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MOLECULAR CHARACTERIZATION OF PISTILLATE LINES IN CASTOR BEAN (*RICINUS COMMUNIS* L.) THROUGH MOLECULAR MARKERS

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ABSTRACT
Genetic diversity of 13 castor genotypes of pistillate lines (S and NES) was carried out using 33 RAPD and 11 SSR markers. The average polymorphism recorded by the 33 RAPD loci was 70.25%. The mean number of polymorphic bands per primer among 13 castor genotypes was 05 and percent polymorphism ranged from 20 to 100. The dendrogram constructed using pooled RAPD loci data clearly showed that two genotypes from NES line (JP-65 and JP-83) were highly similar and different from other genotypes. The SSR analysis with 11 microsatellite markers produced 36 alleles. The average number of alleles per locus was found to be 3.2. The highest number of alleles (10) and high PIC (0.82) value was found in locus CCat002. Cluster analysis based on SSR data clearly grouped two NES genotypes (JP-65 and JP-83) in to same clusters. For genetic diversity analysis both, RAPD and SSR may be considered reliable molecular techniques.

INTRODUCTION

Castor (*Ricinus communis* L.) is the most important non edible oilseed crop belonging to family *Ephorbiaceae* grown under the tropical, subtropical and temperate climatic region. The major castor growing countries are India, China and Brazil. India ranks first with respect to area and second with respect to productivity. Castor earns good amount of foreign exchange for the nation. Its oil is used in the manufacture of high grade lubricants, paints, varnishes, textile dyeing, printing, perfumes, lubricating greases, polishes, plastics, soaps toothpaste and medical purpose. The flowering in castor occurs in dense terminal clusters (inflorescence) with female flower just above the male flower. The chromosome number of castor is 2n = 20. Monoecious, pistillate, interspersed staminate flower and sex revertant are the most important sex form of castor. Monoeciousis the most natural occurrence of annual and perennial castor, pistillate (P) occurred as a rare recessive mutant with the spike having female flowers throughout the spike, interspersed staminate flower is a variant of pistillate form with male flower interspersed throughout the female flower on the spike and sex revertant is female that turn to monoecious at later stage. Castor has three important pistillate types i.e. N, S, and NES. N typeis based on the form four of the conventional variant as described by Shifrish (1960). It is backcross line that exhibited a segregation ratio of 1:1 monoecious and pistillate plants. Classen and Hoffman (1950) while working with various populations, studied different sex variants as completely pistillate, mostly female, interspersed and pistillate with hermaphrodite. S typeis characterized by sex reverting and environmentally sensitive interspersed staminate flower expression. It has two types as sex revertants and non-reverted female. NES typeis a combination of both N and S type as it carries the homozygous recessive gene for pistillateness and environment sensitive genes for ISF.

Various approaches are available for molecular characterization such as Randomly Amplified Polymorphic DNA (RAPD) (Williams et al. 1990), Simple Sequence Repeat (SSR) (Tautz, 1989), Amplified Fragment length Polymorphism (AFLP) (Zabue and Vos, 1993) and Restriction Fragment Length Polymorphism (RFLP) (Botstein *et al.*, 1980). Among these RAPD is technically simple, robust and useful to characterize the different plant genotypes and are require PCR amplification (Saiki *et al.*, 1985, 1988) of random genomic DNA segment with single primer of arbitrary sequence and it has no need of prior information of the DNA sequence and feasibility of automation (Subudhi and Huang, 1999). The use of such techniques for germplasm characterization facilitates the conservation and utilization of plant genetic resources (Kapteyn and Simon, 2002). Similarly presence of short tandem repeats of varying length is characteristic of microsatellite loci (Akkaya *et al.*, 1992) also called Simple Sequence Repeat (SSR). Powell *et al.* (1996) reported that among different classes of molecular markers, SSR markers are useful for a variety of applications in plant molecular biology, genetics and breeding so present study undertaken to characterize pistillate lines (S and NES) through RAPD and SSR marker.

MATERIALS AND METHODS

Plant material and DNA isolation

The thirteen elite genotypes of castor are come under two pistillate lines of castor. The details of the genotypes have been mentioned below.

