Int.J.Curr.Microbiol.App.Sci (2019) *8*(2): xx-xx

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 02 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.802.xx

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

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Keywords

Castor, EST-SSR, Genetic diversity, Germplasm, Molecular marker

Article Info

Accepted:
xx January 2019
Available Online:
xx February 2019

A B S T R A C T

Castor is a non-edible oilseed crop, primarily grown for oil containing an unusual hydroxy fatty acid and ricinoleic acid (80–90%) of the total fatty acids. 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 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). 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. The genetic diversity information generated in this study would assist in selection of diverse genotypes for breeding to exploit heterosis for development of hybrids.

Introduction

Castor (*Ricinus communis* L.) with 2n=2x=20 of Euphorbiaceae is one of the ancient and important non-edible oil seed crops cultivated in many tropical and subtropical regions. It is a native crop of tropical Africa, mainly grown for castor oil and cake (Weiss, 2000). Castor is a monotypic genus and the classification of subspecies is based on geographical diversity (Moshkin, 1986). The castor oil is primarily used in industry as a lubricant for all types of heavy machinery, locomotive bearings, steam cylinders in railway engines and internal combustion engines in aero planes (Jeong and Park, 2009). Castor oil and cake are also used in farming as a source of high nitrogen fertilizer and in medicine as a purgative and laxative (Suresh, 2009). The seed oil constitutes 50-55% which is unique in terms of its dominance of the single fatty acid Ricinoleic acid (90%) due to which all the special properties of the oil. Because of the presence of toxic constituents such as ricin and allergens, the cake is unfit for edible purposes. India ranks first in area, production

and productivity among the major castor producing countries like Mozambique, China mainland, Ethopia and Brazil. Together, these countries, account for 88.6% (16.44 lakh tons) of the castor seeds produced globally (FAOSTAT, 2013). India with varied eco-systems is one of the centers of castor diversity (Anjani, 2012). There is a lot of scope to increase the productivity by harnessing heterosis existing in the crop to develop improved cultivars and hybrids in castor. In castor, genetic improvement for yield and yield contributing traits was achieved through mutation breeding, recurrent selection, pedigree selection, hybridization (involving single, double, triple crosses), and selection for different traits (Lavanya *et al.,* 2003a, 2003b; Lavanya *et al.,* 2008, Severino *et al.,* 2012). Knowledge on the extent of genetic diversity is critical to assess the variability in the trait of importance, to choose the parents and to estimate the success of a breeding program. The hybrid vigor in castor depends mainly on the genetic diversity and individual combining ability of the parents (Ramana *et al.,* 2003; Lavanya *et al.,* 2006). The prior information on genetic diversity and relatedness is essential for heterosis breeding and hybrid development in any crop. Previously, genetic diversity in castor was studied using agro-morphological and biochemical markers (Athma *et al.,* 1982; Sathaiah and Reddy, 1984; Figueredo *et al.,* 2004; Costa *et al.,* 2006; Milani *et al.,* 2009). Majority of the agronomic characters and sex expression in castor are highly sensitive to environmental conditions like seasons, temperature, day length etc. (Lavanya, 2002; Lavanya and Gopinath, 2008; Lavanya and Solanki, 2010). Absence of sufficient diversity in castor (for isozymes), limited the number of morphological and biochemical markers (Soltis *et al.,* 1992), and environmental factors limited their use in diversity studies. The precise cataloguing of germplasm resources, including genotypes and cultivars by molecular DNA markers has gained a lot of attention in recent times (Wang *et al.,* 2007; Allan *et al.,* 2008; Foster *et al.,* 2010; Kanti *et al.,* 2014; 2015; Senthilvel *et al.,* 2016). Assessment of genetic diversity with DNA markers differentiates the different accessions quickly using only a small quantity of DNA without any environmental influence. In the present study, we examined the genetic diversity of 60 castor genotypes, including 8 pistillate lines and 52 male / varietal / breeding lines that are predominantly used in the breeding programme. EST-SSRs were used to assess the relative diversity between these genotypes to identify diverse lines for crossing programme in castor.

