Morphological and Molecular (ISSR and SCoT) characterization of some grape cultivars in the North Sinai

(Received: 01. 11.2019; Accepted: 15.11.2019)

Nagaty M. A. [1], Ibrahim Sh. D. [2], Basita A. H.[3]
[1]Plant Production Department, Faculty of Environmental Agricultural Sciences, Arish University
[2]Agricultural Genetic Engineering Research Institute (AGERI), ARC.
[3] Department of Genetics, Faculty of Agriculture, Cairo University

ABSTRACT

The present study was focusing on the morphological characterization of six grape cultivars collected from different locations in North Sinai. Also, to assess the genetic diversity using molecular markers (ISSR and SCoT) among these cultivars and four commercial genotypes. Data analysis of the cluster characters showed that highly significant differences were observed among the six cultivars for the average cluster weight and the number of berries/cluster. While, cluster length, and the average of cluster compactness revealed significant differences. Moreover, the results of berry physical characteristics showed a significant variation among the six cultivars. Nine ISSR and ten SCoT primers were used to determine the level of polymorphism, molecular identification of unique markers, and the estimation of genetic distances for the ten grapevine cultivars (6 cultivated in North Sinai and four commercial cultivars). The ISSR primers amplified 93 amplicons of which 51 were polymorphic. While, SCoT primers showed 136 amplicons, the total number of polymorphic amplicons was 52. The two molecular markers revealed positive unique markers only. The number of positive unique markers was 9 and was useful in identifying 5 genotypes out of the 10 cultivars. The similarity indices ranged from 92% to 76% and 94% to 84% for ISSR and SCoT, respectively. The cluster analysis exhibited a tendency the cultivars collected from North Sinai are groups in the same subcluster except Banaty White from the two molecular markers. It is concluded that we need the additional grapevine germplasm were collected from other locations and more morphological characters study to ensure the genetic diversity in grapevine germplasm.

***Key word**: North Sinai, Grapevine, Morphology, Molecular Markers (ISSR, SCoT).*

INTRODUCTION

The grapevine (*Vitis vinifera* L.) is perennial woody fruit plants, which grow in most regions as tropical, subtropical and temperate regions (Anupa *et al*., 2016). It is one of the most economically important crops in worldwide (Wang et *al.,* 2004 and Kurmi *et al.,* 2011).

Egypt production of grapevine was about 1.360.250 tons according to FAOSTAT (2017), the total area cultivated in Egypt is estimated to be about 153.682 feddans. Egypt is the fourth world producer of table grape. Moreover, the Ministry of Agriculture Statistics (2017) showed an increase in the area up to 188.543 feddans produced 1.378.815 tons.

The assessment of genetic diversity within and between plant populations is routinely fulfill using different techniques such as (i) morphological, (ii) biochemical characterization/evaluation (allozyme), in the pregenomic era, and (iii) DNA (or molecular) marker analysis especially single nucleotide polymorphism (SNPs) in postgenomic era (Govindaraj *et al*., 2014).Morphological characterization is the initial step in the description and identification of cultivars. For examples in this description are used shoots, leaves, bunches and berries at different phenological stages (Olv, 1984; Mahmoud *et al*., 2009; Abd El-Wahab, 2011 and Zrinka *et al*., 2017). Genetic improvement of the plant has been done through the development of a new generation of genetic markers. At the same time, the fulfillment of DNA sequence provides information uses in the identification of new species and unknown species (Haq *et al*., 2016).

Molecular markers are useful tools for characterizing and estimating the genetic diversity among different genotypes. Several molecular markers have been used for the assessment of genetic diversity of the grapevine by random amplified polymorphic (Ercisli *et al*., 2009 and Nagaty and El-Assal, 2011), in addition by simple sequence repeat (SSR) (Santana *et al*., 2010; Moreno-Sanz, *et al*., 2011 and Nagaty and El-Assal, 2011). ISSR markers are thought to be particularly useful for studying closely related individuals, which exhibit low levels of polymorphism (Sarwat, 2012). Moreno *et al.* (1998) expected the ISSR markers to be an efficient and reliable tool for distinguishing most grapevine varieties because they can readily differentiate the two closely related varieties. Several studies have been conducted to assess the diversity in grapevine using ISSR (Dhanorkar *et al*. (2005); Alizadeh and Singh (2009) and Seyedimoradi *et al.* (2012). A marker system called Start Codon Targeted Polymorphism (SCoT) quickly gained popularity after being described by Collard and Mackill (2009). Start codon-targeted (SCoT) marker is a new relatively dominant, simple, low-cost, highly polymorphic PCR-based technique, where primers were designed based on plant universal gene composition (Luo *et al*., 2010). These advantages have been validated through studies on genetic diversity in grape (Zhang *et al*., 2011), potato (Gorji *et al*., 2011), date palm (Adawy *et al*., 2014) and in olive (Al Samman *et al.*, 2017). The present study was focusing on the morphological characterization of six grape cultivars collected from different locations in North Sinai. Also, to assess the genetic diversity using two molecular markers (ISSR and SCoT) between these cultivars and four commercial genotypes.

MATERIALS AND METHODS

Plant materials

The plant material (fruits and leaves) used in the present investigation was comprised of the ten grapevine cultivars (Khalily Red, Balady White, Banaty White, Balady Red, Unnamed Red, Khalily White, Early Superior, Thompson, Flame and Red Globe). The studied grape cultivars from one to six are grown in different locations in Rafah and El Sheikh Zewaid cities in North Sinai Governorate (Table 1) and the rest cultivars (from 7 to 10) are the commercial cultivars (Early Superior, Thompson, Flame and Red Globe) which grown and distributed in the different farms in different locations in Egypt. Early Superior, Thompson, Flame and Red Globe cultivars are Table grapes, which are used for fruit production. Early Superior and Thompson produce fruits with white color, while Flame and Red Globe produce fruits with red color. Early Superior, Thompson and Flame cultivars used in this study are seedless cultivars.

