eISSN: 2357-044X *Taeckholmia 39 (2019): 44-66*

Assessment of genetic diversity in different geographically isolated *Alhagi graecorum* Boiss. populations using SCoT marker

Ghada M. Abd El- hak[1], Shafik D. Ibrahim[2], Atif Halem[1], Hussam Zaki Hassan[1], Walaa A. Rayan[1*]

[1]Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.
[2]Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center, Giza, Egypt.

***Corresponding author: walaagenetics@yahoo.com**

Abstract

The *Alhagi* genus (commonly known as Camel thorn) belongs to family Fabaceae, has various medicinal properties as revealed by several studies in some diseases treatment. The assessment of genetic diversity within and among *Alhagi graecorum* populations was carried out using ten SCoT primers. In this study, 25 individuals (five individuals from each population) were sampled, amplified separately and as bulked DNA (DNA from the five samples gathered from each population were bulked together to identify polymorphism among the studied populations). In total, 140 and 156 bands were scored with 71.3% and 63.7% polymorphism, respectively. Average polymorphism information content (PIC) for amplified DNA individual of samples was 0.87 and bulked- DNA samples was 0.75**.** Analysis of molecular variance (AMOVA) showed that 52% of the total variation was observed within populations while variation among populations was 48%. The UPGMA cluster analysis divided the 25 individuals and bulked samples into two main groups, each group was further subdivided into many sub-clusters. PCoA was also carried out and in agreement with the UPGMA**.** The GC values of sequencing for three clearly unique bulked- DNA bands from (SCoT5, SCoT20 and SCoT21) were 54.7, 45.5 and 60.6%, respectively with average percentage 53.6%**.** The present results showed the efficiency of SCoT markers for the evaluation of genetic relationships between and among populations due to their efficiency in revealing polymorphism.

Key words: Medicinal plant, *Alhagi graecorum*, Genetic variations, sequencing, SCoT.

INTRODUCTION

The *Alhagi* genus belongs to family Fabaceae, which commonly known as Camel thorn or Manna trees. It is wild plant which grows in salty soils and dry environment; it is native in Mediterranean and central Asia regions (**Boulos, 1966**). This genus comprises different species in the world such as *A. pseudalhagi* M. Mieb. Desv., *A. graecorum* Boiss., and *A. sparsifolia* Shap., etc. **(Khan, 2009; Badshah and Hussain, 2011; Xue *et al*., 2012)**, these different species of *Alhagi* have been explored for their antioxidant potential and nutritive value along with various medicinal properties **(Muhammad *et al*., 2015; Ahmed, 2019)**. Several studies have revealed the use of *Alhagi* plants in treating a wide spectrum of diseases including gastroenteritis, ulcers, fever, inflammations hypertension and cancer **(Zou *et al*., 2012; Laghari *et al*., 2012)**.

Natural products have interesting and useful biological activities and they also perform various functions (**Biswas *et al*., 2010)**. Researchers are increasingly turning their attention toward natural products in order to develop better drugs against cancer, as well as viral and microbial infections **(Revathi and Parimelazhghan, 2010)**. Since the early days of mankind, plants with secondary metabolites have been used by humans to treat or even protect against infections, health disorders and illness **(Wyk and Wink, 2004)**. *Alhagi*

Received on 13/11/2019 and accepted on 03/12/2019

graecorum is one of these plants which have active constituents such as, flavonoids, fatty acids, coumarins, glycosides, sterols, steroids, resins, vitamins, alkaloids, carbohydrates, tannins, unsaturated sterols and triterpenes (**Jack - Masquelier, 1996)**.

Detection of genetic variation and determination of genetic relationships between individuals and populations is an important consideration for the efficient conservation and utilization of plant genetic resources **(Henry, 1998; Semagn, *et al*., 2006**). Some studies which were conducted during the last decade of the 20th century reported numerous DNA markers that have been utilized in plant breeding programs **(Kordrostami and Rahimi, 2015)**. Once the generated molecular marker profiles have been evaluated, there are different strategies how to estimate the similarity between the analysed individuals. Similarity indices measure the amount of closeness between two individuals; the larger values are more similar between the two individuals. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics. The presence of various types of molecular markers, and differences in their principles, methodologies, and applications require careful consideration in choosing one or more of such methods **(Semagn *et al*., 2006)**.

PCR based molecular markers; such as Random Amplified Polymorphic DNA [RAPD **(Williams *et al*., 1990** and **Javouhey *et al*., 2000)**], Microsatellites or Simple Sequence Repeat [SSR, **(Adawy, *et al*., 2010** and **Ma *et al*., 2015)**], Inter-Simple Sequence Repeat [ISSR, (**Zietkiewicz *et al*., 1994; Bornet and Branchard, 2001**)] are quick, reliable, lower cost and highly informative techniques. So that these techniques are increasingly practical for routine applications to tropical or subtropical species for which very limited research resources are available **(Semagn, *et al*., 2006)**. DNA marker as SCoT is used efficiently for studying genetic diversity of plants **(Collard and Mackill, 2009; Sadek and Ibrahim, 2018)**. Initiating a trend away from random DNA markers towards gene-targeted markers, this marker system called Start Codon Targeted (SCoT) Polymorphism 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 **(Collard and Mackill, 2009)**.

SCoT markers have been successfully used to evaluate genetic diversity and structure, identify cultivars, and for quantitative trait loci (QTL) mapping and DNA fingerprinting in different species, including wheat, rice, check pea, sugarcane and grape (**Collard and Mackill, 2009; Amirmoradi *et al*., 2012; Guo *et al*., 2012; Adawy, *et al*., 2013; Hamidi *et al*., 2014; Ibrahim *et al*.,2016)**. These markers are used with various plant material, among them were; *Andrographis paniculata* **(Tiwari *et al*., 2016)**, *Vigna unguiculata* **(Igwe *et al*., 2017)**, wheat **(Abd El-Lateif and Hewedy, 2018)**, *Trichosanthes dioica* Roxb. (**Kumar and Agrawal, 2019)**. Currently, there is no report on genomic DNA isolation and the use of SCoT markers for molecular identification and genetic diversity characterization of collected *Alhagi graecorum* from Egypt. Therefore, in this study isolation, amplification, and characterization of genomic DNA of twenty five *A. graecorum* genotypes (different five habitats) in Egypt using ten SCoT molecular markers were carried out.

MATERIALS AND METHODS

Plant material:

The material comprises 25 genotypes of *Alhagi graecorum* were collected from different five regions; (1-5) =El-Dakhla Oasis, (6-10) = Botanical garden, Botany

Department, Ain Shams University, (11-15) = Wadi –El Rayan, (16-20) = Qarun Lake and (21-25) = Siwa Oasis.

DNA extraction:

Genomic DNA was extracted and purified from young leaves of the samples by using 2% CTAB extraction buffer according to (**Doyle and Doyle, 1990**). DNA concentrations of total genomic DNA in each sample were estimated using a spectrophotometer (TU 1880 Double Beam UV-VIS). All the DNA samples were stored at −20 °C.

SCoT -PCR analysis:

SCoT assay was performed as described in **(Collard and Mackill, 2009)**, for screening was carried out using 10 primers, Table (1).

Table 1: List of primers and their nucleotide
Sequences used for SCoT marker in *A. graecorum* amplification.

No.	Primer code	Primer nucleotide sequence (5'→3')
1	SCoT-4	5` ACCATGGCTACCACCGCA 3`
2	SCoT-5	5` CAATGGCTACCACTAGCG 3`
3	SCoT-6	5` CAATGGCTACCACTACAG 3`
4	SCoT-7	5` ACAATGGCTACCACTGAC 3`
5	SCoT-18	5` CCATGGCTACCACTAGCA 3`
6	SCoT-19	5` CCATGGCTACCACCGGCG 3`
7	SCoT-20	5` CAACAATGGCTACCACGC 3`
8	SCoT-21	5` CCATGGCTACCACCGGCC 3`
9	SCoT-23	5` CATGGCTACCACCGGCCC 3`
10	SCoT-24	5` CCATGGCTACCACCGCAG 3`

SCoT-PCR reactions were conducted using anchored primers, which were synthesized by Eurofins, Germany. Amplification was performed in a Gene Amp® PCR System 9700 thermal cycler (Applied Bio-systems) programmed using the temperature conditions as shown in Table (2). Electrophoresis of DNA samples were performed on 1.7 % agarose gel and visualized with 0.5 mg/ml ethidium bromide. DNA bands were visualized on a UV transilluminator at 302 nm and photographed by Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software, Bio-Rad™. Only the clearest and strongest SCoT bands were scored manually as present (1) or absent (0) to be used for further analysis.

Table 2: The SCoT -PCR reaction parameters.

Steps	Temperature	Time	Cycles
Initial denaturation	94 °C	5 min	1
Denaturation	94 °C	1 min	40
Annealing	50 °C	1 min	
Extension	72 °C	1.5 min	
Final extension	72 °C	7 min	1

Bulking DNA

Aliquots (10 µl) of the same concentration of the DNA individuals were mixed into a bulk of DNA representing each region. Hence, the outcome was 5 bulked DNA samples representing the different populations of *A.graecorum* included in this study. Nine SCoT primers (the same primers applied before except SCoT-7) were used to amplify the bulked DNA samples with the same conditions.

Statistical analysis

Genetic variation by using AMOVA analysis:

This performed to estimate the variance components and their significance levels among and within populations using GenALEx program.

Genetic relatedness and cluster analysis:

The scored binary data generated by SCoT marker was compared to determine the genetic relatedness of the 25 *A. graecorum* genotypes. Similarity matrices and cluster analyzing for SCoT markers were performed individually as well as collectively. The SCoT binary matrices were processed using the Bio-Rad diversity database software package and converted into similarity matrices according to Dice

coefficient (**Dice, 1945; Sneath and Sokal, 1973**). The formula used by Dice to estimate the genetic similarity coefficient (GS) between two genotypes was as follows:

Dice formula: GSij = 2a/ (2a+b+c)

Where GSij 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.

Principal coordinates analysis (PCoA):

Also known as classical multidimensional scaling which is a set of data analyses that show the structure of complex distance-like data represented in a high dimensional space into the lowest possible dimensional space. Prinicipal coordinates analysis (PCoA) resembles principal component analysis (PCA) but it takes dissimilarity as input (**Pavoine *et al*., 2004; Gower, 2015**). PCoA was performed based on the matrix calculated for each marker using PAST software (**Hammer *et al*., 2001**).

