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Assessment of Genetic Diversity in Castor (*Ricinuscommunis* L.) using Microsatellite Markers

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Due to expanded usage and a primary raw material for various industrial applications and biofuel production, variability is necessity to develop high yielding castor (*Ricinuscommunis* L.) genotypes. Therefore, it is essential to document the germplasm of this important crop to increase its production. With this view, a study was conducted with 15 diverse castor genotypes to evaluate diversity using 14 microsatellite/simple sequence repeats (SSRs) markers. In SSR analysis, 14 primers generated a total of 31 amplicons with mean number of 2.21 polymorphic amplicons per primer. The range of molecular weight of these amplicond/bands was from 120 to 300 bp.The polymorphic information content (PIC) ranged from 0.231 (SSR-8) to 0.684 (SSR-10) with an average of 0.413. The primers SSR-7 and SSR-10 possess higher PIC value above 0.6. The highest genetic similarity was noticed between the genotypes, GEETA and 48-1 whereas; least genetic similarity was reported between the genotypes namely, JI-96 and SH-72. Clustering pattern of dendrogram generated by pooled SSR data showed two main clusters. The cluster A was the largest with eight genotypes and cluster B contained seven genotypes. Accessions, JI-96 and SH-72 were identified as highly diverse based on molecular studies and can be exploited to develop biparental mapping populations as well as in castor improvement program to wider the genetic background of various castor genotypes.

Keywords: *Ricinuscommunis* L., microsatellite markers, SSR, genetic diversity**.**

Castor (*Ricinuscommunis* L.) with chromosome number 2n = 2X = 20, resides one of the most important non-edible oilseed crop which is found across all the tropical and semi-tropical areas of the world. It belongs to mono-specific genus *Ricinus* of *Euphorbiaceae* family. It has cross pollination up to the extent of 50 per cent due to its monoecious nature[1]. It is believed that castor's center of origin of should be Ethiopian-East African region as Ethiopia has maximum diversity of castor[2]. It is a major source of ricinoleic acid which is an unusual hydroxyl fatty acid found in castor seeds[3]. Castor oil is mainly used as a laxative, but it is also used for the production of several industrial products like plasticizers, grease, paints, lubricants, drying oil, cosmetics, lipsticks, plastics, surfactants, insulators *etc*.[4]. Due to its high demand, castor is a commercial crop in various countries

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like India, China, Brazil, Russia, Philippines and Thailand and it has around 19% of the total area in the global oilseed scenarios and 9% of the total phyto-oil production[5]. Globally, India is leading producer and exporter of castor seed oil with export of 80% of its total castor oil. Even though, the castor is important export product of agriculture sector, it is grown merely on 1.5 million ha to a limited level. By considering the stable demand for castor oil in numerous industries, there is crucial need to prominently improve the area, production and yield of castor crop[6].

Knowledge of the genetic diversity and relationship among the varieties are very useful in recognition of gene pools, identification of germplasm and its management strategies. Identification of genetic diversity using DNA markers can provide insights into the genetic structure and diversity among varieties from different geographical origins. When the magnitude and nature of genetic diversity is estimated in advance, a suitable selection strategy is planned to accord with heritability of genetic traits. A combination of passport data and genetic diversity information from molecular markers would therefore enhance the formation of germplasm stocks[7]. Among the various DNA-based markers, SSR (microsatellite) is a class of genetic marker that has proven to be abundant and well distributed throughout the genome of plants[8]. The simple sequence repeats markers require only small amounts of DNA sample without involving radioactive labels and are simpler as well as faster. Moreover, SSR markers have ability to discriminate genotypes into homozygotes and heterozygotes due to co-dominant nature, high level of polymorphism as compared to most of the molecular markers[9], robustness, multi-allelic in nature, highly reproducible, hyper-variability, high information containing and amenability to automation[10]. Microsatellite/SSR markers can be used as a powerful tool for genotyping and diversity study. Hence, the present research was carried out to assess the genetic diversity among diverse genotypes of castor using SSR markers.

