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Evaluation of molecular diversity of new castor lines (*Ricinus communis* L.) using random amplified polymorphic DNA markers

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ABSTRACT

The aim of this work was to detect genetic variability among the set of 32 castor genotypes using five random amplified polymorphic DNA (RAPD) markers. Amplification of genomic DNA of 32 genotypes, using RAPD analysis, yielded 41 fragments, with an average of 8.20 polymorphic fragments per primer. Number of amplified fragments ranged from 5 to 11, with the size of amplicons varied from 100 to 1200 bp. The polymorphic information content value ranged from 0.598 (RLZ 9) to 0.811 (RLZ 6) with an average of 0.746 and diversity index value ranged from 0.557 (RLZ 9) to 0.889 (RLZ 7) with an average of 0.784. The dendrogram based on hierarchical cluster analysis using unweighted pair group method with arithmetic average algorithm was prepared.

KEY WORDS: Castor, genetic diversity, molecular markers, random amplified polymorphic DNA

INTRODUCTION

Castor (*Ricinus communis* L., $2n = 2\times = 20$, Euphorbiaceae) is an industrially important non-edible oilseed crop widely cultivated in the arid and semi-arid regions of the world.[1] The seed of castor contains more than 45% of oil-rich (80-90%) in an unusual hydroxyl fatty acid, ricinoleic acid.[2] Castor oil is the only vegetable oil soluble in alcohol, presenting high viscosity and requiring less heating than other oils during the production of biodiesel.[2]

DNA-based molecular analysis tools are ideal for germplasm characterization and phylogenetic studies. Among the various DNA-based markers, amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism have been used to study genetic diversity. These markers elucidate the phylogenetic relationships among various lines for their efficient use in breeding and genetic resource management. These methods, however, involve the use of expensive enzymes, radioactive labeling, and are cumbersome and hence, appear unsuitable. Random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) markers, on the other hand, require only small amounts of DNA sample without involving radioactive labels and are simpler as well as faster. RAPD has proven to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of plant species.[3-8]

The aim of this study was to detect genetic variability among the set of 32 new castor genotypes using five RAPD markers.

MATERIAL AND METHODS

Plant Material and DNA Extraction

A total of 32 castor genotypes were obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia). DNA of 32 genotypes of castor was extracted from leaves of 10 days old seedlings using the Gene JET Plant Genomic DNA Purification Mini Kit. Each sample was diluted to 20 ng with TE buffer (10 mM Tris-HCl, pH 8.0 and 0.1 mM EDTA, pH 8.0) and stored at −20°C.

RAPD Amplification

Amplification of RAPD fragments was performed according to Gajeraa *et al*. (2010)[3] [Table 1] using 5 decamer arbitrary primers obtained from Genomed, Warsaw, Poland. Amplifications were performed in a 25 µl reaction volume containing 5 µl of DNA (100 ng), 12.5 µl of Master Mix (Genei, Bengaluru, India), and 1 µl of primer (10 pmol). Amplification was performed in programed thermocycler (Biometra, Germany) with initial denaturation at 94°C for 5 min, 42 cycles of denaturation at 94°C for 1 min, primer annealing at 38°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 5 min. Amplified products were separated in 1.5% agarose in ×1 tris-borate-EDTA buffer. The gels were stained with ethidium bromide and documented using gel documentation system Grab-It 1D preWindows.

Data Analysis

The RAPD bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. A dendrogram was constructed based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) in SPSS professional statistics version 17 software package. For the assessment of the polymorphism between ricin genotypes and usability of RAPD markers in their differentiation diversity index (DI),[9] the probability of identity,[10] and polymorphic information content (PIC)[11] were used.

RESULTS

Polymerase chain reaction amplifications using five RAPD primers produced 41 DNA fragments that could be scored in all genotypes [Figure 1]. The selected primers amplified DNA fragments across 32 genotypes studied, with the number of amplified fragments varied from 5 (RLZ 7) to 11 (RLZ 8), and the amplicon size ranged from 100 to 1200 bp. Among 41 amplified bands, all were polymorphic, with an average of 8.20 polymorphic bands per primer. The PIC value ranged from 0.598 (RLZ 9) to 0.811 (RLZ 6) with an average of 0.746 and DI value ranged from 0.557 (RLZ 9) to 0.889 (RLZ 7) with an average of 0.784 [Table 2].

