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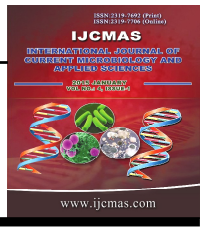

Original Research Article

RAPD analysis of genetic diversity of castor bean (*Ricinus communis L.*)

H.N.Lakhani, S.V.Patel*, N.P.Bodar and B.A.Golakiya

Department of Biotechnology, Junagadh Agricultural University, Junagadh-362001, India
**Corresponding author*

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A B S T R A C T

Castor (*Ricinus communis* L.) is a plant that is commercially very important to the world. It is produced in about 30 countries lying in the tropical belt of the world. It is an important plant for production of industrial oil. Assessment of genetic diversity of a crop species is a prerequisite to its improvement; hence it is important to identify the genetic diversity of castor genetic resources for development of improved cultivars. The aim of the present study was to study the molecular diversity for varietal identification and phylogenetic relationships among thirteen castor genotypes and identify those with distinct DNA profiles. Twenty-seven RAPDs primers were used, out of which 16 polymorphic primers revealed 100% polymorphism among the castor genotypes. Dendrogram was constructed using UPGMA method which revealed distinct clusters. Values of the polymorphic information content (PIC) value ranged from 0.423 to 0.883 with an average of 0.705. Knowledge of the genetic diversity of castor can be used in future breeding programs for increased oil production to meet the ever increasing demand of castor oil for industrial uses as well as for biodiesel production.

Introduction

Castor (*Ricinus communis* L.) native to the tropical and sub-tropical regions continues to be an important non edible oil seed crop of arid and semi-arid regions of the world, for its high utilitarian value oil (Govaerts *et al.*, 2000). India ranks 1st in terms of production of Castor oil seed in the world. In India, castor is grown on nearly 1.1 million ha with the total production of 1.64 million tons and an average yield of 1500 kg/ha in the year 2013 (FAO, 2013). Despite its name, castor is not a true bean, as it contains more than 45% oil and the oil is rich (80–90%) in an unusual hydroxyl fatty acid, ricinoleic acid (Jeong and Park, 2009).

Castor oil is used as raw material for numerous and varied industrial applications, such as: manufacture of polymers, coatings, lubricants for aircrafts, cosmetics etc. and also for the production of biofuel. Due to this increasing demand in the global market, there is a short supply of castor oil and this trend seems to get worst every year. Hence, new varieties need to be developed with high hectarage and productivity.

Castor (*Ricinus communis* L.) belongs to the family Euphorbiaceae. It has chromosome number 2n = 20.

Castor is indigenous to Eastern Africa and most probably originated in Ethiopia. Castor is a highly polymorphic species; normally monoecious with pistillate flowers are situated on the upper part and staminate flowers on the lower part of raceme. Production of female and male flowers is highly influenced by environmental conditions. Though it is a cross-pollinated crop, most of the cultivars have been developed through hybridization followed by selection, as hybrids give significantly greater yields than pure lines or varieties (Moll *et al.,* 1962; Birchler *et al.,* 2003; Reif *et al.,* 2007).

Castor has a high degree of variation and offers the breeder ample scope to undertake screening and selection of seed sources for the desired traits. The previous studies paid more attention on morphological and agronomic trait variations and traits identification, e.g. diseases resistance, stress resistance. Knowledge of the genetic diversity is a fundamental aspect in the improvement of a crop species.

Selection is the most important activity in all plant breeding programmes (Zobel *et al.,* 1984). Since, variability is a prerequisite for selection programmes, new genetic approaches like molecular marker technologies have been adopted to map genomes, in order to assess better cross combinations in developing better hybrids.

Assessment of genetic variation using molecular markers is crucial for the efficient management and biodiversity conservation of plant genetic resources in gene banks. A large number of polymorphic markers are required to determine genetic relationships and genetic diversity in a reliable manner (Santalla *et al.,* 1998). This limits the use of morphological and biochemical characters, which are limited in number or lack ample diversity in castor. Further, these analyses have intrinsic disadvantages such as limited numbers of markers, and are often less efficient due to their variation and sensitivity to short-term environmental fluctuations (Crawford *et al.,* 1995; Francisco *et al.,* 1996; Essilman *et al.,* 1997; Lesica *et al.,* 1998). DNA based molecular analysis tools are ideal for germplasm characterization and phylogenetic studies.

RAPD has proven to be helpful in detecting genetic variations and evaluation of genetic diversity and also for identifying germplasm in a number of plant species (Gwanama *et al.,* 2000; Kapteyn and Simon, 2002). The utility of PCR-based marker variations as phylogenetic markers for investigating evolutionary relationships among plants has been clearly established. These techniques are independent of environmental factors and offer significant advantage for species identification in that they are rapid, relatively cheap and eliminate the need to grow plants up to maturity.

