

Genetic profiling system optimization for *Ricinus communis* L. using microsatellite markers

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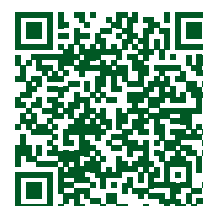
Abstract: The objective of this study was to assess the genetic diversity of *Ricinus communis* L., a species with a mixed mating system that requires optimized molecular markers for accurate characterization, within the set of 108 castor genotypes using 20 SSR primers. A total of 59 alleles were identified, averaging 3.7 per locus. Observed heterozygosity (0.169) was lower than expected (0.400), suggesting an excess of homozygotes due to selection or inbreeding. Shannon's diversity index ranged from 0.397 to 1.176, averaging 0.674. The polymorphism information content values varied from 0.045 to 0.628, indicating moderate to high marker informativeness. Markers Rc05, Rc85A, Rc85B, and Rc251 were the most informative, allowing genotype discrimination and paternity testing (> 90%). These SSR markers proved efficient for the genetic characterization of *R. communis* and can support breeding programs. Future studies should focus on refining marker selection for better discrimination and genetic diversity conservation.

Keywords: Castor bean, genetic diversity, probability of identity, paternity test

INTRODUCTION

The castor bean (*Ricinus communis* L.), from the *Euphorbiaceae* family, is native to the Ethiopian region of East Africa and is cultivated in tropical and subtropical regions (Priyanka et al. 2021). With a chromosome number of $2n = 2x = 20$, it is a fast-growing plant that reaches heights of 1 to 6 meters (Chaudhari et al. 2021). Its pollination, both cross-pollination and self-pollination, occurs through wind (anemophily), with up to 50% cross-pollination (Allan et al. 2008, Oliveira et al. 2012, Chaudhary et al. 2021). This species has high commercial value due to its oil and is cultivated in more than 30 countries, such as India, Brazil, and China, representing about 19% of the global oilseed area and contributing 9% of global vegetable oil production (Andreazza et al. 2013, Manjunath and Sannappa 2014, Landoni et al. 2023).

With domestication, many ancient and wild varieties are being lost in favor of modern ones, raising the need to conserve cultivated plant germplasm. The collection, preservation, and evaluation of germplasm resources are essential for plant breeding (Cuevas and Prom 2020). However, with the increase in collected germplasm, the screening and utilization process becomes difficult (Chen et al. 2017). Phenotypic traits are valuable for the construction of germplasms but are easily affected by environmental conditions and can be difficult to measure



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accurately (Chen et al. 2017). In this regard, DNA molecular markers, developed based on polymorphisms, represent a direct method for measuring genetic diversity, rarely being affected by environmental interactions (Wang et al. 2023).

Among the various markers, microsatellites (SSRs) are widely accepted for their discriminative power and genome-wide distribution (Phumichai et al. 2015, Dharajiya et al. 2020, Koltun et al. 2024). SSRs are useful in multiple investigations, both in cultivated and wild species, including diversity analysis, evolutionary studies, gene flow estimation, variety/hybrid identification, DNA fingerprinting, linkage map construction, gene tagging, association mapping, and identification of quantitative trait loci (Miah et al. 2013). Once polymorphic molecular markers are identified, they can be used to assess genetic diversity in collections and promote unequivocal varietal identification, phylogenetic analysis, and marker-assisted breeding programs (Vivodík et al. 2020, Kim et al. 2021, Anggraeni et al. 2022, Landoni et al. 2023).

SSR markers have been applied to evaluate castor bean diversity (Phumichai et al. 2015, Dharajiya et al. 2020). Kim et al. (2021) demonstrated the utility of ISSR and RAPD markers in differentiating wild and cultivated genotypes, while Vivodík et al. (2020) highlighted the effectiveness of SSR markers in analyzing Tunisian castor accessions. Anggraeni et al. (2022) reported SSR marker associations with agronomic traits, suggesting their potential for marker-assisted selection. This study aimed to assess the genetic diversity among 108 *R. communis* L. accessions from a breeding program germplasm bank using 20 SSR markers.

MATERIAL AND METHODS

Plant material

The study was conducted at the São Manuel Experimental Farm, located in the municipality of São Manuel, State of São Paulo, belonging to the College of Agriculture (FCA) of the São Paulo State University “Júlio de Mesquita Filho” (UNESP), Botucatu-SP Campus. A total of 108 castor bean accessions from the FCA-UNESP germplasm bank were used in the study. Fully expanded leaves were collected, cleaned, and placed in paper bags for cold drying in a refrigerator. The samples were labeled and sent to genetic analysis.

