

GENETIC VARIABILITY STUDIES IN *ALOE VERA* USING RAPD MARKERS

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Abstract: Studies were conducted to evaluate the genetic diversity among twenty genotypes of Aloe vera using the RAPD markers. RAPD analysis with ten primers generated all polymorphic bands and no monomorphic band was observed. The number of polymorphic allele ranged from 2 to 6 with different primers. Genetic diversity of twenty genotypes as estimated by polymorphic information content (PIC) value ranged from 0.52 to 0.96. The cluster dendrogram of RAPD showed similarity values from 0.65 to 0.92. Dendrogram generated using RAPD data showed two major clusters. Cluster I consist of two genotypes however, cluster II included eighteen genotypes. Dendrogram revealed that Rishakesh Aloe-1 was distinctly related with home Aloe at a similarity coefficient 0.54. PIC values of RAPD primers namely MAP-4, MAP-1 and MAP-9 were 0.96, 0.88, 0.86, respectively and provides maximum accessions coverage in the aloe vera genome. These RAPD primers are useful for genetic variability studies in aloe vera.

Keywords: RAPD, *Aloe vera*, Genetic variability

INTRODUCTION

Aloe vera is an important medicinal plant. It belongs to the family Liliaceae. It is mostly grown in hot and dry climates (Reynolds and Dweck; 1999). There are over 300 species of aloe vera grown around the world. However, only two species are grown today commercially namely *Aloe barbadensis* Miller and *aborescens*. An understanding of germplasm diversity and genetic relationships in a germplasm collection is a valuable aid for crop improvement strategies. The past limitations associated with morphological, biochemical and cytological markers for assessing genetic diversity in cultivated and wild plant species have largely been solved by the development of DNA markers such as RFLP, AFLP and SSR. However, these molecular markers have technical differences in terms of cost, speed, amount of DNA needed, technical skills, and degrees of polymorphism, precision of genetic distance estimates and the statistical power of tests. RAPDs which are simple to use and do not require the use of radioactive materials (Williams *et al.*, 1990). The technical ease of RAPD markers and the facility of their application in forest trees, crops, medicinal plants and lower plants for genetic linkage mapping, phylogeny and systematic (Caetano Anolles *et al.*, 1991) have opened new avenues. There are very scanty reports in the literature on genetic variability studies in aloe vera using molecular techniques. Thus the present study was conducted to characterize the aloe vera germplasm accessions collected from different parts of Uttar Pradesh, Uttarakhand and Rajasthan for investigating genetic diversity using RAPD markers.

MATERIAL AND METHOD

In current studies plant material (leaf samples) were collected from Horticulture Research Centre of S.V.P. University of Agriculture & Technology, Meerut, U.P. India. The details of a twenty genotypes

included in the present study are presented in table 1. Total DNA was extracted from fresh leaves by the cetyl tri-methyl ammonium bromide (CTAB) method as described by Murray and Thompson (1980). The quality and concentration of extracted DNA were estimated by using a UV-Vis spectrophotometer. The details of primers used for RAPD analysis is presented in Table 2. DNA amplification reaction for RAPD was performed in a total volume of 25 μ l containing 2 μ l of genomic DNA, 1 μ l primer, 0.5 μ l dNTPs, 3 μ l MgCl₂, 2.5 μ l PCR buffer, 1 μ l tag polymerase and 15 μ l sterile distilled water. Ten random primers (obtained from Bangalore Genei, India) were selected for analysis. Amplification was performed in a thermal cycler (BIO-RAD Cyclor™) with the following profile: 95°C for 5 min (initial denaturation), 94°C for 1 min, 30-32°C for 1 minutes, 72°C for 2 minutes for 44 cycles with a final extension at 72°C for 10 minutes. The RAPD-PCR products were analyzed directly on 2% agarose gels in TAE buffer and were visualized by staining with ethidium bromide and transillumination under short-wave UV light. Pair wise comparison of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic products was used to generate similarity coefficients of Jaccard's coefficient by NTSYS-pc version 2.1 software (Rohlf 2000). Dendrogram was constructed by the unweighted pair group method with arithmetic averages (UPGMA) according to (Rohlf 1993). Analysis was performed by using dendrogram along with Jaccard's coefficient. The polymorphism information content (PIC) values were computed as described by (Botstein *et al.*, 1980).

RESULT AND DISCUSSION

Number of alleles

Among ten RAPD primers, all primers produced polymorphic loci which were fully distributed on genome. A total of 37 alleles were detected. The overall size of amplified products ranged from 200

bp to 1000bp. The number of alleles per locus varied from 2 to 6. The result on genetic variability analysis, number of polymorphic alleles, monomorphic alleles for each RAPD locus and PIC values are presented in Table 3. The highest number of alleles were observed in MAP-1 (six alleles) followed by MAP-7 (five alleles), MAP-2, MAP-3, MAP-9 (four alleles each), MAP-5, MAP-6, MAP-8, MAP-10 (three alleles each) and MAP-4 (two alleles each) that provides the summarized data regarding the number of distinctive alleles and their distribution in various genotypes.