Table (1): Grape (Vitis vinifera L.) cultivars and their locations in Rafah and El Sheikh Zewaied cities, North Sinai, Egypt.

No.	Cultivar	Location
1	Khalily Red	Rafah (Dawar El Tank, El Safa District)
2	Balady White	Rafah (Salah El Din Street, El Rasm District)
3	Banaty White	Rafah (Abo shnar District)
4	Balady Red	El Sheikh Zewaied (El Skadra District)
5	Unnamed Red	El Sheikh Zewaied (El Azhar District)
6	Khalily White	El Sheikh Zewaied (El Kawthar District)

Methods

Fruit physical characteristics

Some of fruit physical characteristics were recorded for the local Egyptian grape cultivars which grown and distributed in Rafah and El Sheikh Zewaid cities in North Sinai Governorate namely (Khalily Red, Balady White, Banaty White, Balady Red, Unnamed Red and Khalily White). The physical characteristics was carried out in 2018 season. The chosen vines were healthy and grown in a sandy loam soil in the private garden and with no irrigation (rainfall only). Three replicates for each cultivar were taken. Three clusters from each cultivar were harvested and immediately transported to the fruit laboratory of Plant production department, Arish University and kept in -20°C until fruit physical parameters recorded. The harvesting date was determined when soluble solids reached about 16–18 % and when berries attain full color stage according to Tourky *et al*. (1995).

In the present study, two different approaches were employed for the characterization (fruit physical characteristics and molecular characterization based on ISSR and SCoT markers). Fruit physical characteristics (cluster length, cluster weight, cluster compact, number of berries/cluster, weight of five berries, berry length, berry width, berry size and berry flesh thickness) were recorded for the local Egyptian grape cultivars which grown and distributed in Rafah and El Sheikh Zewaied cities in North Sinai Governorate namely: Khalily Red, Balady White, Banaty White, Balady Red, Unnamed Red and Khalily White

Cluster characteristics

Clusters were collected from upper, middle and lower position of the grapevine to record cluster Length (cm), cluster weight (g), number of berries/cluster and cluster compactness. Cluster compactness was measured using score range from 1 to 3 where 1 is very compact and 3 is less compact (Table 5) (Christodulou *et al*., 1968).

Berry characteristics

From the harvested grapes one Kg berries were chosen randomly for analyzing berry weight, berry size, berry length, berry width and berry flesh thickness. Berry length, width and flesh thickness measured using Vernier caliper whereas berry weight was measured using a digital top load balance with an accuracy of two decimal units.

Statistical analysis of fruit characteristics

Data was subjected to an analysis of variance (ANOVA) based on a randomized complete block design (RCBD) with six cultivars and three replicates for each cultivar. Data were analyzed using Co-STAT 6.13 software for windows to compare the cultivars. Means were compared using Duncan's multiple range test at the 0.05% level probability (Duncan, 1955).

Molecular characteristics

Thirty Leaf samples of Egyptian grapes belong to ten cultivars (three replicates for each cultivar) with different morphological traits were collected for molecular studies. Fresh young leaves samples of grape local cultivars located in North Sinai Governorate were collected from different locations in Rafah and El Sheikh Zewaid cities (Table 2). In the meantime, eight seedlings (two for each cultivar) (one-year-old) of four commercial Egyptian grapevine cultivars (Early Superior, Thompson, Flame and Red Globe) collected from Horticulture Research Institute farm, Agricultural Research Center (ARC), Giza, Egypt and these cultivars have different morphological traits were kindly provided by the Horticultural Research Institute – ARC. and the fresh young leaves samples were collected from these seedlings for molecular studies.

Table (2): Sampling details of six locations in North Sinai governorate, Egypt.

Location	City	Latitude(N)	Longitude(E)
١	Rafah (Dawar El Tank, Safa District)	31° 15' 10.8"	34° 13' 58.6"
2	Rafah (Salah el Din Street, El-Rasm District)	31° 16' 24.5"	34° 13' 01.3"
3	Rafah (Yamit, Abo shnar District)	31° 16' 53.2"	34° 10' 54.9"
4	El Sheikh Zewaied (El Skadra District)	31° ١5' 05.0"	34° 06' 55.2"
5	El Sheikh Zewaied (El Azhar District)	31° ١٣' 02.9"	34° 07' 32.2"
6	El Sheikh Zewaied (El Kawthar District)	31° 12' 59.2"	34° 06' 54.4"

DNA extraction and purification

Total DNA was extracted from fresh young leaves using DNeasy Kit (Qiagen) according to the manufacturer's protocol. DNA quality was determined visually on 1% agarose gel in comparison to 10 µl of a DNA size marker (100bp DNA). To estimate DNA concentration, compare the degree of fluorescence of the DNA sample with the different bands in DNA size marker.

ISSR-PCR Reactions

A set of 9 primers ISSR (Table 3) was used in the detection of polymorphism. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM primer, 1 U *Taq* DNA polymerase and 30 ng DNA templates.

Table (3): Sequence of ISSR primers.

Primer	Sequence 5'-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR- 6	5'-CGCGATAGATAGATAGATA-3'
ISSR- 8	5'-AGACAGACAGACAGACGC-3'
ISSR- 9	5'-GATAGATAGATAGATAGC-3'
ISSR- 10	5'-GACAGACAGACAGACAAT-3'
ISSR- 13	5'-AGAGAGAGAGAGAGAGYT-3'
ISSR- 14	5'-CTCCTCCTCCTCCTCTT-3'-
ISSR- 15	5'-CTCTCTCTCTCTCTCTRG-3'
ISSR- 18	5'-HVHCACACACACACACAT-3'

A: Adenine, T: Thymine, G: Guanine and C: Cytosine Y: (C or T), V: (A or C or G), H: (A or C or T)

Thermocycling Profile and Detection of the PCR Products

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 35 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 50ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. A 100bp DNA ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000).