The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. In dendrogram, unique genotype RM-32 separated from other 31 genotypes which were further subdivided into 2 clusters. Cluster 1 contained 16 castor new lines and cluster 2 contains 15 lines of ricin. Only four genotypes (RM-48, RM-51 and RM-57, RM-58, respectively) were not distinguished [Figure 2]. Using more polymorphic RAPD markers genetically close genotypes can be distinguished.

Table 1: List of RAPD primers

RAPD primers	Primer sequence (5′-3′)	Chromosomal location
RLZ 6	5′GTGATCGCAG 3′	7RL
RLZ 7	5′GTCCACACGG 3′	2RL
RLZ 8	5′GTCCCGACGA 3′	7RL
RLZ 9	5′TGCGGCTGAG 3′	2RS
RLZ 10	5′ACGCGCATGT 3′	4RL

RAPD: Random amplified polymorphic DNA

Table 2: The statistical characteristics of the RAPD markers used in castor

Primers	Number of alleles	DI	PIC	PI
RLZ 6	9	0.791	0.811	0.016
RLZ 7	5	0.889	0.723	0.010
RLZ 8	11	0.831	0.810	0.009
RLZ 9	7	0.557	0.598	0.071
RLZ 10	9	0.854	0.789	0.019
Average	8.20	0.784	0.746	0.025

DI: Diversity index, PIC: Polymorphic information content,
PI: Probability of identity, RAPD: Random amplified polymorphic DNA

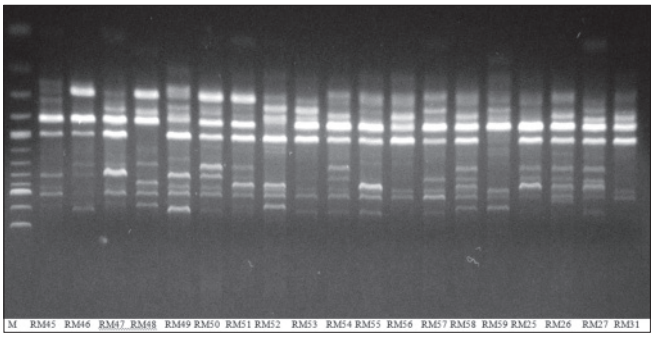

Figure 1: Polymerase chain reaction amplification products of 19 genotypes of castor produced with random amplified polymorphic DNA primer RLZ 6. Lane M is 1-kb DNA ladder and lanes RM45-RM31 are castor new genotypes

DISCUSSION

Similar values of DI and the PIC were detected by other authors,[3,12-18] and these values presented a high level of polymorphism of castor genotypes detected by RAPD markers. Gajeraa *et al*. (2010)[3] used 30 RAPD polymorphic primers for the analysis of 22 castor bean genotypes. RAPD analysis yielded 256 fragments, of which 205 were polymorphic, with an average of 6.83 polymorphic fragments per primer. Dhingani *et al*. (2012)[12] used three DNA-based molecular marker techniques, *viz*., RAPD, ISSR, and SSR to assess the genetic diversity in castor genotypes. Number of amplified fragments with RAPD primers ranged from 4 to 13, with the size of amplicons ranging from 100 to 2650 bp in size. The polymorphism

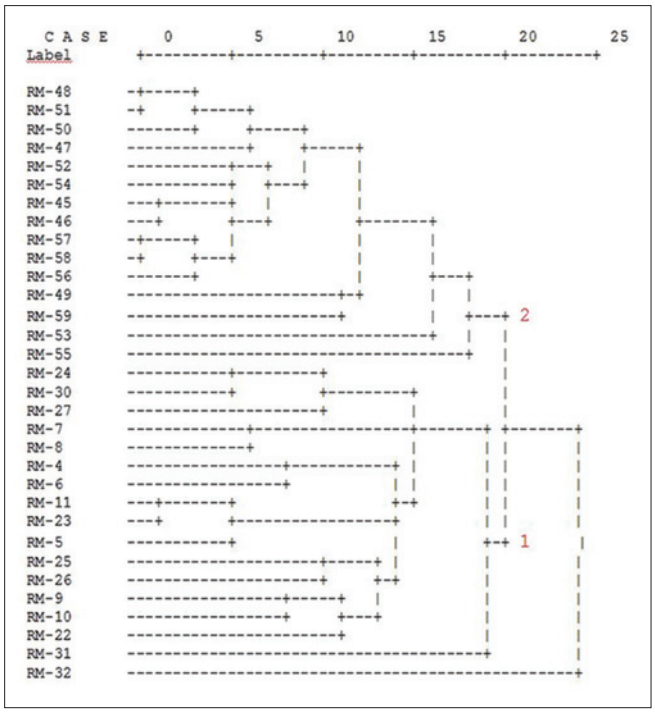

Figure 2: Dendrogram of 32 castor genotypes prepared based on five random amplified polymorphic DNA markers

