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**Harshita J Kachhadiya**
Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India

**RB Madaria**
Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh, Gujarat, India.

**Rushita V Bhadani**
Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India.

**Abhijeeta K Nandha**
Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India.

**Manthan Kapuria**
Shree P.M. Patel Institute of Integrated M.Sc. in Biotechnology, Anand, Gujarat, India.

**HP Gajera**
Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India.

**Correspondence**
**Harshita J Kachhadiya**
Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India

# Genetic diversity among castor (*Ricinus communis* L.) genotypes as revealed by RAPD and ISSR markers

**Harshita J Kachhadiya, RB Madaria, Rushita V Bhadani, Abhijeeta K Nandha, Manthan Kapuria and HP Gajera**

**Abstract**
Castor (*Ricinus communis* L.) is an important plant for production of industrial oil. The systematic evaluation of the molecular diversity encompassed in castor inbreds or parental lines offers an efficient means of exploiting the heterosis in castor as well as for management of biodiversity. Two DNA-based molecular marker techniques, *viz.,* random amplified polymorphism DNA (RAPD) and inter simple sequence repeat (ISSR), were used to assess the genetic diversity in castor genotypes. Out of the 40 RAPD and 40 ISSR primers screened, a total of 35 polymorphic primers (24 RAPDs and 23 ISSRs), were used in this study. Amplification of genomic DNA of 20 castor genotypes, using RAPD analysis, yielded 147 fragments, of which 96 were polymorphic, with an average of 4.0 polymorphic fragments per primer. Number of amplified fragments with RAPD primers ranged from 2 to 13, with the size of amplicons ranging from 102 to 2548 bp in size. The polymorphism ranged from 0.0 to 100.0, with an average of 66.68%. The 23 ISSR primers produced 142 bands across 20 genotypes, of which 118 were polymorphic, with an average of 5.13 polymorphic fragments per primer. The number of amplified bands varied from 2 to 11, with size of amplicons ranging from 100 to 2342 bp. The percentage of polymorphism using ISSR primers ranged from 0.0 to 100.0, with an average of 78.54%. The Mantel test between the two Jaccard's similarity matrices gave r = 0.78, showing correlation between RAPD- and ISSR-based similarities. Clustering of genotypes within the groups was not similar when RAPD and ISSR derived dendrograms were compared, whereas, the pattern of clustering of the genotypes remained akin in RAPD and combined data of RAPD and ISSR. The similarity coefficient ranged from 0.58 to 0.88, 0.41 to 0.77, and 0.69 to 0.93 with RAPD, ISSR, and combined dendrogram, respectively. Knowledge on the genetic diversity of castor can be used to future breeding programs for increased oil production to meet the ever increasing demand of castor oil for industrial uses as well as for biodiesel production.

**Keywords:** Castor, molecular marker, RAPD, ISSR, genetic diversity, polymorphism

## Introduction
Castor (*Ricinus communis* L., 2n=2x = 20, Euphorbiaceae), is industrially important non-edible oilseed crop widely cultivated in the arid and semi-arid regions of the world (Govaerts *et al.,* 2000) [9]. It is cultivated around the world because of the commercial importance of its oil. India is the world's largest producer of castor seed and meets most of the global demand for castor oil. The seed of castor contain more than 45% oil and this oil is rich (80–90%) in an unusual hydroxyl fatty acid, ricinoleic acid (Jeong and Park, 2009) [11]. Castor oil is the only vegetable oil soluble in alcohol, presenting high viscosity, and requiring less heating than others oils during the production of biodiesel. Due to its unique chemical and physical properties, the oil from castor seed is used as raw material for numerous and varied industrial applications, such as: manufacture of polymers, coatings, lubricants for aircrafts, cosmetics, etc, and for the production of biodiesel. (Jeong and Park, 2009) [11]. Due to the presence of "Ricin" (poisonous alkaloids), it is highly poisonous to man and for animal feed. The castor cake is a good source of organic manure as it contains nitrogen 4.5 %, $P_2O_5$ 1.75 % and $K_2O$ 1.5 % and also controls white ants and nematodes. It is useful as a trap crop because root contain "ricin" (poisonous alkaloids) which kills nematodes entered into roots (Bozza *et al.*, 2014) [4]. Ricin, a poisonous substance found in castor, is state-of-art tool in neurobiology for selectively destroying neuronal populations (Singh, 1976) [23]. With more than 95 per cent of the world's castor production concentrated in limited parts of India

China, and Brazil (Sailaja *et al*., 2008) [21], and because of the ever increasing world- wide demand of castor.

Castor is a cross pollinated crop and is usually cultivated as a hybrid in India, as hybrids give significantly greater yields than pure lines or varieties (Moll *et al*., 1962 [15]; Birchler *et al.,* 2003 [3]). Genetic diversity assessment prior to developing hybrids can aid in better exploitation of heterosis (Reif *et al*., 2007) [19]. Knowledge about germplasm diversity and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies (Mohammadi and Prasanna, 2003) [14]. New molecular tools hold the promise of allowing the identification of genes involved in a number of traits including adaptive traits, and polymorphisms causing functional genetic variation. Conventional breeding techniques which are based on the processes of crossing, back-crossing and selection, proved to be time consuming.

Therefore, molecular technology is increasingly becoming popular as a powerful tool for unambiguous authentication. A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner (Santalla *et al.,* 1998) [22]. Molecular techniques for detecting differences in the DNA of individual plants to examine variability in cultivar are useful for identification of potential parental lines. These differences in general are called molecular marker. These molecular markers used for characterization as well as phylogenic analysis in various plant species with reliable and authentic results (Behera *et al.,* 2008) [1]. DNA markers provide a direct measure of genetic diversity and go beyond diversity based on agronomic traits or geographic origin (Dreisigacker *et al.,* 2005) [5], thus help in better germplasm management and develop more efficient strategies for crop improvement.

Among the various molecular marker techniques available, polymerase chain reaction (PCR)-based markers, such as randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) have been most popular because of speed, low cost, does not require prior knowledge of DNA sequence and the use of only minute amounts of DNA template for analysis (Bhat, 2002) [2]. RAPD has been the most employed technique in diversity analysis, mapping and genotype identification in number of plant species but low reproducibility is the limit of this technique. ISSR markers overcome the shortcomings of the low reproducibility of RAPD; they produce more reliable and reproducible bands because of the higher annealing temperature and longer sequence of ISSR primers. ISSRs are ideal as markers for genetic mapping and population studies because of their abundance, and the high degree of polymorphism between individuals within a population of closely related genotypes (Singh *et al.,* 2011) [24]. Those properties indicate their potential role as good supplements for RAPD-based genome analysis.

## Materials and Methods

**Plant material:** Twenty genotypes of castor (*Ricinus communis* L.) were collected from the Main Oilseeds Research Station, JAU, Junagadh, Gujarat, India, to study molecular diversity by RAPD, ISSRand SSR assay. The name and origin of selected castor genotypes is given in Table 1. Seeds of each genotype were sown in pots and young leaves of two weeks old plants were collected from each genotype for the DNA isolation.

