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ASSESSMENT OF RAPD POLYMORPHISM IN RICIN GENOTYPES

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ABSTRACT

The aim of this work was to detect genetic variability among the set of 30 castor genotypes using 6 RAPD markers. Amplification of genomic DNA of 30 genotypes using RAPD analysis yielded 50 polymorphic fragments with an average of 8.33 fragments per primer. Number of amplified fragments varied from 5 (RLZ7) to 11 (RLZ8) and the amplicon size ranged from 330 to 1200 bp. All 50 amplified bands were polymorphic. The polymorphic information content (PIC) values ranged from 0.774 (RLZ7) to 0.870 (RLZ8) with an average of 0.825 and index diversity (DI) value ranged from 0.786 (RLZ7) to 0.872 (RLZ8) with an average of 0.831. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. Dendrogram separated ricin genotypes into three main clusters. Two genotypes (RM-72 and RM-73) were genetically the closest. 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.

Keywords: Castor, genetic variability, molecular markers, RAPD technique

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. The seed of castor contains more than 45% of oil and this oil is rich (80–90%) in an unusual hydroxyl fatty acid, ricinoleic acid. 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 and Park, 2009**). Due to its unique chemical and physical properties, the oil from castor seed is used as raw material for numerous and varied industrial applications, such as: manufacture of polymers, coatings, lubricants for aircrafts, cosmetics, etc, and for the production of biodiesel (**Jeong and Park, 2009**) with more than 95% of the world's castor production concentrated in limited parts of India, China, and Brazil (**Sailaja *et al.*, 2008**). Because of the ever increasing world-wide demand of castor for industrial use, there is a pressing need to increase the hectarage and productivity of castor (**Gajeraa *et al.*, 2010)**.
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; Reif *et al.*, 2007**). Higher magnitude of heterosis and genetically superior hybrids can be obtained by combining diverse parents in hybrid development. Conventional diversity analysis methods, in the field, are time consuming, laborious, resource intensive and drastically affected by environmental factors, therefore, a technique that is rapid and not affected by environment is needed for assessment of genetic diversity and selection of parental lines for use in hybrid development programmes (**Santalla et al., 1998**). 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. A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner (**Santalla *et al.*, 1998**). 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. 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 which are cumbersome and hence they appear unsuitable (**Gajeraa *et al.*, 2010)**. 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 been also proven to be simple and quite efficient in detecting genetic variations used for diversity assessment and for identifying germplasm in a number of plant species (**Welsh and McClelland, 1990, Gwanama *et al.*, 2000, Kapteyn and Simon, 2002, Gajeraa *et al.*, 2010**). 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 (**Gonzalez *et al.*, 2002, Labajová *et al.*, 2011**). 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**).
The aim of this study was to detect genetic variability among the set of 30 castor genotypes using 6 RAPD markers.

MATERIAL AND METHODS

Plant material and DNA extraction

Ricin lines (30) were obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia). DNA of 30 castor genotypes was extracted from 10 day old leaves using the Gene JET Plant Genomic DNA Purification Mini Kit (Thermo Scientific).

RAPD amplification

Amplification of RAPD fragments was performed according to **Gajeraa *et al.* (2010)** (Table 1) using 6 decamer arbitrary primers. Amplifications were performed in a 25 µl reaction volume containing 5 µl DNA (100 ng), 12.5 µl Master Mix (Promega, USA) and 1 µl of 10 pmol of primer. Amplification was performed in a 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 UVP PhotoDoc-t®. The size of alleles was determined by comparing with 100 bp standard lenght marker (ThermoFisher Scientific).

Table 1 List of used RAPD primers

Primers	Primer sequence (5´-3´)	Expecting molecular weight range (bp)	Localization
RLZ3	5´TGTCCAGCTT 3´	1200	2RL
RLZ6	5´GTGATCGCAG 3´	330	7RL
RLZ7	5´GTCCACACGG 3´	750	2RL
RLZ8	5´GTCCCGACGA 3´	350	7RL
RLZ9	5´TGCGGCTGAG 3´	650	2RS
RLZ10	5´ACGCGCATGT 3´	1100	4RL

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)

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

where pi and pj are frequencies of ith and jth fragment of given genotype.

