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STUDIES ON GENETIC DIVERGENCE OF RAPESEED GENOTYPES USING SSR MARKERS

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Abstract

Thirty five *Brassica napus* genotypes were evaluated to check their genetic diversity on the basis of mapped 20 microsatellite markers. The genetic similarity values of coefficient ranged from 0.28 to 0.85. A dendrogram was generated based on UPGMA which showed that genotypes were divided into five major clusters. Cluster A had genotypes with higher similarity coefficient of 0.85 while Cluster E had only one genotype (pop 35) genetically distinct from other genotypes. PIC values were calculated for each of the 20 SSR primer pairs. Ten primers PIC value ranged from 0.30 (O110-A05) to 0.59 (Na10-D09 markers). This study would be helpful in assessing the genetic diversity. It was revealed that there was no correlation between the geographical distribution and genetic diversity of the studied germplasm, depicting their close genetic relationship.

Key words: Genetic divergence, Rapeseed, SSR markers.

Introduction

At global level Rapeseed (*Brassica napus*) is the 3rd most common source of cooking oil, providing about 16% of the whole world's supply (Anon., 2017). Canada is the largest producer of oilseed rape with production of 18.4 million tonnes (Anon., 2016a).Pakistan is facing acute shortage of edible oil due to continuously increasing population and per capita consumption owing to improved living standards. Only one-fourth of local demand is fulfilled by edible oil produced from all traditional and non-traditional oilseeds. The remaining is fulfilled by huge import of edible oil from other countries. During 2016-17 total estimated availability of edible oil was 2.426 million tonnes. About 152.5 billion rupees were spent on the import of 1.98 million tonnes of edible oil while the local production was about 0.446 million tonnes during 2016. The expenditure on the import of edible oil showed decrease of 4.07 percent against the previous year. Sunflower, Canola, Rapeseed-Mustard and Cotton are considered as major oilseed crops in Pakistan (Anon., 2016b). Rapeseed-Mustard is important source of edible oil from traditional oilseed crops.

Breeding programme can efficiently use the genetic divergence present in the germplasm after the evaluation of genetic diversity (Ilyas *et al.*, 2018; Ibrahim *et al.*, 2016; Khan *et al.*, 2016a). Cultivated varieties can be analyzed for genetic diversity, helpful in selection of diverse and best parental combinations which can be used for creating genetically diverse segregating progenies for crop improvement and incorporation of desirable traits into the cultivated variety (Thompson *et al*., 1998). Use of diverse genetic resources is indispensable to improve the genetic potential of modern variety. Genetically diverse germplasm helps to create favorable combination of genes and facilitates production of a unique genotype through increase in share to the number of favorable gene by which the parents diverge (Talebi *et al*., 2008).

It is difficult to differentiate modern *B. napus* cultivars because their parent's genetic background is very narrow (Zhou, 2001). Yet, limited polymorphism among the closely related genotypes has been revealed by the use of traditional techniques such as phenological, morphological and isozyme analysis and environmental conditions influence the results. Therefore, an effective technique should be explored for the estimation of level of polymorphism among *B. napus* genotypes. In recent years, DNA polymorphism based molecular genetic analysis technique is used for the identification and characterization of novel germplasm. These novel genotypes are used in breeding programme to bring about crop improvement (O'Neill *et al.*, 2003). Various kinds of molecular markers have already been tested as important apparatus for genetic divergence studies (Khan *et al*., 2016b).

Salient features of SSR markers like co-dominance, high polymorphism and abundance in the genome, make it prominent among various molecular marker systems (Hasan *et al.*, 2006). The utilization of SSR primer pairs for genetic divergence studies of rapeseed is common. The present experiment was performed to estimate the genetic divergence and similarity among 35 *B. napus* genotypes based on objectives: (i) to investigate the usefulness of microsatellite markers in identifying different rapeseed genotypes on the basis of polymorphism; (ii) to identify effective SSR markers for *B. napus* germplasm.

Materials and Methods

Plant material:A collection of 35 *B. napus* genotypes, obtained from gene bank of Bio Resources Conservation Institute, National Agriculture Research Center, Islamabad were used in this study. Genotypes with their codes and origin are presented in Table 1. Genotypes were grown in nursery tray and kept in growth chamber (25±1°C) of the department Plant Breeding and Genetics, PMAS-AAUR during 2015.

Table 1. Detail of local and exotic *B. napus* genotypes along with their origin and code.

Sr. No.	Genotype	Origin	Code	Sr. No.	Genotype	Origin	Code
1.	1696	Pakistan	Pop1	19.	24881	Pakistan	Pop19
2.	27441	Australia	Pop2	20.	24878	Pakistan	Pop20
3.	24257	Unknown	Pop3	21.	28183	Canada	Pop21
4.	1499	Pakistan	Pop4	22.	26818	Unknown	Pop22
5.	1500	Pakistan	Pop5	23.	28163	Canada	Pop23
6.	1692	Pakistan	Pop6	24.	24892	Pakistan	Pop24
7.	27420	China	Pop7	25.	28175	Canada	Pop25
8.	1716	Unknown	Pop8	26.	28161	Canada	Pop26
9.	27418	China	Pop9	27.	28170	Canada	Pop27
10.	24906	Pakistan	Pop10	28.	28178	Canada	Pop28
11.	24883	Pakistan	Pop11	29.	28187	Canada	Pop29
12.	24864	Pakistan	Pop12	30.	26811	Unknown	Pop30
13.	27438	Australia	Pop13	31.	26813	Unknown	Pop31
14.	25020	Pakistan	Pop14	32.	26812	Unknown	Pop32
15.	24207	Unknown	Pop15	33.	28184	Canada	Pop33
16.	24256	Unknown	Pop16	34.	26820	Unknown	Pop34
17.	27432	China	Pop17	35.	28165	Canada	Pop35
18.	24191	Unknown	Pop18				

Table 2. Name, sequence and repeat motif of 20 SSR primer pairs (http://ukcrop.net/perl /search/Brassica DB).