Sequencing:

Three unique fragments were selected from bulked DNA for sequencing. DNA sequencing reactions were performed using ABI PRISM Big Dye terminator cycle sequencing ready reaction kit (PE Applied Biosystems, Foster City, California, USA), in conjunction with ABI PRISM (310 Genetic Analyzer). Cycle sequencing was performed using the GeneAmp® PCR System 9700 instrument, and the reaction was conducted in a total volume of 20 µl, containing 8 µl of terminator ready reaction mix, 100-500 ng of PCR product, and 2 pmol of M13 universal forward primer (provided with the kit). The cycle sequencing program was set at 96°C for 2 min (1 cycle); (96°C for 10 sec, 50°C for 10 sec, and 60°C for 4 min, repeated for 25 cycles); 60°C for 2 min (1 cycle), with rapid thermal ramping. The nucleotide sequence was determined automatically by the electrophoresis of the cycle sequencing reaction product on 310 Genetic Analyzer. The data were provided as fluorometric scans from which the sequence was assembled using the sequence analysis software**.**

Sequence analysis:

Nucleotide sequences were analysed for homology to nucleotide sequences in GenBank non-redundant databases using the BLAST program (http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Characterization of the capability of each SCoT primer to detect polymorphism

The percentage of polymorphism detected among *Alhagi* samples gathered from different five regions using the ten SCoT primers was shown in Table (3) and Figure (1). The maximum polymorphism percentage for SCoT-4, SCoT-5 and SCoT-6 reached to 68.75%, 83.33% and 77.78%**,** in Qarun Lake, Wadi El-Rayan and Siwa Oasis, respectively when compared with other regions, also in SCoT-7 the maximum polymorphism was in Qarun Lake (66.67%). On the other hand, the highest values of polymorphism of each primers; SCoT-18, SCoT-19 and SCoT-20 were recorded 36.36%, 58.3% and 54.5% in Siwa Oasis, Wadi El-Rayan and Qarun Lake**,** respectively but the polymorphism percentage for SCoT-21 and SCoT-23 reached to 57.1% and 50% for both Siwa Oasis and Qarun Lake respectively. SCoT-24 recorded 50% in all different regions except El-Dakhla Oasis which showing no polymorphism (Table 3**)**. SCoTs are important markers which more stable, produce more reproducible and reliable bands and can be used effectively for population studies, genetic mapping in different plants and in the marker assisted selection programs **(Collard and Mackill, 2009)**. These markers are directly involved in relation of gene function and can be

Table 3: The percentage of polymorphism recorded by the ten SCoT primers of *A. graecorum* genotypes collected from five locations.

Primer code	Location	Total no. of bands	Monomorphic bands	Polymorphic bands	Percentage of polymorphism (%)
SCoT-4	El-Dakhla oasis	12	6	5	50.00
	Ain Shams university	15	13	2	13.33
	Wadi El-Rayan	16	7	9	56.25
	Qarun lake	16	5	11	68.75
	Siwa Oasis	17	6	11	64.71
SCoT-5	El-Dakhla oasis	5	2	3	60.00
	Ain Shams university	12	6	6	50.00
	Wadi El-Rayan	12	2	10	83.33
	Qarun lake	15	3	12	80.00
	Siwa Oasis	11	4	7	63.64
SCoT-6	El-Dakhla oasis	5	4	1	20.00
	Ain Shams university	10	9	1	10.00
	Wadi El-Rayan	8	4	4	50.00
	Qarun lake	9	5	4	44.44
	Siwa Oasis	9	2	7	77.78
SCoT-7	El-Dakhla oasis	7	3	2	57.00
	Ain Shams university	8	8	0	00.00
	Wadi El-Rayan	9	4	5	55.56
	Qarun lake	12	4	8	66.67
	Siwa Oasis	9	6	3	33.33
SCoT-18	El-Dakhla oasis	6	5	1	16.67
	Ain Shams university	11	9	2	18.18
	Wadi El-Rayan	10	7	3	30.00
	Qarun lake	9	6	3	33.33
	Siwa Oasis	11	7	4	36.36
SCoT-19	El-Dakhla oasis	8	5	3	37.50
	Ain Shams university	10	6	4	40.00
	Wadi El-Rayan	12	5	7	58.30
	Qarun lake	13	6	7	53.9
	Siwa Oasis	9	7	2	22.22
SCoT-20	El-Dakhla oasis	6	5	1	16.70
	Ain Shams university	10	9	1	10.00
	Wadi El-Rayan	10	10	0	0.00
	Qarun lake	11	5	6	54.55
	Siwa Oasis	11	8	3	27.27
SCoT-21	El-Dakhla oasis	7	6	1	14.29
	Ain Shams university	7	7	0	00.00
	Wadi El-Rayan	8	4	4	50.00
	Qarun lake	8	4	4	50.00
	Siwa Oasis	14	6	8	57.14
SCoT-23	El-Dakhla oasis	7	5	2	28.57
	Ain Shams university	10	10	0	00.00
	Wadi El-Rayan	10	9	1	10.00
	Qarun lake	10	5	5	50.00
	Siwa Oasis	10	7	3	30.00
SCoT-24	El-Dakhla oasis	4	4	0	00.00
	Ain Shams university	8	4	4	50.00
	Wadi El-Rayan	10	5	5	50.00
	Qarun lake	8	4	4	50.00
	Siwa Oasis	8	4	4	50.00

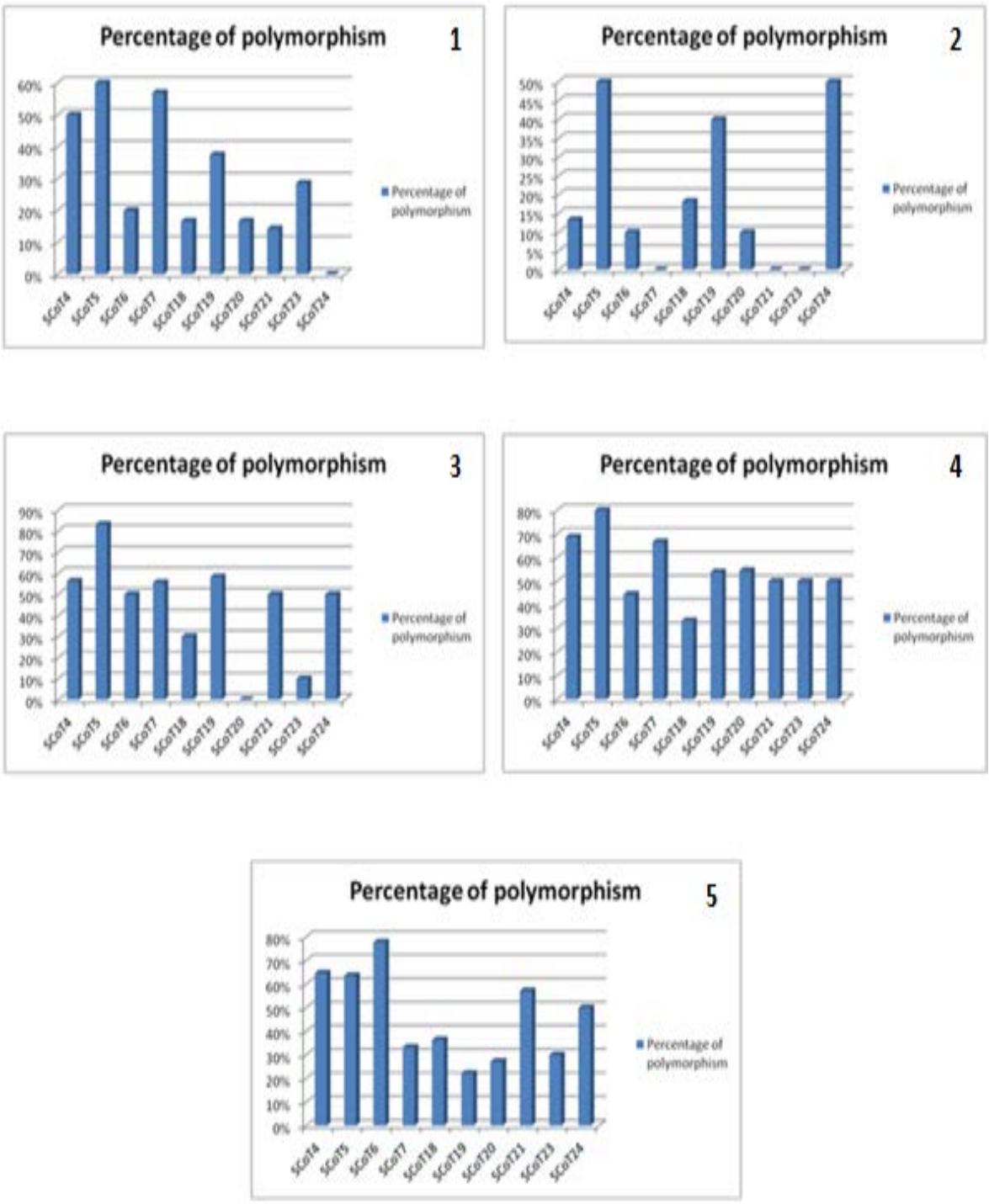

Figure 1: The polymorphism values detected by the ten SCoTprimers among *Alhagi* samples gathered from 1=El-Dakhla Oasis; 2=Ain Shams University; 3=Wadi El-Rayan; 4=Qarun Lake and 5= Siwa Oasis.

utilized in genotyping and to explore polymorphism **(Gorji *et al.*, 2011 and Poczai *et al.*, 2013)**. Start codon targeted polymorphism technique can be employed to identify DNA polymorphisms, studying genetic diversity and relationships among *Alhagi* genotypes as used for *Arachis hypogaea* **(Xiong *et al.*, 2011)**; they ranged polymorphism per primer from 14.29 to 66.67% with an average of 36.76%. Also, in mango germplasm generated 273 bands with an average of 8.27 bands per primer among the 50 accessions, of which 208 bands were polymorphic **(Luo *et al*., 2010)**. In another study, a total of 289 bands were generated in *Dactylis glomerata* and 272 bands were polymorphic, with an average value of 12.95 per primers which ranged between seven to 20 bands (**Yan *et al*., 2016**). As well as, ten SCoT primers were screened by **Talebi *et al*. (2018)**, the total of 73 bands were generated in *Cartamus tinctorious*, with 83% polymorphism out of which 61 bands. The maximum and minimum number of polymorphic bands were obtained using SCoT35 (11 bands) and SCoT22 (3 bands), respectively. Similar work, 27 SCoT primers were used by **Yang *et al.* (2019)**, 419 fragments from a total 429 were polymorphic in *Miscanthus lutarioriparius* populations. For each SCoT primer, the number of polymorphic fragments ranged from 7 (ST15) to 21 (ST27, 32), with an average of 15.52, whereas, the percentage of polymorphic fragments was f1`rom 88.89% to 100.00%. Furthermore, many other studies were employed different SCoT primers to study the genetic diversity for many plant populations such as *Dendrobium nobile* **(Bhattacharyya *et al*., 2013)** and *Jojoba* genotypes **(Heikrujam *et al*., 2015)**.