Materials and Methods

Genomic DNA extraction and SSR analysis

A set of 60 commonly used, constitutionally different breeding lines of castor developed at the Indian Institute of Oilseeds Research (IIOR) and other castor ACRIP centre's were used in the present study. The pedigree and major morphological characters of the genotypes were given in Table 1. In this study, a representative plant of each genotype was selected and the total genomic DNA was extracted from fresh leaf samples as described by Doyle and Doyle (1990) with slight modifications. The quality and quantity were measured through 0.8% agarose gel electrophoresis. EST-SSR markers were developed in the IIOR from the publicly available ESTs (64, 756); a set of 35 primer pairs designed was used for genotyping. The PCR reactions were performed in 10 µl reaction volume containing 1 × PCR buffer with 1.5 mM MgCl2 (Genei, India), 0.08 mM each of dNTPs (Genei, India), 5 pm of each forward and reverse primer, 0.2 U Taq DNA polymerase (Genei, India) and 25 ng template DNA. DNA amplification was performed in

the Master cycler Gradient Eppendorf version 2.1 (Eppendorf, USA). DNA was pre-denatured at 94 °C for 5 min followed by 30 cycles of denaturation at 92 °C for 30 sec, primer annealing at 56 °C for 30 sec and primer extension at 72 °C for 30 sec followed by a final extension at 72 °C for 7 min. The PCR product was separated in 6% polyacrylamide gels on a Sequi-Gen (BioRad, USA) sequencing unit for 3 h in 1×TBE at 100W, 50 mA. After electrophoresis, the bands were visualized by silver-staining as reported by Tegelstrom (1992) with slight modifications

Genetic analysis

The genetic diversity estimates viz., number of alleles, gene diversity (expected heterozygosity; He) and polymorphic information content (PIC) were obtained using Power Marker version 3.25 (Liu and Muse, 2005). The SSR allelic data were used to construct neighbor-joining (NJ) based on pair-wise simple matching coefficients using DARwin version.5.0.158 (Perrier and Jacquemoud-Collet, 2006) to understand the genetic relationships among genotypes. Principal coordinates analysis (PCoA) was also performed to visualise the overall representation of diversity in the genotypes.

Structure analysis

The genetic structure of the accessions was also investigated using a model-based clustering algorithm (STRUCTURE v.2.3.4) that genetically separates groups according to allele frequencies (Pritchard *et al.,* 2000). The possible number of K was assumed from 1 to 10 in order to determine the optimal K. Each run consisted of a burn-in period of 100,000 steps followed by 200,000 Monte Carlo Markov chain replicates, assuming an admixture model and correlated allele frequencies. The mean posterior probability (LnP(D)) values per K were obtained based on 10 replications. The delta K measure (Evanno *et al.,* 2005) was used to determine the K as implemented in the online version of STRUCTURE HARVESTER (http://tayloro. biologyucla.edu/Struct_harvest) (Earl and VonHoldt, 2012).

Results and Discussion

Genetic diversity

Genetic diversity in the genotypes is the foundation for any breeding program for crop improvement. In the present study, a set of 60 breeding lines used generally in breeding program was characterized for the extent of genetic diversity, genetic relatedness and population structure using 35 EST-SSR markers developed in IIOR. Microsatellites markers are considered ideal for characterizing genetic diversity and relatedness among the genotypes due to co-dominant nature and high reliability. SSR markers are mostly used in castor for genetic diversity studies (Allan *et al.,* 2008; Bajay *et al.,* 2009; Qiu *et al.,* 2010; Kanti *et al.,* 2014, 2015; Senthilvel *et al.,* 2016). Even though, SNPs are widely used to study genetic diversity in crops now-a-days, SSRs are preferred due to their multi-allelic nature, which provides more information per locus (Remington *et al.,* 2001). For this study 35 EST- SSR markers were selected randomly from the designed EST-SSR primer pairs based on the amplification, amplicon size and polymorphism to characterize 60 breeding lines out of which, five were monomorphic. A total of 85 alleles were observed with 30 polymorphic SSR markers. The number of alleles per locus ranged from 2 to 4 with a mean of 2.8 (Table 2). The major allele frequency ranged from 0.38 to 0.68 with an average of 0.54. SSR allelic diversity in the genotypes studied were low (NA=2.8, PIC=0.45), which could be because of using