RESULTS AND DISCUSSION

PCR amplifications using 6 RAPD primers produced 50 DNA fragments that could be scored in all genotypes. The selected primers amplified DNA fragments across the 30 genotypes studied with the number of amplified fragments varying from 5 (RLZ7) to 11 (RLZ8) and the amplicon size varied from 330 to 1200 bp. Of the 50 amplified bands, all 50 were polymorphic with an average of 8.33 fragments per primer (Table 2). The polymorphic information content (PIC) values varied from 0.774 (RLZ7) to 0.870 (RLZ8) with an average of 0.825 and index diversity (DI) value ranged from 0.786 (RLZ7) to 0.872 (RLZ8) with an average of 0.831 (Table 2). Similar values of DI and the PIC were detected by other authors (**Gajeraa *et al*., 2010; Machado *et al*., 2013; Tomar Rukam *et al*., 2014; Vivodík *et al*., 2014; Kallamadi *et al*., 2015**). Dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. In constructed dendrogram genotypes were divided into three main clusters (1, 2, 3). Cluster 1 contained unique genotype (RM-76), cluster 2 included two genotypes (RM-62, RM-68) and cluster 3 contained 27 genotypes of ricin. Cluster 3 subdivided 27 genotypes of ricin into two subclusters, subcluster 3a with unique genotype RM-70 and subcluster 3b with 26 genotypes of ricin. Two genotypes of subcluster 3b (RM-72 and RM-73) were genetically the closest (Figure 1). Using more polymorphic RAPD markers genetically close genotypes can be distinguished.
In our study we used 6 RAPD primers to study genetic polymorphism of 30 ricin lines and similar PIC values we obtained as **Gajeraa *et al.* (2010)** who used 30 RAPD polymorphic primers for the analysis of 22 castor bean genotypes. We can consider used RAPD primers as sufficiently polymorphic. RAPD analysis of **Gajeraa *et al.* (2010)** yielded 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. (2012).** Using RAPD markers, together they detected 122 fragments, of which 71 were polymorphic, representing the percentage of polymorphism fragments 58.20%. Dendrogram constructed using UPGMA algorithm divided 37 analyzed ricin genotypes into 4 main clusters. 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 the unweighted pair-group method with arithmetic mean (UPGMA). Authors identified 552 fragments, of which 311 were polymorphic (56.3%). The cultivars were clustered in five groups, evidence that there is genetic difference among them. Authors of their work confirmed, that RAPD markers are efficient in the study of genetic dissimilarity in castor bean.

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

RAPD primer	number of fragments	DI	PIC	PI
RLZ3	9	0.862	0.859	0.003
RLZ6	9	0.792	0.792	0.013
RLZ7	5	0.786	0.774	0.036
RLZ8	11	0.872	0.870	0.002
RLZ9	7	0.828	0.821	0.006
RLZ10	9	0.842	0.837	0.005
average	8.33	0.831	0.825	0.011

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

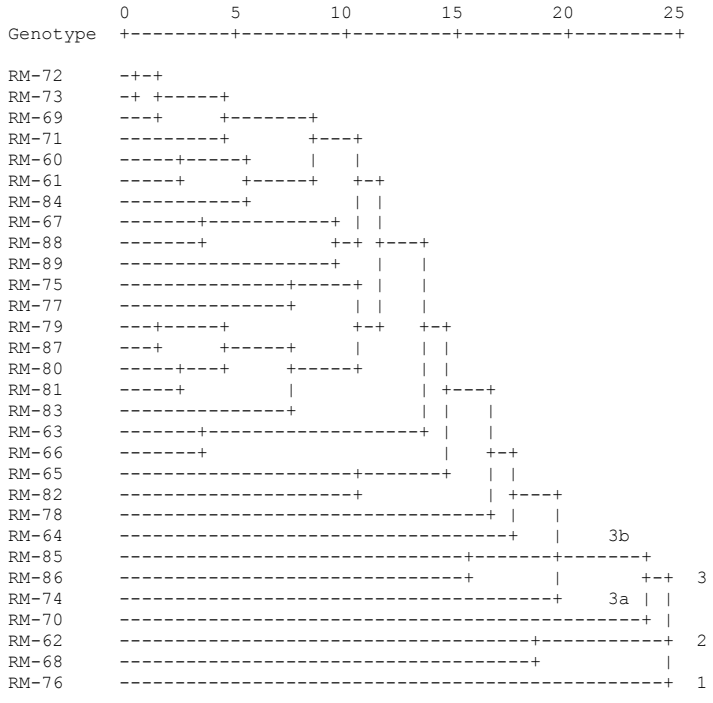

Figure 1 Dendrogram of 30 castor genotypes prepared based on 6 RAPD markers

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 two 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.

RAPD molecular markers have been used in population genetic studies (**Parsons *et al.*, 1997, Esselman *et al.*, 1999**). Some researchers have considered RAPD markers to represent segments of DNA with noncoding regions (**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 divided 30 analyzed genotypes into three main clusters. Using 6 RAPD markers only two castor bean genotypes have not been distinguished (RM-72 and RM-73). For better discrimination of analyzed genotypes of ricin, 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. We can consider used RAPD primers as sufficiently polymorphic. RAPD markers are useful in the assessment of castor bean diversity, for 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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