S No.	Pistillate lines	Genotypes	
1.	S - line	1	SKP-35
		2	SKP-84
		3	SKP-93
		4	SKP-116
		5	VP-1
		6	Geeta
		7	DPC-9
		8	LRES-17
		9	MCP-1
		10	M-619
		11	TSP-10R
2.	NES - line	1	JP-83
		2	JP-65

Above mentioned genotypes were procured from Castor and Mustard Research Station, SDAU, Sardarkrushinagar Gujarat. DNA extraction protocol has been standardized for molecular characterization of thirteen genotypes of pistillate lines in castor with the help of CTAB(Cetyltrimethylethyl Ammonium Bromide) method of Doyle and Doyle (1990) and SDS method of Dellaporta *et al* (1983). Nine days after germination (DAG) seedlings (approximately 8 cm in height) were used for genomic DNA isolation. The quantification of DNA was carried out at 260 nm in spectrophotometer and stock DNA was diluted to make final solution of 20ng/µl for PCR analysis.

RAPD assay

For molecular characterization through RAPD followed the method given by Mathews *et al*. (2007) with minor modifications. The genomic DNA extracted from each genotype was subjected to polymerase chain reaction using 40 random decamer primers from OPA and OPB series. Among forty primers 33 were selected for further analysis. Amplification was carried out in a 200 µl thin walled PCR tube containing a 25 µl reaction mix (1X Taq buffer, 16µl sterile DDH2O, 2mM MgCl2, 1mM dNTP, 20pmol Primer, 1U Taq DNA Polymerase, 20 ng DNA). Amplification reactions were carried out for 40 cycles. Each cycle comprised of 1 min at 94ºC, 1:30 min at 37

Table 1: Results of RAPD analysis in thirteen genotypes of castor

S. No.	Primers	Primer sequence (5′-3′)	Molecular weight range (bp)	Total no. of bands	No. of polymorphic bands	No. of monomorphic bands	Percent polymorphism	PIC value
1	OPA-1	CAGGCCCTTC	146-1429	11	7	4	63.63	0.89
2	OPA-4	AATCGGGCTG	240-2201	13	7	6	53.84	0.90
3	OPA-5	AGGGGTCTTG	236-2667	8	5	3	62.50	0.85
4	OPA-6	GGTCCCTGAC	449-1515	6	3	3	50.00	0.76
5	OPA-7	GAAACGGGTG	139-1024	10	5	5	50.00	0.88
6	OPA-9	GGGTAACGCC	803-1336	4	4	0	100.00	0.51
7	OPA-11	CAATCGCCGT	486-1241	5	5	0	100.00	0.67
8	OPA-12	TCGGCGATAG	154-2249	11	9	2	81.81	0.80
9	OPA-13	CAGCACCCAC	365-1278	5	4	1	80.00	0.78
10	OPA-14	TCTGTGCTGG	429-1128	8	6	2	75.00	0.81
11	OPA-15	TTCCGAACCC	205-1330	5	5	0	100.00	0.73
12	OPA-16	AGCCAGCGAA	336-1104	4	3	1	75.00	0.49
13	OPA-17	GACCGCTTGT	95-1424	9	4	5	44.44	0.84
14	OPA-18	AGGTGACCGT	386-1006	4	3	1	75.00	0.56
15	OPA-20	GTTGCGATCC	317-2037	9	7	2	77.77	0.85
16	OPB-2	TGATCCCTGG	349-2431	8	3	5	37.50	0.85
17	OPB-3	CATCCCCCTG	618-1599	4	2	2	50.00	0.70
18	OPB-4	GGACTGGAGT	375-1961	7	6	1	85.72	0.82
19	OPB-5	TGCGCCCTTC	357-1350	5	4	1	80.00	0.79
20	OPB-6	TGCTCTGCCC	304-1216	10	6	4	60.00	0.88
21	OPB-7	GGTGACGCAG	444-2023	8	5	3	62.50	0.85
22	OPB-8	GTCCACACGG	444-1366	5	5	0	100.00	0.74
23	OPB-10	CTGCTGGGAC	236-2271	8	3	5	37.50	0.84
24	OPB-11	GTAGACCCGT	287-1626	8	6	2	75.00	0.84
25	OPB-12	CCTTGACGCA	756-1440	5	3	2	60.00	0.75
26	OPB-13	TTCCCCCGCT	679-1984	5	4	1	80.00	0.67
27	OPB-14	TCCGCTCTGG	429-1327	5	5	0	100.00	0.67
28	OPB-15	GGAGGGTGTT	320-1239	8	7	1	87.50	0.87
29	OPB-16	TTTGCCCGGA	366-1867	7	6	1	85.71	0.78
30	OPB-17	AGGGAACGAG	285-2674	11	8	3	72.72	0.84
31	OPB-18	CCACAGCAGT	125-665	5	1	4	20.00	0.75
32	OPB-19	ACCCCCGAAG	259-2019	11	8	3	72.72	0.86
33	OPB-20	GGACCCTTAC	274-1829	12	6	6	50.00	0.90
			Total	244	165	79	2318.36	25.72
			Average	7.39	5.00	2.3	70.25	0.77

