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Research Article

Molecular Diversity Analysis in Dhaincha (*Sesbania cannabina* L.) Using RAPD Primers

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ABSTRACT

Dhaincha belonging to family fabaceae is a widely grown green manure crop. In present study random amplified polymorphic DNA (RAPD) markers were used (A total of eight primers generated reproducible RAPD patterns from twenty screened primers). PCR amplification using these RAPD primers yielded seventy scorable bands, of which sixty seven were polymorphic, with an average of 8.7 polymorphic fragments per primer. Number of amplified fragments ranged from 4 (OPA-11) to 16 (OPA-13). Percentage polymorphism ranged from 75% (OPA-11) to 100 % (OPA-1, OPA-2, OPA-4, OPA-8 and OPA-11), with an average of 95.71%. The PIC value ranges from 0.203 (OPA-11) to 0.762 (OPA-3). The Jaccard's similarity indices based on RAPD profiles were subjected to UPGMA cluster analysis and genotypes grouped in two major clusters and seven sub clusters. Clustering pattern was correlated with geographical distribution of the genotypes. Twenty nine out of thirty genotypes grouped to cluster II. This indicated the presence of narrow genetic base of the genotypes used in the study.

Keywords: *Dhaincha, RAPD, Polymorphic.*

INTRODUCTION

Dhaincha (*Sesbania cannabina* L.) (2n=2x=24) is an important green manuring crop belonging to family fabaceae sub family papilionaceae. Nearly twenty four species of genus *Sesbania* are found throughout tropics. *S. cannabina* is predominant in Indian sub-continent among these species. It has wide range of adaptability in varying agro-climatic conditions which has made possible its cultivation throughout tropical and sub-tropical regions of the world. It is multipurpose leguminous crop and is widely used as green manure crop to increase the yield of rice, wheat, maize and sugarcane etc. Being a leguminous crop it produce root nodules which in association with symbiotic bacteria such as *Rhizobium* spp. fix considerable amount of nitrogen in soil.

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Now-a-days use of inappropriate technologies have resulted in the deterioration of soil quality leading to soil organic matter losses and structure degradation, affecting water, air and nutrients flows, and consequently plant growth. For this reason, the application of organic matter including green manure to the soil has become a common agricultural practice for soil quality restoration, maintaining soil organic matter, reclaiming degraded soils and supplying the plant nutrients**.** Keeping in view this there is a great need to start a breeding programme for improvement of green manuring crops, but very less efforts have been made in this direction.

Genetic diversity evaluation is an important and a prerequisite for any breeding program (Dwivedi et al., 2001). The assessment of genetic diversity is important not only for crop improvement but also for efficient management and conservation of germplasm resources (Tahir & Karim, 2011). Several approaches such as morphological, protein, isozyme and molecular markers have been employed to assess genetic diversity in crop plants. Among all of these the DNA based molecular marker approach has been found to be superior, because of its capability to reveal more polymorphism (Mignouna et al., 1998). Nowadays, molecular markers are avaluable tools in the characterization and evaluation of genetic diversity within and between species and population.

RAPD markers have many advantages such as higher frequency of polymorphism, rapidity, technical simplicity, requirement of a few nanograms of DNA, no requirement of prior information of any DNA sequence and feasibility of automation; and hence widely used for diversity analysis in many crops. RAPD markers have been used for molecular diversity analysis in dhaincha by Bisoyi et al. (2010). In this work total thirty genotypes were characterized using RAPD primers and phylogenic relationship was also determined.

