RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (3) 929-939, 2024
http://doi.org/10.54910/sabrao2024.56.3.3
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

GENETIC DIVERSITY AMONG SEEDED DATE PALM GENOTYPES USING START CODON TARGETED (SCoT) MARKERS

A.H. ANANY[1], KH.A. SOLIMAN[2], A.M. ABUSHADY[2,3], A.A.H. ELHALWAGI[1], S.G. THABET[4], and A.M. ALQUDAH[5*]

[1]National Gene Bank (NGB), Agricultural Research Center (ARC), Giza, Egypt
[2]Department of Genetics, Faculty of Agriculture, Ain-Shams University, Cairo, Egypt
[3]Biotechnology School, Nile University, Giza, Egypt
[4]Department of Botany, Faculty of Science, Fayoum University, Fayoum, Egypt
[5]Biological Science Program, Department of Biological and Environmental Sciences, Qatar University, Doha, Qatar
*Corresponding author's email: aalqudah@qu.edu.qa; ahqudah@gmail.com
Email addresses of co-authors: Ali.anany@post.agr.cu.edu.eg, aalqudah@qu.edu.qa, khaled346@agr.asu.edu.eg, aabushady@nu.edu.eg, abeerelhalwagi@gmail.com, sgs03@fayoum.edu.eg

SUMMARY

Discovering its genetic diversity and background is crucial for the date palm (*Phoenix dactylifera* L.) genetic resources management and conservation. In the latest study, the start codon targeted (SCoT) molecular marker utilization determined the genetic diversity and set the distinctive genotypes in 10 seeded date palm (*P. dactylifera* L.) genotypes. The genotyping attained through SCoT markers assessed the genetic variations in the date palm genotypes by the dendrogram. The highest genetic similarity of 89% was evident between the date palm genotypes G8 and G9, while the lowest similarity of 71% was between the genotypes G10 and G1 and G1 and G8. Overall, 160 bands amplified through 10 seeded date palm trees ranged from 150 bp to 1600 bp. Having the lowest value of PIC grouped the markers SCoT-02, SCoT-03, SCoT-04, and SCoT-09 into a cluster based on the heat map plot. However, the genotypes G4 and G5 are in a cluster along with genotype G6. The genetic association stemmed through genetic parameters using PCoA, heat map, and genetic distance. These findings can help identify the genetic diversity of anonymous date palm genotypes.

Keywords: Date palm (*P. dactylifera* L.), date palm genotypes, genetic distance, genetic diversity, heat mapping, PCoA, SCoT markers

Communicating Editor: Dr. Kamile Ulukapi

Manuscript received: October 30, 2023; Accepted: January 12, 2024.

Citation: Anany AH, Soliman KA, Abushady AM, Elhalwagi AAH, Thabet SG, Alqudah AM (2024). Genetic diversity among seeded date palm genotypes using start codon targeted (SCoT) markers. *SABRAO J. Breed. Genet.* 56(3): 929-939. http://doi.org/10.54910/sabrao2024.56.3.3.

Key findings: The results enunciated that the highest genetic similarity of 89% was between the date palm (*P. dactylifera* L.) genotypes G8 and G9, while the lowest value of 71% was between the genotypes G10 and G1, and G1 and G8. Overall, 160 bands amplified through 10 seeded date palm trees ranged from 150 bp to 1600 bp. The markers of SCoT-02, SCoT-03, SCoT-04, and SCoT-09 were in a cluster. The genotypes G4 and G5 were in a cluster along with G6.

INTRODUCTION

The date palm (*P. dactylifera* L.; Family Arecaceae) is one of the oldest staple food crops that can grow worldwide (Ahmed and Al-Qaradawi, 2009). It is a dioecious and monocotyledon, characterized by having 18 pairs of diploid chromosomes in its genome (2n = 2x = 36), ranking second economically among monocotyledons with an approximately estimated size of 670 Mb (Al-Dous *et al.,* 2011).

The genetic studies of date palms attract little attention compared with other crops like cereals and fruits. Dates daily consumption worldwide is due to their exceptional nutritional value and associated health benefits (Bekheet, 2013). In Middle Eastern countries, especially Egypt, the estimated number of date palm trees is approximately 62 million. Egypt leads global date production, generating 1,694,813 tons in 2015–2016, followed by Iran, Algeria, Saudi Arabia, and others (FAO, 2016) (http://www.fao.org).

The identification of date palm genotypes depends mainly on morphological, biochemical, and molecular markers (El-Sharabasy and Rizk, 2019; Mahdy and Ahmad, 2023). The genetic studies explored the high diversity of the date palm genome, including stress resistance and sugar metabolism-related genes; hence, date palms have a considerable role in evolution. Recent technological proceedings enabled researchers to produce a preliminary sequence of commercial date palm cultivars that refined to a genome size of roughly 670 Mb. Although this draft sequence is vital for grasping gene variation, it does not offer detailed structural data about the genome. This sequence identified the scaffolds with polymorphisms associated with date palm gender (Hazzouri *et al.,* 2019)**.** However, further studies, like searching for selective sweeps and quantitative trait loci (QTL), would greatly benefit from having the genetic map (Hazzouri *et al.,* 2019). A few genome-wide studies focusing on *P. dactylifera*, with a recent study conducted in Qatar, presented a draft genome assembly using the data from the Illumina GAII sequencing platform (Al-Dous *et al.,* 2011). Their research estimated the genome size at 658 Mb, assembled about 58% of that genome (amounting to 382 Mb), and predicted 25,059 genes.