Sr. No.	Name	Forward primer	Reverse primer	Repeat motif
1.	BRMS-001	GGTGGCTCTAATTCCTCTGA	ATCTTTCTCTCACCAACCCC	$(GA)_{25}$
2.	BRMS-019	CCCAAACGCTTTTGACACAT	GGCACAATCCACTCAGCTTT	$(GT)_{10}$
3.	BRMS-020	AACAAGAGAAGGAGAGCCACCG	CGCTTATAAAATGGCAGTCGCA	$(TC)_{21}$
4.	BRMS-037	CTGCTCGCATTTTTTATCATAC	TACGCTTGGGAGAGAAAACTAT	$(CA)_{10}$
5.	BRMS-042	GGATCAGTTATCTGCACCACAA	TCGGAATTGGATAAGAATTCAA	$(AAT)_4$, $(CT)_{4,}$ $(T)_{2,}$ $(CT)_4$
6.	Na10-B08	AGA GAA AAA CAC TTC CCG CC	GTG AGC TTT GCG AAA CAC G	$(CT)_{38}$
7.	Na10-D03	ATGATTTGCCTTGAAATGCC	GATGAAACAATAACCTGAGACACAC	di GT/CA
8.	Na10-D09	AAGAACGTCAAGATCCTCGC	ACCACCACGGTAGTAGCG	di GT/CA
9.	Na10-D11	GAGACATAGATGAGTGAATCTGGC	CATTAGTTGTGGACGGTCGG	di GA/CT
10.	Na10-F06	CTCTTCGGTTCGATCCTCG	TTTTTAACAGGAACGGTGGC	tri GGC/CCG
11.	Na10-G10	TGGAAACATTGGTGTTAAGGC	CATAGATTCCATCTCAAATCCG	di GA/CT
12.	Na12-A02	AGCCTTGTTGCTTTTCAACG	AGTGAATCGATGATCTCGCC	$(CT)_{16}$
13.	Na12-A07	TCAAAGCCATAAAGCAGGTG	CATCTTCAACACGCATACCG	$(GT)_{11}$
14.	Na12-B09	ACGGAAGATCAAACAGCTCC	TGAGCGACCCATTCTTTAGG	tri GGC/CCG
15.	Na12-C07	ACTCAACCCCACAAACCTG	AGTTCCCCGGATCCGATTAG	di GA/CT
16.	Na12-C08	GCAAACGATTTGTTTACCCG	CGTGTAGGGTGATCTAGATGGG	di GA/CT
17.	Na12-D04	ACGGAGTGATGATGGGTCTC	CCTCAATGAAACTGAAATATGTGTG	di GT/CA
18.	Na14-D07	GCATAACGTCAGCGTCAAAC	CTGCGGGACACATAACTTTG	tri GGC/CCG
19.	Ni2-A12	AACAAACAAGAGTCGAATACGG	AATGCCCTCTAACTGAGCCC	dis. di GT/CA
20.	O110-A05	TGTAATAACCCGACCCATCC	CTCTCTCGCTCTCTCGATCC	di GA/CT

SSR marker analysis: The *Brassica napus* genotypes were genetically characterized by using 20 mapped SSR primers. The primer information was taken from the publicly available domain (Lowe *et al*., 2002, 2004; www.brassica.info/ssr/SSRinfo.htm). Table 2 shows the sequence of primers along with expected size of their fragments. The DNA was extracted from *B. napus* leaves by following a protocol developed by Doyle & Doyle (1990). Gel Electrophoresis was used for DNA quantification. One µl of 6X Bromophenol Blue Loading Dye was mixed with 5 µl of DNA. All the samples were loaded in separate wells on a 0.5% concentrated Agarose gel. PCR was performed using DNA as template and SSR primers in a Thermal Cycler. The amplified product was further analyzed on agarose gel or stored at -20°C until further analysis.

The PCR products were resolved and checked on a 2% concentration of agarose gel. The gel was run with 1X TBE buffer. A constant power of 100 V was applied for 45 minutes. Gel was stained with Ethidium Bromide Then the result was visualized by Gel Documentation System (Fig. 1). Score able bands obtained on the photograph of Gel Documentation were considered as a single allele.