DNA extraction and SSR markers

Genomic DNA extraction was performed following the protocol used by Vivodík et al. (2020), ensuring high-quality DNA for SSR amplification. The extracted DNA was quantified using a spectrophotometer, and PCR amplification followed the methodology of Kim et al. (2021). The 20 markers used for genotype characterization (Table 1) were selected based on informative content parameters and the size of the amplified products from a list of 118 markers developed from expressed gene sequences of *R. communis* (ESTs) by Qiu et al. (2010).

Data analysis

The genetic diversity parameters were calculated for each SSR locus using the GeneALEX software, version 6.51b2 (Peakall and Smouse 2012). The parameters included: the mean number of alleles per locus (N_a), the effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), Shannon’s information index (I), and the fixation index (F). Allele frequency was also calculated using the same software.

To estimate the marker combinations required to discriminate the genotypes, the probability of identity (PI) and the combined probability of identity (CPI) were calculated using GenAlex. Considering the possibility of relatedness among samples, the probability of identity for siblings (Pisibs) and the combined probability of identity for siblings (CPisibs) were also calculated. PI applies to genetically unrelated individuals, while Pisibs is specific to full siblings. Additionally, the discrimination power (CPD), which indicates the average probability of two individuals having different genotypes, and the combined discrimination power for siblings (PDSibs) were assessed.

The probability of paternity exclusion (PE) was calculated using the same software, estimating Values for three situations: trio paternity exclusion probability (PEtrio) and duo paternity exclusion. probability (PEduo), and pair paternity exclusion probability (PEpar). For each situation, the percentage of combined paternity exclusion probability (CPEtrio, CPEduo, CPEpar) was calculated.

Table 1. Summary of microsatellites data analysis. Calculation of observed Heterozygosity (Ho), Expected Heterozygosity (He), Unbiased Expected Heterozygosity (uHe), Shannon's Information Index (I) and Fixation Index (F)

Loci	N	N _a	N _e	I	H _o	H _e	uH _e	F
Rc05-1	108	6	2.691	1.176	0.269	0.628	0.631	0.573
Rc158-1	108	3	1.810	0.661	0.176	0.447	0.450	0.607
Rc176-1	108	4	1.792	0.778	0.231	0.442	0.444	0.476
Rc182-1	108	3	1.509	0.540	0.111	0.337	0.339	0.670
Rc25-1	108	4	2.109	0.819	0.287	0.526	0.528	0.454
Rc35-1	108	2	1.552	0.541	0.093	0.356	0.357	0.740
Rc45-1	108	3	1.941	0.699	0.213	0.485	0.487	0.561
Rc16-1	108	4	2.091	0.813	0.231	0.522	0.524	0.556
Rc186-1	108	3	1.048	0.126	0.028	0.045	0.046	0.389
Rc20-1	107	5	1.165	0.346	0.037	0.142	0.142	0.736
Rc251-1	108	4	1.938	0.924	0.130	0.484	0.486	0.732
Rc258-1	108	1	1.000	0.000	0.000	0.000	0.000	N/A
Rc167-1	108	2	1.726	0.612	0.139	0.421	0.423	0.670
Rc167-1	108	6	1.705	0.730	0.157	0.413	0.415	0.619
Rc256-1	108	3	2.034	0.739	0.269	0.508	0.511	0.472
Rc61-1	108	2	1.997	0.692	0.333	0.499	0.502	0.332
Rc65-1	108	3	1.282	0.397	0.111	0.220	0.221	0.495
Rc85A-1	108	6	2.327	1.136	0.204	0.570	0.573	0.643
Rc85B-1	108	4	2.196	1.017	0.204	0.545	0.547	0.626
Rc90-1	108	6	1.705	0.730	0.157	0.413	0.415	0.619
Mean	107.95	3.7	1.781	0.674	0.169	0.400	0.402	0.577
SE	0.050	0.333	0.097	0.067	0.020	0.039	0.039	0.026

N: number of individuals; Na: number of different Alleles; Ne: number of effective alleles = $1/\sum p_i^2$; p_i : relative frequency of the i th allele; I: Shannon's Information Index = $1 * \sum (p_i * \ln(p_i))$; Ho: Observed Heterozygosity = $\frac{n^2 \text{hets}}{N}$; He = Expected Heterozygosity = $1 - \sum p_i^2$; uHe: Unbiased Expected Heterozygosity = $(\frac{2N}{2N-1})$; F: Fixation Index = $1 - \frac{H_o}{H_e}$.