EST-SSR markers. In general, EST-SSR markers were observed to be less polymorphic, but as functional markers the polymorphism is associated with the coding regions and detects the true genetic diversity available inside or adjacent to the genes (Eujayl *et al.,* 2002; Maestri *et al.,* 2002; Thiel *et al.,* 2003). Low SSR polymorphism in castor is also evident from previous studies. Qiu *et al.,* (2010) reported that the EST-SSR alleles ranged from 2 to 6 with an average of 2.97 alleles per locus among 24 genotypes. Similarly, Bajay *et al.,* (2009) reported an average of 3.3 alleles per locus using 38 germplasm accessions. Allan *et al.,* (2008) reported an average of 3.1 alleles per locus among 200 genotypes. Senthilvel *et al.,* (2016) reported an average of 2.97 alleles in a collection of inbred lines (144) from the core collection of castor. Gene diversity (He) per locus ranging from 0.44 to 0.63 with an average of 0.53 was observed in this study. These values are, slightly higher than the moderate levels of gene diversity per locus (0.38 – 0.42) reported by Bajay *et al.,* (2014); Kanti *et al.,* (2014) and Senthilvel *et al.,* (2016). Allan *et al.,* (2008), on the other hand reported very low level of gene diversity (0.188) in worldwide genotyping of castor germplasm accessions. The relatively low levels of He revealed by molecular markers in castor can be due to breeding bottlenecks, where only a small proportion of the variability of the gene pools was funneled through. The PIC value ranged from 0.35 to 0.62 with an average of 0.45 (Table 2). Kanti *et al.,* (2014) reported PIC value ranging from 0.12 to 0.35 with an average of 0.37, comparable to the observed PIC value in this study, in castor germplasm collected from Andaman and Nicobar Islands, India. However, large range of PIC values (0.07 - 0.73; 0.01- 0.62) but with a low mean value of 0.32 and 0.36 was observed by Qiu *et al.,* (2010) and Senthilvel *et al.,* (2016) respectively. The PIC value is indicative of the effectiveness and usefulness of SSR loci and measures the information about a given marker locus for the pool of genotypes (Kupper *et al.,* 2011). The level of polymorphism is influenced by the number of genotypes, type of plant material used in the study. For instance, Allan *et al.,* (2008) studied genetic diversity of 200 genotypes using gSSR markers and observed an average PIC value of 0.4. Whereas, Senthilvel *et al.,* (2016) studied 144 diverse inbred lines derived from core collection of castor germplasm and found slightly lower mean PIC value (0.36). In our study, nine markers showed > 0.5 PIC value (mRcDOR49, mRCDOR55, mRcDOR69, mRcDOR76, mRcDOR106, mRcDOR153, mRCDOR177, mRcDOR203 and mRcDOR240) indicating their usefulness for applications in diversity analysis. In this study low genetic diversity at the molecular level is observed, which confirmed the previous findings. Nevertheless, low SSR polymorphism in castor is a concern that would limit their use for mapping important traits. Many studies on assessment of genetic diversity in castor germplasm showed low levels of variability regardless of the marker systems employed (Allan *et al.,* 2008; Gajeria *et al.,* 2010; Foster *et al.,* 2010; Qiu *et al.,* 2010; Bajay *et al.,* 2010; Rivarola *et al.,* 2011; Pecina-Quintero *et al.,* 2013; Wang *et al.,* 2013; Vivodik *et al.,* 2014; Kanti *et al.,* 2014, 2015; Senthilvel *et al.,* 2016). The extensive agro-morphological diversity for vegetative, reproductive and seed traits observed in the castor genotypes has not reflected at molecular level genetic variability. However, use of few markers, different marker systems and plant material for evaluation of genetic variation might be the reason for detecting contradictory levels of diversity in castor. The low genetic variation in castor could probably be due to selected cultivation, domestication and long term propagation of few varieties (Sujatha *et al.,* 2008).

Genetic relationship

Cluster analysis showed three major clusters (I, II, III) and sub-groups within the major clusters (Ia, Ib, IIa, IIb, IIIa, IIb, IIIc). Cluster I included 20 genotypes, Cluster II included 21 and Cluster III consisted of 19 genotypes. A neighbor- joining (NJ) tree depicting genetic relationships between 60 castor genotypes based on pair-wise dissimilarity coefficients is shown in Figure 1. Overall, pair-wise simple matching coefficients ranged from 0.00 (DPC 14 and DPC 16) to 0.88 (DCS 25 and DCS 102; 48-1 and DPC 9) with an average of 0.48.

