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Assessment of Molecular Diversity in Castor Genotypes Using SSR Markers

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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 twenty castor genotypes and identify those with distinct DNA profiles. Thirty SSRs primers were used, out of which 25 polymorphic primers revealed 53.33 % 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 0.0 to 0.66 with an average of 0.11. 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.) belongs to the family Euphorbiaceae. It has chromosome number 2n = 20. It is 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. 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 (Lakhani *et al.,* 2015).

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). 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 (Reif *et al.,* 2007). 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.

Genetic markers have polymorphic genetic properties which can be used to distinguish the parental origin of alleles (Andersen and Lubberstedt, 2003). 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 (Lesica *et al.,* 1998). DNA based molecular analysis tools are ideal for germplasm characterization and phylogenetic studies (Masjoc, 2002).

Simple sequence repeats (SSR) or microsatellite marker on other hand require less amount of DNA sample without involvement of radioactive labels and is simpler as well as faster. Also SSR markers have ability to discriminate genotypes into homozygotes and heterozygotes due to the co-dominant nature. The objective of the present study was to investigated and characterized the genetic diversity present in the Indian genotypes of castor using SSR markers with an aim of accurate assessment of genetic diversity and select better cross combinations to develop popular hybrids with higher production potentials.

The information gathered here would be helpful in genomic mapping studies and for the development of castor genotypes with wider and diverse genetic background to obtained improved crop productivity. The identified polymorphic markers could also be exploited for improvement of castor through MAS and breeding as well as in future germplasm conservation strategies.

Materials and Methods

Collection and assemblage of plant materials

The experimental material for the present study consisted of twenty castor genotypes collected from different parts of Gujarat. The list of species and their collection sites are furnished in Table 1.

DNA isolation

Seeds of each castor genotype were sown in pots and young leaves of 2 weeks old plants were collected from each genotype for DNA isolation. Genomic DNA was extracted from freshly harvested leaves of each castor genotype by adopting the procedure outlined by Purohit *et al.,* (2013) with necessary modification.

Quantification and electrophoresis of genomic DNA

Agarose of 0.8% was melted and the gel was casted using gel casting plate. DNA samples were loaded in each well along with 5 µl of loading dye and run at a constant voltage of 100 V. The gel was placed over an UV-trans-illuminator and viewed at 300 nm. The nucleic acid appeared as orange coloured intact band owing to the fluorescence of ethidium bromide and the gel was photographed using Alpha Innotech multiimage Digital 120 Zoom digital camera. 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.

SSR analysis

Total thirty SSR primers were used for PCR amplification. Out of thirty SSR primers twenty-five were amplified and were used for further analysis. The PCR reactions were performed as per Galova *et al.,* (2015) with some modifications. The PCR mixture comprised of 50 ng genomic DNA, 10 mM Tris-HCl (pH 8.0), 2.5 mM $MgCl_2$, 1U *Tag* polymerase, 10 pmole primer and 200 µM of dNTPs mixture (Bangalore Genei, India). The volume was made 15 µl with sterile distilled water. The temperature cycle profiles were: an initial denaturation step for 5 min at 94°C, followed by 35 cycles of denaturation step at 94°C for 1 min, annealing at 57-60°C for 45 sec and primer extension at 72°C for 45 sec; final extension cycle of 7 min at 72°C was performed on Veriti 96-well Thermal Cycler.

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. Faint bands were not recorded for analysis. By comparing the banding patterns of genotypes for a specific primer, genotype-specific bands were identified.

Statistical analysis

The molecular size of each fragment was estimated using Alpha Ease FC software (Alpha Innotech Corporation). SSR 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). The polymorphism information content

(PIC) was calculated by the formula: PIC = 2Pi (1−Pi) (Bhat, 2002) where, Pi is the frequency of occurrence of polymorphic bands in different primers. Pairwise similarity matrices were generated by Jaccard's coefficient of similarity (Jaccard, 1908) by using the SIMQUAL format of NTSYSpc 2.1(Rohlf, 2000). Correlation between the two matrices obtained with two-marker types was estimated by means of the Mantel matrix correspondence test (Mantel, 1967). This test yields a product moment correlation (r) that is one measure of the relatedness between the two matrices. A dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSYS-pc to show a phonetic representation of genetic relationships as revealed by the similarity coefficient (Sneath and Sokal, 1973).