SCoT-PCR Reactions

Ten primers of SCoT Table (4) were used in the detection of polymorphism among the ten grapevine. SCoT primers were designed as previously described by Collard and Mackill (2009). Synthesis of SCoT primers is carried out by HVD Vertriebs-Ges. m.b.H. (Vienna, Austria). The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM primer, 1 U *Taq* DNA polymerase and 30 ng DNA templates.

Table (4): Sequence of SCoT primers.

Primer	Sequence 5'-3'
SCoT-2	5'- CAACAATGGCTACCACCC -3'
SCoT-3	5'- CAACAATGGCTACCACCG -3'
SCoT-4	5'- CAACAATGGCTACCACCT -3'
SCoT-5	5'- CAACAATGGCTACCACGA -3'
SCoT-6	5'-CAACAATGGCTACCACGC-3'
SCoT-7	5'-CAACAATGGCTACCACGG-3'
SCoT-8	5'-CAACAATGGCTACCACGT-3'
SCoT-9	5'-CAACAATGGCTACCAGCA-3'
SCoT-17	5'-ACCATGGCTACCACCGAG-3'
SCoT-19	5'-ACCATGGCTACCACCGGC-3'

Thermocycling Profile and Detection of the PCR Products

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems). Cycler programmed at 94ºC for 5 min as an initial denaturation cycle. This was followed by 35 cycles, Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 50ºC for 1 min, and an elongation step at 72ºC for 1min. The primer extension segment was extended to 7 min at 72ºC in the final cycle. PCR products were visualized using the same procedure as in ISSR markers.

Molecular markers analysis

The banding patterns generated by ISSR-PCR and SCoT marker analyses were compared to determine the genetic relatedness of the samples under study. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated

according to Dice coefficient (Sneath and Sokal, 1973).

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

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. The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA) (Sneath and Sokal, 1973).

RESULTS AND DISCUSSION

Morphological character (Fruit physical characteristics)

Cluster characteristics

Data concerning cluster characteristics are presented in Table (5). Cluster length, cluster weight, cluster compactness and average number of berries/cluster were recorded as quantitative traits. With regard to cluster length, the results in Table (5) found that the differences among the grape cultivars were significant. Balady red and Balady white had the highest recorded values for cluster length (15.76 and 14.06 cm) respectively. For cluster weight and average number of berries/cluster, data illustrated in Table (5) showed that highly significant differences among sampled grape cultivars. Unnamed Red cultivar had the highest cluster weight (326.66 cm), while Khalily White had the lowest one (153.46 cm). As for average number of berries/cluster values, it was observed that Balady Red had the highest record (126), while Khalily Red cultivar had the lowest one (75). With regard to cluster compactness there were significant differences among sampled local grape cultivars for cluster compactness, Khalily Red, Balady White and Balady Red had the highest values (2) as compared to Banaty White, Unnamed Red and Khalily White which had the lowest records (1). It is clear that in Khalily Red, Balady White and Balady Red cultivars the cluster was well filled and compacted. The result in this respect is agreed with many investigators worked on different cultivars (Aisha *et al*., 1998 and Marwad, 2002).

Table (5): Average cluster length (cm), cluster weight (g), cluster compactness and number of berries/cluster of studied grape (Vitis vinifera L.) cultivars.

No.	Cultivar	Average cluster length (cm)	Average cluster weight (g)	Average cluster compactness	Average number of berries/cluster
1	Khalily Red	11.06 d	264.04 b	2	75.00 e
2	Balady White	14.06 b	175.93 d	2	91.00 d
3	Banaty White	13.27 c	163.85 e	1	109.00 c
4	Balady Red	15.76 a	223.88 c	2	126.00 a
5	Unnamed Red	13.56 bc	326.66 a	1	91.00 d
6	Khalily White	10.86 d	153.46 f	1	122.00 b

Means having the same alphabetical letters within each column are not significantly different at P≤0.05 according to Duncan's multiple range tests.

Berry characteristics

Data concerning berry physical characteristics are presented in Tables 6. The result showed a highly significant variation among sampled local grape cultivars for physical parameter records. Unnamed Red cultivar had the highest values for average weight of five berries, average berry size, average berry length, average Berry width and average berry flesh thickness (20.63g, 4.00 cm^3, 2.10 cm, 1.83 cm and 0.91 cm) respectively. In the meantime, Khalily White had the lowest values for average weight of five berries, average berry length, average berry width and average berry flesh thickness (7.44 g, 1.53 cm, 1.26 cm and 0.63 cm) respectively. While, Balady White had the lowest value in the average berry size (1.33 cm^3). The result is in regularity with many investigators worked on different cultivars (Mahmoud *et al*., 2009 and Abd El-Wahab, 2011).

Table (6): Average weight of five berries (g), average berry size (cm^3), average berry length (cm), average berry width (cm) and average berry flesh thickness of studied grape (Vitis vinifera L.) cultivar.

No.	Cultivar	Average weight of five berries (g)	Average berry size (cm^3)	Average berry length (cm)	Average berry width (cm)	Average berry flesh thickness (cm)
1	Khalily Red	19.35 b	3.66 a	2.10 a	1.76 a	0.88 a
2	Balady White	10.26 c	2.00 b	1.63 bc	1.36 c	0.68 c
3	Banaty White	8.24 d	1.33 c	1.63 bc	1.30 c	0.65 c
4	Balady Red	10.68 c	2.00 b	1.76 b	1.50 b	0.75 b
5	Unnamed Red	20.63 a	4.00 a	2.10 a	1.83 a	0.91 a
6	Khalily White	7.44 d	2.00 b	1.53 c	1.26 c	0.63 c

Means having the same alphabetical letters within each column are not significantly different at P≤0.05 according to Duncan's multiple range tests.