**DNA isolation:** Total plant genomic DNA was extracted from young leaves of each genotype using Cetyl Trimethyl Ammonium Bromide (CTAB) method as described in Purohit *et al.* (2012) [17]. The quantity and quality of the isolated DNA was determined by using Pico Drop (Qiagen). Dilutions of 25 ng/µl of each genotype were prepared and stored at 4 °C for further use in PCR analysis.

**RAPD analysis:** Fourty oligonucleotide primers of10-mer, each with at least 60% G+C content (Table 2), were obtained from OperonTechnologies, Inc., Alameda, CA. PCR reactions were performed as per Williams *et al.* (1990) [29] with some modifications. The PCR master mix (15 µl) contained 10x PCR buffer (10 mM Tris-HCl, pH 8.3), 2.5 mM each dNTPs, 25 pmoles primer, 50 ng of genomic DNA and 3 unit of Taq DNA polymerase (Invitrogen). The samples were subjected to 35 repeats of the following cycle: 94 °C for 1 min, 37 °C for 1.5 min, 72 °C for 2 min with an initial denaturation of 4 min and a final extension of 7 min. All the above PCR amplification was performed in 0.2 ml thin-walled PCR tubes placed in a thermal cycler (Veriti®, Applied Biosystems). The products were analysed by electrophoresis in 1.5 % agarose gel stained in ethidium bromide (10 mg/ml) and run in 1x TBE buffer at 100 V for 2 h. The separated bands were visualized under UV transilluminator and photographed using a gel documentation system (BioRad).

**ISSR analysis:** Fourty oligonucleotide primers were obtained from Operon Technologies, Inc., Alameda, CA (Table 3). PCR reactions were performed as per Gajera *et al.* (2010) [7] with some modifications. ISSR amplification were carried out in 15 µl volume containing 1 µl DNA, 12.5 µl master mix and 1 µlof 10 pmol primer. The amplification reaction consisted of consisted of an initial denaturation step at 94°C for 5min, followed by 35 cycles of 1 min at 94°C (denaturation), 1min at a specific annealing temperatures (Table 3), and 2 min at 72°C (extension) followed by a final extension step at 72°C for 5min. Amplificationproducts were electrophoresed in 1.5% agarose in 1× TBE buffer. The gels were stained with ethidium bromide and documentedusing gel documentation system.

**Reproducibility of amplification patterns:** DNA amplifications with each RAPD and ISSR primers were repeated at least thrice to ensure reproducibility. The bands were considered reproducible and scorable only after observing and comparing them in three separate amplifications for each primer. Clear and intense bands were scored while faint bands against background smear were not considered for further analysis.

**Scoring and data analysis:** The molecular size of each fragment was estimated using AlphaEase FC software (Alpha Innotech Corporation). The RAPD, ISSR and SSR markers were scored as present (1) or absent (0) of a band, and the data obtained were used in a rectangular matrix. The data matrix was then used to generate a genetic similarity index (Nei and Li, 1979) [16] using NTSYS 2.1 (Rohlf, 2000) [20]. By comparing the banding patterns of genotypesfor a specific primer, genotype-specific bands were identified. Faint or unclear bands were not considered. The binary data generatedwere used to estimate levels of polymorphism by dividingthe polymorphic bands by the total number of scored bands. The polymorphism information content (PIC) was calculated by the formula: PIC = 2Pi (1−Pi) (Bhat, 2002) [2] where, Pi is the frequencyof occurrence of polymorphic bands in different primers. Pairwise similarity matrices were

generated by Jaccard's coefficient of similarity (Jaccard, 1908) [10] by using the SIMQUAL format of NTSYSpc 2.1(Rohlf, 2000) [20]. Correlation between the two matrices obtainedwith two-marker types was estimated by means of the Mantel matrix correspondence test (Mantel, 1967) [13]. This test yields a product moment correlation (r) that is one measure of the relatedness between the two matrices. A dendrogram was constructed by using the unweighted pair group method with arithmetic average (UPGMA) with the SAHN module of NTSYS-pc to show a phonetic representation of genetic relationships as revealed by the similarity coefficient (Sneath and Sokal, 1973) [25].

**Result and Discussion:** Total plant genomic DNA was extractedfrom young leaves by Cetyl Trimethyl Ammonium Bromide (CTAB) method withsome modifications. The absorbance ratio of DNA at A260/A280 ranged from 1.72 to1.89 and the concentration ranged from 136.09 to 223.04 ng/µl.

**RAPD band pattern:** Genetic diversity studies can identify alleles that might affect the ability of the organism to survive in its existing habitat, or might enable it to survive in more diverse habitats. This knowledge is valuable for germplasm conservation, individual, population, variety or breed identification and genetic improvement (Duran *et al.,* 2009) [6]. Various types of markers such as morphological, biochemical and molecular markers are used for this purpose (Vivodik *et al.,* 2015) [28].

Fourty RAPD primers having 60% or more GC content were used for the present investigation. Out of 40 primers, 24 primers were amplified and showed 100% polymorphism. A total 147 amplified bands were obtained of which 96 bands were polymorphic. The DNA amplicon size and polymorphism generated among various genotypes of *R. communis* L. using RAPD primers are presented in Table 4. The total number of bands observed for every primer was recorded separately and polymorphic bands was checked subsequently. The total number of amplified bands varied between 2 (primer OPM-02) and 13 (primer OPM-07) with an average of 4.0 bands per primer. The polymorphism of all 20 genotypes *R. communis* L. were 66.68% and the overall size of PCR amplified products ranged between 102 bp to 2548 bp. Similar to present finding, Lakhani *et al.* (2015) [12] used RAPD molecular markers to assessed genetic diversity between 13 castor genotypes. Out of 27 primers, 16 primers amplified a total number of 99 bands with 100 % polymorphism. Earlier, Vivodik *et al.,* (2014) [27] used RAPD molecular markers to analyze genetic diversity between 40 castor genotypes. A total number of 66 bands were amplified having 8.25 polymorphic bands per primer. Earlier, Ram *et al.,* (2008) [18] obtained high level of polymorphism of 78.69 per cent among *Jatropha* species.

Based on RAPD similarity matrix data (Table 5), the value of similarity coefficient ranged from 0.58 to 0.88 i.e. 58-88%. Maximum similarity value of 0.58 was observed between genotypes JI-338 and RG-111. Similarly minimum similarity value of 0.88 was observed between genotypes RG-2821 and RG-3017 and these genotypes are highly diverse at genetic level.