ºC and 2 min at 72 ºC. Amplified product were separated on 1.6% agarose gel, stained with ethidium bromide and photographed under UV light.

SSR assay

Twenty universal primers were used to screen all 13 genotypes. Among 20 primers 11 were used for further analysis. The PCR reactions consisted of 1X Taq buffer, 16.5µl sterile DDH2O, 1.5mM MgCl2, 1mM dNTP, 5-10pmol Primer (FP&RP), 1U Taq DNA Polymerase and 20 ng DNA for 40 cycles. Cyclic condition were consisted of 94ºC for 1min, 55-65 ºC (depends on melting temperature) for 1:30 min and 72 ºC for 2min. Amplified product were separated on 2.2% agarose gel.

Data analysis

Data was scored for computer analysis on the basis of the presence or absence of the PCR products. If a product was present in a genotype, it was designated as '1' and if absent; it was designated as '0'.The data generated by RAPD and SSR loci were analyzed with the software NTSYSpc version 2.02 (Rohlf, 1994).

RESULTS AND DISCUSSION

RAPD markers

Thirteen pistillate lines genotypes (eleven from S line and two from NES line) of castor were analyzed by using 40 RAPD primers (OPA and OPB series). Among forty primers 33 (15 from OPA and 18 from OPB series) were selected for final analysis. A total of 244 bands 165 were polymorphic (70.25%). The number of DNA fragment varied from four to thirteen and mean number of polymorphic bands per primer among 13 castor genotypes was 05 and the size of PCR amplified DNA fragment ranged from 95 to 2674 bp (table no 1). The highest polymorphism (100%) was exhibited by primers OPA 9, 11, 15 from OPA series and OPB 8, 14 (plate -1) from OPB series and the PIC value varied from 0.49 to 0.90 (table no 1). Higher polymorphism detected by the RAPD markers in this study is comparable with other crop of *Ephorbiaceae* family (Ganesh Ram *et al.,* 2007). The primer OPA-10 was used for PCR amplification with five castor genotypes i.e. Geeta, DPC-9, SKP-35, VP-1 from S line and JP-83 from NES line. OPA-10 was not included in analysis because it was used only for differentiate S and NES lines according to these it gave one unique band (size 756bp) in JP-83 genotype which was absent in all other S line genotypes (Plate 1).

Genetic diversity

Dendrogram constructed with the data generated by all 33 RAPD primers and their amplicons grouped the 13 castor genotypes into one cluster *i.e.* A (Fig. 1). This cluster was further divided into two sub-clusters *i.e.* A1 and A2. Sub-cluster A1aa grouped all two NES line genotypes *i.e.* JP-83 and JP-65 which was very near to each other along with another one genotype from S line i.e. Geeta (Fig. 1). The genetic similarities ranged from 0.72 to 0.86. The average genetic similarity among these 13 genotypes was 0.78. The highest similarity index value of 0.86 was found between JP-83 and JP-65 (Table 2). The dendrogram constructed using pooled RAPD primers series data showed higher similarity in the genotypes of NES line. The goodness of RAPD primers was checked by MXCOMP function in the NTSYSpc version 2.02 and got 0.82 Matrix correlations (r).