ranged from 54.54 to 100.0, with an average of 79.54%. Genetic diversity of 37 ricin genotypes grown in China using RAPD markers was studied by Li *et al*. (2012).[13] Using RAPD markers, together they detected 122 alleles, of which 71 were polymorphic, representing the percentage of polymorphism alleles 58.20%. In the study Machado *et al*. (2013)[14] used 58 RAPD primers for the analysis of 15 castor bean cultivars. Authors identified 552 fragments, of which 311 were polymorphic (56.3%). Authors confirmed that RAPD markers are efficient in the study of genetic dissimilarity in castor bean. Pecina-Quinteroa *et al*. (2013)[15] studied the diversity and genetic relationships among accessions of *R. communis* from the state of Chiapas, Mexico using AFLP and SSR markers. In addition, 100% of the SSR primers were polymorphic, with an average of 5.5 alleles per locus. Tomar Rukam *et al*. (2014)[16] investigated the fingerprinting and phenotyping of 25 castor genotypes available in Gujarat and other States of India. The UPGMA dendrogram obtained using morphological characters clearly separated the 25 genotypes of castor into three groups. The present investigation of Kallamadia *et al*. (2015)[17] was to assess the extent of genetic diversity in 31 accessions of castor representing seven geographic areas in the world using RAPD, ISSR, and start codon targeted (SCoT) polymorphism primers. Among the three marker systems, RAPD had revealed the highest average percentage of polymorphism (54) while SCoT markers disclosed the lowest average percentage of polymorphism (21). The aim of the present study was to analyze the molecular diversity of varietal identification and phylogenetic relationships among 13 castor genotypes and identify those with distinct DNA profiles. Values of the PIC value ranged from 0.423 to 0.883 with an average of 0.705.[18]

RAPD molecular markers have been used in population genetic studies.[19-22] Some researchers have considered RAPD markers to represent segments of DNA with non-coding regions and to be selectively neutral[23,24] and some studies have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci.[21,25,26]

CONCLUSION

The analysis showed that the RAPD markers are very effective molecular markers for the assessment of the genetic diversity in castor bean. In dendrogram, unique genotype RM-32 separated from other 31 genotypes which were further subdivided into 2 clusters. Cluster 1 contained 16 castor new lines and cluster 2 contains 15 lines of ricin. Only four genotypes (RM-48, RM-51 and RM-57, RM-58, respectively) were not distinguished. Using more polymorphic RAPD markers genetically, close genotypes can be distinguished.

RAPD markers are useful in the assessment of castor bean diversity, the detection of duplicate sample in genotype collection, and the selection of a core collection to enhance the efficiency of genotype management for use in castor bean breeding and conservation.

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REFERENCES

1. Govaerts R, Frodin DG, Radcliffe-Smith A. World Checklist and Bibliography of Euphorbiaceae (with Pandaceae). Trowbridge, Wiltshire: Redwood Books Limited; 2000.
2. Jeong GT, Park DH. Optimization of biodiesel production from castor oil using response surface methodology. Appl Biochem Biotechnol 2009;156:1-11.
3. Gajeraa BB, Kumara N, Singha AS, Punvara BS, Ravikirana R, Subhasha N, *et al*. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. Ind Crops Prod 2010;32:491-8.
4. El Kichaoui A, Abu Zayed MA, Ayesh BM. Genotyping