Genetic diversity of individuals and bulked-samples DNA in *A. graecorum* as revealed by SCoT markers

The percentage of polymorphism was recorded by the ten SCoT primers among the different *A. graecorum* genotypes (1-25) collected from different five locations was shown in Table (4) and Figure (2). A total of 140 bands were amplified among the 25 individuals including 37 monomorphic DNA fragments and 103 were polymorphic with percentage 71.3%, four of them are unique bands, whereas the number of polymorphic amplicons varied from 5 to 18 for SCoT-23 and SCoT-5, respectively; but the percentage of the polymorphism were varied between 50% and 90% with SCoT-23 and SCoT-5, respectively (Table 4**)**. The SCoT fingerprinting patterns revealed by the nine primers used among the five studied *Alhagi* populations (bulked- DNA samples) are showed in Figure (3). The genetic polymorphism revealed by the SCoT-primers between the five *Alhagi* populations, which yielded total number of 156 bands; 54 of them were monomorphic bands and 102 were polymorphic with 39 unique bands and average percentage 63.7% (Table 5**)**. The number of polymorphic bands varied from 7 to 23 with SCoT-19, SCoT-23 and SCoT-5, respectively but the percentage of the polymorphism were varied between 42% and 85% with SCoT-18 and SCoT-5, respectively. Start Codon Targeted (SCoT) markers were used for studying the genetic diversity and relationships within and among *Alhagi* populations. The level of polymorphism produced in this study was relatively higher than that reported in *Lycopersicum esculentum* accessions (36.14%) by using 10 SCoT primers **(Shahlaei *et al*., 2014)** and 49.4% in *Jojoba* genotypes by using 15 SCoT primers **(Heikrujam *et al*., 2015)**. The present results in agreement with **Luo *et al*. (2010,**

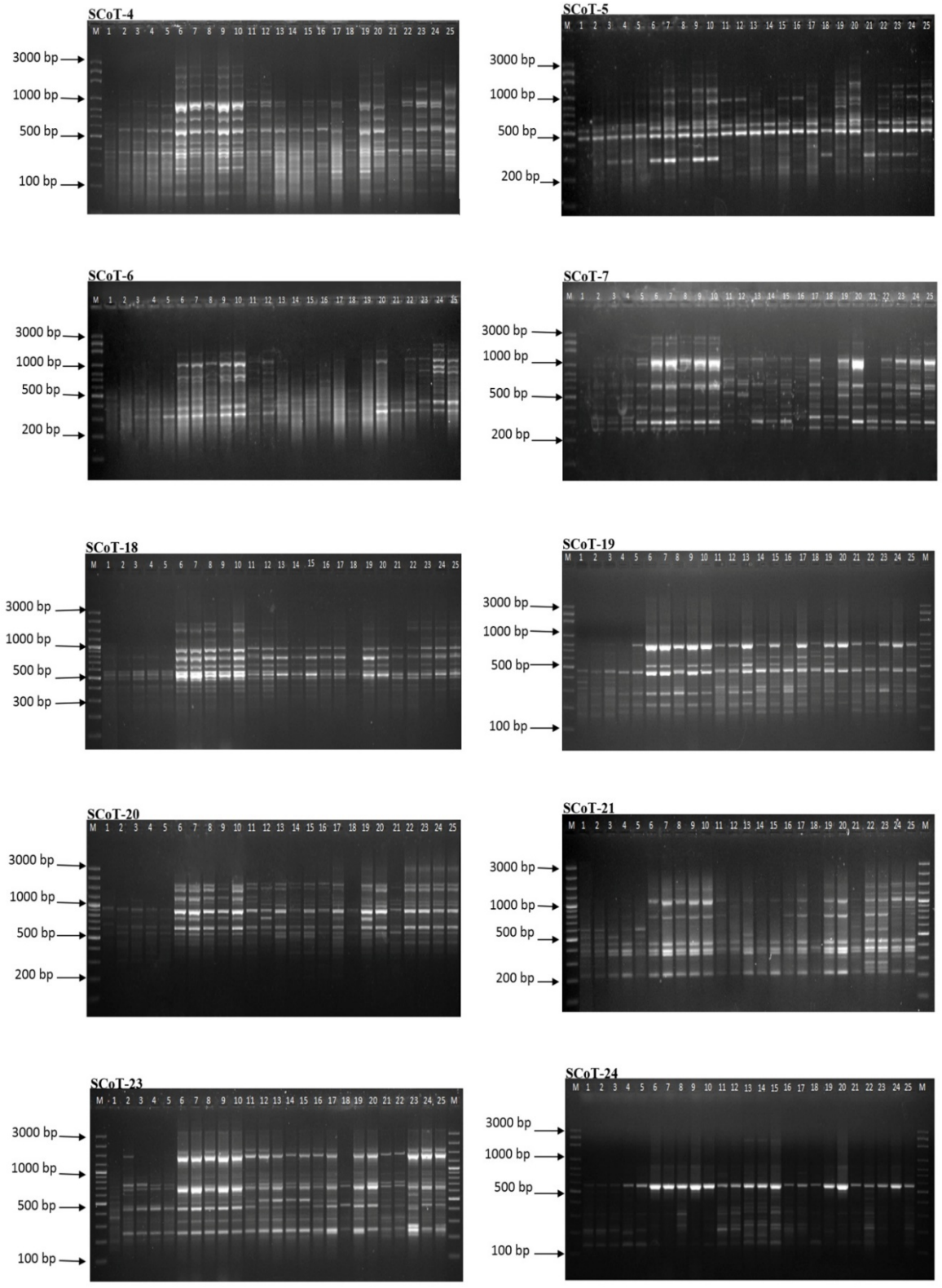

Figure 2: **Agarose gel electrophoresis of PCR amplicons using ten SCoT primers to genetically characterize *A. graecorum* genotypes numbered (1-25). M refers to DNA marker 100 bp plus.**

2011) they reported that, the percentage of polymorphism were 76.19% and 65.82%, respectively in mango germplasm accessions. Also, many investigators recorded higher percentages of polymorphism for different plant populations such as 96.21% on *Dendrobium nobile* (**Bhattacharyya *et al.*, 2013**), 92.20% on *Trichosanthes dioic*a (**Kumar and Agrawal, 2019**) and 97.67% on *Miscanthus lutarioriparius* (**Yang *et al.*, 2019**).

The polymorphic information content (PIC) varied from 0.654 to 0.955 values where the SCoT-23 had the lowest PIC value while primer SCoT-5 had the highest value with average 0.87 (Table 4). On the other hand, the lowest value of polymorphic information content (PIC) among different SCoT primers in bulked DNA samples was 0. 672 in both SCoT 20 and SCoT 23, but the largest value was recorded in the other primers 0.768 with average 0.75 (Table 5). Polymorphic information content (PIC) values of a primer help in determining its effectiveness in genetic diversity analysis **(Sivaprakash *et al.*, 2004)**. They suggested that the ability of the marker system to resolve genetic variation may be more directly related to the degree of polymorphism. Their efficiency was evident from high values of polymorphic percentage and average number of polymorphic bands per primer. The efficiency of SCoT markers has been tested to determine the polymorphic information content for *Alhagi* genotypes within and among populations then compared with different studies. Similar to the present results, higher mean of PIC (> 0.5) ranged from 0.42 to 0.92 with an average of 0.78 were produced by **Agarwal *et al.* (2018)** for 29 rose germplasms. In *Dendrobium nobile*, **Bhattacharyya *et al.* (2013)** observed higher PIC value of 0.78 using SCoT marker system, also **Satya *et al.* (2015)** recorded higher PIC value of 0.69 using the same marker to assess genetic diversity of *Boehmeria nivea*. Furthermore, the PIC value of *Alhagi* was higher when compared with *Trichosanthes dioica* of 0.45 (**Kumar and Agrawal, 2019**), *Miscanthus lutarioriparius;* the (PIC) values were from 0.22 to 0.29, with an average of 0.26 (**Yang *et al.*, 2019**). Other plants were recorded lower PIC than the present results, such as **Tiwari *et al.* (2016)** studied the genetic relationships among *Andrographis paniculata* collected from five agro-ecological regions using SCoT markers; the PIC ranged from 0.09 to 0.48, with an average value of 0.34. **Alikhani *et al.* (2014)** and **Heikrujam *et al.* (2015)** showed the PIC by using SCoT markers for *Quercus brantii* and *Simmondsia chinensis*, respectively was 0.38. Furthermore, **Talebi *et al.* (2018)** recorded that**;** the PIC average value was 0.39 for safflower genotypes using SCoT technique.

The molecular variance (AMOVA) was used to estimate the genetic variability within and among populations (Table 6), being most of the genetic diversity found within the populations (52%). The genetic variation levels among different populations is due to environmental change conditions **(Lovejoy and Hannah, 2005)** and populations with low variability are generally considered less adapted under adverse circumstances. Therefore, the assessment of genetic diversity in geographically isolated populations is very valuable for identification of low diverse populations. The histories of species and reproductive systems have direct influence on the distribution and levels of genetic variation, genetic divergence, and genetic structure **(Hamrick and Godt, 1996; Segarra-Moragues and Catalan, 2002)**. In this study, the genetic variation within and among populations by using the AMOVA analysis and SCoT technique is similar to many studies such as **Zhang *et al.* (2015)** who estimated the genetic diversity and relationships among Chinese *Elymus sibiricus* accessions and showed greater genetic variation within geographical regions (50.99%) than among them (49.01%). Furthermore, **Jiang *et al.* (2014)** demonstrated that, orchard grass

Table 4: SCoT primer names, total number of bands, polymorphic bands, monomorphic bands, percentage of polymorphism, unique bands, fragment size range and polymorphic information content (PIC) as revealed by SCoT analysis of 25 *A. graecorum* genotypes.