SSR markers

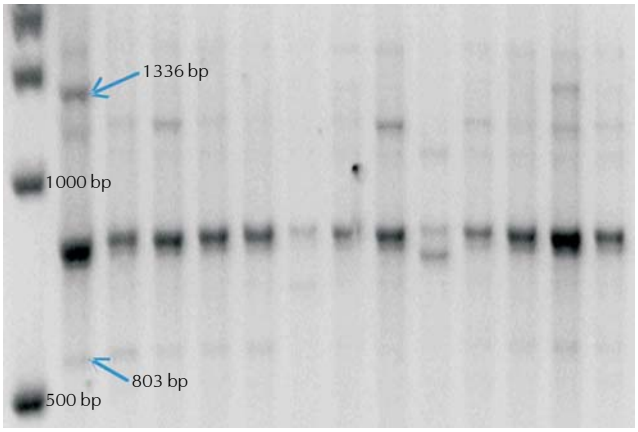
Twenty SSRs were amplified to analyze the genetic variation among 13 genotypes of diploid castor at molecular level and out of twenty; eleven were selected for further analysis. The alleles are classified simply as 'fragments' and as with RAPD fingerprints, are scored across all genotypes (Aranzana *et al.*, 2001). Eleven SSR primers given 36 alleles in the size rangedfrom 115 to 1927 bp and the total number of alleles were varying from one to ten. The PIC value varied from 00 to 0.82 with a mean 0.34 (Table 3). The average number of alleles per locus was 3.2.The identical results were also

Table 3: Allele size range, allele number and PIC values of polymorphic SSR loci in castor genotypes

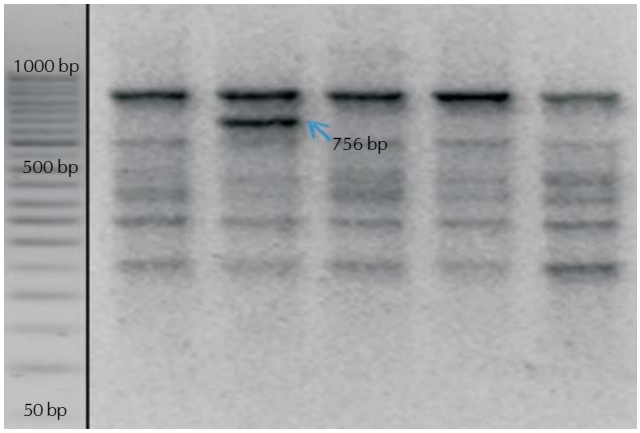
S. No.	Locus	Size Range	Alleles	PIC
1	CCB-2	132-353	3	0.24
2	CCB-4	153-312	2	0.15
3	CCB-5	141-182	2	0.50
4	CCB-7	156	1	00
5	CCB-9	334-708	2	0.13
6	CCttc001	136-1555	7	0.69
7	CCat002	115-948	10	0.82
8	CCat003	217	1	00
9	Cccat001	150-1927	4	0.68
10	CCtta001	185-437	3	0.60
11	RM-283	204	1	00
		Total	36	3.81
		Mean	3.2	0.34

Table 2: Jaccard's similarity coefficient for different castor genotypes based on RAPD data analysis