DNA-based molecular marker development has targeted genetic diversity, sex determination, cultivar identification, phylogenetic study, and the latest various innovative and potential marker techniques (Bahraminejad and Mohammadi-Nejad, 2015; Al-Haidari and Al-Tamimi, 2023). The data from the genome sequencing holds great promise for developing new markers across various crop plant species (Elmeer and Mattat, 2012). The DNA markers derived from genetic variations within the DNA have significantly established new findings in different biological fields, including taxonomy, phylogenetic relationships, and genetics (Dorado *et al.,* 2017).

Start codon targeted polymorphism based on the short-conserved region in plant genes surrounding the ATG translation start codon has been well characterized in previous studies (Zhang *et al.,* 2015). SCoT primers' design comes from the short conserved region flanking the ATG start codon and serves as a single primer amplification reaction for the forward and reverse directions (Zhao *et al.,* 2020). DNA-based markers' wide use is prevalent nowadays in date palm cultivars (Chen *et al.,* 2013), with the recent development of the Start Codon Targeted (SCoT) marker (Poczai *et al.,* 2013; Zhang *et al.,* 2015). The application of SCoT markers to numerous plant species has included rice (Collard and Mackill, 2009), beans (Hromadová

et al., 2023), chicory (El-Taher *et al.,* 2023), and soybean (Rayan and Osman, 2019).

In recent decades, the employment of molecular techniques has assessed the genetic diversity among date palm cultivars. Saboori *et al.* (2020) studied 113 date palm trees of 13 cultivars grown in different regions of Iran using SCoT markers to examine the genetic diversity among and within these date palm cultivars. Their results indicated the efficiency of SCoT markers in analyzing the date palms' genetic diversity and fingerprinting, specifically revealing a high genetic variability within the population.

Therefore, it suggested utilizing modern molecular breeding and biotechnology approaches to get detailed information based on the genetic content and structure of existing germplasm (Al-Khayri *et al.,* 2015). The presented study aimed to use the start codon targeted (SCoT) molecular markers to determine genetic diversity and recognize the distinctive genotypes in 10 seeded date palm (*Phoenix dactylifera* L.) genotypes.

MATERIALS AND METHODS

The latest research carried out in 2021-22 proceeded on 10 date palm genotypes procured from the Central Laboratory for Research and Development of Date Palm, Agricultural Research Centre (ARC), Giza, Egypt (Table 1). The collected fresh and healthy leaf tissues (5 g) of each genotype in the Eppendorf tube underwent a study at the Agriculture Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.

Table 1. List of studied date palm genotypes.

Genotypes	Genotype ID
Type 01	G1
Type 02	G2
Type 03	G3
Type 04	G4
Type 05	G5
Type 06	G6
Type 07	G7
Type 08	G8
Type 09	G9
Type 10	G10

Table 2. SCoT primer name, sequence, and polymorphism information derived from 10 SCoT markers.

Primers	Sequence	MW	Mb	Ub	Pb	Tb	P%	PIC
SCoT-02	CAACAATGGCTACCACCC	150-750	11	1	6	17	35	0.11
SCoT-03	CAACAATGGCTACCACCG	170-690	13	0	5	18	28	0.106
SCoT-04	CAACAATGGCTACCACCT	210-1600	5	3	15	20	75	0.226
SCoT-06	CAACAATGGCTACCACGC	170-1100	2	1	9	11	82	0.23
SCoT-08	CAACAATGGCTACCACGT	180-810	6	2	6	12	50	0.168
SCoT-09	CAACAATGGCTACCAGCA	210-870	9	1	7	16	44	0.157
SCoT-11	AAGCAATGGCTACCACCA	190-1100	6	1	11	17	65	0.208
SCoT-12	ACGACATGGCGACCAACG	210-1500	4	0	13	17	76	0.261
SCoT-13	ACGACATGGCGACCATCG	220-1400	5	2	9	14	64	0.181
SCoT-14	ACGACATGGCGACCACGC	180-840	2	3	16	18	89	0.182
Total		150-1600	63	14	97	160	60	0.193
Means			6	1	10	16		

Mb= monomorphic, MW= molecular weight, Ub= unique band, Pb= polymorphic band, P%= polymorphism percent, Tb= total number of bands, and PIC= polymorphic information content.

DNA extraction and start codon targeted (SCoT)

Total DNA extraction followed the Zymo Research Kit. Electrophoresis checking on 1.2% agarose gel electrophoresis was at 100 Volt/30 min. The total DNA, diluted to 10 ng/µl, gained PCR analysis. Ten SCoT primers' selection followed the method according to Collard and Mackill (2009) (Table 2).

PCR amplification and product electrophoresis

The PCR reaction performed in a 20 µl reaction mixture contained 10 ng template DNA, 200 µM dNTPs, 250 nM of each primer, 1.5 mM MgCl2, 1x PCR buffer, and 1-unit Taq DNA polymerase. The PCR amplification ran through the Techno thermocycler (Germany) with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 50 °C to 60 °C based on the primers (Table 2) for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 5 min before cooling at 4 °C. Amplification results visualization run on 2% agarose gel, followed by the ethidium bromide staining. A 100-base pair (bp) molecular size ladder (Fermentas, Germany) helped determine the fragment size.

