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Estimation of Genetic Diversity among Canola Accessions using Simple Sequence Repeat Markers

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ESTIMATION OF GENETIC DIVERSITY AMONG CANOLA ACCESSIONS USING SIMPLE SEQUENCE REPEAT MARKERS

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ABSTRACT

Genetic studies through molecular markers proved important to find out the genetic diversity of canola. In this study, 50 lines of canola were used to find the polymorphism using 15 SSR primers and investigated the genetic diversity, PIC values, frequency-based genetic distance, and allelic frequencies. Mean gene diversity, frequency-based genetic distance, and PIC values were 0.8777, 0.233 and 0.8666, respectively for the canola lines. A good range of genetic diversity was found among studied canola lines with value 85.91% polymorphism. Maximum and minimum genetic distances among 50 lines were 1 and 0.26, respectively. Accessions ACC-26068, ACC-24241, ACC-24244, ACC-24233, ACC-24423 and ACC-24224 have maximum genetic distance. Accessions ACC-24879 and ACC-24169 had minimum genetic distance i.e., 0.26. Dendrogram based on genetic distances showed four main clusters that were further dividing into several sub-clusters. The primers utilized in the present study, were valuable to identify different accessions of canola to find the variability present. This variability will be helpful to initiate the breeding program with their molecular genetic basis.

Keywords: Molecular diversity, simple sequence repeat, dendrogram, polymorphism information content, canola.

INTRODUCTION

Canola has been cultivated for many years, used as a resource of edible oil and have a wide range of usage in industries to manufacture bioproducts. Canola is taken as the third crop after soybean oil and palm oil and it is also considered economically the fifth important crop after wheat, rice, cotton, and maize (Bhardwaj & Hamama, 2003; Liu et al., 2016). After the cotton crop canola is the major source of edible oil in Pakistan. It is estimated that 307,000 hectares are cultivated and annul production of canola is 233000 tons. Canola seed is the major source of protein and oil. Canola seed contain 46 to 48 % of oil (Ali et al., 2011). Allopolyploid species of *Brassica* are originated by hybridization between two same species of *Brassica* by doubling the chromosome number. Some of the crops are economically important like cotton (*Gossypium hirsutum*), wheat (*Triticum aestivum*), and canola (*Brassica napus*) are allopolyploids produced by two different genomes. Polyploid plant has significant role in plants evolution. There are number of advantages of polyploid plant as compared to diploid plants, like polyploidy plant has ability to survive in the severe condition and resistant against the pests and other diseases.

An increasing demand for canola oil for domestic consumption and export market is also realized through the production of naturally high monounsaturated fat to take place high contents of fats of palm oil (Moghaieb et al., 2014). Unsaturated fatty acids are more beneficial as compare to saturated fatty

acids. Saturated fatty acids are helpful to increase the level of blood cholesterol (Moghaieb et al., 2006). Molecular breeding has played a key role to obtain high yield and good quality of oilseed plants and a reduction in genomic change in some essential plants (Korzekwa, 2019).

Archeology records estimate that *brassica* plants were cultivated 1500BC ago. In 1935 UK has projected the evolution of main crop species. *B.oleracea*, *B. nigra* and *B. rapa* were originated from *B. napus*, *B. juncea* and *B. carinata*. There are three amphidiploid species like *B. carinata* (BBCC), *B. juncea* (AABB) and *B. napus* (AACC), developed through the interbreeding between individuals of the same species. This genetic change has increased the diversity of morphotypes in *brassica* species which are used as food in various forms like seeds, roots, stem, leaf, and flower (Khurshid et al., 2019; Summanwar, 2021).

Molecular markers have been developed and show a wide range of applications in molecular biology that involve ecology, population genetics, evolution, phylogenetic study, and study genomic traits like hair and skin color in both animal and plants. These various applications can be achieved because of more advances in molecular techniques (Morin et al., 2004; Park et al., 2021). DNA based markers are used as a functional gene in genetic engineering (Agrama & Tuinstra, 2003). Various markers may be used to evaluate plant's genetic diversity such as restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), random amplified polymorphic DNA (RAPD, simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP). Various markers have been used to select the desired plant. Molecular markers are used to assist plant breeders to select the plant of desire characteristics (Hoshino et al., 2012).

Simple sequence repeats (SSR) or microsatellites commonly used to estimate the genetic diversity for crop plants. because of their robust nature, codominant, multi-allelic, hyper-variable, and locus-specific type (Kong et al., 2000; Oraguzie et al., 2007; Murray et al., 2009). The objective of this investigation was to evaluate genetic diversity and polymorphic information contents (PIC) between canola cultivars by using SSR markers.