MATERIALS AND METHODS

Genomic DNA extraction

A set of 15 genotypes of castor having diverse morphological qualitative characters (Table 1) was procured from Main Castor-Mustard Research Station, S. D. Agricultural University, Sardarkrushinagar, Gujarat, India was used for the assessment of genetic diversity using SSR markers in the present study. Genomic DNA of 15 castor genotypes was isolated from bulked fresh leaf tissues with modified CTAB method[11]. The integrity and quality of extracted DNA was confirmed on 0.8% agarose gel followed by quantification of DNA using spectrophotometer (BioSpectrometer, Eppendorf, Germany). Finally, each DNA sample was diluted to a working concentration of 20 ng/µl and kept at 4°C.

PCR Amplification and electrophoresis

Total 14 SSR primers, developed at SDAU[12]were used for PCR amplification in the present study (Table 2). The PCR was carried out in 15 µl reaction mixture which contained 1.0 µl template DNA, 1.5 µl 10X PCR buffer, 2.0 µl primer (reverse and forward), 1.5 µl Taq polymerase, 1.2 µl dNTPs (2.5 mM each), 1.2 µl 25 mM $MgCl_2$ and 6.6 µl sterile nuclease free water. The PCR reaction was carried out by following cycling conditions: an initial denaturation at 94°C for 5 min., followed by 35 cycles of denaturation at 94°C for 1 min., primer annealing at Tm " 2°C (primer specific) for 30 sec., amplification at 72°C for 1:30 min., and a final extension at 72°C for 7 min.in (Eppendorf, Germany).For the separation of amplified SSR products, electrophoresis was performed in 3.0% agarose gel[13]. After electrophoresis, the gel was carefully taken out from unit and photographed using FluroChem FC_2 gel documentation system (Alpha Innotech Corporation, USA).

Scoring and analysis of data

Data were 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 genetic association between genotypes evaluated was made by calculating the Jaccard's similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers[14]. The similarity coefficients were used for cluster analysis using the Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) and Principal Component Analysis (PCA) were performed with a bootstrap frequency of n=500 in software PAST version 3.18[15]. The

Table 1. Castor genotypes used in present study

Sr. No.	Genotypes	Qualitative characters								
		Stem color	Bloom	Capsule type	Types of internodes	Leaf shape	Spike type	Inflorescence spike types	Plant type	Seed shape
1	VP-1	Green	Triple	Spiny	Condensed	Deep cup	Compact	Pistillate	Dwarf	Oval
2	GEETA	Red	Double	Non-spiny	Elongated	Flat	Loose	Pistillate	Tall	Round
3	JP-65	Red	Zero	Spiny	Elongated	Flat	Compact	Pistillate	Medium	Oval
4	SKP-84	Mahogany	Triple	Spiny	Condensed	Deep cup	Semi-compact	Pistillate	Dwarf	Oval
5	VI-9	Green	Double	Spiny	Elongated	Flat	Compact	Monoecious	Medium	Oval
6	JI-35	Green	Double	Spiny	Elongated	Flat	Compact	Interspersed	Tall	Round
7	48-1	Red	Double	Non-spiny	Elongated	Flat	Loose	Interspersed	Tall	Oval
8	SH-72	Green	Double	Spiny	Elongated	Flat	Compact	Interspersed	Tall	Oval
9	JI-96	Mahogany	Triple	Spiny	Elongated	Flat	Loose	Monoecious	Tall	Oval
10	SKI-215	Mahogany	Double	Non-spiny	Elongated	Flat	Semi-compact	Interspersed	Tall	Oval
11	SKI-352	Mahogany	Triple	Spiny	Elongated	Flat	Compact	Interspersed	Medium	Oval
12	SKI-370	Mahogany	Triple	Spiny	Elongated	Flat	Compact	Interspersed	Medium	Oval
13	SKI-372	Mahogany	Triple	Spiny	Elongated	Flat	Semi-loose	Interspersed	Medium	Oval
14	SKI-373	Mahogany	Double	Spiny	Elongated	Flat	Semi-loose	Interspersed	Medium	Oval
15	DCS-94	Green	Triple	Spiny	Elongated	Flat	Semi-loose	Interspersed	Medium	Oval