The dendrogram was constructed using UPGMA based on Jaccard's similarity coefficient through NTSYSpc-2.02i software for RAPD data of twenty castor genotypes (Table 5 and Fig. 1). The RAPD cluster tree analysis of twenty *R. communis* L. genotype showed that they were mainly divided into main two clusters at a similarity of 66% (Fig 1). The genotypes were grouped into two main clusters: cluster-I and cluster-II which shared 66% similarity. The cluster-I was divided into two subclusters-A and B both contained a total of 17 genotypes. Subcluster-A was further bifurcated into two groups A1 and A2 which has nearly 72% likeness. Group A1 was further divided into subgroup A1(a) and A1(b). Subgroup A1(a) consist of 7 genotypes such as JI-344**,** JI-384, JI-386, RG-2022**,** RG-2787**,** RG-2819**,** RG-2719 and RG-1963 having nearly 76% similarity while subgroup A1(b) consist of 6 genotypes such as RG-109, RG-1631, RG-2821, RG-3017, RG-3018 and RG-2829 having nearly 76% similarity. Subgroup A2 consists of only two genotypes such as JI-357 and RG-18 having nearly 76% similarity. Subcluster B consists of only one genotype RG-111 having nearly 70% similarity with subcluster A. The cluster-II consisted of only three genotypes such as JI-259, JI-338 and JI-342 and were the most diverse genotypes among all twenty genotypes.

**ISSR band pattern:** Fourty ISSR primers were used for the present investigation, twenty-three primers showed 78.54% amplification in all genotypes. The 10 ISSR primers, total 142 amplified bands were observed of which 118 were polymorphic. Out of 118 polymorphic bands, 109 were shared polymorphic and 9 bands were unique polymorphic (Table 6). The total number of bands observed for every primer was recorded separately and polymorphic bands percentage was calculated subsequently (Table 6). The total number of amplified bands varied between 2 (UBC-828) and 11 (UBC-848) which an average 5.13 per primer. The polymorphism percentage ranged from as low as 0% (UBC-853) to as high as 100% in six primers (UBC-809, UBC-811, UBC-823, UBC-825, UBC-829, UBC-830, UBC-843, UBC-847 and UBC-848). Average polymorphism across all the 20 genotypes of *R. communis* L. was found to be 78.54%. Overall size of PCR amplified products ranged between 100 bp to 2342 bp. Similar to present finding, Goodarzi *et al.* (2015) [8] evaluated 12 castor accessions using ISSR markers of UBC series. A total of 166 bands showed amplification and out of that 116 bands were polymorphic with 68.89 % polymorphism. Earlier, Tomar *et al.* (2014) [26] screened 25 castor genotypes using 60 ISSR primers of UBC series, out of which 27 primers were amplified a total of 256 fragments with an average of 9.4 fragments per primer.

ISSR similarity matrices of 20 *R, communis* L. genotypes revealed the relationship among them (Table 7). The similarity indices between different genotypes ranged from 0.41 to 0.77 i.e. 41-77%. Maximum similarity value of 0.77 was observed in JI-386 and RG-2819. While minimum similarity value of 0.41 was observed in JI-259 and RG-1631. The low ranged of similarity showed that genotypes are genetically more diverse and highly polymorphic.

Similarity index and cluster analysis for ISSR data of twenty castor genotypes was done by Jaccard's coefficient and UPGMA using NTSYSpc-2.02i software. The ISSR cluster analysis of 20 *R. communis* L. genotype showed that they were mainly divided into two major clusters at similarity coefficient of 0.51 (Fig. 2). The genotypes were grouped into two main clusters-I and II with an average similarity of 51%. The cluster-I comprised of two subclusters A and B with 55% likeness. Subcluster A was further divided into group A1 and A2 having 56% relatedness. Group A1 was further divided into subgroups A1 (a) and A1(b) having nearly 56% relatedness. Subgroup A1(a) consisted of 14 genotypes *viz.*, JI-342**,** JI-344**,** RG-18, JI-357, RG-1963, RG-2787, RG-2022,

RG-2829, RG-3017, RG-3018, JI-386, RG-2819, RG-2719 and RG-2821 while, subgroup A1(b) consisted of only one genotype which was RG-1631. Subgroup A2 consists of only two genotypes such as RG-109 and RG-111 having nearly 67% similarity. Subcluster B consisted of one genotype which was JI-384. Cluster-II consisted of two genotypes JI-259 and JI-338 and were the most diverse among all genotypes.

**Combined RAPD and ISSR analysis:** The ISSR and RAPD data were combined for UPGMA cluster analysis. The UPGMA dendrogram thus obtained from the cluster analysis of ISSR and RAPD data is shown in Fig. 3. Jaccard's similarity coefficient ranged from 0.69 to 0.93. The matrices for RAPD and ISSR markers were also compared using Mantel's test (Mantel, 1967) [13] for matrix correspondence. The correlation between the matrices of cophenetic correlation values for the dendrogram based on RAPD and ISSR data was low (r = 0.79). The clustering pattern of the genotypes in the combined analysis remained akin to the RAPD dendrogram, while the ISSR-based dendrogram showed some variation in the clustering of castor genotypes.

Cluster analysis performed from combining data of both markers generated a dendrogram that separated the genotypes into two distinct clusters. Cluster-I was divided into two subclusters A and B with nearly 77% similarity (Table 8). Subcluster A was divided into two groups A1 and A2 with nearly 67% similarity. Subcluster A1 consisted of seven genotypes *viz.*, RG-109, RG-1631, RG-2719, RG-2821, RG-3017, RG-3018 and RG-2829 having nearly 83% similarity. Subcluster A2 was divided into two groups A2(a) and A2(b) with nearly 81% similarity. Group A2(a) consisted of five genotypes JI-344, RG-2022, RG-2787, RG-2819 and RG-1963 while, group A2(b) consisted of four genotypes JI-357, JI-384, RG-18, and JI-386. Subcluster B consisted of one genotype RG-111 having 77% similarity. The cluster-II consisted of three genotypes JI-259, JI-338 and JI-342 and were the most diverse genotypes among all the twenty genotypes.

## Conclusion

Based on the molecular markers for castor genotypes, it was concluded that RAPD, ISSR and SSR are most reliable to distinguish castor genotypes. RAPD primers OPF-07 (544), OPF-09 (973, 503 and 438), OPM-07 (2378), OPM-09 (416), OPM-10 (1128), OPN-08 (172), OPO-04 (108), OPS-07 (308) and OPT-01 (601) and ISSR primers ISSR-3 (1578), UBC-809 (1432. 1293 and 599), UBC-826 (930 and 637), UBC-867 (414 and 167) and UBC-874 (742) amplified unique and genotype specific bands to discriminate genotypes. The results of the present study showed that, castor genotypes constitute a broad genetic base rich for a breeding and improvement program. From the clustering patterns and the genetic relationship obtained, selection for breeding programmes can be done from the different clusters realized to capture in entirety the available gene pool.

