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GENETIC DIVERSITY AND POPULATION STRUCTURE IN TUNISIAN CASTOR GENOTYPES (*RICINUS COMMUNIS* L.) DETECTED USING SCOT MARKERS

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ABSTRACT

Due to the chemical and physical properties of castor oil (*Ricinus communis* L.) that make it a valuable raw material for numerous industrial applications, including the production of biofuel, interest to develop more and better varieties has been increased. In the present study, the representatives of the genus castor collected from 12 different parts of Tunisia were differentiated by the DNA fingerprinting patterns using 37 SCoT primers. PCR amplification of DNA using 37 primers for SCoT analysis produced 268 DNA fragments that could be scored in all 56 genotypes of Tunisian castor. The number of amplified fragments varied from 4 (SCoT 45, SCoT 31 and ScoT 17) to 10 (SCoT 3, SCoT 11, SCoT 14, SCoT 18 and SCoT 12). Of the 268 amplified bands 230 were polymorphic, with an average of 6.22 polymorphic bands per primer. 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 SCoT 17 (0.411) and the the highest PIC values were detected for SCoT 14 (0.868) with an average of 0.751. 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 56 Tunisian castor genotypes were clustered into two main clusters (1 and 2). Of the 56 genotypes of Tunisian castor, 2 unique genotypes were separated (BA-5 and K-4). Genetically the closest were two genotypes from Tunisian region Souassi (S-2 and S-5) in subclaster 2bc. Results showed the utility of SCoT markers for estimation of genetic diversity of castor genotypes leading to genotype identification.

Keywords: castor; DNA; PCR; dendrogram; SCoT marker

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)**. Due to the distinctive characteristics of castor oil, such as its high percentage of ricinoleic acid, unusual chemical structure and low freezing point, castor oil is widely utilised in paints, nylon, aviation oil, lubricants, soaps, inks, dyes, cosmetics, adhesives, biodiesel, and other novel castor-bean-derived 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 products **(Scholz and Silva, 2008)**. Castor is cultivated on commercial scale in an area of 1,525,000 ha in 30 countries with 1,581,000 MT seed production. India, China, Brazil, USSR, Thailand, Ethiopia and Philippines are the major castor growing countries in the world **(Damodaram and Hegde, 2010)**.

Knowledge of genetic variability is important for breeding programs to provide the basis for developing desirable genotypes. Genetic variability in castor bean has been

(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 **(Cheema et al., 2010; 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)**.

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 **(Kallamadi et al., 2015)**, sugarcane **(Que et al., 2014)** and mango (**Gajera et al., 2014)**.

Scientific hypothesis

The present study is focused on estimation of genetic distance between 56 Tunisian 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.

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.

SCoT amplification: 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.

Statisic analysis

A dendrogram was constructed based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA). For the assessment of the polymorphism between genotypes maize and usability SCoT 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 37 SCoT primers. The efficacy of the SCoT technique in this study is further supported by the obtained PIC values of the primers used in the analysis. The PIC value of the SCoT marker system was found to be 0.751 which are at par with the optimal PIC. PCR amplification of DNA using 37 primers (Table 1) for SCoT analysis produced 268 DNA fragments that could be scored in all 56 genotypes of Tunisian castor (Figure 1). The number of amplified fragments varied from 4 (SCoT 45, SCoT 31 and ScoT 17) to 10 (SCoT 3, SCoT 11, SCoT 14, SCoT 18 and SCoT 12), and the amplicon size ranged from 200 to 2500 bp. Of the 268 amplified bands 230 were polymorphic, with an average of 6.22 polymorphic bands per primer. Results indicated the presence of wide genetic variability among different genotypes of Tunisian castor. From these 37 primers, primers SCoT 3, SCoT 14 and SCoT 15 were the most polymorphic, where 9 polymorphic amplification products were detected. The lowest number of amplified polymorphic fragments (3) were detected by primers SCoT 2, SCoT 17, SCoT 31 and SCoT 45. The percentage of polymorphism ranged from 50.00% (SCoT 2) to 100% (SCoT 13, SCoT 15, SCoT 20, SCoT 30, SCoT 44, SCoT 65 and SCoT 66). 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 SCoT 17 (0.411) and the the highest PIC values were detected for SCoT 14 (0.868) with an average of 0.751.