Assessment of the genetic diversity among the ten grapevine cultivars

The ISSR primers are based on SSR sequences (di, tri-, tetra-, or penta- nucleotides repeats), anchored to genomic sequence repeat (SSRs), (5' or 3') using two or four arbitrary, often degenerate nucleotides. Polymorphisms occur whenever one genome lacks the repeated sequence or has a deletion or insertion that modifies the distance between repeats. Compared to the SSR technique, ISSR does not require prior sequence information and generates a high number of polymorphisms, which makes it quick and easy to develop (Goulao *et al*., 2001). This technique is based on the observation that the short conserved regions of plant genes are surrounded by the ATG translation start codon (Sawant *et al*.,1999). The SCoT markers can be used either in isolation or in combination with other techniques to assess genetic diversity and to obtain reliable information about population processes and structure across different plant families

As shown in Tables (7 and 8) and Fig. (1) The total number of amplicons produced by the nine ISSR and ten SCoT primers was 93and 136 amplicons with averages of 9.3 and 13.6 amplicons/primer respectively. The total number of polymorphic bands was 51 (ISSR) with an average of 5.1 amplicons/primer and 52 (SCoT) with an average of 5.2 amplicons/primer. The number of polymorphic amplicons per primer ranged from one (ISSR-18) to 9(ISSR-6 and ISSR- 13) in ISSR and 5 to 10 in SCoT (Tables 7 and 8). The different primers revealed the different levels of polymorphism in the two molecular markers.

The highest average of marker polymorphism percentage (75.00 %,) was obtained by two ISSR primers (ISSR-6 and ISSR-8) and the lowest average of polymorphism (14.28%) was revealed by ISSR primer (ISSR-18). The two primers (SCoT-5 and SCoT-4) had no visible polymorphic bands in the SCoT (Tables 7 and 8). On the other hand**,** the percent of polymorphism reflects the genetic differences among the tested ten cultivars. The percentage of polymorphism revealed by SCoT and ISSR analysis was 38.24% and 54.83% (Tables 7and 8).

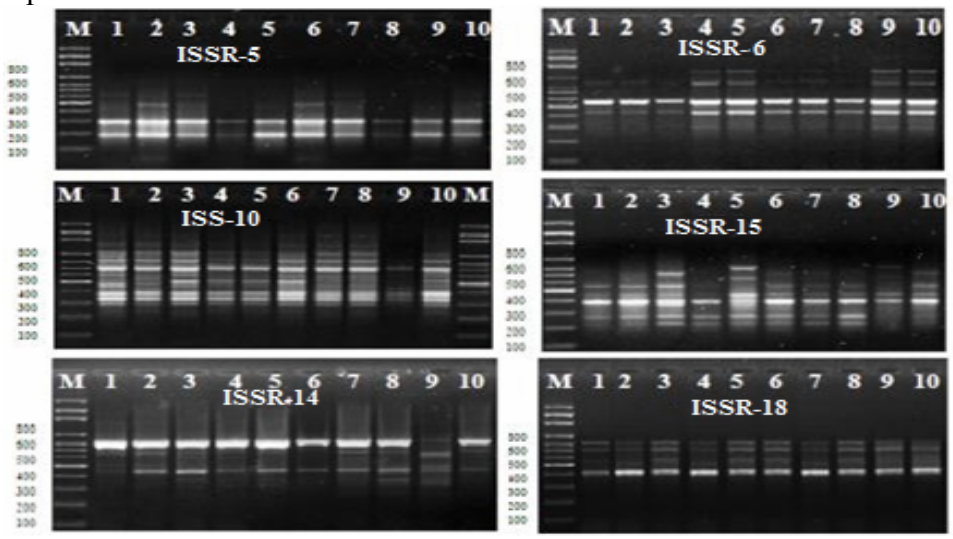

Fig.(1):ISSR profiles of the ten grapevine genotype. Lane 1 to 10 represent: Khalily Red, Balady White, Banaty White, Balady Red, Unnamed Red and Khalily White, Early Superior,Thompson, Flame and Red Globe respectively. M: molecular marker (100bp ladder).

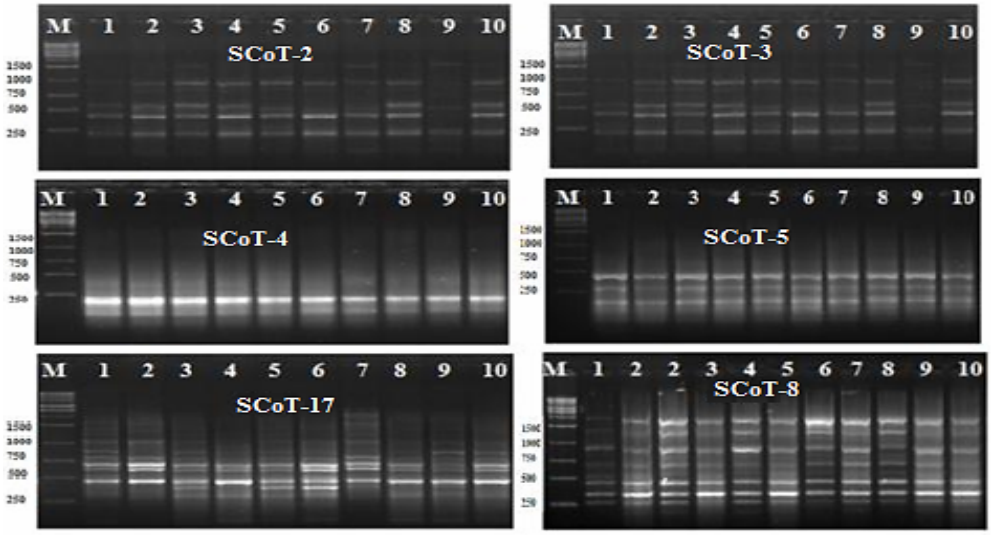

Fig. (2): SCoT profiles of the ten grapevine genotype. Lane 1 to 10 represent: Khalily Red, Balady White, Banaty White, Balady Red, Unnamed Red and Khalily White, Early Superior, Thompson, Flame and Red Globe respectively. M: Molecular Marker (1Kb ladder).