**Table 1:** List of castor genotypes with its origin

| Sr. No. | Name of the genotypes | Source |
|---|---|---|
| 1 | JI-259 | JAU, Junagadh |
| 2 | JI-338 | JAU, Junagadh |
| 3 | JI-342 | JAU, Junagadh |
| 4 | JI-344 | JAU, Junagadh |
| 5 | JI-357 | JAU, Junagadh |
| 6 | JI-384 | JAU, Junagadh |
| 7 | JI-386 | JAU, Junagadh |
| 8 | RG-18 | IIOR, Hyderabad |
| 9 | RG-109 | IIOR, Hyderabad |
| 10 | RG-111 | IIOR, Hyderabad |
| 11 | RG-1631 | IIOR, Hyderabad |
| 12 | RG-1963 | IIOR, Hyderabad |
| 13 | RG-2022 | IIOR, Hyderabad |
| 14 | RG-2719 | IIOR, Hyderabad |
| 15 | RG-2787 | IIOR, Hyderabad |
| 16 | RG-2819 | IIOR, Hyderabad |
| 17 | RG-2821 | IIOR, Hyderabad |
| 18 | RG-2829 | IIOR, Hyderabad |
| 19 | RG-3017 | IIOR, Hyderabad |
| 20 | RG-3018 | IIOR, Hyderabad |

**Table 2:** Details of RAPD primers used in molecular analysis of castor genotypes

| Sr. No. | RAPD Primer | Sequence 5'- 3' | GC (%) | Tm (ºC) |
|---|---|---|---|---|
| 1 | OPF-04 | 5'-GGTGATCAGG-3' | 60 | 25.0 |
| 2 | OPF-06 | 5'-GTGGGCTGAC-3' | 60 | 27.0 |
| 3 | OPF-09 | 5'-CCAAGTTACC-3' | 70 | 25.0 |
| 4 | OPM-02 | 5'-GTTGGTGGCT-3' | 60 | 25.0 |
| 5 | OPM-04 | 5'-ACAACGCCTC-3' | 70 | 25.0 |
| 6 | OPM-07 | 5'-GGCGGTTGTC-3' | 60 | 27.0 |
| 7 | OPM-09 | 5'-CCGTGACTCA-3' | 60 | 25.0 |
| 8 | OPM-10 | 5'-GTCTTGCGGA-3' | 70 | 25.0 |
| 9 | OPN-01 | 5'-TCTGGCGCAC-3' | 70 | 27.0 |
| 10 | OPN-02 | 5'-CTCACGTTGG-3' | 60 | 25.0 |
| 11 | OPN-06 | 5'-ACCAGGGGCA-3' | 60 | 27.0 |
| 12 | OPN-08 | 5'-GGTACTCCCC-3' | 70 | 27.0 |
| 13 | OPO-04 | 5'-GAGACGCGCA-3' | 70 | 25.0 |
| 14 | OPO-09 | 5'-ACCTCAGCTC-3' | 60 | 25.0 |
| 15 | OPO-10 | 5'-TGCCGGCTTG-3' | 60 | 27.0 |
| 16 | OPP-07 | 5'-AAGTCCGCTC-3' | 60 | 25.0 |
| 17 | OPR-05 | 5'-TCCCACGCAA-3' | 60 | 25.0 |
| 18 | OPR-08 | 5'-TCAGAGCGCC-3' | 70 | 27.0 |
| 19 | OPS-01 | 5'-GTCCATGCCA-3' | 70 | 25.0 |
| 20 | OPS-03 | 5'-GACCTAGTCC-3' | 60 | 25.0 |
| 21 | OPS-07 | 5'-CCC GTTGCCT -3' | 60 | 27.0 |
| 22 | OPT-01 | 5'-TCCGATGCTG-3' | 60 | 25.0 |
| 23 | OPT-03 | 5'-CAGAGGTCTT-3' | 70 | 27.0 |
| 24 | OPT-09 | 5'-TCCGATGTGA-3' | 70 | 25.0 |

**Table 3:** Details of ISSR primers used in molecular analysis of castor genotypes

| Sr. No. | ISSR Primer | Sequence (5'→3') | GC (%) | Tm (ºC) |
|---|---|---|---|---|
| 1 | ISSR 3 | AGCAGCAGCAGCAGCAGCT | 67.0 | 68.6 |
| 2 | ISSR 6 | AGAGAGAGAGAGAGAGG | 53.0 | 46.6 |
| 3 | ISSR 7 | GAGAGAGAGAGAGAGAT | 47.0 | 42.9 |
| 4 | UBC-807 | AGAGAGAGAGAGAGAGT | 47.0 | 42.4 |
| 5 | UBC-809 | AGAGAGAGAGAGAGAGA | 52.9 | 46.5 |
| 6 | UBC-811 | GAGAGAGAGAGAGAGAC | 52.9 | 43.2 |
| 7 | UBC-818 | CACACACACACACACAG | 53.0 | 52.0 |
| 8 | UBC-823 | TCTCTCTCTCTCTCTCC | 47.4 | 52.9 |
| 9 | UBC-825 | ACACACACACACACACT | 47.0 | 49.2 |
| 10 | UBC-826 | ACACACACACACACACC | 53.0 | 53.3 |
| 11 | UBC-828 | TGTGTGTGTGTGTGTGA | 47.1 | 50.0 |
| 12 | UBC-829 | TGTGTGTGTGTGTGTGC | 53.0 | 56.3 |
| 13 | UBC-830 | TGTGTGTGTGTGTGTGG | 56.1 | 52.9 |
| 14 | UBC-843 | CTCTCTCTCTCTCTCTA | 44.4 | 37.6 |
| 15 | UBC-847 | CACACACACACACACAT | 44.4 | 53.7 |
| 16 | UBC-848 | CACACACACACACACAC | 50.0 | 46.0 |
| 17 | UBC-853 | TCTCTCTCTCTCTCTCT | 53.0 | 53.7 |
| 18 | UBC-855 | ACACACACACACACACT | 44.44 | 51.9 |
| 19 | UBC-857 | ACACACACACACACACG | 44.4 | 52.0 |
| 20 | UBC-858 | TGTGTGTGTGTGTGTGT | 53 | 60.5 |
| 21 | UBC-866 | CTCCTCCTCCTCCTCCTC | 67.0 | 60.4 |
| 22 | UBC-867 | GCGGCGGCGGCGGCGGCG | 100.0 | 88.5 |
| 23 | UBC-874 | CCCTCCCTCCCTCCCT | 75 | 59.4 |