MATERIALS AND METHODS

In this study, 50 lines of canola were used. Germplasm of canola was collected from Pakistan Genetic Resource Institute (PGRI), Islamabad, and germplasm were planted in the field at Bahauddin Zakariya University Multan, Pakistan. Row length was 4 m with interplant spaces and 0.5 cm gap between rows. Each line was planted in one row as shown in Table 1. Healthy and vigorous plants were gained.

Sample Collection and Extraction of Genomic DNA

Five to seven young and fresh leaves were collected as a sample and kept in the polythene bags and these bags were placed on ice to prevent the leaves from environmental stress. Leaves samples were stored in the freezer at -80 ^{o}C in the lab. The genomic DNA of each sample was isolated from leaves by using CTAB (cetyl trimethyl ammonium bromide) method illustrated by Doyle and Doyle (1990). DNA pellet of each sample was diluted in 20 ul of double distilled water and stored at -20 ^{o}C. DNA of each sample was quantified by 0.8 % agarose gel electrophoresis. For each sample DNA quality was estimated by using spectrophotometer.

SSR Markers Analysis

A total 15 SSR markers were used to evaluate the 50 canola lines. These

markers assisted to evaluate the genetic diversity among the canola cultivars. SSR primers for this study were obtained from http://www.brassica.info/resource/markers.php.

PCR (Polymerase Chain Reaction)

PCR was conducted to analyze the SSR markers (Table 2). Total reaction volume in the PCR tube was 20 ul, consisting of 5 ul template DNA, Buffer (2.5 ul), dNTPs (2.5ul), forward and reverse primer (1.5 ul), *Taq polymerase* (0.5 ul), and PCR water 8 ul in each sample. PCR was conducted by using the Gene Amp® system (applied Biosystems). The PCR profile consisting of initial denaturation for 5 minutes at 94 ^{o}C using 35 cycles for denaturing at 95 ^{o}C for 1 minutes, annealing temperature at 55 ℃ for 1minute, initial extension at 72 ^{o}C for 1 minute, and a final extension at 72 ^{o}C for 2 minutes. PCR products were analyzed on 1.8 % agarose gel to check the amplification of PCR products and product bands were visualized under the ultraviolet (UV) light. Ladder of 100 base pair was also loaded in the gel well to evaluate the band's size of canola lines.

Table 1: selected lines of canola used for genetic analysis.

Sr No.	ACCESSION	Sr No	ACCESSION
1	ACC-24211	26	ACC-26068
2	ACC-24212	27	ACC-24244
3	ACC-24213	28	ACC-24241
4	ACC-24214	29	ACC-27391
5	ACC-24215	30	ACC-24240
6	ACC-24216	31	ACC-24875
7	ACC-24217	32	ACC-27405
8	ACC-24218	33	ACC-24880
9	ACC-24219	34	ACC-24214
10	ACC-24220	35	ACC-27396
11	ACC-24221	36	ACC-24210
12	ACC-24222	37	ACC-24254
13	ACC-24223	38	ACC-24889
14	ACC-24224	39	ACC-24217
15	ACC-24225	40	ACC-23633
16	ACC-24226	41	ACC-24169
17	ACC-24227	42	ACC-24879
18	ACC-24228	43	ACC-27403
19	ACC-24229	44	ACC-24715
20	ACC-24230	45	ACC-24876
21	ACC-24231	46	ACC-27396
22	ACC-24232	47	ACC-27388
23	ACC-24233	48	ACC-24884
24	ACC-24234	49	ACC-24848
25	ACC-24235	50	ACC-24881

Table 2: SSR primers used for genetic evolution in canola.