S.	Primer code	Total no. of bands	Polymorphic bands	Monomorphic bands	Percentage of polymorphism (POL %)	Unique bands	Fragment size range (bp)	PIC
1	**SCoT-4**	21	17	4	80.9	-	2312-154	0.948
2	**SCoT-5**	20	18	2	90	2	2713-201	0.955
3	**SCoT-6**	11	9	2	81.8	-	2138-247	0.884
4	**SCoT-7**	13	10	3	76.9	1	2351-255	0.9
5	**SCoT-18**	12	7	5	58	-	2047-320	0.88
6	**SCoT-19**	15	11	4	73	1	1634-150	0.92
7	**SCoT-20**	11	6	5	54.5	-	2757-289	0.789
8	**SCoT-21**	16	12	4	75	-	1848-214	0.869
9	**SCoT-23**	10	5	5	50	-	3063-196	0.654
10	**SCoT-24**	11	8	3	72.7	-	2044-119	0.868
Total		**140**	103	37		**4**		
Average		14	9.9	3.7	**71.3**			**0.87**

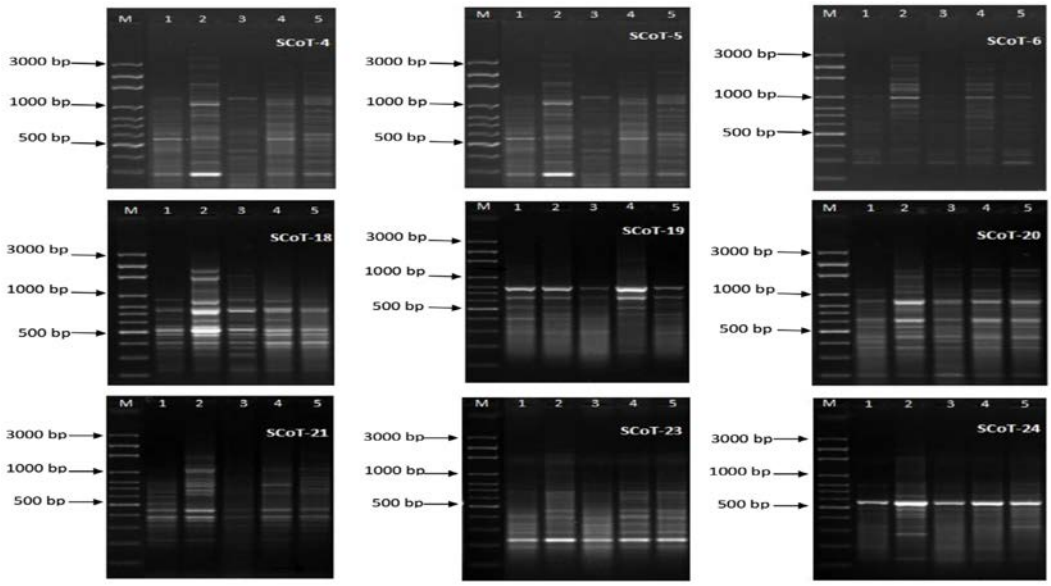

Figure 3: SCoTs **profiles (4, 5, 6, 18, 19, 20, 21, 23 and 24) for *A. graecorum* populations (DNA bulked samples). M refers to DNA ladder (100 bp) plus. *A. graecorum* populations are 1 = El-Dakhla Oasis; 2 = Ain Shams University; 3 = Wadi El-Rayan; 4 = Qarun Lake; 5 = Siwa Oasis.**

Table 5: Total number of bands, polymorphic bands, monomorphic bands, percentage of polymorphism, unique bands, fragment size range and polymorphic information content (PIC) as revealed by SCoT analysis among *A. graecorum* populations (DNA bulked samples).

S.	Primer name	Total no. of bands	Polymorphic bands	Monomorphic bands	Percentage of polymorphism (POL %)	Unique bands	Fragment size range (bp)	PIC
1	SCoT-4	18	12	6	66.7	4	3082-229	0.768
2	SCoT-5	27	23	4	85	7	2790-278	0.768
3	SCoT-6	20	16	4	80	6	2030-329	0.768
4	SCoT-18	19	8	11	42	4	1572-244	0.768
5	SCoT-19	11	7	4	63.6	4	1251-203	0.768
6	SCoT-20	17	8	9	47	6	1526-218	0.672
7	SCoT-21	17	12	5	70.6	2	1324-203	0.768
8	SCoT-23	14	7	7	50	3	1464-216	0.672
9	SCoT-24	13	9	4	69.2	3	549-161	0.768
	Total	156	102	54		39		
	Average	17.3	11.3	6	63.7			0.75

Table 6: Analysis of molecular variance (AMOVA) for nine SCoT markers among *A. graecorum* populations.

Source of variations	Degree of freedom	Sum of squares	Mean square	Variance components	% of total variance
Among populations	4	230.16	57.54	9.48	48%
Within populations	20	202.00	10.10	10.100	52%
Total	24	432.16		19.58	100

germplasms had a higher level of genetic variance within groups (69.13) than among geographical regions and distributions (30.87%), suggesting a high discriminating ability of the SCoT technique for this plant. Similar to the present results, **Yang *et al*. (2019)** showed that variation by using SCoT primers was more abundant within *Miscanthus lutarioriparius* populations (84.91%) than among them (15.09**).** In contrast, there are many investigators observed higher molecular variation among populations than within them; **Al-Qurainy *et al*. (2015)** showed higher molecular variation (52%) among the date palm populations, whereas 48% was found within the populations. Also, **Bhattacharyya *et al.* (2013)** analyzed the genetic variation among the *Dendrobium nobile* populations 56.63%, whereas 43.37% was recorded within them. Different factors such as mating system **Charlesworth and Wright (2001)**, gene flow **Hamrick and Godt (1996)**, population size, selection, etc. influence genetic diversity levels within populations. Out- crossing plant species keep together new genes combinations rapidly and commonly have high genetic diversity. Short lived perennials plant species are considered to be less genetically diverse than long lived ones.

Genetic similarities matrix of *A. graecorum* as revealed by SCoT markers

Table (7) showed that, the estimated similarities among the 25 *Alhagi* genotypes ranged from 0.6 to 0.99. The highest genetic similarity (0.99) was between genotypes 6 and 7 (both of them from Ain Shams University), while the lowest genetic similarity (0.6) was between each of the following genotypes 19 (individual from Qarun Lake) and 4 (individual from El-Dakhla Oasis), 1 (individual from El-Dakhla Oasis) and 23 (individual from Siwa Oasis), and 1 (individual from El-Dakhla Oasis) and 24 (individual from Siwa Oasis). The similarity index resulted from SCoT scoring for the five *A. graecorum* populations were shown in Table (8). The estimated similarities ranged from 0.65 to 0.87. The highest similarity value (0.87) was recorded between both Qarun Lake and Siwa Oasis populations; this indicated that these two populations closely related to each other. On the other hand, the lowest similarity value (0.65) was recorded between Ain Shams University and Wadi El-Rayan populations, indicating that these were distantly related variety. Similar result obtained by **Heikrujam *et al.* *(*2015)** who recorded similarity co-efficient of SCoT markers ranged from 0.733 to 0.922 in female *Simmondsia chinensis* genotypes and 0.941 to 0.746 in the male genotype populations, indicating high levels of genetic similarity among the genotypes studied. Also, similarity values according to Jaccard's coefficients of the safflower genotypes based on SCoT molecular markers ranged from 0.49 to 0.93 were reported by **Talebi *et al*. (2018)**.

Cluster analysis as revealed by SCoT markers and principal coordinates analysis

The dendrogram obtained from UPGMA cluster analysis of genetic distance based on 10 SCoT primers is shown in Figure (4A). The cluster analysis resolved 25 *Alhagi* individuals into two main clusters. The first main cluster consisted of population coming from El-Dakhla Oasis (1-5), one individual from Siwa Oasis population (21) and other individual from Qarun Lake population (18). Where this cluster can be subdivided into two sub-clusters, the first sub-cluster included all individuals of El-Dakhla Oasis population (1-5) while the second sub-cluster included the two individuals from Siwa Oasis population (21) and Qarun Lake population (18).The second main cluster consisted of populations coming from Ain

Table 7: Genetic similarity matrix among the 25 *A. graecorum* genotypes as computed according to Dice's coefficient as revealed by SCoT markers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1																								
2	0.90	1																							
3	0.94	0.93	1																						
4	0.88	0.89	0.89	1																					
5	0.86	0.91	0.88	0.93	1																				
6	0.62	0.68	0.66	0.63	0.66	1																			
7	0.62	0.67	0.65	0.62	0.65	0.99																			
8	0.64	0.69	0.68	0.64	0.68	0.96	0.95	1																	
9	0.61	0.66	0.64	0.61	0.64	0.94	0.94	0.94	1																
10	0.61	0.66	0.64	0.61	0.63	0.95	0.95	0.92	0.94	1															
11	0.69	0.71	0.68	0.66	0.69	0.82	0.82	0.82	0.81	0.84	1														
12	0.67	0.73	0.69	0.67	0.71	0.81	0.81	0.81	0.81	0.82	0.89	1													
13	0.68	0.74	0.71	0.67	0.73	0.80	0.80	0.80	0.80	0.81	0.82	0.84	1												
14	0.72	0.74	0.74	0.67	0.72	0.76	0.75	0.75	0.76	0.77	0.84	0.84	0.88	1											
15	0.67	0.69	0.69	0.65	0.70	0.79	0.79	0.78	0.77	0.78	0.86	0.86	0.86	0.89	1										
16	0.72	0.77	0.74	0.69	0.73	0.78	0.78	0.77	0.76	0.77	0.87	0.88	0.81	0.86	0.90	1									
17	0.67	0.71	0.70	0.67	0.70	0.79	0.79	0.79	0.79	0.79	0.82	0.85	0.88	0.86	0.88	0.87	1								
18	0.79	0.84	0.83	0.83	0.83	0.70	0.69	0.70	0.70	0.70	0.71	0.74	0.74	0.78	0.73	0.78	0.76	1							
19	0.63	0.65	0.66	0.60	0.63	0.89	0.89	0.86	0.87	0.87	0.79	0.81	0.81	0.78	0.81	0.79	0.86	0.70	1						
20	0.62	0.65	0.66	0.61	0.63	0.87	0.87	0.83	0.84	0.84	0.80	0.81	0.77	0.73	0.78	0.79	0.80	0.67	0.91	1					
21	0.71	0.77	0.76	0.74	0.76	0.73	0.73	0.72	0.72	0.72	0.75	0.77	0.80	0.80	0.80	0.79	0.80	0.84	0.77	0.74	1				
22	0.63	0.67	0.68	0.64	0.68	0.79	0.79	0.77	0.76	0.77	0.80	0.80	0.77	0.76	0.77	0.76	0.79	0.72	0.81	0.78	0.81	1			
23	0.60	0.65	0.65	0.63	0.64	0.81	0.81	0.79	0.78	0.80	0.75	0.81	0.76	0.69	0.75	0.75	0.77	0.70	0.83	0.81	0.76	0.89	1		
24	0.60	0.65	0.65	0.63	0.65	0.84	0.85	0.81	0.81	0.82	0.77	0.80	0.77	0.73	0.77	0.76	0.80	0.72	0.86	0.83	0.78	0.87	0.90	1	
25	0.61	0.64	0.65	0.59	0.63	0.84	0.84	0.82	0.80	0.81	0.79	0.80	0.77	0.75	0.77	0.76	0.80	0.71	0.85	0.84	0.78	0.87	0.90	0.93	1

