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Molecular marker-based genetic diversity analysis in castor genotypes (*Ricinus communis* L.)

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ABSTRACT

Castor is an important oilseed in the industrial sector for its application in various fields. The study was conducted with the twenty selected genotypes. 15 RAPD primers were amplified a total of 112 bands. Among these 106 bands were polymorphic, and 6 were monomorphic. The average per cent polymorphism obtained for RAPD primers was 96.67 %. Average PIC values for RAPD markers were 0.81 per primer and RPI with an average value of 6.16. 12 ISSR primers amplified a total of 54 bands out of which 49 were polymorphic and 5 bands were monomorphic. The average PIC value for ISSR was 0.67. The average IPI was 3.14. The similarity coefficient of cluster analysis was in the range of 15.0 % - 76.31 % for RAPD, 40.74 % - 92.59 % for ISSR and 28.8 % - 80.7 % for the pooled study. Dendrogram construction with UPGMA analysis shows that JI-456 for RAPD, SKP-84 for ISSR and JI-456 for pooled analysis as the most diverse genotypes among the twenty genotypes. These results will be helpful in the selection of the genotypes for future breeding programs to increase the specific traits. The important challenge faced during the research work were the isolation of genomic DNA from the leaf sample, as it was highly prone to pigmentation issue and this was overcome by slightly changing the composition of extraction buffer. The another difficulty faced during the work were setting up of the PCR protocol for marker amplification the minimum reaction volume for PCR leads to amplification issues and this overcome by increasing reaction volume.

Keywords: *Castor, RAPD, ISSR, Polymorphism, PIC, RPI, IPI, Genetic diversity, UPGMA.*

Introduction

The castor bean (also known as castor, castor-oil plant), scientifically named *Ricinus communis* L. (2n = 20, X = 10), belongs to the Euphorbiaceae family of flowering plants. This plant belongs to the monotypic genus *Ricinus* of the *Euphorbiaceae*. It is primarily cultivated for its oil in countries like India, Mozambique, Brazil, and China [6]. Castor is one of the oldest cultivated plants. Castor oil was extensively used in medicine in ancient Egypt. The castor seed contains more than 45% oil and this oil is rich (80-90%) in an unusual hydroxyl fatty acid called ricinoleic acid [7]. India stands out as the largest producer, accounting for 1.842 million tons from 1.046 million hectares, which represents over 95% of global output. Mozambique, China, and Myanmar follow as significant contributors [14]. Within India, Gujarat is the top producer, with 0.737 million hectares yielding 1.432 million tons, followed by Rajasthan, Andhra Pradesh, and Telangana [16].Biodiesel from castor oil is highly valued for its excellent lubricating ability, high energy, and favorable fuel properties [15].

Genetic diversity in castor plants (*Ricinus communis*) is analyzed using various molecular markers to study genetic variation, population structure, and the relationships between different varieties or populations. This research employs PCR-based molecular markers, such as Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR), to genetically characterize castor genotypes.

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Molecular markers are highly effective tools for analyzing genetic variability [19]. This helps in identifying genetically diverse parents for breeding programs, expanding the gene pool, and developing hybrids with improved traits. Marker analysis also helps detect unique alleles or genetic variations linked to essential agronomic traits, providing valuable information for targeted breeding or genetic engineering efforts. These efforts can enhance traits such as drought tolerance, pest resistance, or oil content in castor plants. By identifying and conserving genetically distinct castor genotypes, researchers can preserve a diverse gene pool, which holds potential for future breeding programs and environmental adaptation. Marker-based genetic diversity analysis in castor provides critical insights for guiding breeding programs, improving crop management, conserving genetic resources, and boosting the resilience and productivity of castor cultivation.

Materials and Methods

Plant Material: The 20 genotypes of castor were collected from the Main Oilseed Research Station, Junagadh Agricultural University, Junagadh, Gujarat to study the molecular markers-based diversity analysis by using RAPD and ISSR markers. Seeds of each genotype were sown in separate pots and the DNA extraction was carried out from the sample collected 12-15 days after germination.

Extraction of DNA

The DNA of each sample was extracted using the modified CTAB method [12]. The presence of intense band was observed in Agarose gel electrophoresis and the concentration of each sample was measured by using Nanodrop (Qiagen) and the

A_{260} / A_{280} ratio were checked. The samples were stored at 4°C for further use.

