potravinárstvo®
Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 13, 2019, no. 1, p. 294-300
https://doi.org/10.5219/1116
Received: 12 March 2019. Accepted: 12 March 2019.
Available online: 28 May 2019 at www.potravinarstvo.com

ISSN 1337-0960 (online)

GENETIC DIVERSITY IN TUNISIAN CASTOR GENOTYPES (*RICINUS COMMUNIS* L.) DETECTED USING RAPD MARKERS

Martin Vivodík, Ezzeddine Saadaoui, Želmíra Balážová, Zdenka Gálová and Lenka Petrovičová

ABSTRACT

Castor (*Ricinus communis* L.) is a plant that is commercially very important to the world. It is produced in about 30 countries lying in the tropical belt of the world. It is an important plant for production of industrial oil. Assessment of genetic diversity of a crop species is a prerequisite to its improvement; hence it is important to identify the genetic diversity of castor genetic resources for development of improved cultivars. The present study is focused on estimation of genetic distance between 56 Tunisian castor genotypes, based on 18 RAPD markers. Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, Tunisia. The ricin genotypes were obtained from 12 regions of Tunisia. The efficacy of the RAPD technique in this study is further supported by the obtained PIC values of the primers used in the analysis. PCR amplification of DNA using 18 primers for RAPD analysis produced 145 DNA fragments that could be scored in all 56 genotypes of Tunisian castor. The number of amplified fragments varied from 3 (OPE-07) to 13 (SIGMA-D-01), and the amplicon size ranged from 100 to 1500 bp. Of the 145 amplified bands, 145 were polymorphic, with an average of 8.11 polymorphic bands per primer. The lowest values of polymorphic information content were recorded for RLZ 9 (0.618) and the the highest PIC values were detected for OPD-08 (0.846) with an average of 0.761. A dendrogram was constructed from a genetic distance matrix based on profiles of the 18 RAPD primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 56 Tunisian castor genotypes were clustered into five main clusters. Genetically the closest were four genotypes from cluster 1 (BT-1 – S-5 and K-1 – N-3). Knowledge of the genetic diversity of castor can be used in 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; DNA; dendrogram; PCR; PIC

INTRODUCTION

Castor bean (syn. castorbean, castor, castor-oil-plant), *Ricinus communis* L. (2n = 20, X = 10), is a species of flowering plant in the spurge family, *Euphorbiaceae*. It is an oilseed crop cultivated mainly in India, Mozambique, Brazil, and China **(FAOSTAT, 2014)**.

Genetic diversity in a germplasm is the fundamental requirement for crop improvement programs. There are several genetic markers available for assessment of genetic diversity among the genotypes and accessions **(Kole and Rabinowicz, 2018)**. Though castor bean is a monotypic, it exhibits wide phenotypic diversity. In castor bean, genetic markers such as agro-morphological characters, biochemical and cytological markers were widely used in characterization of genetic variation in the germplasm **(Kole and Rabinowicz, 2018).**

Since 1990, random amplified polymorphic DNA (RAPD) markers have been successfully applied for identification of DNA polymorphism in various plant species **(Williams et al., 1990)**. They are often used for screening of a wide range of genetic stocks in order to find linkage with traits of agronomic significance **(Masojć, Myśków and Milczarski, 2001)**. Genetic diversity in castor bean was assessed by using both dominant and codominant molecular markers (random amplified polymorphic DNA, RAPD) **(Reddy, Nadigatla and Mulpuri, 2015; Vivodík et al., 2015a)**, inter-simple sequence repeats (ISSR) **(Wang et al., 2013; Vasconcelos et al., 2016)**, start codon targeted (SCoT)

(Kallamadi et al., 2015; Reddy, Nadigatla and Mulpuri, 2015), amplified fragment length polymorphism (AFLP) **(Allan et al., 2008; Quintero et al., 2013)**, simple sequence repeat (SSR) **(Gálová et al., 2015; Rukhsar et al., 2017)**, expressed sequence tag-simple sequence repeats (EST-SSR) **(Kanti et al., 2015; Wang et al., 2017)**, and random microsatellite amplified polymorphic DNA (RMAPD) **(Dong et al. 2012)**, and also advanced molecular markers, such as single nucleotide polymorphism (SNP) **(Foster et al. 2010)**, sequencerelated amplification polymorphism (SRAP) **(Lu et al., 2010)**, target region amplification polymorphism (TRAP) **(Simões et al., 2017a)**, and methylation-sensitive amplification polymorphism (MSAP) **(He et al., 2017)**. The polymerase chain reaction (PCR) has been used by many authors, such as **Žiarovská et. al., (2015)**; **Vyhnánek et. al., (2015)**; **Bošeľová and Žiarovská (2016)**; **Ražná et. al., (2016)**; **Žiarovská et. al., (2017)**; **Simões et. al., (2017b); Žiarovská et. al., (2018)**; **Ansari et al., (2018); Balážová et al., (2018); El-Fiki and Adly, (2019).**

Scientific hypothesis

The present study is focused on estimation of genetic distance between 56 Tunisian castor genotypes, based on 18 RAPD markers.