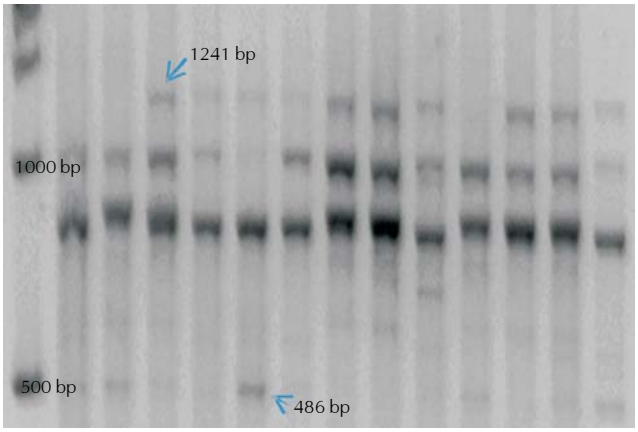
Genotypes	Geeta	JP-83	DPC-9	SKP-35	VP-1	SKP-116	TSP-10R	SKP-84	JP-65	M-619	SKP-93	LRES-17	MCP-1
Geeta	1												
JP-83	0.8502	1											
DPC-9	0.7877	0.8370	1										
SKP-35	0.8342	0.8636	0.8524	1									
VP-1	0.7206	0.7403	0.7433	0.7771	1								
SKP-116	0.7321	0.7529	0.7262	0.7816	0.7068	1							
TSP-10R	0.6753	0.7210	0.7604	0.7474	0.7157	0.6978	1						
SKP-84	0.6974	0.7157	0.7715	0.7676	0.7282	0.6666	0.7277	1					
JP-65	0.8333	0.8639	0.8011	0.8268	0.7252	0.7167	0.7068	0.7106	1				
M-619	0.7200	0.7500	0.7527	0.7877	0.7150	0.7682	0.7150	0.7187	0.7643	1			
SKP-93	0.7158	0.7165	0.7382	0.7526	0.6931	0.6927	0.6938	0.7772	0.7486	0.7388	1		
LRES-17	0.7210	0.7578	0.7783	0.8020	0.7164	0.7267	0.7250	0.7711	0.7526	0.7814	0.8031	1	
MCP-1	0.7000	0.7486	0.7419	0.7567	0.7142	0.7455	0.7052	0.7268	0.7430	0.7528	0.7472	0.8087	1