The pairwise simple matching coefficients of Cluster I ranged from 0.2 (JI 336 and DCS 1)) to 0.75 (DCS 45 and DCS 92), cluster II ranged from 0.13 (DCS79 and DCS 80) to 0.84 (DCS 60 and DPC 17) and cluster III ranged from 0.00 (DPC 14 and DPC 16) to 0.8 (JI 220 and DCS 38; DCS 16 and DCS 81). Cluster Ia consists of four male lines: DCS 1, DCS 2, DCS 3and DCS 5 which were derivatives involving Bhagya variety as the common parent. Cluster IIb consists of 8 male lines and one non- revertant pistillate line DPC 9 with distinct morphological characters like green stem colour, single bloom, spiny capsules, early duration (110-120 days), resistant to *Fusarium* wilt used in the development of two hybrids like DCH 177 and YRCH1. DCS 92, DCS 94 are derivatives from NES type of line NES19. DPC-9 and DCS 103 has VP-1 background. Cluster IIa is the major sub cluster consisting of 15 genotypes. It consists of one pistillate line JP-81 was also closely related to male lines derived by involving an S type of pistillate lines like LRES 17, M 584. This cluster contains two best male lines 48-1 and DCS 9 which are the parental lines of the popular hybrids GCH 4 and DCH 177 respectively. 48-1 is a male line with a red stem, non-spiny capsules, zero bloom, *Fusarium* wilt resistant, moderately resistant to *Botryotinia* grey mold is largely grown as a variety. DCS 86 and DCS 86-1 are the cross derived male lines with non-spiny capsule from 48-1. Cluster IIb includes one new pistillate line, DPC 17, a cross derivative of M-619 XJI 225 with red stem colour, double bloom, spiny capsules is revertant type of pistillate line. Cluster IIIa includes three male lines (DCS 102, DCS 100, DCS 49) and five pistillate lines. Among the 5 pistillate lines DPC 13 and DPC 14 were derivatives of VP-1 based 'S' type of pistillate source while DPC 15 and DPC 16 were developed using 'NES' source of pistillate line. (Lavanya, 2002; Lavanya and Gopinath, 2008) and DPC 11 was developed from a different source of pistillate expression (163-1-11 X 1501-4). Cluster IIIb includes five genotypes (DCS 68, DCS 59, DCS 78, DCS 107 and DCS 99). DCS 78 is the male line involved in the development of prominent hybrid DCH 215 and the newly released variety DCS 107 was derived from cross of DCH 177 and JI 133. Custer IIIc included one pistillate line VP-1, which is the first pistillate line developed in India and five male lines. Among five male lines, DCS 38 and DCS 81 are cross derivatives involving VP-1 while DCS 106 derived from a multiple cross involving four F1s and six different parents is highly diverse the cluster. Principal coordinate analysis (PCoA) was carried out on the same SSR data set. The results of PCoA showed that the first two axes captured only 10.7 % and 8.3 % of total variance, respectively and did not show any strong groupings (Figure 2).

Population structure

To further verify the results of the cluster and PCoA analyses, the programme structure was used. Population structure means a non-random distribution of the genetic diversity, which changes over time in species between groups (Hamrick and Loveless, 1989).

The Structure uses a model based on a Bayesian clustering approach to infer the population structure (Pritchard *et al.,* 2000). The structure analysis was performed by setting number of clusters (k) from 1 to 10 with 10 replications for each K. The LnP(D) showed a constant increase with the increasing subpopulation number (K) and no significant clear cut-off was observed based on the LnP(D) (Figure 3a). However, delta-K (DK) analysis of LnP(D) (Evanno *et al.,* 2005), showed a sharp peak at K =3, suggesting three populations within the collection of 60 genotypes (Figure 3b). Based on the threshold value of the membership coefficient (≥0.75), 40 accessions were assigned to three populations (namely, P1, P2, P3 and P4) and the remaining 20 accessions to the admixture group. The bar plot showing the population structure for K=3 also indicated e populations with clear admixture in the individuals (Figure 4). P1 comprised of 21 (52.5%), P2 comprised of 11 (27.5) and P3 comprised of 8 genotypes (20%). The average gene diversity between individuals in the same cluster was 0.445, 0.427 and 0.347 for P1, P2 and P3 respectively. The mean Fst values within P1, P2 and P3 were 0.272, 0.270 and 0.46. AMOVA partitioned the total genetic variance into two components: among and within populations. Maximum of genetic variation was explained by individuals within the populations (84.79%) but not by individuals among the populations (13.21%). STRUCTURE is one of the most widely used software for population analysis, which helps to assess the patterns of genetic structure in a subset of samples (Porras-Hurtado *et al.,* 2013). The average distances and Fst values within the main populations were low and 33.3% of the genotypes are admixtures. The populations are not further subdivided into sub populations. The Fst values among major genotypic groups were low (Fst < 0.2) suggesting low genetic divergence and the genetic structuring was weak. Senthilvel *et al.,* (2016) found that there was no marked genetic structuring within the collection of 144 inbred lines derived from a core collection of castor.