MATERIAL AND METHODOLOGY

Fifty-six castor (*Ricinus communis* L.) genotypes were used in the present study. Seeds of castor were obtained from the University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, Tunisia. The ricin genotypes were obtained from 12 regions of Tunisia: S- Souassi (5 genotypes), BT- Bouthay (4 genotypes), GH- Ghomrassen (5 genotypes), BA- Sidi bou ali (5 genotypes), MT- Matmata (4 genotypes), AG- Mateur (5 genotypes), N- Nefza (4 genotypes), MD- Mednine (5 genotypes), M- Mornag (5 genotypes), G- Gabes (4 genotypes), K- Kebili (5 genotypes), KJ- Ksar jedid (5 genotypes). Genomic DNA of castor cultivars was extracted from leaves of 14-day old plantlets with GeneJET Plant Genomic DNA Purification Mini Kit according to the manufacturer's instructions. DNA concentrations were estimated by UV-Vis spectrophotometer Q5000, Quawell.

Figure 1 Photo of *Ricinus communis* L. (Spanishalex, Dreamstime.com)

Amplification of RAPD fragments was performed according to **Gajeraa et al. (2010)** using decamer arbitrary primers (Table 1). Amplifications were performed in a 25 μL reaction volume containing 100 ng of DNA, 12.5 μL of Master Mix (Genei, Bangalore, India) and 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 electrophoresed in 1.5% agarose in 1× TBE buffer. The gels were stained with ethidium bromide and documented using gel documentation system.

Statisic analysis

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 ricin and usability RAPD markers in their differentiation we used polymorphic information content (PIC) **(Weber, 1990)**.

RESULTS AND DISCUSSION

In the present study, the representatives of the genus *Ricinus communis* collected from 12 different parts of Tunisia were differentiated by the DNA fingerprinting patterns using 18 RAPD primers. The efficacy of the RAPD technique in this study is further supported by the obtained PIC values of the primers used in the analysis. PCR amplification of DNA using 18 primers (Table 1) for RAPD analysis produced 145 DNA fragments that could be scored in all 56 genotypes of Tunisian castor (Figure 2). The number of amplified fragments varied from 3 (OPE-07) to 13 (SIGMA-D-01), and the amplicon size ranged from 100 to 1500 bp. Of the 145 amplified bands, 145 were polymorphic, with an average of 8.11 polymorphic bands per primer. Results indicated the presence of wide genetic variability among different genotypes of Tunisian castor. To determine the level of polymorphism in the analysed group of Tunisian castor genotypes polymorphic information content (PIC) was calculated. The lowest values of polymorphic information content were recorded for RLZ 9 (0.618) and the the highest PIC values were detected for OPD-08 (0.846) with an average of 0.761.

A dendrogram was constructed from a genetic distance matrix based on profiles of the 18 RAPD primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 56 Tunisian castor genotypes were clustered into five main clusters (Figure 3). Cluster 1 contained 10 genotypes of castor from different regions of Tunisia and cluster 2 contained 10 genotypes of castor from different regions of Tunisia. Cluster 3 contained 6 genotypes of Tunisian castor and cluster 4 contained 17 genotypes of Tunisian castor and cluster 5 contained 13 genotypes of tunisian castor. Genetically the closest

were four genotypes from cluster 1 (BT-1 – S-5 and K-1 – N-3) (Figure 2).

