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Original scientific paper

START CODON TARGETED POLYMORPHISM FOR EVALUATION OF FUNCTIONAL GENETIC VARIATION AND RELATIONSHIPS IN CULTIVATED CASTOR (*Ricinus communis* L.) GENOTYPES

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In the present study, 111 castor genotypes were differentiated by the DNA fingerprinting patterns using 37 SCoT primers. The selected primers amplified DNA fragments across the 111 genotypes studied with the number of amplified fragments varying from 3 (SCoT 14) to 10 (SCoT 30 and SCoT 44) and the amplicon size varied from 100 to 3000 bp. Of the 246 amplified bands, 186 were polymorphic with an average of 5.03 fragments per primer. The percentage of polymorphic bands ranged from 57.14 % (SCoT 34) to 100.00 % (SCoT 28 and SCoT 33) with an average of 77.50%. The polymorphic information content (PIC) values varied from 0.372 (SCoT 14) to 0.818 (SCoT 30) with an average of 0.677. A dendrogram was constructed from a genetic distance matrix based on profiles of the 37 SCoT primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 111 diverse accessions of castor was clustered into two main clusters (1 and 2). The first cluster were subdivided into two subclusters (1a and 1b). Subclaster 1a contained 11 genotypes of castor and subclaster 1b contained 6 genotypes of castor. Subclaster 2 were subdivided into two subclusters (2a and 2b). Subclaster 2a contained 44 castor genotypes and subclaster 2b contained 50 castor genotypes. Results showed the utility of SCoT markers for estimation of genetic diversity of castor genotypes leading to genotype identification.

Keywords: ricin, SCoT markers, polymorphism, dendrogram**,** PIC.

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INTRODUCTION

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 (ANJANI, 2012). 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 random amplified polymorphism DNA (RAPD) (VIVODÍK *et al*., 2015), amplified fragment length polymorphism (AFLP) (ALLAN *et al*. 2008), simple sequence repeat (SSR) (GÁLOVÁ *et al*., 2015), inter-simple sequence repeat (ISSR) (WANG *et al*., 2013), single nucleotide polymorphism (SNP) markers (FOSTER *et al*., 2010), start codon targeted polymorphism (SCoT) (KALLAMADI *et al.*, 2015)**,** target region amplification polymorphism (TRAP) (SIMÕES *et al*., 2017) and using protein markers (MALOOK *et al.*, 2016). The polymerase chain reaction (PCR) has been used by many authors, such as ŽIAROVSKÁ *et al*. (2015); KANTI *et al*. (2015); VYHNÁNEK *et al*. (2015); BOŠEĽOVÁ and ŽIAROVSKÁ (2016); RAŽNÁ *et al*. (2016); ŽIAROVSKÁ *et al*. (2017); SIMÕES *et al*. (2017); AZIZI *et al*. (2017); POPOVIĆ *et al*. (2017); SENKOVÁ *et al*. (2017); BAYAT *et al.* (2018); REZAEI *et al*. (2018); NIKOLIĆ *et al*. (2018); SALEHI *et al*. (2018).

With initiating a trend away from random DNA markers towards gene-targeted markers, a novel marker system called SCoT (COLLARD and MACKILL, 2009) was developed based on the short conserved region flanking the ATG start codon in plant genes. SCoT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility. They are dominant markers like RAPDs and could be used for genetic analysis, quantitative trait loci (QTL) mapping and bulk segregation analysis. In principle, SCoT is similar to RAPD and ISSR because the same single primer is used as the forward and reverse primer (COLLARD and MACKILL, 2009). Suitability of SCoT markers system has been successfully employed in genetic diversity analysis and fingerprinting of a number of agricultural and horticultural crop species, such as oat (BALÁŽOVÁ *et al*., 2017), rye (PETROVIČOVÁ *et al*., 2017), maize (VIVODÍK *et al*., 2016), date palm (AL-QURAINY *et al*., 2015), orchardgrass (JIANG *et al*., 2014), pepper (TSABALLA *et al.*, 2015), ramie (SATYA *et al.*, 2015), castor (KALLAMADIA *et al*., 2015), sugarcane (QUE *et al*., 2014) and mango (GAJERA *et al.*, 2014).