Thus, the present study has been undertaken with the objective of assessing the genetic diversity and molecular characterization of important castor genotypes using RAPDs markers. The observed polymorphism may be useful for genetic enhancement of castor for desirable traits.

Material and Methods

Plant material

Thirteen genotypes of castor (*Ricinus communis* L.) including male, female and hybrids were collected from the Main Castor Research Station, SDAU, Dantiwada, Gujarat, India, to study molecular diversity by RAPD assay. The pedigree and origin of the selected castor genotypes is given in Table 1. Seeds of each genotype were sown

in pots and young leaves of 2 weeks old plants were collected from each genotype for DNA isolation.

DNA isolation

Total plant genomic DNA was extracted from young leaves of each genotype using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method as described in Doyle and Doyle (1990). The quantity and quality of the isolated DNA was determined by using PicoDrop N.D.1000 (Software Ver. 3.3.2, Thermo Scientific, USA). Dilutions of 25 ng/µl of each genotype were prepared and stored at 4 °C for further use in PCR analysis.

RAPD analysis

Twenty seven oligonucleotide primers of 10-mer, each with at least 60% G+C content (Table 2), were obtained from Operon Technologies, Inc., Alameda, CA. PCR reactions were performed as per Williams *et al.* (1990) with some modifications. The PCR master mix (25µl) contained 10x PCR buffer (10 mM Tris-HCl, pH 8.3), 100 mM each dNTPs, 25 pmoles primer, 25 ng of genomic DNA and 1 unit of Taq DNA polymerase (Invitrogen). The samples were subjected to 35 repeats of the following cycle: 94°C 1 min, 38°C for 1 min, 72°C for 2 min with an initial denaturation of 3 minutes and a final extension of 10 minutes.

All the above PCR amplification was performed in 0.2 ml thin-walled PCR tubes placed in a thermal cycler (Veriti®, Applied Biosystems). The products were analysed by electrophoresis in 1.5% agarose gel stained in ethidium bromide (10 mg/ml) and run in 1x TBE buffer at 100 V for 2 h. The separated bands were visualized under UV transilluminator and photographed using a gel documentation system (BioRad). The amplified fragments were scored as '1' for the presence and '0' for the absence of a band from higher to lower molecular weight products.

Statistical analysis

The molecular size of each fragment was estimated using AlphaEase FC software (Alpha Innotech Corporation). RAPD markers were scored as present (1) or absent (0) of a band, and the data obtained were used in a rectangular matrix. The data matrix was then used to generate a genetic similarity index (Nei and Li, 1979) using NTSYS 2.1 (Rohlf, 2000).

Cluster analysis was carried out based on genetic distance (GD = 1 – GS), using UPGMA (unweighted pair-group method using arithmetic averages) (Sneath and Sokal, 1973). The resulting clusters were represented as dendrograms and viewed in the program Tree View ver. 1.5. A polymorphic index (PIC) was calculated as $PIC = 1 - p^2 - q^2$, where p is the frequency of bands present bands and q is frequency of bands absent (Ghislain *et al.*, 1995).

Results and Discussion

Total plant genomic DNA was extracted from young leaves by Cetyl Trimethyl Ammonium Bromide (CTAB) method with some modifications. The absorbance ratio of DNA at A260/A280 ranged from 1.72 to 1.89 and the concentration ranged from 119.5 to 218.9 ng/µl.

Sixteen RAPD primers showed polymorphism, out of the twenty seven used, producing a total of 99 bands/alleles, all of which were polymorphic representing 100% polymorphism with the number of amplified fragment varying with size ranging from 60 to 1814 bp. The average bands per primer were 4.75. The PIC values varied between

0.423 (OPF-12) and 0.883 (OPA-05) with an average of 0.705 per primer (Table 2). The dendrogram constructed by UPGMA method generated two main clusters that consists all the varieties grouped together in their respective sub-cluster. The RAPD profile was visualized on 1.2 per cent agarose gel and photographed. Only those fragments consistently amplified were scored for analysis. Similar results have also been reported in castor (Gajera *et al.,* 2010)**,** Azuki bean (Yee *et al.,* 1999) and in groundnut (Dwivedi *et al.,* 2001).

The UPGMA dendrogram (Rohlf, 2000), based on the Jaccard's coefficient of similarity (Dwivedi *et al.,* 2001), allowed for two main clusters to be distinguished, clusters I and II (Figure 1). Cluster I was again divided into two sub-clusters IA and IB. Sub-cluster IA further divided in to A1 and A2; A1 further bifurcated into two groups i.e. A_{1a} and A_{1b}.