Dhingani et al. (2012) used 25 RAPD primers in this study. Amplification of genomic DNA of 8 genotypes, using RAPD analysis, yielded 92 fragments, of which 72 were polymorphic, with an average PIC value of 0.29. Number of amplified fragments with RAPD primers ranged from 4 to 13, with the size of amplicons ranging from 100 to 2650 bp in size. The polymorphism ranged from 54.54 to 100.0, with an average of 79.54 percent. The objective of work **Machado et al. (2013)** was to identify genetically different cultivars of castor bean (*Ricinus communis*) using RAPD markers. A total of 58 RAPD primers were used to genotype 15 cultivars. The genetic dissimilarity between cultivars was calculated by Jaccard's index, using the unweighted pair-group method with arithmetic mean (UPGMA). Five hundred and fifty-two fragments were identified, of which 311 were polymorphic (56.3%). The cultivars were clustered in five groups, evidence that there is genetic difference among them. RAPD markers are efficient in the study of genetic dissimilarity in castor bean. The aim of the present study of **Lakhani et al. (2015)** was to study the molecular diversity for varietal identification and phylogenetic relationships among thirteen castor genotypes and identify those with distinct DNA profiles. Twenty-seven RAPDs primers were used, out of which 16 polymorphic primers revealed 100% polymorphism among the castor genotypes. Dendrogram was constructed using UPGMA method which revealed distinct clusters. Values of the polymorphic information content (PIC) value ranged from 0.423 to 0.883 with an average of 0.705. This work of **Tomar Rukam et al. (2014)** investigated the fingerprinting and phenotyping of 25 castor genotypes available in Gujarat and other States of India. An integrated approach based on the exploitation of morphological traits and molecular markers, such as RAPD and ISSR fingerprints was employed. Morphological trait analysis and statistical analysis of markers were useful for reconstructing a castor varietal dendrogram. The results of the morphological and molecular analyses allowed us to confirm a remarkable differentiation among castor genotypes. The UPGMA dendrogram obtained using morphological characters clearly separated the 25 genotypes of castor into three groups.

The aim of study **Vivodík et al. (2015a)** was to assess genetic diversity within the set of 111 ricin genotypes using 13 RAPD primers. For differentiation of 111 ricin genotypes 13 RAPD primers were used. Amplification of genomic DNA of 111 genotypes using RAPD analysis yielded 102 fragments, with an average of 7.85 polymorphic fragments per primer. Number of amplified fragments with RAPD primers ranged from 3 to 13, with the size of amplicons ranging from 100 to 1500 bp. The polymorphism information content (PIC) value ranged from 0.491 to 0.898 with an average of 0.764 and diversity index (DI) value ranged from 0.576 to 0.900 with an average of 0.776. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. In dendrogram separated unique genotype RM-32 from other 110 genotypes which were further grouped into 3 subclusters (1, 2, 3). Only four genotypes were not distinguished. The aim of work **Vivodík et al. (2015b)** was to detect genetic variability among the set of 32 castor genotypes using five random amplified polymorphic DNA (RAPD) markers. Amplification of genomic DNA of 32 genotypes, using RAPD analysis, yielded 41 fragments, with an average of 8.20 polymorphic fragments per primer. Number of amplified fragments ranged from 5 to 11, with the size of amplicons varied from 100 to 1200 bp. The polymorphic information content value ranged from 0.598 (RLZ 9) to 0.811 (RLZ 6) with an average of 0.746 and diversity index value ranged from 0.557 (RLZ 9) to 0.889 (RLZ 7) with an average of 0.784. The dendrogram based on hierarchical cluster analysis using unweighted pair group method with arithmetic average algorithm was prepared. The aim of work **Balážová, Vivodík and Gálová (2016)** was to detect genetic variability among the set of 30 castor genotypes using 6 RAPD markers. Amplification of genomic DNA of 30 genotypes using RAPD analysis yielded 50 polymorphic fragments with an average of 8.33 fragments per primer. Number of amplified fragments varied from 5 (RLZ7) to 11 (RLZ8) and the amplicon size ranged from 330 to 1200 bp. All 50 amplified bands were polymorphic. The polymorphic information content (PIC) values ranged from 0.774 (RLZ7) to 0.870 (RLZ8) with an average of 0.825 and index diversity (DI) value ranged from 0.786 (RLZ7) to 0.872 (RLZ8) with an average of 0.831. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. Dendrogram separated ricin genotypes into three main clusters. Two genotypes (RM-72 and RM-73) were genetically the closest. 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. **Vivodík et al. (2015c)** analyzed seventeen castor genotypes for genetic variability using Random Amplified Polymorphic DNA (RAPD) markers. Thirteen polymorphic RAPD primers amplified 102 DNA fragments, with an average of 7.85 fragments per primer. Number of amplified fragments ranged from 3 (OPE-07) to 13 (SIGMA-D-01), with the size of amplicons ranging from 100 to 1200 bp. The polymorphic information content (PIC) value ranged from 0.450 (OPE-07) to 0.892 (SIGMA-D-01) with an average of 0.771 and diversity index (DI) value ranged from 0.551 (OPE-07) to 0.894 (SIGMA-D-01) with an average of 0.787. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared and analyzed genotypes were grouped into two main clusters and only two genotypes (RM-5 and RM-23) could not be distinguished.