RAPD analysis

The PCR process for RAPD was performed according to the method given by [21] with some modifications. The RAPD assays were performed using random 10-mer oligonucleotide primers. The PCR master mix (20 µl) contains PCR Buffer (10X) - 2 µl, Taq polymerase (3U/µl) - 0.2 µl, dNTPs Mix (2.5 mM each) - 0.1 µl, Primer (25 pM/µl) - 2.0 µl, Template DNA (50 ng/µl) - 1.0 µl, Millipore sterile D/W - 17.40 µl respectively. The samples were subjected to 40 repeated PCR cycles having 94 °C for 1 min, 37 °C for 1 min and 72 °C for 2 min with 4 min of initial denaturation and 5 min of final extension by using a thermal cycler. All of this was performed using 0.2 ml 96 welled PCR plates. After completion of the PCR the samples were loaded in 2.0 % of agarose gel electrophoresis for 1:30 hr. and the bands were visualized by using gel documentation system and images were captured.

ISSR analysis

The PCR process for ISSR was performed according to the method given by [4] with some modifications. The genomic DNA was amplified using UBC- ISSR primer series. The PCR master mix (10 µl) contains PCR Buffer (10X) - 2 µl, Taq polymerase (3U/µl) - 0.2 µl, dNTPs Mix (2.5 mM each) - 0.1 µl, Primer (25 pM/µl) - 1.2 µl, Template DNA (50 ng/µl) - 1.2 µl, Millipore sterile D/W - 5.3 µl respectively. The samples were subjected to 40 repeated PCR cycles having 94 °C for 1 min, 46-48 °C for 1 min based on the Tm of each primer and 72 °C for 2 min with 4 min of initial denaturation and 5 min of final extension by using thermal cycler. All of this was performed using 0.2 ml 96 welled PCR plates. After completion of the PCR the samples were loaded in 1.2 % of Agarose gel electrophoresis for 1:15 hr. and the bands were visualized by using gel documentation system and images were captured.

Scoring and data analysis

PIC for RAPD and ISSR was calculated on the basis of allele frequency [1]. PIC values were then used to calculate a RAPD Primer Index (RPI) and ISSR Primer Index (IPI) which were generated by multiplying the PIC values of all the markers amplified by the same primer. The molecular size of each fragment was estimated using AlphaEase FC software. Clear and distinct bands amplified by RAPD and ISSR primers were scored for the presence (1) and absence (0) of the corresponding band among the castor genotypes. The data was entered into MS-Excel data sheet and subsequently analyzed using NTSYS-pc version 2.02 [13]. The data matrix was read by NTSYS-pc version 2.02 (Numerical Taxonomy and Multivariate Analysis System for personal computers, Exeter software) and analyzed by the SIMQUAL (similarity for qualitative data) program with jaccard's similarity coefficient. SIMQUAL is a program for computing a variety of similarity and dissimilarity coefficients for qualitative data. The qualitative nature of the absence (0) or presence (1) state of a marker was used as the basis for similarity analysis among various castor genotypes. A matrix of 0 and 1 act as the input, and the output is a matrix of similarity or dissimilarity coefficients. The resultant similarity matrix was entered into SAHN (sequential, agglomerative, hierarchical and nested clustering method) clustering program, a tree matrix was produced and a dendrogram constructed using UPGMA (unweighted pair-group method with arithmetic averages).