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 56 Tunisian castor genotypes were clustered into two main clusters (1 and 2) (Figure 2). Of the 56 genotypes of Tunisian castor, 2 unique genotypes were separated: BA-5 – genotypes from Tunisian region Sidi bou ali and K-4 – genotypes from Tunisian region Kebili. Cluster 1 was divided into subclasters 1a and 1b. Subclaster 1a contained 5 genotypes of castor from different regions of Tunisia and subclaster 1b contained 6 genotypes of castor from different regions of Tunisia. Cluster 2 was divided into subclasters 2a and 2b. Subclaster 2a contained 6 genotypes of Tunisian castor, all 5 genotypes from the region Ksar jedid (KJ-1, KJ-2, KJ-3, KJ-4, KJ-5) and one genotype

from Tunisian region Kebili (K-5). Subclaster 2b was divided into 3 subclasters (2ba, 2bb and 2bc). Subclaster 2 ba contained 2 genotypes from Tunisian region Ghomrassen (GH-2 and GH-5). Subclaster 2bb contained 6 genotypes from different regions of Tunisia and subclaster 2 bc contained 29 gentypes of castor from different regions of Tunisia. Genetically the closest were two genotypes from Tunisian region Souassi (S-2 and S-5) in subclaster 2bc (Figure 2).

Lower average polymorphism (21%) obtained by SCoT technique was detected by **Kallamadi et al. (2015)** who analysed molecular diversity of castor (*Ricinus communis* L.). Out of a total of 108 bands, 23 (21%) were polymorphic with an average of 2.1 polymorphic bands per primer. The total number of bands per primer varied from 5 and 20 in the molecular size range of 100 – 3000 bp. The PIC/DI varied from 0.06 for SCoT28 to 0.45 for SCoT12 with an average of 0.24. On the other side, higher polymorphism with SCoT primers has been reported in crops like peanut **(Xiong et al., 2011)**, cicer **(Amirmoradi et al., 2012)**, mango **(Luo et al., 2010)**, ramie **(Satya et al., 2015)**, sugarcane **(Que et al., 2014)**, Chinese bayberry (**Fang-Yong and Ji-Hong, 2014)**, pepper **(Tsaballa et al., 2015)**, castor **(Kallamadi et al., 2015)**, maize **(Vivodík et al., 2016)**, durum wheat **(Etminan et al., 2016)**, oat **(Balážová et al., 2017)**, rye **(Petrovičová et al., 2017)**, vetch **(Chai et al., 2017)**.

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. Cluster analysis by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and structure placed the 240 individuals into four distinct clusters. **Balážová et al. (2017)** used eighteen primers to study polymorfism of 8 oat genotypes.

