

DNA ANALYSIS OF RICIN USING RAPD TECHNIQUE

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ABSTRACT

Castor (*Ricinus communis* L.) is an important plant for production of industrial oil. 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. The aim of this work was to detect genetic variability among the set of 30 castor genotypes using 5 RAPD markers. Amplification of genomic DNA of 30 genotypes, using RAPD analysis, yielded 35 fragments, with an average of 7,00 polymorphic fragments per primer. Number of amplified fragments ranged from 5 to 9, with the size of amplicons ranging from 100 to 1200 bp. The polymorphic information content (PIC) value ranged from 0,662 to 0,855 with an average of 0,780 and diversity index (DI) value ranged from 0,669 to 0,857 with an average of 0,785. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. 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: ricin, RAPD technique, genetic diversity

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 and 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 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), and because of the ever increasing world-wide demand of castor for industrial use, there is a pressing need to increase the hectare and productivity of castor.

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 (Moll *et al.*, 1962, Birchler *et al.*, 2003 and 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. 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). This limits the use of morphological characters and isozymes, which are limited in number or lack adequate diversity in castor. 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 (Lowrey and Crawford, 1985, Soltis *et al.*, 1992, Crawford *et al.*, 1995, Francisco *et al.*, 1996, Essilman *et al.*, 1997 and Lesica *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, 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 (Welsh and McClelland, 1990, Gwanama *et al.*, 2000 and 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 5 RAPD markers.

MATERIAL AND METHODS

Plant material and DNA extraction

DNA was extracted from 30 genotypes of castor, using the Gene JET Plant Genomic DNA Purification Mini Kit. Fresh leaf tissues (0.1 g) were ground in liquid nitrogen and taken into a 2 ml microcentrifuge tube. To the ground sample 0.35 ml of lysis buffer A, 0,05 ml of lysis buffer B and 0,02 ml RNase was added, and mixed by vortexing and incubate the sample for 10 min at 65 °C. Next, we add 0,13 ml of the Precipitation solution and incubated 5 min on ice and centrifuged for 5 min at 14 000 rpm. The supernatant after centrifugation was taken and transferred to a clean microcentrifuge tube and we added 0,4 ml of Plant gDNA Binding Solution and 0,4 ml of 96% ethanol. Prepared mixture was transferred to the spin column and centrifuged for 1 min at 8 000 rpm and we added 0,5 ml Wash buffer 1 and 0,5 ml Wash buffer 2 and centrifuged. To elute the genomic DNA, we added 0,1 ml Elution Buffer and incubated for 5 min at room temperature and centrifuged for 1 min at 10 000 rpm. Each sample was

diluted to 20 ng with TE buffer (10 mM Tris-HCl, pH 8.0 and 0.1 mM EDTA, pH8.0) and stored at -20 °C.

RAPD amplification

Amplification of RAPD fragments was performed according to Gajeraa et al. (2010) 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.

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.

Table 1 List of primers, the number of amplified products, the number of polymorphic and monomorphic bands

sr. no.	primers	primer sequence (5'-3')	molecular weight range (bp)	total number of bands	number of polymorphic bands	number of monomorphic bands	% polymorphism
1.	OPA-03	AGTCAGCCAC	100-800	9	9	0	100,0
2.	OPD-02	GGACCCAACC	200-1000	6	6	0	100,0
3.	OPD-07	TTGGCACGGG	150-900	8	8	0	100,0
4.	OPF-14	TGCTGCAGGT	200-1200	5	5	0	100,0
5.	SIGMA-D-14	TCTCGCTCCA	250-1000	7	7	0	100,0
		total	-	35	35	0	-
		average	-	7,00	7,00	0	100,0

RESULTS AND DISCUSSION

PCR amplifications using 5 RAPD primers produced 35 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 (OPF-14) to 9 (OPA-03), and the amplicon size varying from 100 to 1200 bp. Of the 35 amplified bands, all 35 were polymorphic, with an average of 7,00 polymorphic bands per primer. The polymorphic information content (PIC) value varied from 0.662 (OPF-14) to 0.855 (OPA-03), with an average of 0,780 and index diversity (DI) value varied from 0,669 (OPF-14) to 0,857 (OPA-03) with an average of 0,785. A dendrogram based on UPGMA analysis separated 30 genotypes into two groups. One ricin genotype RM-62 (cluster I) separated from others. Second group of 29 ricin genotypes (II) was divided into 3 main clusters (1, 2, 3). Cluster 1 contained 11 ricin genotypes, cluster 2 contained 4 ricin genotypes (RM-61, RM-63, RM-71 and RM-57) and cluster 3 contained 14 ricin genotypes. We could not distinguish two genotypes, RM-65 and RM-66, which can be caused due the same genetic background.