The present study is focused on estimation of genetic distance between 111 castor genotypes, based on 37 SCoT markers. Although the information gathered here would be helpful in future for genomic mapping studies leading to development of castor cultivars with broader genetic background to obtain improved crop productivity.

MATERIALS AND METHODS

Plant material and DNA isolation

Ricin lines (111) (Table 2) were obtained from the breeding station Zeainvent Trnava Ltd. (Slovakia). DNA of 111 genotypes of castor was extracted from 10 day old leaves using the Gene JET Plant Genomic DNA Purification Mini Kit. The concentration and quality of DNA was checked up on 1.0% agarose gel coloured by ethidium bromide and detecting by comparing to λ-DNA with known concentration.

PCR conditions

A total of 37 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 UVP PhotoDoc-t® camera system. 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 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 genotypes castor and usability SCoT markers in their differentiation we used polymorphic information content (PIC) (WEBER, 1990).

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

where p_i and p_j are frequencies of i^{th} and j^{th} fragment of given genotype.

RESULTS AND DISCUSSION

The development of molecular markers has opened up numerous possibilities for their application in plant breeding. For detecting polymorphisms new molecular marker system called SCoT (COLLARD and MACKILL, 2009) was developed which TAG coding sequences of the genome. SCoT marker system had initially been validated in the model species rice (*Oryza sativa*) (COLLARD and MACKILL, 2009).

For the molecular analysis of 111 castor genotypes 37 SCoT primers were used. PCR amplifications using 37 SCoT primers produced total 246 DNA fragments that could be scored in all genotypes. The selected primers amplified DNA fragments across the 111 genotypes studied with the number of amplified fragments varying from 3 (SCoT 14) to 10 (SCoT 30 and SCoT 44) and the amplicon size varied from 100 to 3000 bp. Of the 246 amplified bands, 186 were polymorphic with an average of 5.03 fragments per primer (Table 1). The percentage of polymorphic bands ranged from 57.14 % (SCoT 34) to 100.00 % (SCoT 28 and SCoT 33) with an average of 77.50 %. The polymorphic information content (PIC) values varied from 0.372 (SCoT 14) to 0.818 (SCoT 30) with an average of 0.677 (Tab.1).

A dendrogram was constructed from a genetic distance matrix based on profiles of the 37 SCoT primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 111 diverse accessions of castor was clustered into two main clusters (1 and 2) (Figure 1). The first cluster were subdivided into two subclusters (1a and 1b). Subclaster 1a contained 11 genotypes of castor and subclaster 1b contained 6 genotypes of castor. Subclaster 2 were subdivided into two subclusters (2a and 2b). Subclaster 2a contained 44 castor genotypes and subclaster 2b contained 50 castor genotypes. Using 37 SCoT markers we were able to distinguish all 111 genotypes of the ricin.