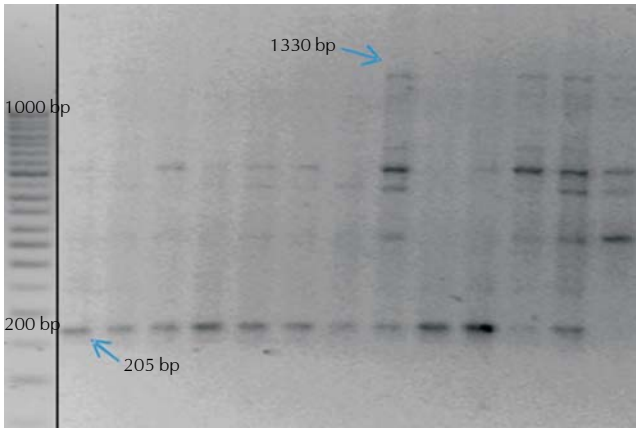
OPA-09



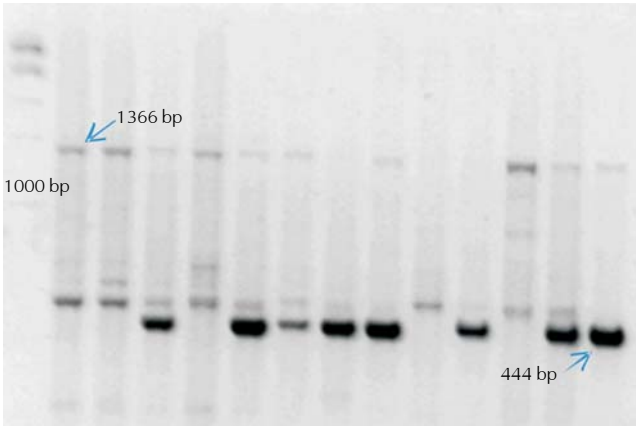
OPA-10



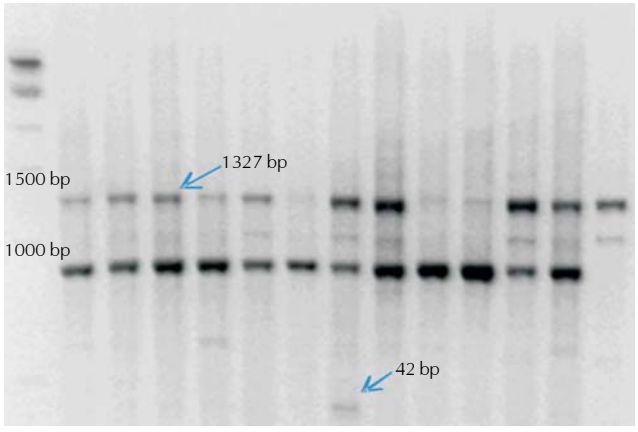
OPA-11



OPA-15



OPA-08



OPA-14

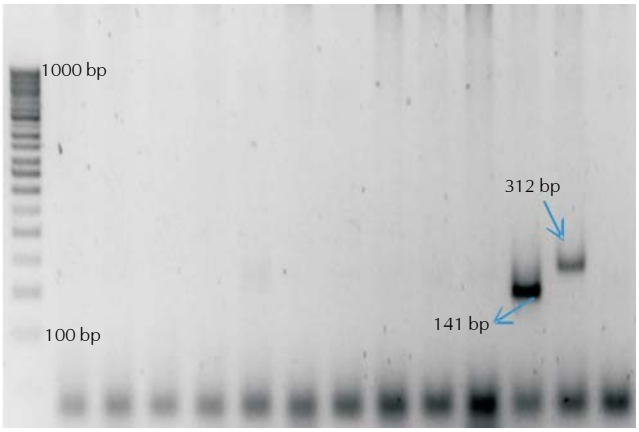
Genotype sequence in all above gel except OPA-10- M-500 bp Ladder, 1-Geeta, 2-JP-83, 3-DPC-9, 4-SKP-35, 5-VP-1, 6-SKP-116, 7-TSP-10R, 8-SKP-84, 9-JP-65, 10-M-619, 11-SKP-93, 12-LRES-17, 13-MCP-1. Genotype sequence for OPA-10 primer- M-50 bp Ladder, 1-Geeta, 2-JP-83, 3-DPC-9, 4-SKP-35, 5-VP-1

Plate 1: RAPD patterns of different castor genotypes produced by primer OPA-09, OPA-10, OPA-11, OPA-15, OPB-08 and OPB-14

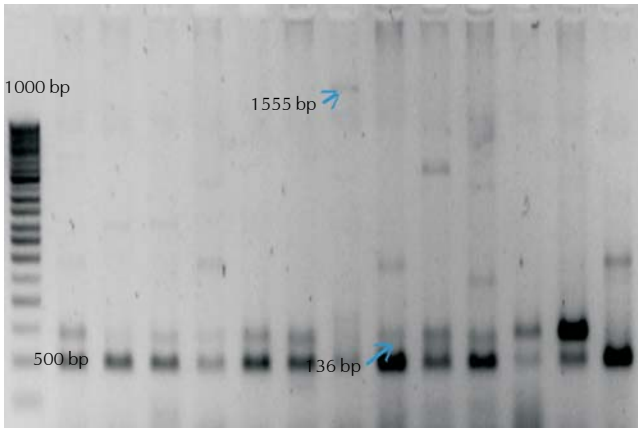
reported by Gerard *et al.* (2008) they used nine SSR primers for castor genotyping and got average 3.1 alleles per locus. The highest number of alleles (10) and high PIC (0.82) value was found in locus CCat002 and five loci (CCB-5, CCttc001, CCat002, Cccat001 and CCtta001) (Plate-2) identified as more informative (*i.e.* PIC > 0.5).

Genetic diversity

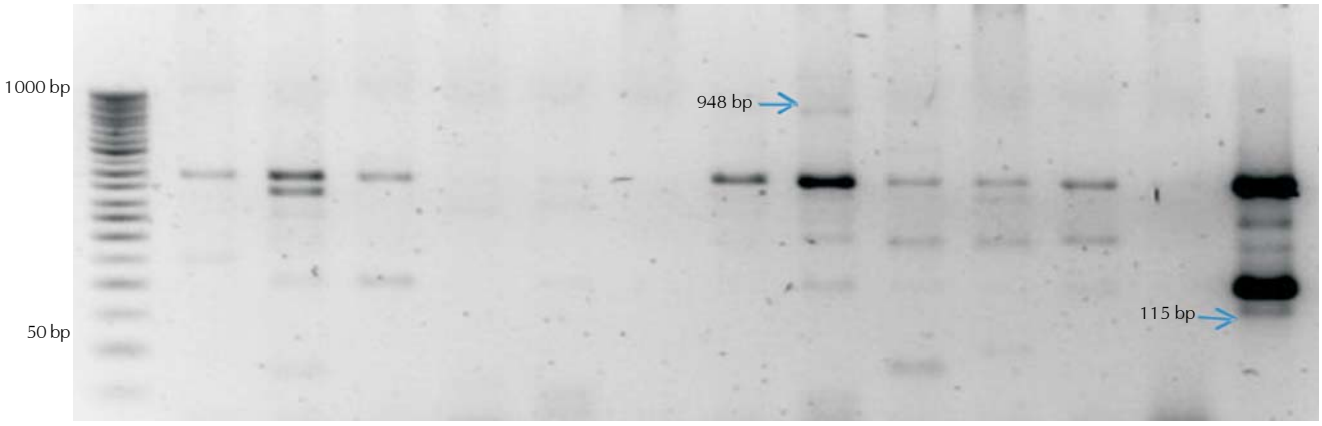
A dendrogram based on UPGMA analysis grouped the 13 castor genotypes into one main cluster *i.e.* cluster A. Cluster A



