IDENTIFICATION AND DIFFERENTIATION OF RICINUS COMMUNIS L. USING SSR MARKERS

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ABSTRACT

The castor-oil plant (*Ricinus communis* L.), a member of the spurge family (Euphorbiaceae), is a versatile industrial oil crop that is cultivated in many tropical and subtropical regions of the world. Castor oil is of continuing importance to the global specialty chemical industry because it is the only commercial source of a hydroxylated fatty acid. Castor also has tremendous future potential as an industrial oilseed crop because of its high seed oil content, unique fatty acid composition, potentially high oil yields and ability to be grown under drought and saline conditions. Knowledge of genetic variability is important for breeding programs to provide the basis for developing desirable genotypes. The aim of this study was to assess genetic diversity within the set of 60 ricin genotypes using 10 SSR primers. Ten SSR primers revealed a total of 67 alleles ranging from 4 to 9 alleles per locus with a mean value of 6.70 alleles per locus. The PIC values ranged from 0.719 to 0.860 with an average value of 0.813 and the DI value ranged from 0.745 to 0.862 with an average value of 0.821. Probability of identity (PI) was low ranged from 0.004 to 0.018 with an average of 0.008. A dendrogram was constructed from a genetic distance matrix based on profiles of the 10 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 six clusters. We could not distinguish 2 genotypes grouped in cluster 1, RM-96 and RM-98, which are genetically the closest. Knowledge on the genetic diversity of castor can be used to future breeding programs of castor.

Keywords: castor; genetic diversity; molecular markers; 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. Castor is an important industrial oilseed crop. 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 in castor bean has been studied using molecular techniques, including amplified fragment length polymorphism (AFLP) **(Pecina-Quintero et al., 2013)**, random amplified polymorphism DNA (RAPD) **(Vivodík et al., 2014)**, 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. Amplification of genomic DNA of 40 genotypes, using RAPD analysis, yielded in 66 fragments, with an average of 8.25 polymorphic fragments per primer. **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. Among the DNA markers, SSR markers have been used intensively to analyse genetic diversity. 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 10 SSRs markers.

MATERIAL AND METHODOLOGY

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. DNA of 60 genotypes of castor was extracted from leaves of 10 day old seedlings using the Gene JET Plant Genomic DNA Purification Mini Kit. Each sample was diluted to 20 ng with TE buffer (10 mM Tris–HCl, pH 8.0 and 0.1 mM EDTA, pH 8.0), stored at -20 °C and resolved on agarose gel with the standard lambda DNA for determining the DNA concentration.

SSR 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 analysis:

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.

RESULTS AND DISCUSSION

Ten SSR primers were used for cultivar identification and estimation of the genetic relations among 60 ricin genotypes. We analyzed 60 genotypes of ricin, because the company Zeainvent Trnava gave us such a database of genotypes. All 10 SSR primers generated clear banding patterns with high polymorphism (Figure 1.). Ten SSR primers revealed a total of 67 alleles ranging from 4 (Rco15) to 9 (Rco05) alleles per locus with a mean value of 6.70 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 0.719 (Rco15) to 0.860 (Rco05) with an average value of 0.813 and the DI value ranged from 0.745 (Rco15) to 0.862 (Rco05) with an average value of 0.821 (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 0.004 (Rco05) to 0.018 (Rco15) with an average of 0.008 (Table 2).

