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DIFFERENTIATION OF RICIN USING RAPD MARKERS

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Abstract

The aim of this study was to assess genetic diversity within the set of 111 ricin genotypes using 13 RAPD primers. For differentiation of 111 ricin genotypes 13 RAPD primers were used. Amplification of genomic DNA of 111 genotypes using RAPD analysis yielded 102 fragments, with an average of 7.85 polymorphic fragments per primer. Number of amplified fragments with RAPD primers ranged from 3 to 13, with the size of amplicons ranging from 100 to 1500 bp. The polymorphism information content (PIC) value ranged from 0.491 to 0.898 with an average of 0.764 and diversity index (DI) value ranged from 0.576 to 0.900 with an average of 0.776. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. In dendrogram separated unique genotype RM-32 from other 110 genotypes which were further grouped into 3 subclusters (1, 2, 3). Only four genotypes were not distinguished. Using more polymorphic RAPD markers genetically close genotypes can be distinguished. Knowledge on the genetic diversity of castor can be used for 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.

Key words: *Ricinus communis,* Ricin, Genetic diversity, RAPD markers

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). 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 & Park, 2009). 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 (Jeong & Park, 2009). 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 (Birchler *et al*., 2003 and Reif *et al*., 2007). A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner (Santalla *et al*., 1998; Pervaiz *et al*., 2010). DNA-based molecular analysis tools are ideal for germplasm characterization and phylogenetic studies (Shinwari *et al*., 2011). Among the various DNA-based markers, microsatellites, amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) have been used to study genetic diversity (Rabbani *et al*., 2010). 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 (Turi *et al*., 2012). 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 (Kapteyn & Simon, 2002, Jan *et al*., 2011). RAPD has proven to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of species (Bežo *et al*., 2005; Bakht *et al*., 2013; Rasool, 2013; Sultan *et al*., 2013; Petrovičová *et al*., 2014; Yilmaz *et al*., 2014; Sharawy *et al.,* 2015). 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 (Labajová *et al*., 2011; Žiarovská *et al*., 2013; Štefúnová *et al*., 2014). Limited studies have been carried out on the genetic diversity and phylogenetics of castor using molecular markers. Recently, studies have been initiated on assessment of genetic variation in castor germplasm using AFLP and SSR markers (Allan *et al*., 2008).

Materials and Methods

Plant material and RAPD amplification: Ricin lines (111) were obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia). DNA of 111 genotypes of castor was extracted from 10 day old leaves using the Gene JET Plant Genomic DNA Purification Mini Kit. Amplification of RAPD fragments was performed according to Gajeraa *et al*., (2010) using decamer arbitrary primers (Table 1). Amplifications were performed in a 25 µl reaction volume containing 100 ng of DNA, 12.5 µl of Master Mix (Genei, Bangalore, India) and 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 electrophoresed in 1.5% agarose in 1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system.

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 genotypes ricin and usability RAPD markers in their 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)

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

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

Table 1. List of RAPD primers (Gajeraa *et al*., 2010).

Primers	Primer sequence (5´-3´)	Molecular weight range (bp)
OPA-02	TGCCGAGCTG	400-2800
OPA-03	AGTCAGCCAC	330-870
OPA-13	CAGCACCCAC	370-1800
OPB-08	GTCCACACGG	530-1550
OPD-02	GGACCCAACC	280-1850
OPD-07	TTGGCACGGG	360-1440
OPD-08	GTGTGCCCCA	260-1700
OPD-13	GGGGTGACGA	160-1800
OPE-07	AGATGCAGCC	300-1940
OPF-14	TGCTGCAGGT	190-1850
SIGMA-D-01	AAACGCCGCC	280-1350
SIGMA-D-14	TCTCGCTCCA	350-900
SIGMA-D-P	TGGACCGGTG	300-3000

Results

PCR amplification of DNA using 13 primers (Table 1) for RAPD analysis produced 102 DNA fragments that could be scored in all 111 genotypes of ricinus (Fig. 1). The number of amplified fragments varied from 3 (OPE-07) to 13 (SIGMA-D-01), and the amplicon size ranged from 100 to 1500 bp (Table 2). Of the 102 amplified bands, all 102 were polymorphic, with an average of 7.85 polymorphic bands per primer. Results indicated the presence of wide genetic variability among different genotypes of castor. Variations in DNA sequences lead to polymorphism. Greater polymorphism is indicative of greater genetic diversity. Using of parents with greater genetic diversity results in broad genetic base of the hybrids.