**Table 4:** Size, number of amplified bands, per cent polymorphism and PIC obtained by RAPD primers in 20 castor genotypes

| Sr. No. | RAPD Primer | Band Size (bp) | Total No. of Bands (A) | Polymorphic Bands (B) | | | Mono-Morphic Band | % Poly-Morphism (B/A) | PIC* | RPI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | S | U | T | | | | |
| 1 | OPF-04 | 123-889 | 5 | 3 | 1 | 4 | 1 | 80.0 | 0.70 | 3.50 |
| 2 | OPF-06 | 102-422 | 3 | 2 | 0 | 2 | 1 | 66.6 | 0.48 | 1.44 |
| 3 | OPF-09 | 542-973 | 6 | 3 | 3 | 6 | 0 | 100.0 | 0.65 | 3.90 |
| 4 | OPM-02 | 783-986 | 2 | 2 | 0 | 2 | 0 | 100.0 | 0.44 | 0.88 |
| 5 | OPM-04 | 592-1432 | 5 | 5 | 0 | 5 | 0 | 100.0 | 0.76 | 3.80 |
| 6 | OPM-07 | 369-2378 | 13 | 8 | 1 | 9 | 4 | 69.2 | 0.90 | 11.70 |
| 7 | OPM-09 | 244-2219 | 4 | 1 | 1 | 2 | 2 | 50.0 | 0.67 | 2.68 |
| 8 | OPM-10 | 496-1810 | 6 | 1 | 1 | 2 | 4 | 33.3 | 0.78 | 4.68 |
| 9 | OPN-01 | 358-1982 | 7 | 7 | 0 | 7 | 0 | 100.0 | 0.79 | 5.53 |
| 10 | OPN-02 | 231-1215 | 5 | 0 | 0 | 0 | 5 | 0.0 | 0.77 | 3.85 |
| 11 | OPN-06 | 169-1102 | 5 | 4 | 0 | 4 | 1 | 80.0 | 0.73 | 3.65 |
| 12 | OPN-08 | 172-504 | 6 | 1 | 1 | 2 | 4 | 33.3 | 0.78 | 3.90 |
| 13 | OPO-04 | 108-653 | 7 | 4 | 1 | 5 | 2 | 71.4 | 0.79 | 5.53 |
| 14 | OPO-09 | 356-1073 | 5 | 5 | 0 | 5 | 0 | 100.0 | 0.62 | 3.10 |
| 15 | OPO-10 | 532-1549 | 3 | 3 | 0 | 3 | 0 | 100.0 | 0.55 | 1.65 |
| 16 | OPP-07 | 313-2548 | 9 | 9 | 0 | 9 | 0 | 100.0 | 0.83 | 7.47 |
| 17 | OPR-05 | 143-2365 | 9 | 1 | 0 | 1 | 8 | 11.1 | 0.85 | 7.65 |
| 18 | OPR-08 | 275-1925 | 6 | 0 | 0 | 0 | 6 | 0.0 | 0.82 | 4.92 |
| 19 | OPS-01 | 139-1025 | 7 | 1 | 0 | 1 | 6 | 14.2 | 0.80 | 5.60 |
| 20 | OPS-03 | 426-1453 | 7 | 7 | 0 | 7 | 0 | 100.0 | 0.81 | 5.67 |
| 21 | OPS-07 | 299-2337 | 9 | 6 | 1 | 7 | 2 | 77.7 | 0.83 | 7.47 |
| 22 | OPT-01 | 159-1021 | 5 | 2 | 1 | 3 | 2 | 60.0 | 0.73 | 3.65 |
| 23 | OPT-03 | 205-929 | 4 | 4 | 0 | 4 | 0 | 100.0 | 0.67 | 2.68 |
| 24 | OPT-09 | 250-1576 | 9 | 6 | 0 | 6 | 3 | 66.6 | 0.64 | 5.76 |
| **Total** | | | 147 | 85 | 11 | 96 | 51 | - | - | - |
| **Average** | | | - | - | - | 4.0 | 2.12 | 66.68 | 0.72 | 4.61 |

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

**Table 5:** Jaccard's similarity coefficient of 20 castor genotypes based on RAPD data analysis

| | JI-259 | JI-338 | JI-342 | JI-344 | JI-357 | JI-384 | JI-386 | RG-18 | RG-109 | RG-111 | RG-1631 | RG-1963 | RG-2022 | RG-2719 | RG-2787 | RG-2819 | RG-2821 | RG-2829 | RG-3017 | RG-3018 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JI-259 | 1.00 | | | | | | | | | | | | | | | | | | | |
| JI-338 | 0.88 | 1.00 | | | | | | | | | | | | | | | | | | |
| JI-342 | 0.82 | 0.79 | 1.00 | | | | | | | | | | | | | | | | | |
| JI-344 | 0.71 | 0.70 | 0.75 | 1.00 | | | | | | | | | | | | | | | | |
| JI-357 | 0.68 | 0.74 | 0.68 | 0.75 | 1.00 | | | | | | | | | | | | | | | |
| JI-384 | 0.69 | 0.70 | 0.66 | 0.77 | 0.74 | 1.00 | | | | | | | | | | | | | | |
| JI-386 | 0.66 | 0.66 | 0.63 | 0.75 | 0.77 | 0.80 | 1.00 | | | | | | | | | | | | | |
| RG-18 | 0.67 | 0.68 | 0.67 | 0.68 | 0.76 | 0.79 | 0.75 | 1.00 | | | | | | | | | | | | |
| RG-109 | 0.62 | 0.64 | 0.61 | 0.68 | 0.71 | 0.66 | 0.77 | 0.70 | 1.00 | | | | | | | | | | | |
| RG-111 | 0.59 | 0.58 | 0.60 | 0.67 | 0.69 | 0.65 | 0.71 | 0.68 | 0.69 | 1.00 | | | | | | | | | | |
| RG-1631 | 0.60 | 0.62 | 0.62 | 0.73 | 0.72 | 0.72 | 0.73 | 0.70 | 0.76 | 0.76 | 1.00 | | | | | | | | | |
| RG-1963 | 0.63 | 0.60 | 0.63 | 0.74 | 0.75 | 0.73 | 0.74 | 0.69 | 0.67 | 0.71 | 0.79 | 1.00 | | | | | | | | |
| RG-2022 | 0.68 | 0.69 | 0.72 | 0.77 | 0.72 | 0.82 | 0.77 | 0.72 | 0.71 | 0.72 | 0.76 | 0.75 | 1.00 | | | | | | | |
| RG-2719 | 0.65 | 0.65 | 0.65 | 0.73 | 0.75 | 0.75 | 0.81 | 0.72 | 0.80 | 0.73 | 0.81 | 0.74 | 0.82 | 1.00 | | | | | | |
| RG-2787 | 0.70 | 0.67 | 0.71 | 0.79 | 0.74 | 0.77 | 0.75 | 0.69 | 0.70 | 0.70 | 0.75 | 0.76 | 0.84 | 0.79 | 1.00 | | | | | |
| RG-2819 | 0.68 | 0.65 | 0.68 | 0.79 | 0.74 | 0.82 | 0.80 | 0.75 | 0.72 | 0.73 | 0.78 | 0.83 | 0.83 | 0.85 | 0.85 | 1.00 | | | | |
| RG-2821 | 0.64 | 0.68 | 0.64 | 0.70 | 0.72 | 0.75 | 0.73 | 0.77 | 0.75 | 0.71 | 0.79 | 0.72 | 0.73 | 0.82 | 0.71 | 0.82 | 1.00 | | | |
| RG-2829 | 0.65 | 0.66 | 0.67 | 0.76 | 0.72 | 0.78 | 0.76 | 0.69 | 0.75 | 0.71 | 0.77 | 0.80 | 0.79 | 0.77 | 0.73 | 0.83 | 0.81 | 1.00 | | |
| RG-3017 | 0.60 | 0.62 | 0.62 | 0.66 | 0.69 | 0.71 | 0.72 | 0.70 | 0.77 | 0.67 | 0.78 | 0.72 | 0.73 | 0.80 | 0.71 | 0.76 | 0.88 | 0.81 | 1.00 | |
| RG-3018 | 0.64 | 0.68 | 0.64 | 0.70 | 0.75 | 0.77 | 0.78 | 0.77 | 0.77 | 0.73 | 0.81 | 0.74 | 0.80 | 0.82 | 0.72 | 0.82 | 0.86 | 0.81 | 0.81 | 1.00 |