Table (7): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and polymorphism percentage as revealed by ISSR analysis among the 10 grapevine genotypes.

Name Primer	Total Amplicons	Monomorphic Amplicons	Polymorphic Amplicons	Polymorphism %
ISSR5	7	3	4	57.14
ISSR6	12	3	9	75.00
ISSR8	4	1	3	75.00
ISSR9	9	3	6	66.66
ISSR10	18	11	7	38.88
ISSR13	13	4	9	69.23
ISSR14	10	3	7	70.00
ISSR15	13	8	5	38.46
ISSR18	7	6	1	14.28
Total	93	42	51	54.83
Average	9.3	4.2	5.1	-

Table (8): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and polymorphism percentage as revealed by SCoT analysis among the 10 grapevine genotypes.

Name Primer	Total Amplicons	Monomorphic Amplicons	Polymorphic Amplicons	Polymorphism %
SCoT-2	10	4	6	60
SCoT-3	9	4	5	55.56
SCoT-4	8	8	-	0
SCoT-5	10	10	-	0
SCoT-6	14	9	5	35.71
SCoT-7	16	10	6	37.50
SCoT-8	19	12	7	36.84
SCoT-9	18	9	9	50
SCoT-17	18	8	10	55.56
SCoT-19	14	10	4	28.57
Total	136	84	52	38.24
Average	13.6	8.4	5.2	-

In this respect, Guo *et al.* (2012) found that in grapevine the total of 131 amplicons were produced 93.1% of them were polymorphic the average polymorphism information content was 0.82. The results also indicated that SCoT markers are informative and could be used to detect polymorphism for grape varieties. Ibrahim *et al.* (2016) used 24 SCoT primers generated 362 total fragments with 77.10% of polymorphism and 0.04 of average PIC. In seven grape varieties. The level of genetic similarity among the ten grapevine genotypes to Dice coefficient in the two molecular markers (ISSR and SCoT) is presented in Tables (9 and 10). The present results showed that the two types of markers ISSR and SCoT exhibited the different levels of similarity among the ten genotypes. The highest genetic similarity (94%) was revealed by two markers between Balady Red and Unnamed in ISSR and between Balady Red and Thompson in SCoT .While, the lowest genetic similarity (76%) was detected by ISSR between Balady White and Flame.

In this respect, Ibrahim *et al*., (2016) found that the similarity values between the varieties Thompson and Early Superior was (85%). This was followed by 84% of similarity between the two varieties Flame and Red Globe based on SCoT markers. Moreover, Mahmoud *et al*.(2009) found that the highest similarity index was recorded (1.0), between Black Monukka and Rich Baba cultivars, while the lowest similarity index was recorded (0.0) between Rich Baba and Queen cultivars

Table (9): Genetic similarity (GS) matrices computed according to Dice coefficient from ISSR.

	Khalily Red	**Balady White**	**Banaty White**	**Balady Red**	**Unnamed Red**	**Khalily White**	**Early Superior**	**Thompson**	**Flame**	**Red Globe**
Khalily Red	1.00									
Balady White	0.88	1.00								
Banaty White	0.88	0.85	1.00							
Balady Red	0.81	0.80	0.82	1.00						
Unnamed Red	0.79	0.78	0.81	0.92	1.00					
Khalily White	0.87	0.86	0.89	0.82	0.79	1.00				
Early Superior	0.88	0.87	0.88	0.83	0.79	0.86	1.00			
Thompson	0.86	0.86	0.87	0.85	0.81	0.87	0.90	1.00		
Flame	0.78	0.76	0.79	0.82	0.83	0.77	0.80	0.83	1.00	
Red Globe	0.82	0.84	0.85	0.82	0.80	0.87	0.87	0.86	0.81	1.00

Table (10): Genetic similarity (GS) matrices computed according to Dice coefficient from SCoT.

	Khalily Red	**Balady White**	**Banaty White**	**Balady Red**	**Unnamed Red**	**Khalily White**	**Early Superior**	**Thompson**	**Flame**	**Red Globe**
Khalily Red	1.00									
Balady White	0.89	1.00								
Banaty White	0.87	0.90	1.00							
Balady Red	0.90	0.91	0.88	1.00						
Unnamed Red	0.86	0.87	0.90	0.91	1.00					
Khalily White	0.89	0.93	0.88	0.92	0.88	1.00				
Early Superior, ,	0.88	0.89	0.88	0.87	0.87	0.88	1.00			
Thompson	0.90	0.91	0.91	0.94	0.89	0.91	0.88	1.00		
Flame	0.86	0.87	0.93	0.84	0.87	0.85	0.91	0.89	1.00	
Red Globe	0.89	0.93	0.90	0.92	0.90	0.93	0.88	0.93	0.87	1.00

In the present investigation the cluster analysis using the two molecular markers (ISSR and SCoT) revealed the dendrograms classified the genotypes into two main clusters (Fig.3). The first cluster included the three cultivars where Banaty White, Early Superior showed closer relationship than Flame. The Second cluster comprised 5 genotypes cultivated in North Sinai with two commercial cultivars Red Globe and Thompson cultivars for ISSR and SCoT respectively. This cluster divided into two subclusters the first one revealed Red Globe and Unnamed Red Red separated from the other genotypes for ISSR and SCoT respectively. The other subcluster included two groups in ISSR. The first one contains three cultivars, where unnamed Red and Thompson showed closer relationship than Balady White. The second group included 3 genotypes, where Khalily Red and Balady Red showed a closer relationship than Khalily White. While, for the SCoT markers the subcluster was divided into three groups the first one contain Khalily Red only, and the second group included Balady Red and Thompson and the third group contains the three remaining genotypes, where Balady White and Khalily White, showed the closest relationship. These discrepancies in the genetic similarity revealed by the different marker types could be attributed to the mechanism of detecting the polymorphism and genome coverage by the two different markers (Alsamman *et al*., 2017). In this regard, Mahmoud *et al*. (2009) determined the genetic diversity among the four grape cultivars using ISSR, which separated the four grape cultivars into two major groups.

