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RAPD Analysis of Genetic Diversity of Castor Bean

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***Abstract*—**The aim of this work was to detect genetic variability among the set of 40 castor genotypes using 8 RAPD markers. Amplification of genomic DNA of 40 genotypes, using RAPD analysis, yielded in 66 fragments, with an average of 8.25 polymorphic fragments per primer. Number of amplified fragments ranged from 3 to 13, with the size of amplicons ranging from 100 to 1200 bp. Values of the polymorphic information content (PIC) value ranged from 0.556 to 0.895 with an average of 0.784 and diversity index (DI) value ranged from 0.621 to 0.896 with an average of 0.798. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared and analyzed genotypes were grouped into two main clusters and only two genotypes could not be distinguished. Knowledge on the genetic diversity of castor can be used for future breeding programs for increased oil production for industrial uses.

***Keywords*—**Dendrogram, polymorphism, RAPD technique, *Ricinus communis* L.

I. Introduction

CASTOR (*Ricinus communis* L.) is an important industrial oilseed crop. Its seed oil has multifarious applications in production of wide industrial products ranging from medicines to lower molecular weight aviation fuels, fuel additives, biopolymers and biodiesel [1], [2]. Castor oil is the only vegetable oil that contains up to 85% of the unique hydroxy fatty acid, ricinoleic acid, which confers distinctive industrial properties to the oil. Castor grows as an indeterminate annual or perennial depending on climate and soil types in tropical, sub-tropical and warm temperate regions in the world [3]. The major castor oil consuming countries are the European Union countries, USA and Japan. China and India are also emerging fast as major consumers. Most of the global castor is credited with 48% oil content out of which 42% could be extracted. Castor is an ideal candidate for bio-oil production [4]. It is not a food crop and can be grown productively on underutilized marginal uplands. Several high yielding varieties and hybrids were evolved in the last four decades. However, to meet the tremendous global demand for castor oil, cultivars with further enhanced yield and oil percentage, disease and insect resistance and drought tolerance are needed [5].

Genetic diversity assessment prior to developing hybrids can aid in better exploitation of heterosis [6]. Assessment of genetic variation using molecular markers appears to be an attractive alternative to the conventional diversity analyses and can also aid in management and conservation of biodiversity [7]. A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner [6]. This limits the use of morphological characters and isozymes, which are limited in number or lack adequate diversity in castor [8]. Further, isozyme analyses have inherent disadvantages such as limited numbers of markers, and are often less effective due to their inconsistency and sensitivity to short-term environmental fluctuations [9]-[11].

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 (RFLP) have been used to study genetic diversity [12]. 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 [13]. 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 [14]. RAPD was proven to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of plant species [15]-[17]. ISSR has been shown to provide a powerful, rapid, simple, reproducible and inexpensive means to assess genetic diversity and identify differences between closely related cultivars in many species [18], [19]. Recently, studies have been initiated on assessment of genetic variation in castor germplasm using AFLP and SSR markers [20].

The aim of this study was to assess genetic diversity within the set of 40 ricin genotypes using 8 RAPD primers.

II. Material and Methods

A. Plant Material and DNA Extraction

Ricin lines (40) were obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia). DNA of 40 genotypes of castor was extracted from 10 day old leaves using the Gene JET Plant Genomic DNA Purification Mini Kit (Fermentas^{TM}).

B. RAPD Amplification

Amplification of RAPD fragments was performed as [16] (Table I) using decamer arbitrary primers (Operon technologies Inc, USA; SIGMA-D, USA). Amplifications were performed in a 25 µl reaction volume containing 5 µl DNA (100 ng), 12.5 µl Master Mix (Genei, Bangalore, India),

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and 1 µl of 10 pmol of primer. Amplification was performed in a programmed 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× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system Grab-It 1D for Windows.