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

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	CTCCTTTCACGTCCATCATC	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	TCTTCCTGGGCCATACACTA	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	CCCTGTCAAAACATTCTTCG	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	ACAACTGACGGGAAACTGAA	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	CCAATCTGTTCGATTTCACC	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	CACCAGGACAACTCAATTCC	GGATTACGGAGACGAGGTTT	2	0.58	0.49	0.37
mRCDOR130	(ACA)10	GAAGCTACGTCTGTCCCAAA	GTGGGTATTGTAGAGGGGCT	3	0.64	0.51	0.47
mRCDOR147	(GCT)9	GCTTAGCTTTGTGTCTCCCA	ACCAACCCTGCATAGCATTA	3	0.60	0.54	0.47
mRCDOR153	(CAC)8	TCCCTGTTAAACCTGATTGC	CAGAAGTTGGGGTTATCGTG	4	0.52	0.61	0.55*
mRCDOR166	(CAC)9	ACCCCACACGACCTTTCTAC	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	CCCGCATTAATGTTCCTATG	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

*Indicates PIC values > 0.5.

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

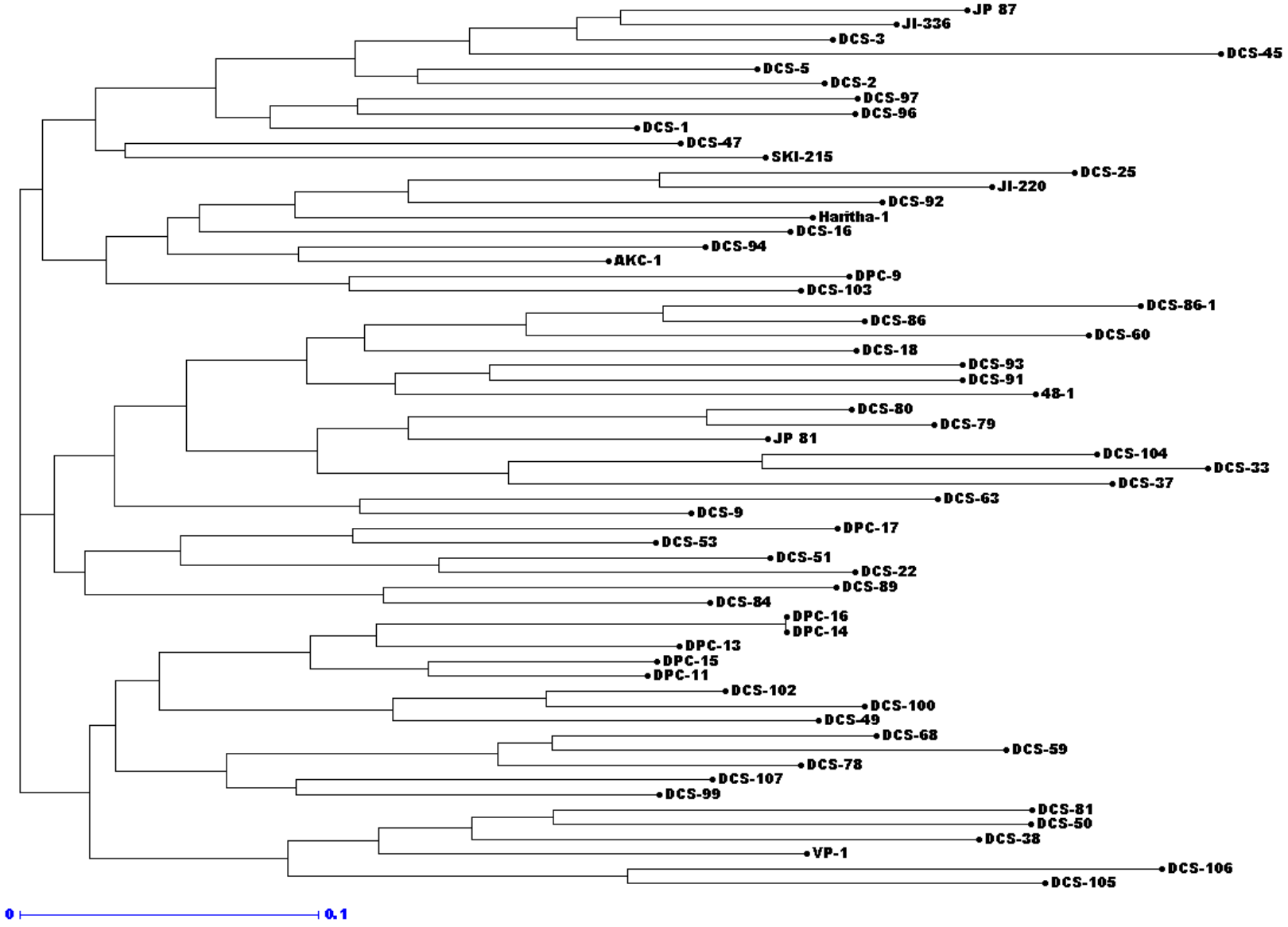


Fig.2 Principal coordinate analysis (PCoA) of 60 genotypes of castor. Axes- 1 (10.7%) and Axes-2 (8.3%) did not separate the genotypes into major groups