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 1.71 to 1.89 and the concentration ranged from 136.09 to 223.04 ng/µl.

The twenty five primers showed polymorphism, out of the thirty used, producing a total of 36 bands/alleles, all of which were polymorphic representing 53.33 % polymorphism with the number of amplified fragment varying with size ranging from 89 to 2190 bp. The number of amplified fragments produced per primer varying from 1 (Castor 6, Castor 7, Castor 19, Castor 20, Castor 25, Castor 34, Castor 38, Castor 48, Castor 53, Castor 62, Castor 72, Castor 82, Castor 97, Castor 98, Castor 111, Castor 114, Castor 117 and Castor 123) to 5 (Castor 73) with average of 0.84 bands per primer (Table 1). The PIC values varied between 0.0 (Castor 6, Castor 7, Castor 19, Castor 20, Castor 25, Castor 34, Castor 38, Castor 48, Castor 53, Castor 62, Castor72, Castor 82, Castor 97, Castor 98, Castor 111, Castor 114, Castor 117 and Castor 123) and 0.66 (Castor 8) with an average of 0.11 per primer (Table 1).

The dendrogram constructed by UPGMA method generated two main clusters that consists all the varieties grouped together in their respective sub-cluster. The SSR profile was visualized on 1.5 % agarose gel and photographed. Only those fragments consistently amplified were scored for analysis. Similar to present finding, Maurya and Yadav (2016) studied the level of heterozygosity in *Jatropha curcas* using SSR markers. Out of 56 SSR primers, 18 primers were amplified. The polymorphic SSRs showed allele variation from 2 to 9 with an average of 3.56 alleles per primer. Earlier, Galova *et al.,* (2015) analyzed 60 genotypes of castor using 10 SSR primers of Rco series and amplified 67 alleles ranging from 4 (Rco15) to 9 (Rco05) alleles with a mean value of 6.70 alleles per locus. Earlier, Costa *et al.,* (2013) analyzed 66 sweet cassava accessions with 13 SSR primers of GA and SSRY series which amplified a total of 33 alleles with an average of 2.53 alleles per marker. Sakure *et al.,* (2012) studied 22 castor genotypes using 20 SSR primers of SSRY series, out of which 10 SSR primers amplified, a total number of 353 fragments, out of which 309 were polymorphic, with an average of 9.8 polymorphic bands per primer.

The dendrogram obtain from UPGMA analysis grouped twenty genotypes into two main clusters with Jaccard similarity coefficient ranging from 0.62 to 1.00 (Table 2). Cluster one further divided into two subcluster-A and B both contained a total of 18 genotypes (Fig. 1). Subcluster-A was further bifurcated into two groups A1 and A2 having nearly 79 % likeness.

Table.1 Size, number of amplified bands, per cent polymorphism and PIC obtained by SSR primers in the 20 castor genotypes