RESULTS AND DISCUSSION

A total of 59 alleles were identified across 20 SSR markers, with an average of 3.7 alleles per locus. The percentage of polymorphic alleles was 95%, with only one locus (Rc258) considered monomorphic. These results align with previous findings in genetic diversity assessments of castor bean (Alhaji et al. 2019, Kim et al. 2021, Anggraeni et al. 2022).

The variation in the number of alleles (Na) ranged from 1 to 6, and the effective number of alleles (Ne) ranged from 1.048 to 2.691, with an average of 1.781. Shannon's index (I) ranged from 0.397 to 1.176, with an average of 0.674, indicating considerable genetic diversity, though not uniformly distributed among the loci. These results are consistent with previous studies, such as Rukhsar et al. (2017), who, using 14 markers, identified 44 alleles with an average of 3.14 alleles per locus, and Seo et al. (2011), who analyzed 72 castor bean accessions with 28 SSR markers, finding 73 alleles with an average of 3.18 alleles per locus. Bajay et al. (2009) also reported similar averages, with 3.3 alleles per locus.

Observed heterozygosity (Ho) ranged from 0.028 to 0.333, with an average of 0.169, indicating moderate to low genetic diversity. In contrast, expected heterozygosity (He) ranged from 0.045 to 0.628, with an average of 0.400, suggesting a higher theoretical expectation of diversity compared to what was observed. Unbiased heterozygosity (uHe) was similar to He, reinforcing the discrepancy. The fixation index of 0.577 indicates an excess of homozygotes in the population, possibly resulting from inbreeding or selective pressures.

These results show low Ho values, similar to those found by Seo et al. (2011), who reported a Ho of 0.11 and He of 0.31, and by Bajay et al. (2009), with an average Ho of 0.06 and He of 0.416. The predominance of homozygotes suggests the presence of self-pollination, although castor bean has a mixed mating system (Allan et al. 2008).

The variation in the number of alleles at each locus (Na) reflects the genetic heterogeneity of the population studied. Despite the diversity of alleles, only a significant fraction contributes to effective genetic variability, as observed in the

number of effective alleles (N_e) and Shannon's index (I). This indicates that few alleles are frequent enough to maintain genetic diversity, while others may be subject to selective pressure or genetic drift, affecting their frequency (Bajay et al. 2009, Seo et al. 2011).

The probability of identity (PI) was high for most SSRs, ranging from 0.210 (Rc05) to 1.00 (Rc258), indicating a high likelihood that two random individuals share the same genetic profile. Pisibs' values were also high for related individuals (Table 2). Markers such as Rc05, Rc85A, Rc85B, and Rc251 were the most informative, allowing discrimination of all the genotypes evaluated ($CPI = 3.62 \times 10^{-3}$). To exclude individuals with the same multilocus genotype, considering relatedness among samples, eight markers were required ($CPI_{sibs} = 9.59 \times 10^{-3}$) (Table 2).

In Table 3, the 20 markers evaluated in the population of 108 *R. communis* samples are ranked in ascending order of informative content for the probability of paternity exclusion (PE). PE corresponds to the ability of a marker to exclude a randomly selected individual in the population, erroneously indicated as a parent, from the possibility of being the true parent of an offspring. When analyzing three distinct situations, it was observed that the combined analysis of the 10 most informative markers provides a trio combined paternity exclusion probability (CPETrio) of 94.8%. In contrast, the analysis using all 20 markers provides a CPETrio of 98.6%.

The CPEpar stood out as the most informative, reaching over 99% accuracy with nine markers, and reaching 99.9% with all 20 markers combined. In contrast, the CPEduo, even with 20 markers, only reached 87.4%, highlighting the difficulty in identifying the male parent without knowing the female parent. The probability of identity (PI) estimates the chance of two unrelated individuals sharing the same genotype. A CPI of 99% was achieved with four markers, while a CPisibs of 99% required eight of the most representative markers (Rc05, Rc85A, Rc85B, Rc251, Rc25, Rc16, Rc256, and Rc176). These markers were also the most informative for paternity testing, confirming 98.6% of the parents.