Data scoring and analysis

The analysis of the gel of the primers had the bands scored with the coding of 0 and 1 as absent or present bands, respectively. Using the Polymorphism Information Content (PIC) calculated for each marker, the basic information about molecular markers determines their application in genetic mapping. The PIC provides an estimate of the discriminatory power of a locus by considering not only the number of alleles expressed but also the relative frequencies of those alleles. The PIC values calculation employed by Anderson *et al.* (1993) relied on the following equation:

$$PIC = 1-p^2-q^2$$

Where:

Pi is the frequency of the i^{th} alleles, and q is the null allele frequency.

PIC values range from 0 (no discriminatory power) to 1 (very high discriminatory power).

The genetic similarity coefficient (GS) between the two genotypes received estimation according to the Dice coefficient (Sneath and Sokal, 1973).

$$\text{Dice formula: } GS_{ij} = 2a/(2a+b+c)$$

Where GS_{ij} is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i.

The similarity matrix used in performing the cluster analysis organized the observed data into meaningful structures to develop taxonomies. The cluster analysis computation as the Unweighted Pair Group Method with Arithmetic Average (UPGMA) method generated a dendrogram for studying the relationship among the date palm genotypes based on the Dice coefficient (Sneath and Sokal, 1973) (Table 3). Heatmap plot estimate based on ascendant hierarchical clustering depended on Dice distances. Feeding all the recorded data to the XLSTAT helped run the statistical analysis (Addinsoft, 2023).

RESULTS AND DISCUSSION

In this study, the date palm (*P. dactylifera* L.) genotype morphological description followed Stearn (1973). This step was critical to authenticating these unknown date palm genotypes still undocumented before as cultivars, which subjects to describe and compare derived morphological observations to the atlas of the date palm in Egypt (El-Sharabasy and Rizk, 2019).

Table 3. Similarity matrix between 10 date palm genotypes based on Dice coefficient.

Genotypes	1	2	3	4	5	6	7	8	9
2	0.80								
3	0.83	0.84							
4	0.79	0.82	0.82						
5	0.80	0.80	0.78	0.81					
6	0.73	0.76	0.72	0.79	0.84				
7	0.73	0.73	0.73	0.77	0.81	0.84			
8	0.71	0.75	0.74	0.77	0.81	0.84	0.88		
9	0.72	0.77	0.78	0.81	0.78	0.83	0.84	0.89	
10	0.71	0.74	0.73	0.81	0.77	0.81	0.83	0.84	0.83

Red color indicates low values and green indicates high values.