Results and Discussion

RAPD analysis

Total 35 RAPD primers were screened, out of which 15 primers amplified a total of 112 bands. Out of 112 bands, 106 bands were polymorphic with an average of 7 bands per primer while 6 bands were monomorphic (Table 1.1; Image 1&2). The per cent polymorphism obtained for RAPD primers ranged from 50 % to 100 % with an average value of 96.67 % per primer. The unique bands were produced by the primers OPA-03 (243 bp, 621 bp), OPA-04 (601 bp 2918 bp), OPA-05 (209bp, 388 bp, 741 bp, 2023 bp), OPB-01 (238bp, 2460 bp), OPB-04 (374 bp), OPC-01 (183 bp) and OPE-02 (210 bp, 4025 bp) respectively. Jaccard's similarity coefficient which revealed that the lowest similarity of 15.0 % was noticed between JI-456 and JI-473, while the highest similarity of 76.31 % was noticed between the genotypes ANDCP 16-1 and JI-456 (Table 1.2). The dendrogram was constructed using UPGMA based on Jaccard's similarity coefficient through NTSYSpc-2.02i software for RAPD binomial data of twenty castor genotypes (Table 1.2 and Fig. 1.1). The 20 castor genotypes were grouped into two main clusters: cluster-I and cluster-II, which shared 30 % similarity. The cluster-I was divided into two subclusters-A and B both contained a total of 19 genotypes with 42 % similarity (Fig. 1.1). Subcluster-A was further subdivided into two groups A1 and A2 which had nearly 44 % likeness. Group A1 was further cleaved into subgroups A1 (a) and A1 (b) having nearly 45 % relatedness. Subgroup A1 (a) consisted of 11 genotypes such as SKP-84, JP-96, JI-509, JI-471, ANDCP 16-1, SKP-126, JI-531, JI-528, JP-105, JI-476 and JI-522. Subgroup A1 (b) consisted of three genotypes such as JI-449, JI-491 and JI-527 and had nearly 50% similarity. While group A2 were further divided into A2 (a) and A2 (b) which is having 55 % likeliness. SubgroupA2 (a) consisted of two genotypes such as JP-108 and JI-473, which have 69 % similarity. The subgroup A2 (b) consisted of only one genotype such asJI-523having nearly 54 % similarity with subgroup A2 (a). The subcluster-B consisted of 2 genotypes such as JI-454 and JI-516 with nearly 42 % similarity. The cluster-II consisted of only one genotype which named JI-456, which was the most diverse genotype among all twenty genotypes of castor.

RAPD amplification yields a total of 6,011 amplification products, from which only 1,859 bands (30.92 %) were found to be polymorphic and the size of bands ranged from 300 to 2,500 bp [10]. Similarly the RAPD showed highest genetic similarity (92%) was between SKI 336 and SKI 343. However, minimum similarity was found between JI 362 and SKI 336 (41%). The dendrogram was divided into three main clusters; cluster one included 19 genotypes while cluster II and III included 5 and 1 genotype, respectively [18]. Likewise, genetic diversity among wilt-resistant lines through RAPD shows the similarity coefficient in the range of 0.61 to 0.98 [3]. Likewise the RAPD analysis showed that UPGMA analysis allowed for two main clusters to be distinguished. Cluster I was having ten genotypes viz. VP-1, GAUCH-1, VI-9, GEETA, JI-35, GCH-2, GCH-4, SH-72, GCH-5, SKP-84 and GCH-7. Major cluster II comprised of SKI-215 and 48-1. The Jaccard's similarity coefficient was observed from 0.454 to 0.969 [11]. Similarly PCR amplification of RAPD primers yielded 145 DNA fragments. The number and size of amplified fragments ranged from 3 to 13 and 100 to 1500 bp. All the amplified bands were found to be polymorphic. The PIC value varied, with the lowest values observed for RLZ 9 (0.618) and the highest for OPD-08 (0.846) [20]. The RAPD analysis of castor genotypes gave genetic similarity range from 0.75 to 0.99 with an average of 0.85 [9].

ISSR analysis

Total 12 ISSR primers were used which generated 54 fragments from which 49 bands were polymorphic, having 45 shared and 4 unique bands with an average of 4.08 bands per primer. The per cent polymorphism obtained for ISSR primers ranged between 50-100 % with an average of 90.59 % per primer (Table 1.3; Image 3&4). Out of 12 ISSR primers, 4 primers were able to produce unique bands *viz*. UBC-825 (570 bp), UBC-826 (386 bp), UBC-840 (262 bp) and UBC-851 (407 bp) respectively. Jaccard's similarity coefficient reveals that the lowest similarity of 40.74 % was noticed between SKP-84 and ANDCP 16-1, while the highest similarity of 92.59 % was noticed between the genotypes JI-509 and JI-527 and also between JI-531 and JI-527 (Table 1.4).