Table 1. Statistical characteristics of the SCoT markers used in castor

SCoT Primers	Primer sequence (5′-3′)	TNoB	NoPB	PoPB	PIC
ScoT 2	CAACAATGGCTACCACCC	4	3	75.00	0.592
ScoT 3	CAACAATGGCTACCACCG	7	5	71.43	0.723
SCoT 6	CAACAATGGCTACCACGC	8	7	87.50	0.734
SCoT 8	CAACAATGGCTACCACGT	7	5	71.43	0.740
SCoT 9	CAACAATGGCTACCAGCA	6	5	83.33	0.706
SCoT 11	AAGCAATGGCTACCACCA	6	5	83.33	0.730
SCoT 13	ACGACATGGCGACCATCG	9	6	66.67	0.566
SCoT 14	ACGACATGGCGACCACGC	3	2	66.67	0.372
SCoT 15	ACGACATGGCGACCGCGA	5	4	80.00	0.637
SCoT 16	ACCATGGCTACCACCGAC	6	5	83.33	0.774
SCoT 17	ACCATGGCTACCACCGAG	8	5	62.50	0.646
SCoT 18	ACCATGGCTACCACCGCC	4	3	75.00	0.573
SCoT 19	ACCATGGCTACCACCGGC	7	5	71.43	0.703
SCoT 20	ACCATGGCTACCACCGCG	6	5	83.33	0.641
SCoT 21	ACGACATGGCGACCCACA	9	7	77.78	0.700
SCoT 22	AACCATGGCTACCACCAC	4	3	75.00	0.573
SCoT 12	ACGACATGGCGACCAACG	9	6	66.67	0.663
SCoT 23	CACCATGGCTACCACCAG	5	3	60.00	0.607
SCoT 26	ACCATGGCTACCACCGTC	8	5	62.50	0.718
SCoT 28	CCATGGCTACCACCGCCA	7	7	100.00	0.744
SCoT 29	CCATGGCTACCACCGGCC	4	3	75.00	0.616
SCoT 30	CCATGGCTACCACCGGCG	10	8	80.00	0.818
SCoT 31	CCATGGCTACCACCGCCT	5	4	80.00	0.521
ScoT 33	CCATGGCTACCACCGCAG	6	6	100.00	0.773
SCoT 34	ACCATGGCTACCACCGCA	7	4	57.14	0.702
SCoT 36	GCAACAATGGCTACCACC	7	5	71.43	0.752
SCoT 40	CAATGGCTACCACTACAG	6	5	83.33	0.730
SCoT 44	CAATGGCTACCATTAGCC	10	8	80.00	0.694
SCoT 45	ACAATGGCTACCACTGAC	6	4	66.67	0.569
SCoT 54	ACAATGGCTACCACCAGC	9	7	77.78	0.800
SCoT 59	ACAATGGCTACCACCATC	6	4	66.67	0.641
SCoT 60	ACAATGGCTACCACCACA	9	6	66.67	0.767
SCoT 61	CAACAATGGCTACCACCG	7	6	85.71	0.773
SCoT 62	ACCATGGCTACCACGGAG	5	4	80.00	0.713
SCoT 63	ACCATGGCTACCACGGGC	8	6	75.00	0.748
SCoT 65	ACCATGGCTACCACGGCA	9	7	77.78	0.791
SCoT 66	ACCATGGCTACCAGCGAG	4	3	75.00	0.512
Average		**6.65**	**5.03**	**77.50**	**0.677**
Total		**246**	**186**	-	-

TNoB-Total number of bands, NoPB- Number of polymorphic bands, PoPB- Percentage of polymorphic bands (%), PIC-Polymorphic information content