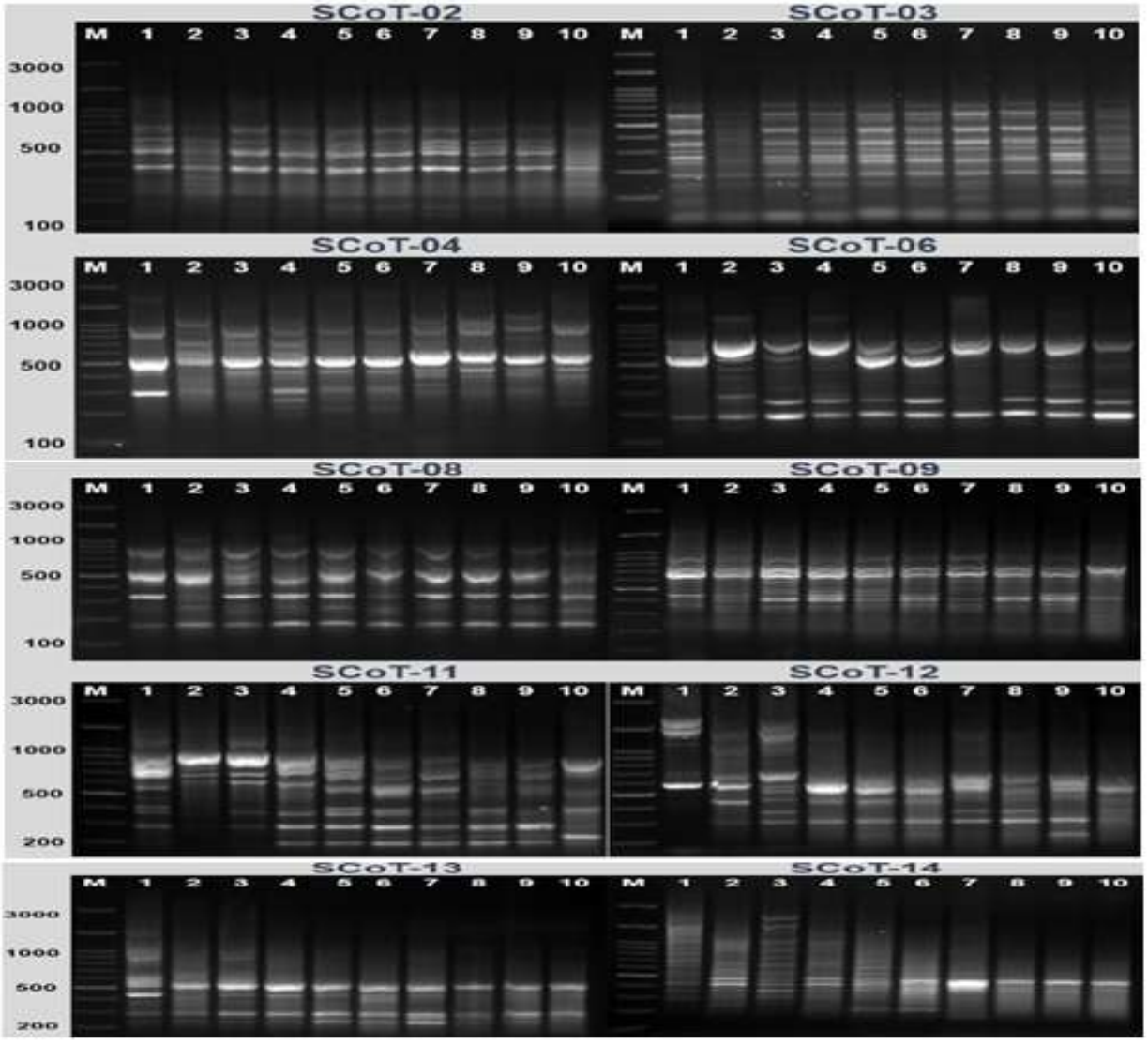

Figure 1. Photographs showing SCoT products of the 10 different seeded date palms using 10 primers. (From left to right): M= DNA ladder (100bp), 1 to 10= genotypes.

Genetic diversity

For further identification, analyzing the 10 seeded date palm genotypes used the PCR-based marker technique, SCoT (Figure 1). The polymorphism information content (PIC) derived from SCoT markers appears in Table 1. In total, 160 bands attained amplification through 10 seeded date palm genotype trees ranging from 150 bp to 1600 bp in size. The amplicons ranged from 11 (SCoT-6) to 20 (SCoT-4), averaging 16 bands per primer. The results further revealed that 97 bands scored several polymorphic bands, oscillating from five bands (SCoT-3) to 16 bands (SCoT-14), with an average of 10 bands per primer. The polymorphism percent estimated for the primer SCoT-14 revealed the highest polymorphism (89%), followed by 82%, 76%, and 75% scored in the primers SCoT-6, SCoT-12, and SCoT-4, respectively. Adawy and Atia (2014) reported higher averages for SCoT bands/primer than the present investigation (16) at 10.1 and 7.1, respectively.

Estimating the PIC, 10 SCoT primers became samples because of the high quality and low nonspecific amplification banding patterns (Table 2), reflecting the polymorphic content of the markers and similar allele frequency distribution by comparing with the other primers. The presence and quality of the amplification products sustained verification on agarose gels. The primers SCoT-02 and SCoT-03 showed the lowest value of PIC (0.11). However, the primers SCoT-12, SCoT-04, SCoT-06, and SCoT-11 revealed the PIC highest values, scoring 0.26, 0.226, 0.23, and 0.208, respectively. Higher PIC values than with the current study came from Akkak *et al.* (2009), ranging from 0.15 to 0.79 in Algerian and Californian date palm cultivars. Congruently, reports of an average PIC value of 0.60 among date palm genotypes from Iran, Iraq, and Africa emerged (Arabnezhad *et al.*, 2011). However, Jaskani *et al.* (2016) reported a PIC mean value of 0.39, which agrees with the PIC (0.193**)** obtained in this research.

The obtained data enunciated the polymorphism nature of the seeded date palm genotypes. The results further revealed this in the four primers, SCoT-4, SCoT-6, SCoT-12, and SCoT-4, by their percentage of polymorphism. These primers have the potential for identification and are more valuable when converting them into a simple sequence PCR-based marker for large-scale screening and evaluation. These markers could assess the detection of unknown genotypes based on the laboratory conditions compared with field screening, which is arduous and less accurate (Mahdy *et al.,* 2021).

Genetic distance and similarity indices

The genetic similarity estimates with the Dice coefficient relied on SCoT markers (Table 2). The similarity among the date palm genotypes ranged from 0.71 (between G1 and G10) to 0.89 (between G8 and G9). The phylogenetic tree revealed that all the genotypes fall into three classes (Figure 2). The first class (CI) includes the date palm genotypes G5, G6, G7, G8, G9, and G10. The second class (CII) contains the genotypes G2, G3, and G4, while the third class (CIII) only includes genotype GI. The CI falls into one group, while the rest of the classes (CII and CIII) fall into a separate group. As shown in Figure 2, the cluster divided the seeded date palm genotypes into two distinct groups at 0.76. Group I (right cluster) splits into two subgroups at 0.81. The first includes G1, while the second subgroup comprises genotypes G2, G3, and G4. Group II (left cluster) further incurs division into two subgroups at 0.811. The first subgroup includes G5 and G6, while the second has the rest of the date palm genotypes. Similarly, Adawy and Atia (2014) reported high genetic similarity among three Egyptian cultivars (Zaghloul, Hayani, and Samany) using SCoT primers that ranged from 0.85 to 0.95. This high genetic similarity among Egyptian date palms also acquired confirmation by other investigations using different marker systems, i.e., RAPD (Soliman *et al.,* 2003), AFLP (El-Khishin *et al.,* 2003; Adawy *et al.*, 2005), and ISSR (Hussein *et al.,* 2005).