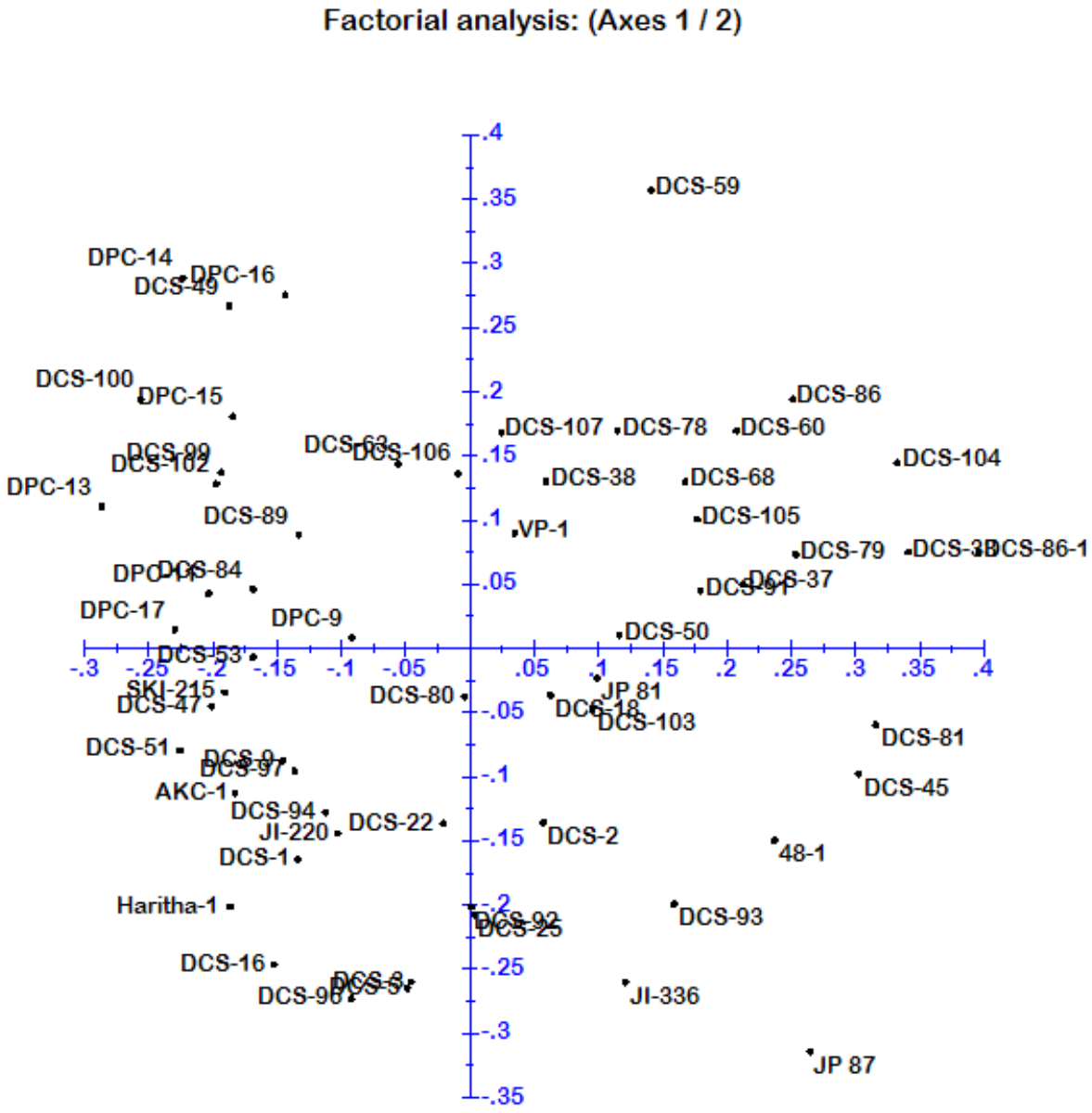


Fig.3 Determination of the optimal value of K for the genotypes.
(a) Log probability of data, L(K) averaged over the replicates (b) Plot of Delta K calculated as the mean of