RESEARCH PAPER

Discrimination of castor (*Ricinus communis* L.) genotypes through SSR marker

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Castor (*Ricinus communis* L.) is one of the most ancient, non-edible oilseed crop. It is a highly polymorphic due to higher percentage of cross pollination. New genetic approaches have been developed, like molecular marker technology which adopted to map the castor genome, in order to select better cross combinations for popular hybrids. Genetic diversity in twenty two castor genotypes were assessed using SSR marker which gave higher percentage (90.90%) of polymorphic loci, higher heterozygosity (52%) and a greater range of genetic diversification for discriminating among them. In SSR analysis, a total 29 alleles were generated.The data revealed from ten markers, that genotypes JI-220, JI-353, JP-102 and JI-377 showed high genetic dissimilarity so, it is very important to improve the castor crop with the help of hybridization and crop improvement programme. The information gathered here would be helpful in genomic mapping studies with wider and diverse genetic background to obtain improved crop productivity.

Key words **:** Castor, SSR, Genetic diversity, Molecular marker

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INTRODUCTION

Castor (*Ricinus communis* L., 2n = 2x = 20, Euphorbiaceae), is one of the most ancient, non-edible oilseed crops of the world valued for its medicinal and industrial application. Though castor oil is the chief commercial commodity, castor cake is also a good source of nitrogen (5.5 %) and widely used as manure but it is unfit for cattle feed due to presence of ricin. Dried stalks of castor are used for fuel in rural areas. Castor oil is chiefly used as a lubricant in high-speed engines, for the production of paints and varnishes, synthetic resins, fibre and nylon and in pharmaceutical due to its laxative properties and has many other industrial uses. The area, production and productivity of castor in India 10.54 lakh tones during 2007-2008 from area 7.87 lakh hectares with productivity level of 1339 kilogram per hectare contributing about 40 per cent of the world requirement (Anonymous, 2009). The other castor growing countries in the world are Brazil, China, Russia, Thailand, U.S.A. and a group of African countries. Castor is a highly polymorphic normally monoecious with pistillate flowers are situated on the upper part and staminate flowers on the lower part of raceme. Though it is a cross-pollinated crop, most of the cultivars have been developed through hybridization followed by selection. New genetic approaches like molecular marker technology have been adopted to map the castor genome, in order to select better cross combinations to develop popular hybrids. Genetic markers have polymorphic genetic properties which can be used to distinguish the parental origin of alleles (Andersen and Lubberstedt, 2003). This genetic marker can be used to identify the genes that are responsible for genetic variation of economically important traits. Most traits and of interest in plant breeding such as productivity and resistance to biotic and abiotic stresses are determined by more than one gene or even large number of genes that are called quantitative traits. The identification of the major loci influencing these traits can be used to assist in the selection of desired plants (Masojc, 2002). Among the various DNA-based markers, amplified fragment length polymorphism (AFLP), random polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) have been used to study genetic diversity. These markers elucidate the phylogenetic relationships among various lines, for their efficient use in breeding and genetic

resource management. AFLP and RFLP methods, however, involve the use of expensive enzymes, radioactive labelling, and are cumbersome while RAPD marker is non-reproducible and hence, appear unsuitable. 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 investigate 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.

RESEARCH METHODOLOGY

Plant materials and DNA extraction:

The experimental material comprised of twenty two diverse castor genotypes including eight inbreeds, eight pistillate, one staminate and remaining were hybrids (Table A). The experiment was conducted at Biotech Lab, Department of Agricultural Botany, Junagadh Agriculture University, Junagadh, Gujarat, India. The genotypes were raised in field in Randomized Block Design (RBD) with two replications at Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh, Gujrat, India. Randomly five plants in each genotype were collected and mixed in equal proportion for DNA extraction. DNA was extracted from fresh newly expanded leaves using CTAB protocol adapted from Doyle and Doyle (1987) with minor modification (*viz.*, concentration of NaCl, Sodium acetate incubation duration and temperature).

SSR assays:

Total twenty SSR primers were used for PCR amplification. Out of twenty SSR primers ten were amplified and were used for further analysis (Table A). 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 pico mole primer and 200 µM of dNTPs mixture (Bangalore Genei, India). The volume was made 25 µl with sterile distilled water. The temperature cycle profiles were: an initial denaturation step for 5 min at 94^{0}C, followed by 35 cycles of denaturation step at 94^{0}C for 1 min, annealing at 58^{0}C for 2 min and primer extension at 72^{0}C for 2 min; final extension cycle of 5 min at 72^{0}C was performed on Eppendorf Mastercycler. SSR bands were designated on the basis of their molecular size (length of polynucleotide amplified). 100 bp DNA ladder for SSR loaded simultaneously with primer products in the gel was used to estimate the molecular size. The distance run by amplified fragments from the well was translated to molecular size with reference to molecular weight of marker. The presence of each band was scored as '1' and its absence as '0'.