**Table 6:** Size, number of amplified bands, per cent polymorphism and PIC obtained by ISSR primers in the 20 castor genotypes

| Sr. No. | ISSR Primer | Band Size (bp) | Total No. of Bands (A) | Polymorphic Bands (B) | | | Mono-Morphic Band | % Poly-Morphism (B/A) | PIC* | IPI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | S | U | T | | | | |
| 1 | ISSR 3 | 216-1578 | 7 | 5 | 1 | 6 | 1 | 85.7 | 0.75 | 5.25 |
| 2 | ISSR 6 | 156-1813 | 7 | 6 | 0 | 6 | 1 | 85.7 | 0.81 | 5.67 |
| 3 | ISSR 7 | 190-2078 | 6 | 5 | 0 | 5 | 1 | 83.3 | 0.76 | 4.56 |
| 4 | UBC-807 | 145-707 | 5 | 1 | 0 | 1 | 4 | 20.0 | 0.78 | 3.90 |
| 5 | UBC-809 | 278-1432 | 6 | 3 | 3 | 6 | 0 | 100.0 | 0.70 | 4.20 |
| 6 | UBC-811 | 330-1600 | 4 | 4 | 0 | 4 | 0 | 100.0 | 0.50 | 2.00 |
| 7 | UBC-818 | 335-971 | 3 | 2 | 0 | 2 | 1 | 66.6 | 0.58 | 1.74 |
| 8 | UBC-823 | 640-1032 | 3 | 3 | 0 | 3 | 0 | 100.0 | 0.42 | 1.26 |
| 9 | UBC-825 | 391-1895 | 3 | 3 | 0 | 3 | 0 | 100.0 | 0.59 | 1.77 |
| 10 | UBC-826 | 315-1686 | 9 | 6 | 2 | 8 | 1 | 88.8 | 0.83 | 7.47 |
| 11 | UBC-828 | 452-849 | 2 | 1 | 0 | 1 | 1 | 50.0 | 0.42 | 0.84 |
| 12 | UBC-829 | 151-1411 | 8 | 8 | 0 | 8 | 0 | 100.0 | 0.83 | 6.64 |
| 13 | UBC-830 | 182-1008 | 5 | 5 | 0 | 5 | 0 | 100.0 | 0.79 | 3.95 |
| 14 | UBC-843 | 153-2252 | 10 | 10 | 0 | 10 | 0 | 100.0 | 0.86 | 8.60 |
| 15 | UBC-847 | 100-1306 | 10 | 10 | 0 | 10 | 0 | 100.0 | 0.88 | 8.80 |
| 16 | UBC-848 | 168-2342 | 11 | 11 | 0 | 11 | 0 | 100.0 | 0.86 | 9.46 |
| 17 | UBC-853 | 181-985 | 3 | 0 | 0 | 0 | 3 | 0.0 | 0.66 | 1.98 |
| 18 | UBC-855 | 384-1112 | 4 | 2 | 0 | 2 | 2 | 50.0 | 0.69 | 2.76 |
| 19 | UBC-857 | 118-911 | 8 | 6 | 0 | 6 | 2 | 75.0 | 0.83 | 6.64 |
| 20 | UBC-858 | 128-995 | 6 | 5 | 0 | 5 | 1 | 83.3 | 0.82 | 4.92 |
| 21 | UBC-866 | 202-1377 | 7 | 4 | 0 | 4 | 3 | 57.1 | 0.83 | 5.81 |
| 22 | UBC-867 | 312-1146 | 7 | 4 | 2 | 6 | 1 | 85.7 | 0.76 | 5.32 |
| 23 | UBC-874 | 153-958 | 8 | 5 | 1 | 6 | 2 | 75.0 | 0.80 | 6.40 |
| Total | | | 142 | 109 | 9 | 118 | 24 | - | - | - |
| Average | | | - | - | - | 5.13 | 1.04 | 78.54 | 0.72 | 4.78 |