MATERIALS AND METHODS

Total genomic DNA was extracted from the 3 weeks old leaves by Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1990) with some modifications from thirty dhaincha genotypes (Table 1). DNA quantification was done by spectrophotometer at 260/280 nm. To check the quality of DNA, DNA was electrophoresed on 0.8% agarose gel. The dilution of extracted DNA was verified again by spectrophotometry. A total of 20 decamer primers of OPA series were screened for PCR amplification (Imperial Life Science Pvt. Ltd.), out of which 8 primers were used for analysis. PCR was performed using 25 µl reaction mixture containing 2 µl of DNA (50ng/µl), 0.60 µl of Taq DNA polymerase (5U), 2 µl of primers, 2.5 µl of dNTPs, 2.5 µl of 10X PCR buffer and remaining 15.60 µl of ddH_2O. Amplification was programmed for 40 cycles with initial denaturation at 94°C for 5 min., followed by cycling conditions of denaturation at 94°C for 30 sec., annealing at 36°C at 30 sec. and extension at 72°C for 1 min. After 40 cycles, there was a final extension step of 5 min at 72 °C. Submerged gel electrophoresis unit was used for fractionating amplified PCR products on 1.5 per cent agarose gel. The gel was prepared in 1X TAE buffer (Sambrook et al. 1989) containing (50µl/100ml) of ethidium bromide. The ratio of samples and loading dye was 4:1 and it was loaded with the help of micropipette. Electrophoresis was carried out at 50 V for 3 hours in 1X TAE buffer. After separation the gel was viewed under UV transilluminator and photographed with the help of gel documentation system (Biorad). The amplified products were scored for presence (1), absence (0). Band size was determined by comparison with 100 bp DNA ladder (Genei, Bangalore) as standard. A rectangular data matrix (qualitative data matrix) was obtained by the scores (0 or 1) for each band obtained from photograph. The data was used for similarity based analysis using programme NTSYS-PC (Version 2.02) developed by (Rolf et al., 1993) Jaccard's similarity coefficients was calculated using the programme SIMQUAL. Similarity coefficients were used to construct UPGMA (Unweighted pair group method with average) to produce

dendrogram. The polymorphic percentage and PIC value of the obtained bands were calculated by using following formula for each primer.

$$\text{Polymorphic \%} = (\text{no. of polymorphic bands} / \text{Total bands}) \times 100$$

$$\text{PIC value} = 1 - \sum (Pi)^2$$

Table 1: List of dhaincha genotypes used in the study

No.	Genotype	No.	Genotype	No.	Genotype
01	NSB -1	11	NSB -11	21	NSB -21
02	NSB -2	12	NSB -12	22	NSB -22
03	NSB -3	13	NSB -13	23	NSB -23
04	NSB -4	14	NSB -14	24	NSB -24
05	NSB -5	15	NSB -15	25	NSB -25
06	NSB -6	16	NSB -16	26	NSB -26
07	NSB -7	17	NSB -17	27	NSB -27
08	NSB -8	18	NSB -18	28	NSB -28
09	NSB -09	19	NSB -19	29	CSD-123
10	NSB -10	20	NSB -20	30	CSD-137

Table 2: Detail of RAPD primers used in molecular analysis of dhaincha genotypes

No.	Primer	Sequence 5' to 3'	GC- Content (%)
1.	OPA-01	CAGGCCCTTC	70
2.	OPA-02	TGCCGAGCTG	70
3.	OPA-03	AGTCAGCCAC	60
4.	OPA-04	AATCGGGCTG	60
5.	OPA-08	GTGACGTAGG	60
6.	OPA-11	CAATCGCCGT	60
7.	OPA-13	CAGCACCCAC	70
8.	OPA-17	GACCGCTTGT	60

RESULT AND DISCUSSION

RAPD has been standardized and employed successfully by Bisoyi et al. (2010) to analyze samples of Sesbania species. A wide range of polymorphic loci generation depends on the proper choice of primers for DNA amplification. The optimum number of primers required to differentiate two or more cultivars may vary with the test materials used. If the amount of variation present in the cultivars is high the use of only few primers can serve the purpose of generating useful information.

In the present investigation, initially twenty random primers were examined out of which nine primers did not produce any amplified products of DNA and three primers gave sub optimal result without distinct fragments. As a result, eight primers were used for genomic DNA amplification of thirty test genotypes. The list of such primers used in this investigation is presented in Table 2.

A total of 70 band were obtained out of which 67 are polymorphic. The number of bands per primer ranged from 4-16 with an average of 8.75 bands. Out of eight primers five primers produced 100% polymorphic loci and three primers viz., OPA-3, OPA-11 and OPA-13 produce 85.50%, 75% and 93.75% polymorphic loci, respectively. Thus, the present set of materials revealed extremely higher level of polymorphism (95.71 %) of RAPD markers. The level of polymorphism obtained in the present investigation may be ascribed to higher genetic variation in the selected thirty test genotypes. Mir et al. (2008) and Chandana et al. (2013) also reported high level (96% and 66%) of polymorphism,

respectively. PIC value of primers ranges from 0.203 to 0.762 with an average of 0.628. The highest PIC value showed by primer OPA-3 followed by OPA-1 (0.753). PIC value for OPA-11 (0.203) was lowest among all primers. High PIC value was reported by Sanghani et al. (2015) in primer OPA-1.