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

Marker name	*Ta* (°C)	Repeat motif	Sequence of the primer (5´ - 3´)
Rco02	60	$(AC)_{12}$	F: CTAGCTTTGGGGCACAGTC R: GGAAAATAGGTGCGTATGAAAC
Rco05	60	$(TG)_6(GA)_{22}(GAA)_4$	F: AGCCCAGAAATTGGAAAAGA R: CAAACCCAAGCAAACCTCA
Rco06	60	$(TG)_{11}$	F: GGGTGAAAATGAAGAGATTGG R: ATAACCCGTGAAGCATGGAC
Rco08	60	$(TG)_{10}$	F: CGTGTGTCTGTGTGCATGTC R: CCTCAACCCTTTGCTGTTTC
Rco09	60	$(AC)_{11}$	F: CCAACTCCCTTGTCTGCAA R: GTGAATGGCAAGCAGCAAT
Rco13	62	$(GA)_{23}$	F: GGTGCTTCCAGAAATTCAGTT R: GGAGGGGAAAGACAGGATTC
Rco15	60	$(AG)_{18}$	F: CACGCACGTTAAAGCAAACT R: GCGAAGAAACCAAAATGGAG
Rco18	60	$(CA)_{17}$	F: AGGGGGATAAGCGTGATATG R: CCGTTATGAAAAGGAAAGCA
Rco20	60	$(TC)_{23}$	F: CCAAAAGGAATGTGGGACTC R: TGTGGAGAGGATGAAGAGGAA
Rco22	62	$(AAAC)_3(AC)_9(TC)_5$	F: ATCCGCCGACAATAGCAG R: GCAACACTCTCTTCCCTGAA

Note: *Ta*- annealing temperature.

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
Rco02	7	0.834	0.827	0.007
Rco05	9	0.862	0.860	0.004
Rco06	5	0.792	0.778	0.010
Rco08	7	0.834	0.830	0.006
Rco09	6	0.814	0.806	0.007
Rco13	8	0.839	0.835	0.006
Rco15	4	0.745	0.719	0.018
Rco18	8	0.841	0.838	0.006
Rco20	6	0.818	0.809	0.008
Rco22	7	0.832	0.826	0.006
Average	**6,70**	**0.821**	**0. 813**	**0.008**

Note: DI- diversity index, PIC- polymorphic information content, PI- probability of identity.
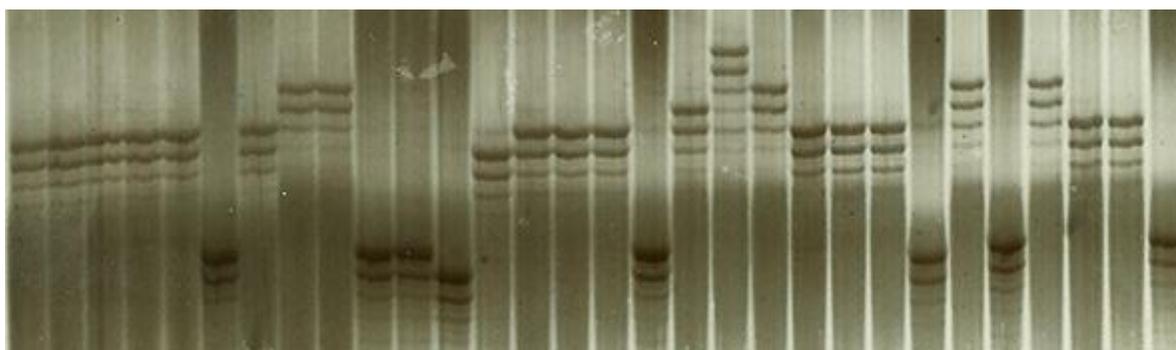
Figure 1. PCR amplification products of 30 genotypes of castor produced with SSR marker Rco-13. Lanes 1 - 30 are castor genotypes RM-45 – RM-74.

A dendrogram was constructed from a genetic distance matrix based on profiles of the 10 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 six clusters. Cluster 1 contained 13 genotypes, cluster 2 included 7 genotypes of ricin and cluster 3 contained 4 genotypes of ricin- RM-45, RM-46, RM-47 and RM-74. Cluster 4 included 15 genotypes and cluster 5 contained 8 genotypes. The last cluster 6 included 13 genotypes of ricin (Figure 2.). We could not distinguish 2 genotypes grouped in cluster 1, RM-96 and RM-98, which are genetically the closest.