The matrix standardization helped elaborate the linkage among the studied seeded date palm genotypes based on SCoT attributes, with the coordinates computed for bi-plot mapping using the principal coordinate analysis (Figure 3), confirming the results derived from the cladogram (Figure 2). The results further authenticated that the plotting

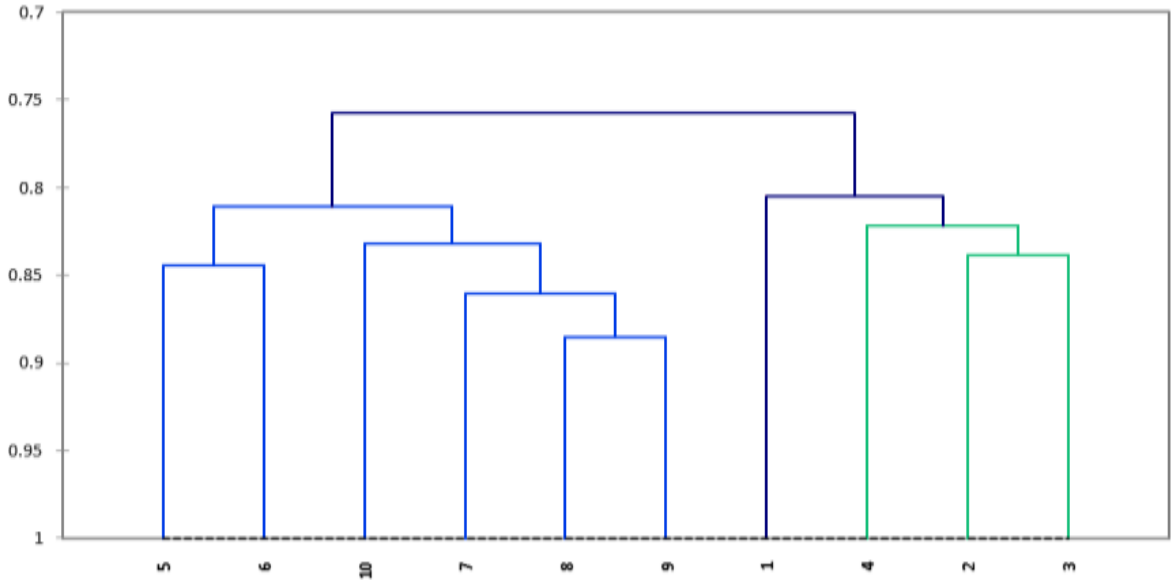

Figure 2. Phylogenetic tree obtained from SCoT markers for 10 date palm genotypes based on the UPGMA. From 1 to 10= ID of genotypes (for more details, see Table 1).

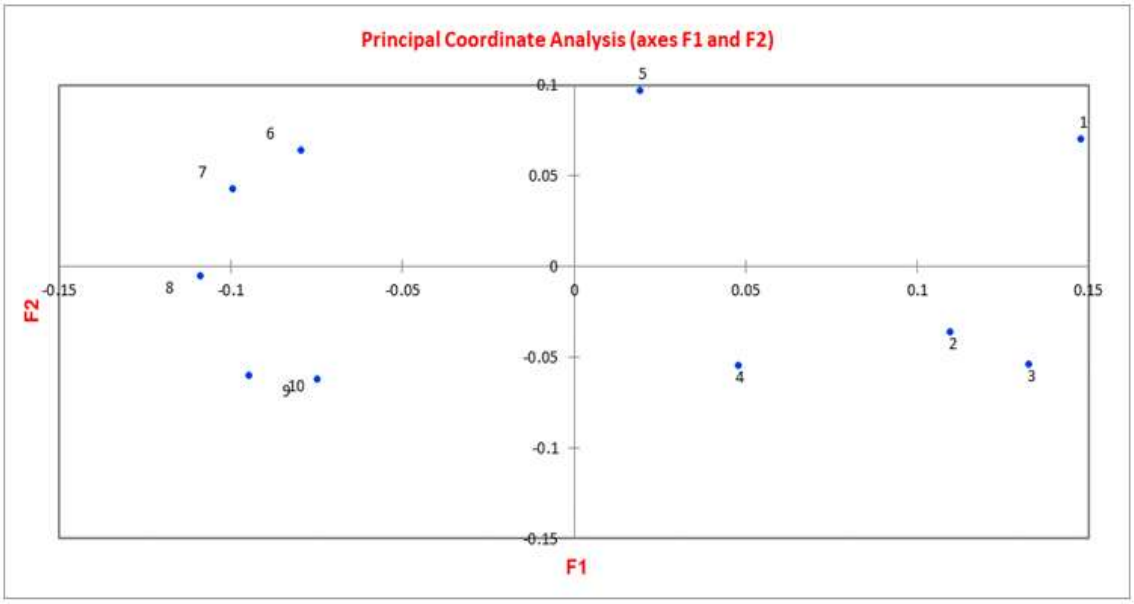

Figure 3. PCoA based on SCoT markers. The matrix plot received processing by XLSTAT 2019. From 1 to 10= ID of genotypes (for more details, see Table 1).