the second-order rate of change in likelihood of K divided by the standard deviation of the likelihood of K as per Evannoet al. (2005)

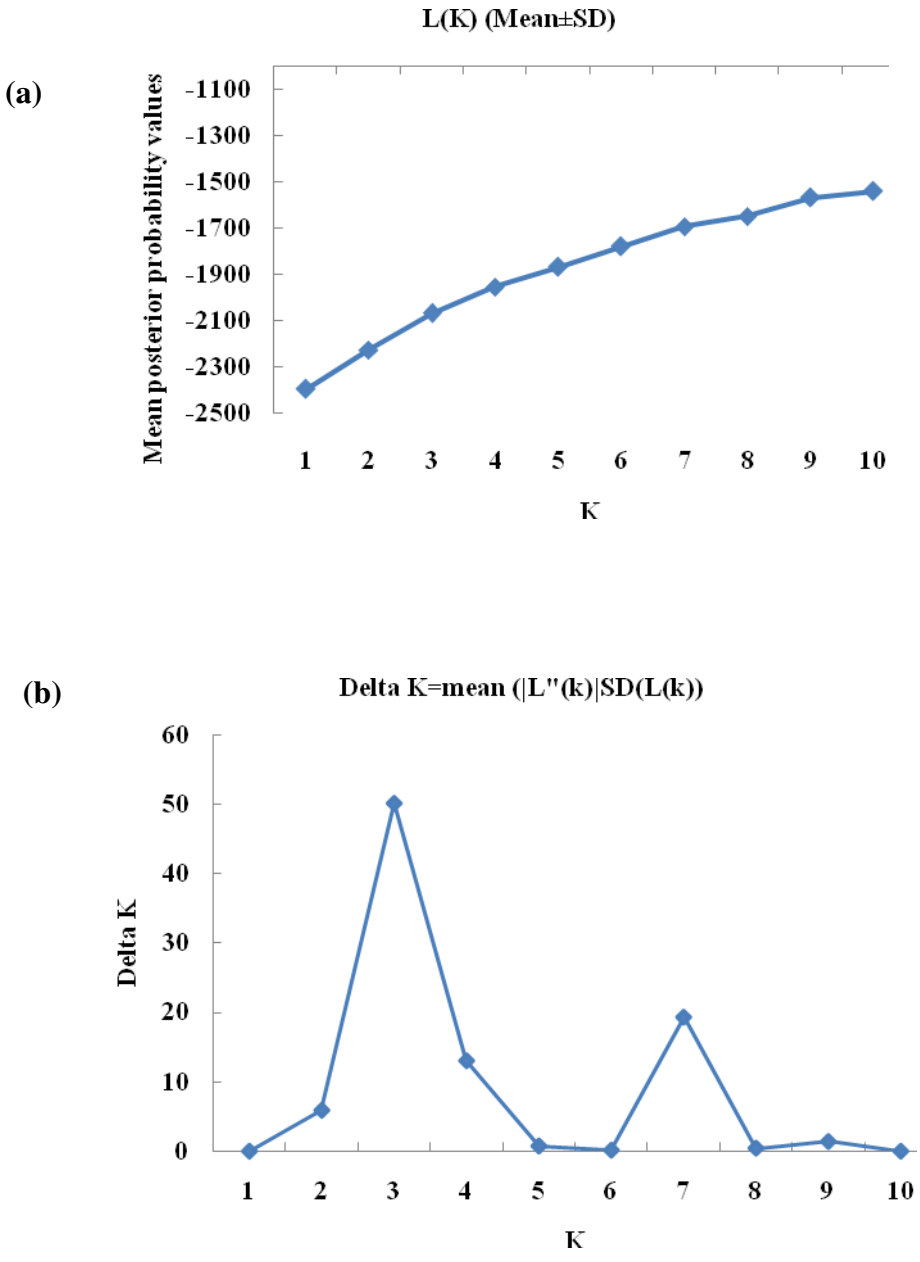
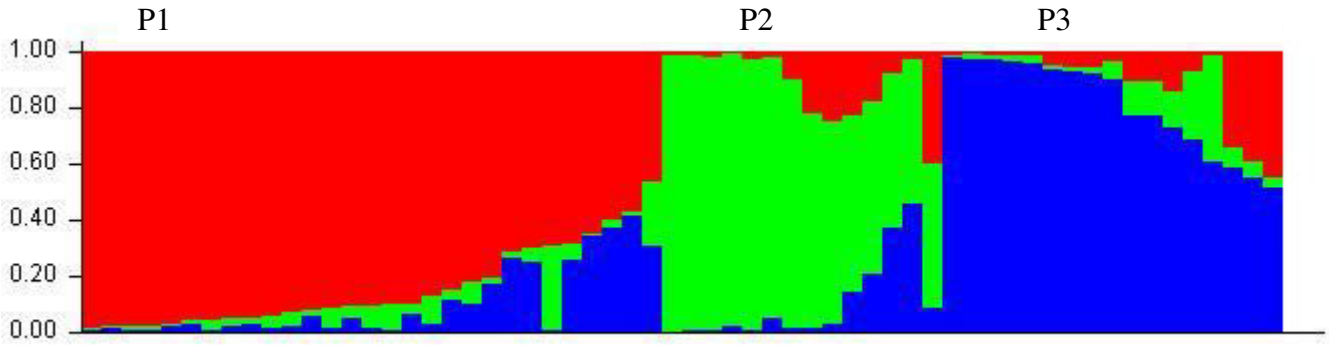