Table 2. Particulars of polymorphic SSR primer pairs used in the study

Sr. No.	Primer Name		Sequence	Tm (°C)	TA	PA	% P	PIC	Range of MW (bp)
1	Castor_SSR_1	FP	CGTAATGGGTCCGAGTCATC	58	02	02	100	0.408	185 to 191
		RP	AGAAACTCGGTGGTCTTCCA	58					
2	Castor_SSR_2	FP	TTTTGTTGCATTTCGTTTGC	52	02	02	100	0.391	254 to 278
		RP	TGATGGGCTTCTCAATGATG	54					
3	Castor_SSR_3	FP	TACCTCCAGGTCCTCCTCCT	62	02	02	100	0.337	127 to 151
		RP	ATTGTGCCACATGGTTGAGA	56					
4	Castor_SSR_4	FP	AAGTAAAATTTGCAAACACATGC	52	02	02	100	0.320	251 to 266
		RP	GCACGTGAAACATATGGCTG	57					
5	Castor_SSR_5	FP	TCGCAAATGGAAATTACGTG	53	02	02	100	0.459	262 to 274
		RP	CAGAACGGCTAGCAATGAAA	55					
6	Castor_SSR_6	FP	CCGAGACTAAGAATGGACCAA	56	02	02	100	0.495	221 to 259
		RP	TCAAACTCAGCGAGTAGGCA	58					
7	Castor_SSR_7	FP	GGTTGCAGAAGCTCCAAAAA	56	03	03	100	0.665	217 to 249
		RP	TAATACGCGGATGCAGACAG	57					
8	Castor_SSR_8	FP	GTGCCGTTGAAGGATCAGTT	58	02	02	100	0.231	165 to 193
		RP	GGCTTTGGTGAAGGTTTTGA	55					
9	Castor_SSR_9	FP	TGCAACAGGCAACCATCTAC	58	02	02	100	0.320	120 to 136
		RP	TCCATCTTTGTCTCTTCCTTCA	55					
10	Castor_SSR_10	FP	GAATTTGCAACGATGGCTTT	54	04	04	100	0.684	136 to 158
		RP	TGGGACCTTGGTCAAGTGAT	58					
11	Castor_SSR_11	FP	TCCTTATGAACAAAGTGGAGAATG	54	02	02	100	0.391	261 to 297
		RP	CAGCTTGAGGGGGAGTGTTA	59					
12	Castor_SSR_12	FP	CACATGCTTGGCTTCTTTCA	55	02	02	100	0.492	167 to 184
		RP	CGAGCATCACCAGAGTGTTT	57					
13	Castor_SSR_13	FP	CAGAGCCCATGGTGATTTCT	57	02	02	100	0.337	234 to 288
		RP	CCACAAAAAGCAGCCAAATC	55					
14	Castor_SSR_14	FP	GGACACGTGTGTTGCTGTAAG	59	02	02	100	0.245	271 to 300
			Total		31	31	-	-	-
			Mean		02.21	02.21	100	0.413	-
			Range		02-04	02-04	-	0.231 to 0.684	120 to 300

FP: Forward Primer; RP: Reverse Primer;Tm: Melting Temperature; TA: Number of Total Alleles; PA: Number of Polymorphic Alleles; %P: Per cent Polymorphism; PIC: Polymorphism Information Content;MW: Molecular Weight; bp: Base Pairs

Table 3. Jaccard's similarity coefficientsfor castor genotypes based on SSR markers