Table A : List of twenty two castor genotypes with their location

Sr. No.	Genotypes	Location
1.	VP-1*	Vijapur (Gujarat)
2.	48-1[@]	Vijapur (Gujarat)
3.	SKP-84*	S.K. Nagar (Gujarat)
4.	SKI-215[@]	S.K. Nagar (Gujarat)
5.	GC-3[@]	Junagadh (Gujarat)
6.	GCH-4** (VP-1 x 48-1)	Junagadh (Gujarat)
7.	GCH-6** (JP-65 x JI-96)	Junagadh (Gujarat)
8.	GCH-7**(SKP-84 x SKI-215)	S.K. Nagar (Gujarat)
9.	JP-65*	Junagadh (Gujarat)
10.	JP-91*	Junagadh (Gujarat)
11.	JP-92*	Junagadh (Gujarat)
12.	JP-95*	Junagadh (Gujarat)
13.	JP-101*	Junagadh (Gujarat)
14.	JP-102*	Junagadh (Gujarat)
15.	JI-96[@]	Junagadh (Gujarat)
16.	JI-220[@]	Junagadh (Gujarat)
17.	JI-344[@]	Junagadh (Gujarat)
18.	JI-353[@]	Junagadh (Gujarat)
19.	JI-377[@]	Junagadh (Gujarat)
20.	JI-385[@]	Junagadh (Gujarat)
21.	DCS-9[#]	DOR (Hyderabad)
22.	PCS-124[#]	Palem (Andhra Pradesh)

* Pistillate, [@]Inbreeds, #Staminate, **Hybrids

Statistical analysis:

Difference in the banding pattern was quantitatively scored from gel for presence 1 and absence 0 of bands. The binary data generated were used to estimate levels of polymorphism information content (PIC) was calculated by formula:- PIC = 2Pi(1-Pi) (Bhat, 2002), where Pi is the frequency of occurrence of polymorphic bands in different primers. Pair-wise matrices were generated by Jaccard's coefficient of similarity (Jaccard, 1980) by using SIMQUAL format of NYSYSS-pc version 2.02 Exter Software (Rohlf, 1998). A dendrogram was constructed by using the unweight pair group method with arithmetic average (UPGMA) with SHAN module of NTSYS-pc to show phenotypic representation of genetic relationships as revealed by similarity coefficient (Sneath and Sokal, 1973).Genetic interpretations of SSR markers variation in banding patterns were also subjected to genotypic data

based on co-dominance nature of marker to interpret the observed homozygosity (Ho) and heterozygosity (He) within genotypes (Chamberlain, 1998).

RESEARCH FINDINGS AND ANALYSIS

The results are summarized below according to objectives of the study:

SSR analysis:

PCR amplification of DNA, using ten SSR primers, produced a total 29 alleles and 353 DNA fragments that could be scored in all genotypes (Table 1). The number of amplified fragments produced per primer varying from 2 (SSRY-83, SSRY-61, SSRY-52, SSRY-47 and SSRY-40) to 5 (SSRY-203) with average of 3 bands per primer (Table 1). Out of 353 amplified fragments, 309 were polymorphic, with an average of 9.8 polymorphic bands per primer. Banding pattern of different genotypes with respect of ten primers showed that SSRY-21 produced maximum polymorphic bands while primer SSRY-324 produced different banding pattern in JP-102 (Fig. 1A and 1B). The size of amplified fragment ranged from 38 to 845 bp which reflected remarkable difference in the number of repeats between the different alleles. Calculated values for PIC ranged from 0.2097 (SSRY-40) to 0.840 (SSRY-203) with four loci identified as informative markers (PIC > 0.5). These values corresponded with Shannon information index value for gene diversity, which varied from 0.5860 (SSRY-52) to 1.1891 (SSRY-26). The percentage of polymorphism ranged from 47.61 per cent (SSRY-61) and 100 per cent in eight primers with the average of 90.90 per cent. The average observed heterozygosity (Hos) was 0.5202 (Table 1). Highest number of observed heterozygosity (Hop) was found with SSRY-61 (0.9091) marker followed by SSRY-203 (0.8000) whereas lowest was found with SSRY-21 (0.1053).

