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Evaluation of Yield, Fruit Quality and Molecular Diversity for Three Grape Cultivars under New Valley Conditions

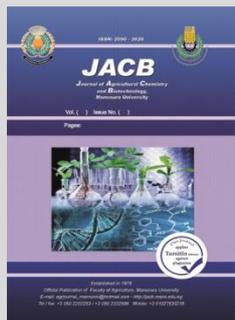
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Cross Mark



ABSTRACT

The current investigation was performed to evaluate the performance and DNA molecular analysis of three grape cultivars under the New Valley governorate, Egypt conditions. Most of the evaluated traits significantly varied among the cultivars. Superior cultivar had the best performance in cluster trait (weight, length and width), berries trait (number per cluster and 100 berries weight) and the total yield per vine. On the other hand, Flame cultivar overtopped in some quality traits (Total Soluble Solids, reducing and total reducing sugars). Molecular analysis was performed by using start codon targeted (SCoT) and inter-simple sequence repeats (ISSR) markers. The detected polymorphism was 68.38% and 41.84%, respectively. The polymorphism information content (PIC) for SCoT and ISSR markers was 0.29 and 0.19, respectively. Thus, SCoT marker was more informative. In contrast, the obtained dendrogram by ISSR showed a better clustering pattern than the SCoT marker.

Keywords: cultivar; genetic diversity; grape; molecular markers.

INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the world's most important fruit crops. It is the second fruit after citrus regarding its productivity but classified as the third major crop according to its cultivated area after citrus and banana. In Egypt, the grape productivity is about 1.7 million tons and the cultivated area is around 196,993 feddans (FAO, 2017). In the past decade, it was almost cultivated with Thompson seedless and Romi Ahmar cultivars. Recently, many cultivars have been introduced to Egypt (El-Morsy *et al.*, 2017).

New valley governorate is 1000 km fare from Cairo in the western desert. Grape is the second favorite fruit after Dates in it. Nevertheless, grape cultivation is still limited although the grape is one of the most successful fruit crops that can be cultivated in the newly reclaimed desert lands. Therefore, much grape quantity is imported from other governorates. However, this work could be contributed to the availability of cultural information regarding the cultivation of grapes in the New Valley governorate.

Phenotypic characterization has widely been used to evaluate the performance of different genotypes under unfavorable conditions (Cantini *et al.*, 1999). However, the obtained results often including the environmental effects too (Kumar, 1999). DNA Molecular markers are a good method to improve the ability of genetic diversity evaluation among genetic resources. They are not influenced by environmental conditions. During the last decade, ISSR PCR-based molecular marker has been developed to use a single primer from a sequence repeats usually 16 to 25 pb long, to amplify the region inter microsatellite sequence (Zietkiewicz *et al.*, 1994). Furthermore, at the beginning of the 21st century Start codon targeted (SCoT) markers have been used, based on the short conserved region flanking the starting codon ATG of a gene (Collard and Mackill, 2008; Xiong *et al.*, 2011). Both ISSR

and SCoT are dominant markers. They have widely been used in the detection of genetic diversity among different genotypes (Guo *et al.*, 2012; Bashandy, 2016).

The main aim of the current investigation is to evaluate the performance of three grape cultivars under the natural conditions of New valley governorate, Egypt using field evaluation and DNA molecular marker analysis.

MATERIALS AND METHODS

The current study was conducted during two successive seasons of 2017 and 2018 on six years-old grapevines of three seedless grape cultivars namely Early Sweet, Flame and Superior (Table 1). They were grown at Afak farm located at Balat, New Valley governorate, Egypt. The vines were planted at 3x1.5 meters apart in silty clay soil and the irrigation was by a drip irrigation system and supported by Gable system. Each cultivar carry 30 cluster per vine after manual slipping of clusters. The experiment was conducted in a randomized complete block design with three replicates for each cultivar. All farming management practices were carried out as recommended. At ripening stage, three clusters were randomly picked up from each vine and we estimated some morphological traits (i.e., cluster weight (g), cluster length (cm), cluster width (cm), number of berries per each cluster, 100 berries weight (g), berry weight (g) yield per vine (kg) and some quality traits (i.e., total soluble solids (TSS), reducing sugars (RS), non-reducing sugars (NRS), total reducing sugars (TRS) according to A.O.A.C. (1985) and pH).