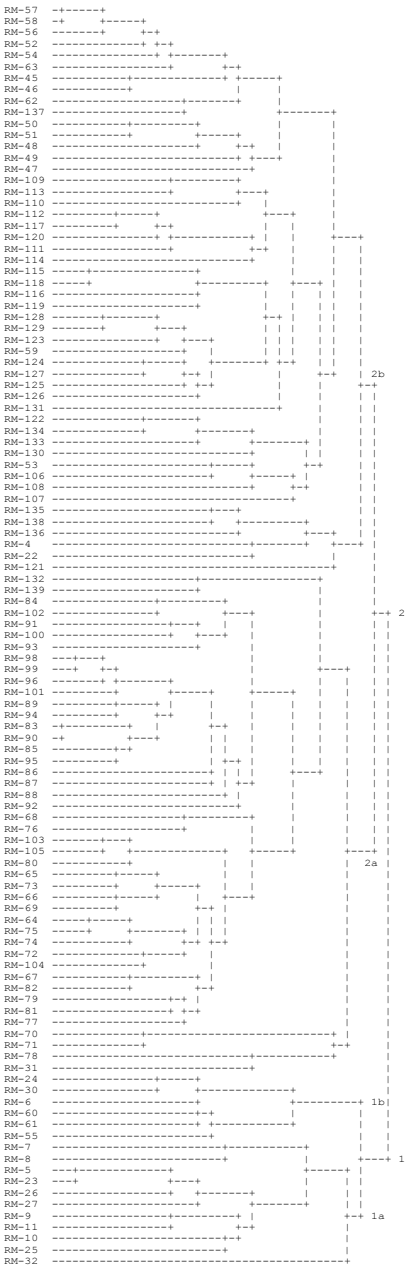

Fig. 1. Dendrogram of 111 castor genotypes based on data from 37 SCoT markers

Table 2. List of 111 ricin genotypes analyzed

1.	RM-4	41.	RM-66	81.	RM-107
2.	RM-5	42.	RM-67	82.	RM-108
3.	RM-6	43.	RM-68	83.	RM-109
4.	RM-7	44.	RM-69	84.	RM-110
5.	RM-8	45.	RM-70	85.	RM-111
6.	RM-9	46.	RM-71	86.	RM-112
7.	RM-10	47.	RM-72	87.	RM-113
8.	RM-11	48.	RM-73	88.	RM-114
9.	RM-22	49.	RM-74	89.	RM-115
10.	RM-23	50.	RM-75	90.	RM-116
11.	RM-24	51.	RM-76	91.	RM-117
12.	RM-25	52.	RM-77	92.	RM-118
13.	RM-26	53.	RM-78	93.	RM-119
14.	RM-27	54.	RM-79	94.	RM-120
15.	RM-28	55.	RM-80	95.	RM-121
16.	RM-29	56.	RM-81	96.	RM-122
17.	RM-30	57.	RM-82	97.	RM-123
18.	RM-31	58.	RM-83	98.	RM-124
19.	RM-32	59.	RM-84	99.	RM-125
20.	RM-45	60.	RM-85	100.	RM-126
21.	RM-46	61.	RM-86	101.	RM-127
22.	RM-47	62.	RM-87	102.	RM-128
23.	RM-48	63.	RM-88	103.	RM-129
24.	RM-49	64.	RM-89	104.	RM-130
25.	RM-50	65.	RM-90	105.	RM-131
26.	RM-51	66.	RM-91	106.	RM-132
27.	RM-52	67.	RM-92	107.	RM-133
28.	RM-53	68.	RM-93	108.	RM-134
29.	RM-54	69.	RM-94	109.	RM-135
30.	RM-55	70.	RM-95	110.	RM-136
31.	RM-56	71.	RM-96	111.	RM-137
32.	RM-57	72.	RM-98		
33.	RM-58	73.	RM-99		
34.	RM-59	74.	RM-100		
35.	RM-60	75.	RM-101		
36.	RM-61	76.	RM-102		
37.	RM-62	77.	RM-103		
38.	RM-63	78.	RM-104		
39.	RM-64	79.	RM-105		
40.	RM-65	80.	RM-106		

Level of polymorphism in analysed castor genotypes was determined by calculated polymorphic information content (PIC) (Table 1). Similar values of PIC were detected by other authors (LUO *et al*., 2012; ARYA *et al*., 2014; GAJERA *et al*., 2014; QUE *et al*., 2014; GAO *et al*.,