and identification of six date palm (*Phoenix dactylifera* L.) cultivars of the Gaza Strip by random amplification of polymorphic DNA. Emirates J Food Agric 2013;25:916-25. doi: 10.9755/ejfa.v25i11.15918.
5. Srivashtav VS, Kapadia CV, Mahatma MK, Jha SK, Jha S, Ahmad T. Genetic diversity analysis of date palm (*Phoenix dactylifera* L.) in the kutch region of India using RAPD and ISSR markers. Emirates J Food Agric 2013;25:907-15. doi: 10.9755/ejfa.v25i11.14325.
6. Omalsaad AK, Islam A, Jahan MA, Yaakob Z, Osman M. Genetic relationship between roselle (*Hibiscus sabdariffa* L.) and kenaf (*Hibiscus cannabinus* L.) accessions through optimization of PCR based RAPD method. Emirates J Food Agric 2014;26:247-58. doi: 10.9755/ejfa.v26i3.16498.
7. Vivodík M, Balážová Ž, Gálová Z. RAPD analysis of the genetic diversity of castor bean. Int J Biol Vet Agric Food Eng 2014;8:583-6.
8. Žiarovská J, Ražná K, Labajová M. Using of inter microsatellite polymorphism to evaluate gamma-irradiated Amaranth mutants. Emirates J Food Agric 2013;25:673-81. DOI: 10.9755/ejfa.v25i9.15879.
9. Weir BS. Genetic Data Analysis. Methods for Discrete Population Genetic Data. Sunderland, MA: Sinauer Associates; 1990. p. 377.
10. Paetkau D, Calvert W, Stirling I, Strobeck C. Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 1995;4:347-54.
11. Weber JL. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. Genomics 1990;7:524-30.
12. Dhingani RM, Tomar RS, Parakhia MV, Patel SV, Golakiya BA. Analysis of genetic diversity among different *Ricinus communis* genotypes for macrophomina root rot through RAPD and microsatellite markers. Int J Plant Prot 2012;5:1-7.
13. Li FJ, Wang CL, He D, Liu ZQ, Chen MH, Wang YR, *et al*. Evaluation of genetic diversity in castor (*Ricinus communis* L.) using RAPD markers. Adv Mater Res 2012;343-344:981-7.
14. Machado EL, Silva SA, Santos AS, Bastos LA, Pestana CN, dos Santos KS, *et al*. Genetic dissimilarity between castor bean cultivars using RAPD markers. Pesqui Agropecu Bras 2013;48:342-5.
15. Pecina-Quinteroa V, Anaya-Lópeza JL, Núnez-Colína CA, Zamarripa-Colmenerob A, Montes-Garcíac N, Solís-Bonilla JL, *et al*. Assessing the genetic diversity of castor bean from Chiapas, México using SSR and AFLP markers. Ind Crops Prod 2013;41:134-43.
16. Tomar Rukam S, Parakhia MV, Kavani RH, Dobariya KL, Thakkar JR, Rathod VM, *et al*. Characterization of castor (*Ricinus communis* L.) genotypes using different markers. Res J Biotechnol 2014;9:6-13.
17. Kallamadia PR, Ganga Rao Nadigatlab VP, Mulpurib S. Molecular diversity in castor (*Ricinus communis* L). Ind Crops Prod 2015;66:271-81. Available from: 10.1016/j.indcrop.2014.12.061.
18. Lakhani HN, Patel SV, Bodar NP, Golakiya BA. RAPD analysis of genetic diversity of castor bean (*Ricinus communis* L.). Int J Curr Microbiol Appl Sci 2015;4:696-703.
19. Žiarovská J, Bežo M, Hrdlicková M, Fernandéz E. Standardization and reproducibility of random marker based analysis of micropropagated crimson beebalm. Genetika 2014;4:855-64. DOI: 10.2298/GENSR1403855Z.
20. Pawar UR, Baskaran J, Ajithkumar IP, Panneerselvam R. Genetic variation between *Xylocarpus spp*. (*Meliaceae*) as revealed by random amplified polymorphic DNA (RAPD) markers. Emirates J Food Agric 2013;25:597-604. doi: 10.9755/ejfa.v25i8.15403.
21. Štefúnová V, Bežo M, Labajová M, Senková S. Genetic analysis of three Amaranth species using ISSR markers. Emirates J Food Agric 2014;26:35-43. DOI: 10.9755/ejfa.v26i1.15911.
22. Vivodík M, Balážová Ž, Gálová Z. DNA analysis of ricin using RAPD technique. J Microbiol Biotechnol Food Sci 2014;3:181-3. doi: 10.15414/jmbfs.2015.4.special2.125-127.
23. Bachmann K. Nuclear DNA markers in plant biosystematics research. Opera Bot 1997;132:137-48.
24. Landergott U, Holderegger R, Kozlowski G, Schneller JJ. Historical bottlenecks decrease genetic diversity in natural populations of *Dryopteris cristata*. Heredity (Edinb) 2001;87(Pt 3):344-55.
25. Penner GA. RAPD analysis of plant genomes. In: Jauhar PP, editor. Methods of Genome Analysis in Plants. Boca Raton, FL: CRC; 1996. p. 251-68.
26. Labajová M, Senková S, Žiarovská J, Ražná K, Bežo M, Štefúnová V, *et al*. The potential of ISSR markers in Amaranth gamma-radiance mutants genotypying. J Microbiol Biotechnol Food Sci 2011;1:507-21.