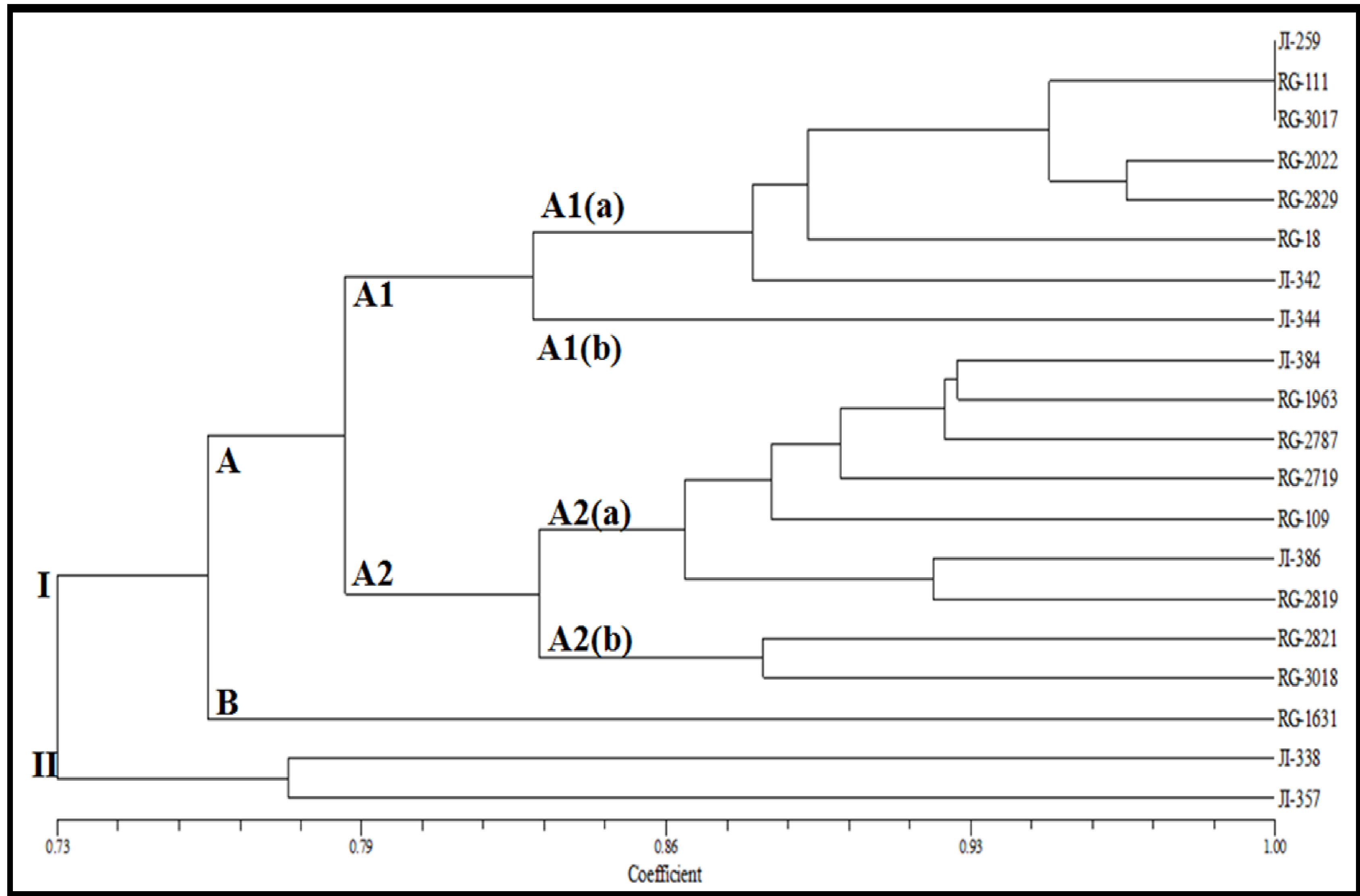
Sr. No.	SSR Primer	Band Size (bp)	Total No. of Bands (A)	Polymorphic Bands (B)			Mono-Morphic Band	% Poly-Morphism (B/A)	PIC*	SPI
				S	U	T				
1	Castor 6	139	1	0	0	0	1	0.0	0.0	0.0
2	Castor 7	228	1	0	0	0	1	0.0	0.0	0.0
3	Castor 8	175-432	3	1	0	1	2	33.33	0.66	1.98
4	Castor 19	222	1	0	0	0	1	0.0	0.0	0.0
5	Castor 20	233	1	1	0	1	0	100.0	0.0	0.0
6	Castor 25	212	1	0	0	0	1	0.0	0.0	0.0
7	Castor 26	89-240	2	0	1	1	1	50.0	0.09	0.18
8	Castor 34	236	1	1	0	1	0	100.0	0.0	0.0
9	Castor 38	153	1	1	0	1	0	100.0	0.0	0.0
10	Castor 42	254-306	2	2	0	2	0	100.0	0.49	0.98
11	Castor 45	173-243	2	2	0	2	0	100.0	0.50	1.0
12	Castor 48	205	1	1	0	1	0	100.0	0.0	0.0
13	Castor 53	252	1	0	0	0	1	0.0	0.0	0.0
14	Castor 62	237	1	0	0	0	1	0.0	0.0	0.0
15	Castor 72	245	1	1	0	1	0	100.0	0.0	0.0
16	Castor 73	191-2190	5	2	3	5	0	100.0	0.53	2.65
17	Castor 78	93-183	2	1	0	1	1	50.0	0.16	0.32
18	Castor 79	175-256	2	1	0	1	1	50.0	0.49	0.98
19	Castor 82	222	1	0	0	0	1	0.0	0.0	0.0
20	Castor 97	192	1	1	0	1	0	100.0	0.0	0.0
21	Castor 98	135	1	0	0	0	1	0.0	0.0	0.0
22	Castor 111	231	1	1	0	1	0	100.0	0.0	0.0
23	Castor 114	214	1	1	0	1	0	100.0	0.0	0.0
24	Castor 117	206	1	1	0	1	0	100.0	0.0	0.0
25	Castor 123	208	1	0	0	0	1	0.0	0.0	0.0
TOTAL			36	18	4	22	14	-	-	-
AVERAGE			-	-	-	0.84	0.56	53.33	0.11	0.32

