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GENETIC DIVERSITY ANALYSIS OF CASTOR (*RICINUS COMMUNIS* L.) USING SSR MARKERS

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ABSTRACT

The aim of this study was to assess genetic diversity within the set of 60 ricin genotypes using 5 SSR primers. Ten SSR primers revealed a total of 36 alleles ranging from 5 to 10 alleles per locus with a mean value of 7.20 alleles per locus. The PIC values ranged from 0.758 (Rco30) to 0.879 (Rco29) with an average value of 0.829 and the DI value ranged from 0.774 (Rco30) to 0.881 (Rco29) with an average value of 0.836. Probability of identity (PI) was low ranged from 0.002 (Rco29) to 0.015 (Rco30) with an average of 0.006. A dendrogram was constructed from a genetic distance matrix based on profiles of the 5 SSR loci using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 60 diverse accessions of castor bean was clustered into five clusters. Cluster 1 contained 14 genotypes, cluster 2 included 7 genotypes of ricin and cluster 3 contained 8 genotypes of ricin. Cluster 4 included 10 genotypes and cluster 5 contained 21 genotypes. We could not distinguish 4 genotypes grouped in cluster 1, RM-103 and RM-104 and genotypes RM-100 and RM-101, which are genetically the closest. Knowledge on the genetic diversity of castor can be used to 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; Dendrogram; Genetic diversity; Simple sequence repeat (SSR)

INTRODUCTION

Castor (*Ricinus communis* L.) is a cross-pollinated diploid (2n = 2x = 20) species belonging to the family *Euphorbiaceae* and genus *Ricinus*. 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 (**Ogunniyi, 2006**). Castor seeds contain around 50–55% oil which is rich in an unusual hydroxy fatty acid, ricinoleicacid which constitutes about 80–90% of the total fatty acids (**Jeong and Park, 2009**).

Knowledge of genetic variability is important for breeding programs to provide the basis for developing desirable genotypes. Genetic variability has been studied using molecular techniques, including amplified fragment length polymorphism (AFLP) (**Pecina-Quintero *et al*., 2013**), random amplified polymorphism DNA (RAPD) (**Petrovičová *et al*., 2015; Vivodík *et al*., 2015**), single nucleotide polymorphism (SNP) markers (**Foster et al., 2010**), simple sequence repeat (SSR) (**Tan *et al*., 2014**), start codon targeted polymorphism (SCoT) and inter simple sequencerepeat (ISSR) (**Kallamadi *et al*., 2015**). **Pecina-Quintero *et al*., (2013)** used four different AFLP primer pairs. In total, the four combinations of selective primers amplified 430 products, of which 228 were polymorphic. **Vivodík *et al*., (2014)** used 8 RAPD markers to detect genetic variability among the set of 40 castor genotypes. **Foster *et al*., (2010)** analyzed the population genetics of *R. communis* in a worldwide collection of plants from germplasm and determined the population genetic structure of 676 samples using single nucleotide polymorphisms (SNPs) at 48 loci. The goal of **Tan *et al*., (2014)** was to develop a more complete panel of SSR markers that can be used to construct a genetic map of castor bean and to examine genetic variation in this plant. The present investigation of **Kallamadi *et al*., (2015)** has been undertaken to assess the extent of genetic diversity in 31 accessions of castor using ISSR and SCoT primers. These markers are favourable as they exhibit high locus-specificity, high levels of variability, robustness towards genotyping, and a co-dominant mode of inheritance (**Woodhead *et al*., 2005**). So far, several investigations on the discrimination between crop genotypes using SSR markers have been carried out by **Siripiyasing *et al*., (2013); Fayyaz *et al*., (2014); Kanwal *et al*., (2014); Polat *et al*., (2015); Yousaf *et al*., (2015)**.

This study investigates the genetic diversity among 60 castor genotypes using 5 SSRs markers for the purpose of further breeding ricin.

MATERIAL AND METHODS

Plant material and DNA extraction

A total 60 castor genotypes (called RM-45 – RM-105) obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia), were used in this study. Genotype of castor were grown in a cultivation box at temperature 27 °C and photoperiod 12 hours light and 12 hours dark. DNA of 60 genotypes of castor was extracted from leaves of 10 day old seedlings using the GeneJET Plant Genomic DNA Purification Mini Kit. Each sample was diluted to 20 ng in TE buffer (10 mmol Tris–HCl, pH 8.0 and 0.1 mmol EDTA, pH8.0), stored at -20 °C and resolved on agarose gel with the standard lambda DNA for determining the DNA concentration.