Altogether 153 different fragments were amplified of which 67 were polymorphic with an average number of 3.72 polymorphic fragments per genotype. The number of polymorphic fragments ranged from one (SCoT9, SCoT62) to nine (SCoT40). The polymorphic information content ranged from 0 (SCoT9, SCoT62) to 0.876 (SCoT40) with an average of 0.524. **Petrovičová et al. (2017)** study genetic variability among the set of 45 rye genotypes using 8 SCoT markers. Amplification of genomic DNA of 45 genotypes, using SCoT analysis, yielded 114 fragments, with an average of 14.25 polymorphic fragments per primer. The hierarchical cluster analysis showed that the rye genotypes were divided into 2 main clusters. In the present study, **Etminan et al. (2016)** analyzed genetic variation in a mini-core collection of durum wheat germplasm, including 25 breeding lines and 18 landraces, using six start codon targeted (SCoT) markers. High levels of polymorphism were observed; 98.70% (ISSR) and 100% (SCoT), which indicated that these markers are useful tools for detection of genetic variation in the collection. In the present investigation, **Vivodík et al. (2016)** analyzed 40 genotypes of maize from Czechoslovakia, Hungary, Poland, Union of Soviet Socialist Republics, Slovakia and Yugoslavia using 20 Start codon targeted (SCoT) markers. These primers produced total 114 fragments across 40 maize genotypes, of which 86 (76.43%) were polymorphic with an average of 4.30 polymorphic fragments per primer and number of amplified fragments ranged from 2 (SCoT 45) to 8 (SCoT 28 and SCoT 63). The polymorphic information content (PIC) value ranged from 0.374 (ScoT 45) to 0.846 (SCoT 28) with an average of 0.739. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. The hierarchical cluster analysis showed that the maize genotypes were divided into two main clusters.

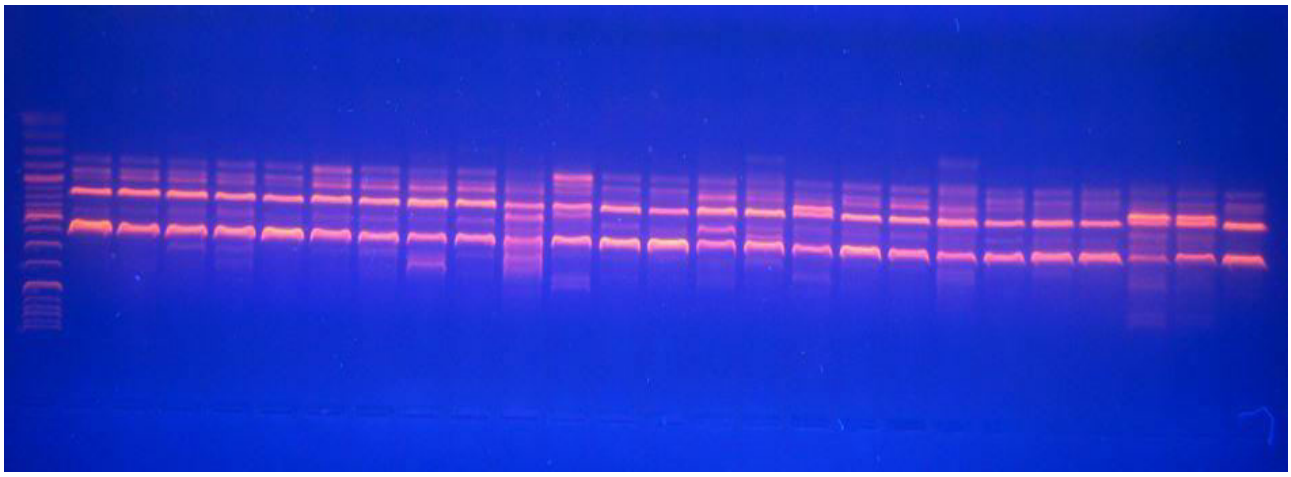

Figure 1 PCR amplification products of 25 genotypes of Tunisian castor produced with primer SCoT-12. Lane M is Quick-Load® 100 bp DNA ladder and lanes 1-25 are Tunisian castor genotypes.