The dendrogram constructed using UPGMA reveals that the 20 genotypes were divided into two clusters. Cluster I was having only one genotype SKP-84, which was the most diverse among other castor genotypes studied. The cluster-II comprised two subclusters A and B with 64 % similarity. Subcluster A was further divided into group A1 and A2 having 72 % likeness. Group A1 was further divided into subgroups A1 (a) and A1 (b) having nearly 74 % relatedness. Subgroup A1 (a) consisted of 15genotypes *viz.,* JP-96, JI-476, JI-523, JI-471, JP-105, JP-108, JI-527, JI-509, JI-531, JI-528, ANDCP 16-1, SKP-126, JI-456, JI-449 and JI-522. Subgroup A1 (b) consisted of two genotypes which were JI-491 and JI-454. Likewise, group A2 was having only one genotype JI-516. The subcluster B was having only one genotype which was JI-473 (Fig. 1.2).

Studies showed among the 169 ISSR amplified bands, 127 (75.15 %) were polymorphic for Chinese *Jatropha* which had high genetic diversity [2]. Inter Simple Sequence Repeat (ISSR) marker analysis to assess the genetic diversity among 22 castor genotypes. The number of bands produced by the different markers ranged from 8 (UBC – 841 and UBC - 890) to 13 (UBC - 840). PIC values ranged from 0.87 (UBC – 841 and UBC - 890) to 0.92 (UBC - 840) and polymorphism percentage ranged from33.3 (UBC - 888) to 100 (UBC – UBC – 841) [4]. Similarly, genetic relationships were studied using 27 ISSR markers, yielding 307 polymorphic bands with polymorphism contents ranging from 0.76 to 0.95 for IMPN 1 and UBC 807 markers, respectively [17]. ISSR analysis showed that the genetic distance among accessions ranged from 0.2 to 0.056. A model-based Bayesian approach subdivided 60 genotypes from 12 accessions into 6 subgroups. UPGMA dendrogram based on Nei's genetic distance classified 12 accessions into 4 groups [5]. Similarly, the genetic diversity analysis of 20 castor genotypes was carried out by using the Inter Simple Sequence Repeat molecular marker technique. The number of bands produced by different markers ranged from 2 to 11 and the polymorphism percentage ranged from 0 to 100. The overall size of amplified PCR products ranged from 100 bp to 2342 bp [8].

Pooled study of RAPD and ISSR

Genetic similarity of both molecular markers was determined for each pair of twenty genotypes of castor which revealed that the lowest similarity of 28.8 % was noticed between JI-456 and JI-473, while highest similarity of 80.7 % was noticed between ANDCP 16-1 and SKP-126 (Table 1.5). The dendrogram (Fig. 1.3) was divided the genotypes into two main clusters I and II with an average resemblance of 44 %. Cluster I divided into two subclusters A and B both contained 19 genotypes which nearly 46 % likeness. Subcluster-A were having only one genotype SKP-84. Subcluster B was further divided into B1 and B2 which have nearly 50 % similarity. Group B1were further divided into B1 (a) and B1 (b) having nearly 53 % similarity. B1(a) consisted of 15 genotypes such as JP-96, JP-105, JI-522, JI-476, JI-523, JP-108, JI-473, JI-491, JI-471, ANDCP 16-1, SKP-126, JI-527, JI-509, JI-531 and JI-528 while group B1(b) consisted of only one genotype JI-449. Group B2 consisted of two genotypes such as JI-454 and JI-516.The cluster-II consisted of only one genotype JI-456 and was the most diverse among all twenty genotypes.

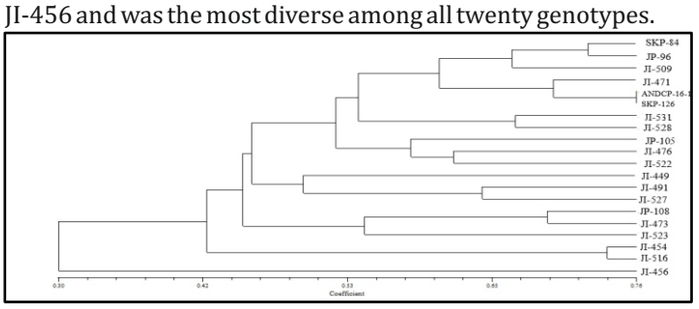

Figure 1.1 Dendrogram depicting the genetic relationship among 20 castor genotypes based on data of RAPD