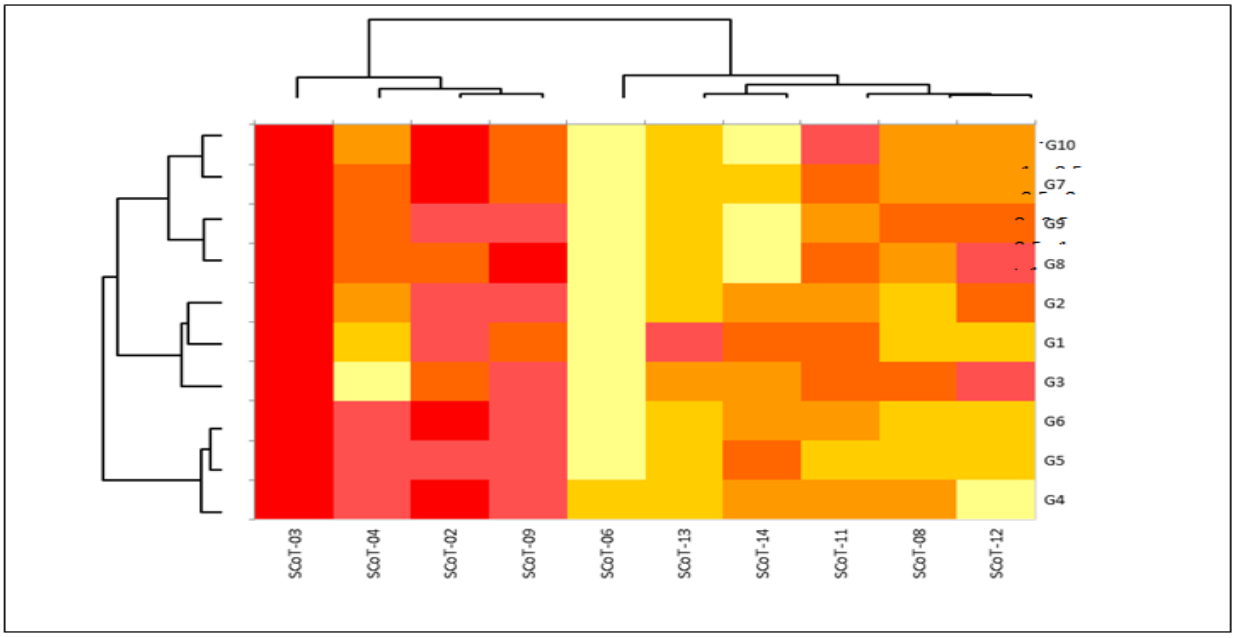

Figure 4. Heatmap plot of 10 unknown date palm genotypes derived from SCoT markers using ascendant hierarchical clustering based on Euclidian distances. From G1 to G10= ID of genotypes (for more details, see Table 1).

of date palm genotypes G2, G3, and G4 was in a separate group. The genotypes G9 and G10 occurred close to each other; however, these genotypes could be beneficial to generate new promising genotypes with genetic improvement by G1 and G5 toward the 'X' axis and Class II (Figure 2) and the 'Y' axis. In agreement with the cluster results, Adawy *et al*. (2005) grouped Sewi cultivars in one subcluster, and Eissa *et al*. (2009) grouped Zaghloul and Hayani in the same cluster. In contrast, other studies that used SSR loci showed diverse genetic distances among Sudanese, Moroccan, Qatari, Iraqi, Mauritanian, Nigerian, and Saudi date palm genotypes (Ahmed and Al-Qaradawi, 2009).

The results implied that SCoT-14 was the leading marker by showing the highest polymorphism (89%), while the least polymorphic marker was SCoT-03, with only 28% polymorphism (Figure 4). According to these findings, the SCot-14 marker can efficiently study genetic diversity and identify different unknown date palm cultivars. Additionally, grouping the markers SCoT-02, SCoT-03, SCoT-04, and SCoT-09 into a separate cluster was due to having the lowest value of PIC (Table 1). However, the date palm genotypes G4 and G5 attained grouping into a cluster along with genotype G6. The rest of the genotypes underwent division into subgroups. It was remarkable that a correlation appeared from the PCoA, heat map, genetic distance, and genetic parameters. The derived results indicated that the SCoT markers technology is very effective in utilizing the identification and genetic diversity of unknown date palm genotypes. Saboori *et al.* (2020) revealed the efficiency of SCoT markers in genetic diversity analysis and genetic fingerprinting of date palms.

The scattering of the date palm accessions confirmed the highest genetic variation in the genotypes. Selection of the proper molecular marker for identification is very crucial to consider. SCoT markers have great potential in germplasm identification and can reproduce polymorphisms. The presented results indicated that the used markers ably and effectively identify the unknown date palm genotypes through DNA-based markers. SCoT markers have the highest capability to identify

specific germplasm. Past studies reported that the genetic polymorphism percentage ranged from 32% to 37% using various molecular markers like ISSR, cp-DNA, and genomic DNA, which agrees with SCoT molecular marker results reported here (Wu *et al.,* 2013). Based on the nature of the SCoT marker, these regions could show variations in the coding regions and may linked to a functional gene (Luo *et al.,* 2011). Therefore, the SCOT private bands may be valuable in date palm genetic fingerprinting and cultivar identification.

Therefore, the approach proved more effective in distinguishing the studied date palm germplasm and constructing unique DNA profiles of each genotype for future use. The presented study revealed a broad genetic base and suggested that geneticists must review the genotypes using various approaches. The cultivars' identification is vital as more than 500 different registered date palm cultivars and unknown genotypes grow in Egypt, and their cultivation still depends on the traditional approaches. However, detailed information on the genetic content can be reproducible using molecular markers and biotechnology (Mahdy and El-Sharabasy, 2021).

Genetic identification is imperative for the conservation of the different plant species. Therefore, developing a different marker system has benefited the assessment of genetic diversity and identification of the date palm germplasm, as discussed using the reproducible SCoT molecular markers for seeded date palm genotypes. The SCoT marker has potential applications, viz., genetic diversity studies, QTL mapping, and bulked segregant analysis (Collard and Mackill, 2009). However, some studies reported that the polymorphism detected by these markers was low compared with other markers (Bahraminejad and Mohammadi-Nejad, 2015). The latest study findings would help molecular fingerprinting for germplasm screening, identification, and conservation in the future. The results show that the date palm genotypes have a broad genetic diversity range and, therefore, have allocations in numerous agroclimatic regions.