Statistical analysis:

Analysis of variance (ANOVA) for a randomized complete block design according to Gomez and Gomez (1984) was conducted. Least significant differences (L.S.D) were used in mean comparisons. The recorded data were analyzed by using SAS statistical software program.

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Table 1. The name, origin and specific traits of the studied grape cultivars.

Cultivars	Origin	Traits description	Harvest date
Early Sweet	Foreign	Creamy white color, a large round seedless berry and has a highly sweet flavor.	1 May to 15 May
Flame	Foreign	Deep red color, round, sweet, vine-ripened by the sun and early season cultivar.	15 May to 30 June
Superior	Foreign	Obvious green color, a large round seedless berry tends to oval, Bunches abound with berries, crisp texture with a refreshingly sweet taste and early season cultivar.	15 May to 15 June

Molecular characterization:

All the molecular procedures were achieved at the Department of Genetics, Faculty of Agriculture, New Valley University, Egypt.

DNA extraction:

Genomic DNA was isolated from fresh immature leaves of each of the three grape cultures using plant DNA isolation kit (Favorgenv Biotech Corp. Cat. No. FAPGK001) as mentioned in the manufacturer manual. DNA quality and quantity were assessed by spectrophotometer, and then their final concentration was adjusted to 50 ng/μl.

PCR amplification and electrophoresis:

SCoT and ISSR reactions were prepared in a total volume of 25 μl containing: 12.5 μl of green PCR Master Mix, 2X (50 units/ml Taq DNA polymerase, 400 μM of each the four dNTPs and 3 mM MgCl₂), 2 μl of both 10 μM primer and DNA (50 ng) and finally 8.5 μl of ddH₂O. Amplifications were done in a thermal cycler (Labocon, U.K.). Twelve primers (by metabion) for each marker were used (Table 2). The used program was 94°C for 5 min as initial denaturation, 38 cycles including denaturation at 94°C for 45 sec, annealing for 1 min at 50°C and 48°C for SCoT and ISSR marker, respectively, extension at 72°C for 2 min and a final extension at 72°C for 7 min. PCR amplification products were separated on 1.5% agarose gels in 1×TBE (Tris-Borate-EDTA) buffer at 5 V/cm. Then, they were stained with ethidium bromide.

Data analysis:

We scored 1 for the present band and 0 for the absent band. Jaccard's similarity coefficient was used to estimate genetic similarity. A dendrogram was constructed based on the similarity matrix data by unweighted pair group method with arithmetic average (UPGMA), cluster analysis was calculated using the software computational package MVSP 3.1. program. Some indices namely, polymorphic information contents (PIC), Resolving power (Rp) and Marker Index (MI) were calculated according to Anderson *et al.* (1993), Prevost and Wilkinson (1999) and Powell *et al.*, (1996), respectively.

Table 2. ID and sequences of both SCoT and ISSR Primers.

SCoT Primers	Sequence (5' to 3')	ISSR Primers	Sequence (5' to 3')
SCoT 1	CAACAATGGCTACCACCA	UBC 807	(AG) ₈ T
SCoT 2	CAACAATGGCTACCACCC	UBC 808	(AG) ₈ C
SCoT 8	CAACAATGGCTACCACGT	UBC 810	(GA) ₈ T
SCoT 9	CAACAATGGCTACCAGCA	UBC 811	(GA) ₈ C
SCoT 14	ACGACATGGCGACCACGC	UBC 812	(GA) ₈ A
SCoT 18	ACCATGGCTACCACCGCC	UBC 814	(CT) ₈ A
SCoT 20	ACCATGGCTACCACCGCG	UBC 815	(CT) ₈ G
SCoT 25	ACCATGGCTACCACCGGG	UBC 823	(TC) ₈ C
SCoT 28	CCATGGCTACCACCGCCA	UBC 826	(AC) ₈ C
SCoT 33	CCATGGCTACCACCGCAG	UBC 834	(AG) ₈ TT
SCoT 35	CATGGCTACCACCGGCC	UBC 840	(GA) ₈ TT
SCoT 36	GCAACAATGGCTACCACC	UBC 862	(AGC) ₆

RESULTS AND DISCUSSION

The performance of the three seedless grapes was evaluated during two seasons. The results in Table (3)

showed that all the studied traits in both the two seasons of evaluations are varied significantly among them exemption the pH, also NRS in the second season.