TABLE I
LIST OF RAPD PRIMERS

RAPD primers	primer sequence (5´-3´)
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-13	CAGCACCCAC
OPD-07	TTGGCACGGG
OPD-13	GGGGTGACGA
OPE-07	AGATGCAGCC
SIGMA-D-01	AAACGCCGCC
SIGMA-D-14	TCTCGCTCCA

C. 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. The binary data generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands and to prepare a dendrogram. A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the SPSS professional statistics version 17 software package was constructed.

For the assessment of the polymorphism between ricin genotypes and usability RAPD markers in their differentiation we used diversity index (DI) [21], the probability of identity (PI) [22] and polymorphic information content (PIC) [23]. They were calculated according to formulas:

Diversity index (DI):

$$DI = 1 - \sum p_i^2 \quad (1)$$

Polymorphic information content (PIC):

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 \cdot p_j^2 \quad (2)$$

Probability of identity (PI):

$$PI = \sum p_i^4 + \sum_{i=1}^{i=n-1} \sum_{j=i+1}^{n} \left(2 p_i p_j \right)^2 \quad (3)$$

where p_i and $p_j$are frequencies of i^{th} and j^{th} fragment of given genotype.

III. RESULTS AND DISCUSSION

PCR amplifications using 8 RAPD primers produced 66 DNA fragments that could be scored in all genotypes (Fig. 1). The selected primers amplified DNA fragments across the 40 genotypes studied, with the number of amplified fragments ranged from 3 (OPE-07) to 13 (SIGMA-D-01).

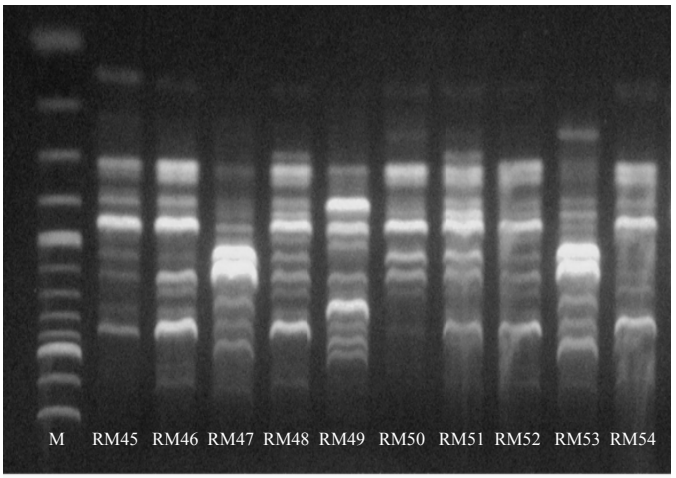

Fig 1 PCR amplification products of 10 genotypes of castor produced with RAPD primer SIGMA-D-01. Lane M is 1-kb DNA ladder and lanes RM45- RM54 are castor genotypes

The amplicon size ranged from 100 to 1200 bp (Table II). Of the 66 amplified bands, all 66 were polymorphic, with an average of 8.25 polymorphic bands per primer. To determine the level of polymorphism in the analyzed set of ricin lines, diversity index (1), polymorphic information content (2) and probability of identity (3) were calculated. All three features were calculated for all used RAPD primers by using individual frequencies of the fragments for each marker. The polymorphism information content value ranged from 0.556 (OPE-07) to 0.895 (OPD-13), with an average of 0.784 and index diversity value ranged from 0.621 (OPE-07) to 0.896 (OPD-13) with an average of 0.798. Probability of identity was low ranged from 0.001 to 0.074 with an average of 0.027 that indicates the possibility to differentiate genetically close genotypes (Table II).