Genotype	VP-1	GEETA	JP-65	SKP-84	VI-9	JI-35	48-1	SH-72	JI-96	SKI-215	SKI-352	SKI-370	SKI-372	SKI-373	DCS-94
VP-1	1.000														
GEETA	0.476	1.000													
JP-65	0.579	0.476	1.000												
SKP-84	0.632	0.455	0.476	1.000											
VI-9	0.450	0.429	0.318	0.364	1.000										
JI-35	0.318	0.304	0.450	0.250	0.474	1.000									
48-1	0.550	0.882	0.550	0.391	0.500	0.364	1.000								
SH-72	0.304	0.476	0.304	0.292	0.611	0.706	0.550	1.000							
JI-96	0.600	0.333	0.455	0.455	0.231	0.231	0.333	0.143	1.000						
SKI-215	0.364	0.632	0.500	0.240	0.450	0.526	0.722	0.579	0.231	1.000					
SKI-352	0.563	0.471	0.563	0.389	0.333	0.600	0.563	0.600	0.556	0.412	1.000				
SKI-370	0.364	0.476	0.500	0.292	0.381	0.261	0.550	0.364	0.231	0.364	0.389	1.000			
SKI-372	0.429	0.292	0.429	0.550	0.261	0.208	0.348	0.250	0.455	0.200	0.412	0.500	1.000		
SKI-373	0.550	0.524	0.409	0.391	0.364	0.250	0.600	0.348	0.545	0.409	0.471	0.476	0.550	1.000	
DCS-94	0.450	0.304	0.318	0.364	0.333	0.400	0.364	0.381	0.455	0.261	0.412	0.381	0.450	0.364	1.000

software program AlphaEase FC version 6.0.0 was used to determine the molecular weight of bands separated on the gel. The Polymorphism Information Content (PIC) value was calculated on the basis of allele frequency by the given formula[16]. The polymorphism percentage was calculated by the following formula. Polymorphism (%) = (Number of polymorphic bands/Total number of bands) × 100.

RESULT AND DISCUSSION

The inherent problem of genotype × environment interaction associated phenotype based variability can be easily conquered through DNA markers[17]. In the present study, out of 14 SSR primers, all the fourteen primers were polymorphic. The molecular weight of the amplicons produced by SSR primers ranged from 120 bp (SSR-9) to 300 bp (SSR-14). A total of 31 alleles were amplified and average number of allele was 2.21 with a range of 2 to 4 alleles/amplicons per primer (Table 2). The past reports showed 1.8[18], 2.30[19], 3.0[20], 3.14[6], 3.18[21], 4.9[22], 5.5[23] and 6.7[24] mean numbers of alleles per primer.

The PIC for SSRs used in the present study ranged from 0.231 (SSR-8) to 0.684 (SSR-10) with an average of 0.413 (Table 2, Fig. 1). Some of the past reports on the utilization of SSR markers for the genetic diversity analysis in castor contained lower PIC values *viz*., 0.26[21] and 0.37[25]. However, some reports have higher PIC values as compared to present study like, 0.43[6], 0.43[20], 0.51[19], 0.70[23], 0.76[22], 0.81[24] and 0.87[18]. It is important to be mentioned that most of the past studies having higher PIC values were carried out using less number of primers as compared to the present study. Overall, the PIC values found in the present study are appropriate to assess the genetic diversity among castor genotypes.

The genetic similarity coefficient ranged from 0.143 to 0.882. The highest similarity coefficient (0.882) was noticed between the genotypes GEETA and 48-1 indicating that they are genetically more similar as compared to other genotypes, whereas the lowest similarity (0.143) was noticed between the genotypes namely, JI-96 and SH-72 showing that these two genotypes had least genetic similarity (Table 3). According to the similarity coefficients, genotypes used in the

present study possessed higher variation among the set of genotypes as compared to few previous studies which had range of similarity coefficients from 0.57 to 1.00[18] and 0.44 to 0.88[26] and less variation as compared to the results of some other reports *viz*., 0.07 to 1.0[23], 0.26 to 1.0[19] and 0.05 to 0.96[21]. According to the SSR analysis, GEETA and 48-1 were most similar genotypes which is also supported by the qualitative morphological characters which are similar in both of these genotypes *viz*., red stem, double bloom, non-spiny spike, elongated internodes, flat leaf shape, loose spike, tall plant type and oval seed shape. The little variation present between GEETA and 48-1 might be due to few different characters like GEETA contains pistillate inflorescence whereas 48-1 possesses interspersed type of inflorescence. JI-96 and SH-72 genotypes were most diverse genotypes revealed by SSR amplification which might be due to presence of variations in morphological characters *viz*., JI-96 has green stem whereas SH-72 has green stem color, JI-96 has triple bloom whereas SH-72 has double bloom, JI-96 has loose spike whereas SH-72 has compact spike and JI-96 has monocious inflorescence spike whereas SH-72 has interspersed inflorescence spike.