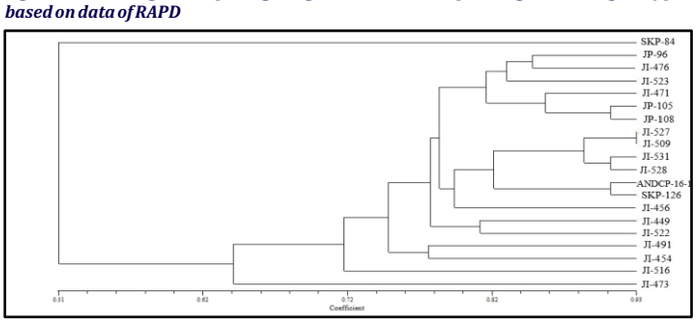

Figure 1.2 Dendrogram depicting the genetic relationship among 20 castor genotypes based on data of ISSR

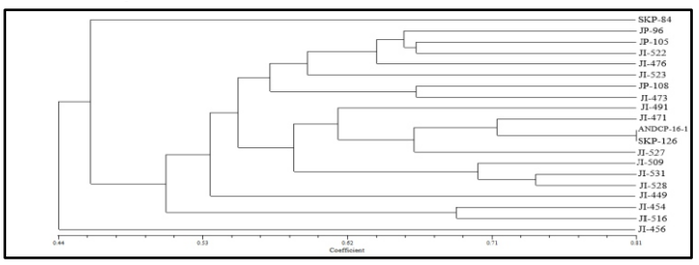

Figure 1.3 Dendrogram depicting the genetic relationship among 20 castor genotypes based on pooled data of RAPD and ISSR

Table 1.1 Size, number of amplified bands, per cent polymorphism and PIC obtained by RAPD primers in 20 Castor genotypes

Sr. No.	RAPD Primer	Size of amplified fragment	Total No. of Bands (A)	Polymorphic Bands (B)			% Polymorphism (B/A)	PIC	RPI
				S	U	T			
1	OPA 01	254-1843 bp	8	8	0	8	100	0.87	6.94
2	OPA 02	355-5865 bp	7	7	0	7	100	0.83	5.81
3	OPA 03	243-2514 bp	12	4	2	6	50	0.89	10.74
4	OPA 04	302-2918 bp	9	7	2	9	100	0.84	7.53
5	OPA 05	209-2023 bp	9	5	4	9	100	0.82	7.36
6	OPB 01	238-2461 bp	8	6	2	8	100	0.83	6.64
7	OPB 03	373-2650 bp	4	4	0	4	100	0.67	2.69
8	OPB 04	261-4072 bp	7	6	1	7	100	0.78	5.43
9	OPB 05	355-1723bp	5	5	0	5	100	0.76	3.82
10	OPC 01	183-3149 bp	9	8	1	9	100	0.85	7.65
11	OPC 05	389-1178 bp.	4	4	0	4	100	0.74	2.94
12	OPD 02	265-3024 bp	11	11	0	11	100	0.90	9.89
13	OPE 02	211-4025 bp	9	7	2	9	100	0.80	7.20
14	OPE 03	225-1485 bp	6	6	0	6	100	0.80	4.78
15	OPE 05	341- 1070 bp	4	4	0	4	100	0.74	2.98
TOTAL			112	92	14	106			
AVERAGE			-	-	-	7.07	96.67	0.81	6.16

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

Table 1.2 Jaccard's similarity coefficient of castor genotypes based on RAPD data analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.72	1.00																		
3	0.57	0.52	1.00																	
4	0.45	0.44	0.53	1.00																
5	0.56	0.55	0.49	0.61	1.00															
6	0.51	0.42	0.47	0.64	0.66	1.00														
7	0.71	0.60	0.46	0.53	0.70	0.58	1.00													
8	0.67	0.68	0.50	0.53	0.70	0.58	**0.76**	1.00												
9	0.38	0.35	0.29	0.15	0.23	0.18	0.32	0.29	1.00											
10	0.69	0.64	0.49	0.40	0.46	0.39	0.59	0.63	0.45	1.00										
11	0.57	0.44	0.42	0.44	0.48	0.48	0.60	0.56	0.36	0.64	1.00									
12	0.49	0.50	0.37	0.34	0.46	0.41	0.51	0.59	0.35	0.65	0.67	1.00								
13	0.57	0.61	0.38	0.31	0.48	0.37	0.51	0.58	0.37	0.58	0.47	0.53	1.00							
14	0.48	0.16	0.34	0.34	0.37	0.37	0.42	0.45	0.35	0.45	0.47	0.49	0.44	1.00						
15	0.47	0.51	0.40	0.48	0.51	0.58	0.43	0.52	**0.15**	0.35	0.33	0.34	0.44	0.69	1.00					
16	0.51	0.53	0.39	0.42	0.57	0.49	0.45	0.62	0.33	0.51	0.48	0.54	0.56	0.52	0.54	1.00				
17	0.54	0.67	0.44	0.35	0.53	0.36	0.46	0.53	0.33	0.51	0.38	0.43	0.61	0.48	0.56	0.62	1.00			
18	0.46	0.52	0.38	0.49	0.52	0.50	0.49	0.51	0.32	0.41	0.36	0.32	0.47	0.50	0.59	0.52	0.56	1.00		
19	0.47	0.54	0.36	0.33	0.45	0.34	0.46	0.46	0.37	0.43	0.38	0.36	0.44	0.35	0.36	0.47	0.48	0.44	1.00	
20	0.41	0.57	0.31	0.30	0.44	0.29	0.39	0.47	0.39	0.44	0.36	0.42	0.44	0.42	0.40	0.55	0.52	0.48	0.74	1.00