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

**Table 7:** Jaccard's similarity coefficient of 20 castor genotypes based on ISSR data analysis

| | JI-259 | JI-338 | JI-342 | JI-344 | JI-357 | JI-384 | JI-386 | RG-18 | RG-109 | RG-111 | RG-1631 | RG-1963 | RG-2022 | RG-2719 | RG-2787 | RG-2819 | RG-2821 | RG-2829 | RG-3017 | RG-3018 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JI-259 | 1.00 | | | | | | | | | | | | | | | | | | | |
| JI-338 | 0.57 | 1.00 | | | | | | | | | | | | | | | | | | |
| JI-342 | 0.56 | 0.62 | 1.00 | | | | | | | | | | | | | | | | | |
| JI-344 | 0.57 | 0.53 | 0.73 | 1.00 | | | | | | | | | | | | | | | | |
| JI-357 | 0.59 | 0.60 | 0.59 | 0.64 | 1.00 | | | | | | | | | | | | | | | |
| JI-384 | 0.49 | 0.43 | 0.58 | 0.53 | 0.60 | 1.00 | | | | | | | | | | | | | | |
| JI-386 | 0.50 | 0.47 | 0.53 | 0.66 | 0.60 | 0.61 | 1.00 | | | | | | | | | | | | | |
| RG-18 | 0.52 | 0.50 | 0.60 | 0.71 | 0.57 | 0.53 | 0.67 | 1.00 | | | | | | | | | | | | |
| RG-109 | 0.49 | 0.49 | 0.60 | 0.58 | 0.55 | 0.55 | 0.55 | 0.58 | 1.00 | | | | | | | | | | | |
| RG-111 | 0.51 | 0.46 | 0.49 | 0.54 | 0.56 | 0.52 | 0.59 | 0.62 | 0.67 | 1.00 | | | | | | | | | | |
| RG-1631 | 0.41 | 0.51 | 0.58 | 0.58 | 0.61 | 0.51 | 0.53 | 0.55 | 0.57 | 0.52 | 1.00 | | | | | | | | | |
| RG-1963 | 0.50 | 0.58 | 0.68 | 0.60 | 0.65 | 0.51 | 0.52 | 0.56 | 0.59 | 0.48 | 0.65 | 1.00 | | | | | | | | |
| RG-2022 | 0.48 | 0.49 | 0.58 | 0.58 | 0.62 | 0.60 | 0.72 | 0.53 | 0.54 | 0.55 | 0.56 | 0.56 | 1.00 | | | | | | | |
| RG-2719 | 0.51 | 0.49 | 0.60 | 0.63 | 0.56 | 0.57 | 0.75 | 0.69 | 0.59 | 0.58 | 0.51 | 0.62 | 0.66 | 1.00 | | | | | | |
| RG-2787 | 0.52 | 0.55 | 0.63 | 0.57 | 0.66 | 0.52 | 0.58 | 0.58 | 0.58 | 0.56 | 0.59 | 0.68 | 0.69 | 0.67 | 1.00 | | | | | |
| RG-2819 | 0.45 | 0.44 | 0.53 | 0.59 | 0.50 | 0.57 | 0.77 | 0.60 | 0.55 | 0.49 | 0.50 | 0.53 | 0.67 | 0.71 | 0.55 | 1.00 | | | | |
| RG-2821 | 0.51 | 0.41 | 0.45 | 0.52 | 0.51 | 0.52 | 0.64 | 0.53 | 0.51 | 0.51 | 0.44 | 0.48 | 0.60 | 0.61 | 0.54 | 0.60 | 1.00 | | | |
| RG-2829 | 0.52 | 0.55 | 0.61 | 0.60 | 0.65 | 0.51 | 0.61 | 0.59 | 0.58 | 0.54 | 0.55 | 0.68 | 0.67 | 0.70 | 0.67 | 0.61 | 0.57 | 1.00 | | |
| RG-3017 | 0.51 | 0.47 | 0.55 | 0.53 | 0.62 | 0.53 | 0.60 | 0.51 | 0.57 | 0.55 | 0.54 | 0.64 | 0.69 | 0.62 | 0.63 | 0.56 | 0.60 | 0.63 | 1.00 | |
| RG-3018 | 0.52 | 0.60 | 0.58 | 0.53 | 0.65 | 0.57 | 0.63 | 0.53 | 0.57 | 0.50 | 0.55 | 0.65 | 0.71 | 0.65 | 0.65 | 0.63 | 0.57 | 0.74 | 0.66 | 1.00 |