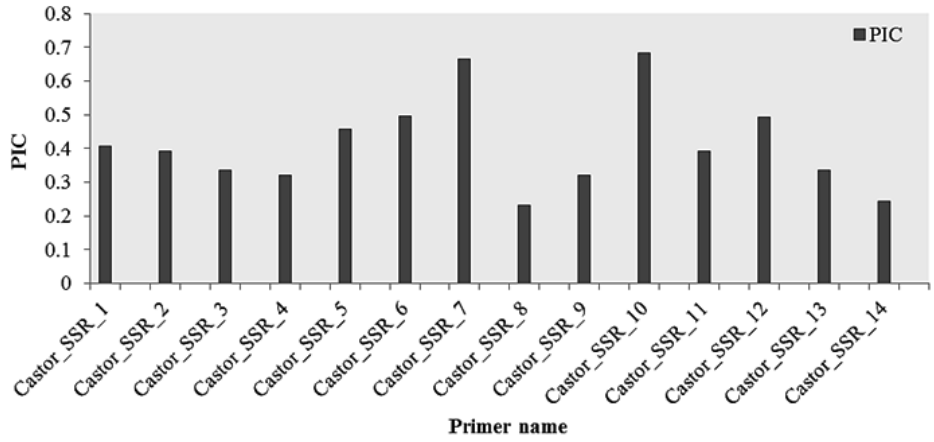

Fig. 1. PIC values of SSR primers

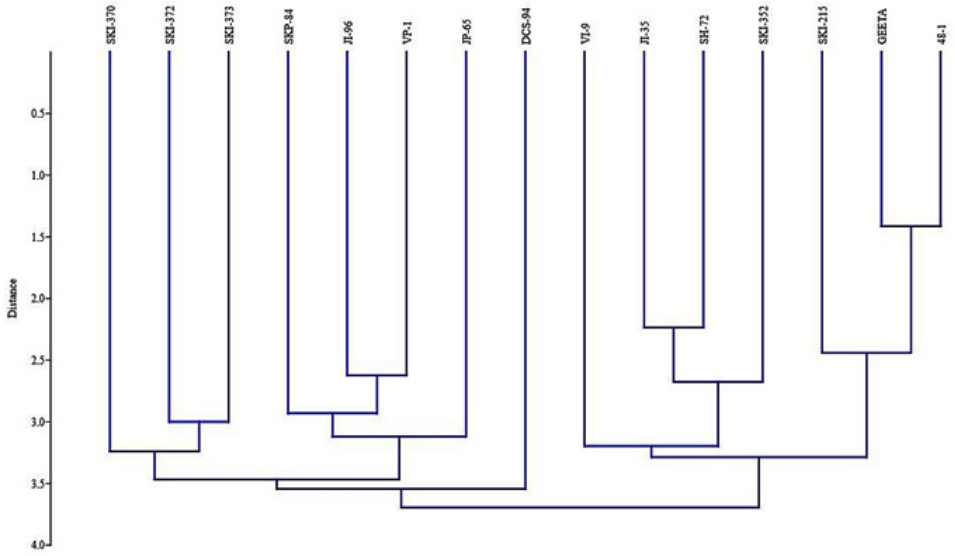

Fig. 2. UPGMA based dendrogramof castor genotypes based on SSR markers

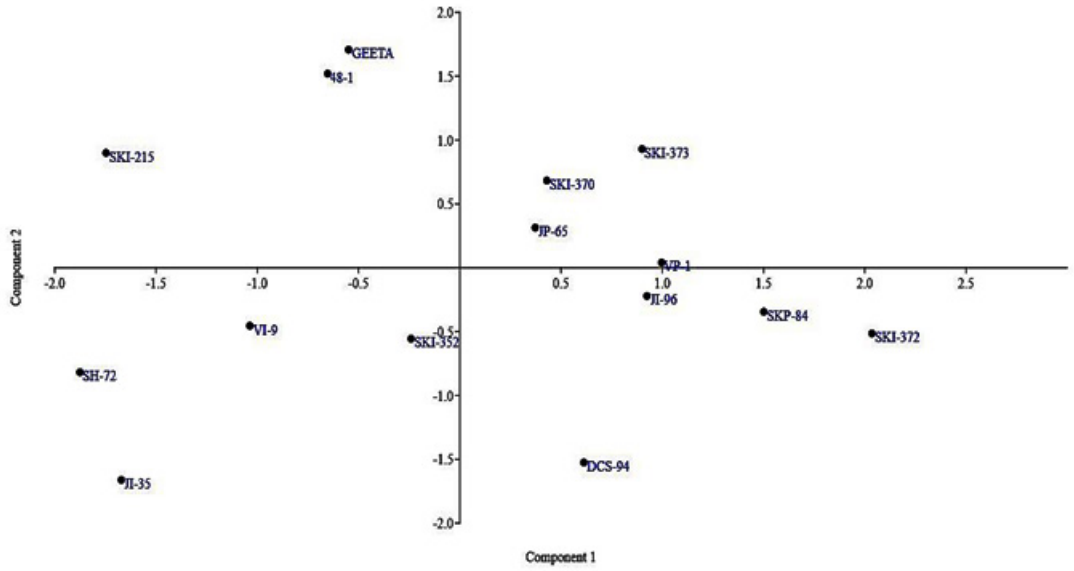

Fig. 3. 2-D plot of Principle Component Analysis (PCA) of castor genotypes based on SSR markers

The dendrogram was prepared based on UPGMA by using Jaccard's similarity co-efficients through PAST (version 3.18) (Fig. 2). The dendrogram (Jaccard's distance, paired-group) clustered with the data generated by all primers and their amplicons grouped the 15 castor genotypes into two major clusters *i.e.,* cluster A and cluster B as shown in fig. 2. Among the two major clusters, the cluster A contained eight genotype and the cluster B contained seven genotypes. The cluster A was further divided into two sub-clusters *viz*., A1 and A2. The sub-cluster A1 contained seven genotypes *viz.,* SKI-370, SKI-372, SKI-373, SKP-84, JI-96, VP-1 and JP-65, while sub-cluster A2 contained only one genotype namely, DCS-94. The cluster B also further divided into two sub-clusters B1 and B2. The sub-cluster B1 contained four genotypes *viz.,* VI-9, JI-35, SH-72 and SKI-352, whereas sub-cluster B2 containing three genotypes namely, SKI-215, GEETA and 48-1. Similarly, grouping of the genotypes in two major clusters were observed in the past studies[21-26]. Further, more number of genotypes can be used for the diversity assessment in castor using more number of polymorphic primers for more accurate estimation of diversity and population structure analysis. Recently, this kind of works have been published[27,28.]

The 2-D PCA plot was derived from the two major components after analyzing SSR genotyping data. In the PCA plot, it can be observed that GEETA followed by JI-35 were placed farthest in the 2nd component (Y- axis), while SKI-372 and SH-72 were placed farthest in the 1st component (X-axis)(Fig. 3).This grouping pattern of 2-D PCA plot matched with the dendrogram obtained through UPGMA based cluster analysis. The genotypes scattered throughout the 2-D plot. The genotypes namely, GEETA and JI-35 contained round seed shape hence, they might be placed far from other genotypes on 2-D PCA plot. This grouping pattern of PCA matched with the dendrogram obtained through UPGMA based cluster analysis.

CONCLUSION

Castor is important industrial crop which exhibits outstanding diversity in the India. Our study shows that there is amazing genetic variation existing among castor genotypes, however this is primitive effort, the study exhibited potential of SSR marker for elucidation of genetic diversity. Hence, result from the present study will help breeders for the assessment the diversity at molecular and better utilization of germplasm for the variety development. Among all the genotypes

used in the study, JI-96 and SH-72 were most diverse genotypes which can be utilized for further breeding experiments.

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