Table 8: Genetic similarity matrix of five *A. graecorum* populations (bulked DNA samples) as computed according to Dice's coefficient from SCoT data. *Alhagi* populations are numbered (1-5). 1 = El-Dakhla oasis; 2 = Ain Shams university; 3 = Wadi El-Rayan; 4 = Qarun Lake; 5 = Siwa Oasis.

	1	**2**	**3**	**4**	**5**
1	1				
2	0.68	1			
3	0.78	**0.65**	1		
4	0.75	0.76	0.73	1	
5	0.77	0.79	0.72	**0.87**	1

Shams University (6-10), Wadi El-Rayan (11-15) and the remaining individuals coming from Qarun Lake (16,17,19,20) and Siwa Oasis (22-25) populations. This cluster can be subdivided into two sub-clusters, the first included all Wadi El-Rayan population (11-15) and two individuals from Qarun Lake population (16 and 17), while the second sub-cluster included Ain Shams University population (6-10) and the remaining individuals from Qarun Lake (19 and 20) and Siwa Oasis (22-25) populations.

On the other hand, the dendrogram obtained from UPGMA cluster analysis of genetic distance based on nine SCoT markers between the different five populations is showed in Figure (4B). The resulting SCoT dendrogram showed that the studied populations were differentiated into two clusters. In the first cluster, Ain Shams University population was delimited as a single population in a sub-cluster, while Qarun Lake and Siwa Oasis populations were grouped together as another clade. The second cluster divided into two sub-clusters, Wadi El-Rayan and El-Dakhla populations, each represented by a single clade.

To well comprehend and obtain an alternative view of the relationships among *Alhagi* genotypes, PCoA was performed using the genetic similarity data sets Figure (5a) showed the scatter plots for PCoA based on SCoT marker data. PCoA was basically similar to the grouping created by UPGMA clustering. The PCoA resulted from SCoT marker classified the 25 *Alhagi* individuals into 4 groups. Group A comprises four individuals from Siwa Oasis population (22-25), group B comprising Ain Shams University population (6-10) and two individuals from Qarun Lake population (19 and 20), group C having all individuals coming from Wadi El-Rayan (11-15) and two individuals from Qarun Lake population (16 and 17), while the last group D contains all El-Dakhla Oasis individuals (1-5) in addition to individual from Qarun Lake (18) and individual from Siwa Oasis (21) populations. The PCoA was basically similar to the grouping created by UPGMA clustering resulted from SCoT marker Figure (5b). This analysis was classifying the five *Alhagi* populations into 3 groups; the first one comprising the Ain Shams University population (A) as indicated by cluster analysis, and the second group having El-Dakhla Oasis and Wadi El-Rayan populations (B) while the latter (C) comprised Qarun Lake and Siwa Oasis populations. Similar to our results, clustering based on geographical regions has also been seen in different plants such as *Arabidopsis thaliana* **(Tyagi *et al.*, 2016)** and *Pulsatilla patens* **(Szczecinska *ta al.*, 2016)**. The pattern of genetic subdivision can be clearly demonstrated in the UPGMA cluster analysis, in which our results were similar to the *Jojoba* **(Heikrujam *et al.*, 2015)** and switchgrass **(Zhang *et al.*, 2016)** populations which divided into two groups according to their habitat.

Also, **Yan *et al.* (2016)** reported that, results from the UPGMA were relative to the geographical distribution of the orchardgrass (*Dactylis glomerata* L.). Furthermore**, Kumar and Agrawal (2019)** divided the *Trichosanthes dioica* accessions into three main clusters by using UPGMA tree. The results of PCoA analysis also support this habitat-specific genetic clustering model obtained by UPGMA similar to many researchers; **Talebi *et al*. (2018)** grouped the safflower genotypes into three major groups using SCoT molecular dataset. Also, **Zhang *et al*. (2015)** reported that, cluster analysis separated the *Elymus sibiricus* accessions into two major clusters and three sub-clusters, similar to results of principal coordinate analysis (PCoA). In addition, **Yang *et al.* (2019)** recovered five clear clusters using principal coordinate analysis (PCoA) and an unweighted pair group method with arithmetic mean (UPGMA)

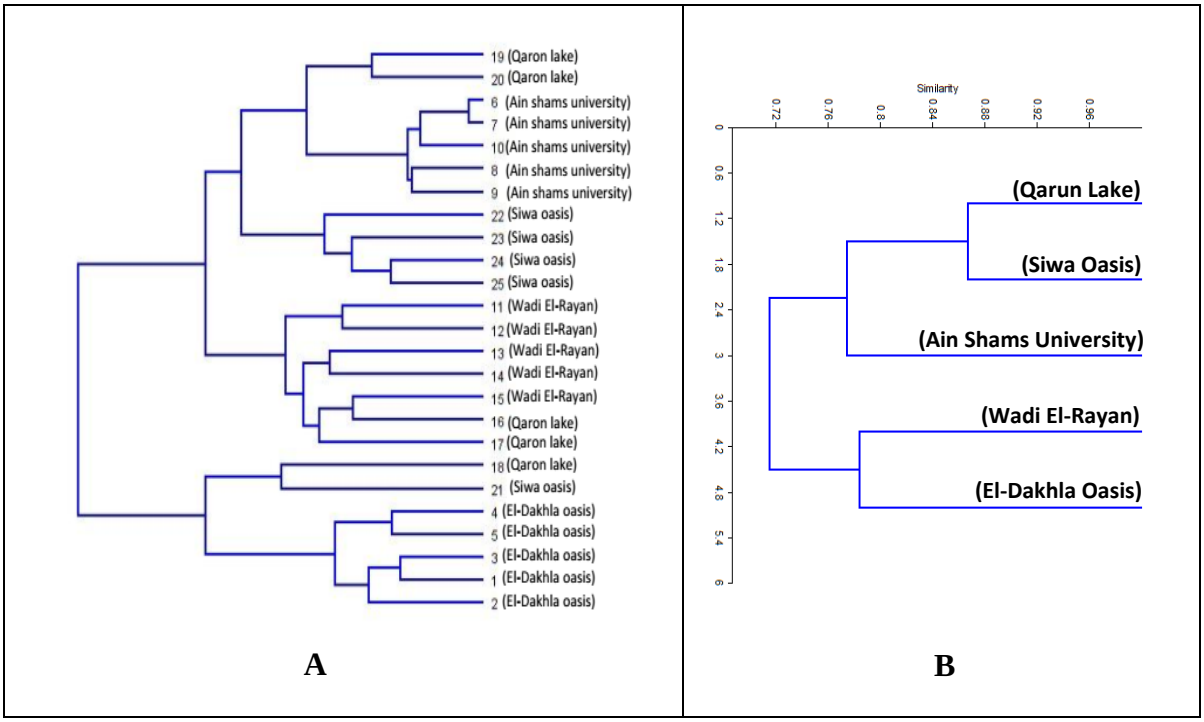

Figure 4: Dendrogram: (A) the 25 *A. graecorum* genotypes 1-25. (B) the five *A. graecorum* populations (bulked DNA samples) constructed from the SCoT data using Unweighed Pair-group Arithmetic Average (UPGMA) and similarity matrix computed according to Dice's coefficient. Populations: 1 = El-Dakhla Oasis; 2 = Ain Shams University; 3 = Wadi El-Rayan; 4 = Qarun Lake; 5 = Siwa Oasis.

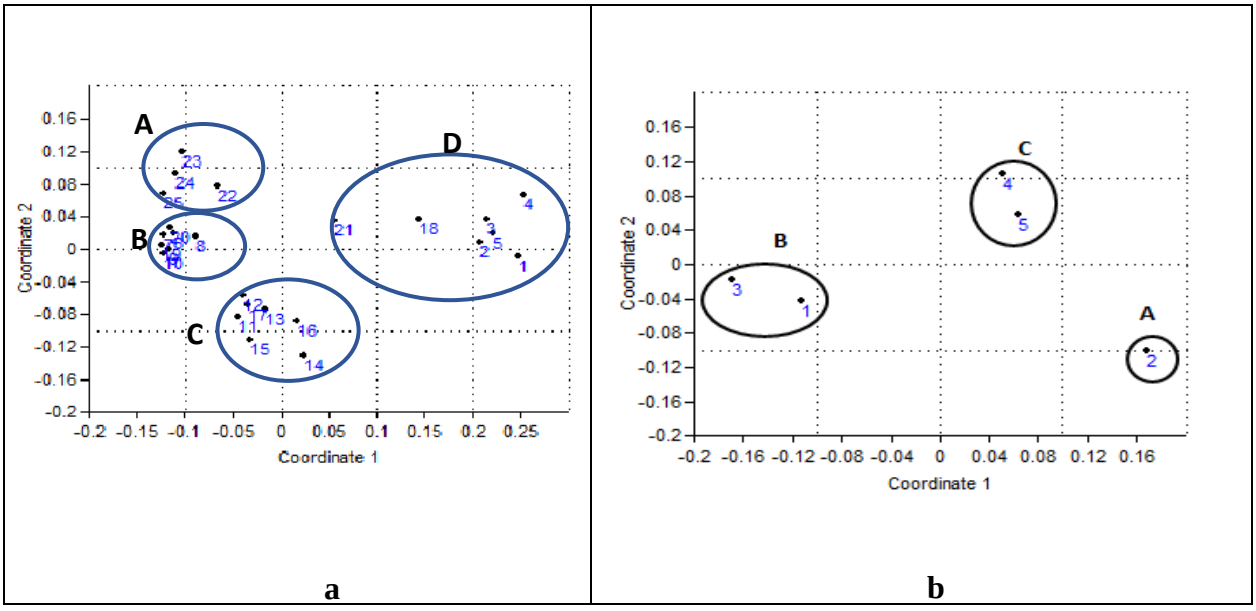

Figure 5: Scatter plot of principal coordinate analysis (PCoA) of a) 25 *Alhagi* individuals b) 5 *Alhagi* populations (bulked DNA samples) based on SCoT marker data: 1=El-Dakhla Oasis; 2=Ain Shams University; 3=Wadi El-Rayan; 4=Qarun Lake; 5= Siwa Oasis.

cluster analysis on *Miscanthus lutarioriparius* populations.