Genetic relatedness:

The dendrogram obtain from UPGMA analysis grouped 22 genotypes into two main clusters with Jaccard similarity coefficient

Table 1 : Detail of SSR primers, fragment size, observed number of allele (NA), total number of polymorphic bands, percentage of polymorphism (%), polymorphism information content (PIC), Shannon's information index (I) and Observed heterozygosity (Hos) revealed by SSR analysis of twenty two castor genotypes

Sr. No.	SSR Locus		Primer Sequence (5'-3')	Annealing temperature (°C)	Fragment size	NA	(MB)	(PB)	(TB)	PB/TB x 100 (%)	PIC value	(I)	Hos
1.	SSRY-40	F	TGCATCATGGTCCACTCACT	64.3	57-81	2	0	26	26	100	0.2097	0.4101	0.2857
		R	CATTCTTTTCGGCATTCCAT	63.5									
2.	SSRY-47	F	GGACCACCTTTTGCTGAGTT	63.2	70-80	2	0	36	36	100	0.4932	0.6838	0.5909
		R	TTGGAACAAAGCAGCATCAC	63.8									
3.	SSRY-52	F	GCCAGCAAGGTTTTGCTACAT	63.9	49-93	2	0	32	32	100	0.3966	0.5860	0.4545
		R	AACTGTCAAACCATTCTACTTGC	60.9									
4.	SSRY-26	F	TGCTAATTGCAGGAAATAGGAT	61.3	56-167	3	22	35	57	61.40	0.6549	1.1891	0.5429
		R	GCAGCTTTTTTAGCATAACAATCAA	62.9									
5.	SSRY-61	F	GGCTGCTTTACCTTCTACTCADA	62.6	49-89	2	22	20	42	47.61	0.4958	0.6890	0.9091
		R	CAAGAACGCCAATATGCTGA	63.6									
6.	SSRY-83	F	TGCCTAGATGGTGATTATTGCTT	63.3	47-79	2	0	24	24	100	0.3750	0.5882	0.2500
		R	TGCTTACTCTTTGATTCCACG	61.7									
7.	SSRY-21	F	CCTGCCACAATATTGAAATGG	63.8	49-52	4	0	22	22	100	0.2748	0.6538	0.1053
		R	CAACAATTGGACTAAGCAGCA	62.7									
8.	SSRY-39	F	TCAATGCATAGGATTTTGAAAGTTA	61.1	38-75	3	0	43	43	100	0.5540	1.0123	0.6923
		R	AATGAAATGTAGGTCATGCT	56.3									
9.	SSRY-324	F	CGCTTACAACACCACCTTCA	63.6	51-512	4	0	35	35	100	0.5224	0.9275	0.5714
		R	GCTTGATCTCAGCCATGTCA	64.0									
10.	SSRY-203	F	AGGAAGCTGTCCCCTATTCA	62.2	42-845	5	0	36	36	100	0.8400	1.1577	0.8000
		R	TACCAGCAAGACCATCACCA	64.1									
Total						29	44	309	353				
Mean										90.90	0.5047	0.7891	0.5202

* F- Forward primer * R- Reverse primer

Table 2 : Genetic similarity matrices computed according to Jaccard coefficient from ten SSR banding pattern of castor genotypes