Sr	Primer name	Forward (5`-3`)	Reserve (5`-3`)
1	BRAS011	TGGGACGTAGTCAGTCAACAA	CCAAGTGCGAGAAGAGGAAG
2	BRAS014	CCCAITGACAACTCTTCTCTT	CTGTGTTCGCCCATTATG
3	BRAS072	GCCATCTACACATTTATCCC	CACTAACCTTCTTGCTACCGT
4	BRAS078	ATTGGGTTCTGACCTTITCTC	CTTTTCCTCATCGCTACCAC
5	BRAS084	ATTGGGTTCTGACCTIITCTC	TTTTCCTTCATCGCTACCAC
6	CB10026	TCGTTCTGACCTGTCGTTAT	GGAAATGGCTGCTCATGTT
7	CB10028	CTGCACATTTGAAA TIGGTC	AAATCAACGCTTACCCACT
8	CB10092	TTGATCCGAAATTCTCTGG	AGGCAAGCAATAGATAAAGG
9	CB10143	CATGGGAGGCTGTCTAAA	TTGCACCCATACGTTTTC
10	CB10369	CATTCACAGGACCAGAGC	CAAAGCCAAGACAACCA T
11	CB10427	TCCCAACAAAAGAGTCCA	CAGCGAACCGAGTCTAAA
12	CB10545	CTCGCAATAGTCGCAGAT	TGCCCTACTGTCTCCTCA
13	CB10587	TTGTGTTTTGCCTTCTGA	TTTGCGCACAAACAATAA
14	CB10594	ACTGCAGGGAAGTTCGAT	TTTGGCTCCGTTACACAT
15	Na 12E02	TTGAAGTAGTTGGAGTAATTGGAGG	CAGCAGCCACAACCTTACG

Data Analysis

Bands produced by SSR markers were scored as 0 and 1 indicating the absence and presence of bands, respectively. Only visible bands were scored. Polymorphism among selected canola lines was calculated on the basis of absence and presence of band. Genetic distance between canola lines was calculated by the power marker version 3.2.

RESULTS

Total 15 SSR primers were used to analyze the genetic diversity in 50 canola cultivars. Primers showed different polymorphism levels between the 50 cultivars of canola. Selected markers generated bands in the size of 100 to 1200 bp. PCR amplification by using 15 SSR primers produced total 620 alleles out of which 533 alleles were considered as polymorphic showing 85.96 % polymorphism. Maximum loci were generated by BRAS072A, CB10369, CB10545, CB10587, Na12_E02, and CB10594 primers and minimum loci were examined by CB10092 and CB10143 primers.

genetic dissimilarity between canola lines was identified by estimating the identities and genetic distance between canola lines as per Nei's coefficient. Analysis of clusters was done by using UPGMA (unweighted pair group method with arithmetic averages). The taxonomic relationship was constructed based on Nei's pairwise genetic distance by using a power marker. The UPGMA genetic tree was constructed by using the power marker as described by Lee et al., (2008).

Polymorphic Information Content

The quantity of amplified and polymorphic alleles and the ratio of polymorphic alleles were calculated. Polymorphisms among 50 cultivars of canola were confirmed by observing the PIC values for fifteen SSR primers. Out of 620 alleles, 533 alleles were polymorphic with 85.96 % polymorphism and 20 alleles were monomorphic with 14.04 % monomorphism. The estimated size of the amplified alleles ranges from 100 to 1200bp. Each single genotype dis-similarity was calculated with the help of PIC values.

Table 3: Genetic diversity parameters of 50 canola lines with 15 SSR markers.

Markers	Major Allele Frequency	Allele No.	Gene Diversity	PIC
BRAS011	0.06	39	0.9696	0.968724
BRAS014	0.22	13	0.8488	0.831360
BRAS072	0.14	39	0.9568	0.955370
BRAS078	0.62	4	0.5320	0.466901
BRAS084	0.24	11	0.8504	0.834085
CB10026	0.06	43	0.9728	0.972100
CB10028	0.46	12	0.7304	0.704207
CB10092	0.18	22	0.9176	0.912254
CB10143	0.26	32	0.9096	0.906059
CB10369	0.06	38	0.9688	0.967877
CB10427	0.14	34	0.9504	0.948444
CB10545	0.3	15	0.8544	0.841976
CB10587	0.23	26	0.9144	0.910459
CB10594	0.18	32	0.9384	0.935774
Na 12-E02	0.34	20	0.8512	0.842598
Mean	0.233	25.33	0.8777	0.866546

Polymorphic information content (PIC) values for different primers varied from 0.467 to 0.97 with an average value of 0.87. Nine SSR marker showed PIC values greater than 0.9, it means there is much genetic divergence is present among the 50 cultivars of canola (Table 3). Polymorphism information content (PIC) values showed that SSR markers are equally distributed in population.

Genetic Distance Matrix

Genetic dissimilarity is helpful to identify the yield of cultivars. The genetic distance among 15 SSR markers of canola is shown in the Table 3. The major allele frequency was ranges from 0.06 to 0.62 with mean value 0.233. The maximum and minimum number of alleles involves were 43 and 4 for marker CB10026 and BRAS078 respectively. The average number of alleles for 15 SSR markers was 25.33. Marker CB10026 showed maximum genetic diversity with value 0.9728 and PIC value 0.972100. While, marker BRAS078 showed minimum genetic diversity with value 0.5320 and PIC value 0.466901.