SCoTs sequencing analysis resulted from bulked DNA samples of *Alhagi graecorum*

The amplified unique products obtained using SCoT-5, SCoT-20 and SCoT-21 primers (only primers showing clear and reproducible band patterns) were selected for further analysis were purified and sequenced then aligned. The amplified bands were resulted at molecular sizes 300, 400 and 700 bp with SCoT-5, SCoT-20 and SCoT-21, respectively (Figure 6). The sequence of these fragments from genomic *Alhagi graecorum* DNA collected from Wadi EL-Rayan, Ain Shams University and EL-Dakhla Oasis regions, respectively were 578 bp for SCoT-5, 585 bp for SCoT-20 and 553 bp for SCoT-21 (Figure 7). Also, **Hao *et al*. (2018)** deposited the sequence of the *Taxus* media-specific fragment of 530 bp fragment in GenBank (GenBank accession number MF447807).The sequence contained 49.25% A-T and 50.75% G-C. Based on this sequence, a SCoT-SCAR primer pair was designed, The *T.* media-specific sequence of 530 bp had no homology with other sequences in GenBank. **Abdel-maksoud *et al*. (2009)** showed the significant sequence homology of the fragments Vms2-9 & Vms2-17 of *Vicia monantha* with ESTs from *Saccharum officinarum* cDNA under drought stress with E-values 3e-10 & 3e-11, respectively whereas Fragment Vms5-50 was homology with an EST from Groundnut drought stress with E-value 4e-23, but Fragments Vms1-1 & Vms2-21 showed homology with *Cucumis sativus* C-repeat/DRE binding factor 1 (cbf1) with E-values 2e-06 & 1e-06, respectively.

The comparison between nucleotide sequences of the fragments isolated from genomic *Alhagi graecorum* DNA and another plants sequence in the Genbank, by alignment the isolated sequence in Genbank by nucleotide blast and blastx (http//blast.ncbi.nlm) was shown in Table (9). The identity percentage of SCoT-5 with a large value in homology with *Vigna unguiculata* cultivar Xiabao 2 chromosome Vu03 (89.47%) with accession number CP039346.1 and coverage 8%, while the large coverage was 12% in homology with *Lupinus angustifolius* cultivar Tanjil chromosome LG-11 with accession number CP023123.1 and identity 82.61%. On the other hand, the identity percentage of SCoT-20 with a large value in homology with *Lotus japonicus* genomic DNA, chromosome 5, clone: LjT47L09, TM0218, complete sequence (85.71%) with accession number AP006374.1 and coverage 51%, while the higher coverage recorded 56% in homology with *Lupinus angustifolius* cultivar Tanjil chromosome LG-04 with accession number CP023116.1 and identity 82.52%. In case of SCoT-21 the percentage of identity was in higher value in homology with Cytochrome b6 apoprotein of *Pisum sativum* (28.89 %) with accession number AAD41888.1 and coverage 48%, while the minimum value of identity was found in homology with cytochrome b6 of *Medicago truncatula* (25%) with accession number RHN38456.1. **Mohamed *et al.* (2017)**, studied the sequence analysis of highly pronounced unique polymorphic SCoT amplicons **(**SCoT-2, SCoT-3, SCoT-11, SCoT-47, and SCoT-48 primers) among the *Triticum aestivum* L. cultivars followed by analyze unique monomorphic SCoT amplicon obtained with SCoT-8. Forward sequences of the latter SCoT amplicons were aligned and compared by their BLAST scores to published available

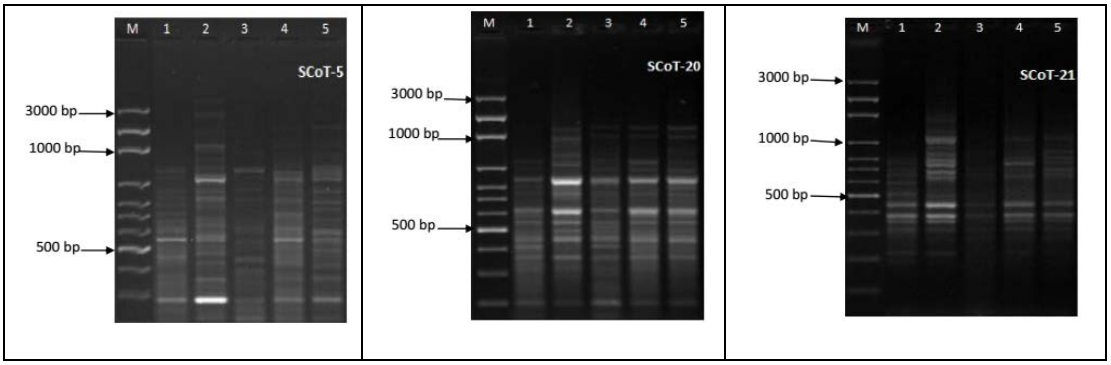

Figure 6: SCoT profiles (SCoTs 5, 20 and 21) for *A. graecorum* populations. M refers to DNA ladder. 100 bp plus. *A. graecorum* populations are 1=El-Dakhla Oasis; 2=Ain Shams University; 3=Wadi El-Rayan; 4=Qarun Lake; 5= Siwa Oasis.

GGAAGGCGGGAACCACACATCTCTTTTTTCCCCCCCCCCCCTTTAATATTTTTGTTAGACCCC
CCCTTCCTTCCTCTTCGCCACCTTTTTCATCCTCTTCCGGACCGTCTCTGCCCCCCCTGAGGA
ATGCGAGGGACAAGCTCAACGCCGTGACCGCTATCCCCACAAGTTTGCGCCTCTTCTGGAAT
GGACCGGCTTTTCCTTAGGGGCTCCTTTACCCCGAAAAAATACGCTTCCGGTCGAGGGTGGG
AGATGAGGACGCCGGGGGGAGAAGTGATCGGCCGCGTGGGGAGGAGGGGGGCGCAAGTTG
GGCTGGGACTGTCTATGCCGACACTACTTTATGGTGGTGGAGCCACAGAGGGGCGGAACAA
TGTGATGCCACAATCCACTCAATAAACATTTTCTGGTCCGCATACGGGCACACTCAATTTAC
ACAGAATTTGTTCCACAGCCTCTCACCTCCTTCTAGGGCGCATTTTTCAACACTGGCCCTTCA
AATTTAAAGCCTACGGAATACGGACGGTCGAGAAAGCGAGGTGGTCGGATTTGCCCTGGAA
GGCAAAGAAAGAGTAGAGAAC

a

GCCATATTGAGTCGCCTCTCTCATCAGATTTACATTGACCATGTCCCCACCACTTATGGCATC
CTTTAACTTTTCACCAAAACTTTTTTCAATGAGAGCAGATGGTATTGTTATGTTCTCTATGTA
TTTTGCTGACGATCCATCCTCTTCAGGTGTGTCCATAGTTATTAAGGGTTCCTCAATATCATC
TGCAACAAGAACCGCAGAAGCTCCAGCCTTCTGTGCATTCCAGACCTTGAGAGCAAAGAAA
CAATCTGAAAAGGAACACACGAAAGCACAGGGAATACACACATGAGATAACATTTGAAGG
CGTGGTAGCCATTGTTGAGTGGAAAGGGGGAGAAACAACGTGAACTGCCGAGGCTGACGG
GTAGCTACTAGGAAAAAGGTGACCCGCAGTATGCTCAGCCAATCTTTTAATATAAGAATTAT
GATTCAGTGATTTATCTCGGATATTTGACACAAACACGCACCTCACACGAGAAGTGCACTCT
CCAAGAAAAGTGCCCTTGAATAGCTGCTGGTTCCAGTATACGCGGCCTCCGCGGAGTCGGA
GTGGGCGAAGGTGGAACGGGACCTCGAATT

b

GCATTATCGTCCGCGTGTCGCAAGCTTCTTTTCTCTTTGGACCTCTCCCTCGATGCGGGTGGC
GCTTGGGTAATACGACCAATCCATCACTGGCGCGCACTTCTCTTCGGAGCAGGGCTGACCGG
ACCCATGCTGGGACCCTGCTTCACCGGCGCTTTCCTGCGGCCTCCGGAAATTCACTGGTTGA
TACGACTGGTCTTGCCCATGCTCTACCTGGGCAATGGCTTCCCCCGCTACTCCCTGCCCCATG
ACATGCTGTCCGGACCCGGGCTCCCGTTTGCCGACTACCTGATGAATGCCTCTCCCATGGCC
AGCACTAGCGATGACTTCTTCATCTTTCACGTGATCTTCCCGGCTTACGGATTGTCCCCCGCC
TGTAATTGGCCCACATCCTGCTCATCCCGCTGCTGCTCCTAGGGTGGCGGGCCGCTCATCTC
TCTCTGGACTTCTACCGCCGCCACACCCCTCACCCGAGAGGCGGACGCACCAAGAAAAAGG
CTCCCACATGCCCGCTGTTTTGAGTATATGCGATGGAGGGCGTGGCCCCCGGTGG

c

Figure 7: DNA sequences: a) SCoT-5 fragment collected from Wadi El-Rayan region (578 bp), b) SCoT-20 fragment collected from Ain Shams University region (585 bp) and c) SCoT-21 fragment collected from El-Dakhla Oasis region (553 bp) **of *Alhagi graecorum*.**

Table 9: Showing the homology, identity, coverage and accession numbers of some SCoT markers.