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. The number of alleles ranged from four to eight (Rco23) with an average of 5.5 per marker. **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). Values for observed (HO) and expected (HE) heterozygosity ranged from 0.00 to 0.19 (mean = 0.11) and from 0.04 to 0.54 (mean = 0.31), respectively. **Gedil et al., (2009)** used only six primers (SSRY26, SSRY40, SSRY47, SSRY 61, SSRY52, and SSRY 189) 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 theirdonors, with an average of 2.1 alleles per locus. Each genotype had one or two alleles per locus. Shannon's information index ranged from 0.050 to 0.954 (mean 0.578) and Nei's expected heterozygosity values ranged from 0.017 to 0.587 (mean 0.439). Based on the profiles of the SSR loci, a dendrogram was constructed using the unweighted pair-group method with an arithmetic average (UPGMA). The 53 somaclones and five donors were divergent and clustered into six groups with a similarity coefficient of 0.878. **Dhingani et al., (2012)** used 9 SSR primers for

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RM-96  -+-----------------------------+
RM-98  -+                             +-------+
RM-95  -----------------------------+        +---------+
RM-99  ----------------------------------+        |
RM-72  ---------------------------------+------+   |
RM-73  ---------------------------+         +---+  |
RM-71  --------------------------------+    +----+---+---+1
RM-81  ---------------------------------+   |   |
RM-100 --------------------------------+------+  |   |
RM-101 --------------------------------+      +---+  |
RM-93  -------------------------------+--------+  |     |
RM-94  ----------------------------+       +---+     |
RM-60  ----------------------------------+          |
RM-85  ---------------------------+------+          |
RM-86  ---------------------------+      +-+        |
RM-59  -----------------------------+---+  |        |
RM-87  ----------------------------+      +--------+2
RM-69  ----------------------------+--------+  |     |
RM-70  ----------------------------+      +-+     |
RM-68  ----------------------------+              |
RM-45  ------------------+------+                 |
RM-46  ----------------+       +----------------+   |