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

SCoT Primers	Primer sequence (5´-3´)	TNoB	NoPB	PoPB	PIC
ScoT 2	CAACAATGGCTACCACCC	6	3	50.00	0.605
ScoT 3	CAACAATGGCTACCACCG	10	9	90.00	0.850
SCoT 6	CAACAATGGCTACCACGC	8	6	75.00	0.758
SCoT 8	CAACAATGGCTACCACGT	8	7	87.50	0.815
SCoT 9	CAACAATGGCTACCAGCA	8	6	75.00	0.772
SCoT 11	AAGCAATGGCTACCACCA	10	8	80.00	0.839
SCoT 13	ACGACATGGCGACCATCG	7	7	100.00	0.793
SCoT 14	ACGACATGGCGACCACGC	10	9	90.00	0.868
SCoT 15	ACGACATGGCGACCGCGA	9	9	100.00	0.843
SCoT 16	ACCATGGCTACCACCGAC	8	7	87.50	0.822
SCoT 17	ACCATGGCTACCACCGAG	4	3	75.00	0.411
SCoT 18	ACCATGGCTACCACCGCC	10	8	80.00	0.852
SCoT 19	ACCATGGCTACCACCGGC	9	8	88.89	0.854
SCoT 20	ACCATGGCTACCACCGCG	8	8	100.00	0.841
SCoT 21	ACGACATGGCGACCCACA	8	7	87.50	0.772
SCoT 22	AACCATGGCTACCACCAC	6	4	66.67	0.717
SCoT 12	ACGACATGGCGACCAACG	10	8	80.00	0.811
SCoT 23	CACCATGGCTACCACCAG	7	6	85.71	0.822
SCoT 26	ACCATGGCTACCACCGTC	7	6	85.71	0.731
SCoT 28	CCATGGCTACCACCGCCA	6	5	83.33	0.731
SCoT 29	CCATGGCTACCACCGGCC	7	6	85.71	0.816
SCoT 30	CCATGGCTACCACCGGCG	8	8	100.00	0.851
SCoT 31	CCATGGCTACCACCGCCT	4	3	75.00	0.438
SCoT 33	CCATGGCTACCACCGCAG	8	7	87.50	0.828
SCoT 34	ACCATGGCTACCACCGCA	6	5	83.33	0.706
SCoT 36	GCAACAATGGCTACCACC	6	5	83.33	0.742
SCoT 40	CAATGGCTACCACTACAG	7	6	85.71	0.726
SCoT 44	CAATGGCTACCATTAGCC	5	5	100.00	0.765
SCoT 45	ACAATGGCTACCACTGAC	4	3	75.00	0.477
SCoT 54	ACAATGGCTACCACCAGC	8	7	87.50	0.830
SCoT 59	ACAATGGCTACCACCATC	6	5	83.33	0.705
SCoT 60	ACAATGGCTACCACCACA	7	6	85.71	0.726
SCoT 61	CAACAATGGCTACCACCG	9	8	88.89	0.815
SCoT 62	ACCATGGCTACCACGGAG	6	5	83.33	0.742
SCoT 63	ACCATGGCTACCACGGGC	5	4	80.00	0.533
SCoT 65	ACCATGGCTACCACGGCA	8	8	100.00	0.834
SCoT 66	ACCATGGCTACCAGCGAG	5	5	100.00	0.739
Average		**7.24**	**6.22**	**85.20**	**0.751**
Total		**268**	**230**	**-**	**-**