Table 1 Statistical characteristics of the RAPD markers used in Tunisian castor genotypes.

Sr. no.	Primers	Primer sequence (5´-3´)	Molecular weight range (bp)	Total number of bands	PIC value
1.	OPA-02	TGCCGAGCTG	200 – 1000	7	0.729
2.	OPA-03	AGTCAGCCAC	100 – 800	9	0.652
3.	OPA-13	CAGCACCCAC	100 – 1500	7	0.780
4.	OPB-08	GTCCACACGG	200 – 800	8	0.715
5.	OPD-02	GGACCCAACC	200 – 1000	6	0.816
6.	OPD-07	TTGGCACGGG	100 – 900	8	0.714
7.	OPD-08	GTGTGCCCCA	200 – 600	7	0.846
8.	OPD-13	GGGGTGACGA	100 – 1500	12	0.810
9.	OPE-07	AGATGCAGCC	300 – 800	3	0.825
10.	OPF-14	TGCTGCAGGT	200 – 1200	5	0.812
11.	SIGMA-D-01	AAACGCCGCC	100 – 1200	13	0.731
12.	SIGMA-D-14	TCTCGCTCCA	200 – 1000	7	0.710
13.	SIGMA-D-P	TGGACCGGTG	200 – 1500	10	0.717
14.	RLZ 6	GTGATCGCAG	200 – 1500	12	0.794
15.	RLZ 7	GTCCACACGG	100 – 1000	10	0.790
16.	RLZ 8	GTCCCGACGA	200 – 1200	7	0.808
17.	RLZ 9	TGCGGCTGAG	200 – 1200	7	0.618
18.	RLZ 10	ACGCGCATGT	100 – 1500	8	0.832
		Total	-	146	
		Average	-	8.11	0.761

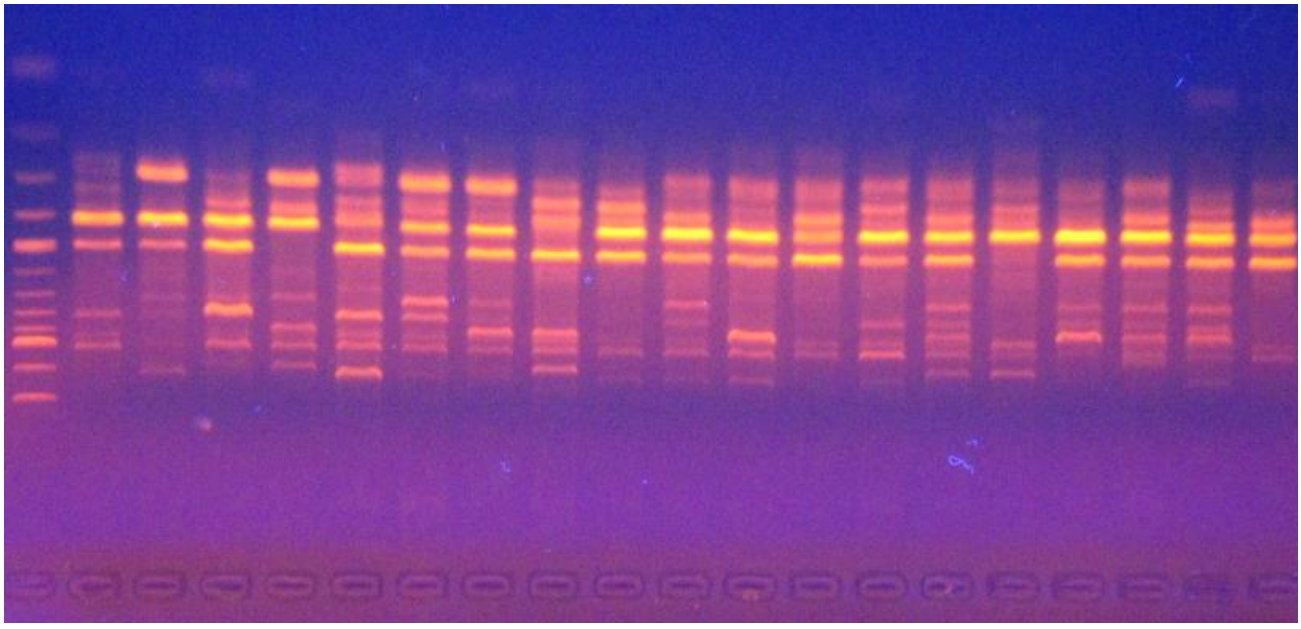

Figure 2 PCR amplification products of 19 genotypes of Tunisian castor produced with primer OPA-02.
Note: Lane M is Quick-Load® 100 bp DNA ladder and lanes 1-19 are Tunisian castor genotypes.