(1:SKP-84, 2:JP-96, 3:JI-449, 4: JI-491, 5: JI-471, 6:JI-527, 7: ANDCP-16-1, 8:SKP-126, 9:JI-456, 10:JI-509, 11: JI-531, 12:JI-528, 13:JP-105, 14:JP-108, 15:JI-473, 16:JI-476, 17:JI-522, 18:JI-523, 19:JI-454, 20:JI-516)

Table 1.3 Size, number of amplified bands, per cent polymorphism and PIC obtained by ISSR primers in 20 castor genotypes

Sr. No.	ISSR Primer	Size of amplified fragment	Total No. of Bands (A)	Polymorphic Bands (B)			% Polymorphism (B/A)	PIC	IPI
				S	U	T			
1	UBC 807	252-1341 bp	5	5	0	5	100	0.77	3.86
2	UBC 808	265-741 bp	4	4	0	4	100	0.74	2.97
3	UBC 809	235-1370 bp	7	6	0	6	85.71	0.85	5.94
4	UBC 813	349-1845 bp	3	3	0	3	100	0.50	1.50
5	UBC 818	278-2105 bp	5	4	0	4	80	0.76	3.78
6	UBC 825	411-1732 bp	4	3	1	4	100	0.67	2.00
7	UBC 826	386-861 bp	4	3	1	4	100	0.60	2.42
8	UBC 840	184-530 bp	4	3	1	4	100	0.55	2.19
9	UBC 841	246-1527 bp	2	1	0	1	50	0.47	0.94
10	UBC 842	153-853 bp	7	5	0	5	71.43	0.84	5.91
11	UBC 844	391-2945 bp	3	3	0	3	100	0.48	1.45
12	UBC 851	220-1528 bp	6	5	1	6	100	0.79	4.74
TOTAL			54	45	4	49	-	-	-
AVERAGE			-	-	-	4.08	90.59	0.67	3.14

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

Table 1.4 Jaccard's similarity coefficient of castor genotypes based on ISSR data analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.57	1.00																		
3	0.59	0.80	1.00																	
4	0.52	0.76	0.74	1.00																
5	0.44	0.76	0.74	0.78	1.00															
6	0.52	0.76	0.78	0.78	0.81	1.00														
7	**0.41**	0.76	0.74	0.74	0.78	0.81	1.00													
8	0.50	0.81	0.80	0.72	0.80	0.83	0.91	1.00												
9	0.52	0.80	0.78	0.70	0.74	0.81	0.78	0.80	1.00											
10	0.56	0.83	0.81	0.74	0.81	**0.93**	0.81	0.83	0.85	1.00										
11	0.52	0.69	0.74	0.78	0.78	**0.93**	0.85	0.80	0.78	0.85	1.00									
12	0.54	0.78	0.76	0.80	0.83	0.91	0.83	0.81	0.76	0.87	0.91	1.00								
13	0.48	0.80	0.81	0.81	0.85	0.81	0.81	0.83	0.81	0.78	0.78	0.83	1.00							
14	0.46	0.81	0.83	0.76	0.87	0.83	0.83	0.85	0.80	0.83	0.76	0.81	0.91	1.00						
15	0.50	0.74	0.65	0.57	0.65	0.61	0.61	0.70	0.61	0.65	0.54	0.59	0.65	0.70	1.00					
16	0.43	0.85	0.76	0.72	0.83	0.76	0.83	0.85	0.80	0.80	0.72	0.81	0.83	0.89	0.74	1.00				
17	0.52	0.72	0.81	0.74	0.78	0.85	0.74	0.76	0.78	0.81	0.81	0.80	0.81	0.80	0.61	0.72	1.00			
18	0.57	0.85	0.80	0.80	0.80	0.72	0.69	0.70	0.76	0.76	0.69	0.74	0.83	0.81	0.63	0.81	0.72	1.00		
19	0.59	0.76	0.70	0.78	0.78	0.70	0.70	0.76	0.70	0.70	0.70	0.76	0.78	0.76	0.57	0.76	0.70	0.83	1.00	
20	0.54	0.78	0.65	0.61	0.72	0.69	0.72	0.74	0.69	0.72	0.65	0.74	0.72	0.74	0.67	0.85	0.65	0.81	0.72	1.00