Fragment name	Homology	Identity	Coverage	Accession number
SCoT-5	*Lupinus angustifolius* cultivar Tanjil chromosome LG-11	82.61%	12%	CP023123.1
	Vigna unguiculata cultivar Xiabao 2 chromosome Vu03	89.47%	8%	CP039346.1
	Vigna unguiculata cultivar Xiabao 2 chromosome Vu10	82.93%	7%	CP039352.1
SCoT-20	*Lupinus angustifolius* cultivar Tanjil chromosome LG-04	82.52%	56%	CP023116.1
	Lotus japonicus genomic DNA, chromosome 5, clone: LjT47L09, TM0218, complete sequence	85.71%	51%	AP006374.1
	Lupinus angustifolius cultivar Tanjil chromosome LG-08	85.67%	51%	CP023120.1
SCoT-21	Cytochrome b6 [*Medicago truncatula*]	25%	56%	RHN38456.1
	Cytochrome b6 apoprotein [*Pisum sativum*]	28.89%	48%	AAD41888.1
	hypothetical protein GLYMA_01G058600 [Glycine max]	27.78%	48%	KRH75043.1

Table 10: Base frequency and length of sequenced region of SCoT-5, SCoT-20 and SCoT-21 markers for the studied genotypes obtained from bulked DNA samples for different geographic regions.

Fragments name	A		T		G		C		AT	GC	Total length
	No.	%	No.	%	No.	%	No.	%	%	%	base
SCoT5	128	22.1	134	23.1	151	26.1	165	28.5	45.3	54.7	578
SCoT20	171	29.2	148	25.3	133	22.7	133	22.7	54.5	45.5	585
SCoT21	84	15.2	134	24.2	139	25.1	196	33.5	39.4	60.6	553
Mean									**46.4**	**53.6**	**572**

sequences *via* GenBank and they reported that sequencing results of both mono- and some polymorphic amplicons have shown high identity between examined cultivars and the American *T. aestivum* L. cultivar Chinese spring (query cover 81-95%). Also, **Dhawan *et al*. (2013)** compared the male-specific fragment of date palm (*Phoenix dactylifera* L.) which cloned and sequenced (GenBank accession number JN123357) and didn't show homology to any database sequence on BLASTn search (http://www.ncbi. nlm.nih.gov/Blast.cgi).

The GC content of SCoT5, SCoT20 and SCoT21 primers are 54.7, 45.5 and 60.6%, respectively with average percentage 53.6%; while the AT content in all fragments was 45.3, 54.5 and 39.4%, respectively with average percentage 46.4, also the total length of SCoT5, SCoT20 and SCoT21 was varied 578, 585 and 553 bp, respectively with a mean length 572 bp (Table 10). The average GC content was higher than many previous studies for different plants, such as *Echinochloa phyllopogon* **(Chen *et al*., 2017),** *Phoenix dactylifera* **(Dhawan *et al*., 2013)** and *Pogostemon cablin* **(Kumar *et al*., 2016)** was 45.8%, 34.5 % and 28.49%, respectively. Also, **Smarda *et al.* (2014)** studied GC content in 239 different plant genomes, finding that the GC content of monocots varied between 33.6% and 48.9%, and increased GC content was documented in species able to grow in seasonally cold and/or dry climates. Furthermore, **Wang *et al.* (2019)** observed that a scatterplot of the genomic GC of *Acer truncatum* content and sequencing depth can provide information on sequencing data bias. The GC content of *Acer truncatum* genome was 35.04%. In the present results at the 3 alignment levels it can be concluded that; the highest values of identity SCoT-5 (89.47%) followed by SCoT-20 (85.71%) were recorded but the minimum value was in SCoT-21 (25%). The largest percentage of coverage was found in both SCoT-20 and SCoT-21 (56%), while the lowest percentage of coverage was 7% in SCoT-5 (Table 9). The mean percentage of AT and GC content for SCoT sequences primers was recorded 46.4 and 53.6, respectively with a mean total length 572bp (Table 10).

CONCLUSION

The present study is the first detailed report of genetic relationships among and within *Alhagi* genotypes collected from different geographical regions using gene-targeted SCoT markers. The present results revealed a high polymorphic percentage with high molecular variation within populations (52%) more than among them (48%). In addition, the GC average percentage of three clearly unique bulked-DNA bands was 53.6%. In conclusion, the SCoT marker system provides a highly efficient, reproducible and powerful tool for studying the genetic diversity for *A. graecorum* populations.

REFERENCES

Abdel-Lateif, K. S. and Hewedy, O. A. (2018) Genetic diversity among Egyptian wheat cultivars using SCoT and ISSR markers. *SABRAO Journal of Breed Genet*ics, *50*, 36-45.

Abdel-maksoud, R., Ageez, A., El-Khishin, D., Fahmy, E. and Abdel-Tawab, F. (2009) Differential Gene Expression in Response to Salt Stress in *Vicia monantha. Egyptian Journal of Genetics and Cytology, 38:* 137-152.

Adawy, S.S., Ibrahim, S.D. Sayed, A.I. and El-Morsy S.I. (2010) Construction of Microsatellite Library from Date Palm (*Phoenix dactylifera* L.) for Isolation and Characterization of SSR Markers. *Journal of Biotechnology Research, 10,* 23-38.

Adawy, S.S., Diab, A.A., Sayed, A.I., Ibrahim, S.D., El-Morsy, S.I. and Saker, M.M. (2013) construction of genetic linkage map and qtl analysis of net blotch

resistance in barley. *International Journal of Advanced Biotechnology and Research, 4* (3) 348-363.

Agarwal, A., Gupta, V., Haq, S. U., Jatav, P. K., Kothari, S. L. and Kachhwaha, S. (2018) Assessment of genetic diversity in 29 rose germplasms using SCoT marker. *Journal of King Saud University-Science*, In press, corrected proof, Available online 17 April 2018.

Ahmed, M. A. (2019) Protective effect of aqueous extract of *Alhagi maurorum* in spermatogenesis and antioxidant status of adult rats exposed to carbon tetrachloride. *Iraqi Journal of Veterinary Sciences, 33*(1), 1-7.

Alikhani, L., Rahmani, M. S., Shabanian, N., Badakhshan, H. and Khadivi-Khub, A. (2014) Genetic variability and structure of *Quercus brantii* assessed by ISSR, IRAP and SCoT markers. *Gene*, *552*(1), 176-183.

Al-Qurainy, F., Khan, S., Nadeem, M. and Tarroum, M. (2015) SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm cultivars. *Pakistan Journal of Botany*, *47*(2), 637-643.

Amirmoradi, B., Talebi, R. and Karami, E. (2012) Comparison of genetic variation and differentiation among annual *Cicer* species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. *Plant systematics and evolution*, *298*(9), 1679-1688.

Badshah, L. and Hussain, F. (2011) People preferences and use of local medicinal flora in District Tank, Pakistan. *Journal of Medicinal Plants Research, 5*(1), 22-29.

Bhattacharyya, P., Kumaria, S., Kumar, S. and Tandon, P. (2013) Start Codon Targeted (SCoT) marker reveals genetic diversity of *Dendrobium nobile* Lindl., an endangered medicinal orchid species. *Gene, 529*(1), 21-26.

Biswas, A., Bari, M. A., Roy, M. and Bhadra, S. K. (2010) Inherited folk pharmaceutical knowledge of tribal people in the Chittagong hill tracts. Bangladesh, ***9***(1): 77-89

Bornet, B. and Branchard, M. (2001) Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. *Plant molecular biology reporter*, *19*(3), 209-215.

Boulos, L. (1966) Flora of the Nile Region in Egyptian Nubia. *Journal of Botanical Taxonomy and Geobotany*, *73*(3), 184-215.

Charlesworth, D. and Wright, S. I. (2001) Breeding systems and genome evolution. *Current opinion in genetics & development*, *11*(6), 685-690.

Chen, G., Zhang, W., Fang, J. and Dong, L. (2017) Identification of massive molecular markers in *Echinochloa phyllopogon* using a restriction-site associated DNA approach. *Plant diversity*, *39*(5), 287-293.

Collard, B. C. and Mackill, D. J. (2009) Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant molecular biology reporter*, *27*(1), 86.

Dhawan, C., Kharb, P., Sharma, R., Uppal, S. and Aggarwal, R. K. (2013) Development of male-specific SCAR marker in date palm (*Phoenix dactylifera* L.). *Tree genetics & genomes*, *9*(5), 1143-1150.

Dice, L.R. (1945) Measures of the amount of ecologic association between species. *Ecology* 26:297-302.

Doyle, J.J. and Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus*, 12: 13-15.

Gorji, A. M., Poczai, P., Polgar, Z. and Taller, J. (2011) Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. *American journal of potato research*, *88*(3), 226-237.

Gower, J.C. (2015) Principle coordinates analysis. Wiley StatsRef: Statistics Reference Online.

Guo, D. L., Zhang, J. Y. and Liu, C. H. (2012) Genetic diversity in some grape varieties revealed by SCoT analyses. *Molecular biology reports*, *39*(5), 5307-5313.

Hamidi, H., Talebi, R. and Keshavarzi, F. (2014) Comparative efficiency of functional gene-based markers, start codon targeted polymorphism (SCoT) and conserved DNA-derived polymorphism (CDDP) with ISSR markers for diagnostic fingerprinting in wheat (*Triticum aestivum* L.). *Cereal research communications*, *42*(4), 558-567.

Hammer, Ø., Harper, D. A. T. and Ryan, P. D. (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*(1), 4-9.

Hamrick, J.L. and Godt, M.J.W. (1996) Effects of life history traits on genetic diversity in plant species. *Phil Trans R Soc Lond*, 351:1291-1298.

Lovejoy, T. E. and Hannah, L. J. (2005) Climate change and biodiversity. (Eds.) Yale university press, New Haven and London.

Hao, J., Jiao, K., Yu, C., Guo, H., Zhu, Y., Yang, X. and Dong, M. (2018) Development of SCoT-Based SCAR marker for rapid authentication of *Taxus* Media. *Biochemical genetics*, *56*(3), 255-266.