TABLE II
STATISTICAL CHARACTERISTICS OF THE RAPD MARKERS USED IN CASTOR ANALYSIS

RAPD Primers	Number of fragments	molecular weight range (bp)	DDI	PIC	PI
OPA-02	7	100-800	0.788	0.776	0.013
OPA-03	9	250-1000	0.861	0.858	0.010
OPA-13	7	200-1000	0.818	0.809	0.025
OPD-07	8	200-1000	0.741	0.740	0.025
OPD-13	12	150-900	0.896	0.895	0.001
OPE-07	3	200-1200	0.621	0.556	0.074
SIGMA-D-01	13	250-1000	0.886	0.884	0.002
SIGMA-D14	7	200-1100	0.767	0.749	0.055
Average	**8.25**		**0.798**	**0.784**	**0.027**

DI- diversity index
PIC-polymorphic information content
PI- probability of identity

Reference [16] shows analysis of 22 castor bean genotypes by thirty RAPD polymorphic primers. RAPD analysis yielded

in 256 fragments, of which 205 were polymorphic, with an average of 6.83 polymorphic fragments per primer. Number of amplified fragments with RAPD primers ranged from 6 to 12, with the size of amplicons ranging from 160 to 3000 bp in size. The polymorphism ranged from 27.2 to 100.0, with an average of 80.2%.Genetic diversity of 37 ricin genotypes grown in China using RAPD markers was studied [24]. Using RAPD markers, together they detected 122 alleles, of which 71 were polymorphic, representing the percentage of polymorphism alleles 58.20%.

A dendrogram based on hierarchical cluster analysis using UPGMA algorithm (Fig. 2) separated 40 genotypes into two main groups. Cluster I contained four genotypes, in which a single ricin genotype RM-53 separated from other three genotypes (RM-55, RM-61 and RM-84). In cluster II separated unique ricin genotype RM-76 (2a) and subcluster 2b was further subdivided into two subclusters (2bi, 2bii). Subcluster 2bi contained three ricin genotypes and subcluster 2bii 32 genotypes of ricin. We could not distinguish 2 genotypes, RM-64 and RM-75grouped in 2bii subcluster, which can be caused due the same genetic background. For better differentiation of analyzed ricin genotypes, it is necessary to use a higher number of RAPD markers.

Reference [24] shows dendrogram constructed based on RAPD markers using UPGMA algorithm that divided 37 analyzed ricin genotypes from China into 4 main clusters.

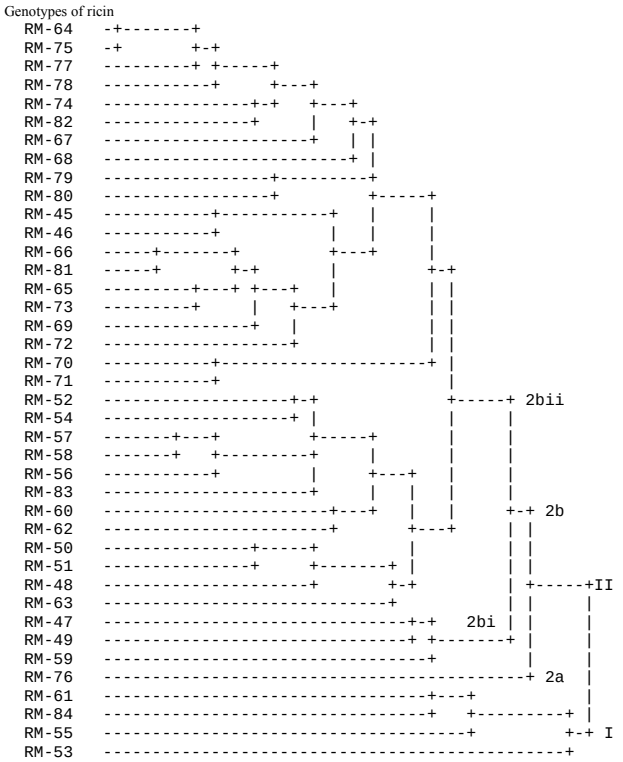

Fig 2 Dendrogram of 40 castor genotypes prepared based on 8 RAPD markers

Reference [20] shows using amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) for analysis of 41 castor bean accessions. They found out that despite surveying five continents and 35 countries, genetic diversity in castor bean germplasm is relatively low (overall He = 0.126 for AFLPs and 0.188 for SSRs) compared to estimates of genetic diversity in other plant species.