1- Fruit and cluster traits evaluation:

According to the studied cluster characteristics, Superior cultivar had the highest value (645.9 g, 24.44 cm and 15.78 cm) in cluster weight, length and width, respectively in the first season. For the second season, as well as Superior cultivar was the best and the values were 654.6 gm, 24.11 cm and 15.21 cm, respectively. It followed by both Flame and Early Sweet in both the two seasons. Furthermore, Superior cultivar recorded the highest values of the berries number per cluster and 100 berries weight in both the first season (175.8, 356.0 gm, respectively) and the second season (177.3, 356.9 gm, respectively). Moreover, it was superior over the other cultivars in the total yield per vine in the first season (19.38 kg) and the second season (19.64 kg). All these evaluated characters are the most important indicators for comparing among different grape cultivars and very helpful for crop improvement (Viana *et al.*, 2011). The superiority of superior cultivar in yield is not only due to its superiority in all the above traits but also may be related to its high ability for biotic and abiotic adaptation (Soni *et al.*, 2019). The yield of Flame cultivar per vine was higher than the finding of Soni *et al.* (2019) who found that its production approximately 4.03 kg. on the other hand, the yield of Early Sweet cultivar was close to finding of El-Salhy *et al.*, 2019 (7.98 kg).

2- Quality parameters assessment:

Some quality traits were assessed for deep comparing among the studied cultivars (Table 3). The TSS significantly differed among the genotypes; all the cultivars had a value higher than the 12.5°Brix (minimum maturity reference value for table grapes, OIV 2008). The maximum value was recorded by Flame cultivar in both seasons (19.80, 20.50°Brix, respectively). Furthermore, the Flame cultivar had the highest value of reducing sugars and TRS in both seasons. These values were 18.83 for RS and 19.18 for TRS in the first season, while in the second season they were 19.17 and 19.58, respectively. Concerning NRS, Superior cultivar had the highest value (0.5167) in the first season, while in the second season the difference among the genotypes was not significant. For the acidity (pH), all the genotypes did not significantly differ. Its value ranged from 3.68 to 3.85 in both seasons. The chemical composition of the grapes is influenced by various factors such as age, genotype and growing conditions (Liu *et al.*, 2006). Flame cultivar had the maximum quantity of TSS and this finding is consistent with Soni *et al.* (2019). Thus, it is a preferable cultivar, because most the consumers prefer cultivars that have a lower level of acidity and a higher level of TSS (Jayasena and Cameron, 2008). On the other hand, El-Salhy *et al.* (2019) reported that TSS and reducing sugar values in Early sweet cultivar were 14.9 and 12.11, respectively, these results are close to our results.

Table 3. Mean performance of some agronomic and quality characteristics of the studied grape cultivars in the two seasons.

Traits Cultivars	Cluster Weight (g)	Cluster Length (cm)	Cluster width (cm)	No.berries /cluster	100 berries weight (g)	Yield/vine (kg)	TSS (°Brix)	RS %	TRS	NRS	pH
First season											
Flame	244.0 b	18.59 b	12.26 ab	136.1 b	164.2 b	7.320 b	19.80 a	18.83 A	19.18 a	0.2533 b	3.85 a
Superior	645.9 a	24.44 a	15.78 a	175.8 a	356.0 a	19.38 a	14.70 b	13.63 b	14.23 b	0.5167 a	3.74 a
Early Sweet	236.7 b	18.81 b	11.48 b	124.3 b	176.2 b	7.103 b	14.90 b	14.15 b	14.64 b	0.1500 b	3.78 a
LSD at 5%	42.44	3.536	4.143	21.46	18.97	1.276	1.885	2.093	1.692	0.222	0.6185
p-value	**	**	*	**	**	**	**	**	**	**	NS
Second season											
Flame	240.4 b	17.36 b	11.91 b	136.4 b	163.6 b	7.213 b	20.50 a	19.17 a	19.58 a	0.2833 a	3.76 a
Superior	654.6 a	24.11 a	15.21 a	177.3 a	356.9 a	19.64 a	15.40 b	14.26 b	14.95 b	0.5267 a	3.68 a
Early Sweet	238.5 b	19.22 b	10.89 b	126.2 b	174.6 b	7.140 b	15.81 b	14.39 b	15.20 b	0.7000 a	3.84 a
LSD at 5%	25.87	3.962	1.133	12.70	15.18	0.7285	0.7535	0.9998	0.7285	0.8088	0.3142
p-value	**	**	**	**	**	**	**	**	**	NS	NS

Note: ^{ns} = not significant, * = significant at 5 % probability level, ** = significant at 1 % probability level, LSD= Least significant difference, TSS= Total soluble solids, RS= Reducing sugars, TRS= Total reducing sugars, NRS= Non-reducing sugars, pH= Potential hydrogen.