OPA-13 and OPA-08 gave maximum number of polymorphic bands i.e. 15 and 12 bands, respectively. Total 1021 amplicons were produced by all primers together. The maximum number of amplicons were produced by the primers OPA-13 (280) followed by OPA-8 (153), whereas lowest amplicons was produced by primer OPA-1 (59). In brief primer OPA-08 was found most informative as it produces total 12 polymorphic bands with 100% polymorphism and PIC value of 0.730 (Table 3). The obtained results are in conformity with the results of Tripathi et al. (2015) and Bisoyi et al. (2010). Thus, from following discussion it is proved that RAPD assay is efficient in identifying polymorphism at DNA level if suitable primers are used.

The use of appropriate statistical method is very important to make genetic variation more definitive. The UPGMA is calculated based on the assumption that mutation rate among different genotypes is constant and this has been widely used for analysis of genetic variation in plants. This method was used in the present study during analysis of RAPD polymorphism and thus the clusters obtained are reproducible.

All the fragments scored were used for genetic diversity analysis. Jaccard's similarity coefficients was calculated to assess the genetic resemblances among the cultivars and the similarity coefficient matrix was used for UPGMA cluster analysis. The similarity matrix was ranged between 0.17 to 0.97 means diversity between genotypes is 3 to 83%. The mean similarity was 0.60 indicating that genotypes are diverse from each other. The maximum similarity coefficient (0.97) was observed between NSB-6 and NSB-7 and NSB-6 and NSB-10. The minimum similarity coefficient 0.17 was noted between NSB-1. The cluster analysis separated 30 genotypes in only two distinct groups. The dendrogram obtained by the UPGMA method (Fig. 2) show correlation between the genetic divergence and geographical distribution. The accessions belonging to the same region were grouped in a same cluster. For example the genotypes viz., NSB-2 to NSB-16 collected from south Gujarat region distributed into sub clusters IIA, IIB and IIC. Whereas cultivar CSD-123 and CSD-137 developed by CSSRI, Karnal, Haryana were fall in sub cluster-IID. Chandana et al. (2013) reported same results as they obtained two cluster one having single genotype and second cluster consisting of remaining all genotypes.

Table 3: DNA amplification profile and polymorphism generated in dhaincha by 8 RAPD primers

No.	Primer	Total No. of bands(a)	Total No. of polymorphic bands(b)	Polymorphism (%) (b/a) × 100	PIC value
1.	OPA-1	05	05	100.00	0.753
2.	OPA-2	07	07	100.00	0.586
3.	OPA-3	08	07	87.50	0.762
4.	OPA-4	09	09	100.00	0.731
5.	OPA-8	12	12	100.00	0.730
6.	OPA-11	04	03	75.00	0.203
7.	OPA-13	16	15	93.75	0.556
8.	OPA-17	09	09	100.00	0.704
		70	67	95.71	