RAPD molecular markers have been used in population genetic studies [25]-[27]. Some researchers have considered RAPD markers to represent segments of DNA with noncoding regions and to be selectively neutral [28], [29], and some studies have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci [30].

IV. Conclusion

The analysis showed that the RAPD markers are very effective molecular markers for the assessment of the genetic diversity in castor bean. The dendrogram prepared based on UPGMA algorithm divided 40 analyzed genotypes into two main clusters (I, II). Cluster I contain 4 genotypes of ricin and cluster II contain 36 ricin genotypes. Using 8 RAPD markers only two castor bean genotypes have not been distinguished. For better discrimination of the analyzed ricin genotypes, it is necessary to use a higher number of RAPD markers. Our analysis proved utilization of RAPD markers for differentiation of used set of castor genotypes. 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.

References

[1] O. Y. Sarıbıyık, M. Özcanlı, H. Serin, S. Serin, K. Aydın, "Biodiesel Production from *Ricinus communis* Oil and Its Blends with Soybean Biodiesel," *Strojniškivestnik - Journal of Mechanical Engineering,* vol. 56, no.12, pp. 811-816, 2010.

[2] D. S.Ogunniyi, "Castor oil: a vital industrial raw material," *Bioresour. Technol*., vol. 97, pp. 1086–1091,2006.

[3] H. J. Caupin, "Products from castor oil: past, present and future," in: Gunstone, F.D., Padley, F.B. (Eds.), Lipid Technologies and Applications. *Marcel Dekker, New York*, 1997, pp. 787–795.

[4] D. L.Auld, M. D. Zanotto, T. A. McKeon, J. B. Morris, "Castor," in: Vollmann, J.Rajcan, I. (Eds.), Oil Crops, *Handbook of Plant Breeding,* vol.4, pp. 317–332,2009.

[5] J. D. Bilbro, "Castor: an old crop with new potential" Southwest Farm Press Newslett. (http://southwestfarmpress.com),2007.

[6] M.Santalla, J. B. Power, M. R. Davey, "Genetic diversity in mungbean germplasm revealed by RAPD markers," *Plant Breed*., vol. 117, pp. 473–478,1998.

[7] E.J.Essilman, D.J. Crawford, S.Brauner, T.F. Stuessy, G. J. Anderson, O. M. Silva, "RAPD marker diversity within and divergence among species of Dendroseris (*Asteraceae: Lactuceae*),"*Am. J. Bot.,* vol. 4, pp. 591–596,1997.

[8] P.S. Soltis, D.E. Soltis, T. L. Tucker, A.Lang, "Allozyme variability is absent in the narrow endemic Bensoniellaoregona (Saifragacear)," *Conserv. Biol*., vol. 6, pp. 131–134,1992.

[9] T. K.Lowrey, D. J. Crawford, "Allozyme divergence and evolution in Tertramolopium (Compositae: Astereae) on the Hawaiian Islands," *Syst. Bot*., vol. 10, pp. 64–72, 1985.

[10] A.M.Crawford, K.G.Dodds, A.J.Ede, "An autosomal genetic linkage map of the sheep genome," *Genetics,* vol. 140,pp. 703–724.1995.

[11] P.Lesica, R.F.Leary, F.R. Allendort, D.E. Bilderbecl, "Lack of genetic diversity within and among populations of an endangered plant, Hawellia aquatilis," *Conserv. Biol.,* vol. 2,pp. 275–282,1998.

[12] J.Welsh, M. McClelland, "Fingerprinting genomes using PCR with arbitrary primers," *Nucleic Acids Res*., vol. 18, pp. 7213–7218,1990.