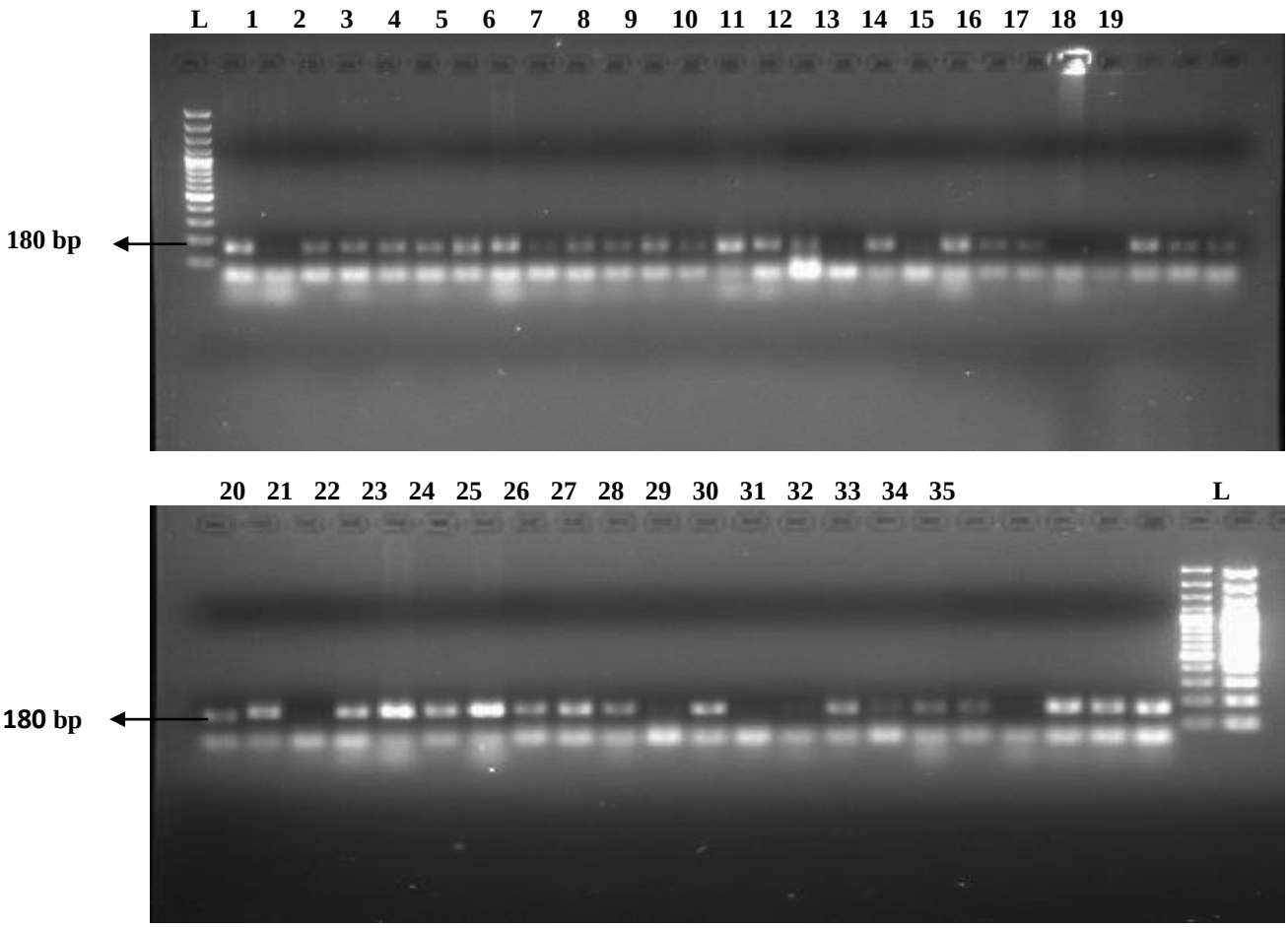

Fig. 1. Agarose Gel (2%) showing Amplification pattern of 35 genotypes produced by Primer Na10-D03. The lane L represents molecular weight marker (100 bp DNA Ladder, Fermentas) and Lane 1-35 represents the genotypes listed in table.

Data analysis: Bands were scored as bivariate (1-0) data matrix. A standard equation was used to compute Polymorphic Information Content of SSR markers (Botstein *et al*., 1980; Anderson *et al.*, 1993).

$$PIC = 1 - \Sigma (pi)^2$$

In a population, frequency of ith allele is represented as pi.

For genetic similarity calculations among 35 *B. napus* exotic and local genotypes, Dice similarity index (Nei & Li, 1979) was used. The computer program "POPGENE" v. 1.31 (Yeh *et al.*, 1999) was used to construct dendrogram (cluster analysis) based on the UPGMA algorithm.

Results and Discussion

Twenty SSR primers were selected from public domain due to high polymorphism and amplification strength. These were used to ascertain the genetic diversity of the 35 *B. napus* genotypes. The selected primers generated characteristic bands in the range of 100 to 600 bp. An assortment of 580 bands were generated and recorded 44 score able alleles from the 20 polymorphic SSR primers in the 35 *B. napus* genotypes (Table 3).

Amphidiploids species exhibit less polymorphism than diploid species. Polymorphism reported for *Brassica napus* is less than 45% (Kresovich *et al*., 1995). In amphidiploids like *Brassica napus*, due to meager self-incompatibility, there is always a low level of out-crossing which leads to low percentage of polymorphism (Weerakoon *et al.*, 2010). While all primer used in this study showed 100 % polymorphism rate. This might be due to the conserve genetic base of the plant material used.