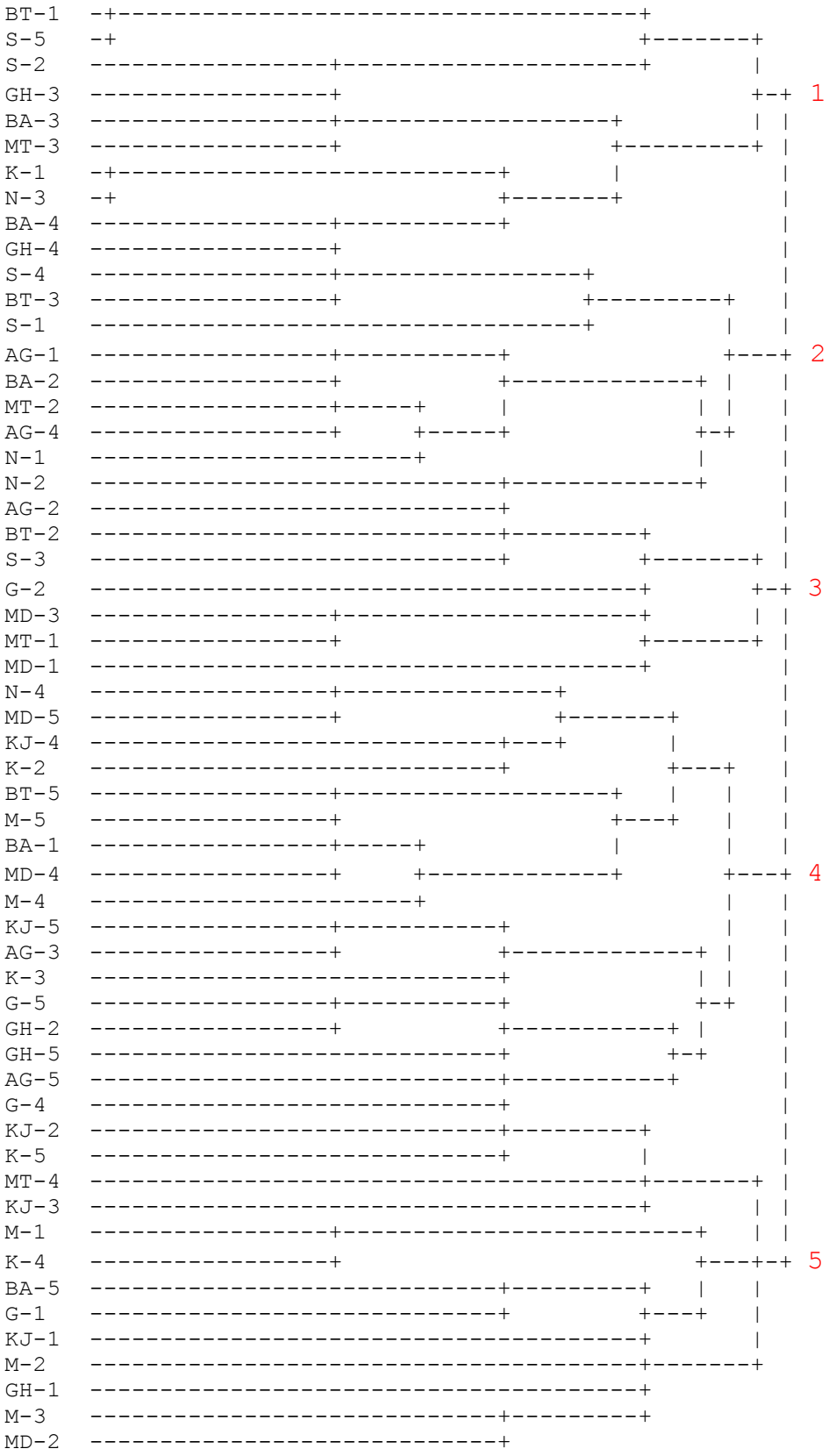

Figure 3 Dendrogram of 56 Tunisian castor genotypes prepared based on 18 RAPD markers.
Note: S – Souassi (5 genotypes), BT – Bouthay (4 genotypes), GH – Ghomrassen (5 genotypes), BA – Sidi bou ali (5 genotypes), MT – Matmata (4 genotypes), AG – Mateur (5 genotypes), N – Nefza (4 genotypes), MD – Mednine (5 genotypes), M – Mornag (5 genotypes), G – Gabes (4 genotypes), K – Kebili (5 genotypes), KJ – Ksar jedid (5 genotypes).