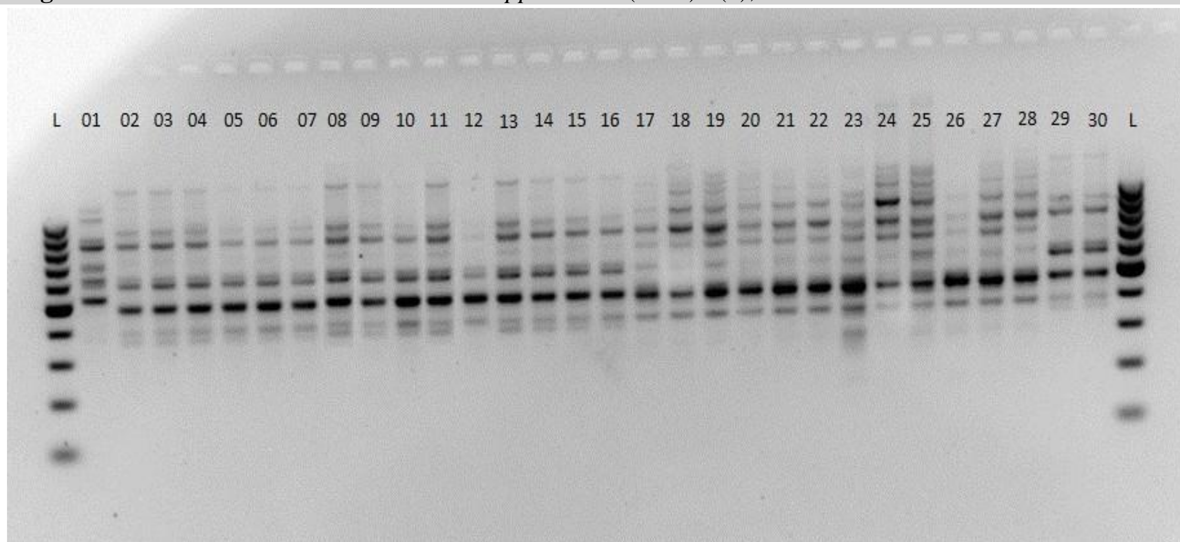

Fig. 1: RAPD Profile generated through OPA-13. L-Ladder (100 bp), 1-30 different dhaincha genotypes

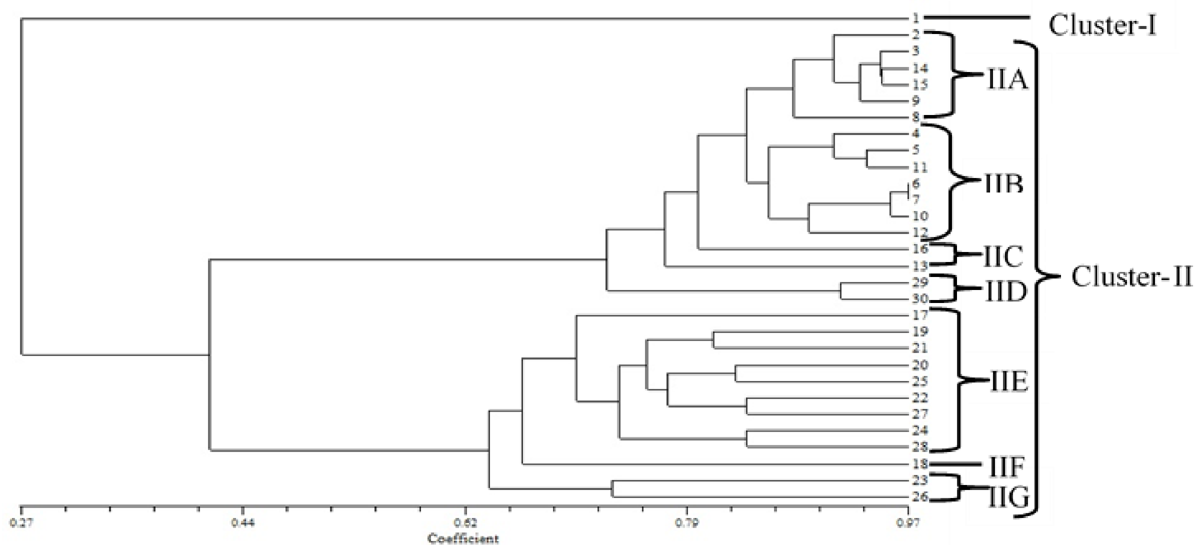

Fig. 2: Dendrogram generated for thirty dhaincha genotypes using UPGMA cluster analysis based on Jaccard's similarity coefficient

Table 4: Jaccard's similarity coefficient matrix between thirty genotypes of dhaincha

	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
1	1.00																													
2	0.31	1.00																												
3	0.31	0.95	1.00																											
4	0.33	0.87	0.87	1.00																										
5	0.29	0.84	0.84	0.91	1.00																									
6	0.25	0.89	0.89	0.86	0.88	1.00																								
7	0.25	0.87	0.87	0.84	0.86	0.97	1.00																							
8	0.28	0.85	0.90	0.82	0.84	0.84	0.82	1.00																						
9	0.28	0.90	0.95	0.87	0.84	0.89	0.87	0.90	1.00																					
10	0.27	0.92	0.92	0.89	0.91	0.97	0.94	0.87	0.92	1.00																				
11	0.27	0.84	0.84	0.91	0.94	0.88	0.86	0.79	0.84	0.91	1.00																			
12	0.22	0.81	0.81	0.78	0.85	0.91	0.88	0.81	0.81	0.88	0.79	1.00																		