S = Shared; U = Unique; T = Total polymorphic bands; PIC = Polymorphism information content; SPI = SSR primer index = Number of bands x PIC

Table.2 Jaccard's similarity coefficient of 20 castor genotypes based on SSR

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1	1.00																			
2	0.73	1.00																		
3	0.87	0.80	1.00																	
4	0.84	0.66	0.84	1.00																
5	0.79	0.78	0.80	0.71	1.00															
6	0.83	0.76	0.84	0.87	0.76	1.00														
7	0.74	0.67	0.75	0.77	0.72	0.89	1.00													
8	0.90	0.71	0.84	0.81	0.77	0.81	0.72	1.00												
9	0.86	0.66	0.74	0.83	0.71	0.89	0.85	0.77	1.00											
10	**1.00**	0.73	0.87	0.84	0.79	0.83	0.74	0.90	0.86	1.00										
11	0.81	0.73	0.76	0.68	0.68	0.77	0.69	0.78	0.73	0.81	1.00									
12	0.77	0.70	0.84	0.87	0.70	0.93	0.89	0.75	0.89	0.77	0.72	1.00								
13	0.93	0.80	0.94	0.84	0.80	0.90	0.81	0.90	0.80	0.93	0.81	0.84	1.00							
14	0.83	0.69	0.77	0.80	0.69	0.93	0.82	0.80	0.88	0.83	0.83	0.86	0.83	1.00						
15	0.77	0.69	0.77	0.80	0.69	0.93	0.89	0.80	0.88	0.77	0.77	0.93	0.83	0.92	1.00					
16	0.73	0.66	0.74	0.77	0.71	0.89	0.92	0.71	0.85	0.73	0.68	0.89	0.80	0.81	0.88	1.00				
17	0.80	0.67	0.75	0.77	0.72	0.89	0.86	0.77	0.85	0.80	0.74	0.83	0.81	0.89	0.82	0.85	1.00			
18	0.97	0.77	0.90	0.81	0.77	0.87	0.77	0.87	0.83	0.97	0.84	0.81	0.97	0.86	0.80	0.77	0.83	1.00		
19	**1.00**	0.73	0.87	0.84	0.79	0.83	0.74	0.90	0.86	**1.00**	0.81	0.77	0.93	0.83	0.77	0.73	0.80	0.97	1.00	
20	0.76	**0.62**	0.71	0.73	0.74	0.85	0.81	0.68	0.81	0.76	0.70	0.79	0.77	0.85	0.78	0.81	0.88	0.79	0.76	1.00

Fig.1 Dendrogram depicting the genetic relationship among 20 castor genotypes based on SSR markers.



Group A1 was further divided into subgroup A1(a) and A1(b). Subgroup A1(a) consist of 7 genotypes JI-259, RG-111, RG-3017, RG-2022, RG-2829, RG-18 having nearly 88 % similarity while subgroup A1(b) consist of only one genotype JI-342. Group A2 was further divided into subgroup A2(a) and A2(b). Subgroup A2(a) consist of 7 genotypes JI-384, RG-1963, RG-2787, RG-2719, RG-109. JI-386 and RG-2819 having nearly 87 % similarity while subgroup A2(b) consist of two genotypes RG-2821 and RG-3018 having 88 % similarity. Subcluster B consists of only one genotype RG-1631.

The technique used in the present investigation for analyzing the genetic diversity classified the twenty castor genotypes into distinct clusters based on the dendrogram prepared by UPGMA algorithm. Our analysis proved the utility of SSR markers for distinguishing the used set of castor genotypes. In conclusion, the present study indicated the prevalence of ample DNA polymorphism in selected castor genotypes which could be further utilized for breeding programs. The information gathered here would be helpful in genomic mapping studies with wider and diverse genetic background to obtained improved crop productivity.

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