2014; FANG-YONG *et al*., 2014; JIANG *et al*., 2014; HUANG *et al*., 2014; SATYA *et al*., 2015) and these values presented a high level of polymorphism of genotypes detected by SCoT markers**.** HUANG *et al*. (2014) assessed the genetic diversity of six *Hemarthria* cultivars using seven SCoT primers, which together amplified 105 bands with an average of 15 bands per sample. Start codon-targeted markers were utilized by GAJERA *et al*. (2014) who used 19 SCoT markers for characterization and genetic comparison among 20 mango cultivars. These primers produced total 117 loci across 20 cultivars, of which 96 (79.57 %) were polymorphic. In the study QUE *et al*. (2014), used 20 start codon targeted (SCoT) marker primers to assess the genetic diversity among 107 sugarcane accessions within a local sugarcane germplasm collection. These primers amplified 176 DNA fragments, of which 163 were polymorphic (92.85%). The aim of GAO *et al*., (2014) was to estimate the genetic diversity across 43 varieties of *Lycoris*. Of 57 SCoT primers screened, 23 SCoT primers were identified to be high polymorphism. FANG-YONG *et al*. (2014) assessed the genetic diversity of 31 germplasm resources of *Myrica rubra* from Zhejiang Province, the major gathering site and the largest producer of *M. rubra* in China using start codon-targeted polymorphism (SCoT) markers. Authors used 38 primers to perform PCR amplification of 31 genotypes, from which 298 reproducible bands were obtained, including 251 polymorphic bands (84.23%). SATYA *et al*. (2015) used 24 start codon targeted (SCoT) markers to assess genetic diversity and population structure of indigenous, introduced and domesticated ramie (*Boehmeria nivea* L. Gaudich.). JIANG *et al*. (2014) used start codon-targeted (SCoT) markers to analyze the diversity and genetic relationships among 95 orchardgrass accessions. In total, 273 polymorphic bands were detected with an average of 11.4 bands per primer. In the study ZHANG *et al*. (2015) used SCoT markers to study the genetic diversity and relationships among 53 *Elymus sibiricus* accessions. KHAN *et al.* (2016) studied genetic differences to evaluate the level and distribution of diversity among genotypes of *Mentha* using SCoT markers. The results showed significant differences among 13 genotypes. The 10 SCoT primers chosen for molecular analysis revealed 171 bands, of which 117 were polymorphic. Percentage of polymorphic bands ranged from 75 to 100% according to primers tested. The generated dendrogram based on SCoT profiles divided the genotypes into 5 groups. Cluster analysis based on these traits grouped the genotypes into 8 separate clusters. CHAI *et al.* (2017) investigate the optimal number of individuals that may represent the genetic diversity of a single population, using Start Codon Targeted (SCoT) markers. Two cultivated varieties and two wild accessions were evaluated using five SCoT primers, also testing different sampling sizes: 1, 2, 3, 5, 8, 10, 20, 30, 40, 50, and 60 individuals. The results showed that the number of alleles and the Polymorphism Information Content (PIC) were different among the four accessions. Cluster analysis by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and structure placed the 240 individuals into four distinct clusters.

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REFERENCES

AL-QURAINY, F., S., KHAN, M., NADEEM, M.,TARROUM (2015): SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm cultivars. Pakistan J. Bot., *47*: 637-640.

ALLAN, G., A., WILLIAMS, P.D., RABINOWICZ, A.P., CHAN, J., RAVEL, P., KEIM (2008): Worldwide genotyping of castor bean germplasm (*Ricinus communis* L.) using AFLPs and SSRs. Genet. Resour. Crop Evol., *55*: 365-378.

ANJANI, K. (2012): Castor genetic resources: a primary gene pool for exploitation. Ind. Crops Prod., *35*: 1–14.

ARYA, L., R.K., NARAYANAN, M., VERMA, A.K., SINGH, V., GUPTA (2014): Genetic diversity and population structure analyses of *Morinda tomentosa* Heyne, with neutral and gene based markers. Gen. Res. Crop Evol., *61*: 1469-1479.

AZIZI, H., M., SHEIDAI, V., MOZAFFARIAN, Z., NOORMOHAMMADI (2017): Molecular studies (ISSR, cpDNA) in *Tragopogon buphthalmoides* (Asteraceae): Population genetics and taxonomic implication. Genetika, *49* (3): 775-789.

BALÁŽOVÁ, Ž., Z., GÁLOVÁ, M., VIVODÍK, L., PETROVIČOVÁ, R., HORNYÁK GREGÁŇOVÁ (2017): Molecular variability of oat based on gene specific markers. Potravinarstvo Slovak Journal of Food Sciences, *11*: 332-337.

BAYAT, M., R., AMIRNIA, H., ÖZKAN, A., GEDIK, D., ATES, B., TANYULAC, M., RAHIMI (2018): Diversity and phylogeny of saffron (*Crocus sativus* L.) accessions based on IPBS markers, Genetika, *50* (1): 33-44.