CONCLUSIONS

The pertinent results showed the efficiency of SCoT markers concerning date palm genotype identification, their genetic diversity and evaluation, and conservation in future generations. These markers yield adequate detailed data on genetic make-up and can also identify the unknown date palm genotypes. Thus, assessing genetic diversity among and within cultivars would be more beneficial for their conservation. The derived results indicated that the SCoT markers technology was more effective in identifying the genetic diversity of the date palm germplasm.

REFERENCES

Adawy SS, Atia MA (2014) A multidisciplinary molecular marker approaches to assess the genetic diversity in Egyptian date palm. *Int. J. Biotechnol. Res*. 4: 1-12.

Adawy SS, Hussein EH, Ismail SEME, El-Itriby HA (2005). Genomic diversity in date palm (*Phoenix dactylifera* L.) as revealed by AFLPs in comparison to RAPDs and ISSRs. *Arab J. Biotechnol*. 8: 99-114.

Addinsoft (2023). XLSTAT statistical and data analysis solution. Boston, USA. https://www.xlstat.com.

Ahmed TA, Al-Qaradawi AY (2009). Molecular phylogeny of Qatari date palm genotypes using simple sequence repeats markers. *Biotechnol*ogy 8: 126-131. doi: 10.3923/biotech.2009.126.131.

Akkak A, Scariot V, Torello Marinoni D, Boccacci P, Beltramo C, Botta R. (2009). Development and evaluation of microsatellite markers in *Phoenix dactylifera* L. and their transferability to other *Phoenix* species. *Biol. Plant.* 53: 164-166. https://doi.org/ 10.1007/s10535-009-0026-y.

Al-Dous EK, George B, Al-Mahmoud ME, Al-Jaber MY, Wang H, Salameh YM, Al-Azwani EK, Chaluvadi S, Pontaroli AC, DeBarry J, Arondel V, Ohlrogge J, Saie IJ, Elmeer KM, Bennetzen JL, Kruegger RR, Malek JA (2011). De novo genome sequencing and comparative genomics of date palm (*Phoenix dactylifera*). *Nat. Biotechnol.* 29: 521-527. https://doi.org/10.1038/nbt.1860.

Al-Haidari HAM, Al-Tamimi AJT (2023). Physical and chemical mutagenesis in *Catharanthus roseus* plant using SCoT markers. *SABRAO J. Breed. Genet*. 55(2): 399-406. http://doi.org/10.54910/sabrao2023.55.2.12.

Al-Khayri JM, Jain SM, Johnson DV (2015) Date palm genetic resources and utilization. *Springer*. https://doi.org/10.1007/978-94-017-9707-8.

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993). Optimizing parental selection for genetic-linkage maps. *Genome* 36(1): 181-186. https://doi.org/10.1139/g93-024.

Arabnezhad H, Bahar M, Mohammadi HR, Latifian M (2011). Development, characterization, and use of microsatellite markers for germplasm analysis in date palm (*Phoenix dactylifera* L.). *Scien. Hortic.* 134: 150-156. https://doi.org/10.1016/j.scienta.2011.11.032.

Bahraminejad A, Mohammadi-Nejad G (2015). Genetic diversity analysis of date palm (*Phoenix dactylifera* L.) genotypes using RAPD markers. *Ann. Res. Rev. Biol.* 5(1): 41-47. 10.9734/ARRB/2015/11683.

Bekheet S (2013). Direct organogenesis of date palm (*Phoenix dactylifera* L.) for propagation of true-to-type plants. *Scien. Agric*. 4: 85-92.

Chen F, Shi J, Luo YQ, Sun SY, Pu M (2013). Genetic characterization of the gypsy moth from China (*Lepidoptera, Lymantriidae*) using inter simple sequence repeats markers. *PLoS One* 8(8): e73017. https://doi.org/10.1371/journal.pone.0073017.

Collard BCY, Mackill DJ (2009). Start Codon Targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol. Biol. Rep*. 27: 86-93. https://doi.org/10.1007/s11105-008-0060-5.

Dorado G, Unver T, Budak H, Hernández MP (2017). Molecular markers. In: *Reference Module in Biomedical Sciences*; Elsevier. 10.1016/B978-0-12-801238-3.08996-0. 10.1016/B978-0-12-801238-3.08996-0.

Eissa E, El-Razek AA, El-Sharabasy S, Rizk R (2009). Morphological and molecular genetic characterization of soft date palm (*Phoenix dactylifera* L.) cultivars in Egypt. *Egy. J. Genet. Cytol.* 38: 269-284.

El-Khishin DA, Adawy SS, Hussein EH, El-Itriby HA (2003). AFLP fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars. *Arab J. Biotechnol.* 6: 223-234.

Elmeer K, Mattat I (2012). Marker-assisted sex differentiation in date palm using simple sequence repeats. *3 Biotechnol*. 2(3): 241-247. https://doi.org/10.1007/s13205-012-0052-x.

El-Sharabasy SF, Rizk RM (2019). Atlas of date palm in Egypt. FAO, Egypt.