Genotypes	VP 1	GCH 4	48 1	SKP 84	GCH 7	SKI 215	GC 3	DCS 9	PCS 124	GCH 6	JP 65	JP 91	JP 92	JP 95	JP 101	JP 102	JI 96	JI 220	JI 344	JI 353	JI 377	JI 385
VP 1	1.00																					
GCH 4	0.63	1.00																				
48 1	0.63	0.88	1.00																			
SKP 84	0.71	0.76	0.76	1.00																		
GCH 7	0.60	0.74	0.74	0.72	1.00																	
SKI 215	0.69	0.56	0.56	0.63	0.61	1.00																
GC 3	0.55	0.68	0.78	0.67	0.74	0.56	1.00															
DCS 9	0.60	0.74	0.74	0.63	0.70	0.53	0.74	1.00														
PCS 124	0.62	0.75	0.84	0.65	0.80	0.55	0.75	0.71	1.00													
GCH 6	0.52	0.74	0.74	0.63	0.79	0.53	0.65	0.70	0.80	1.00												
JP 65	0.57	0.79	0.79	0.78	0.75	0.58	0.70	0.75	0.76	0.75	1.00											
JP 91	0.75	0.71	0.71	0.70	0.76	0.60	0.64	0.76	0.86	0.76	0.73	1.00										
JP 92	0.67	0.63	0.63	0.53	0.68	0.69	0.55	0.68	0.62	0.60	0.65	0.67	1.00									
JP 95	0.60	0.74	0.83	0.63	0.79	0.53	0.74	0.79	0.80	0.70	0.75	0.76	0.78	1.00								
JP 101	0.65	0.61	0.71	0.59	0.67	0.79	0.71	0.58	0.68	0.58	0.63	0.57	0.75	0.67	1.00							
JP 102	0.55	0.52	0.60	0.50	0.57	0.47	0.52	0.43	0.59	0.57	0.48	0.50	0.48	0.50	0.61	1.00						
JI 96	0.55	0.68	0.78	0.67	0.74	0.56	0.78	0.74	0.75	0.65	0.70	0.64	0.55	0.74	0.71	0.52	1.00					
JI 220	0.58	0.72	0.82	0.61	0.68	0.59	0.72	0.68	0.79	0.78	0.74	0.67	0.67	0.78	0.75	0.63	0.72	1.00				
JI 344	0.55	0.78	0.88	0.67	0.74	0.56	0.88	0.83	0.84	0.74	0.79	0.71	0.63	0.83	0.71	0.52	0.78	0.82	1.00			
JI 353	0.50	0.68	0.68	0.59	0.58	0.43	0.61	0.58	0.60	0.65	0.63	0.58	0.50	0.58	0.48	0.54	0.61	0.64	0.61	1.00		
JI 377	0.53	0.59	0.59	0.47	0.40	0.44	0.42	0.47	0.50	0.47	0.45	0.55	0.53	0.56	0.50	0.42	0.42	0.63	0.50	0.45	1.00	
JI 385	0.55	0.68	0.78	0.58	0.65	0.56	0.68	0.83	0.75	0.65	0.70	0.71	0.72	0.83	0.71	0.45	0.78	0.72	0.78	0.54	0.59	1.00

ranging from 0.44 to 0.88 (Table 2). Cluster one further divided into two subcluster (I_a and I_b). Subcluster-Ia comprised of JP-101 and SKI-215, subcluster-Ib comprised JP-92 and VP-1. Genotypes within cluster II further grouped into three subcluster (II_a, II_b and II_c). Subcluster-II_a comprised 48-1 and GCH-4 with 0.88 similarity coefficient, SubclusterII_b comprised GCH-6, GCH-7, JP-91 and PCS-124. Genotype JP-91 inbreeds showed higher similarity coefficient (0.88) with PCS-124 staminate genotype. Subcluster-II_c comprised JI-344, GC-3, JI-96, JI-385, DCS-9 and JP-95. Genotypes JI-220,JI-353,JP-102 and JI-377 showed diverse relationship as compared to other genotypes.

Similarity coefficient revealed that the similarity with parents and their hybrids namely GCH-4 hybrid showed 88 per cent similarity with 48-1 staminate and 63 per cent with VP-1 pistillate genotypes. Similarly, hybrid GCH-7 showed 72 per cent similarity with SKP-84 pistillate and 61 per cent with SKI-215 inbreed genotype.

Wide ranges of genetic discrimination among all genotypes were observed showing that it is possible to both classify the elite genotypes and select genotypes or cultivars for the highest genetic diversity using SSRs, as indicated by cluster analysis. Due to co-dominant nature of SSR marker, it has great potential to discriminate the hybrid and their parent genotypes. In the present investigation, ten SSR markers produced 309 polymorphic that unambiguously discriminate twenty two castor genotypes into two major cluster and separate out those which have diverse genetic background. The polymorphism detected in present investigation showed 90.90 per cent (Table 1) and provides typically large number of polymorphic loci with respect to genetic diversity reported in plant measurements by Allan *et al.* (2008); Nybom (2004) and Melaku *et al.* (2009). Highest number of observed heterozygosity (Hop) was found with SSRY-61 (0.9091) marker followed by SSRY-203 (0.8000) whereas lowest was found with SSRY-83 (0.2500) marker followed by SSRY-SSRY-21 (0.1053). The reason for higher value heterozygosity might be due to most significant factors is mobility, as greater mobility of an individual tends to give it greater migratory potential. Castor is highly cross-pollinated which maintained gene flow between two populations can also lead to a combination of the two gene pools, increasing the genetic variation between