SSR and data analysis

Amplification of SSR fragments was performed according to **Bajay *et al*., (2009, 2011)** (Table 1). Polymerase chain reaction (PCR) were performed in 25 µl of a mixture containing 10.5 µl H_2O, 12.0 µl Master Mix (Genei, Bangalore, India), 0.75 µl of each primer (10 pmol) and 1 µl DNA (100 ng). Amplification was performed in a programmed thermocycler (Biometra, Germany) and amplification program consisted of an initial denaturing step at 94 °C for 1 min, followed by 35 cycles of amplification [94 °C (1 min), 1 min at the specific annealing temperature of each primer pair (Table 1), 72 °C (1 min)] and a final elongation step at 72 °C for 10 min. Amplification products were confirmed by electrophoresis in 7% denaturing polyacrylamide gels and silver stained and documented using gel documentation system Grab-It 1D for Windows. Data obtained from SSR analysis were scored as presence (1) or absence (0) of fragments for each castor genotype and entered into a matrix. Based on the similarity matrix, a dendogram showing the genetic relationships between genotypes was constructed using unweighted pair group method with arithmetic mean (UPGMA) by using the SPSS professional statistics version 17 software package. For the assessment of the polymorphism between castor genotypes and usability of SSR markers in their differentiation diversity index (DI) (**Weir, 1990**), the probability of identity (PI) (**Paetkau *et al*., 1995**) and polymorphic information content (PIC) (**Weber, 1990**) were used.

Table 1 List of SSR primers **(Bajay *et al.*, 2009, 2011)**

Marker name	*Ta* (°C)	Repeat motif	Sequence of the primer (5´ - 3´)
Rco23	62	$(GA)_{15}(AG)_8$	F: CATGGATGTAGAGGGTCGAT R: CAGCCAAGCCAAAGATTTTC
Rco26	62	$(CT)_{19}$	F: TTGCTTGTCAAAGGGGAGTT R: TCATTTTGAGGGAGAAACCA
Rco29	60	$(GA)_7$	F: GGAGAAAAGAAAGGGAGAAGG R: GCCAAAAGCACACTTAATTTGA
Rco30	60	$(AG)_{19}$	F: TGAAACTTTGGAGCTTGGAGA R: GGTCCCACACATTCATACACA
Rco31	60	$(TC)_{12}(TCTA)_4(AC)_{10}$	F: ACAATGCGTGTGTCTGTGTG R: CCTCAACCCTTTGCTGTTTC

Ta- annealing temperature

RESULTS AND DISCUSSION

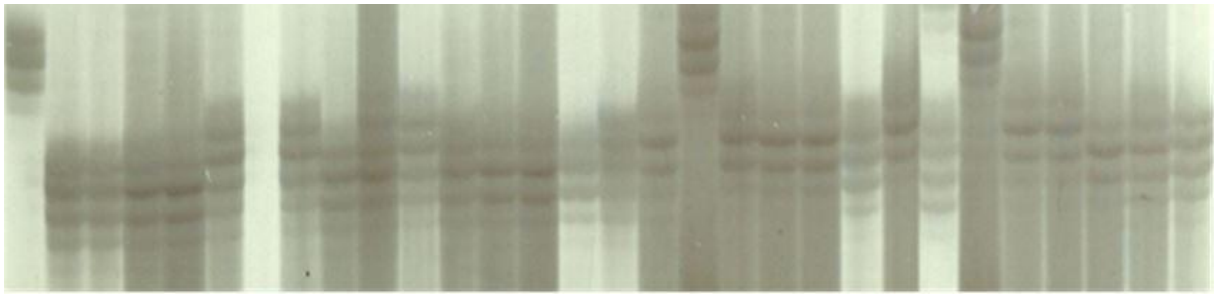
Five SSR primers were used for cultivar identification and estimation of the genetic relations among 60 ricin genotypes. All 5 SSR primers generated clear banding patterns with high polymorphism (Figure 1). Five SSR primers revealed a total of 36 alleles ranging from 5 (Rco30) to 10 (Rco29) alleles per locus with a mean value of 7.20 alleles per locus (Table 2). 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. The PIC values ranged from 0.758 (Rco30) to 0.879 (Rco29) with an average value of 0.829 and the DI value ranged from 0.774 (Rco30) to 0.881 (Rco29) with an average value of 0.836 (Table2). 100% of used SSR markers had PIC and DI values higher than 0.7 that means high polymorphism of chosen markers used for analysis. Probability of identity (PI) was low ranged from 0.002 (Rco29) to 0.015 (Rco30) with an average of 0.006 (Table 2).

A dendrogram was constructed from a genetic distance matrix based on profiles of the 5 SSR loci using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 60 diverse accessions of castor bean was clustered into five clusters. Cluster 1 contained 14 genotypes, cluster 2 included 7 genotypes of ricin and cluster 3 contained 8 genotypes of ricin. Cluster 4 included 10 genotypes and cluster 5 contained 21 genotypes (Figure 2). We could not distinguish 4 genotypes grouped in cluster 1, RM-103 - RM-104 and RM-100 - RM-101, which are genetically the closest.