Polymorphic information content: The Polymorphism Information Content (PIC) is known as one of the important uses of the SSR markers to evaluate their delineation power (Junjian *et al.*, 2002). The polymorphism among the 35 genotypes was checked by finding PIC values for the 20 SSR markers. The number of alleles per SSR primer was found to be 1-3 with an average of 2.2. Ten SSR primers detected a single allele and thus their PIC value was recorded as zero. PIC value of the remaining 10 SSR primers ranged from 0.30 to 0.59. SSR primer Na10-D03 generated the highest PIC value (0.59), whereas, O110-A05 recorded the lowest PIC value (0.30). The average PIC of all the SSR markers was 0.23 per locus inferring the ability of the used primers to differentiate the *B. napus* genotypes (Table 3).

Table 3. SSR markers, annealing temperature, allele size (bp), no. of polymorphic alleles and PIC value.

Sr. #	SSR markers	Annealing temp.	Allele size (bp)	No. of polymorphic allele	PIC value
1.	BRMS-001	60	115-190	2	0.34
2.	BRMS-019	55	100-250	1	0.00
3.	BRMS-020	56	143-560	3	0.57
4.	BRMS-037	55	137-176	1	0.54
5.	BRMS-042	57	112-122	3	0.49
6.	Na10-B08	56	145-100	2	0.00
7.	Na10-D03	57	155-120	1	0.59
8.	Na10-D09	58	240-180	3	0.00
9.	Na10-D11	60	180-135	3	0.39
10.	Na10-F06	55	120-70	1	0.00
11.	Na10-G10	58	220-160	2	0.42
12.	Na12-A02	56	290-240	1	0.49
13.	Na12-A07	57	155-125	2	0.00
14.	Na12-B09	59	208-270	1	0.00
15.	Na12-C07	58	180-120	1	0.00
16.	Na12-C08	56	320-220	1	0.00
17.	Na12-D04	59	290-230	1	0.00
18.	Na14-D07	60	140-90	2	0.50
19.	Ni2-A12	56	250-200	1	0.00
20.	O110-A05	60	210-140	3	0.30
Total				**44**	**4.63**
Average					**0.23**

Genetic similarity analysis: The UPGMA was used to calculate genetic similarity from 580 polymorphic bands. The value of genetic similarity coefficient ranged from 0.28 to 0.85 with a mean value of 0.64. Pop31 and Pop 35 showed the lowest similarity (0.28) while pop1 and pop3; pop3 and pop19; pop13 and pop14; pop13 and pop15; pop 14 and pop24; pop 17 and pop23; pop22 and pop30; pop23 and pop28 were genetically most similar with similarity coefficient of 0.85 (Table 4). In addition, pop3 and pop4: pop3 and pop 34; pop14 and pop16: pop 19 and po25; pop19 and pop29; pop30 and pop31; a very close genetic relationship was found between pop23 and pop33 with higher value of genetic similarity (0.82).

Genetic distance matrixes: Genetic distance is helpful for identifying yield potential and heterosis of intra-specific hybrids. The coefficient of genetic distance among thirty five genotypes of *B. napus* is given in the Table 5. The value of genetic distance varied from 0.15 to 0.99. In contrast to the genetic similarity, the least genetic distance (0.15) was observed between pop1 and pop4; pop3 and pop20: pop13 and pop15; pop13 and pop16; pop 17 and pop24; pop14 and pop25; pop23 and pop29; pop23 and pop34. Maximum genetic distance of 0.99 was observed between Pop35 and pop1; pop29 and pop35: pop31 and pop35.

Genetic relationship among 35 *Brassica napus* genotypes: UPGMA clustering revealed a dendrogram of 35 *Brassica napus* genotypes as shown in Fig. 2. In the dendrogram, genotypes are presented on the vertical axis and the horizontal axis shows the Euclidean distance. The dendrogram elaborate the relative magnitude of resemblance among the genotypes as well as the clusters. At similarity co-efficient of 0.60 all of the genotypes were initially divided into five major clusters (A, B, C, D and E).

Cluster A: Cluster A of consensus tree consisted of 14 genotypes. It is further divided into three sub-clusters A1, A2 and A3. A1 have 5 genotypes in it from which pop1 and pop3 indicated the highest similarity coefficient of 0.85with very similar SSR fingerprint. A2 have five genotypes of which pop28 and pop23: pop17 and pop23 showed the higher similarity coefficient. A3 consist of four genotypes pop18, pop19, pop25 and pop29.

Cluster B: Cluster B consisted of five genotypes. Cluster B is further divided into two sub-clusters B1 and B2. B1 contains only one genotype (pop5) while B2 have four genotypes (pop22, pop30, pop31, pop32) in it.

Cluster C: Cluster C of consensus tree consisted of six genotypes. This cluster is divided into two other sub-clusters C1 and C2. Sub-cluster C1 has two genotypes with similarity coefficient of 0.80. Sub-cluster C2 has four genotypes in two groups with genotype pop7 and po11: pop9 and pop10 having similarity coefficient of 0.80.

Cluster D: Cluster D consist of nine genotypes. It is further divided into sub-cluster D1 and D2. D1 have eight genotypes (pop13, pop14, pop15, pop16, pop20, pop21, pop24 and pop27) while D2 has only one genotype (pop26).