Heikrujam, M., Kumar, J. and Agrawal, V. (2015) Genetic diversity analysis among male and female *Jojoba* genotypes employing gene targeted molecular markers, start codon targeted (SCoT) polymorphism and CAAT box-derived polymorphism (CBDP) markers. *Meta gene*, *5*, 90-97.

Henry, R. J. (1998) Molecular and biochemical characterization of somaclonal variation. In *Somaclonal variation and induced mutations in crop improvement* (pp. 485-499). Springer, Dordrecht.

Ibrahim, S.D., Adawy, S.S., Atia, M.A.M., Alsamman, M.A. and Mokhtar M.M. (2016) Genetic diversity, variety identification and gene detection in some Egyptian grape varieties by SSR and SCoT markers. *POJ*, *9*(5): 311-318.

Igwe, D. O., Afiukwa, C. A., Ubi, B. E., Ogbu, K. I., Ojuederie, O. B. and Ude, G. N. (2017) Assessment of genetic diversity in *Vigna unguiculata* L. (Walp) accessions using inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) polymorphic markers. *BMC genetics*, *18*(1), 98.

Jack Masquelier, E. (1996) Oligomeric proanthocyanidins (OPCs) are the heart of the French paradox. *Townsend Letter for Doctors and Patients*, 46-49.

Javouhey, M., Daguin, F. and Letouze, R. (2000) Somatic embryogenesis, an efficient tool for date palm (*Phoenix dactylifera* L.) industrial micropropagation. Characterization and genetic stability of original offshoots and regenerated plantlets by RAPD markers. In *International Symposium on Methods and Markers for Quality Assurance in Micropropagation 530* (pp. 237-242).

Jiang, L. F., Qi, X., Zhang, X. Q., Huang, L. K., Ma, X. and Xie, W. G. (2014) Analysis of diversity and relationships among orchardgrass (*Dactylis glomerata* L.) accessions using start codon-targeted markers. *Genetics and Molecular Research*, *13*(2), 4406-4418.

Khan, F. M. (2009) Ethno-veterinary medicinal usage of flora of Greater Cholistan desert (Pakistan). *Pakistan Veterinary Journal*, *29*(2), 75-80.

Kordrostami, M. and Rahimi, M. (2015). Molecular markers in plants: concepts and applications. *G3M*, *13*, 4024-4031.

Kumar, D., Kalra, A. B. A. and Dobriyal, M. J. (2016) Effect of Shade and Organic Manure on Growth and Yield of Patchouli [*Pogostemon cablin* (Blanco) Benth.] under Teak (*Tectona grandis* LF) Based Agroforestry System. *Indian Forester*, *142*(11), 1121-1129.

Kumar, J. and Agrawal, V. (2019) Assessment of genetic diversity, population structure and sex identification in dioecious crop, *Trichosanthes dioica* employing ISSR, SCoT and SRAP markers. *Heliyon*, *5*(3), e01346.

Laghari, A. H., Memon, S., Nelofar, A. and Khan, K. M. (2012) Antifungal Ursene-Type Triterpene from the Roots of *Alhagi camelorum*. *Helvetica Chimica Acta*, *95*(9), 1556-1560.

Luo, C., He, X. H., Chen, H., Ou, S. J. and Gao, M. P. (2010) Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers. *Biochemical Systematics and Ecology*, *38*(6), 1176-1184.

Luo, C., He, X. H., Chen, H., Ou, S. J., Gao, M. P., Brown, J. S. and Schnell, R. J. (2011) Genetic diversity of mango cultivars estimated using SCoT and ISSR markers. *Biochemical Systematics and Ecology*, *39*(4-6), 676-684.

Ma, X., Zhang, Q., Zhu, Q., Liu, W., Chen, Y., Qiu, R. and Xie, Y. (2015) A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Molecular plant*, *8*(8), 1274-1284.

Mohamed, A. H., Ibrahim, M., Teleb, S.S. and Tantawy, M.E. (2017) SEM and SCoT Markers Unveil New Taxonomic and Genetic Insights about Some Northern African *Triticum aestivum* L.Cultivars**.** Vegetos- *An International Journal of Plant Res*earch, **30:**1.

Muhammad, G., Hussain, M. A., Anwar, F., Ashraf, M. and Gilani, A. H. (2015) *Alhagi*: a plant genus rich in bioactives for pharmaceuticals. *Phytotherapy research*, *29*(1), 1-13.

Pavoine, S., Dufour, A. B. and Chessel, D. (2004) From dissimilarities among species to dissimilarities among communities: a double principal coordinate analysis. *Journal of theoretical biology*, *228*(4), 523-537.

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

Revathi, P. and Parimelazhagan, T. (2010) Traditional knowledge on medicinal plants used by the Irula tribe of Hasanur Hills, Erode District, Tamil Nadu, India. *Ethnobotanical Leaflets*, *2010*(2),4.

Sadek M.S. and Ibrahim S.D. (2018) Genetic relationships among maize inbred lines as revealed by Start Codon Targeted (SCoT) analysis. *Journal of Innovations in Pharmaceutical and Biological Sciences*, *5* (1), 103-107.

Satya, P., Karan, M., Jana, S., Mitra, S., Sharma, A., Karmakar, P. G. and Ray, D. P. (2015) Start codon targeted (SCoT) polymorphism reveals genetic diversity in wild and domesticated populations of ramie (*Boehmeria nivea* L. Gaudich.), a premium textile fiber producing species. *Meta gene*, *3*, 62-70.

Segarra-Moragues, J. G. and Catalán, P. (2002) Low Allozyme Variability in the Critically Endangered Borderea chouardii and in Its Congener *Borderea pyrenaica* (Dioscoreaceae), Two Paleoendemic Relicts from the Central Pyrenees. *International Journal of Plant Sciences*, *163*(1), 159-166.

Semagn, K., Bjørnstad, Å. and Ndjiondjop, M. N. (2006) An overview of molecular marker methods for plants. *African journal of biotechnology*, *5*(25), 2540-2568.

Shahlaei, A., Torabi, S. and Khosroshahli, M. (2014) Efficacy of SCoT and ISSR markers in assessment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity. *International Journal of Biosciences*, *5*(2), 14-22.

Sivaprakash, K. R., Prashanth, S. R., Mohanty, B. P. and Parida, A. (2004) Genetic diversity of black gram (*Vigna*

mungo) landraces as evaluated by amplified fragment length polymorphism markers. *Current Science*, 1411-1416.

Šmarda, P., Bureš, P., Horová, L., Leitch, I. J., Mucina, L., Pacini, E. and Rotreklová, O. (2014) Ecological and evolutionary significance of genomic GC content diversity in monocots. *Proceedings of the National Academy of Sciences*, *111*(39), E4096-E4102.

Sneath, P. and Sokal, R. (1973) Numerical Taxonomy. San Francisco, California.

Szczecińska, M., Sramko, G., Wołosz, K. and Sawicki, J. (2016) Genetic diversity and population structure of the rare and endangered plant species *Pulsatilla patens* (L.) Mill in East Central Europe. *PLoS One*, *11*(3), e0151730.

Talebi, R., Nosrati, S., Etminan, A. and Naji, A. M. (2018) Genetic diversity and population structure analysis of landrace and improved safflower (*Cartamus tinctorious* L.) germplasm using arbitrary functional gene-based molecular markers. *Biotechnology & Biotechnological Equipment*, *32*(5), 1183-1194.

Tiwari, G., Singh, R., Singh, N., Choudhury, D. R., Paliwal, R., Kumar, A. and Gupta, V. (2016) Study of arbitrarily amplified (RAPD and ISSR) and gene targeted (SCoT and CBDP) markers for genetic diversity and population structure in Kalmegh [*Andrographis paniculata* (Burm. f.) Nees]. *Industrial Crops and Products*, *86*, 1-11.

Tyagi, A., Singh, S., Mishra, P., Singh, A., Tripathi, A. M., Jena, S. N. and Roy, S. (2016) Genetic diversity and population structure of *Arabidopsis thaliana* along an altitudinal gradient. *AoB Plants*, *8*.

Wang, R., Fan, J., Chang, P., Zhu, L., Zhao, M. and Li, L. (2019) Genome Survey Sequencing of *Acer truncatum* Bunge to Identify Genomic Information, Simple Sequence Repeat (SSR) Markers and Complete Chloroplast. *Genome, Forests* 10, 87; doi:10.3390/f10020087.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, *18*(22), 6531-6535.

Wyk, B.E. and Wink, M. (2004) Medicinal Plants of the World. *Timber Press, Portland, OR*, p. 188.

Xiong, F., Zhong, R., Han, Z., Jiang, J., He, L., Zhuang, W. and Tang, R. (2011) Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. *Molecular biology reports*, *38*(5), 3487-3494.

Xue, W., Li, X. Y., Zhu, J. T. and Lin, L. S. (2012) Effects of temperature and irradiance on photosystem activity during *Alhagi sparsifolia* leaf senescence. *Biologia plantarum*, *56*(4), 785-788.

Yan, H., Zhang, Y., Zeng, B., Yin, G., Zhang, X., Ji, Y. and Ma, X. (2016) Genetic diversity and association of EST-SSR and SCoT markers with rust traits in orchardgrass (*Dactylis glomerata* L.). *Molecules*, *21*(1), 66.

Yang, S., Xue, S., Kang, W., Qian, Z. and Yi, Z. (2019) Genetic diversity and population structure of *Miscanthus lutarioriparius*, an endemic plant of China. *PloS one*, *14*(2), e0211471.

Zhang, J., Xie, W., Wang, Y. and 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.

Zhang, Y., Yan, H., Jiang, X., Wang, X., Huang, L., Xu, B. and Zhang, L. (2016) Genetic variation, population structure and linkage disequilibrium in Switchgrass with ISSR, SCoT and EST-SSR markers. *Hereditas*, *153*(1), 4.

Zietkiewicz,E., Rafalski,A. and Labuda,D. (1994) Genome Fingerprinting by Simple Sequence Repeat (SSR)-Anchored Polymerase Chain Reaction Amplification. *Genomics*, *20* (2), 176-183.

Zou, G. A., Mansur, S., Hu, S. C., Aisa, H. A. and Shakhidoyatov, K. M. (2012) Pyrrole alkaloids from *Alhagi sparsifolia*. *Chemistry of natural compounds*, *48*(4), 635-637.