CONCLUSION

Genetic diversity in a germplasm is the fundamental requirement for crop improvement programs. PCR amplification of DNA using 18 primers for RAPD analysis produced 145 DNA fragments that could be scored in all 56 genotypes of Tunisian castor (Figure 1). The number of amplified fragments varied from 3 (OPE-07) to 13 (SIGMA-D-01), and the amplicon size ranged from 100 to 1500 bp. Of the 145 amplified bands, 145 were polymorphic, with an average of 8.11 polymorphic bands per primer. A dendrogram was constructed from a genetic distance matrix based on profiles of the 18 RAPD primers using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 56 Tunisian castor genotypes were clustered into five main clusters. Polymorphism revealed by RAPD technique was 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, construction of linkage maps, conservation of the genetic resources of oat species and QTL mapping.

REFERENCES

Allan, G., Williams, A., Rabinowicz, P. D., Chan, A. .P., Ravel, J., Keim P. 2008. Worldwide genotyping of castor bean germplasm (Ricinus communis L.) using AFLPs and SSRs. *Genetetic Resources Crop Evolution*, vol. 55, no. 3, p. 365-378. https://doi.org/10.1007/s10722-007-9244-3

Ansari, S., Solouki, M., Fakheri, B., Fazeli-Nasab, B., Mahdinezhad, N. 2018. Assesment of molecular diversity of internal transcribed spacer region in some lines and landrace of Persian clover (Trifolium Resupinatum L.). *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 657-666. https://doi.org/10.5219/960

Balážová, Ž., Vivodík, M., Gálová, Z. 2016. Assessment of RAPD polymorphism in ricin genotypes. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 5, no. 4, p. 386-388. https://doi.org/10.15414/jmbfs.2016.5.4.386-388

Balážová, Ž., Gálová, Z., Vivodík, M., Chňapek, M., Hornyák Gregáňová, R. 2018. Molecular analysis of buckwheat using gene specific markers. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 546-552. https://doi.org/10.5219/954

Bošeľová, D., Žiarovská, J. 2016. Direct PCR as the platform of Hedera helix, L. genotypying without the extraction of DNA. *Journal of Central European Agriculture*, vol. 17, no. 4, p. 941-949. https://doi.org/10.5513/JCEA01/17.4.1795

Dhingani, R. M., Tomar, R. S., Parakhia, M. V., Patel, S. V., Golakiya, B. A. 2012. Analysis of genetic diversity among different Ricinus communis genotypes for macrophomina root rot through RAPD and microsatellite markers. *International Journal of Plant Protection*, vol. 5, no. 1, p. 1-7.

Dong, H., Wang, C., Li, W., Yang, G. X., Yang, H., Wang, Y. R., Chen, M. H., Li, F. J., Feng, Y., Chen, G. 2012. Castor germplasm diversity analysis using AP-PCR and RMAPD. *Academic Periodical of Farm Products Processing*, vol. 2012, p. 23- 36.

El-Fiki, A., Adly, M. 2019. Molecular characterization and genetic diversity in some Egyptian wheat (Triticum aestivum L.) using microsatellite markers. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 13, no. 1, p. 100-108. https://doi.org/10.5219/978

FAOSTAT. 2014. Available at: http://www.fao.org/faostat/en/

Foster J. T., Allan, G. J., Chan, A. P., Rabinowicz, P. D., Ravel, J., Jackson, P. J., Keim, P. 2010. Single nucleotide polymorphisms for assessing genetic diversity in castor bean (Ricinus communis). *BMC Plant Biology*, vol. 10, p. 13-18. https://doi.org/10.1186/1471-2229-10-13

Gajeraa, B. B., Kumara, N., Singha, A. S., Punvara, B. S., Ravikirana, R., Subhasha, N., Jadejab, G. C. 2010. Assessment of genetic diversity in castor (Ricinus communis L.) using RAPD and ISSR markers. *Industrial Crops and Products*, vol. 32, no. 3, p. 491-498. https://doi.org/10.1016/j.indcrop.2010.06.021

Gálová, Z., Vivodík, M., Balážová, Ž., Kuťka Hlozáková, T. 2015. Identification and differentiation of Ricinus communis L. using SSR markers. *Potravinarstvo*, vol. 9, no. 1, p. 556-561. https://doi.org/10.5219/516