BOŠEĽOVÁ, D. and J., ŽIAROVSKÁ (2016): Direct PCR as the platform of *Hedera helix*, L. genotypying without the extraction of DNA. Journal of Central European Agriculture, *17*: 941-949.

COLLARD, B.C.Y., D.J., MACKILL (2009): Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Molecular Biology Report, *27*: 86-93.

FANG-YONGA, C.H. and L., JI-HONGA (2014): Germplasm genetic diversity of *Myrica rubra* in Zhejiang Province studied using inter-primer binding site and start codon-targetedpolymorphism markers. Sci. Horticulturae, *170*: 169-175.

FOSTER J.T., G.J., ALLAN, A.P., CHAN, P.D., RABINOWICZ, J., RAVEL, P.J., JACKSON, P., KEIM (2010): Single nucleotide polymorphisms for assessing genetic diversity in castor bean (*Ricinus communis*). BMC Plant Biol., *10*: 13-18.

GAJERA, H.P., R.P., BAMBHAROLIA, R.K., DOMADIYA, S.V., PATEL, B.A., GOLAKIYA (2014): Molecular characterization and genetic variability studies associated with fruit quality of indigenous mango (*Mangifera indica* L.) cultivars. Plant Systematics and Evolution, *300*: 1011-1020.

GAO, Y.H., Y.Q., ZHU, Z.K., TONG, Z.Y., XU, X.F., JIANG, CH.H., HUANG (2014): Analysis of genetic diversity and relationships among genus *Lycoris* based on start codon targeted (SCoT) marker. Biochemical Systematics and Ecology, *57*: 221-226.

GÁLOVÁ, Z., M., VIVODÍK, Ž., BALÁŽOVÁ, T., KUŤKA HLOZÁKOVÁ (2015): Identification and differentiation of *Ricinus communis* L. using SSR markers. Potravinarstvo, *9*: 556-561.

HUANG, L., X., HUANG, H., YAN, G., YIN, X., ZHANG, Y., TIAN, Y., ZHANG, X., JIANG, Y., YAN, X., MA, Y., PENG, J., ZHOU, G., NIE (2014): Constructing DNA fingerprinting of *Hemarthria* cultivars using EST-SSR and SCoT markers. Genetic Resources and Crop Evolution, *61*: 1047-1055.

CHAI, X., R., DONG, W., LIU, Y., WANG, Z., LIU (2017): Optimizing Sample Size to Assess the Genetic Diversity in Common Vetch (*Vicia sativa* L.) Populations Using Start Codon Targeted (SCoT) Markers. Molecules, *22*: 567-577.

JIANG, L.F., X., QI, X.Q., ZHANG, L.K., HUANG, X., MA, W.G., XIE (2014): Analysis of diversity and relationships among orchardgrass (*Dactylis glomerata* L.) accessions using start codon-targeted markers. Genet. Mol. Res., *13*: 4406-4418.

KALLAMADIA, P.R., V.P.R., GANGA RAO NADIGATLAB, S., MULPURIBA (2015): Molecular diversity in castor (*Ricinus communis* L.). Industrial Crops and Products, *66*: 271–281.

KANTI, M., K., ANJANI, B., USHA KIRAN, K., VIVEKANANDA (2015): Agro-morphological and molecular diversity in Castor (*Ricinus communis* L.) germplasm collected from Andaman and Nicobar Islands, India. Czech J. Genet. Plant Breed., *51*: 96–109.

KHAN, N., S., SINGH, S.S., DHAWAN (2016): Development of species specific SCoT markers and analysis of genetic diversity among *Mentha* genotypes. International Journal of Innovative Science, Engineering & Technology, *4* (2): 244-264.

LUO, C., X.H., HE, H., CHEN, Y., HU, S.J., OU (2012): Genetic relationship and diversity of *Mangifera indica* L.: revealed through SCoT analysis. Genetic Resources and Crop Evolution, *59*: 1505-1515.