Polymorphism of RAPD markers

The alleles revealed by RAPD markers showed a high degree of polymorphism, all 10 primers produced 100% polymorphic bands. The number of polymorphic alleles ranged from 2 to 6. The PIC values, derived from allelic diversity and frequency among the genotypes, were not uniform for all the RAPD loci tested. The PIC value for the RAPDs loci ranged from 0.52 to 0.96. The higher PIC values were observed from primers MAP-4, MAP-1, MAP-9 and their PIC values were 0.96, 0.88 and 0.86 respectively (Table 3). Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus. Similar results were also reported by (DeWoody *et al.*, 1995). Such results further suggested that application of these markers in genotype identification can be used successfully in case of *aloe vera*.

Similarity Vs Dissimilarity Analysis

The cluster dendrogram of RAPD revealed two major clusters that were demarcated at a cut off similarity coefficient level of 0.65, below which the similarity values narrowed conspicuously (Fig.1). Cluster II included eighteen genotypes while clusters I comprising of only two genotypes. Jaccard's coefficient of similarity revealed that high degree of similarity to the extent of 92 % exists between genotypes (AV-4 and AV-8). The cluster I consists of only two genotypes namely AV-3 and AV-5 with similarity coefficient between 0.75 to 0.92. The first subgroup of cluster II consists of 3 genotypes namely, AV-1, AV-18 and AV-10 at a similarity coefficients varied from 0.79 to 0.92. The second subgroup of cluster II consists of 9 genotypes namely AV-2, AV-9, AV-11, AV-4, AV-8, AV-13, AV-16, AV-17 and AV-9 with the similarity coefficients ranged between 0.77 to 0.92. The third subgroup of the cluster II includes four genotypes namely AV-12, AV-19, AV-14 and AV-20 with a similarity coefficient from 0.75 to 0.92. The fourth subgroup of the cluster II include 2 genotypes, namely AV-6, AV-15 with similarity coefficient from 0.65 to 0.9. Based on dendrogram the AV-1, AV-5, AV-15, AV-7, AV-8 and AV-11 genotypes belong to different clusters hence, they are genetically diverse. The results of the present investigations on genetic diversity provide estimates on level of genetic variation among diverse materials that can be used in assessing the purity and variability of genotypes for future breeding programs.

Table1. Details of twenty genotypes of *Aloe vera* included in studies.

S. No.	Name of genotype	Genotype code	Leaves Characteristics	Origin
1	Rishikesh Aleo-1	AV-1	Light green, Linear ovate	Rishikesh
2	Rishikesh Aleo-2	AV-2	Light green, Linear Lanceolate,	Rishikesh
3	Rishikesh Aleo-3	AV-3	Green, Linear Lanceolate	Rishikesh
4	Rishikesh Aleo-4	AV-4	Green, Linear ovate	Rishikesh
5	Home Aloe	AV-5	Green, Linear Lanceolate	Bareilly
6	Pant-1	AV-6	Dark green, Linear Lanceolate	Pantnagar
7	Pant-2	AV-7	Dark green, Linear Lanceolate	Pantnagar
8	Pant-3	AV-8	Green, Linear Lanceolate	Pantnagar
9	Pant-4	AV-9	Light green, Linear Lanceolate	Pantnagar
10	Pant-5	AV-10	Light green, Linear ovate	Pantnagar
11	Sahjahanpur Aleo-1	AV-11	Dark green, Linear Lanceolate	Sahjahanpur
12	Sahjahanpur Aleo-2	AV-12	Dark green, Linear ovate	Sahjahanpur
13	Sahjahanpur Aleo-3	AV-13	Green, Linear Lanceolate	Sahjahanpur
14	Bikaner Sweet Aleo	AV-14	Light green, Linear Lanceolate	Bikaner
15	Bikaner Bitter Aleo-1	AV-15	Dark green, Linear Lanceolate	Bikaner

16	Bikaner Bitter Aleo-2	AV-16	Dark green, Linear ovate	Bikaner
17	Meerut Aleo-1	AV-17	Green, Linear ovate	Meerut
18	Meerut Aleo-2	AV-18	Dark green, Linear ovate	Meerut
19	Meerut Aleo-3	AV-19	Dark green, Linear Lanceolate	Meerut
20	Meerut Aleo-4	AV-20	Green, Linear Lanceolate	Meerut

Table2. Details of RAPD primers along with sequences used for the analysis of twenty *Aloe vera* genotypes.