Most alleles (52%) had a frequency greater than 5%, while 47% were considered rare, with a frequency lower than 5%. Only the allele with 196 bp occurred in two loci (Rc182 and Rc186), which may indicate some genetic proximity. The loci with the highest number of alleles were Rc05, Rc167, Rc85A, and Rc90, each containing six alleles (Table 2).

Table 2. Estimates of the information content of 20 microsatellite markers (SSR) evaluated for identity analysis, derived from 108 samples of *Ricinus communis* L.

Rank	Loci	pb	PI	PI_{sibs}	CPI	CPI_{sibs}	CPD (%)	CPD_{sibs} (%)
1	Rc05	196-218	0.210	0.488	2.10×10^{-01}	4.88×10^{-01}	78.99	51.17
2	Rc85A	178-268	0.225	0.521	4.72×10^{-02}	2.54×10^{-01}	95.28	74.55
3	Rc85B	281-364	0.252	0.541	1.19×10^{-02}	1.38×10^{-01}	98.81	86.24
4	Rc251	183-195	0.305	0.584	3.62×10^{-03}	8.04×10^{-02}	99.64	91.96
5	Rc25	243-251	0.336	0.571	1.22×10^{-03}	4.59×10^{-02}	99.88	95.41
6	Rc16	240-256	0.338	0.574	4.12×10^{-04}	2.63×10^{-02}	99.96	97.37
7	Rc256	182-190	0.362	0.586	1.49×10^{-04}	1.54×10^{-02}	99.99	98.46
8	Rc176	223-232	0.368	0.621	5.49×10^{-05}	9.59×10^{-03}	99.99	99.04
9	Rc61	206-212	0.375	0.594	2.06×10^{-05}	5.70×10^{-03}	100	99.43
10	Rc45	190-203	0.379	0.602	7.80×10^{-06}	3.43×10^{-03}	100	99.66
11	Rc158	106-153	0.401	0.627	3.13×10^{-06}	2.15×10^{-03}	100	99.79
12	Rc167	159-165	0.406	0.645	1.27×10^{-06}	1.39×10^{-03}	100	99.86
13	Rc90	203-225	0.406	0.645	5.15×10^{-07}	8.93×10^{-04}	100	99.91
14	Rc167	217-238	0.424	0.646	2.18×10^{-07}	5.77×10^{-04}	100	99.94
15	Rc35	245-249	0.478	0.692	1.04×10^{-07}	3.99×10^{-04}	100	99.96
16	Rc182	169-181	0.493	0.705	5.15×10^{-08}	2.81×10^{-04}	100	99.97
17	Rc65	274-291	0.631	0.798	3.25×10^{-08}	2.24×10^{-04}	100	99.98
18	Rc20	269-284	0.740	0.864	2.41×10^{-08}	1.94×10^{-04}	100	99.98
19	Rc186	169-179	0.912	0.955	2.19×10^{-08}	1.85×10^{-04}	100	99.98
20	Rc258	198-198	1	1	2.19×10^{-08}	1.85×10^{-04}	100	99.98

pb: allele base pairs; PI: identity probability; PI_{sibs} : identity probability for siblings; CPI: combined identity probability; CPI_{sibs} : combined identity probability for siblings; CPD (%): combined discrimination power; CPD_{sibs} (%): combined discrimination power for siblings.

Table 3. Estimates of the information content of the 20 microsatellite markers (SSR) for kinship analyzes derived from the 108 samples of *Ricinus communis* L.