Genetic Tree and Cluster Analysis

A dendrogram of 50 lines of canola was constructed by using of Power marker as shown in the figure1. In the dendrogram vertical axis are represented the lines and the horizontal axis are represents genetic distance among cultivars. The dendrogram demonstrates the similarity between the cultivars of canola. Based on similarity all the genotypes were divided into four main clusters.

Cluster A

Cluster A consisted of 6 genotypes. Cluster A further grouped into A1 and A2. A1 sub-cluster is consisting of 3 genotypes including ACC-26068, ACC-24241, and ACC-24244. A2 sub-cluster is also consists of 3 genotypes ACC-24234, ACC-24223, and ACC-24224.

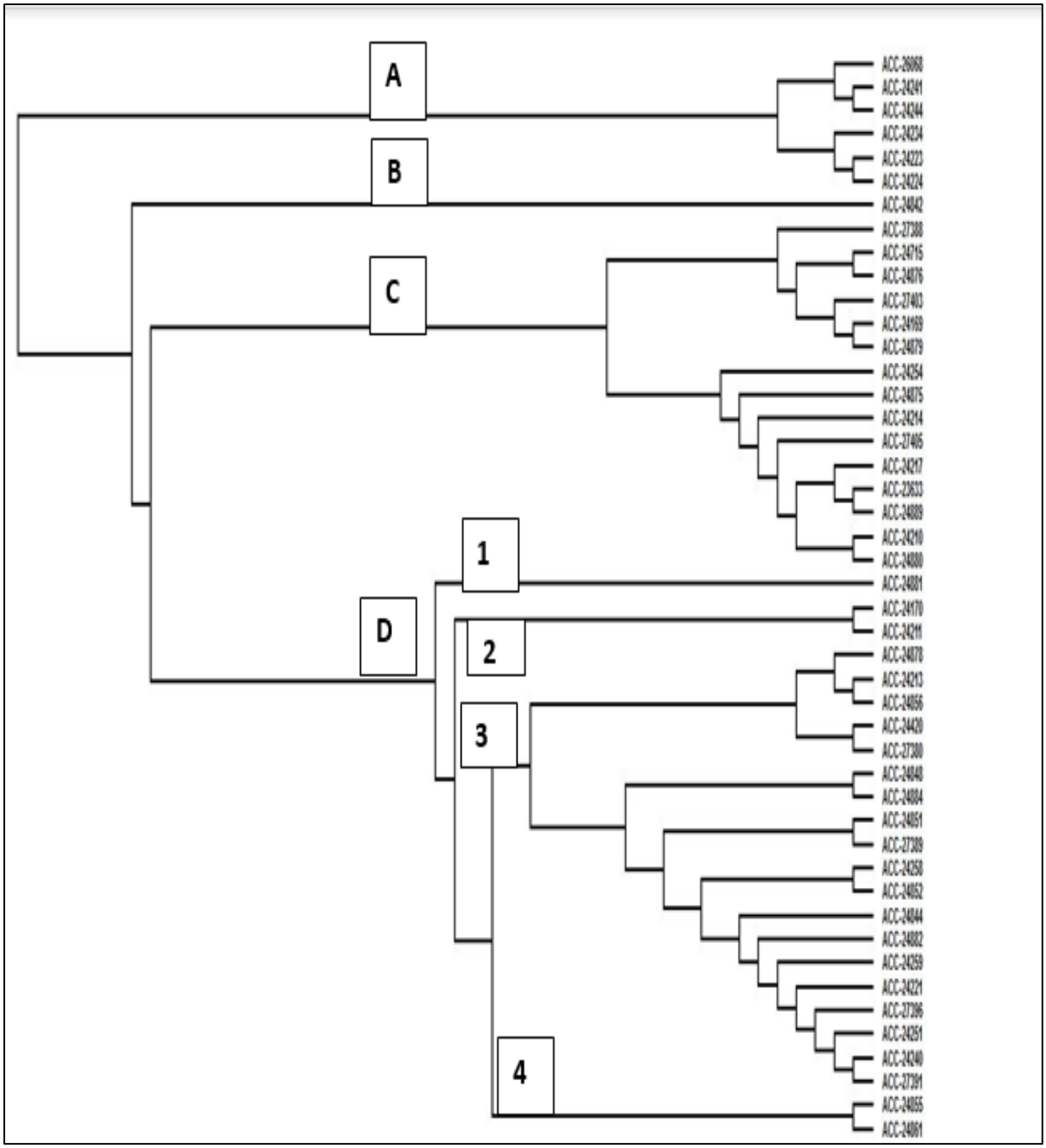

Figure 1: UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram representing the genetic relations between 50 canola cultivars.

