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STUDY OF DNA POLYMORPHISM OF THE CASTOR NEW LINES BASED ON RAPD MARKERS

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ABSTRACT

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. Seventeen castor genotypes were assessed for genetic variability using Random Amplified Polymorphic DNA (RAPD) markers. Thirteen polymorphic RAPD primers amplified 102 DNA fragments, with an average of 7.85 fragments per primer. Number of amplified fragments ranged from 3 (OPE-07) to 13 (SIGMA-D-01), with the size of amplicons ranging from 100 to 1200 bp. The polymorphic information content (PIC) value ranged from 0.450 (OPE-07) to 0.892 (SIGMA-D-01) with an average of 0.771 and diversity index (DI) value ranged from 0.551 (OPE-07) to 0.894 (SIGMA-D-01) with an average of 0.787. 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 (RM-5 and RM-23) could not be distinguished.

Keywords: *Ricinus communis* L.**,** DNA markers, genetic diversity, RAPD technique

INTRODUCTION

Castor (*Ricinus communis* L.) belongs to a monotypic genus, *Ricinus* and subtribe, *Ricininae* with chromosome number 2n =2x = 20. Its seed is the castor bean which despite its name is not a true bean and contains more than 45% of oil (**Penner *et al.*, 1996**). Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa and India but is widespread throughout tropical regions (and widely grown elsewhere as an ornamental plant). Castor seed oil is used as raw material for numerous and varied industrial applications such as manufacture of polymers, coatings, lubricants for aircrafts, cosmetics and for the production of biodiesel (**Jeong *et al.*, 2009**).

Characterization of crop genetic diversity is essential for increasing the productivity which for long has been based on morphological traits that often have notable advantages such as straightforward detection and measurement and relevance to characters of importance to germplasm users (**Bhat *et al.*, 2004**). However, they can also have serious limitations. Many of them are controlled by multiple alleles and loci, making it difficult to relate patterns of phenotypic variation to their genetic bases and their expression may be strongly affected by the environment (**Brown *et al.*, 1996**). In contrast to morphological and biochemical traits, molecular markers based on DNA polymorphisms are generally not affected by environment. Important classes of DNA-based molecular markers include Amplified Fragment Length Polymorphisms AFLPs as well as Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR) and Single Sequence Repeats (SSR) (**Heslop Harrison and Schwarzacher, 1996; Penner et al., 1996; Reiter, 2001 and Vienne et al., 2003)**. RAPD markers require only small amounts of DNA sample without involving radioactive labels and are simpler as well as faster. RAPD has proved to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of plant species (**Gwanama *et al.*, 2000**). RAPD 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 (**Gonzalez *et al.*, 2002, Labajová *et al.*, 2011**). Higher magnitude of heterosis and genetically superior hybrids can be obtained by combining diverse parents in hybrid development. Genetic diversity assessment prior to developing hybrids can aid in better exploitation of heterosis. 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. Limited studies have been carried out on the genetic diversity and phylogenetics of castor using molecular markers (**Welsh and Mcclelland, 1990; Kapteyn and Simon, 2002**).

The objective of the present study was to detect genetic variability among the set of 17 castor new lines using 13 RAPD markers.

MATERIAL AND METHODS

Plant material and DNA extraction

Castor new lines (17) were obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia). DNA of 17 genotypes of castor was extracted from 10 day old leaves (planted in the soil) using the Gene JET Plant Genomic DNA Purification Mini Kit.

RAPD amplification

Amplification of RAPD fragments was performed according to **Gajera et al. (2010),** as shown in the table 1, 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), 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 pre Windows.

Table 1 List of 13 RAPD primers

RAPD Primers	primer sequence (5´-3´)	molecular weight range (bp) of amplified DNA fragments
OPA-02	TGCCGAGCTG	100-800
OPA-03	AGTCAGCCAC	250-1000
OPA-13	CAGCACCCAC	200-1000
OPB-08	GTCCACACGG	100-1200
OPD-02	GGACCCAACC	200-1200
OPD-07	TTGGCACGGG	200-1000
OPD-08	GTGTGCCCCA	100-1100
OPD-13	GGGGTGACGA	150-900

OPE-07	AGATGCAGCC	200-1200
OPF-14	TGCTGCAGGT	100-850
SIGMA-D-01	AAACGCCGCC	250-1000
SIGMA-D-14	TCTCGCTCCA	200-1100
SIGMA-D-P	TGGACCGGTG	250-1100

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 of RAPD markers for differentiation we used diversity index (DI) **(Weir, 1990**), the probability of identity (PI) (**Paetkau et al., 1995**) and polymorphic information content (PIC) (**Weber, 1990**).
They were calculated according to formulas:

Diversity index (DI)

$$DI = 1 - \sum p_i^2$$

Probability of identity (PI)

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

Polymorphic information content (PIC)