Table 4. Genetic similarity coefficient among 35 *Brassica napus* genotypes based on Dice similarity coefficient.

pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	****	0.7429	0.8571	0.7429	0.5429	0.6286	0.6571	0.6286	0.5429	0.5143	0.6857	0.6	0.4286	0.4571	0.5714	0.4	0.6857	0.6857	0.7143	0.4286	0.4	0.6	0.6571	0.4286	0.6	0.5143	0.5143	0.6857	0.7143	0.6857	0.6286	0.4571	0.6286	0.7429	0.3714
2		****	0.7714	0.7143	0.6857	0.6571	0.6286	0.6571	0.5714	0.6	0.7714	0.6286	0.4571	0.5429	0.5429	0.6	0.7143	0.6571	0.6857	0.5143	0.4286	0.6857	0.6857	0.4571	0.6286	0.4857	0.5429	0.6571	0.6857	0.7143	0.5429	0.5429	0.6571	0.7714	0.4571
3			****	0.8286	0.6857	0.5429	0.6286	0.6571	0.6286	0.4857	0.7143	0.5143	0.4571	0.5429	0.6	0.5429	0.7714	0.7714	0.8571	0.5143	0.4286	0.7429	0.7429	0.5143	0.7429	0.5429	0.6	0.7143	0.8	0.8286	0.7143	0.6	0.7143	0.8286	0.4
4				****	0.6857	0.5429	0.6286	0.6571	0.6857	0.5429	0.7143	0.5143	0.5714	0.5429	0.6571	0.5429	0.7714	0.6571	0.6857	0.4571	0.4286	0.6857	0.6857	0.4571	0.6286	0.5429	0.6	0.7143	0.6857	0.7714	0.6571	0.6571	0.6	0.7714	0.4571
5					****	0.7429	0.6571	0.6857	0.7143	0.6286	0.6286	0.6	0.6	0.6286	0.6857	0.6857	0.6857	0.7429	0.6571	0.4857	0.5143	0.7143	0.7714	0.6571	0.7143	0.5143	0.5714	0.6857	0.6	0.6857	0.6857	0.6857	0.7429	0.7429	0.4857
6						****	0.6857	0.7143	0.6286	0.6571	0.6571	0.8	0.5714	0.6	0.6	0.5429	0.6	0.6571	0.5714	0.4571	0.4857	0.6857	0.6857	0.6286	0.6286	0.4857	0.5429	0.7143	0.4571	0.6	0.6	0.6	0.6571	0.6	0.5143
7							****	0.7429	0.7714	0.7429	0.8	0.6	0.5429	0.6286	0.6286	0.5143	0.7429	0.6286	0.7143	0.4857	0.4571	0.6571	0.7143	0.6	0.6571	0.5143	0.5714	0.6286	0.6571	0.6286	0.5143	0.5143	0.5714	0.6286	0.4286
8								****	0.7429	0.6	0.7143	0.6286	0.6857	0.6571	0.7143	0.6	0.7714	0.7143	0.6857	0.6857	0.6571	0.7429	0.8	0.6286	0.6857	0.5429	0.6571	0.7714	0.6857	0.6571	0.6	0.5429	0.7143	0.7143	0.5143
9									****	0.8	0.8	0.6571	0.7143	0.6857	0.7429	0.6286	0.8	0.6286	0.7714	0.6	0.6286	0.7143	0.7143	0.7143	0.6571	0.5714	0.7429	0.6857	0.7143	0.6857	0.6286	0.6286	0.6857	0.6857	0.5429
10										****	0.7714	0.6857	0.6857	0.7143	0.7143	0.7143	0.7143	0.5429	0.6286	0.5714	0.6	0.5714	0.6857	0.6857	0.5714	0.4857	0.7143	0.6571	0.5714	0.5429	0.4857	0.4857	0.6571	0.6	0.5714
11											****	0.6857	0.6286	0.6	0.6571	0.6571	0.8286	0.7143	0.8	0.6286	0.6	0.7429	0.6857	0.5714	0.7429	0.6	0.6571	0.7143	0.7429	0.7714	0.6	0.6	0.6571	0.7714	0.4
12												****	0.7143	0.7429	0.6857	0.6286	0.6286	0.6286	0.6	0.4857	0.5714	0.6571	0.6	0.7143	0.6	0.5143	0.6286	0.6857	0.4857	0.5714	0.5143	0.6286	0.5714	0.5143	0.6
13													****	0.8571	0.8571	0.8	0.6286	0.6857	0.6	0.7143	0.8	0.6571	0.5429	0.7714	0.6571	0.6286	0.8	0.6286	0.6	0.5714	0.5714	0.6286	0.5714	0.5714	0.6
14														****	0.7714	0.8286	0.6571	0.6571	0.6857	0.6286	0.7143	0.7429	0.6286	0.8571	0.6286	0.6	0.7714	0.6	0.5714	0.6	0.4857	0.6571	0.5429	0.5429	0.6857
15															****	0.7143	0.7714	0.7143	0.6857	0.6857	0.6571	0.6286	0.6857	0.7429	0.6857	0.6	0.7714	0.7143	0.6286	0.6	0.6	0.5429	0.6571	0.6571	0.5714
16																****	0.7143	0.7143	0.6286	0.6857	0.7143	0.6857	0.6857	0.7429	0.6286	0.6571	0.7714	0.6571	0.6286	0.6571	0.6	0.6571	0.7143	0.6571	0.5714
17																	****	0.7714	0.8	0.5714	0.5429	0.7429	0.8571	0.6286	0.7429	0.6571	0.7143	0.7714	0.7429	0.7714	0.6571	0.5429	0.7143	0.7714	0.4571
18																		****	0.7429	0.5714	0.6571	0.6857	0.7429	0.6857	0.8	0.6571	0.7143	0.7714	0.7429	0.7143	0.6571	0.6	0.7143	0.7714	0.4
19																			****	0.6571	0.5714	0.8286	0.7143	0.6571	0.8286	0.5714	0.6857	0.6857	0.8286	0.8	0.7429	0.6857	0.7429	0.7429	0.4286
20																				****	0.8	0.6	0.4857	0.6	0.6	0.6286	0.6286	0.5714	0.6571	0.5143	0.4571	0.6286	0.6286	0.4857
21																					****	0.6286	0.4571	0.6286	0.5714	0.6	0.7143	0.5429	0.5714	0.5429	0.4857	0.4857	0.5429	0.6	0.5714
22																						****	0.6571	0.6	0.7143	0.5714	0.6286	0.6286	0.7143	0.8571	0.7429	0.7429	0.6286	0.6857	0.5429
23																							****	0.7143	0.7143	0.5714	0.6857	0.8571	0.6571	0.6857	0.6857	0.6286	0.8571	0.7429	0.4857
24																								****	0.6571	0.6286	0.7429	0.6286	0.4857	0.5143	0.5143	0.6857	0.6286	0.5143	0.6
25																									****	0.6286	0.6857	0.7429	0.7143	0.7429	0.6857	0.6286	0.6857	0.7429	0.4286
26																										****	0.6	0.6	0.5714	0.7143	0.5429	0.5429	0.5429	0.7143	0.5714
27																											****	0.7714	0.6857	0.6	0.5429	0.5429	0.6571	0.6571	0.6286
28																												****	0.7429	0.7143	0.6571	0.6	0.8286	0.7714	0.5143
29																													****	0.8	0.7429	0.5714	0.7429	0.7429	0.3714
30																														****	0.8286	0.7143	0.7143	0.8286	0.4
31																															****	0.7714	0.8286	0.6571	0.2857
32																																****	0.6571	0.5429	0.5143
33																																	****	0.7714	0.4
34																																		****	0.4571
35																																			****