The sub cluster A_{1a} contained seven genotypes *viz*. VP-1, GAUCH-1, VI-9, GEETA, JI-35, GCH-2 and GCH-4. Sub-cluster A_{1b} was simplicifolious containing SH-72. Cluster A2 comprised of staminate lines GCH-5 and SKP-84. Cluster IB included the hybrid GCH-7. Major cluster II was bifolious comprising of SKI-215 and 48-1. The lowest value of Jaccard's similarity coefficient observed was 0.454, between GCH-4 and SKI-215, hence, both of these genotypes i.e. GCH-4 and SKI-215 can be exploited for hybrid vigour. Since these genotypes are genetically diverse. Also, GCH-5 and 48-1(0.484) and VP-1 and 48-1 (0.505) could be used as parental lines for breeding programs. The highest similarity coefficient 0.969 was found between GAUCH-1 and VI-9 (Table 3).

Table.1 The pedigree and origin (develop location) of 13 genotypes of castor evaluated for genetic diversity using RAPD

Sr. No	Genotype	Pedigree	Origin
1	VP-1	TSP-10-R × JI-15) F_2 × (JP-5 × 26006) F_2	Vijapur, Gujarat
2	GAUCH-1	VBH-44 (VP-1 x VI-9)	S.K. Nagar, Gujarat
3	VI-9	Selection from S-20	Vijapur, Gujarat
4	GCH-2	VP-1 x JI-35	S.K. Nagar, Gujarat
5	JI-35	Local selection from Junagadh, India	Junagadh, Gujarat
6	GCH-4	VP-1 x 48-1	S.K. Nagar, Gujarat
7	SKI-215	(SKI-8A × SA-2) F10	S.K. Nagar, Gujarat
8	GEETA	Selection from 48-1	S.K. Nagar, Gujarat
9	GCH-5	GEETA x SH-72	S.K. Nagar, Gujarat
10	SH-72	Parental Line	S.K. Nagar, Gujarat
11	SKP-84	KP-1×VP-1	S.K. Nagar, Gujarat
12	GCH-7	SKP 84 x SKI-215	S.K. Nagar, Gujarat
13	48-1	Parental Line	S.K. Nagar, Gujarat

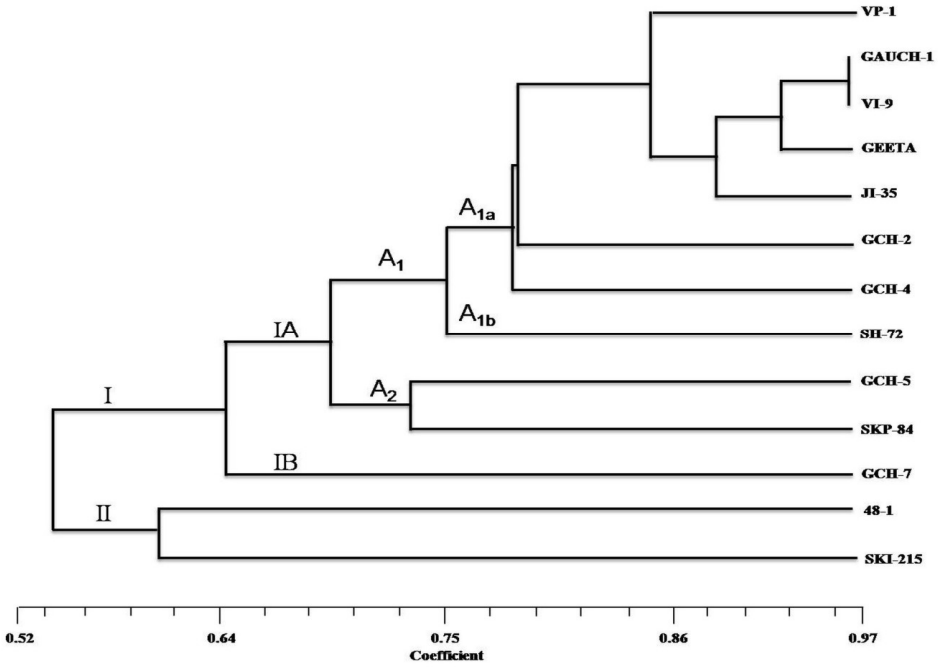
Table.2 List of primers used, number of amplified products, number of polymorphic bands, and percentage of polymorphism and PIC value obtained by analyzing 13 genotype of castor