El-Taher AM, Elzilal HA, Abd El-Raouf HS, Mady E, Mahdy EMB, Ragab OG, Emam EA, Alaraidh IA, Randhir TO, Ibrahim MFM (2023). Characterization of some Cichorium taxa grown under Mediterranean climate using morphological traits and molecular markers. *Plants* 12: 388. https://doi.org/10.3390/plants12020388.

FAO (2016). Crop Production. Statistics Division, Food and Agriculture Organization of the United Nations, Rome, Italy.

Hazzouri KM, Gros-Balthazard M, Flowers JM, Copetti D, Lemansour A, Lebrun M, Masmoudi K, Ferrand S, Dhar MI, Fresquez ZA, Rosas U, Zhang J, Talag J, Lee S, Kudrna D, Powell RF, Leitch IJ, Krueger RR, Wing RA, Amiri KMA, Purugganan MD (2019). Genome-wide association mapping of date palm fruit traits. *Nat. Commun.* 10:4680. https://doi.org/10.1038/s41467-019-12604-9.

Hromadová Z, Gálová Z, Mikolášová L, Balážová Ž, Vivodík M, Chňapek M (2023). Efficiency of RAPD and SCoT markers in the genetic diversity assessment of the common bean. *Plants* 12(15): 2763. https://doi.org/10.3390/plants12152763.

Hussein EH, Adawy SS, Ismail SEME, El-Itriby HA (2005). Molecular characterization of some Egyptian date palm germplasm using RAPD and ISSR markers. *Arab J. Biotechnol.* 8: 83-98.

Jaskani MJ, Awan FS, Ahmad S, Khan IA (2016). Development of molecular method for sex identification in date palm (*Phoenix dactylifera* L.) plantlets using novel sex-linked microsatellite markers. *3 Biotechnology* 6: 22. https://doi.org/10.1007/s13205-015-0321-6.

Luo C, He X-H, Chen H, Ou S-J, Gao M-P, Brown JS, Tondo CT, Schnell RJJBS (2011). Genetic diversity of mango cultivars estimated using SCoT and ISSR markers. *Biochem. Syst. Ecol*. 39: 676-684. 10.1016/j.bse.2011.05.023. https://doi.org/10.1016/j.bse.2011.05.023.

Mahdy EM, Ahmad H (2023). A study of Corchorus L. diversity in Egypt using high-throughput phenotyping platform (HTPP): An Egyptian genebank example. *Genet. Resour. Crop Evol.* 1-11. doi:10.1007/s10722-023-01551-6.

Mahdy EM, El-Sharabasy SF (2021). Date palm genetic identification and improvement utilizing molecular markers and DNA barcoding. In: The Date Palm Genome, Vol.

1: Phylogeny, Biodiversity and Mapping. pp. 101-134. *Cham: Springer International* Publishing. https://doi.org/10.1007/978-3-030-73746-7_5.

Mahdy EMB, El-Shaer HFA, Sayed AIH, El-Halwagi (2021). Genetic diversity of local cowpea (*Vigna spp* [L.] Walp.) accessions cultivated in some regions of Egypt. *Jordan J. Biol. Sci.* 14(4):775-789. https://doi.org/10.54319/jjbs/140419.

Poczai P, Varga I, Laos M, Cseh A, Bell N, Valkonen JP, Hyvönen J (2013). Advances in plant gene-targeted and functional markers: A review. *Plant Methods* 9: 1-32. https://doi.org/10.1186/1746-4811-9-6.

Rayan WA, Osman SA (2019). Phylogenetic relationships of some Egyptian soybean cultivars (*Glycine max* L.) using SCoT marker and protein pattern. *Bull. Nat. Res. Center* 43, 161, doi:10.1186/s42269-019-0197-4.

Saboori S, Noormohammadi Z, Sheidai M, Marashi S (2020). SCoT molecular markers and genetic fingerprinting of date palm (*Phoenix dactylifera* L.) cultivars. *Genet. Resour. Crop Evol.* 67: 73-82. https://doi.org/10.1007/s10722-019-00854-x.

Sneath PH, Sokal RR (1973). Numerical Taxonomy. The Principles and Practice of Numerical Classification.

Soliman SS, Ali BA, Ahmed MMM (2003). Genetic comparisons of Egyptian date palm cultivars *(Phoenix dactylifera* L*.)* by RAPD-PCR*. Afr. J. Biotechnol*. 2: 86-87**.** doi: 10.5897/AJB2003.000-1017.

Stearn WT (1973). Botanical Latin. Newton Abbott: David and Charles.

Wu J-M, Li Y-R, Yang L-T, Fang F-X, Song H-Z, Tang H-Q, Wang M, Weng M-L (2013). cDNA-SCoT: A novel rapid method for analysis of gene differential expression in sugarcane and other plants. *Aust. J. Crop Sci*. 7: 659-664.

Zhang J, Xie W, Wang Y, Zhao X (2015). Potential of start codon targeted (SCoT) markers to estimate genetic diversity and relationships among Chinese *Elymus sibiricus* accessions. *Molecules* 20(4): 5987-6001. 10.3390/ molecules20045987. https://doi.org/ 10.3390/molecules20045987.

Zhao X, Zou G, Zhao J, Hu L, Lan Y, He J (2020). Genetic relationships and diversity among populations of *Paris polyphylla* assessed using SCoT and SRAP markers. *Physiol. Mol. Biol. Plants* 26: 1281-1293. https://doi.org/10.1007/s12298-020-00808-z.