

Molecular Diversity and Population Structure in Breeding Lines of Castor (*Ricinus communis* L.) Using SSR Markers

Commercial exploitation of heterosis in castor was successful in India due to the development of stable pistillate lines from a dominant and epistatic “S” type pistillate source. Diversification of pistillate sources using NES and other new sources necessitated the need for identification of diverse male combiners among the existing pool of male combiners. In this study, 60 breeding lines/genotypes (Table 1) were characterized for genetic diversity and population structure using EST-SSRs primers. SSR allelic variation was low as indicated by the average number of alleles (2.8), gene diversity (0.53) and polymorphic information content (0.45) (Table 2). Cluster analysis (neighbor joining tree) revealed 3 major genotypic groups. The genotypes showed weak population structure (membership coefficients (≥ 0.75)) and 66.7% genotypes were classified into 3 populations (K=3) and the remaining 33.3 % genotypes into admixture group in STRUCTURE analysis (Fig 1, 2, 3). The genetic diversity information generated in this study would assist in selection of diverse genotypes for breeding to exploit heterosis for development of hybrids.

Table 1. List of castor genotypes used for genetic diversity studies

S.No	Genotype	Pedigree	Morphological characters
1	VP-1	(JP-5 x 28006) x TSP10R x JI-15) F ₂	Green, triple bloom, spiny, dwarf condensed nodes, cup shaped leaves, pistillate line
2	48-1	HO x MD	Red, double bloom, non-spiny
3	AKC-1	-	Red, double bloom, spiny
4	Haritha	PPL-4 X 48-1	Green, double bloom, spiny
5	SKI 215	-	Red, double bloom, non-spiny,
6	JI-220	-	Green, spiny, triple bloom
7	JI-336	Geeta x JI -226	Red, spiny, triple bloom
8	JP-81	SKP-4 x 48-1	Red, spiny, double bloom
9	JP-87	JP-68 x SKI-73	Red, spiny, Triple bloom
10	DCS-1	240 x Bhagya	Red, non-spiny, double bloom
11	DCS-2	Bhagya x CO-1	Red, spiny, double bloom
12	DCS-3	Bhagya X H-86	Red, spiny, double bloom
13	DCS-5	240 x Bhagya	Red, spiny, double bloom
14	DCS-9	240 x Bhagya	Red, spiny, double bloom
15	DCS-16	Selection from HC-8	Green, spiny, double bloom
16	DCS-18	Bhagya X HC-8	Red, non-spiny, triple bloom
17	DCS-22	T-3 x 279	Green, spiny, triple bloom
18	DCS-25	EC-169803 x Aruna	Red, spiny, double bloom
19	DCS-33	EC-169803 x Aruna	Green, spiny, double bloom
20	DCS-37	163-1 x 43-3	Red, spiny, double bloom