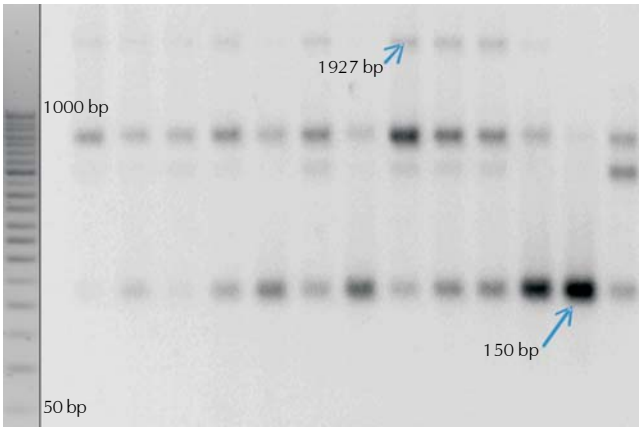
CCB-5



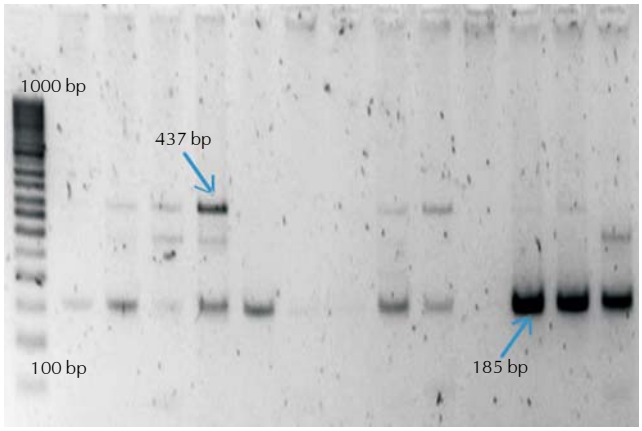
CCttc001



CCat002



Cccat001



CCtta001

Genotype sequence in all following gel- M-50 bp Ladder, 1-Geeta, 2-JP-83, 3-DPC-9, 4-SKP-35, 5-VP-1, 6-SKP-116, 7-TSP-10R, 8-SKP-84, 9-JP-65, 10-M-619, 11-SKP-93, 12-LRES-17, 13-MCP-1

Plate 2: SSR patterns of different castor genotypes produced by primer CCB-5, CCttc001, CCat002, Cccat001 and CCtta001

was divided into two sub-clusters A1 and A2. Sub-cluster A2 grouped total four NES and S line genotypes i.e. JP-83, JP-65 and SKP-84, DPC-9 respectively within these genotypes, two (JP-83 and JP-65) from NES line showed near to each other (Fig 2). Jaccard's pair-wise similarity coefficient values for 13 genotypes were calculated and are presented in Table 4. The genetic similarities ranged from 0.33 to 0.78. The average genetic similarity among these 13 genotypes was 0.57 (Table 4). Matrix correlation was r = 0.87 checked by MXCOMP function in the NTSYSpc version 2.02.