MALOOK, I., M., JAMIL, M., FAYYAZ, H., REHMAN, M., IRSHAD, K.U., BASHAR, S., KHAN, N., AKHTER, M.M., ASLAM, E.S., RHA (2016): Biochemical and molecular characterization of Castor bean (*Ricinus Communis* L.) collected from different climatic zones of Pakistan. Journal of Bio-Molecular Sciences, *4*: 16-23.

NIKOLIĆ B., D., KOVAČEVIĆ, S., MLADENOVIĆ DRINIĆ, A., NIKOLIĆ, Z.S., MITIĆ, S., BOJOVIĆ, P.D., MARIN (2018): Relationships among some pines from subgenera *pinus* and *strobus* revealed by nuclear EST-microsatellites. Genetika, *50* (1): 69-84.

PETROVIČOVÁ, L., Ž., BALÁŽOVÁ, M., VIVODÍK, Z., GÁLOVÁ (2017): Detection genetic variability of *Secale cereale* L. by SCoT markers. Potravinarstvo Slovak Journal of Food Sciences, *11*: 197-202.

POPOVIĆ, V., A., LUČIĆ, L.J., RAKONJAC, B., CVJETKOVIĆ, S., MLADENOVIĆ DRINIĆ, D., RISTIĆ (2017): Assessment of genetic diversity of silver fir (*Abies alba* mill.) in Serbia using SSR markers. Genetika, *49* (3): 979-988.

RAŽNÁ, K., M., BEŽO, L., HLAVAČKOVÁ, J., ŽIAROVSKÁ, M., MIKO, J., GAŽO, M., HABÁN (2016): MicroRNA (miRNA) in food resources and medicinal plant. Potravinarstvo, *10*: 188-194.

REZAEI, J., M., ZARE MEHRJERDI, H., MASTALI (2018): ISSR based analysis of genetic diversity in some endangered species of *Allium* subg. *Melanocrommyum.* Genetika, *50* (1): 59-68.

SALEHI, M., A., ARZANI, M., TALEBI, A., ROKHZADI (2018): Genetic diversity of wheat wild relatives using SSR markers. Genetika, *50* (1): 131-141.

SATYA, P., M., KARANA, S., JANA, S., MITRAA, A., SHARMA, P.G., KARMAKAR, D.P., RAYB (2015): Start codon targeted (SCoT) polymorphism reveals genetic diversity in wild and domesticated populations of ramie (*Boehmeria nivea* L. Gaudich.), a premium textile fiber producing species. Meta Gene, *3*: 62-70.

SENKOVÁ, S., J., ŽIAROVSKÁ, E., FERNÁNDEZ, D., BOŠEĽOVÁ, M., BEŽO (2017): IRAP variability in *Prunus domestica* (L.) *Borkh* based on *Cassandra* retrotransposon polymorphism. Genetika, *49* (3): 989-1000.

SIMÕES, K.S., S.A., SILVA, E.L., MACHADO, M.S., SILVA (2017): Genetic divergence in elite castor bean lineages based on TRAP markers. Genetics and Molecular Research, *16*: 80-91.

SIMÕES, K.S., S.A., SILVA, E.L., MACHADO, H.S., BRASILEIRO (2017): Development of TRAP primers for *Ricinus communis* L. Genetics and Molecular Research, *16*: 100-113.

TSABALLA, A., I., GANOPOULOS, A., TIMPLALEXI, X., ALIKI, I., BOSMALI, N., IRINI, T., ATHANASIOS, P., MADESIS (2015): Molecular characterization of Greek pepper (*Capsicum annuum* L.) landraces with neutral (ISSR) and gene-based (SCoT and EST-SSR) molecular markers. Biochemical Systematics and Ecology, *59*: 256-263.

QUE, Y., Y., PAN, Y., LU, C., YANG, Y., YANG, N., HUANG, L., XU (2014): Genetic analysis of diversity within a Chinese local sugarcane germplasm based on Start Codon Targeted Polymorphism. BioMed Res. Int., *2014*: 150-160.