RM-47  ----------------------------+          +---+3
RM-74  -----------------------------------------+   |
RM-83  --------------------------+-------+          |
RM-84  -------------------------+      +-------+    |
RM-82  -------------------------+        |     |
RM-62  --------------------+--------+          +---+ |
RM-63  -----------------+        +-------+   |   | | |
RM-92  ----------------------+        +-----+   | | |
RM-61  ----------------------------+        +-+4
RM-76  --------------------+-----------+      | |
RM-77  -----------------+        +----------+   | |
RM-78  ---------------------+          +      | | |
RM-103 -----------+------+                +-+ |
RM-104 -----------+        +---+           |   |
RM-105 --------------------+   +----------+   |   |
RM-102 --------------------+        +--------+   |
RM-75  ----------------------------------+          |
RM-48  --------------------------+------+          |
RM-49  ---------------------------+   +----------+ |
RM-50  ----------------------------+        +-+5
RM-66  --------------------------------+----------+ |
RM-67  --------------------------+      | |
RM-51  ----------------------------+     | |
RM-52  ----------------------------+   +---+ |
RM-53  ----------------------------+       +   |
RM-79  --------------+--------------------+   |
RM-80  ----------------+        +-----+ |
RM-64  ----------------------+----------+ | |
RM-65  --------------------+        | |
RM-90  -----------------+-----+          +-+6
RM-91  --------------+   +----------+   |
RM-89  ----------------------+        +-----+ |
RM-88  ---------------------------+        +-+
RM-54  -----------------+----------------+   |
RM-55  ----------------+        +---+
RM-57  ----------------+-----+        |
RM-58  ----------------+   +-----------+
RM-56  ---------------------+
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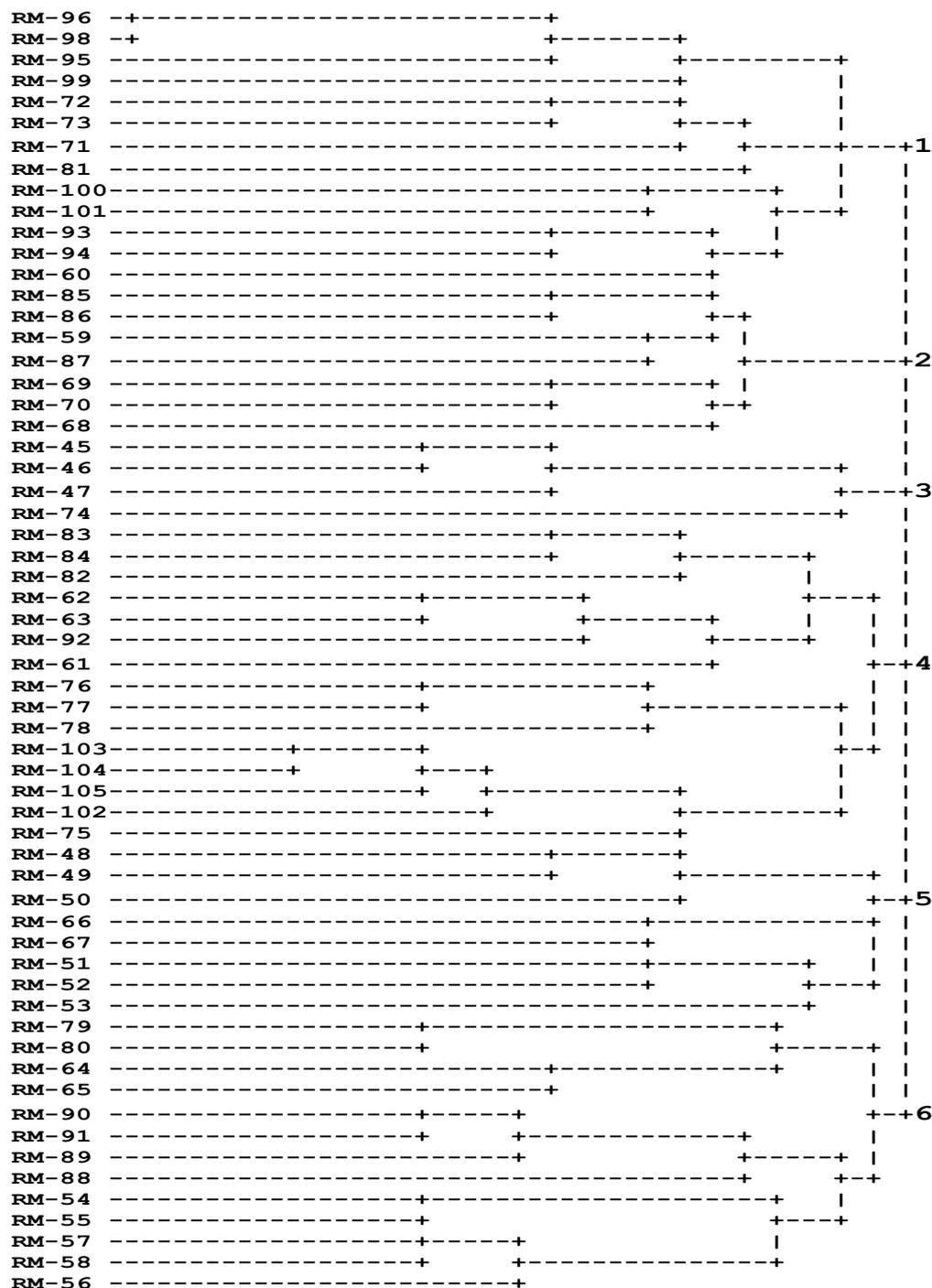
Figure 2. Dendrogram of 60 castor genotypes prepared based on 10 SSR markers.

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)**.

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 six clusters. We could not distinguish 2 genotypes grouped in cluster 1, RM-96 and RM-98 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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