The polymorphism information content (PIC) value varied from 0.491 (OPE-07) to 0.898 (SIGMA-D-01), with an average of 0,764 and index diversity (DI) value varied from 0,576 (OPE-07) to 0,900 (SIGMA-D-01) with an average of 0,776. 92% of used RAPD markers had PIC and DI values higher than 0.6 that means high polymorphism of chosen markers used for analysis. Probability of identity (PI) was low ranged from 0.001 to 0.262 with an average of 0.041 (Table 2). For better differentiation of analysed ricin genotypes, it is necessary to use a higher number of RAPD markers.

A dendrogram based on UPGMA analysis separated unique genotype RM-32 (cluster I) from other 110 genotypes (cluster II) that were further subdivided into 3 subclusters (1, 2, 3) (Fig. 2). Cluster 1 contained unique genotype RM-114 and cluster 2 included three genotypes of ricinus RM-107, RM-108 and RM-113. Cluster 3 with 106 genotypes was divided into two subclusters (3a, 3b), subcluster 3a contained 105 genotypes and in the subcluster 3b separated unique genotype of ricinus RM-93. Subclaster 3a was further subdivided into two big subclusters, subcluster 3aa with 23 genotypes and aubcluster 3ab with 82 genotypes of ricinus. We could not distinguish 4 genotypes grouped in 3aa subcluster, RM-65 and RM-66 and genotypes RM-64 and RM-75, which are genetically the closest.

Table 2. List of RAPD primers, total number of bands and the statistical characteristics of the RAPD markers used in castor.

Primers	Total number of bands	Molecular weight range (bp)	DI	PIC	PI
OPA-02	7	200-1000	0,786	0,775	0,013
OPA-03	9	100-800	0,864	0,861	0,003
OPA-13	7	100-1500	0,833	0,827	0,007
OPB-08	8	250-800	0,817	0,810	0,009
OPD-02	6	200-1000	0,809	0,800	0,028
OPD-07	8	150-900	0,778	0,776	0,016
OPD-08	7	200-600	0,643	0,640	0,062
OPD-13	12	100-1500	0,891	0,889	0,002
OPE-07	3	300-800	0,576	0,491	0,262
OPF-14	5	200-1200	0,642	0,635	0,079
SIGMA-D-01	13	150-1200	0,900	0,898	0,001
SIGMA-D-14	7	250-1000	0,777	0,766	0,019
SIGMA-D-P	10	200-1500	0,770	0,767	0,027
Total	**102**	-	-	-	-
Average	**7,85**	-	**0,776**	**0,764**	**0,041**

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

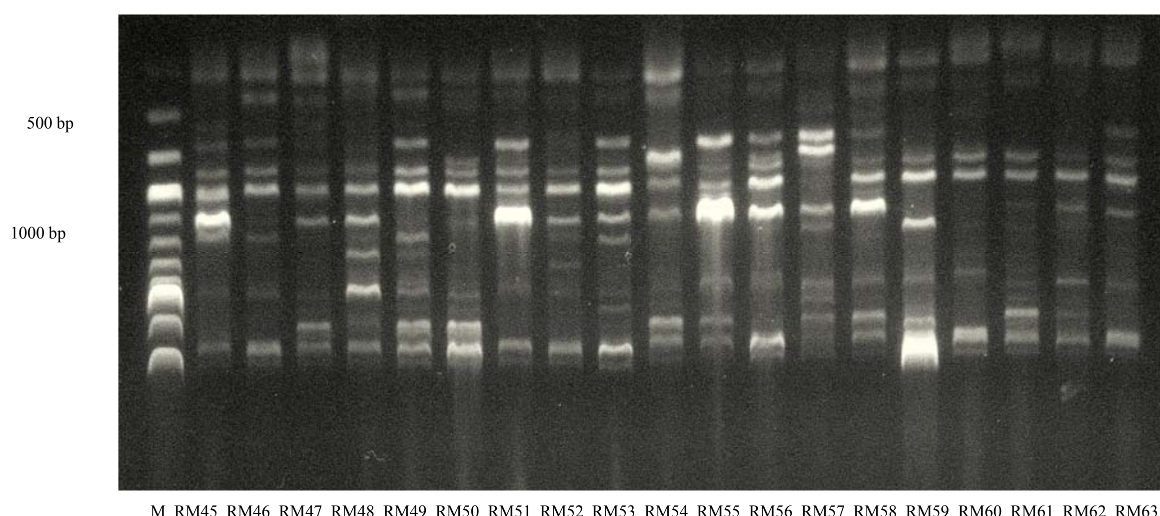

Fig. 1. PCR amplification products of 19 genotypes of castor produced with RAPD primer OPA-13. Lane M is Quick-Load® 100 bp DNA ladder and lanes RM45- RM63 are castor genotypes.