	Highest similarity coefficient
	Lowest similarity coefficient

Table 5. Genetic distance coefficient among 35 *Brassica napus* genotypes based on Dice similarity coefficient.

pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	****																																		
2	0.2973	****																																	
3	0.1542	0.2595	****																																
4	0.2973	0.3365	0.1881	****																															
5	0.6109	0.3773	0.3773	0.3773	****																														
6	0.4643	0.4199	0.6109	0.6109	0.2973	****																													
7	0.4199	0.4643	0.4643	0.4643	0.4199	0.3773	****																												
8	0.4643	0.4199	0.4199	0.4199	0.3773	0.3365	0.2973	****																											
9	0.6109	0.5596	0.4643	0.3773	0.3365	0.4643	0.2595	0.2973	****																										
10	0.665	0.5108	0.7221	0.6109	0.4643	0.4199	0.2973	0.5108	0.2231	****																									
11	0.3773	0.2595	0.3365	0.3365	0.4643	0.4199	0.2231	0.3365	0.2231	0.2595	****																								
12	0.5108	0.4643	0.665	0.665	0.5108	0.2231	0.5108	0.4643	0.4199	0.3773	0.3773	****																							
13	0.8473	0.7828	0.7828	0.5596	0.5108	0.5596	0.6109	0.3773	0.3365	0.3773	0.4643	0.3365	****																						
14	0.7828	0.6109	0.6109	0.6109	0.4643	0.5108	0.4643	0.4199	0.3773	0.3365	0.5108	0.2973	0.1542	****																					
15	0.5596	0.6109	0.5108	0.4199	0.3773	0.5108	0.4643	0.3365	0.2973	0.3365	0.4199	0.3773	0.1542	0.2595	****																				
16	0.9163	0.5108	0.6109	0.6109	0.3773	0.6109	0.665	0.5108	0.4643	0.3365	0.4199	0.4643	0.2231	0.1881	0.3365	****																			
17	0.3773	0.3365	0.2595	0.2595	0.3773	0.5108	0.2973	0.2595	0.2231	0.3365	0.1881	0.4643	0.4643	0.4199	0.2595	0.3365	****																		
18	0.3773	0.4199	0.2595	0.4199	0.2973	0.4199	0.4643	0.3365	0.4643	0.6109	0.3365	0.4643	0.3773	0.4199	0.3365	0.3365	0.2595	****																	
19	0.3365	0.3773	0.1542	0.3773	0.4199	0.5596	0.3365	0.3773	0.2595	0.4643	0.2231	0.5108	0.5108	0.3773	0.3773	0.4643	0.2231	0.2973	****																
20	0.8473	0.665	0.665	0.7828	0.7221	0.7828	0.7221	0.3773	0.5108	0.5596	0.4643	0.7221	0.3365	0.4643	0.3773	0.3773	0.5596	0.5596	0.4199	****															
21	0.9163	0.8473	0.8473	0.8473	0.665	0.7221	0.7828	0.4199	0.4643	0.5108	0.5108	0.5596	0.2231	0.3365	0.4199	0.3365	0.6109	0.4199	0.5596	0.2231	****														
22	0.5108	0.3773	0.2973	0.3773	0.3365	0.3773	0.4199	0.2973	0.3365	0.5596	0.2973	0.4199	0.4199	0.2973	0.4643	0.3773	0.2973	0.3773	0.1881	0.5108	0.4643	****													
23	0.4199	0.3773	0.2973	0.3773	0.2595	0.3773	0.3365	0.2231	0.3365	0.3773	0.3773	0.5108	0.6109	0.4643	0.3773	0.3773	0.1542	0.2973	0.3365	0.7221	0.7828	0.4199	****												