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

RESULTS AND DISCUSSION

PCR amplifications using 13 RAPD primers produced 102 DNA fragments shown in the table 2, with an average of 7.85 fragments per primer, and that could be scored in all genotypes (Fig 1). The selected primers amplified DNA fragments across the 17 genotypes studied, with the number of amplified fragments ranged from 3 (OPE-07) to 13 (SIGMA-D-01) shown in the figure 1, with the size of amplicons ranging from 100 to 1200 bp. The polymorphic information content (PIC) value ranged from 0.450 (OPE-07) to 0.892 (SIGMA-D-01) with an average of 0.771 and diversity index (DI) value ranged from 0.551 (OPE-07) to 0.894 (SIGMA-D-01) with an average of 0.787.
A dendrogram based on hierarchical cluster analysis using UPGMA algorithm, shown in the figure 2, separated 17 genotypes into two clusters. Cluster I contained one genotype (RM-32) and cluster II was divided into two subclusters (2a, 2b). Subcluster 2a contained one ricin genotype (RM-31) and remaining 15 ricin genotypes of 2b were further subdivided into 3 subgroups. We could not distinguish only two genotypes of subcluster 2b, RM-5 and RM-23 that can be caused due to very close genetic background. For better differentiation of analyzed ricin genotypes, it is necessary to use a higher number of RAPD markers.
Gajeraa et al. (2010) used 30 RAPD polymorphic primers for the analysis of 22 castor bean genotypes. 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 by **Li et al. (2011)**. Using RAPD markers, together they detected 122 alleles, of which 71 were polymorphic, representing the percentage of polymorphism alleles 58.20%. Dendrogram constructed using UPGMA algorithm divided 37 analyzed ricin genotypes into 4 main clusters. **Dhingani et al. (2012)** used three DNA-based molecular marker techniques, random amplified polymorphism DNA (RAPD), inter simple sequence repeat (ISSR), and simple sequence repeat (SSR) to assess the genetic diversity in castor genotypes. Amplification of genomic DNA of 8 genotypes, using RAPD analysis, yielded 92 fragments, of which 72 were polymorphic, with an average PIC value of 0.29. 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 ranged from 54.54 to 100.0, with an average of 79.54 percent. In the study **Machado et al. (2013)** used 58 RAPD primers for the analysis of 15 castor bean cultivars. The genetic dissimilarity between cultivars was calculated by Jaccard's index, using UPGMA algorithm. Authors identified 552 DNA fragments, of which 311 were polymorphic (56.3%). The cultivars were clustered in five groups with evidence of genetic difference among them. Authors confirmed that RAPD markers are efficient in the study of genetic dissimilarity in castor bean. **Tomar Rukam et al. (2014)** investigated the fingerprinting and phenotyping of 25 castor genotypes available in Gujarat and other States of India using RAPD and ISSR markers. One hundred thirty decamer RAPD primers from Operon series (OPA to OPZ – five from each series) were screened with the DNA of the 2 castor genotypes. Only fifty-seven primers generated reproducible and scorable RAPD profiles. These produced multiple band profiles with a number of amplified DNA fragments ranging from 4 to 13 with an average of 7.70 fragments per primer. The total number of fragments produced by the fifty seven primers was 439. Maximum number of 13 amplicons was amplified with primer OPG-04 while the minimum number of fragments (4) was amplified with primer OPQ-01. The number of polymorphic fragments ranged from 0 to 7. The total number of polymorphic amplicons obtained by the fifty-seven studied primers was 122. The UPGMA cluster analysis was carried out to represent graphically the genetic distances among the 25 castor genotypes. The obtained dendrogram was divided into three main clusters; cluster one included 19 genotypes while cluster II and III included 5 and 1 genotype, respectively. The main cluster (cluster I) included two subclusters A and B. Subcluster B contained only two genotypes i.e. SKI332 and SKI271 while subcluster A contained 17 genotypes which were further divided into subclusters C and D.

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

RAPD Primers	Number of bands	DI	PIC	PI
OPA-02	7	0,758	0,742	0,024
OPA-03	9	0,820	0,816	0,014
OPA-13	7	0,794	0,780	0,040
OPB-08	8	0,823	0,813	0,008
OPD-02	6	0,763	0,738	0,070
OPD-07	8	0,786	0,783	0,013
OPD-08	7	0,817	0,806	0,010
OPD-13	12	0,887	0,885	0,002
OPE-07	3	0,551	0,450	0,301
OPF-14	5	0,711	0,698	0,055
SIGMA-D-01	13	0,894	0,892	0,002
SIGMA-D-14	7	0,755	0,750	0,025
SIGMA-D-P	10	0,870	0,868	0,004
average	**7,85**	**0,787**	**0,771**	**0,044**

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

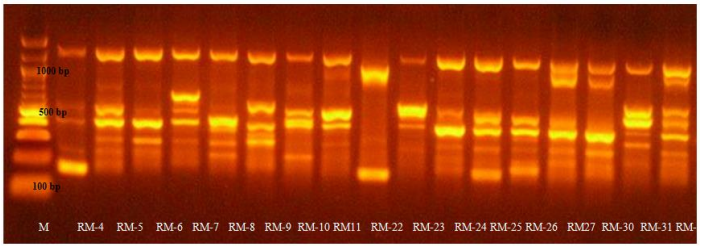

Figure 1 PCR amplification products of 17 genotypes of castor produced with RAPD primer OPA-02. Lane M is 1-kb DNA ladder and lanes RM-4 – RM-32 are castor genotypes.

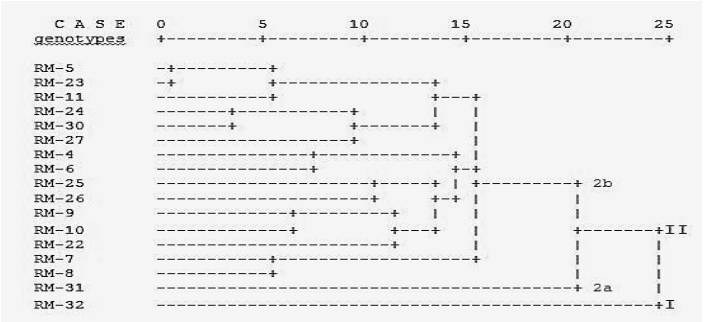

Figure 2 Dendrogram of 17 castor genotypes prepared based on 13 RAPD markers

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 17 analyzed genotypes into two main clusters. Cluster I contain 1 genotypes of ricin and cluster II contain 16 ricin genotypes. Using 13 RAPD markers only two castor bean genotypes have not been distinguished (RM-5 and RM-23). 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.

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