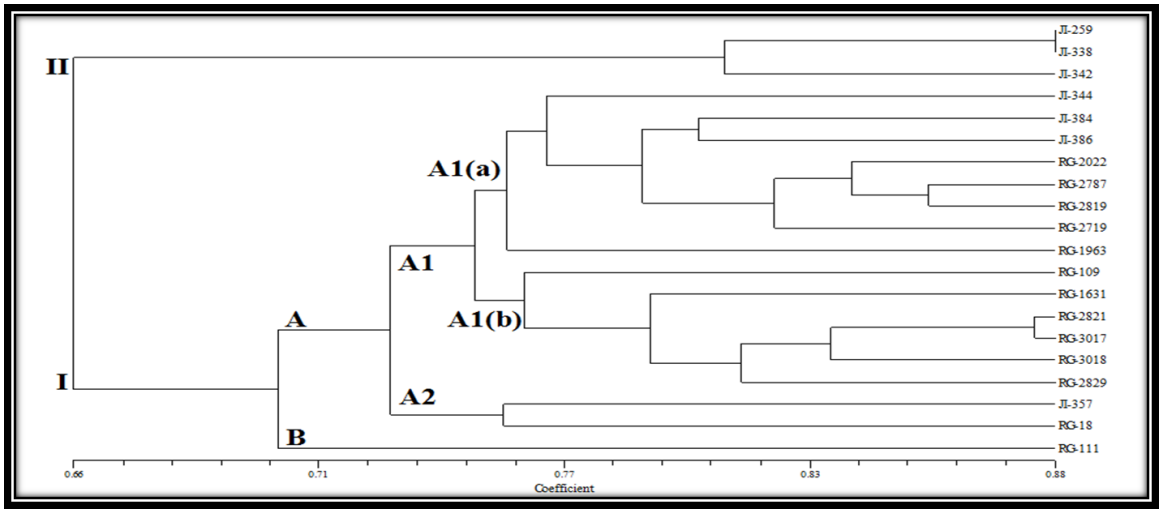


**Fig 1:** Dendrogram depicting the genetic relationship among 20 castor genotypes based on data of RAPD

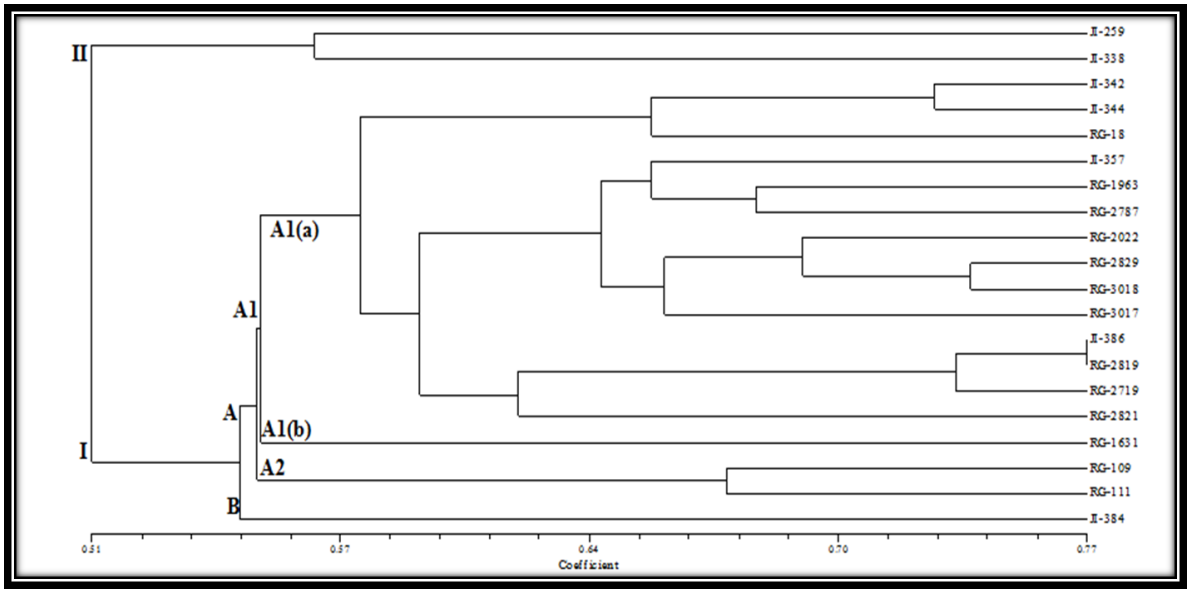


**Fig 2:** Dendrogram depicting the genetic relationship among 20 castor genotypes based on data of ISSR

**Table 8:** Jaccard's similarity coefficient of 20 castor genotypes based on combined RAPD and ISSR data analysis

| | JI-259 | JI-338 | JI-342 | JI-344 | JI-357 | JI-384 | JI-386 | RG-18 | RG-109 | RG-111 | RG-1631 | RG-1963 | RG-2022 | RG-2719 | RG-2787 | RG-2819 | RG-2821 | RG-2829 | RG-3017 | RG-3018 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| JI-259 | 1.00 | | | | | | | | | | | | | | | | | | | |
| JI-338 | 0.93 | 1.00 | | | | | | | | | | | | | | | | | | |
| JI-342 | 0.88 | 0.87 | 1.00 | | | | | | | | | | | | | | | | | |
| JI-344 | 0.80 | 0.80 | 0.83 | 1.00 | | | | | | | | | | | | | | | | |
| JI-357 | 0.78 | 0.83 | 0.77 | 0.83 | 1.00 | | | | | | | | | | | | | | | |
| JI-384 | 0.78 | 0.80 | 0.75 | 0.84 | 0.82 | 1.00 | | | | | | | | | | | | | | |
| JI-386 | 0.75 | 0.76 | 0.71 | 0.82 | 0.84 | 0.86 | 1.00 | | | | | | | | | | | | | |
| RG-18 | 0.78 | 0.79 | 0.77 | 0.78 | 0.84 | 0.86 | 0.82 | 1.00 | | | | | | | | | | | | |
| RG-109 | 0.71 | 0.73 | 0.69 | 0.75 | 0.78 | 0.73 | 0.82 | 0.78 | 1.00 | | | | | | | | | | | |
| RG-111 | 0.69 | 0.69 | 0.69 | 0.75 | 0.77 | 0.73 | 0.78 | 0.77 | 0.76 | 1.00 | | | | | | | | | | |
| RG-1631 | 0.69 | 0.71 | 0.69 | 0.80 | 0.79 | 0.78 | 0.79 | 0.78 | 0.82 | 0.82 | 1.00 | | | | | | | | | |
| RG-1963 | 0.71 | 0.70 | 0.71 | 0.81 | 0.82 | 0.80 | 0.80 | 0.78 | 0.73 | 0.78 | 0.84 | 1.00 | | | | | | | | |
| RG-2022 | 0.76 | 0.78 | 0.80 | 0.83 | 0.80 | 0.87 | 0.82 | 0.80 | 0.77 | 0.78 | 0.82 | 0.80 | 1.00 | | | | | | | |
| RG-2719 | 0.73 | 0.75 | 0.73 | 0.80 | 0.82 | 0.82 | 0.86 | 0.80 | 0.85 | 0.80 | 0.86 | 0.80 | 0.86 | 1.00 | | | | | | |
| RG-2787 | 0.78 | 0.76 | 0.78 | 0.84 | 0.81 | 0.83 | 0.81 | 0.77 | 0.76 | 0.77 | 0.80 | 0.82 | 0.88 | 0.84 | 1.00 | | | | | |
| RG-2819 | 0.76 | 0.75 | 0.76 | 0.84 | 0.81 | 0.87 | 0.85 | 0.82 | 0.78 | 0.80 | 0.83 | 0.87 | 0.88 | 0.89 | 0.89 | 1.00 | | | | |
| RG-2821 | 0.74 | 0.78 | 0.73 | 0.78 | 0.80 | 0.82 | 0.80 | 0.84 | 0.82 | 0.79 | 0.85 | 0.80 | 0.80 | 0.87 | 0.78 | 0.87 | 1.00 | | | |
| RG-2829 | 0.74 | 0.76 | 0.75 | 0.82 | 0.79 | 0.84 | 0.82 | 0.78 | 0.80 | 0.78 | 0.82 | 0.85 | 0.84 | 0.83 | 0.79 | 0.87 | 0.86 | 1.00 | | |
| RG-3017 | 0.70 | 0.73 | 0.71 | 0.74 | 0.78 | 0.78 | 0.79 | 0.79 | 0.83 | 0.75 | 0.84 | 0.78 | 0.79 | 0.86 | 0.78 | 0.82 | 0.92 | 0.86 | 1.00 | |
| RG-3018 | 0.74 | 0.78 | 0.73 | 0.78 | 0.83 | 0.84 | 0.84 | 0.84 | 0.83 | 0.80 | 0.86 | 0.81 | 0.86 | 0.87 | 0.79 | 0.87 | 0.90 | 0.86 | 0.86 | 1.00 |

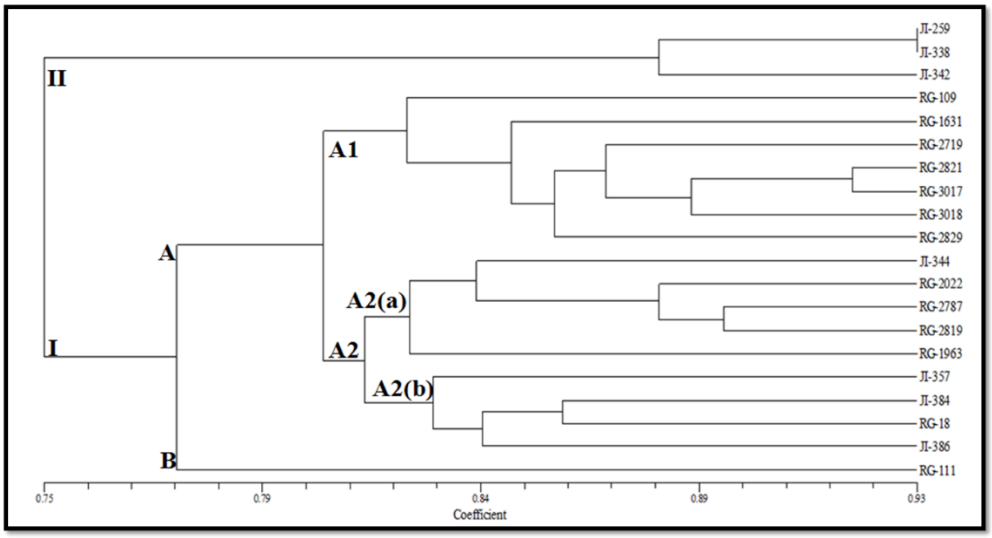


**Fig 3:** Dendrogram depicting the genetic relationship among 20 castor genotypes based on pooled data of RAPD and ISSR molecular markers

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