24	0.8473	0.7828	0.665	0.7828	0.4199	0.4643	0.5108	0.4643	0.3365	0.3773	0.5596	0.3365	0.2595	0.1542	0.2973	0.2973	0.4643	0.3773	0.4199	0.5108	0.4643	0.5108	0.3365	****											
25	0.5108	0.4643	0.2973	0.4643	0.3365	0.4643	0.4199	0.3773	0.4199	0.5596	0.2973	0.5108	0.4199	0.4643	0.3773	0.4643	0.2973	0.2231	0.1881	0.5108	0.5596	0.3365	0.3365	0.4199	****										
26	0.665	0.7221	0.6109	0.6109	0.665	0.7221	0.665	0.6109	0.5596	0.7221	0.5108	0.665	0.4643	0.5108	0.5108	0.4199	0.4199	0.4199	0.5596	0.4643	0.5108	0.5596	0.5596	0.4643	0.4643	****									
27	0.665	0.6109	0.5108	0.5108	0.5596	0.6109	0.5596	0.4199	0.2973	0.3365	0.4199	0.4643	0.2231	0.2595	0.2595	0.2595	0.3365	0.3365	0.3773	0.4643	0.3365	0.4643	0.3773	0.2973	0.3773	0.5108	****								
28	0.3773	0.4199	0.3365	0.3365	0.3773	0.3365	0.4643	0.2595	0.3773	0.4199	0.3365	0.3773	0.4643	0.5108	0.3365	0.4199	0.2595	0.2595	0.3773	0.5596	0.6109	0.4643	0.1542	0.4643	0.2973	0.5108	0.2595	****							
29	0.3365	0.3773	0.2231	0.3773	0.5108	0.7828	0.4199	0.3773	0.3365	0.5596	0.2973	0.7221	0.5108	0.5596	0.4643	0.4643	0.2973	0.2973	0.1881	0.4199	0.5596	0.3365	0.4199	0.7221	0.3365	0.5596	0.3773	0.2973	****						
30	0.3773	0.3365	0.1881	0.2595	0.3773	0.5108	0.4643	0.4199	0.3773	0.6109	0.2595	0.5596	0.5596	0.5108	0.5108	0.4199	0.2595	0.3365	0.2231	0.5596	0.6109	0.1542	0.3773	0.665	0.2973	0.3365	0.5108	0.3365	0.2231	****				
31	0.4643	0.6109	0.3365	0.4199	0.3773	0.5108	0.665	0.5108	0.4643	0.7221	0.5108	0.665	0.5596	0.7221	0.5108	0.5108	0.4199	0.4199	0.2973	0.665	0.7221	0.2973	0.3773	0.665	0.3773	0.6109	0.6109	0.4199	0.2973	0.1881	****			
32	0.7828	0.6109	0.5108	0.4199	0.3773	0.5108	0.665	0.6109	0.4643	0.7221	0.5108	0.4643	0.4643	0.4199	0.6109	0.4199	0.6109	0.5108	0.3773	0.7828	0.7221	0.2973	0.4643	0.3773	0.4643	0.6109	0.6109	0.5108	0.5596	0.3365	0.2595	****		
33	0.4643	0.4199	0.3365	0.5108	0.2973	0.4199	0.5596	0.3365	0.3773	0.4199	0.4199	0.5596	0.5596	0.6109	0.4199	0.3365	0.3365	0.3365	0.2973	0.4643	0.6109	0.4643	0.1542	0.4643	0.3773	0.6109	0.4199	0.1881	0.2973	0.3365	0.1881	0.4199	****	
34	0.2973	0.2595	0.1881	0.2595	0.2973	0.5108	0.4643	0.3365	0.3773	0.5108	0.2595	0.665	0.5596	0.6109	0.4199	0.4199	0.2595	0.2595	0.2973	0.4643	0.5108	0.3773	0.2973	0.665	0.2973	0.3365	0.4199	0.2595	0.2973	0.1881	0.4199	0.6109	0.2595	****	
35	0.9904	0.7828	0.9163	0.7828	0.7221	0.665	0.8473	0.665	0.6109	0.5596	0.9163	0.5108	0.5108	0.3773	0.5596	0.5596	0.7828	0.9163	0.8473	0.7221	0.5596	0.6109	0.7221	0.5108	0.8473	0.5596	0.4643	0.665	0.9904	0.9163	0.99	0.665	0.9163	0.7828	****