3- Molecular characterization:

A- Polymorphism and diversity assessment:

To verify the genetic relationship among the three grape cultivars, SCoT and ISSR molecular analysis were performed (Fig. 1). A total of 117 bands were separated by SCoT marker, their size varied from 140 bp to 1650 bp. The polymorphic bands were 80 bands represented 68.38% polymorphism (Table 4). Regarding ISSR marker, 98 bands were scored and they varied from 130 bp to 1512 pb. Among the total, 41 were polymorphic bands, with 41.84% of polymorphism (Table 4). The SCoT and

ISSR markers have successfully been used in the determination of genetic relationships in plants (Bashandy, 2016, Basheer-Salimia and Mujahed, 2019 and Yue *et al.*, 2019). A significant difference was noted between detected polymorphism by the two markers. This difference is because each marker detects different sequences of the genome (Shahlaei *et al.*, 2014). The SCoT marker produced a higher polymorphism than ISSR, this result confirmed the finding of Paksresht *et al.* (2013), Shahlaei *et al.* (2014), Etminan *et al.* (2016) and Amom *et al.* (2020).

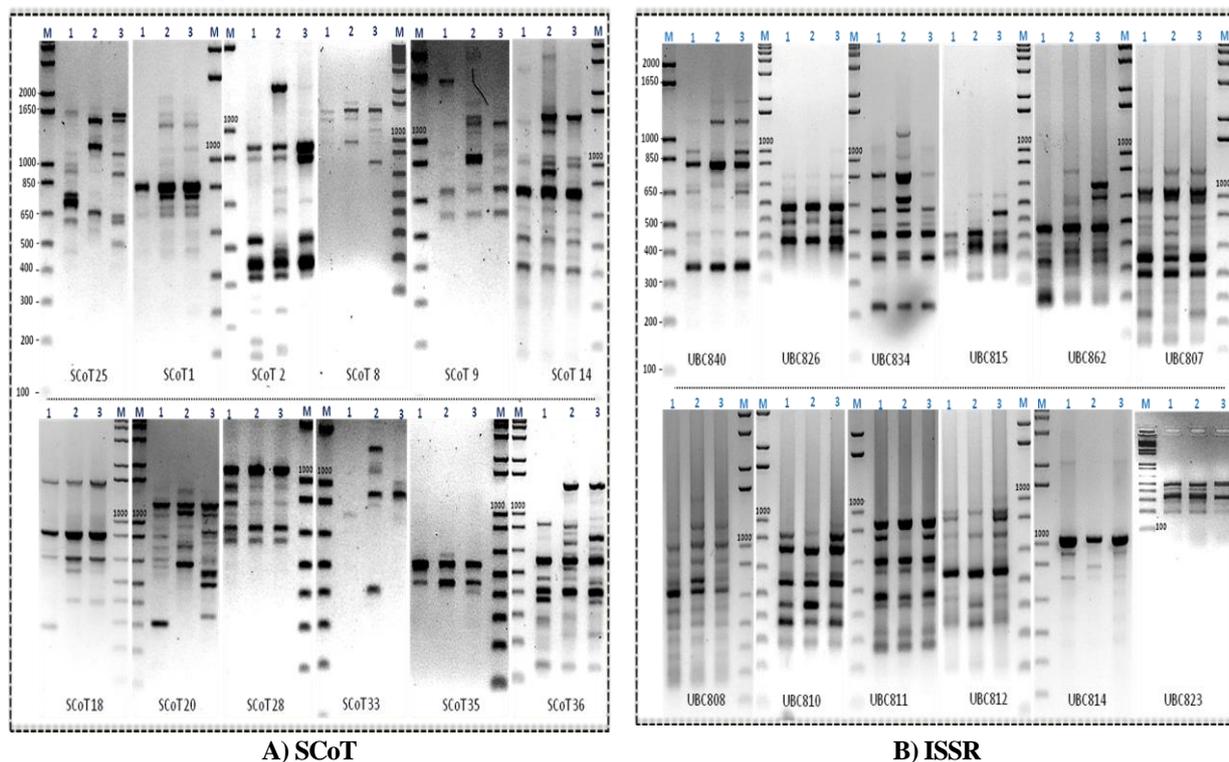


Fig. 1. (A) SCoT banding pattern and (B) ISSR banding pattern of the three grape cultivars. 1, Early Sweet; 2, Flame; 3, Superior, kb DNA marker.