(1:SKP-84, 2:JP-96, 3:JI-449, 4: JI-491, 5: JI-471, 6:JI-527, 7: ANDCP-16-1, 8:SKP-126, 9:JI-456, 10:JI-509, 11: JI-531, 12:JI-528, 13:JP-105, 14:JP-108, 15:JI-473, 16:JI-476, 17:JI-522, 18:JI-523, 19:JI-454, 20:JI-516)

Table 1.5 Jaccard's similarity coefficient of castor genotypes based on combined data analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1.00																			
2	0.59	1.00																		
3	0.50	0.59	1.00																	
4	0.39	0.52	0.57	1.00																
5	0.46	0.61	0.57	0.65	1.00															
6	0.44	0.51	0.56	0.66	0.70	1.00														
7	0.55	0.64	0.55	0.58	0.72	0.65	1.00													
8	0.54	0.71	0.58	0.57	0.65	0.72	**0.81**	1.00												
9	0.36	0.48	0.45	0.31	0.39	0.37	0.47	0.44	1.00											
10	0.57	0.68	0.58	0.49	0.56	0.54	0.66	0.68	0.57	1.00										
11	0.48	0.50	0.51	0.54	0.57	0.62	0.68	0.63	0.49	0.70	1.00									
12	0.43	0.58	0.49	0.47	0.58	0.56	0.61	0.65	0.48	0.71	0.74	1.00								
13	0.47	0.66	0.51	0.46	0.60	0.51	0.61	0.66	0.52	0.63	0.56	0.63	1.00							
14	0.37	0.57	0.52	0.55	0.61	0.61	0.58	0.60	0.38	0.50	0.48	0.52	0.56	1.00						
15	0.39	0.56	0.46	0.47	0.54	0.56	0.48	0.56	**0.29**	0.42	0.38	0.40	0.49	0.67	1.00					
16	0.42	0.63	0.51	0.51	0.66	0.58	0.57	0.69	0.49	0.60	0.56	0.63	0.65	0.65	0.59	1.00				
17	0.45	0.66	0.55	0.46	0.60	0.50	0.54	0.59	0.47	0.59	0.51	0.54	0.67	0.59	0.54	0.64	1.00			
18	0.42	0.61	0.50	0.57	0.60	0.55	0.54	0.54	0.45	0.50	0.45	0.44	0.58	0.60	0.57	0.61	0.59	1.00		
19	0.42	0.58	0.45	0.45	0.55	0.44	0.53	0.54	0.47	0.50	0.47	0.47	0.54	0.49	0.40	0.56	0.53	0.55	1.00	
20	0.38	0.63	0.42	0.39	0.53	0.41	0.50	0.55	0.49	0.52	0.45	0.52	0.53	0.54	0.48	0.65	0.54	0.59	0.69	1.00

(1:SKP-84, 2:JP-96, 3:JI-449, 4:JI-491, 5:JI-471, 6:JI-527, 7:ANDCP-16-1, 8:SKP-126, 9:JI-456, 10:JI-509, 11:JI-531, 12:JI-528, 13:JP-105, 14:JP-108, 15:JI-473, 16:JI-476, 17:JI-522, 18:JI-523, 19:JI-454, 20:JI-516)