He, S., Xu, W., Li, F., Wang, Y., Liu, A. 2017. Intraspecific DNA methylation polymorphism in the non-edible oilseed plant castor bean. *Plant Diversity*, vol. 39, no. 5, p. 300-307. https://doi.org/10.1016/j.pld.2017.05.007

Kallamadi, P. R., Ganga Rao Nadigatlab, V. P. R., Mulpuriba, S. 2015. Molecular diversity in castor (Ricinus communis L.). *Industrial Crops and Products*, vol. 66, p. 271-281. https://doi.org/10.1016/j.indcrop.2014.12.061

Kanti, M., Anjani, K., Usha Kiran, B., Vivekananda, K. 2015. Agro-morphological and molecular diversity in castor (Ricinus communis L.) germplasm collected from Andaman and Nicobar Islands, India. *Czech Journal of Genetics and Plant Breeding*, vol. 51, p. 96-109. https://doi.org/10.17221/205/2014-CJGPB

Kole, Ch., Rabinowicz, P. 2018. *The castor bean genome*. Switzerland: Springer Nature, 272 p. ISBN 978-3-319-97279-4. https://doi.org/10.1007/978-3-319-97280-0

Lakhani, H. N., Patel, S. V., Bodar, N. P., Golakiya, B. A. 2015. RAPD analysis of genetic diversity of castor bean (Ricinus communis L.). *International Journal of Current Microbioly and Applied Sciences*, vol. 4, no. 1, p. 696-703.

Lu, Z., Qi, J. M., Fang, P. P., Su, J. G., Xu, J. T., Tao, A. F. 2010. Genetic diversity and phylogenetic relationship of castor germplasm as revealed by SRAP analysis. *Plant Science Journal*, vol. 28, no. 1, p. 1-6. https://doi.org/10.3724/SP.J.1142.2010.00001

Machado, E. L., Alves Silva, S., de Sousa Santos, A., Andrade Bastos, L., Nogueira Pestana, C., Souza dos Santos, K., Fortes Ferreira, C., Alves Silva Diamantino, M. S. 2013. Genetic dissimilarity between castor bean cultivars using RAPD markers. *Pesquisa Agropecuária Brasileira*, vol. 48, no. 3, p. 342-345. https://doi.org/10.1590/S0100-204X2013000300014

Masojć, P., Myśków, B., Milczarski, P. 2001. Extending a RFLP-based genetic map of rye using random amplified polymorphic DNA (RAPD) and isozyme markers. *Theoretical Applied Genetics*, vol. 102, no. 8, p. 1273-1279. https://doi.org/10.1007/s001220000512

Quintero, V., Anaya-López, J. L., Núñez-Colín, C. A., Zamarripa-Colmenero, A., Montes-García, N., Solís-Bonilla, J. L., Aguilar-Rangel, M. R. 2013. Assessing the genetic diversity of castor bean from Chiapas, México using SSR and AFLP markers. *Industrial Crops and Products*, vol. 41, p. 134-143. https://doi.org/10.1016/j.indcrop.2012.04.033

Ražná, K., Bežo, M., Hlavačková, L., Žiarovská, J., Miko, M., Gažo, J., Habán, M. 2016. MicroRNA (miRNA) in food resources and medicinal plant. *Potravinarstvo*, vol. 10, no. 1, p. 188-194. https://doi.org/10.5219/583

Reddy, K. P., Nadigatla, V. P. R. G. R., Mulpuri, S. 2015. Molecular diversity in castor (Ricinus communis L.). *Industrial Crops and Products*, vol. 66, p. 271-281. https://doi.org/10.1016/j.indcrop.2014.12.061

Rukhsar Patel, M. P., Parmar, D. J., Kalola, A. D., Kumar, S. 2017. Morphological and molecular diversity patterns in castor germplasm accessions. *Industrial Crops and Products*, vol. 97, p. 316-323. https://doi.org/10.1016/j.indcrop.2016.12.036

Simões, K. S., Silva, S. A., Machado, E. L. and Brasileiro, H. S. 2017a. Development of TRAP primers for Ricinus communis L. *Genetics and Molecular Research*, vol. 16, no. 2, p. 100-113. https://doi.org/10.4238/gmr16029647

Simões, K. S., Silva, S. A., Machado, E. L. and Silva, M. S. 2017b. Genetic divergence in elite castor bean lineages based on TRAP markers. *Genetics and Molecular Research*, vol. 16, no. 3, p. 100-112. https://doi.org/10.4238/gmr16039776

Tomar Rukam, S., Parakhia, M. V., Kavani, R. H., Dobariya, K. L., Thakkar, J. R., Rathod, V. M., Dhingani, R. M., Golakiya, B. A. 2014. Characterization of castor (Ricinus communis L.) genotypes using different markers. *Research Journal of Biotechnology*, vol. 9, no. 2, p. 6-13.