Rank	Loci	PE _{trio}	PE _{duo}	PE _{par}	CPE _{trio} (%)	CPE _{duo} (%)	CPE _{par} (%)
1	Rc05	0.3568	0.2131	0.5209	35.68	21.31	52.09
2	Rc85A	0.3464	0.1796	0.5283	57.96	35.44	77.40
3	Rc85B	0.3152	0.1572	0.4847	71.21	45.59	88.35
4	Rc251	0.2734	0.1237	0.4350	79.08	52.32	93.42
5	Rc25	0.2225	0.1388	0.3352	83.74	58.94	95.63
6	Rc16	0.2212	0.1366	0.3337	87.33	64.55	97.09
7	Rc176	0.2174	0.0986	0.3455	90.09	68.05	98.09
8	Rc256	0.1992	0.1293	0.2995	92.06	72.18	98.66
9	Rc167	0.1910	0.0863	0.3040	93.58	74.58	99.07
10	Rc90	0.1910	0.0863	0.3040	94.80	76.77	99.35
11	Rc45	0.1883	0.1175	0.2844	95.78	79.50	99.54
12	Rc61	0.1873	0.1247	0.2810	96.57	82.06	99.67
13	Rc158	0.1781	0.1001	0.2722	97.18	83.85	99.76
14	Rc167	0.1661	0.0885	0.2553	97.65	85.28	99.82
15	Rc35	0.1463	0.0633	0.2313	97.99	86.21	99.86
16	Rc182	0.1436	0.0568	0.2309	98.28	87.00	99.89
17	Rc65	0.1001	0.0242	0.1727	98.45	87.31	99.91
18	Rc20	0.0735	0.0102	0.1381	98.57	87.44	99.92
19	Rc186	0.0228	0.0010	0.0446	98.60	87.45	99.93
20	Rc258	0	0	0	98.60	87.45	99.93

PE_{trio} = probability of exclusion of trio paternity; PE_{duo} = probability of exclusion of dual paternity; PE_{par} = probability of exclusion of even paternity; CPE_{trio} (%): combined probability of exclusion of trio paternity; CPE_{duo} (%) = combined probability of exclusion of dual paternity; CPE_{par} (%) = combined probability of exclusion of even paternity.

The loci with the highest allele frequencies (> 90%) were Rc258, Rc186, and Rc20, with alleles of 198 bp, 179 bp, and 269 bp, showing frequencies of 100%, 98%, and 93%, respectively. This suggests some form of selection or evolutionary pressure on these loci. In all the loci evaluated, a single allele contributed more than 50% of the allele frequency, highlighting a potential scenario of allele predominance or fixation at each specific locus (supplementary file).

The number and distribution of observed allele frequencies followed the expected pattern for these markers, as outlined in the publication by Qiu et al. (2010), who developed these markers.

CONCLUSION

The analysis of microsatellite markers revealed significant genetic heterogeneity in the population, although there is a predominance of homozygotes, suggesting selective processes or inbreeding. The genetic diversity, reflected by the alleles, indicates that only a few contribute to effective variability, yet the markers still allow for the identification of genetic differences among individuals. The high probability of genetic identity among unrelated individuals underscores the need for a specific set of markers for better discrimination. Markers Rc05, Rc85A, Rc85B, and Rc251 are particularly informative, demonstrating high accuracy in genotype discrimination and paternity testing. This study is significant as it provides a genetic framework for future breeding programs, particularly in selecting parental lines with high genetic variability. The findings support the application of SSR markers for genetic conservation and marker-assisted selection in castor bean breeding. Future research should focus on incorporating additional molecular markers to refine genetic differentiation and expanding the study to include more diverse populations to enhance genetic conservation and crop improvement efforts. Additionally, these results highlight the necessity of integrating molecular tools with phenotypic assessments to maximize breeding efficiency and genetic gain in *Ricinus communis* improvement programs.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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REFERENCES