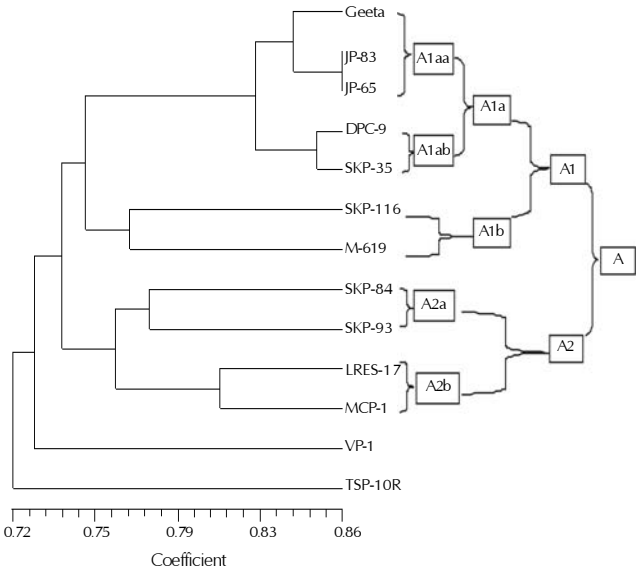


Figure 1: Dendrogram showing clustering of castor genotypes constructed using UPGMA based on Jaccard's similarity coefficient obtained from RAPD primers

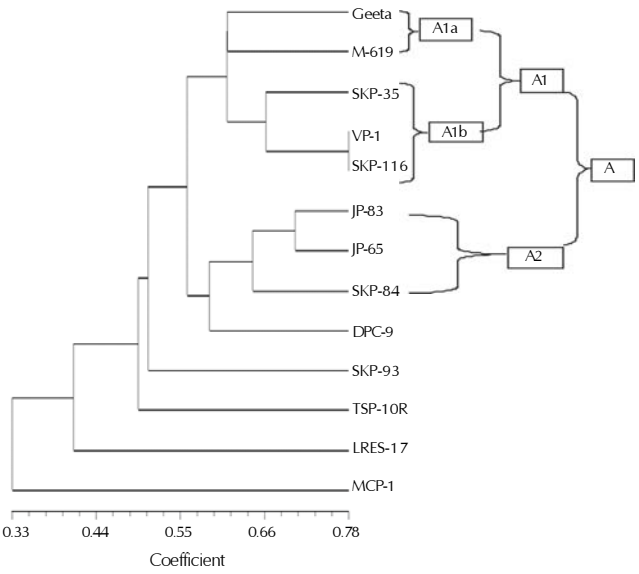


Figure 2: Dendrogram showing clustering of castor genotypes constructed using UPGMA based on Jaccard's similarity coefficient obtained from SSR primers

Table 4: Jaccard's similarity coefficient for different castor genotypes based on SSR data analysis

Genotypes	Geeta	JP-83	DPC-9	SKP-35	VP-1	SKP-116	TSP-10R	SKP-84	JP-65	M-619	SKP-93	LRES-17	MCP-1
Geeta	1												
JP-83	0.5625	1											
DPC-9	0.5714	0.6875	1										
SKP-35	0.5714	0.5882	0.6000	1									
VP-1	0.6363	0.5333	0.4285	0.6666	1								
SKP-116	0.6363	0.5333	0.5384	0.6666	0.7777	1							
TSP-10R	0.5000	0.4375	0.4285	0.4285	0.6000	0.6000	1						
SKP-84	0.5294	0.6315	0.5555	0.6470	0.5000	0.5000	0.4117	1					
JP-65	0.6000	0.7058	0.5294	0.6250	0.5714	0.5714	0.4666	0.6666	1				
M-619	0.6153	0.5294	0.5333	0.5333	0.5833	0.7272	0.5833	0.5882	0.6666	1			
SKP-93	0.5333	0.4736	0.3888	0.4705	0.5000	0.5000	0.5000	0.5263	0.5882	0.6000	1		
LRES-17	0.4000	0.3684	0.2777	0.4375	0.5833	0.4615	0.3571	0.3500	0.3888	0.3750	0.5000	1	
MCP-1	0.3809	0.3076	0.2916	0.4090	0.3500	0.2857	0.2857	0.3461	0.3200	0.3043	0.3333	0.3043	1

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