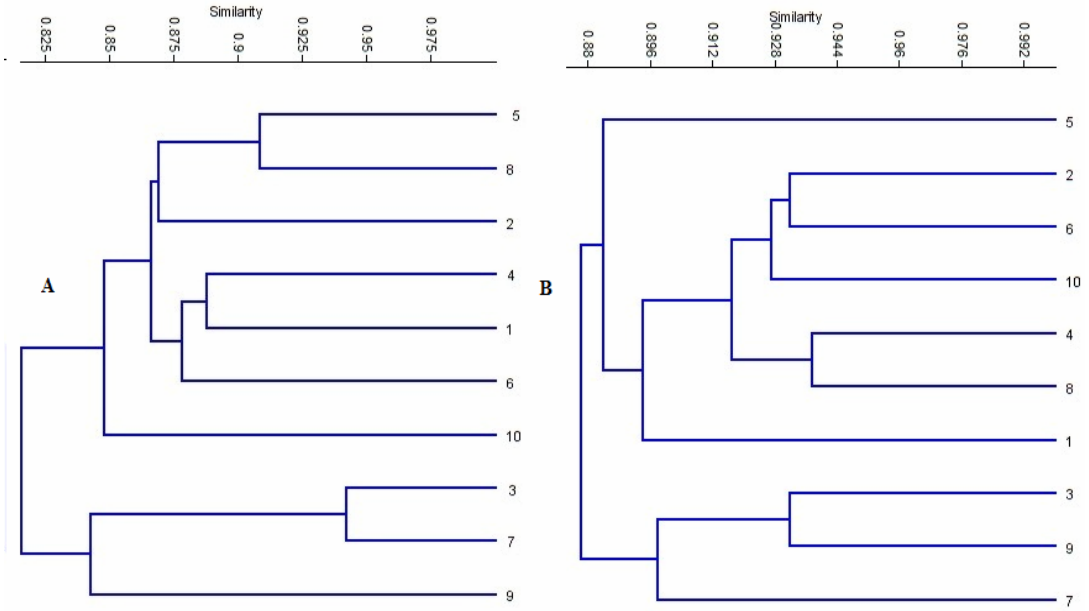

Fig .(3): Dendrograms for ten grapevine cultivars:(A) ISSR and (B) SCoT markers using UPGMA and similarity matrices computed according to Dice coefficient: 1=Khalily Red, 2=Balady White, 3=Banaty White,4= Balady Red,5= Unnamed Red ,6= Khalily White, 7=Early Superior,8=Thompson, 9=Flame and 10=Red Globe.

Unique markers obtained by two markers (ISSR and SCoT) were used the present investigation to characterized the ten grapevine cultivars. Five out of the ten cultivars were characterized by only 9 positive unique markers generated by two molecular markers as shown in Table (11). The Flame cultivar was revealed the highest number (4) of positive unique markers revealed by ISSR and SCoT. Three out of the four generated by ISSR (two with ISSR-14 at 679bp, 305bp and one at 313 bp with ISSR-15) and one with SCoT-2 markers at 767bp .While, Red Globe cultivar was characterized by two positive unique markers generated by ISSR with ISSR-13 at 306bp and ISSR-15 at 200bp. On the other hand, Balady White, Khalily White and Early Superior were characterized by one unique positive marker with ISSR-14, SCoT-9 and SCoT-17 at 500bp, 221bp and 1987bp respectively. In this respect, Ibrahim *et al.* (2016), who showed that SCoT analysis successfully characterized 73 unique positive and negative markers differentiating between the rootstock varieties especially those with green and red fruits. In this respect, Moreno-Sanz *et al*.(2011) presented a key 42 different genotypes have been detected through the SSRs molecular analysis of 293 accessions, 13 of them not being identified in the databases consulted.

Table (11): Genotypes characterized by unique positive ISSR and SCoT markers, marker size and total number of markers identified in each genotype for the five grape cultivars.

Genotypes	ISSR markers	Total# of makers genotypes	SCoT markers	Total# of makers genotypes	Grand total markers
	Unique positive markers		Unique positive markers		
Balady White	ISSR-14(500bp)	1	--		1
Khalily White	-	-	SCoT-9(221bp)	1	1
Early Superior	-	-	SCoT-17(1987bp)	1	1
Flame	ISSR-14(679bp) ISSR-14(305bp) ISSR-15(313bp)	3	SCoT-2(767bp)	1	4
Red Globe	SSR-13 (306bp) ISSR-15(200bp)	2	-	-	2

Our results showed that the different molecular markers ISSR and SCoT estimated the polymorphism among the ten cultivars. The Unnamed cultivar was separated in from the North Sinai genotypes in the SCoT molecular markers, thus maybe revealing that the genetic background different. Yue *et al.* (2019) pointed out the SCoT molecular marker technology can distinguish the materials with close genetic distance, and can be used for early identification techniques of grape mutant materials. On the other hand, Ibrahim *et al*.(2016) pointed out the SCoT technique was successful to target generic regions across grape genome. Moreover, the two makers analysis were more realistic results when the dendrogram analysis has showed two clusters, and this analysis fit together with some fruit characters. One cluster contains the three cultivars Banaty white, Early Superior and Flame were seedless. In this respect, Nagaty and El-Assal (2011) said that RAPD analysis

was more realistic results when the dendrogram analysis showed this analysis fit together with fruit characters. Moreover, SSR's showed very specific PCR products especially cultivars. These products were very specific to the fruit characters of different cultivars. Therefore, we need additional grapevine germplasm to be collected from other locations and more morphological characters' study to ensure the genetic diversity in grapevine germplasm.