21	DCS-38	163-1-11 x 1501-4	Green, non- spiny, double bloom
22	DCS-45	163-1 x 99-2	Red, spiny, double bloom
23	DCS-47	163-1-11 x 1501-4	Red, spiny, double bloom
24	DCS-49	EC-169803 x Aruna	Green, spiny, double bloom
25	DCS-50	EC-169803 x Aruna	Red, spiny, double bloom
26	DCS-51	EC-169803 x Aruna	Red, spiny, double bloom
27	DCS-53	163-1-11 x 1501-4	Red, spiny, double bloom
28	DCS-59	EC-169803 x Aruna	Green, spiny, double bloom, Papaya leaf type
29	DCS-60	EC-169803 x Aruna	Green, spiny, zero bloom
30	DCS-63	EC-169803 x Aruna	Red, spiny, double bloom
31	DCS-68	163-3 x 43-3	Red, spiny, Triple bloom, compact leaf type
32	DCS-78	Male version of DPC-11	Green, spiny, double bloom
33	DCS-79	LRES-17 x REC-9	Red, spiny, double bloom
34	DCS-80	LRES-17 x REC-9	Red, spiny, double bloom
35	DCS-81	LRES-17 x REC-9	Red, spiny, double bloom
36	DCS-84	LRES-19 x TMV-5	Red, spiny, double bloom
37	DCS-86	LRES-19 x 48-1	Green, spiny, double bloom
38	DCS-86-1	LRES-19 x 48-1	Green, spiny, triple bloom
39	DCS-89	163-1-10-2 x 48-1	Red, non-spiny, double bloom
40	DCS-91	163-1-11 x 1501-4	Green, spiny, Triple bloom
41	DCS-92	NES-19 x RMC-3	Green, spiny, Triple bloom
42	DCS-93	NES-19 x RMC-3	Red, spiny, double bloom
43	DCS-94	NES-19 x RMC-3	Green, spiny, triple bloom
44	DCS-96	87-V-2-1 x RMC-3	Green, spiny, triple bloom
45	DCS-97	163-1-10-2 x VI-9	Red, spiny, double bloom
46	DCS-99	DPC 11 x DCS 33	Green, spiny, double bloom
47	DCS-100	DPC 11 x DCS 43	Green, spiny, double bloom
48	DCS-102	DPC 11 x DCS 43	Green, spiny, double bloom
49	DCS-103	M 571 x REC 2	Red, spiny, double bloom
50	DCS-104	M 584 x REC 2	Red, spiny, double bloom
51	DCS-105	NES 19 x RMC 3	Red, spiny, triple bloom
52	DCS-106	DCH 207 x DCH 215	Green, non-spiny, triple bloom
53	DCS-107	DCH-177 x JI-133	Green, spiny, double bloom
54	DPC-9	VP-1 x 128-1 (Bhagya x CO-1)	Green, spiny, zero bloom pistillate line
55	DPC-11	163-1-11 x 1501-4	Green, spiny, double bloom pistillate line
56	DPC-13	VP-1 x REC-128-1	Red, spiny, zero bloom pistillate line
57	DPC-14	VP-1 x REC-128-1	Green, spiny, triple bloom pistillate line
58	DPC-15	NES-6 x DCS-12	Red, spiny, triple bloom, papaya leaf type pistillate line
59	DPC-16	NES-6 x TMV-5	Red, spiny, zero bloom, pistillate line
60	DPC-17	M 619 x JI 225	Red, spiny, single bloom, pistillate line