Furthermore, we calculated the PIC value to measure the informativeness of Polymorphic DNA Markers. The PIC value of the SCoT marker varied from 0.13 to 0.45 with an average of 0.29, but this value ranged from 0.05 to 0.31 with the mean of 0.19 in the ISSR marker. In comparison between the two markers, the SCoT marker gave a higher PIC average than ISSR. Therefore, the SCoT marker has a higher capacity in the detection of polymorphism. Several results are consistent with this result (Paksresht *et al.*, 2013, Shahlaei *et al.*, 2014 and

Amom *et al.*, 2020). On the other hand, the primer resolving power (Rp) was calculated to select the best primer having the highest ability for detection of polymorphism. In the SCoT marker, the Rp value ranged from 1.33 for SCoT28 primer to 8.67 for SCoT25 primers. Regarding ISSR marker, the value of Rp varied from 0.67 to 4.68 for both UBC 810 and UBC 811 and UBC 812, respectively. Moreover, the marker index (MI) was calculated to demonstrate which marker is better at revealing molecular variations. SCoT marker had higher MI

value (2.3) than ISSR (0.77) indicating that the SCoT marker had a higher ability for detecting these variations. This is similar to the result of Gorji *et al.* (2011) who performed fingerprinting in some potato varieties and they found that the SCoT marker was more informative and effective than the ISSR marker.

Table 4. Polymorphism, Polymorphism Information Content, Marker index and Resolving power obtained by SCoT and ISSR markers in the three grape cultivars.

Primer Name	Range of fragment size bp	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	P %	PIC	MI	RP
SCoT								
SCoT 25	465-1650	13	0	13	100	0.45	5.85	8.67
SCoT 1	715-1320	8	3	5	62.5	0.28	1.4	3.34
SCoT 2	238-1285	15	3	12	80	0.36	4.32	8
SCoT 8	750-1650	6	3	3	50	0.22	0.66	2
SCoT 9	595-1650	10	2	8	80	0.36	2.88	5.34
SCoT 14	420-1560	11	7	4	36.4	0.16	0.64	2.67
SCoT 18	280-1640	6	3	3	50	0.22	0.66	2
SCoT 20	300-1480	15	3	12	80	0.35	4.2	8
SCoT 28	600-1080	7	5	2	28.6	0.13	0.26	1.33
SCoT 33	410-1395	7	0	7	100	0.44	3.08	4.67
SCoT 35	415-625	5	2	3	60	0.27	0.81	2
SCoT 36	140-1410	14	6	8	57.1	0.26	2.56	5.34
Total	-	117	37	80	-	-	-	-
Average	-	9.75	3.1	6.67	65.4	0.29	2.3	-
ISSR								
UBC 840	352-1390	10	5	5	50	0.22	1.1	3.34
UBC 826	230-990	7	4	3	50	0.19	0.57	2
UBC 834	220-1300	11	6	5	45.5	0.2	1	3.34
UBC 815	130-545	8	3	5	62.5	0.28	1.4	3.34
UBC 862	200-850	8	4	4	50	0.22	0.88	2.67
UBC 807	250-1030	11	9	2	18.2	0.08	0.16	1.33
UBC 808	495-1512	8	6	2	25	0.11	0.22	1.33
UBC 810	370-942	7	6	1	14.3	0.06	0.06	0.67
UBC 811	280-905	8	7	1	12.5	0.05	0.05	0.67
UBC 812	330-990	10	3	7	70	0.31	2.17	4.67
UBC 814	570-1025	6	2	4	66.7	0.3	1.2	2.67
UBC 823	200-500	4	2	2	50	0.22	0.44	1.33
Total	-	98	57	41	-	-	-	-
Average	-	8.2	4.75	3.42	42.89	0.19	0.77	-

P= Polymorphism, PIC= Polymorphism Information Content, MI= Marker index, PR= Resolving power.