REFERENCES

Abd El-Wahab, M. A. (2011). Description and Evaluation of some Grape cultivars under Egyption conditions. J. American Sci., 7 (10): 10 – 22.

Adawy, S.S.; Jiang, J.; Atia M.A.M. (2014). Identification of novel sex-specific PCR-based markers to distinguish the genders in Egyptian date palm trees. Int. J. Agric. Sci. Res., 4:45–54.

Aisha, S. A. Gaser; El-Mogy, M. M. and Omar, A. H. (1998). Comparative studies on description and evaluation of five new table grape cultivars under Egyptian conditions. Annals of Agric. Sci., 36 (4): 2473 – 2486.

Alizadeh, M. and Singh, S.K. (2009). Molecular assessment of clonal fidelity in micropropagated grape (*Vitis* spp.) rootstock genotypes using RAPD and ISSR markers.Iranian J. of Biotech., 7: 37-44.

Alsamman M. A; Adawy, S. S.; Ibrahim S. D.; Hussein, B. A. and Hussein, E. H. A. (2017). Selective Amplification of Start codon Polymorphic Loci (SASPL): a new PCR-based molecular marker in olive. POJ. :10(02):64-77.

Anupa, T.; Sahijram, L.; Samarth, R. and Rao, B.M. (2016). *In vitro* shoot induction of three grape (*Vitis vinifera* L.) varieties using nodal and axillary explants. Bio. scan., 11(1): 201-204.

Christodulou, A. J.; Weaver, R. J. and Pol, R. M. (1968). Relation of gibberellins treatment to fruit set, berry development and cluster compactness in *Vitis vinifera*. Proc. Am. Soc. Hort. Sci., 92: 301-310.

Collard B.C.Y. and Mackill D.J. (2009). Start codon targeted (SCoT) Polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol Biol Report. 27:86–93.

Dhanorkar, V.M.; Tamhankar S.A.; Patıl S.G.and Rao V.S. (2005).ISSR-PCR for assessment of genetic relationship among grape varieties cultivated in India.Vitis, 44: 127–131.

Duncan,D.B. (1955). Multiple range and multiple F-tests. Biometrics, 11:1-42.

Ercisli, S; Orhan,E ; Hizarci,Y. Yildirim, N. and Agar, G.(2009). Genetic diversity in grapevine germplasm resources in the Coruh Valley revealed by RAPD markers. Biochem Genet, 46:590–597.

FAO STATE (2017). Grapevine Fruit Production. Food and Agriculture Organization (FAO) of the United Nations.

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. Am. J. Potato Res., 88:226–237.

Goulao, L. and Oliveira, C. M. (2001). Molecular characterization of cultivars of apple (*Malus domestica* Borkh.) using microsatellite (SSR and ISSR) markers. Euphytica, 122(1):81-89.

Govindaraj, M; Vetriventhan, M. and Srinivasan, M. (2014). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. Hindwi, 2015:ID431787:1-14.

Guo, D.L.; Zhang,J.Y.and Liu, C.H.(2015). Genetic diversity in some grape varieties revealed by SCoT analyses. Mol.Biol. Rep., 39(5):5307-13.

Haq, Q.M. I; Hussain, T and Kumar A. (2016). Molecular markers: A tool to identify hidden science with especial emphasis on agricultural crops. International Journal of Biology Research, 1(5):50-59.

Ibrahim S.D.; Adawy, S.S.; Atia M.A.M; Alsamman, A.M. 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.

Kurmi, U.S.; Sharma, D.K.; Tripathi, M.K.; Tiwari, R.; Baghel, B.S. and Tiwari, S. (2011). Plant regeneration of 18 *Vitis vinifera* (L.) *via* direct and indirect organogenesis from cultured nodal segments. J. Agric. Technol., 7(3): 721-737.

Luo, C.; He, X-H.; Chen, H.; Ou, S.J. and Gao, M.P. (2010). Analysis of diversity andrelationships among mango cultivars using Start Codon Targeted (SCoT) markers. Biochem.Syst. Ecol., 38:1176–1184.

Mahmoud, Gehan H.S., Rizk-Alla, Mervat, S. and Mohamed, S.Y. (2009). Horticultural and molecular genetic characterization of some grape cultivars under desert land. J. Biol. Chem. Environ. Sci., 4(1):519-544**.**

Marwad, I.A., (2002). Comparative studies of five seedless grape cultivars under conditions of Qalubia governorate, Egypt. Egypt. J. Appl. Sci., 17 (1) 285-306.

Ministry of Agriculture A.R.E. (2017). Economic Agriculture, Department of Agriculture Economic and Statistics.

Moreno-Sanz,P; Loureiro, M.D. and Suárez,B.(2011). Microsatellite character-rization of grapevine (*Vitis vinifera* L.) genetic diversity in Asturias (Northern Spain). Scientia Horticulturae, 129: 433–440.

Moreno,S.; Martin, J.P. and Ortiz ,J. M. (1998). Intersimple sequence repeats PCR for characterization of closely related grapevine germplasm. Euphytica, 101: 117–125.