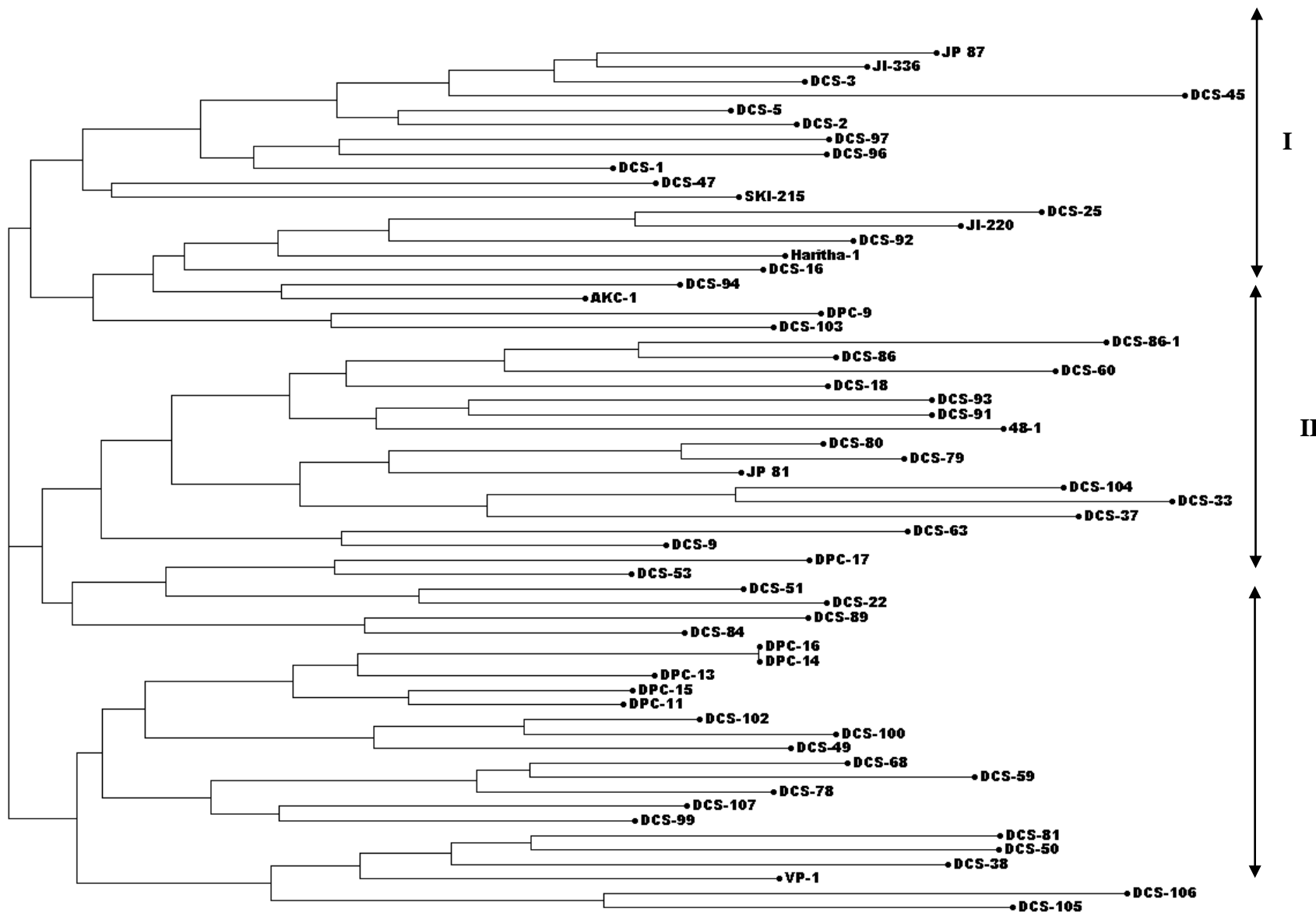
Table 2. Number of alleles (n), major allele frequency (MAF), gene diversity (He), Polymorphic Information content (PIC) calculated for 30 polymorphic EST- SSR primers

*Indicates PIC values > 0.5.

Primer	Motif	Forward primer	Reverse primer	Na	MAF	He	PIC
mRCDOR07	(CT)23	CTTACGCAACAAATCAACCC	GATAGAGGAGGAGAGGTCGC	3	0.63	0.50	0.42
mRCDOR09	(TC)15	CTCCTTTTACGTCCATCATC	GAGAGTGGCATTGTAATGGG	3	0.52	0.52	0.40
mRCDOR13	(AG)12	TCCGCTCCTAGACAAAGAAA	GACCTGTTGTAGCCCATGAC	2	0.67	0.44	0.35
mRCDOR20	(TA)13	CGCAAACCAATCTCTCTCTC	TCTGATCACCTTGCTGCATA	3	0.52	0.58	0.49
mRCDOR22	(TC)13	GCCTCCTTCCTCAACATACA	GGCACCACCATTAACAAAAG	2	0.52	0.50	0.37
mRCDOR24	(TC)13- (TA)12	TCTTCCTGGGCCATACTA	AAGCCTTGGGTTTTGGTATC	3	0.59	0.55	0.47
mRCDOR26	(CT)12	CTCGCCTCTCTCCTTCTCTT	TCTACCATCTCCTCGTAGCG	3	0.65	0.47	0.37
mRCDOR28	(TC)24	ACAGCTCAATTTCTTGCTGC	TAACACAAAACCACTTGGGC	2	0.58	0.48	0.36
mRCDOR49	(CT)14	CCCTGTCAAACATTCTTCG	TGTTGTTGGGATGAATAGGG	3	0.47	0.61	0.53*
mRCDOR50	(AT)15	TCGAAACTCGTCCTCTGTTC	GCAAAACAGATTCGATGCTC	2	0.55	0.54	0.46
mRCDOR55	(TC)12	TCCTCTTCCTCTTCCTCGTT	CGTCAGCCATGGTTAGAGAC	4	0.43	0.62	0.55*
mRCDOR69	(AT)12	GGCAGAAAAGTTGAGATTGC	CAAACACAGTTGGAAAAGGC	3	0.55	0.55	0.50*