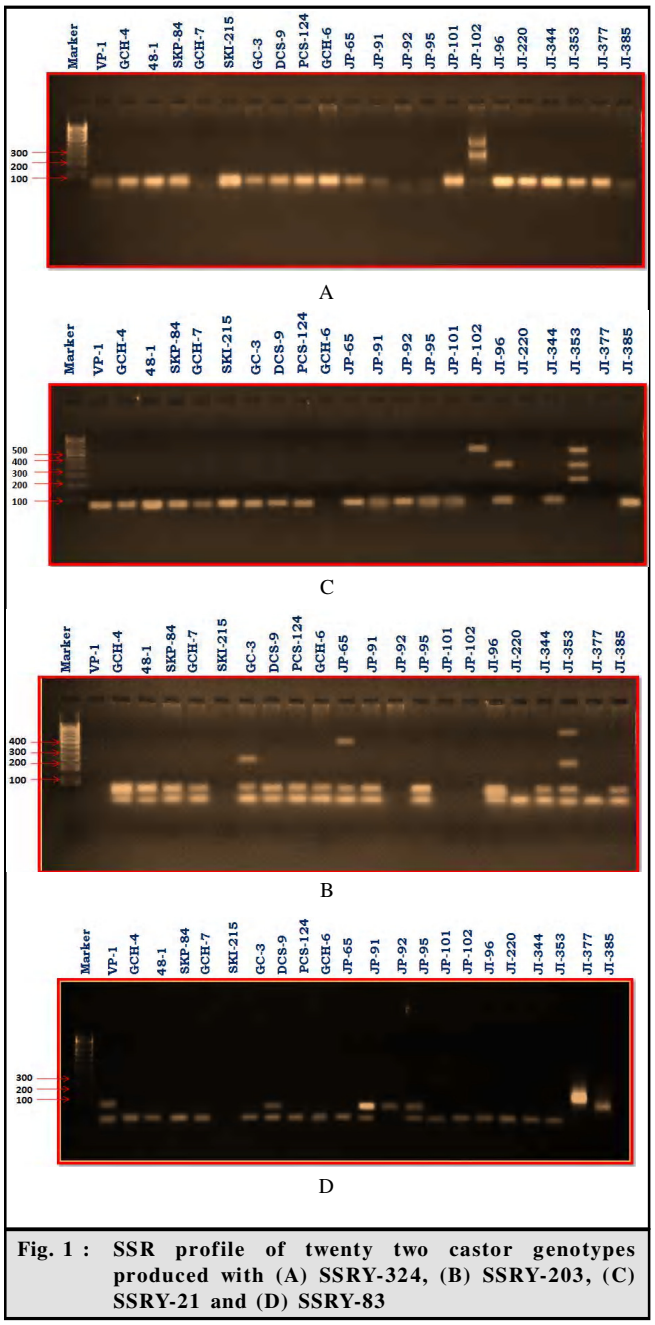

Fig. 1 : SSR profile of twenty two castor genotypes produced with (A) SSRY-324, (B) SSRY-203, (C) SSRY-21 and (D) SSRY-83

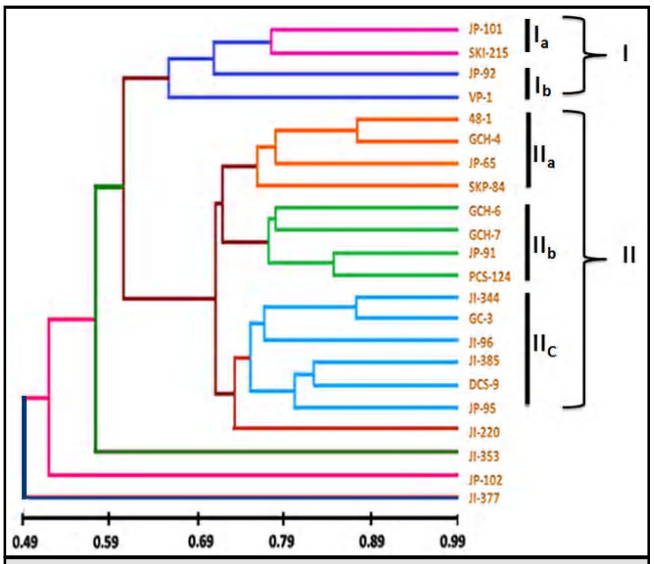

Fig. 2 : Dendrogram based on genetic similarities discriminated all the castor genotypes used in this stud

the two groups. Using SSR marker higher PIC value (SSRY-203 = 0.84), per cent polymorphism (90.90%) and similarity matrix, were found to be more informative in assessment of genetic diversity in castor. Similar results were reported in cassava germplasm (Raghu *et al*., 2007).

It has been concluded that genotypes JI-220, JI-353, JP-102 and JI-377 showed high genetic dissimilarity so, it is very important to improve the castor crop with the help of hybridization and crop improvement program. 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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