Vasconcelos, S., Onofre, A. V. C., Milani, M., Benko-Iseppon, A. M., Brasileiro-Vidal, A. C. 2016. Accessing genetic diversity levels of Brazilian genotypes of castor with AFLP and ISSR markers. *Pesquisa Agropecuária Pernambucana*, vol. 21, no. 1, p. 24-31. https://doi.org/10.12661/pap.2016.005

Vivodík, M., Balážová, Ž., Gálová, Z., Kuťka Hlozáková, T. 2015a. Differentiation of ricin using RAPD markers. *Pakistan Journal of Botany*, vol. 47, no. 4, p. 1341-1345.

Vivodík, M., Balážová, Ž., Gálová, Z., Kuťka Hlozáková, T. 2015b. Evaluation of molecular diversity of new castor lines (Ricinus communis L.) using random amplifi ed polymorphic DNA markers. *Horticultural Biotechnology Research*, vol. 1, p. 1-4.

Vivodík, M., Balážová, Ž., Gálová, Z., Chňapek, M., Petrovičová, L. 2015c. Study of DNA polymorphism of the castor new lines based on RAPD markers. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4 (special issue 2), p. 125-127. https://doi.org/10.15414/jmbfs.2015.4.special2.125-127

Vyhnánek, T., Trojan, V., Štiasna, K., Presinszká, M., Hřivna, L., Mrkvicová, E., Havel, L. 2015. Testing of DNA isolation for the identification of Hemp. *Potravinárstvo*, vol. 9, no. 1, p. 393-397. https://doi.org/10.5219/509

Wang, Ch., Li, G., Zhang, Z., Peng, M., Shang, Y., Luo, R., Chen, Y. 2013. Genetic diversity of castor bean (Ricinus communis L.) in Northeast China revealed by ISSR markers. *Biochemical Systematics and Ecology*, vol. 51, p. 301-307. https://doi.org/10.1016/j.bse.2013.09.017

Wang, M. L., Dzievit, M., Chen, Z., Morris, J. B., Norris, J. E., Barkley, N. A., Tonnis, B., Pederson, G. A., Yu, J. 2017. Genetic diversity and population structure of castor (Ricinus communis L.) germplasm within the US collection assessed with EST-SSR markers. *Genome*, vol. 60, no. 3, p. 193-200. https://doi.org/10.1139/gen-2016-0116

Weber, J. L. 1990. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, vol. 7, no. 4, p. 524-530. https://doi.org/10.1016/0888-7543(90)90195-Z

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, vol. 18, no. 22, p. 6531-6535. https://doi.org/10.1093/nar/18.22.6531

Žiarovská, J., Grygorieva, O., Zeleňáková, L., Bežo, M., Brindza, J. 2015. Identification of sweet chesnut pollen in bee pollen pellet using molecular analysis. *Potravinarstvo*, vol. 9, no. 1, p. 352-358. https://doi.org/10.5219/497

Žiarovská, J., Kyseľ, M., Cimermanová, R., Knoteková, L. 2017. Effect of DNA extraction method in the Rosa Canina L. identification under different processing temperature. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 11, no. 1, p. 190-196. https://doi.org/10.5219/717

Žiarovská, J., Zeleňáková, L., Fernández Cusimamani, E., Kačániová, M. 2018. A thaumatin-like genomic sequence identification in Vitis Vinifera L., stormy wines and musts based on direct PCR. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 12, no. 1, p. 226-232. https://doi.org/10.5219/892

Acknowledgments:

This work was co-funded by VEGA project No. 1/0246/18 (50 %) and KEGA project No. 025SPU-4/2018 (50%).

Contact address:

*Martin Vivodík, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421 37 641 4269,
E-mail: martin.vivodik@uniag.sk
ORCID: https://orcid.org/0000-0001-6265-1616

Ezzeddine Saadaoui, University of Carthage, National Institute of Research in Rural Engineering, Waters and Forests (INRGREF), Regional Station of Gabès, BP 67, Gabès Manara, 6011, Tunisia, +421 37 641 4269, E-mail: saad_ezz@yahoo.fr

Želmíra Balážová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421 37 641 4327, E-mail: zelmira.balazova@uniag.sk

Zdenka Gálová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421 37 641 4596, E-mail: zdenka.galova@uniag.sk

Lenka Petrovičová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, Tel.: +421 37 641 4697, E-mail: lenka.petrovicova@uniag.sk

Corresponding author: *