[13] C.Gwanama, M. T.Labuschagne, A.M. Botha, "Analysis of genetic variation in Cucurbitamoschata by random amplified polymorphic DNA (RAPD) markers," *Euphytica,* vol. 113, pp. 19–24,2000.
[14] O.J.Francisco, D.J.Crawford, A. Santos-Guerra, J.A. Cravalho, "Isozyme differentiation in the endemic genus Argyranthemum (Asteraceae, Anthemideae) in the Macaronesian islands,"*Plant Syst. Evol.,* vol. 202, pp. 137–152,1996.
[15] J.Kapteyn, J.E. Simon, "The use of RAPDs for assessment of identity, diversity and quality of Echinacea," in: *Janick, J., Whipkey, A. (Eds.), Trends in New Crops and New Uses. ASHS Press, Alexandria, VA*, 2002, pp. 509–513.
[16] B.B. Gajera, N. Kumar, A. S. Singh, B.S. Punvar, "Assessment of genetic diversityin castor (Ricinuscommunis L.) using RAPD and ISSR markers," *Ind. Crops Prod.*," vol. 32, pp. 491–498,2010.
[17] M. Bežo, A.Candráková, V. Štefúnová, J.Žiarovská, K.Ražná, M. Bežo, "Identification of flax germplasm collection duplicates using randomly amplified polymorphic DNA markers (RAPD)," *Actafytotechnicaetzootechnica*, vol. 8, no. 3, pp. 62—66, 2005.
[18] A.Gonzalez, A.Coulson, R.Brettell, "Development of DNA markers (ISSRs) inmango," *ActaHortic.,* vol. 575, pp. 139–143,2002.
[19] J. Žiarovská, K. Ražná, M. Labajová, "Using of Inter Microsatellite Polymorphism to evaluate gamma-irradiated Amaranth mutants," *Emir. J. Food Agric.,* vol. 25, no. 9, pp. 673-681, 2013.
[20] G. Allan, A. Williams, P. D. Rabinowicz, A. P. Chan, J. Ravel, P. Keim, "Worldwide genotyping of castor bean germplasm (Ricinus communis L.) using AFLPs and SSRs," *Genet. Resour. Crop. Evol*., vol. 55, pp. 365–378,2008.
[21] B. S. Weir, „Genetic data analysis," Sinauer Associated, Sunderland, Mass,1990.
[22] D. Paetkau, W. Calvert, I. Stirling, C. Strobeck, "Microsatellite analysis of population structure in Canadian polar bears," *Mol. Ecol*., vol. 4, pp. 347-354,1995.
[23] J. L. Weber, "Informativeveness of human (dC-dA)n x (dG-dT)n polymorphism," *Genomics*, vol. 7, pp. 524-530, 1990.
[24] F. J. Li, C.L. Wang, D. He, Z. Q. Liu, M. H. Chen, Y. R. Wang, F. J. Li, Z. H. Yang, G. Chen, "Evaluation of genetic diversity in Castor (Ricinus communis L.) using RAPD markers," *Advanced materials research.,* vol. 343-344, pp. 981-987, 2012.
[25] B.J.Parsons, H. J. Newbury, M.T. Jackson, B.V. Ford-Lloyd, "Contrasting genetic diversity relationships are revealed in rice (Oryza sativa L.) using different marker types," *Mol. Breed.,* vol. 3, pp. 115–125,1997.
[26] E.J.Esselman, J.Q. Li, D.Crawford, J.L. Winduss, A.D. Wolfe, "Clonal diversity in the rare Calamagrostisporteri ssp. Insperata (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers,"*Mol. Ecol.,* vol. 8,pp. 443–451,1999.
[27] A. Li, S.Ge, "Genetic variation and clonal diversity of Psammoc hloavillosa (Poaceae) detected by ISSR markers," *Ann. Bot*., vol. 87, pp. 585–590,2001.
[28] K. Bachmann, "Nuclear DNA markers in plant biosystematics research," *Opera. Bot*., vol. 132, pp. 137–148,1997.
[29] U. Landergott, R. Holderegger, G.Kozlowski, J. J.Schneller, "Historical bottlenecks decrease genetic diversity in natural populations of Dryopteris cristata," *Heredity,* vol. 87,pp. 344–355,2001.
[30] G. A.Penner, "RAPD analysis of plant genomes," in: Jauhar, P.P. (Ed.), Methods of Genome Analysis in Plants. *CRC, Boca Raton*, 1996, pp. 251–268.