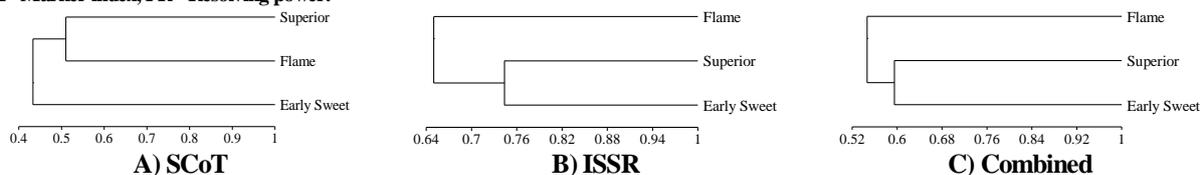


Fig. 2. The dendrograms of genetic distances among the three grape cultivars based on SCoT, ISSR and Combined.

CONCLUSION

The current investigation aimed to evaluate three grape cultivars under the New Valley governorate, Egypt conditions and determine the level of genetic diversity among them. The Superior cultivar surpassed over the two cultivars in the studied agronomic traits, while Flame cultivar was the best in the quality characters. On the other hand, the obtained dendrogram by ISSR was able to classify the cultivars according to their morphological characters.

ACKNOWLEDGMENT

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B- Genetic similarity and relationship:

Furthermore, the degree of similarity among the three cultivars was demonstrated by using Jaccard's similarity coefficient based on SCoT and ISSR data (Table 5). Regarding the SCoT marker, the highest similarity was 0.51 between Superior and Flame, while the lowest similarity (0.40) was between Early Sweet and Flame. Otherwise, the ISSR marker detected the highest similarity (0.74) between Superior and Early Sweet, but Superior and Flame showed the lowest similarity (0.64). The combined results of both markers showed the highest similarity (0.60) between Superior and Early Sweet as the ISSR marker, while Early Sweet and Flame recorded the lowest similarity (0.52) as the SCoT marker.

Also, the dendrograms of genetic distance using SCoT, ISSR markers and combined was constructed (Fig. 2). All the analysis divided the cultivars into two clusters, in SCoT marker the first one contained only Early Sweet, while Superior and Flame were gathered in the second cluster. Regarding both ISSR and combined, the first cluster included Flame only, but the second cluster contained both Superior and Early Sweet cultivars. The constructed dendrograms by both the used markers did not match, because the difference of the detected genetic variations by the two different markers. Many previous works used different markers and exhibited contradictions between resulted dendrograms (Sonia and Gopalakrishna, 2007, Gorji *et al.*, 2011 and Shahlaei *et al.*, 2014).The obtained dendrogram by ISSR marker and the combined gathered both Superior and Early Sweet cultivars in one cluster and this is consistent with that these cultivars are morphologically similar. Therefore, the resulted dendrogram by ISSR exhibited a better clustering pattern than the SCoT marker.

Table 5. The similarity index among the three grape cultivars based on SCoT, ISSR and Combined.

Genotypes	Early Sweet	Flame	Marker type
Flame	0.40	-	SCoT
	0.66	-	ISSR
	0.52	-	Combined
Superior	0.46	0.51	SCoT
	0.74	0.64	ISSR
	0.60	0.57	Combined

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تقييم الناتج وجودة الثمار والتنوع الجيني بين ثلاثة أصناف من العنب تحت ظروف الوادي الجديد

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تم إجراء الدراسة الحالية لتقييم الأداء والتحليل الجيني للحمض النووي لثلاثة أصناف من العنب تحت ظروف محافظة الوادي الجديد ، مصر. تباينت الصفات التي تم تقييمها بشكل معوي بين الأصناف. حيث كان تفوق صنف السوبريور في صفة العقود (الوزن والطول والعرض) ، و صفة الحبوب (العدد لكل عقود ووزن 100 حبة) والمحصول الكلي لكل كرمة. من ناحية أخرى ، فقد تفوق صنف الفليم في بعض صفات الجودة (المواد الصلبة الذائبة الكلية و السكريات المختزلة و السكريات الكلية المختزلة). و قد تم إجراء التحليل الجيني باستخدام الواسمات الجينية (SCoT) و (ISSR). حيث كانت النسبة المئوية لتعدد الأشكال المظهرية 68.38% و 41.84% على التوالي. و كان المحتوى المعلوماتي لتعدد الأشكال المظهرية (PIC) لكل من SCoT و ISSR كان 0.19 و 0.29 على التوالي. وهكذا ، كان واسم ال-SCoT أكثر إفادة. في المقابل، أظهر مخطط التحليل العقودي وقياس القرابة الوراثية للأصناف الذي تم الحصول عليه بواسطة الواسم ISSR نمط تجميع عقودي أفضل من الواسم الجيني SCoT.