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

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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 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, Myškow and Mileczerz, 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)
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.

Statistic 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  | CAGCAACCAC               | 100 – 1500                  | 7                     | 0.780     |
| 4.      | OPB-08  | GTCCACACGG               | 200 – 800                   | 8                     | 0.715     |
| 5.      | OPD-02  | GGACCAACC                | 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   | GTGATGCAGCAG             | 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  | AGCCGCATGT               | 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.

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