S. No.	Primer Code	Sequence
1.	MAP-1	GCACGCCGGA
2.	MAP-2	CACCCTGCGC
3.	MAP-3	CTATCGCCGC
4.	MAP-4	GTGCAATGAG
5.	MAP-5	AAGATAGCGG
6.	MAP-6	GGATCTGAAC
7.	MAP-7	CATCCCGAAC
8.	MAP-8	GCGAATTCCG
9.	MAP-9	GACCCTAGTC
10.	MAP-10	AACCCGGGAA

Table3. Polymorphism Information Content (PIC) of RAPD Loci across different genotypes analyzed in current investigation.

S N	Name of Primers	Annealing temp.	Molecular wt. range (bp)	Total no. of alleles	No. of polymorphic alleles	No. of Monomorphic alleles	% polymorphism	PIC value
1.	MAP-1	32	200-800	6	6	0	100	.89
2.	MAP-2	32	250-800	4	4	0	100	.87
3.	MAP-3	32	200-450	4	4	0	100	.81
4.	MAP-4	32	200-350	2	2	0	100	.96
5.	MAP-5	32	200-500	3	3	0	100	.67
6.	MAP-6	32	200-400	3	3	0	100	.86
7.	MAP-7	32	200-900	5	5	0	100	.78
8.	MAP-8	32	200-700	3	3	0	100	.57
9.	MAP-9	32	200-1000	4	4	0	100	.88
10	MAP-10	32	200-600	3	3	0	100	.52

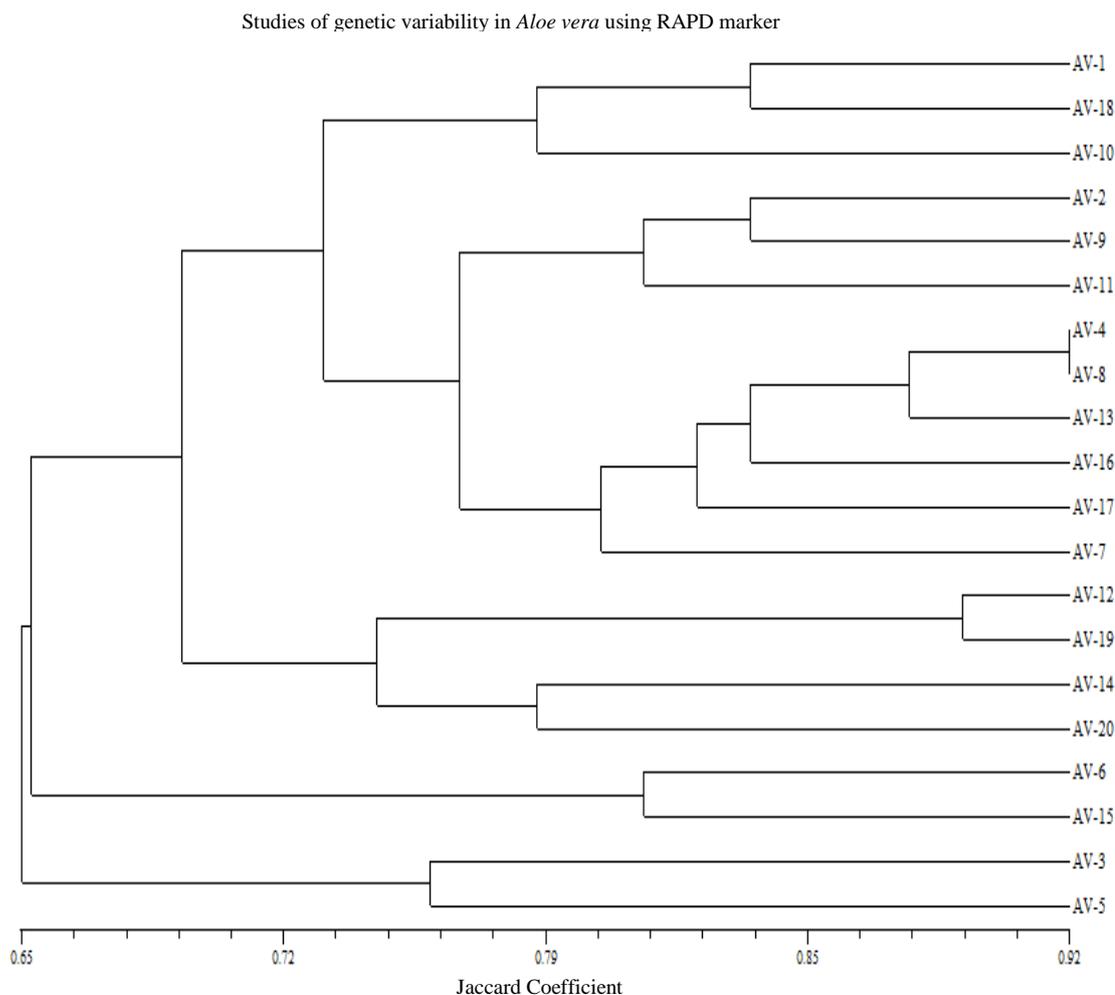


Figure 1. Dendrogram showing clustering of 20 genotypes of *Aloe vera* based on RAPD data

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