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DIFFERENTIATION OF SLOVAK AND TUNISIAN CASTOR GENOTYPES (*RICINUS COMMUNIS* L.) USING SCOT MARKERS

Vivodík Martin*, Petrovičová Lenka, Balážová Želmíra, Gálová Zdenka

Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Nitra, Slovakia

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The characterization of genetic diversity of genotypes is the basic prerequisite for the successful breeding programs of castor like other crops. In the present investigation 40 genotypes of castor were analysed using 10 start codon targeted (SCoT) markers. Ten primers produced 62 DNA fragments with an average of 6.20 bands per primer. From these ten primers, primer SCoT 65, were the most polymorphic, where 7 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (3) was detected by primer SCoT 66. From the 62 amplified bands, 48 (85.94%) were polymorphic, with an average of 4.80 polymorphic bands per primer. To determine the level of polymorphism in the analysed group of castor genotypes, polymorphic information content (PIC) was calculated. The polymorphic information content (PIC) value ranged from 0.652 (ScoT 8) to 0.816 (SCoT 23) with an average of 0.738. The dendrogram of genetic relationships among 40 castor genotypes based on SCoT markers was constructed. The hierarchical cluster analysis showed that the castor genotypes were divided into 3 main clusters. Cluster 1 contained one Tunisian castor genotype KJ-2. Cluster 2 contained 2 castor genotypes RM-84 and RM-94. Cluster 3 was divided into subcluster 3A and subcluster 3B. Subcluster 3A contained 2 Tunisian castor genotypes- KJ-3 and KJ-4. Subcluster 3B contained 35 genotypes of castor. Two Tunisian castor genotypes of 3B subcluster (KJ-1 and KJ-5) were genetically the closest. We can assume that they have close genetic background. The present study shows effectiveness of employing SCoT markers in analysis of castor, and would be useful for further studies in population genetics, conservation genetics and genotypes improvement.

Keywords: Dendrogram, Castor, Molecular marker, SCoT analysis, Polymorphism

Introduction

Castor bean (*Ricinus communis* L.) has recently been highly rated as a source of raw material (oil) for biodiesel production, because beyond its high oil content (25–55%), it is a culture of great social appeal in Brazil by intensive use of workmanship in the field and allows for intercropping with other crops as beans, groundnuts or maize (Madail et al., 2007). In addition, castor bean cultivation is encouraged in areas of low water availability and is genetically improved to produce biofuel (Evangelista et al., 2004).

***Corresponding author:** Vivodík Martin, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Nitra, Slovakia; ✉ vivodikmartin@gmail.com

These types of molecular techniques included random amplified polymorphic DNA (RAPD) (Štefúnová et al., 2015), amplified fragment length polymorphism (AFLP) (Molin et al., 2013), inter-simple sequence repeat (ISSR) (Žiarovská et al., 2013) and simple sequence repeats (SSRs) (Shehata et al., 2009). These marker systems are useful for biodiversity analyses, phylogenetic studies, germplasm management, cultivar identification, and other applications (Luo et al. 2010). Recently, a simple novel DNA marker technique namely start codon targeted (SCoT) polymorphism, was developed by Collard and Mackill (2009). Primers for SCoT marker analysis were designed from the conserved region surrounding the translation initiation codon, ATG (Sawant et al., 1999). Suitability of SCoT markers for the construction of genetic maps, fingerprinting and phylogenetic studies has been proved by many authors. In many crops, such as citrus (Mahjbi et al., 2015) and castor (Kallamadi et al., 2015).

The goals of this study were to examine the effectiveness of SCoT markers for analysis of genetic diversity of castor and to study genetic relationships among 40 castor accessions.

Material and methodology

Plant material and DNA extraction

Ricin lines (20) were obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia) and next 20 ricin lines were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, Tunisia. Regions of origin of analyzed genotypes of Tunisian ricin: KJ- Ksar jedid, K- Kebili, G- Gabes, M- Mornag, MD- Mednine. Genomic DNA was isolated from the 14 days leaves with GeneJET Plant Genomic DNA Purification Mini Kit according to the manufacturer's instructions. Castor genotypes were grown in a growth chamber on humus soil.