	Highest distant coefficient
	Lowest distant coefficient

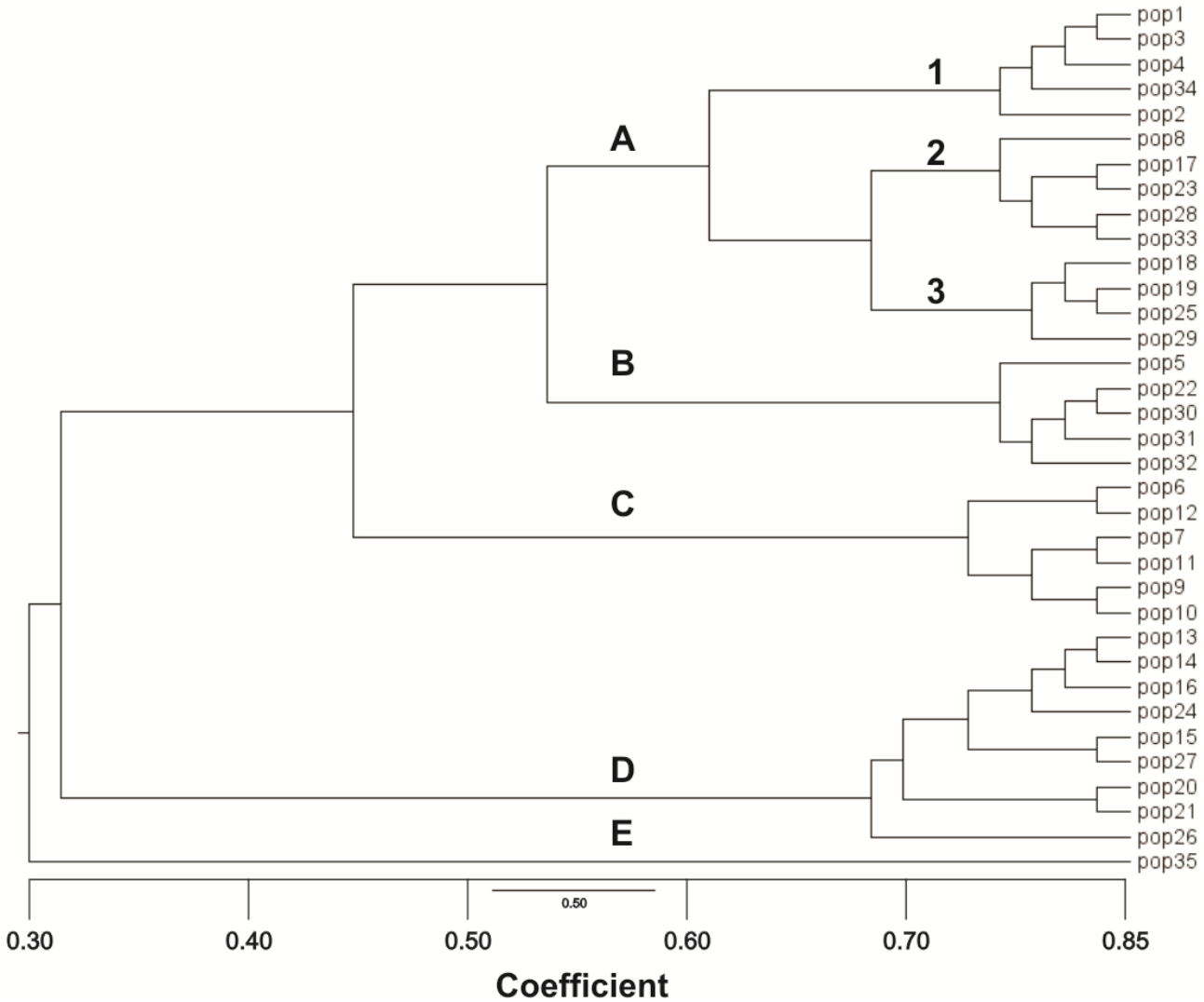

Fig. 2. A dendrogram generated from UPGMA cluster analysis, showing the diversity and relationship among 35 *B. napus* genotypes.

Cluster E: A separate cluster is formed by pop35 (28165) in the dendrogram, which is genetically distinct from other *B. napus* genotypes and may indicate a particular genetic background.

These results demonstrated that SSR has a potential application to evaluate genetic diversity and relationship between *Brassica napus* genotypes. Many other researches supported these results. Genetic relationship and diversity was assessed among 108 winter *B. napus* cultivar by using 18 SSR primers (Xu *et al*., 2008). SSR markers were used to analyze the genetic diversity and relationship among 217 genotypes of *B. napus* (Qu *et al.*, 2012;Zhou *et al.*, 2006). By using SSR markers, potential heterotic group of oilseed rape are identified from the accessions collected from different continents, which are useful for their further use in breeding high yielding rapeseed (Younas *et al.*, 2012).

The results of present experiment indicated the ability of selected SSR markers to differentiate among *B. napus* genotypes. All the genotypes gave unique genetic fingerprints highlighting the peculiarity of this genetic material regarding the genetic diversity. Genotypes from Pakistan showed similarity with genotypes of Australia, China and Canada. This showed that the genotypes cannot be differentiated based on their country of origin and thus the genotypes from similar source country did not completely fit each into separate clusters, which inferred that an widespread gene exchange may have occurred among all genotypes. Therefore, it is proposed to evaluate more diverse germplasm from different continents for estimation of available genetic diversity for further improvement to meet the requirement of today's need.

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