VIVODÍK, M., Ž., BALÁŽOVÁ, Z., GÁLOVÁ, T., KUŤKA HLOZÁKOVÁ (2015): Differentiation of ricin using RAPD markers. Pak. J. Bot., *47*: 1341-1345.

VIVODÍK, M., Z., GÁLOVÁ, Ž., BALÁŽOVÁ, L., PETROVIČOVÁ (2016): Start codon targeted (SCoT) polymorphism reveals genetic diversity in European old maize (*Zea mays* L.) genotypes. Potravinarstvo, *10*: 563-569.

VYHNÁNEK,T., V., TROJAN, K., ŠTIASNA, M., PRESINSZKÁ, L., HŘIVNA, E., MRKVICOVÁ, L., HAVEL (2015): Testing of DNA isolation for the identification of *Hemp*. Potravinárstvo, *9*: 393-397.

WANG, CH., G., LI, Z., ZHANG, M., PENG, Y., SHANG, R., LUO, Y., CHEN (2013): Genetic diversity of castor bean (*Ricinus communis* L.) in Northeast China revealed by ISSR markers. Biochemical Systematics and Ecology, *51*: 301–307.

WEBER, J.L. (1990): Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. Genomics, *7*: 524-530.

ZHANG, J., W., XIE, Y., WANG, X., ZHAO (2015): Potential of Start Codon Targeted (SCoT) Markers to Estimate Genetic Diversity and Relationships among Chinese *Elymus sibiricus* Accessions. Molecules, *20*: 5987-6001.

ŽIAROVSKÁ, J., O., GRYGORIEVA, L., ZELEŇÁKOVÁ, M., BEŽO, J., BRINDZA (2015): Identification of sweet chesnut pollen in bee pollen pellet using molecular analysis. Potravinarstvo, *9*: 352-358.

ŽIAROVSKÁ, J., M., KYSEĽ, R., CIMERMANOVÁ, L., KNOTEKOVÁ (2017): Effect of DNA extraction method in the *Rosa Canina* L. identification under different processing temperature. Potravinarstvo Slovak Journal of Food Sciences, *11*: 190-196.

START KODON CILJANI POLIMORFIZAM ZA OCENU FUNKCIONALNE GENETIČKE VARIJACIJE I ODNOSA GENOTIPOVA GAJENOG RICINUSA (*Ricinus communis* L.)

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Izvod

U ovom radu razdvojeno je 111 genotipova ricinusa pomoću uzoraka DNK sa 37 ScoT prajmera. Odabrani prajmeri su amplifikovali DNK fragmente kod svih 111 genotipova, tako da je broj fragmenata varirao od 3(SCoT 14) do 10 (SCoT 30 i SCoT 44), a veličina amplikona je varirala od 100 do 3000bp. Od ukupno 246 amplifikovanih traka, 186 je bilo polimorfno sa prosečnim brojem od 5.03 fragmenta po prajmeru. Polimorfne trake su bile u opsegu od 57.14 % (SCoT 34) do 100.00 % (SCoT 28 i SCoT 33), sa prosečnom vrednošću od 77.5%. PIC vrednosti su varirale od 0.372 (SCoT 14) do 0.818 (SCoT 30) sa prosekom od 0.677. Dendrogram je konstruisan na osnovu matriksa genetičke distance profila 37 ScoT prajmera, korišćenjem UPGMA metoda. Prema toj analizi, svi uzorci ricinusa su grupisani u dva glavna klastera (1 and 2). Prvi klaster je podeljen na dva podklastera (1a and 1b). Podklaster 1a sastojao se od 11 genotipova ricinusa, a podklaster 1b sadržao je 6 genotipova. Podklaster 2 bio je podeljen u dva podklastera (2a and 2b). Podklaster 2a sastojao se od 44 genotipa ricinusa, a podklaster 2b od 50 genotipova. Rezultati su pokazali da se SCoT markera mogu koristiti za procenu genetičke raznolikosti genotipova ricinusa, na osnovu njihove identifikacije.

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