SCoT amplification and statistical analysis

A total of 10 SCoT primers developed by Collard and Mackill (2009) were selected for the present study (Table 1). Each 15-μL amplification reaction consisted of 1.5 μL (100 ng) template DNA, 7.5 μL Master Mix (Genei, Bangalore, India), 1.5 μL 10 pmol primer, and 4.5 μL distilled water. Amplification was performed in a programmed thermocycler (Biometra, Germany) using the following program: 94 °C for 3 min; 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min; a 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 SCoT 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 the level of polymorphism by dividing the polymorphic bands by the total number of scored bands and to prepare a dendrogram. A dendrogram was constructed based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the help of SPSS professional statistics version 17 software package. For the assessment of the polymorphism between genotypes maize and usability SCoT markers in their differentiation we used polymorphic information content (PIC) (Weber, 1990).

Table 1 List of used 10 SCoT primers (Collard and Mackill, 2009)

SCoT Primers	Primer sequence (5´-3´)
SCoT 6	CAACAATGGCTACCACGC
SCoT 8	CAACAATGGCTACCACGT
SCoT 9	CAACAATGGCTACCAGCA
SCoT 12	ACGACATGGCGACCAACG
SCoT 23	CACCATGGCTACCACCAG
SCoT 66	ACCATGGCTACCAGCGAG
SCoT 65	ACCATGGCTACCACGGCA
SCoT 63	ACCATGGCTACCACGGGC
SCoT 62	ACCATGGCTACCACGGAG
SCoT 61	CAACAATGGCTACCACCG

Results and discussion

In this work, 10 primers were screened for PCR amplification of DNA and SCoT analysis in 40 castor genotypes. Table 1 and Table 2 shows sequences of these primers, total number of amplified fragments from 40 castor genotypes, the number of polymorphic bands and the polymorphic information content for each primer. Ten primers produced 62 DNA fragments (Table 2) with an average of 6.20 bands per primer. From these ten primers, primer SCoT 65, were the most polymorphic, where 7 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (3) was detected by primer SCoT 66.

Table 2 The statistical characteristics of the10 SCoT markers used in castor

SCoT Primers	Total number of bands	Number of polymorphic bands	Percentage of polymorphic bands (%)	PIC
SCoT 6	5	4	80.00	0.729
SCoT 8	4	4	100.00	0.652
SCoT 9	6	4	66.66	0.780
SCoT 12	7	5	71.43	0.715
SCoT 23	7	5	71.43	0.816
SCoT 66	4	3	75.00	0.729
SCoT 65	9	7	77.78	0.651
SCoT 63	8	6	75.00	0.780
SCoT 62	5	4	80.00	0.715
SCoT 61	7	6	85.71	0.815
Average	6.20	4.80	85.94	0.738
Total	62	48	–	–

From the 62 amplified bands, 48 (85.94%) were polymorphic, with an average of 4.80 polymorphic bands per primer. To determine the level of polymorphism in the analysed group of castor genotypes, polymorphic information content (PIC) was calculated (Table 2). The polymorphic information content (PIC) value ranged from 0.652 (ScoT 8) to 0.816 (SCoT 23) with an average of 0.738.