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

Marker name	Number of alleles	DI	PIC	PI
Rco23	8	0.861	0.856	0.003
Rco26	6	0.822	0.816	0.006
Rco29	10	0.881	0.879	0.002
Rco30	5	0.774	0.758	0.015
Rco31	7	0.843	0.837	0.004
Average	7.20	0.836	0. 829	0.006

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



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Figure 1 PCR amplification products of 30 genotypes of castor produced by SSR marker Rco29. Lanes 1 - 30 are castor genotypes RM-45 – RM-74.

Similar results detected **Pecina-Quintero *et al.*, (2013)** who used seven SSR markers and the profiles generated were collectively able to discriminate among 82 *R. communis* accessions and the six controls. **Kyoung-In *et al.*, (2011)** used 28 SSR loci revealed polymorphisms in a castor bean collection consisting of 72 accessions. A total of 73 alleles were detected, with an average of 3.18 alleles per locus, and the polymorphism information content (PIC) ranged from 0.03 to 0.47 (mean = 0.26). **Gedil *et al.*, (2009)** used-six primers for analysis of castor. The 6 SSR primers produced amplification products with alleles ranging from 1 to 2 for the parents and the hybrids. The present investigation of **Tan *et al.*, (2013)** was to assess the genetic diversity in 58 accessions of castor. Seventy alleles were detected among the somaclones and their donors, with an average of 2.1 alleles per locus. Based on the profiles of the SSR loci, a dendrogram was constructed using the unweighted pair-group method with an arithmetic average (UPGMA). **Dhingani *et al.*, (2012)** used 9 SSR primers for analysis of genetic diversity of castor. SSR analysis yielded 16 fragments, of which 11 were polymorphic, with an average PIC value of 0.87. SSR molecular markers have been used in population genetic studies **Yang *et al.*, (2013); Žiarovská *et al.*, (2013); Ahmad *et al.*, (2014); Aslam *et al.*, (2014); Maršálková *et al.*, (2014); Lancíková *et al.*, (2015)**.

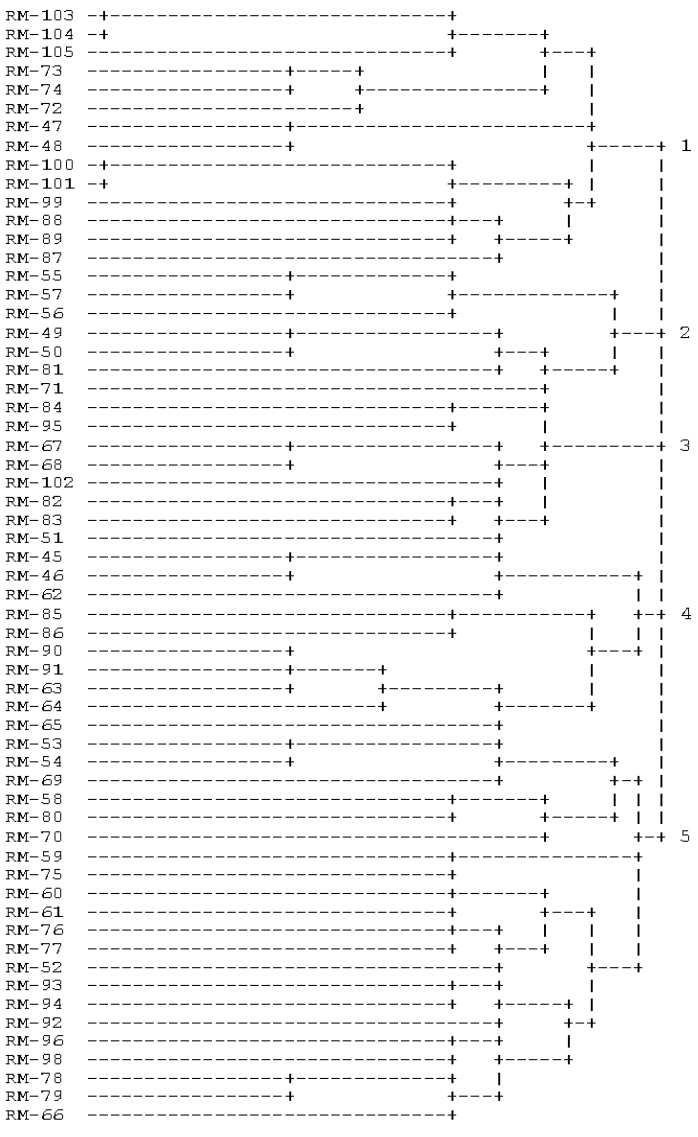


Figure 2 Dendrogram of 60 castor genotypes constructed based on 5 SSR markers.

CONCLUSION

In conclusion, a high level of genetic diversity exists among the castor accessions analyzed. According to analysis, the collection of 60 diverse accessions of castor bean was clustered into five clusters. We could not distinguish 4 genotypes grouped in cluster 1, RM-103 - RM-104 and RM-100 - RM-101, which are genetically the closest. A SSR marker system is a rapid and reliable method for cultivar identification that might also be used in quality control in certified seed production programs, to identify sources of seed contamination, and to maintain pure germplasm collections.

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