Discussion

One hundred eleven genotypes of ricin were assessed using 13 polymorphic RAPD primers. These thirteen primers amplified 102 DNA fragments with an average of 7.85 fragments per primer. All amplified fragments were polymorphic. Similar results achieved also Gajeraa *et al*. (2010) who 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. Authors detected polymorphism from 27.2 to 100.0, with an average of 80.2%. Li *et al*. (2012) analysed genetic diversity of 37 ricin genotypes grown in China using RAPD markers. Using RAPD markers, they detected together 122 fragments, of which 71 were polymorphic, representing the percentage of polymorphic fragments 58.20%. They constructed dendrogram using UPGMA algorithm and divided 37 analysed ricin genotypes into 4 main clusters. 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. Authors divided 15 analysed ricin genotypes into 5 subclusters. Rukam *et al.,* (2014) 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) was to assess the extent of genetic diversity in 31 accessions of castor representing seven geo-graphic areas in the world using RAPD (random amplified polymorphic DNA), ISSR (inter simple sequence repeat) and SCoT (start codon targeted polymorphism) primers. Among the three marker systems, RAPD had revealed highest average percentage of polymorphism (54) while SCoT markers disclosed the lowest average percentage of polymorphism (21).

RAPD molecular markers have been used in population genetic studies Rehman *et al*. (2013), Seema *et al.* (2014), Petrovičová *et al*. (2014), Žiarovská *et al.* (2014). Some researchers have considered RAPD markers to represent segments of DNA with noncoding regions and to be selectively neutral Landergott *et al*. (2001) and some studies have shown that RAPD markers are distributed throughout the genome and may be associated with functionally important loci (Penner, 1996).

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 separated the unique genotype RM-32 from the rest of 110 genotypes which were further subdivided into three main clusters. Using 13 RAPD markers only four 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.

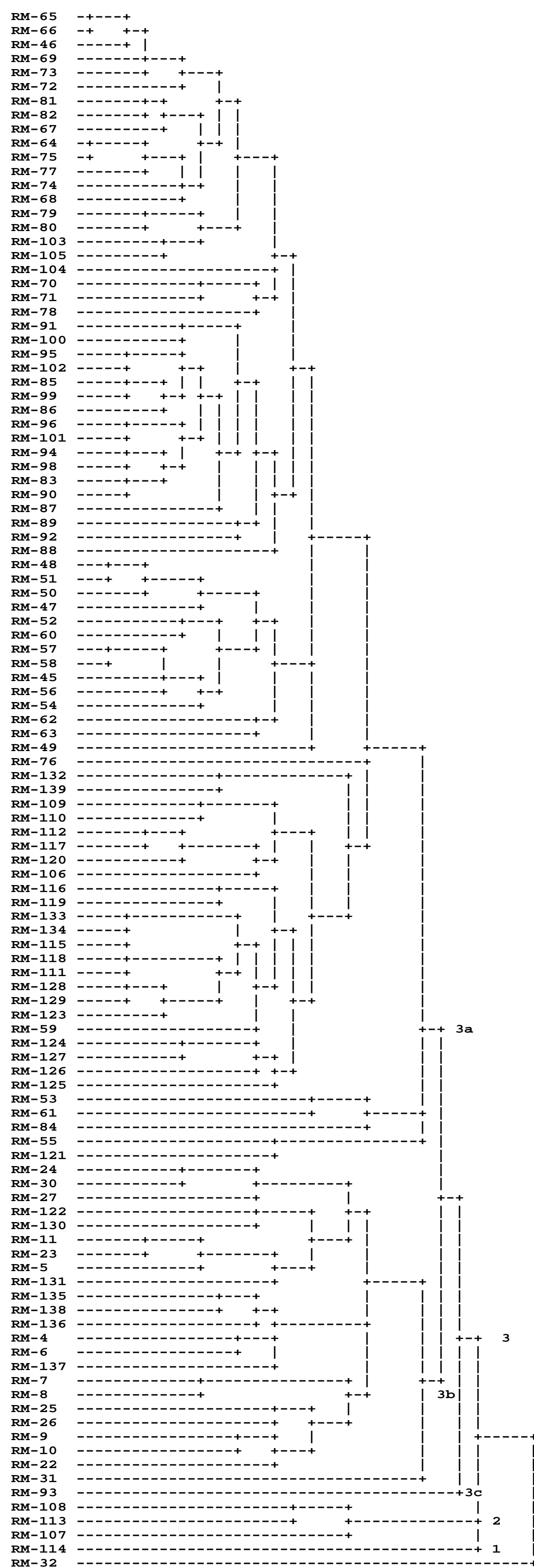

Fig. 2. Dendrogram of 111 castor genotypes prepared based on 13 RAPD markers.

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