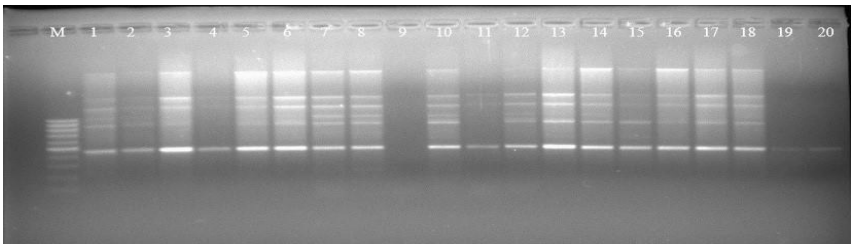

Image 1 - Agarose Gel banding pattern from RAPD-PCR amplification of OPA-02 primer

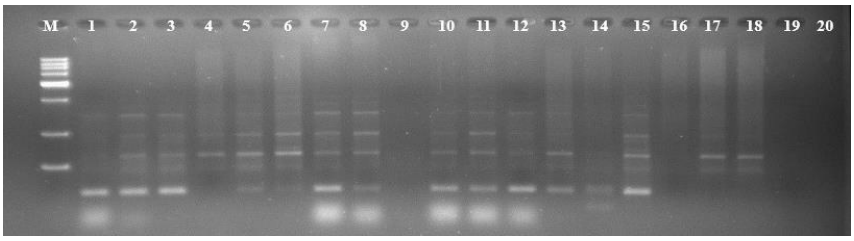

Image 2 - Agarose Gel banding pattern from RAPD-PCR amplification of OPC-01 primer

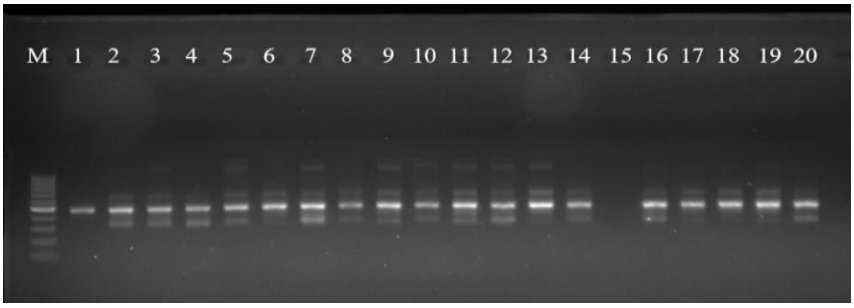

Image 3 - Agarose Gel banding pattern from ISSR-PCR amplification of UBC-807 primer

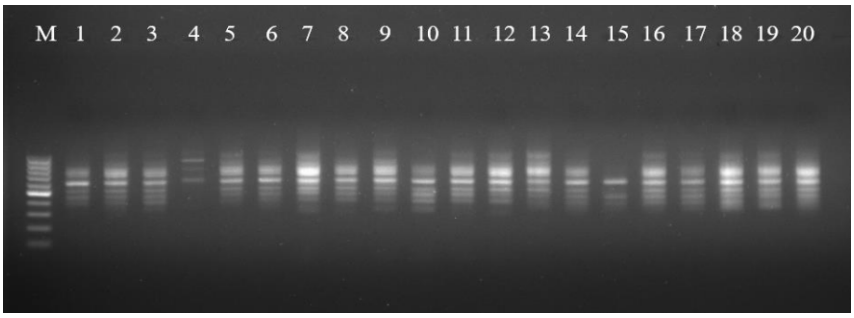

Image 4 - Agarose Gel banding pattern from ISSR-PCR amplification of UBC-809 primer

Conclusion

The molecular analysis showed that the SKP-84 and JI-456 were the most diverse among the other genotypes. The pooled study showed that the genetic similarity of both molecular markers was determined for each pair of twenty genotypes of castor which revealed that the lowest similarity of 28.8 % and the highest similarity of 80.7 % were noticed. With the above results obtained it is possible that the genomic mapping of the castor and molecular studies for the particular trait with the specific primer development or designing in very effective manner.

Future scope of the study

Marker analysis helps to detect unique genetic variations linked to essential traits and providing valuable information for targeted breeding or genetic engineering efforts. Marker-based genetic diversity analysis in castor provides critical insights for guiding breeding programs, improving crop management, conserving genetic resources and boosting the productivity of castor cultivation.

Conflict of interest

The authors declare there is no conflict of interest regarding the preparation of this research manuscript.

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