mRCDOR76	(AG)14	AGGATCAAAAGATGCACAGC	CAATGACAATGGCGACTGAT	3	0.53	0.59	0.51*
mRCDOR88	(TC)13	GGCACGAGGGGATTATCTA	ACAACCTGACGGGAAACTGAA	3	0.64	0.51	0.44
mRCDOR92	(GA)12	GCATGTTTATACCGCTGCTT	TGGAAAGTTTCTCTTGGCTG	3	0.52	0.56	0.46
mRCDOR103	(CAG)8	AATGACAGCGAGTTCAGGAG	GCCATAAACTCACCACAACC	2	0.50	0.50	0.38
mRCDOR106	(CCA)9	CCAATCTGTTCGATTTACC	GAATTGGATTACCACCACCA	3	0.39	0.65	0.57*
mRCDOR113	(AAG)8	TGCCTACTTCTTAAAGGCGA	TCTCGAACATATCGTGAGCA	3	0.68	0.46	0.39
mRCDOR119	(CAC)9	CACCTCACTTTCTCCCTCCT	AACGAGTCGGTTTGATTGAG	4	0.61	0.51	0.43
mRCDOR121	(CTG)9	CACCAGGACAACCTCAATTCC	GGATTACGGAGACGAGGTTT	2	0.58	0.49	0.37
mRCDOR130	(ACA)10	GAAGCTACGTCTGTCCCAA	GTGGGTATTGTAGAGGGGCT	3	0.64	0.51	0.47
mRCDOR147	(GCT)9	GCTTAGCTTTGTGTCTCCCA	ACCAACCCTGCATAGCATT	3	0.60	0.54	0.47
mRCDOR153	(CAC)8	TCCCTGTAAACCTGATTGC	CAGAAGTTGGGGTTATCGTG	4	0.52	0.61	0.55*
mRCDOR166	(CAC)9	ACCCACACGACCTTTCTAC	TGTTGCAGCTTGACACATCT	3	0.43	0.53	0.47
mRCDOR175	(GGA)10	AAATCGGGGAAGAGAATGTC	TGTTGCTGTTGTTGTTACCG	2	0.59	0.48	0.37
mRCDOR177	(TTC)11	CACAGTACGGTCCTTCTGGT	TACAAGAACAAAACGCCACC	3	0.47	0.62	0.59*
mRCDOR181	(GAG)8	TGAGAGGTTGCAAGGTAAGG	CCCGCATTAAATGTTCTATG	3	0.52	0.39	0.43
mRCDOR203	(AAAG)6	ACCTCAAACAAAGCCCAAAC	ACTAAAACAAGGGTGCCTCC	3	0.47	0.60	0.50*

mRCDOR206	(AAAG)6	CGATCGCTCCTTTTCTTTTC	ATCGGTAGCCAAACAAGTGA	2	0.59	0.49	0.37
mRCDOR240	(TC)10	CGTTAAAAGACCAGGAACCA	ATGCTATCTTGCAAAGCCAC	3	0.38	0.56	0.62*
			Average	2.83	0.54	0.53	0.45