Nagaty,M.A and El-Assal,S. (2011). Molecular characterization and genetic relationships among some grape (*Vitis vinifera* L.) cultivars as revealed by RAPD and SSR markers. European Journal of Experimental Biology, 1 (1):71-82.

OIV (1984). Godes des characters des varieties et species de vitis. Paris.

Santana,J.C; Heuertz,M.; Arranz,C; Rubio,J.A; Martínez-Zapater,J.M and Hidalgo,E. (2010). Genetic structure, origins, and relationships of grapevine cultivars from the Castilian Plateau of Spain. Am. J. Enol. Vitic., 61 (2):214-224.

Sarwat,M.(2012). ISSR: A Reliable and Cost-Effective Technique for Detection of DNA. Methods in Molecular Biology (Clifton, N.J.) 862:103-21.

Sawant, S.V.; Singh, P.K.; Gupta, S.K.; Madnala, R. and Tuli, R. (1999). Conserved nucleotide sequences in highly expressed genes in plants. J. Genet., 78:123–131.

Seyedimoradi, H; Talebi, R; Hassani,D. and Karami,F.(2012). Comparative genetic diversity analysis in Iranian local grapevine cultivars using ISSR and DAMD molecular markers. Environmental and Experimental Biology, 10: 125–132.

Sneath, P. H. A. and Sokal,R. R. (1973). Numerical Taxonomy - The Principle and Practice of Numerical Classification. W.H. Freeman and Co., San Francisco.

Tourky, M.N.; El-Shahatand, S.S. and Rizk, M.H. (1995). Evaluation of some new grape cultivars in relation to growth, yield, berry quality and storage life. J. Agric. Sci. Mansoura Univ., 29 (12):153-5167.

Wang, Q.; Mawassi, M.; Sahar, N.; Li, P.; Violeta, C-T.; Gafny, R.; Sela, I.; Tanne, E. and Perl, A. (2004). Cryopreservation of grapevine (*Vitis* spp.) embryogenic cell suspensions by encapsulation–vitrification. Plant Cell, Tissue and Organ Culture, 77: 267–275.

Yue, Q; Zhang, C; Wang, Q; Wang, W; Wang, J and Wu, Y. (2019). Analysis on genetic diversity of 51 grape germplasm resources. Ciência Rural, 49(11): e20190247.

Zhang, J.; Guo, D.; Gong, Y.; Liu, C.H.; Li, M.and Zhang, G.H. (2011). Optimization of Start Codon Targeted Polymorphism PCR (SCoT-PCR) system in *Vitis vinifera*. J. Fruit Sci. 209:214.

Zrinka, K.; Ana, M.; Nikica, P. and Jure; B. (2017). Morphological and genetic characterization of vine grape cultivars of Herzegovina. Croatian Review of Economic, Business and Social Statistics,3 (2):1-9.

الملخص العربي

التوصيف المورفولوجي والجزيئي (ISSR و SCoT) لبعض اصناف العنب في شمال سيناء

محمد أحمد نجاتي[١] - شفيق ابراهيم[٢] – بسيطة عباس حسين[٣]

[١]قسم الإنتاج النباتي – كلية العلوم الزراعية البيئية – جامعة العريش

[٢]معهد بحوث الهندسة الوراثية الزراعية – مركز البحوث الزراعية

[٣]قسم الوراثة- كلية الزراعة – جامعة القاهرة

ركزت الدراسة الحالية على التوصيف المورفولوجي لستة أصناف من العنب تم جمعها من مواقع مختلفة في محافظة شمال سيناء، وكذلك تقييم التنوع الوراثي باستخدام اثنين من المعلمات الجزيئية (ISSR و SCoT) بين هذه الأصناف وأربعة أنماط جينية تجارية. أظهرت الخصائص المورفولوجية للعنقود أن الفروق ذات معنوية عالية قد لوحظت بين الأصناف السته بالنسبة لمتوسط وزن العنقود ومتوسط عدد الحبات / العنقود. بينما كشف طول العنقود ، ومتوسط التحميل للحبات عن اختلافات ذات معنوية. علاوة على ذلك ، أظهرت نتائج الخصائص الفيزيائية لحبات العنب تباينًا كبيرًا بين الأنماط الوراثية الستة. تم استخدام تسعة بوادئ من معلمات ISSR و عشرة من SCoT لتحديد مستوى التشابه الوراثي ، والتعرف علي الواسمات الفريدة ، وتقدير المسافات الوراثية لأصناف العنب العشرة (٦ مزروعة في شمال سيناء وأربعة أصناف تجارية تم جمع شتلاتها من معهد بحوث البساتين، مركز البحوث الزراعية بالجيزة). تم الحصول علي عدد ٩٣ أمبيلكون من ISSRمنها ٥١ كانت متعددة الأشكال. في حين أظهرت SCoT عدد136 أمبليكون ، كان إجمالي عدد أمبليكون ذات التباين الوراثي ٥٢. وقد أظهرت الواسماتين الأثنتين عن علامات ايجابية فريدة فقط . كان عدد الواسمات الفريدة الإيجابية ٩ وكانت مفيدًة في تحديد ٥ تراكيب وراثية من أصل ١٠أصناف . وتراوح التشابه الوراثي بين ٩٢ ٪ إلى ٧٦ ٪ و ٩٤ ٪ إلى ٨٤٪ لـ ISSR و SCoT ، على التوالي. أظهر تحليل االقرابة بناء علي مصفوفات التشابه ميل الأصناف التي تم جمعها من شمال سيناء في نفس المجموعة الفرعية باستثناء صنف البناتي الأبيض في كل من ISSR وSCoT . وخلصنا إلى أننا نحتاج إلى جمع المزيد من المادة الوراثية للعنب من مواقع أخرى ودراسة المزيد من الصفات المورفولوجية لدراسة التنوع الوراثي في المادة الوراثية للعنب على مدى أوسع .