regions. ISSR primers also provide a large quantity of polymorphic information, but this very detailed information only originates from a single hypervariable section of the genome (Meszaros et al., 2007). Also, higher mutation rate in the ISSR loci may be the reason for observing larger number of alleles in this marker (Moghaddam et al., 2010). For this reason, ISSR markers are ideal for distinguishing between genotypes that are genetically very similar. Some studies have shown that RAPD markers are found throughout the genome and may be associated with functionally important loci (Penner, 1996). However, there is little information to indicate that ISSR markers are functionally important (Gajera et al., 2010; Esselman et al., 1999). These differences may also be attributed to marker sampling errors and/or the percent of polymorphism detected by different markers (Gajera et al., 2010), reinforcing the importance of the number of loci and their coverage of the overall genome in obtaining reliable estimates of genetic relationships among cultivars (Souframanien and Gopalakrishna, 2004). However, RAPD and ISSR markers were powerful DNA markers; they are effective and promising markers for assessing genetic variation in *B. napus* species.

In summary, the present findings further strengthened previous reports that the RAPD and ISSR markers can be used effectively to estimate genetic distances among genotypes. However, it is suggested that a greater number of genotypes and molecular markers is required to have better understanding of the presence of genetic variability in *B. napus* germplasm and consequently, more efficient utilization of existing variability for improvement of this important crop.

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