13	0.28	0.76	0.80	0.82	0.79	0.75	0.73	0.76	0.80	0.78	0.84	0.68	1.00																	
14	0.30	0.90	0.95	0.83	0.79	0.85	0.83	0.90	0.90	0.87	0.79	0.77	0.81	1.00																
15	0.30	0.90	0.95	0.83	0.79	0.85	0.83	0.85	0.95	0.87	0.79	0.77	0.81	0.95	1.00															
16	0.25	0.79	0.84	0.76	0.83	0.78	0.76	0.79	0.79	0.81	0.78	0.80	0.75	0.85	0.85	1.00														
17	0.25	0.49	0.49	0.46	0.45	0.43	0.46	0.49	0.52	0.46	0.42	0.47	0.49	0.50	0.53	0.53	1.00													
18	0.17	0.36	0.36	0.35	0.35	0.33	0.35	0.42	0.39	0.33	0.35	0.36	0.42	0.40	0.40	0.39	0.68	1.00												
19	0.25	0.41	0.41	0.38	0.38	0.36	0.38	0.41	0.44	0.38	0.35	0.38	0.41	0.42	0.45	0.42	0.79	0.74	1.00											
20	0.25	0.43	0.46	0.43	0.42	0.43	0.43	0.46	0.49	0.43	0.39	0.43	0.46	0.47	0.50	0.43	0.61	0.64	0.79	1.00										
21	0.25	0.45	0.45	0.42	0.41	0.40	0.42	0.45	0.48	0.42	0.38	0.42	0.45	0.46	0.49	0.46	0.68	0.63	0.82	0.76	1.00									
22	0.27	0.47	0.44	0.44	0.43	0.45	0.47	0.44	0.47	0.44	0.40	0.48	0.44	0.45	0.48	0.45	0.74	0.69	0.79	0.79	0.82	1.00								
23	0.27	0.37	0.37	0.39	0.42	0.37	0.36	0.37	0.40	0.39	0.39	0.40	0.40	0.38	0.41	0.43	0.66	0.53	0.67	0.66	0.64	0.71	1.00							
24	0.20	0.41	0.41	0.38	0.38	0.36	0.38	0.41	0.44	0.38	0.35	0.38	0.41	0.42	0.45	0.42	0.70	0.69	0.75	0.66	0.68	0.71	0.59	1.00						
25	0.25	0.40	0.43	0.40	0.39	0.40	0.40	0.43	0.46	0.40	0.36	0.43	0.43	0.44	0.47	0.43	0.65	0.60	0.74	0.83	0.76	0.79	0.75	0.70	1.00					
26	0.22	0.40	0.40	0.40	0.43	0.41	0.40	0.40	0.43	0.43	0.40	0.44	0.38	0.39	0.42	0.38	0.55	0.50	0.64	0.63	0.62	0.64	0.74	0.56	0.68	1.00				
27	0.27	0.47	0.47	0.47	0.47	0.45	0.47	0.47	0.50	0.47	0.43	0.48	0.47	0.48	0.51	0.48	0.74	0.61	0.71	0.70	0.73	0.84	0.76	0.75	0.84	0.68	1.00			
28	0.20	0.41	0.41	0.38	0.38	0.39	0.41	0.41	0.44	0.38	0.35	0.41	0.41	0.42	0.45	0.42	0.74	0.69	0.75	0.70	0.73	0.79	0.67	0.84	0.84	0.64	0.84	1.00		
29	0.34	0.78	0.83	0.75	0.76	0.73	0.71	0.83	0.78	0.75	0.72	0.69	0.74	0.83	0.83	0.82	0.47	0.37	0.37	0.41	0.40	0.39	0.35	0.37	0.38	0.33	0.42	0.37	1.00	
30	0.33	0.71	0.75	0.68	0.68	0.65	0.63	0.75	0.71	0.68	0.64	0.62	0.67	0.76	0.76	0.74	0.43	0.33	0.33	0.38	0.37	0.36	0.31	0.33	0.35	0.29	0.39	0.33	0.92	1.00

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