Fig.4 Model based clustering of castor genotypes into three populations (P1, P2 and P3)



Both the NJ tree and STRUCTURE grouped the genotypes into 3 major clusters that were almost comparable. The most divergent population P3 detected by STRUCTURE consisted of 14 accessions, which were also present in cluster III. A large number of the genotypes (33.3%) were classified under admixture group suggesting that they have mixed ancestry because of the common parental lines. The results of NJ clustering, PCoA and model based STRUCTURE analyses clearly suggested that there was low allelic diversity and weak genetic structure in the castor genotypes used in the breeding programme of castor. As the castor is highly cross pollinated, low level of population differentiation is expected due to extensive gene flow among individuals.

In conclusion, castor is being cultivated predominantly in India. It is good to go through the breeding programs and selection history to understand the extent of variability existing in the on-going breeding populations. Most of the earlier studies, assessment of the extent of genetic diversity of castor was reported in naturally occurring populations (Wang *et al.,* 2013; Kanti *et al.,* 2014, 2015), land races (Seo *et al.,* 2011); inbreds from core correction (Senthilvel *et al.,* 2016) and germplasm maintained in gene banks (Allan *et al.,* 2008; Foster *et al.,* 2010; Pecina-Quintero *et al.,* 2013). In the present study, a set of 60 elite parental lines commonly used in the castor crossing programme have been molecularly characterized for broadening the genetic base of castor. The molecular data indicated the low SSR allelic diversity with weak or low population structure. Hence, the breeding lines have to be diversified using mutations, crossing diverse lines from different regions or geographically isolated lines for harnessing heterosis, and to develop better hybrids.

Acknowledgements

The authors are thankful to Director, ICAR-Indian Institute of Oilseeds Research (ICAR-IIOR) for extending all the facilities for the research work.

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How to cite this article:

Usha Kiran Betha and Lavanya, C. 2019. Molecular Diversity and Population Structure in Breeding Lines of Castor (*Ricinus communis* L.). *Int.J.Curr.Microbiol.App.Sci.* 8(02): xx-xx. doi: https://doi.org/10.20546/ijcmas.2019.802.xx