0 ————— 0.1
 Fig 1. Neighbour joining tree showing relationship of 60 genotypes of castor

Factorial analysis: (Axes 1 / 2)

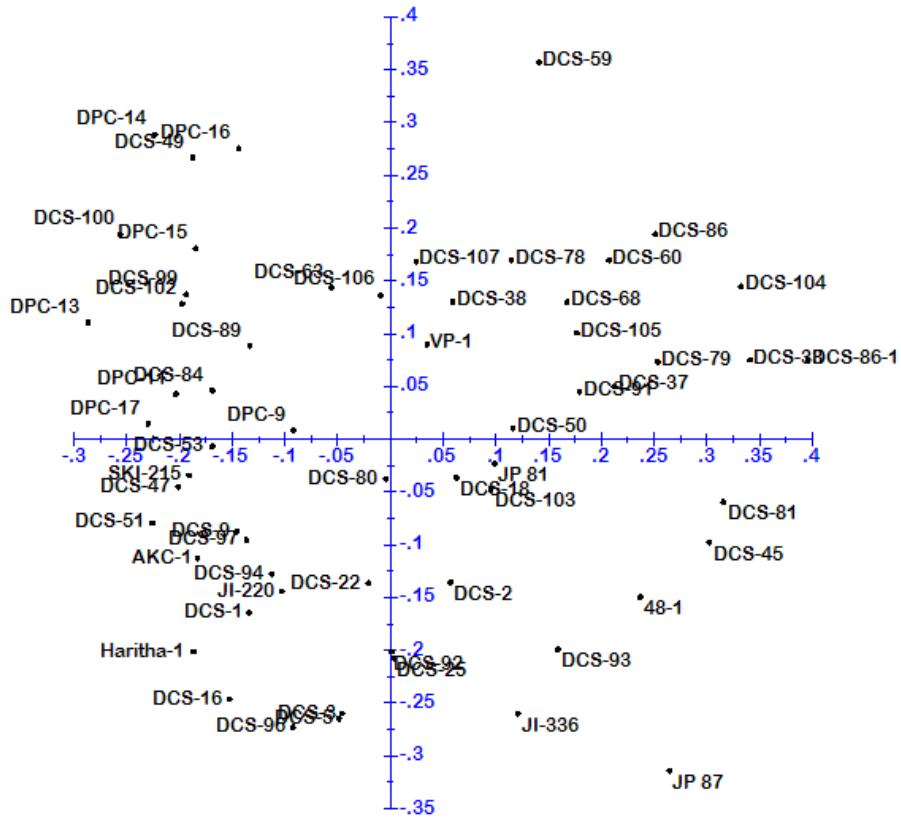


Fig2.Principal coordinate analysis (PCoA) of 60 genotypes of castor. Axes- 1 (10.7%) and Axes- 2 (8.3%) did not separate the genotypes into major groups

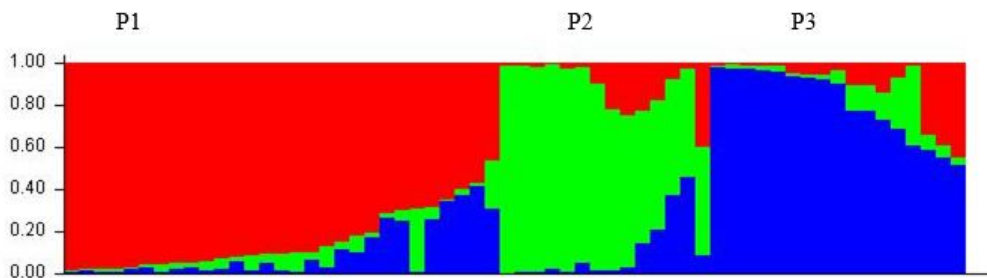


Fig. 4. Model based clustering of castor genotypes into three populations (P1, P2 and P3).