Gajeraa et al. (2010) 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. 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%.

RAPD molecular markers have been used in population genetic studies (Parsons et al., 1997, Esselman et al., 1999 and Li and Ge, 2001). Some researchers have considered RAPD markers to represent segments of DNA with noncoding regions and to be selectively neutral (Bachmann, 1997 and 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).

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

Primers	Number of alleles	DI	PIC	PI
OPD-07	8	0,778	0,776	0,016
SIGMA-D-14	7	0,805	0,794	0,010
OPA-03	9	0,857	0,855	0,009
OPF-14	5	0,669	0,662	0,071
OPD-02	6	0,817	0,811	0,019
Average	7,00	0,785	0,780	0,025

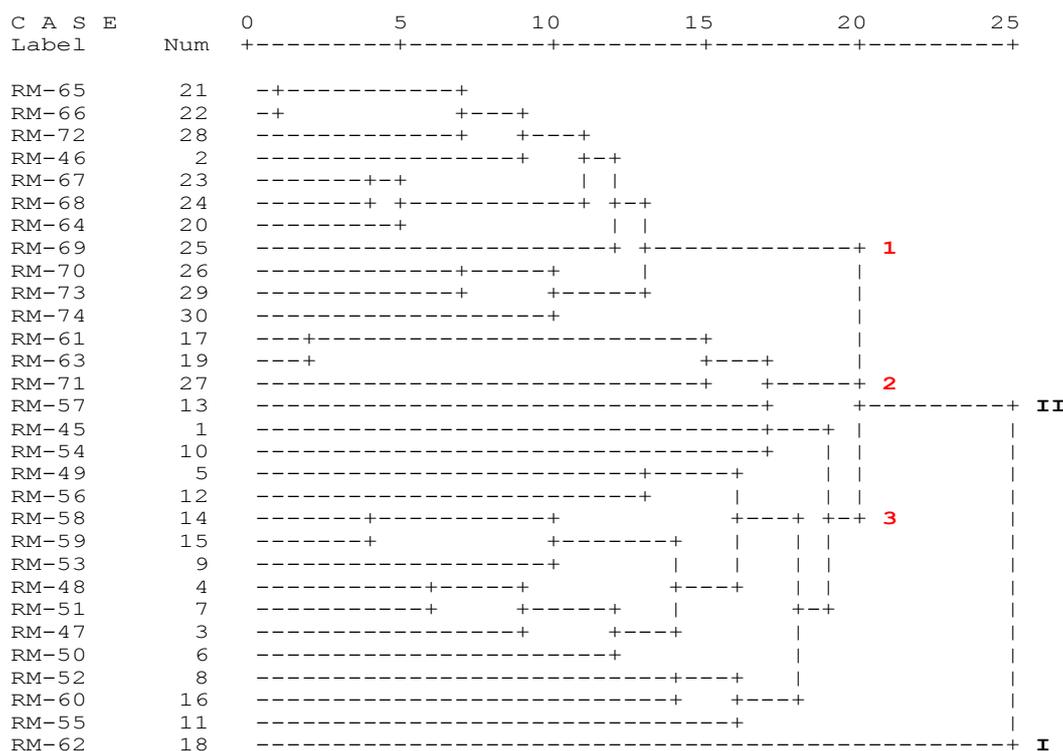


Figure 1 Dendrogram of 30 castor genotypes prepared based on 5 RAPD markers

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 separated unique genotype RM-62 from others and rest (29) of ricin genotypes divided into three main groups. Using 5 RAPD markers only two castor bean genotypes have not been distinguished. 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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