Cluster B

Cluster B is consisting of only one genotype ACC-24842, which genetically different from other genotypes of canola.

Cluster C

Cluster is consisting of 15 genotypes. Cluster C has two sub-clusters C1 and C2. Sub-cluster has 6 genotypes including ACC-27388, ACC-24715, ACC-24876, ACC-27403, ACC-24169 and ACC-24879. Sub-cluster has 9 genotypes including ACC-24254, ACC-24875, ACC-24214, ACC- 27405, ACC-24217, ACC-23633, ACC-24889, ACC-24210 and ACC-24880.

Cluster D

Cluster is consisting of 28 genotypes. Cluster D has four sub-clusters D1, D2, D3, and D4. D1 sub-cluster has only 1 genotype which is ACC-24881. D2 sub-cluster is consists of 2 genotypes including ACC-24170 and ACC-24211. D3 sub-cluster is consist of 19 genotypes including ACC-24878, ACC- 24213, ACC-24856, ACC- ACC-24420, ACC-27380, ACC-24848, ACC-24884, ACC-24851, ACC-27389, ACC-24258, ACC-24852, ACC-24844, ACC-24882, ACC-24259, ACC-24221, ACC-27396, ACC-24251, ACC-24240 and ACC-27391. D4 consist of 4 genotypes including ACC-24855 and ACC-2486.

DISCUSSION

In the present research, we have used 15 SSR markers to evaluate the genetic diversity among 50 cultivars of canola. All the SSR markers were amplified generating 620 different bands. 13 markers were polymorphic out of 15 markers, with CB10026 being the most polymorphic and having a PIC value of 0.9721. These outcomes confirm the efficiency of SSR markers when used in research on genetic diversity. SSR markers are the most recommended markers to evaluate the genetic diversity or resemblances among canola cultivars.

SSR markers are recommended to identify the genetic similarities and dissimilarities between different cultivars of a crop. The purpose of genetic diversity estimation between different lines or species of plants is to obtain informations for crop preservation and to improve the different varieties of the plant (Romero et al., 2009).

Microsatellites or SSR markers are used for the genetic fingerprinting of the number of plant varieties and plant species due to reproducible nature, highly polymorphic and codominant (Gupta and Varshney, 2000).The number of allelic nature primers provide the identification of relative loci allelic changeability across a huge variety of germplasm (Joshi et al., 1999).

One of the newest researches in which RAPD markers was used to reveal 227 alleles, an average of 11.35 alleles per primer were produced, and the percentage of polymorphism was calculated 86.34 % between ten varieties of sunflower (Raza et al., 2018).

Total 38 SSR markers were used to evaluate the genetic diversity between 28 varieties of sunflower which produced total 102 alleles with an average of 2.32 alleles per marker. Genetic similarity between sunflower cultivars was 0.25 to 0.90 and PIC value observed by the researchers were 0.09 minimum and maximum PIC value was 0.62, giving an average of 0.41(Nazarli et al., 2010).

13 SSR markers were used to find the genetic diversity among 30 Pakistani Upland cotton (*Gossypium hirsutum L.*) varieties. These markers produced total 48 bands. Eight markers were polymorphic and five were monomorphic. Among those 13 markers NAU 2083 revealed highest polymorphism that was 68%. The similarity among studied 30 varieties ranged 38.46-100%. 0.6484 was highest PIC value and 0.2833 was the mean pic value. Average major allele frequency, numbers of effective alleles and gene diversity were 0.233, 25.33 and 0.877, respectively (Aslam et al., 2020).

Results of experiments carried out in this study indicated the capability of SSR markers to distinguish between *B. napus* cultivars. All the cultivars provided distinctive genetic fingerprints that enlightened the uniqueness of genetic diversity.

CONCLUSION

An understanding of diversity and similarity between canola cultivars can ease breeders to select parent plants with desired characteristics, these characteristics may be transferred to

offspring for the progress of different cultivars. In present study, genetic diversity was estimated among 50 canola cultivars by using fifteen specific SSR markers. These SSR markers revealed a good range of genetic diversity present among canola cultivars with mean PIC value of 0.866. The diverse lines were explored in this study by using SSR markers can be utilized in further breeding programs by the selection of parent plants having gene of interest, such that these genes of interest with required characteristic may be transferred to offspring to develop novel canola cultivars with improved yield and biotic and abiotic stress tolerance.

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Nil

CONFLICT OF INTEREST

Authors have no conflict of interest

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