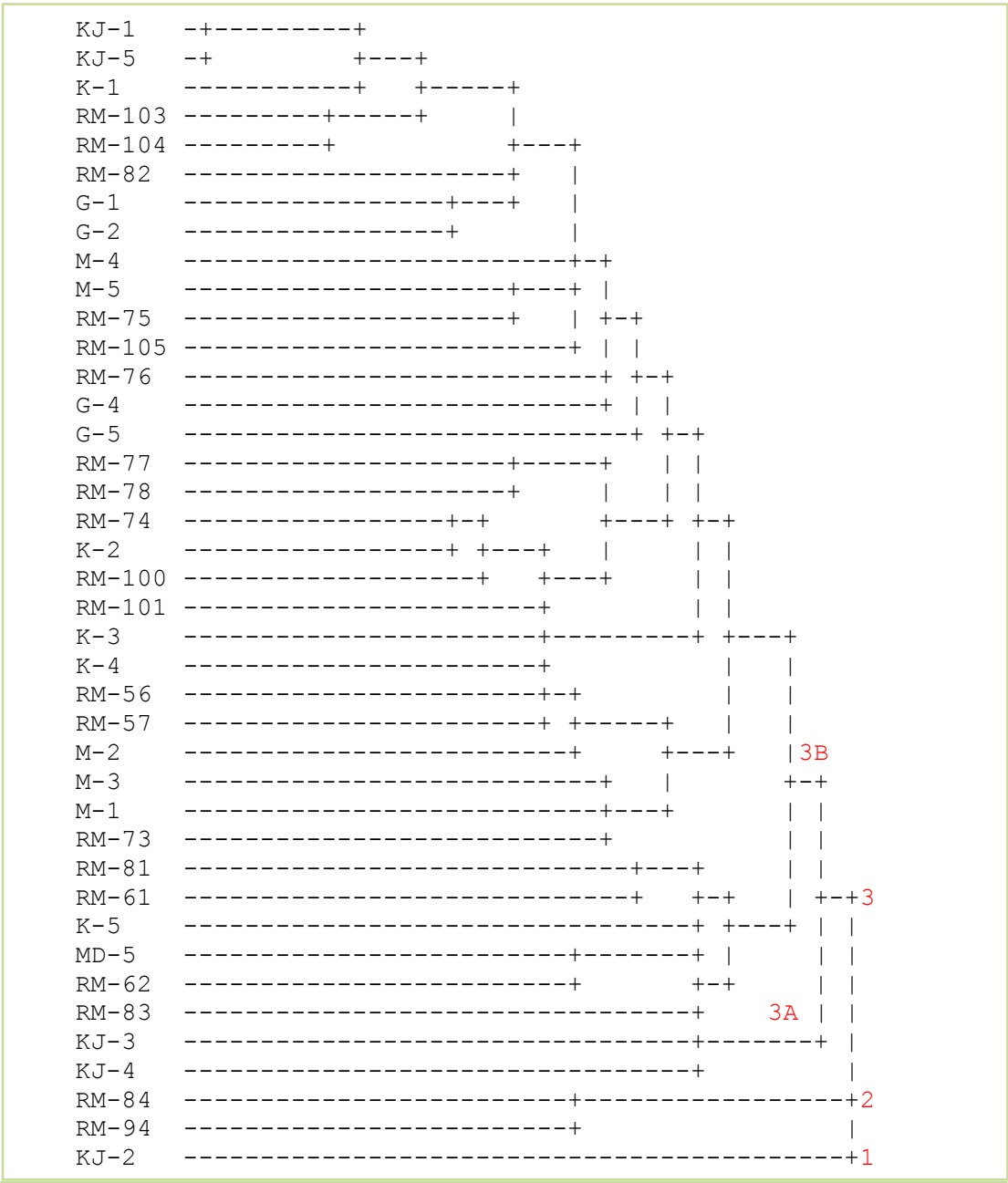

Figure 1 Dendrogram of 40 castor genotypes prepared based on 10 SCoT markers

The dendrogram of genetic relationships among 40 castor genotypes based on SCoT markers was constructed (Figure 1). The hierarchical cluster analysis showed that the castor genotypes were divided into 3 main clusters. Cluster 1 contained one Tunisian castor genotype KJ-2. Cluster 2 contained 2 castor genotypes RM-84 and RM-94. Cluster 3 was divided into subcluster 3A and subcluster 3B. Subcluster 3A contained 2 Tunisian castor genotypes- KJ-3 and KJ-4. Subcluster 3B contained 35 genotypes of castor. Two Tunisian castor genotypes of 3B subcluster (KJ-1 and KJ-5) were genetically the closest. We can assume that they have close genetic background (Figure 1).