Sr. No.	RAPD primers	Primer Sequence (5'-3')	Allele size	Number of bands (A)	Polymorphic bands (B)	Polymorphic % (B/A)	PIC value
1	OPA-02	TGCCGAGCTG	463-988	4	4	100	0.480
2	OPA-04	AATCGGGCTG	387-1062	11	11	100	0.875
3	OPA-05	AGGGGTCTTG	260-1435	10	10	100	0.883
4	OPB-01	GTTTCGCTCC	362-1814	4	4	100	0.699
5	OPB-08	GTCCACACGG	136-799	7	7	100	0.838
6	OPD-02	GTGAGGCGTC	240-1314	7	7	100	0.771
7	OPD-07	GTCCCGACGA	328-1154	5	5	100	0.680
8	OPD-08	TGGACCGGTG	337-917	6	6	100	0.694
9	OPD-11	AAAGCTGCGG	231-1010	10	10	100	0.869
10	OPE-18	GGACTGCAGA	155-1072	7	7	100	0.761
11	OPF-04	GGTGATCAGG	126-560	9	9	100	0.836
12	OPF-12	ACGGTACCAG	269-707	3	3	100	0.423
13	OPF-13	GGCTGCAGAA	251-665	4	4	100	0.740
14	OPF-14	TGCTGCAGGT	60-473	5	5	100	0.778
15	OPH-15	AATGGCGCAG	139-557	4	4	100	0.680
16	OPH-16	TCTCAGCTGG	150-769	5	5	100	0.712
			Total	**99**	**99**	**---**	**---**
			Mean	**4.75**	**4.75**	**100**	**0.705**

Table.1 Genetic distance estimated among the castor genotypes using Jaccard's Coefficient based on the RAPD data (Jaccard, 1908)

	VP-1	GAUCH-1	VI-9	GCH-2	JI-35	GCH-4	SKI 215	GEETA	GCH-5	SH-72	SKP-84	GCH-7	48-1
VP-1	**1.000**	0.898	0.868	0.777	0.838	0.737	0.535	0.848	0.757	0.767	0.696	0.616	0.505
GAUCH-1	0.898	**1.000**	0.969	0.777	0.898	0.777	0.494	0.929	0.737	0.787	0.696	0.636	0.565
VI-9	0.868	0.969	**1.000**	0.808	0.888	0.767	0.525	0.939	0.747	0.797	0.686	0.646	0.575
GCH-2	0.777	0.777	0.808	**1.000**	0.818	0.777	0.636	0.787	0.737	0.686	0.616	0.676	0.525
JI-35	0.838	0.898	0.888	0.818	**1.000**	0.878	0.474	0.900	0.717	0.767	0.656	0.656	0.585
GCH-4	0.737	0.777	0.767	0.777	0.878	**1.000**	0.454	0.808	0.696	0.686	0.616	0.696	0.565
SKI-215	0.535	0.494	0.525	0.636	0.474	0.454	**1.000**	0.484	0.595	0.525	0.494	0.535	0.525
GEETA	0.848	0.929	0.939	0.787	0.900	0.808	0.484	**1.000**	0.747	0.797	0.686	0.626	0.595
GCH-5	0.757	0.737	0.747	0.737	0.717	0.696	0.595	0.747	**1.000**	0.707	0.737	0.636	0.484
SH-72	0.767	0.787	0.797	0.686	0.767	0.686	0.525	0.797	0.707	**1.000**	0.606	0.626	0.575
SKP-84	0.696	0.696	0.686	0.616	0.656	0.616	0.494	0.686	0.737	0.606	**1.000**	0.575	0.545
GCH-7	0.616	0.636	0.646	0.676	0.656	0.696	0.535	0.626	0.636	0.626	0.575	**1.000**	0.505
48-1	0.505	0.565	0.575	0.525	0.585	0.565	0.525	0.595	0.484	0.575	0.545	0.505	**1.000**

Figure.1 Dendrogram depicting the Genetic relationship among the 13 castor genotypes based on RAPD data



Similar work was done in Jatropha species (Ganesh *et al.*, 2008; Subramanyam *et al.*, 2009) and in Cassava (Zacarias *et al.*, 2004) based on RAPD markers. RAPD assay in the present study was useful in the assessment of castor genotypes. This opens up the possibility for the selection of better parents which could be useful for genetic enhancement and development of hybrids in castor genotypes.

The technique used in the present investigation for analyzing the genetic diversity classified the 13 castor genotypes into distinct clusters based on the dendrogram prepared by UPGMA algorithm. For better molecular characterization of the castor genotypes, it is essential to use a higher number of RAPD markers. Our analysis proved the utility of RAPD markers for distinguishing the used set of castor genotypes. RAPD markers are useful in the assessment of castor bean diversity. In conclusion, the present study indicated the prevalence of ample DNA polymorphism in selected castor genotypes which could be further utilized for breeding programs.

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