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

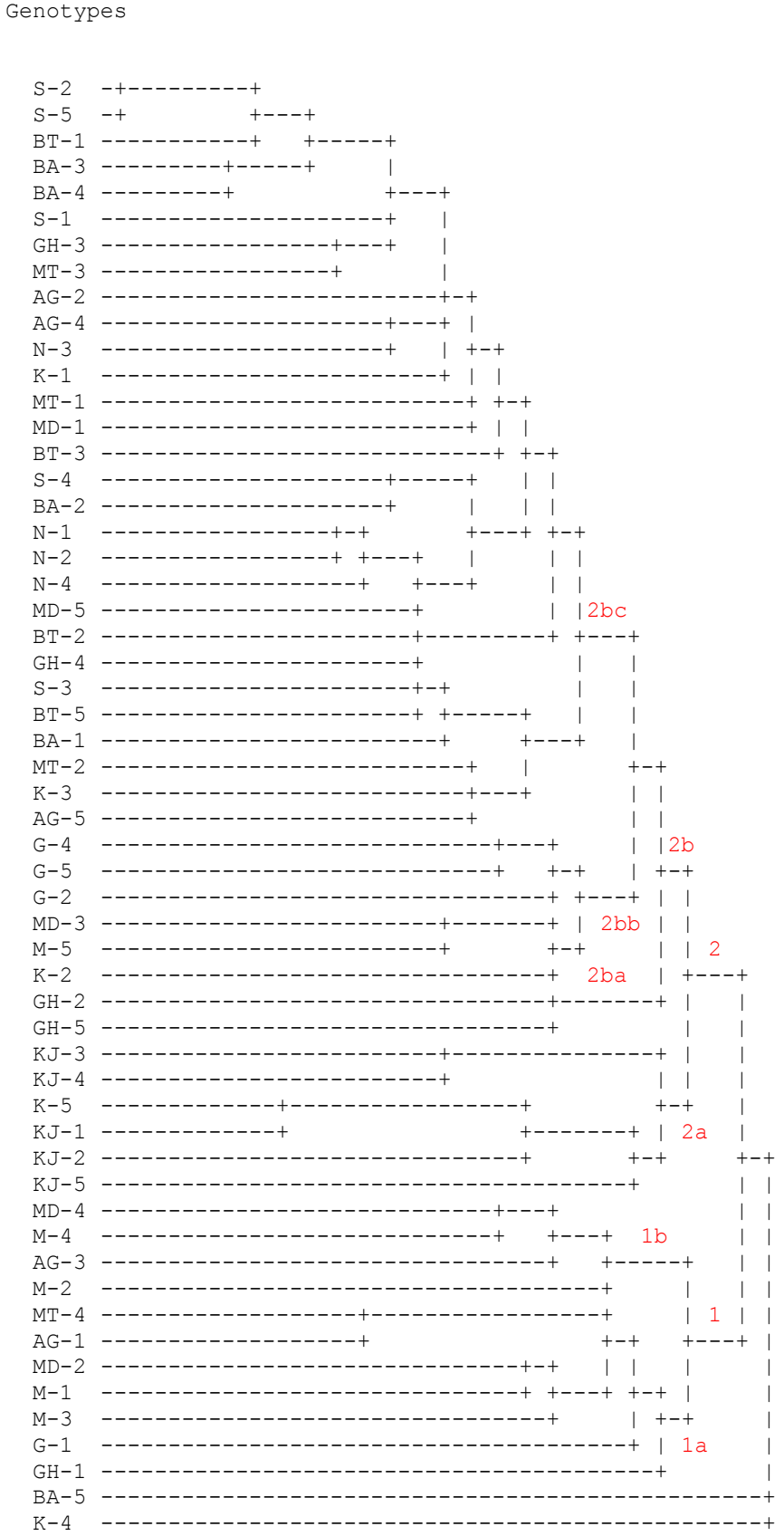

Figure 2 Dendrogram of 56 Tunisian castor genotypes prepared based on 37 SCoT markers. S- Souassi (5 genotypes), BT- Bouthay (4 genotypes), GH- Ghomrassen (5 genotypes), BA- Sidi bou ali (5 genotypes), MT- Matmata (4 genotypes), AG- Mateur (5 genotypes), N- Nefza (4 genotypes), MD- Mednine (5 genotypes), M- Mornag (5 genotypes), G- Gabes (4 genotypes), K- Kebili (5 genotypes), KJ- Ksar jedid (5 genotypes).

CONCLUSION

The present work reported utilization of SCoT markers for the detection of genetic variability of castor genotypes. In summary, SCoT marker analysis was successfully developed to evaluate the genetic relationships among the genus of castor accessions originated from various regions of Tunisia. The hierarchical cluster analysis divided castor genotypes into 2 main clusters. SCoT markers are generated from the functional region of the genome; the genetic analyses using these markers would be more useful for crop improvement programs. Polymorphism revealed by SCoT 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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