- Alhaji KA, Oliseh A, Salihu BZ and Kabaraini MA (2019) Studies on genetic variability and trait relationship in castor (*Ricinus communis* L.). **Journal of Plant Development** **26**: 3-11.
- Allan G, Williams A, Rabinowicz PD, Chan AP, Ravel J and Keim P (2008) Worldwide genotyping of castor bean germplasm (*Ricinus communis* L.) using AFLPs and SSRs. **Genetic Resources and Crop Evolution** **55**: 365-378.
- Andreazza R, Bortolon L, Pieniz S and Camargo FAO (2013) Use of high-yielding bioenergy plant castor bean (*Ricinus communis* L.) as a potential phytoremediator for copper-contaminated soils. **Pedosphere** **23**: 651-661.
- Anggraeni TDA, Waluyo B, Sugiharto AN and Kuswanto (2022) Genetic diversity analysis among 123 accessions of castor (*Ricinus communis* L.) using SSR marker and its association to agronomic traits. **Biodiversitas** **23**: 1211-1221.
- Bajay MM, Pinheiro JB, Batista CEA, Nobrega MBM and Zucchi MI (2009) Development and characterization of microsatellite markers for castor (*Ricinus communis* L.), an important oleaginous species for biodiesel production. **Conservation Genetics Resources** **1**: 237-239.
- Chaudhari BA, Patel MP, Dharajiya DT, Patel AM and Thakur MR (2021) Oil content and fatty acid composition in castor (*Ricinus communis* L.) genotypes. **International Journal of Agriculture, Environment and Biotechnology** **14**: 319-324.
- Chen R, Hara T, Ohsawa R and Yoshioka Y (2017) Analysis of genetic diversity of rapeseed genetic resources in Japan and core collection construction. **Breeding Science** **67**: 239-247.
- Cuevas HE and Prom LK (2020) Evaluation of genetic diversity, agronomic traits, and anthracnose resistance in the NPGS Sudan sorghum core collection. **BMC Genomics** **21**: 1-15.
- Dharajiya DT, Shah A, Galvadiya BP, Patel MP, Srivastava R, Pagi NK, Solanki SD, Parida SK and Tiwari KK (2020) Genome-wide microsatellite markers in castor (*Ricinus communis* L.): Identification, development, characterization, and transferability in *Euphorbiaceae*. **Industrial Crops and Products** **151**: 112461.
- Kim H, Lei P, Wang A, Liu S, Zhao Y, Huang F, Yu Z, Zhu G, He Z, Tan D, Wang H and Meng F (2021) Genetic diversity of castor bean (*Ricinus communis* L.) revealed by ISSR and RAPD markers. **Agronomy** **11**: 457-471.
- Koltun A, Silva PAD, Torres IY, Bonifácio-Anacleto F and Yassitepe JEDCT (2024) Microsatellite markers in maize: challenges and guidelines for implementing multiplex SSR analyses. **Crop Breeding and Applied Biotechnology** **24**: e46392411.
- Landoni M, Bertagnon G, Ghidoli M, Cassani E, Adani F and Pilu R (2023) Opportunities and challenges of castor bean (*Ricinus communis* L.) genetic improvement. **Agronomy** **13**: 2076.
- Manjunath KG and Sannappa B (2014) Identification of castor (*Ricinus communis* L.) ecotypes through molecular characterization in the selected regions of the western ghats of Karnataka, India. **International Journal of Bioassays** **3**: 3492-3498.
- Miah G, Rafii MY, Ismail MR, Puteh AB, Rahim HA, Islam KN and Latif MA (2013) A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. **International Journal of Molecular Sciences** **14**: 22499-22528.
- Oliveira IJ, Zanotto MD, Krieger M and Vencovsky R (2012) Inbreeding depression in castor bean (*Ricinus communis* L.) progenies. **Crop Breeding and Applied Biotechnology** **12**: 269-276.
- Rukhsar, Patel MP, Parmar DJ, Kalola AD and Kumar S (2017) Morphological and molecular diversity patterns in castor germplasm accessions. **Industrial Crops and Products** **97**: 316-323.
- Peakall ROD and Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. **Bioinformatics** **28**: 2537-2539.
- Phumichai C, Phumichai T and Wongkaew A (2015) Novel chloroplast microsatellite (cpSSR) markers for genetic diversity assessment of cultivated and wild Hevea rubber. **Plant Molecular Biology Reporter** **33**: 1486-1498.
- Priyanka U, Dangwal LR and Tarseem L (2021) An ethnomedicinal note on *Ricinus communis* L. (Family *Euphorbiaceae*) in Tehri Garhwal, Uttarakhand. **International Journal of Botany Studies** **6**: 1126-1129.
- Qiu L, Yang C, Tian B, Yang JB and Liu A (2010) Exploiting EST databases for the development and characterization of EST-SSR markers in castor bean (*Ricinus communis* L.). **BMC Plant Biology** **10**: 1-10.
- Seo KI, Lee GA, Ma KH, Hyun DY, Park YJ, Jung JW, Lee SY, Gwag JG, Kim CK and Lee MC (2011) Isolation and characterization of 28 polymorphic SSR loci from castor bean (*Ricinus communis* L.). **Journal of Crop Science and Biotechnology** **14**: 97-103.
- Vivodík M, Saadaoui E, Balázsová Z and Gálová Z (2020) Genetic diversity and relationship of Tunisian castor (*Ricinus communis* L.) genotypes revealed by SSR markers. **Genetika** **52**: 765-776.
- Wang R, Zhong Y, Hong W, Luo H, Li D, Zhao L, Zhang H and Wang J (2023) Genetic diversity evaluation and core collection construction of pomegranate (*Punica granatum* L.) using genomic SSR markers. **Scientia Horticulturae** **319**: 112192.