CONCLUSION

In summary, SCoT marker analysis was successfully developed to evaluate the genetic relationships among the genus castor accessions originated from various area. The dendrogram of genetic relationships among 40 castor genotypes based on SCoT markers was constructed. The hierarchical cluster analysis showed that the castor genotypes were divided into 3 main clusters. Cluster 1 contained one Tunisian castor genotype KJ-2. Cluster 2 contained 2 castor genotypes RM-84 and RM-94. Cluster 3 was divided into subcluster 3A and subcluster 3B. Subcluster 3A contained 2 Tunisian castor genotypes- KJ-3 and KJ-4. Subcluster 3B contained 35 genotypes of castor. Two Tunisian castor genotypes of 3B subcluster (KJ-1 and KJ-5) were genetically the closest. Polymorphism revealed by SCoT technique was so abundant and could be used for molecular genetics study of the castor accessions, providing high-valued information for the management of germplasm, improvement of the current breeding strategies, and conservation of the genetic resources of castor species.

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References

COLLARD, B.C.Y. AND MACKILL, D.J. 2009. Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. In *Plant Mol. Biol. Rep.*, vol. 27, p. 86–93.

EVANGELISTA A.R., ABREU J.G. & PER A.J. 2004. Avaliação da composição química de tortas de mamona e amendoim obtidas por diferentes métodos de extração de óleo. In *Congresso Brasileiro de Mamona,* no. 1, p. 213–218.

KALLAMADI, P.R., GANGA RAO NADIGATLAB, V.P.R., MULPURIB, S. 2015. Molecular diversity in castor (*Ricinus communis* L.). In *Industrial Crops and Products,* vol. 66, p. 271–281. http://dx.doi.org/10.1016/j.indcrop.2014.12.061

LUO, C., HE, X.H., CHEN, H., OU, S.J. et al. 2010. Analysis of diversity and relationships among mango cultivars using start codon targeted (SCoT) markers. In *Biochem. Syst. Ecol.,* vol. 38, p. 1176–1184

MADAIL J.C.M., BELARMINO L.C. & NEUTZLING D.M. 2007. *Aspectos Econômicos da mamona.* Sistemas de Produção. Embrapa, 11 p. ISSN 1806-9207.

MAHJBI, A., BARAKET, G., OUESLATI, A., SALHI-HANNACHI, A. 2015. Start Codon Targeted (SCoT) markers provide new insights into the genetic diversity analysis and characterization of Tunisian Citrus

species. In *Biochemical Systematics and Ecology,* vol. 61, p. 390–398. http://dx.doi.org/10.1016/j.bse.2015.07.017

MOLIN, D., COELHO, C.J., MÁXIMO, D.S., FERREIRA, F.S., GARDINGO, J.R. AND MATIELLO, R.R. 2013. Genetic diversity in the germplasm of tropical maize landraces determined using molecular markers. In *Genet. Mol. Res.*, vol. 12(1), p. 99–114.

SAWANT, S.V., SINGH, P.K., GUPTA, S.K., MADNALA, R., TULI, R. 1999. Conserved nucleotide sequences in highly expressed genes in plants. In *Journal of Genetics,* vol. 78(2), p. 123–131. http://dx.doi.org/10.1007/BF02924562

SHEHATA, A.I., AL-GHETHAR, H.A., AL-HOMAIDAN, A.A. 2009. Application of simple sequence repeat (SSR) markers for molecular diversity and heterozygosity analysis in maize inbred lines. In *Saudi Journal of Biological Sciences,* vol. 16, p. 57–62.

ŠTEFÚNOVÁ, V., BEŽO, M., ŽIAROVSKÁ, J. AND RAŽNÁ, K. 2015. Detection of the genetic variability of *Amaranthus* by RAPD and ISSR markers. In *Pak. J. Bot.,* vol. 47(4), p. 1293–1301.

WEBER, J. L. 1990. Informativeness of human (dC-dA)n × (dG-dT)n polymorphism. In *Genomics,* vol. 7, p. 524–530.

ŽIAROVSKÁ, J., RAŽNÁ, K. AND LABAJOVÁ, M. 2013. Using of inter microsatellite polymorphism to evaluate gamma-irradiated *Amaranth* mutants. In *Emir. J. Food Agric.,* vol. 25(9), p. 673–681.